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SMALL-SCALE VARIABILITY OF METALS IN SOIL

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1. Introduction

The pollutant distribution in different environmental compartments is often characterized by a high variability due to natural, i.e. geogenic, hydrological and meteorological, and anthropogenic influences. Uncertainties arising from all steps of the analytical process also determine the character and degree of the pollutant concentration uncertainty. These influences are generally unknown before starting the investigation. One important question to be answered in soil science is therefore: How accurate, i.e. how precisely and true, can the pollution state of soil be assessed by means of composite samples which are often analyzed to characterize the medium pollution at small scale?

2. Materials and Methods

A case study for investigating the metal concentrations at a small area of 1 m^2 uncultivated pasture, covered by grass and not specifically polluted, was selected to demonstrate the advantage and limitation of results obtained by composite samples.
25 individual samples were taken in a quadratic grid from the topsoil of this small area. The concentrations of 13 metals were determined after digestion with aqua regia by means of different techniques of atomic absorption spectroscopy.

3. Results and Discussion

The results of autocorrelation and semivariogram analysis which are common chemometric tools for describing the spatial relationship between individual sampling points show for all analyzed features that there is no significant correlation between the sampling locations. All measured elements are stochastically distributed in the investigated area.
Applying two-way analysis of variance the homogeneous spatial distribution of the features can be detected. The medium concentrations can then be assessed calculating the mean values. The high standard deviations ranging from 11.4 to 51.2% show the high variable nature of metal concentrations in soil also at this particular sampling „point".

The total measurement uncertainty consists of the sampling uncertainty and the uncertainties resulting from all remaining steps of the analytical process. These sources can be resolved by means of the law of error propagation. Applying subsequent one-way analysis of variance it can be shown that the sampling uncertainty influences the total measurement uncertainty considerably and, therefore, limits the precision of the soil pollution assessment essentially (see Figure).

The minimum mass of a sample to be analyzed must be high enough to avoid significant influence of the distribution inhomogeneity on its properties under investigation. To test this potential influence the whole topsoil of the investigated area was taken as a sample, homogenized and dried. A procedure of mass reduction to the following final sample masses was followed: 5 kg – 500 g – 50 g – 5 g. The Scheffé test as multiple mean comparison shows that

the mean values of the element concentrations in the samples with different masses are not significantly different from each other. Distributional inhomogeneities do not have a significant influence on the mean values in the investigated range of sample masses. Sampling of only 5 g soil does not distort the obtained metal concentrations compared to higher sample masses.

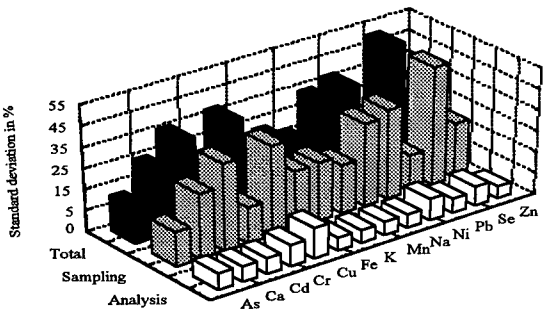
A simple approach based on the assumption of the distribution of the sample data helps to determine the number of increments necessary for the composite sample which reflects the features of interest with an a-priori demanded uncertainty.

Different sampling schemes for obtaining composite samples (which are of common use in soil research and monitoring) are applied. All of these composite samples reflect the same value which does not significantly differ from the true value analyzed in the total soil, i.e. the parent population.

4. Conclusions

Metal distributions in soil have a very high variability arising from different, mostly unknown influences also at small scale. Composite samples have to be recommended for assessing the pollution state also at one particular sampling „point".

Different chemometric methods exist to characterize the homogeneous or heterogeneous distribution, to assess the contribution of the sampling uncertainty to the entire measurement uncertainty, and to determine the sample mass and the number of increments which have to be taken for an undistorted, i.e. representative, pollution assessment.



MERCURY AND OTHER TRACE METAL DISTRIBUTIONS IN THE STRENGBACH BASIN (VOSGES MOUNTAINS, FRANCE).

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1. Introduction

Anthropic activities and particularly industrial waste waters, have become widespread sources of released metals in natural ecosystems. Because mechanisms of metal distribution, transport and accumulation are presently not well known, investigations were developed over the last ten years to study this problem. Most of these studies focus on heavily polluted ecosystems, and few concern 'natural' environments, i.e. ecosystems where anthropic activities are low. The purpose of this study was to evaluate the distribution of metal concentrations in the different compartments of a non-heavily polluted catchment: in the different aquatic phases (solution, suspended matter and bedstream sediments) and in soils. The objective was to relate the changes in trace metal concentrations to other chemical parameters, from along a stream, according to the 'natural' background level and local anthropogenic input influences.

2. Materials and Methods

From the west to the east, the Strengbach stream drains the eastern side of the Vosges mountains (upper Strengbach catchment), the nearby vosgian hills and then joins the Ill-river in the Rhine valley. The upper Strengbach catchment, under long-range atmospheric deposition influence, is forested, mainly laying on granite, sandstones and gneiss. The lower part of the catchment is characterised by winegrowing and the presence of a small town with moderate industrial activities. From up to downstream, the Strengbach and its main tributaries have been sampled (suspended matter, bedstream sediments and water). Six characteristic soil profiles (podzolic, brownic and calcareous brownic soils) as well as the different bedrocks, have also been sampled. Mercury (Hg) was analysed using the CVFAAS method (Messaïfta, 1997), and trace metals (Pb, Cd, Cu, Ni, Zn) by ICP-MS.

3. Results and Discussion

Streamwaters from the Strengbach and its tributaries are circumneutral to basic ($6.06 < pH < 8.22$) and are low to well buffered ($47 <$ alkalinity $< 2390 \mu mol/L$). All trace element concentrations increase from up to downstream, as well as DOC, pH and major element concentrations, except Hg and Al concentrations. The dissolved trace element concentrations ($0.8 < Zn < 7 \mu g/L$; $0.25 < Pb < 1.5 \mu g/L$; $0.3 < Cu < 2 \mu g/L$, $0.3 < Ni < 2 \mu g/L$) are in the same range of values commonly found in the literature for non-polluted sites. In the lower part of the catchment, trace element concentrations can be locally higher, particularly for Zn ($25 \mu g/L$), indicating the influence of anthropogenic activities. The relationships between the different chemical parameters show that DOC and pH seem to be the most important parameters controlling the Hg distribution in the stream (Figure 1). Compared to literature values for natural conditions, Hg concentrations were in the same range or slightly higher: 0-69ng/L in solution, 2-6.7µg/g in suspended matter, and 0.4-8µg/g in the fine fraction sediment (< 50µm). Along the Strengbach stream, the Hg concentration in the suspended matter tends to increase, contrary to Hg in solution, even if the suspended load remains low. This can be interprated as an adsorption of Hg on particulate phases in relation to pH increase. The waters and the suspended matter draining granitic bedrocks present higher Hg concentration than those on gneiss and sandstones, meanwhile Hg concentrations was higher in bedstream sediments from sandstone

bedrocks. According to these observations, the local geochemical background level was found to be 0.2 Hg µg/g. In the soils, Hg concentrations vary according to the type of soil and the profile depth. In all soil profiles, the surface layers were the most enriched one (79–262 Hg ng/g). These concentrations were in the same order of magnitude as values from the literature for non-polluted soils (100-300ng/g of total Hg; Alloway, 1995). However, one soil profile in the alluvion plain presents Hg contents 10 times higher than the others, particularly in the upper layers. These very high values were interpretated as the result of ancient sediment deposits after river flow events on a specific topographic location.

4. Conclusions

The distributions of mercury and other trace elements were characterised in the different compartments of a non-heavily polluted catchment. The most important factors which control their distribution seem to be pH and organic matter content in water and sediments, as well as in the soils. The Hg local background level was estimated to be 0.2µg/g. Hg in bedstream sediments reflects the different bedrock contributions. Moreover, concentrations higher than ambiant could be detected in relation to local anthropogenic inputs. These preliminary results need further detailed investigations, particularly concerning Pb, Cd, Cu, Ni and Zn in soils and sediments.

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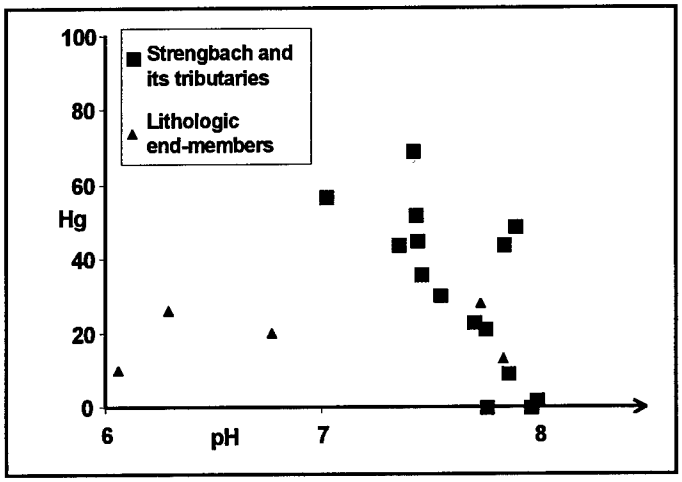

Fig. 1: Relationship between pH and Hg (ng/L)

FRACTIONATION OF CADMIUM IN SOME NEW ZEALAND SOILS

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1. Introduction

Chemical fractionation techniques have been used extensively to fractionate heavy metals such as Cd in soils in an attempt to determine their distribution between their various chemical forms (e.g. Mann and Ritchie 1994). However, in the case of Cd, the vast majority of investigations has involved soils that have been contaminated by sewage-sludge application, mining activity or have had simple Cd salts added. There have been very few Cd fractionation studies using uncontaminated soils or soils with relatively low levels of Cd contamination. The aim of this paper is to use a chemical fractionation technique to determine the forms and concentrations of Cd in a range of contrasting New Zealand soils at low Cd concentrations. In these soils, Cd has been derived from either the soil parent material, or Cd that has accumulated in the soil from the application of phosphate fertiliser.

2. Materials and Methods

Twelve topsoil samples (0-150 mm), which differ substantially in their total Cd content, chemical and physical properties were selected for this study. Total Cd in soils was determined using a nitric acid microwave digestion technique. The chemical fractionation technique adopted was essentially based on the method of Shuman (1985). The procedure separates Cd into five different fractions nominally described as; exchangeable Cd, organic bound Cd, amorphous Fe/Al oxide bound Cd, crystalline Fe/Al oxide bound Cd and residual Cd.

3. Results and Discussion

Total soil Cd concentrations ranged from 0.03 to 1.34 μg Cd g^{-1}. Total Cd was significantly correlated to total phosphorus ($r = 0.93$, $p < 0.001$), which indicates that the Cd in the soils studied was likely to have been derived predominantly from phosphate fertiliser application.

The concentrations of Cd determined in individual fractions for the soils are presented in Table 1. There was a large range in Cd concentrations extracted from individual fractions. For all soils studied, the smallest concentrations of Cd were found in the exchangeable fraction, whilst the largest concentrations of Cd were associated with the residual and organic fractions. Cadmium associated with the two oxide fractions were relatively low. The mean concentrations of Cd present in the different fractions decreased in the order: residual > organic >> amorphous oxide > crystalline oxide > exchangeable.

Table 1 Mean concentrations (μg kg^{-1} soil) of soil Cd in individual fractions

Soils	Cadmium in soil fractions (μg Cd kg^{-1} soil)						
	Exchange-able	Organic	Amorph-ous oxide	Crystalline oxide	Resi-dual	Sum	Tot
Ohura	21.0	113.0	64.3	51.7	100.0	350	312
Te Kuiti	35.1	414.0	42.1	207.7	912.0	1712	1338
Whakatane	23.8	397.0	60.7	52.3	241.0	775	741
Taupo	15.1	152.0	58.0	59.6	421.0	705	447
Rakaia	2.2	38.7	12.7	12.9	92.0	158	183
Temuka	5.4	21.6	8.6	9.7	47.1	92	74
Summit	1.0	8.5	6.7	6.2	9.1	31	31
Waiareka	1.0	73.7	18.9	15.5	29.0	138	125
Tai Tapu	4.5	94.5	16.9	13.8	103.0	232	193
Temuka	3.9	92.9	16.7	7.5	40.0	160	132
Patumahoe	29.6	311.9	124.9	84.2	195.0	746	744
Lismore	0.5	9.3	9.4	11.7	15.8	47	42

Results indicate that a large concentration of Cd is associated with the organic fraction. The large proportion of Cd in the organic fraction compliments the findings Gray *et al.* (1998) in relation to the importance of organic carbon in controlling solubility, sorption and desorption of Cd. The soils studied have medium to high organic carbon contents for New Zealand soils, which may indicate why this fraction is important.

4. Conclusions

Result show there is a wide range in the concentrations of Cd associated with individual soil fractions. The greatest concentration of Cd is associated with the organic and residual fractions, while the lowest concentration of Cd is in the exchangeable form. Results indicate that a substantial proportion of Cd added to the soil in P fertiliser applications ends up in the residual fraction of the soils. This suggest that a substantial proportion of fertiliser applied Cd in these soils has reverted to forms likely to be unavailable for plant uptake.

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Acknowledgments

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HEAVY METALS IN FLOODPLAIN SOILS, NATIONAL PARK "LOWER ODRA VALLEY", NORTHEAST GERMANY

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1. Introduction

River plains with semi-natural flooding regime are highly dynamic landscapes and function as matter sinks. According to nationwide measurement nets for soil state observation these landscapes reach strikingly high concentrations of pollutants. Although the path of suspended matter uptake is relatively clear, the general characterisation of increased contamination can not be justified due to high variation of the measurements.
Therefore a detailed study of the following processes is necessary:

- determination of the most important contaminants in flood plain soils
- detection of the bioavailability of heavy metals with sequential extraction method
- risk analysis of grassland use on the river banks to support the delineation of protected areas
- deduction of methods for a soil observation, which is adequate for this sensitive landscape type

The investigation of the effects of repeated flooding of areas in the southern part of the National Park "Lower Odra Valley" on the potential accumulation of inorganic pollutants was carried out.

2. Materials and Methods

The National Park "Lower Odra Valley" is located between the Polish city of Szczecin in the north and the German city of Angermünde in the south. The study area covers 18 km by 3 km. On three transects, which were set up according to the flood mapping on the basis of optical and radar remote sensing data and relief information, 45 sampling locations were identified. The coordinates were recorded with a DGPS so that the sample points were introduced into a local GIS.
Soil samples were taken at three different depths (2-10 cm, 15-25 cm, 30-40 cm) and analysed for 16 elements after digestion with aqua regia.
On 12 selected locations heavy metal content of plant material was determined after digestion with a mixture of nitric acid and hydrogen peroxide. Parallel to total content the available and mobile heavy metal contents of soil samples were determined according to a sequential extraction procedure developed by ZEIEN & BRÜMMER. All heavy metal analysis were done with ICP- equipment.

3. Results and Discussion

The accumulation of heavy metals was maximum in the upper soil layer (2-10 cm, Table 1). It was found that the average acid soluble heavy metal concentrations are higher than the geochemical background values, but they do not exceed the action values according to the governmental guidelines for soil protection. As expected, a good correlation exists between the heavy metal content and the amount of soil organic matter and clay minerals.

Table1: Average content of heavy metals in floodplain soil samples (2-10 cm, N=45)

Element	Minimum	Maximum	Mean	Median	SD**
As*	2,7	47,8	21,4	21,8	11,3
Cd	0,3	5,7	1,4	1,0	1,1
Co	1,3	22,1	10,1	10,8	4,4
Cr	3,3	130,6	60,2	64,4	31,9
Cu	1,6	161,0	42,6	38,6	32,2
Fe	0,3	5,7	3,1	3,5	1,3
Mn	90,0	4416,0	867,0	730,0	686,0
Ni	1,6	53,3	27,7	31,4	12,5
Pb	5,7	285,0	89,5	76,5	61,6
Zn	23,7	879,0	251.0	217,0	167,0

*all values in mg/kg except Fe in %, ** Standard Deviation

With linear regression analysis the dependencies of heavy metals of the relief position (terrain height) were determined. Every depression in the floodplain landscape acts like a sink and higher amounts of the contaminants can be accumulated in the soil.
For the risk management of soils polluted by heavy metals with regard to the transfer path between soil and plant, the mobile and available heavy metal fractions were determined by sequential extraction. The results show that up to 65% of the Cd-, 43% of the Zn-, 36% of the Mn and 29% of the Ni- acid soluble concentration is mobile and available. These amounts exceed significantly the trigger values, which are based on guidelines of the federal states. The corresponding plant analysis shows the same element distribution pattern with especially high manganese concentrations. Consequently a potential hazard exists for grazing animals in areas with a low topographic relief.
For a regionalisation approach, the relation of DGPS measured terrain heights and remotely sensed flooding frequencies were cross checked with linear regression. The coefficient of determination R^2 reached 0,75 at a 95% level of significance. This fact was taken to be an evidence that the flood mapping is an appropriate means for the regionalisation of the heavy metal measurements. Using 2nd order polynomial models, maps showing the spatial distribution of heavy metals in the soils of the study area were generated.

4. Conclusions

The soils in the flooding areas of the National Park "Lower Odra Valley" are contaminated with heavy metals. The distribution pattern of the contaminants depends on the topographical conditions and the organic matter and clay contents.
Due to high bioavailability of the elements Cd, Zn, Mn and Ni, a potential hazard exists for grazing animals in areas with an intense grassland use.
The regionalisation of point measurements can be achieved by satellite remote sensing data and geostatistics, if enough samples in an appropriate sampling strategy are available. The resulting spatial element distribution maps are useful tools for planning agencies and the National Park authority in their decision making to potential protection action.

AN APPLICATION OF DISJUNCTIVE KRIGING FOR DELINEATION OF HEAVY-METAL CONTAMINATED SOILS

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1. Introduction

Yates and Yates (1988) emphasized that conditional probability can provide a quantitative means of determining whether management actions are necessary. In a contaminated site, the conditional probability of a pollutant's concentration being above a threshold z_c can be coupled with the estimated pollutant's concentration to obtain the risk of false decision making (Journel, 1988). The risk of false decision-making indicate the magnitude of confidence for decision-makers (Juang and Lee, 1998). In disjunctive kriging, the conditional probability of a pollutant's concentration being above a threshold and the pollutant's concentration at an unsampled location can be simultaneously estimated. Moreover, disjunctive kriging is usually based on the semivariogram of normalized data y(x), which can prevent the spatial structure from interference of great variation and skewness of original data z(x). Therefore, we propose a way to obtain the risk of false decision-making via disjunctive kriging. A application to a real data set in a contaminated site in Taiwan is used for illustration.

2. Materials and Methods

A 5 ha paddy field in Taoyuan County, Taiwan (Fig. 1), was studied. The paddy field was irrigated with water, which was contaminated by heavy metals from the discharge of a chemical plant through irrigation channels. The Cd concentrations of soils (0-15 cm) extracted by 0.1N HCl at 78 sampling locations were used as the data set of this study.

To obtain the disjunctive kriging estimator, the original variable, z(x), must be transformed into a new variable, y(x), with a standard normal distribution. The disjunctive kriging estimator can be obtained from a combination of Hermite polynomials. It is shown as follows:

$$z_{DK}^{*}(x_o) = \sum_{k=0}^{K} C_k H_k^{*}[y(x_o)] \text{ and } H_k^{*}[y(x_o)] = \sum_{i=1}^{n} \lambda_{ik} H_k[y(x_i)] \quad , \quad [1]$$

where the series in Eq. [1] is truncated to K terms, and λ_{ik} are the disjunctive kriging weights. Based on Eq. [2], this allows the estimate of the conditional probability of exceeding a cutoff value z_c to be written in terms of $H_k^{*}[y(x_O)]$ as

$$\text{Prob}^{*}[z(x_o) \geq z_c] = \text{Prob}^{*}[y(x_o) \geq y_c] = 1 - G(y_c) + \sum_{k=1}^{K} g(y_c) H_{k-1}(y_c) H_k^{*}[y(x_o)] / k! \quad , \quad [2]$$

where y_c is the inverse value of z_c in the normal scale, and $G(y_c)$ and $g(y_c)$ are the cumulative distribution function (cdf) and probability density function (pdf), respectively.

False decision-making includes two conditions, false positives and false negatives. Based on whether the disjunctive kriging estimate is greater than the threshold ($z_{DK}^{*}(x_O) \geq z_c$) or not ($z_{DK}^{*}(x_O) < z_c$), the risks of false positives $\alpha(x)$ and false negatives $\beta(x)$ can be written in terms of the associated conditional probability. The $\alpha(x)$ and $\beta(x)$ are shown as follows:

$$\alpha(x_O) = \text{prob}[z(x_O) < z_c \mid z_{DK}^{*}(x_O) \geq z_c] = 1\text{-prob}^{*}[z(x_O) \geq z_c] \quad [3]$$

and

$$\square(x_o) = \text{prob}[z(x_o)\text{¡Öz}_c \mid z_{DK}^*(x_o)\text{¡Øz}_c] = \text{prob}^*[z(x_o)\text{¡Öz}_c]. \quad [4]$$

3. Results and Discussion

The semivariograms of the original values z(x) and normalized values y(x) for soil Cd concentrations are shown in Figs. 2 (a) and 2 (b). The experimental semivariogram of z(x) is so erratic that it is difficult to fit reliable models for kriging. The semivariogram of y(x) has obvious spatial-dependence and a reliable fitted-model. This indicates that the normalized values can prevent the spatial structure from interference associated to the great variation and skewness of the original values, and that disjunctive kriging estimation, thus, can be performed.

Based on the conditional probability of Cd concentration being above the threshold, 10 mg/kg, and the estimate of Cd concentration at an unsampled location estimated by using disjunctive kriging, the risks of false positives and false negatives were obtained and are shown in Figs. (3) and (4), respectively. The risk of false decision making can be used for hazardous areas delineation and as the basis for management actions. The areas with high risks also are prime candidates for additional sampling.

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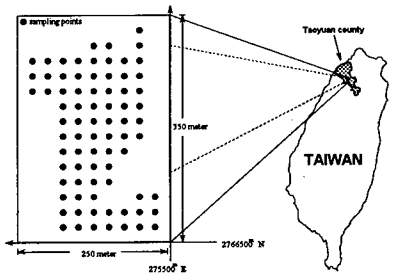

Figure 1: The study site and sampling points.

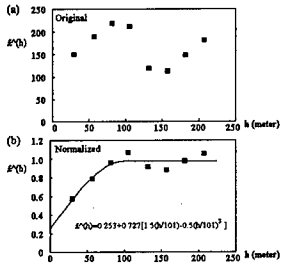

Figure 2: Semivariograms of (a) original and (b) normalized values for soil Cd concentrations.

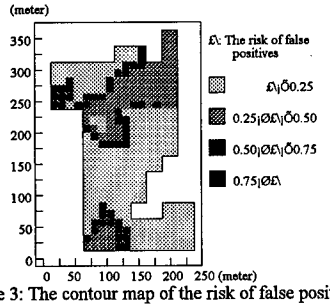

Figure 3: The contour map of the risk of false positives.

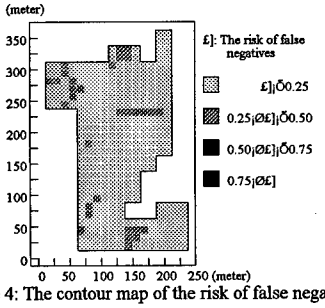

Figure 4: The contour map of the risk of false negatives.

COMPARISON OF THREE NONPARAMETRIC KRIGING METHODS FOR DELINEATING HEAVY-METAL CONTAMINATED SOILS

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1. Introduction

The well known technique of indicator kriging (JOURNEL, 1983) is usually used to predict the conditional cumulative distribution function (CDF) of an unsampled location given observed values. In the indicator approach, an observation z(x) is assigned to a precise measurement without measurement errors. However, in practice, most of the measurements are not exact. Thus, one derivative method, called probability kriging, was proposed. It uses the order relation of observed values to recover the information across all observations (CARR, 1994). The other kriging technique, ordinary kriging with the CDF of order statistics (CDF kriging), also was proposed to reserve the order relation of observations (JUANG et al., 1998). The objective of this study was to investigate whether probability kriging and CDF kriging are better than indicator kriging. A real data set of soil Cd and Pb concentrations in contaminated soils in Taiwan is used for illustration.

2. Materials and Methods

A paddy field about 10 ha in area in Taoyuan county, Taiwan (Fig. 1) was studied. The paddy field was irrigated with water, which was contaminated by heavy metals from the discharge of a chemical plant through irrigation channels. The Cd and Pb concentrations of soils (0-15 cm) extracted by 0.1N HCl at 55 sampling locations were used as the data set of this study.

The indicator function under a desired cutoff value z_k can be written as follows:

$$I(x;z_k) = \begin{cases} 1, & \text{if } z(x) \le z_k \\ 0, & \text{otherwise} \end{cases} \quad [1]$$

Assume the spatial pattern of the indicator coding is stationary and has a stationary mean, $F(z_k)$. Then, the CDF of the rth order statistic at the location x_r is denoted as $F_r(z_k;x_r)$ and defined as follows:

$$F_r(z_k;x_r) = \sum_{i=r}^{n} \binom{n}{i} [F(z_k)]^i [1-F(z_k)]^{n-i} \quad [2]$$

The uniform transformation function $U(x_r)$, also called the standardized rank, is defined as

$$U(x_r) = r/n, \quad [3]$$

where r denotes the rank of the rth order statistic $z(x_r)$.

The indicator kriging estimator, $I^*(x_o;z_k)$, at location x_o can be calculated by

$$I^*(x_o;z_k) = \sum \lambda_i I(x_i;z_k) \quad [4]$$

For CDF kriging, the indicator codes $I(x_i;z_k)$ in Eq. [4] are replaced by the CDF of order statistics $F_r(z_k;x_r)$, and then Eq. [4] also can denote the CDF kriging estimator $F^*(z_k;x_o)$. For probability kriging, the indicator code, $I(x_i;z_k)$, is assigned as the main variable and the uniform value $U(x_r)$ is assigned as the auxiliary variable in the cokriging estimator. Thus, the probability kriging estimator can be defined by

$$I^*(x_o;z_k) = \sum \lambda_i I(x_i;z_k) + \sum \lambda_r U(x_r) \quad [5]$$

For comparison of the three nonparametric kriging methods, the mean squared errors (MSE) of the kriging estimates obtained from cross-validation are used. The MSEs of the indicator kriging, probability kriging, and CDF kriging estimates are calculated using

$$\text{MSE} = \frac{1}{55}\sum_{r=1}^{55}[I(x_r;z_k)-I^*(x_r;z_k)]^2 \text{ or } \frac{1}{55}\sum_{r=1}^{55}[F(z_k;x_r)-F^*(z_k;x_r)]^2 \quad [6]$$

In addition, the relative reduction of MSE (RMSE) to indicator kriging was also calculated to assess the relative improvement of probability kriging and CDF kriging.

3. Results and Discussion

In Table 1, the MSE values of probability kriging and CDF kriging are obviously lower than that of indicator kriging. For soil Cd concentrations, the RMSE values of probability kriging and CDF kriging relative to indicator kriging are 54% and 32%. For soil Pb concentrations, the RMSE values of probability kriging and CDF kriging relative to indicator kriging are 21% and 27%. This indicates that the accuracy of the probability kriging and CDF kriging estimations is much higher than that of the indicator kriging estimation. However, some unreliable estimates were obtained in this case from the probability kriging estimation. Therefore, CDF kriging is more suitable than probability kriging for estimation of the probability of heavy-metal concentrations lower than a cutoff value.

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Table 1: The mean squared errors (MSE) and reduction of mean squared errors (RMSE) of indicator kriging (IK), probability kriging (PK), and CDF kriging (CDFK) via the cross-validation procedure.

	Cd		Pb	
	MSE	RMSE	MSE	RMSE
IK	0.1860		0.1543	
PK	0.0715	54%	0.1209	21%
CDFK	0.1267	32%	0.1122	27%

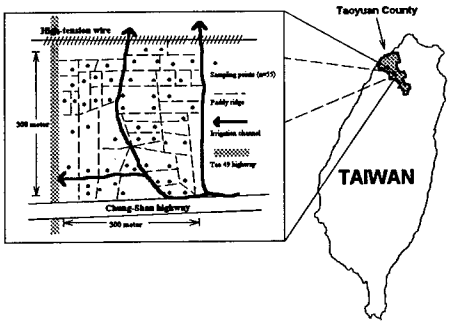

Figure 1: The study site and sampling points.

GEOCHEMICAL BEHAVIOUR OF TOXIC METALS IN THE ROCK-SOIL-PLANT SYSTEM IN AREAS UNDERLAIN BY BLACK SHALES OF THE OKCHON ZONE, KOREA

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1. Introduction

High contents of potentially toxic metals are present in natural geological materials, for example, Cd, Mo and U in some black shales (Thornton, 1983). The Okchon black shale in Korea provides a typical example of this. The high levels of these elements in black shales are of significance in environmental geochemistry, and soils derived from these parent materials tend to reflect their extreme geochemical composition, and may influence human health by determining the elemental composition of crop plants. Environmental geochemical surveys were undertaken in areas underlain by black shales in the Okchon Zone, in order to investigate the enrichment levels and dispersion patterns of toxic metals in rocks, soils and plants, and to evaluate the uptake ratios of trace metals from soils into plants.

2. Materials and Methods

Black shale samples in the Guryongsan and Changri Formations of the Okchon Zone were taken from available outcrops in all the study areas. Surface soil samples (0-15cm depth) were classified into mountain, farmland and paddy soils. Rock and soil samples were analysed for multi-elements by INAA and ICP-AES at Activation Laboratories Ltd. (ACTLAB) in Canada. Plant samples (rice, lettuce, chinese cabbage, red pepper, soybean, sesame, corn) were taken with associated paddy and farmland soils. Plant samples were vigorously washed in deinoised water in order to remove the majority of particles of soil or dust adhering to the outside of plants. Finely milled plant samples were digested with fuming nitric acid and perchloric acid and the resultant solutions were analysed for Cu, Pb, Zn and Cd elements by AAS.

3. Results and Discussion

Black shales : Mean concentrations of Ba, Cd, Mo, V, U and Zn were highly enriched in Okchon black shales, and their mean concentrations were significantly higher than those in average shales. In particular, these element concentrations were the highest in the Duk-Pyung area, and V was also enriched in the Chung-Joo area and Ba in the Bo-Eun and Chu-Bu areas. The highest mean concentrations of trace were found in black shales from the Duk-Pyung area.

Soils : Mean concentrations of As, Ba, Cd, Cu, Mo, Th, U and Zn in soils overlying black shales were significantly higher than those in normal soil reported by Bowen (1979) e.g. 30 mg/g As, 24 mg/g Mo and 50 mg/g U were found in soils from the Duk-Pyung area and 39 mg/g As, 15 mg/g Mo and 27 mg/g U in the Chu-Bu area (Table 1). Arsenic and Mo concentrations in soils from these areas were higher than the permissible level (Kloke, 1979).

Plants : Mean concentrations of Cu, Zn and Cd in rice grains were generally higher than those in normal rice grains grown on uncontaminated soils. The highest mean concentrations of 0.61 mg/g Cd were found in rice grains from the Duk-Pyung area. Mean concentrations of Cu, Zn and Pb in crop were not higher than the upper range values for normal plants (Kabata-Pendias and Pendias, 1984), but that of Cd was higher than the upper limit of 0.2 mg/g. Mean concentration of Cd was the highest in lettuce, and decreased in the order of lettuce > chinese cabbage > red pepper □ soybean = sesame > corn. There were no large differences in Cu and Zn concentrations in crop plant species.

Soil-plant interactions : Significant linear relationships were found between Cu, Zn, Pb and Cd concentrations in paddy soils and rice stalks. Correlation coefficients of Cu and as essential

micronutrients for plant metabolism in soils and rice stalks were higher than those of Pb and Cd as non-essential. Copper, Zn, Pb and Cd concentrations in farmland soils and crop plants were significantly correlated. Copper and Zn showed similar degree of uptake in plant species. However, Cd and Pb showed various degree of uptake in plant species, and higher Cd and Pb uptake were found in chinese cabbage than in corn. The biological absorption coefficients (BAC) of Cu, Cd and Pb in plant species decreased in the order of chinese cabbage > red pepper > rice stalk > rice grain. This indicates that leafy plants such as chinese cabbage and lettuce accumulate trace metals from soil to a greater degree than cereal products such as rice grain (Adriano, 1986).

4. Conclusions

Arsenic, Ba, Cd, Mo, V, U and Zn were highly enriched in Okchon black shales. The highest mean concentrations of these elements were found in black shales from the Duk-Pyung area. Mean concentrations of As, Mo and U in soils derived from black shales occurring in the Duk-Pyung area (30mg/g As, 24mg/g Mo and 50mg/g U) and Chu-Bu area (39mg/g As, 15mg/g Mo and 27mg/g U) were higher than the permissible level suggested by Kloke (1979). Element contents in plants and soils were significantly correlated. The concentration of Cd in plant species decreased in the order of lettuce > chinese cabbage > red pepper > soybean = sesame > rice stalk > corn > rice grain. The BACs in plants were in the order of Cd > Zn = Cu > Pb, which suggests that Cd is more bioavailable to plants than Cu, Pb and Zn.

Table 1. Ranges and mean concentrations of trace elements in soils overlying black shales from the Chung-Joo, Duk-Pyung, Bo-Eun and Chu-Bu area in the Okchon Zone (in mg/g).

Area		As	Ba	Cd	Cu	Mo	Pb	U	Zn
Chung-Joo	Range	3-21	660-2,300	0.6-1.9	18-89	1-15	13-284	2-14	50-350
	Mean	6	938	1.1	27	3	38	4	141
Duk-Pyung	Range	9-113	93-6,381	0.2-7.2	24-403	1-134	12-370	5-780	59-841
	Mean	30	956	0.5	87	24	45	50	160
Bo-Eun	Range	3-93	490-2,300	1.2-3.2	28-127	1-30	22-86	3-13	50-323
	Mean	20	1,239	1.8	52	7	35	6	176
Chu-Bu	Range	6-340	370-15,000	0.2-20.1	20-217	1-240	14-182	1-450	50-1,100
	Mean	39	1,623	1.6	64	15	47	27	184
Normal soil*		6	500	0.35	30	1.2	35	2	90
Permissible level**		20	2,000	3	100	5	100	-	300

*Bowen (1979), **Kloke (1979)

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NATURAL MERCURY EMISSIONS: REVISED ESTIMATES AND THE GLOBAL BALANCE

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1. Introduction

Recent measurements of background mercury emissions have been made in a number of areas of the natural terrestrial environment, and in areas previously subject to disturbance for mining and minerals processing. Separate independent estimates of the regional and continental size of such emissions indicate their potential importance to the global balance of mercury. This paper will discuss implications for that balance of emerging data.

2. Materials and Methods

Measurements have been carried out by both flux chamber analyses and by derivation from gradient measurements at sensor tower arrays of mercury emissions from background areas. These measurements most recently include areas of Tennessee, California and Nevada, USA, and of eastern Canada. Method intercomparisons indicate a slight lower bias in gradient measurements from collocated flux chamber analyses.

3. Results and Discussion

Both flux chamber measurements and vertical fluxes calculated from two level tower measurements indicate larger than expected outgassing of elemental mercury from a background area in west-central Nevada, USA. In addition, unexplained large excursions were noted immediately following local precipitation. It is not known yet whether this is due to water displacement of pore-gas mercury, disassociation of particle-bound mercury, or some other process.

Similar measurements, relying primarily on flux chambers, have also been carried out in Tennessee, USA, and Ontario, Canada. When the measurements from Tennessee were scaled up to continental scale (Lindberg, pers. comm., 1998), resulting values for "natural background" emissions were approximately equal to the more precise U.S. anthropogenic mercury emissions figure of 44 to 48 Mg/yr (Levin et al., 1994; US EPA, 1997). Porcella et al., scaling natural fluxes downward from global estimates to the U.S. land area purely on spatial scaling, arrived at similar figures.

Gustin et al. (1998), however, estimated emissions to air from the land area of Nevada from both natural and disturbed background sources. When these are scaled to the U.S. land area, fluxes of about 92 Mg/yr result. The indication is that mercuriferous soils and geologic formations in the intermontane west of the U.S. offer enrichments of about a factor of 2 from natural background (and highly vegetated) soil areas of the eastern U.S. Measurements in the Canadian Precambrian

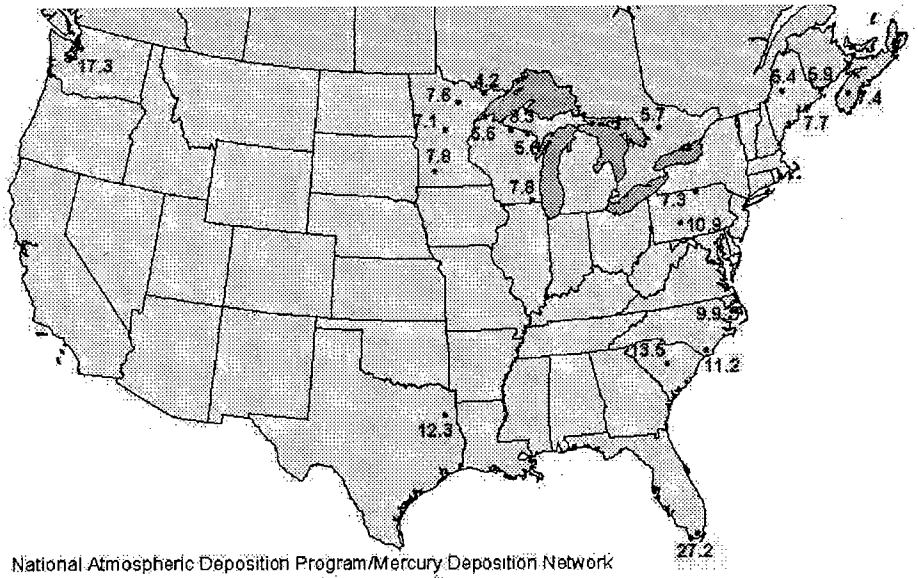
Shield region indicate emissions similar to those of the Nevada sites, in areas with sparse ground cover.

Control of emissions inventories is provided by (relatively sparse) deposition measurements in the U.S. and eastern Canada (Fig. 1). There are to date no evident longitudinal gradients, but data are essentially absent west of the Mississippi Valley. New measurement data expected over the next several years may provide a better estimate.

4. Conclusions

Recent measurements at both natural background and impacted sites in remote areas indicate total mercury emissions to the atmosphere, when scaled to North American geographic scales, may approximate the mass rate of industrial total mercury emissions. To date, model results have not accounted for this additional source category, whose geographic and vertical distribution is somewhat different from those of industrial sources. Evidence that these background emissions at ground level, primarily elemental mercury, impact trajectory calculations of mercury source regions is lacking (J. Keeler, CEC Mercury Experts' Workshop, Las Vegas, NV, October 1998). Future mass balance calculations, and derivation of deposition contributions, must account for these source areas.

Fig. 1. Mercury concentration in precipitation, Mercury Deposition Network, 1997 (Source:



MDN, 4/99)

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TRACE ELEMENTS AVAILABILITY IN WEATHERED SOILS FROM PARANA, BRAZIL

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1. Introduction

In the last 20 years, Brazilian agricultural research institutes have tried to increase the country's productivity and to minimize the degradation of natural resources in areas already cultivated as well as in the new frontiers. For that purpose, great effort has been placed on the evaluation and development of soil conservation and fertilization practices as well as on the understanding of soil degradation processes. However, in spite of the information generated, problems of erosion with exposure of sub-surface horizons are very common and can be easily seen, particularly in regions where intensive mechanization is practiced. This is the case in the state of Paraná in southern Brazil. On the other hand, because of its urban characteristics, the country is facing another serious problem: the pollution of water bodies by soil sediments and by increasing production and inadequate disposal of wastes. At this moment, because of health and environmental concerns, the collection, treatment and disposal of wastes have gained significant momentum in the most densely populated regions of Brazil. Land application is an important option for the disposal and recycling of some wastes in Paraná, as well as for land reclamation. However, there are concerns about the fate of pollutants potentially present in such residues, among which are the following trace elements: Cd, Cu, Cr, Ni, Pb, and Zn. Therefore, in order to evaluate the potential transfer of such elements to the food chain and drinking water, and to allow future decisions concerning this topic, it is a must that representative soils from Paraná are characterized.

2. Materials and Methods

A Rhodic Hapludox, a Quartzipsammentic Haplorthox, and a Typic Acrohumox chosen on the basis of their parent materials, their chemical, physical and mineralogical characteristics, their economic importance, their potential use for wastes recycling, their degree of degradation, requirement for reclamation, and influenced by major urban centers of (Londrina, Paranavaí and Curitiba), were sampled from three important agricultural regions Paraná, Brazil. Soil profiles were opened in units previously described by CASTRO FILHO (1988), CHODUR (1990), and NIETER (1989). The following chemical and physical parameters were determined: texture, pH, potential acidity (H plus Al), exchangeable Ca, Mg, Al and K, extractable P, total organic C, base saturation, and Al saturation. Soil samples taken from different depths, were air dried, ground and sieved through a 2-mm polyethylene sieve. Total and extractable Cd, Cu, Cr, Ni, Pb and Zn were determined. A nitric-perchloric digestion was conducted. Digests were analyzed by flame atomic absorption with a Perkin Elmer 3030B spectrophotometer. Extractions with EDTA, DTPA and Mehlich 3 were proceeded. The results were expressed on an oven dry basis.

3. Results and Discussion

Cadmium was below detection limit both in the total digestions and in the extractions of all soil profiles. Total metals (Cr 0.25-535.86; Cu 9.38-3228.0; Pb 1.03-174.81; Ni 2.79-577.34; Zn

0.13-121.12 mg kg^{-1}) were overwhelmingly greater for the Londrina soil followed by Contenda and then by Paranavaí. This could be explained by their respective parent materials (basalt, migmatite and Caiuá sandstone). The levels of Mehlich 3, EDTA, and DTPA Cu, Pb, Ni and Zn were much lower than their total contents, showing that only a small fraction of the trace elements is potentially bioavailable. In the case of extractable Cu, the results were consistently higher for Londrina and very similar between Paranavaí and Contenda. Mehlich 3 Cu values (0.17-11.22 mg kg^{-1}) tended to be higher than EDTA Cu (<0.01-5.7 mg kg^{-1}) and DTPA Cu (<0.01-6.23 mg kg^{-1}) for all depths. Total Pb fluctuated for the Londrina soil, but remained relatively unchanged with depth for the Paranavaí and Contenda soil profiles. However, total Pb was always the highest for Londrina. On the other hand, Mehlich 3 Pb (<0.01-3.41 mg kg^{-1}) and DTPA Pb (0.04-1.96 mg kg^{-1}) values tended to be highest for the surface horizons of the Contenda soil, fact that could be explained by the location of this profile, within 50 m of a major interstate highway. For all cases, EDTA Pb (<0.01-1.80 mg kg^{-1}) tended to decrease with depth, but differences among the soils for this parameter were not evident. Extractable Ni was below the detection limit when extracted with Mehlich 3 and EDTA (<0.01-0.05 mg kg^{-1}), with the exception of the most surface horizon of the Londrina soil. DTPA, however, detected decreasing amounts of Ni (<0.01-0.27 mg kg^{-1}) with depth for both the Londrina and Contenda soils. Extractable Zn showed the same pattern for all extractants. However, Mehlich 3 Zn values (0.07-2.56 mg kg^{-1}) were higher than EDTA Zn (0.13-2.27 mg kg^{-1}) levels which tended to be greater than the DTPA Zn values (0.08-1.75 mg kg^{-1}). For the three soils, the Zn values were much greater in the most surface layer of the A horizon for all three extractants. Below that, the values were relatively constant. This distribution of Zn in the soil profiles could be explained by the fact that this element is also a micronutrient which could be concentrated in the surface by plant cycling.

4. Conclusions

The characteristics determined for the three soils sampled from Paraná indicate the necessity to use nutrients and liming materials for agricultural purposes. This necessity is mostly evident in the subsurface horizons which have been exposed by erosion and require reclamation. If treated, wastes could be considered an alternative source of nutrients and, or liming to address such problems. This would be possible if some potential contaminants such as trace elements were kept under acceptable levels and applied at agronomic rates. However, a better understanding of trace elements background levels, bioavailability, and behavior in oxisols are required.

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BIOGEOCHEMICAL CYCLING OF SELENIUM: THE OCEAN AS A SOURCE OF SE TO TERRESTRIAL ECOSYSTEMS

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1. Introduction

It has been documented that biological processes in the ocean are a source of selenium in the marine atmosphere (COOKE and BRULAND 1987). This may explain why surface soils in coastal districts of Norway are strongly enriched in selenium compared to similar soils farther away from the ocean, and supports the older hypothesis (LÅG and STEINNES 1974) that there is a marine source of atmospheric selenium supplied to the coastal terrestrial surface. In this paper the presently extensive evidence from Norway of the marine environment as a source area for Se to coastal terrestrial ecosystems is reviewed.

2. Atmospheric Deposition

Analysis of terrestrial mosses is used on a routine basis to monitor atmospheric deposition of a great number of trace elements in Norway, and Se is among the elements that can be monitored in this way. The geographical deposition pattern of Se from surveys in 1977 and 1985 (STEINNES et al. 1994) is shown in Figure 1. In the south there is a contribution from long range transport of pollutants in a similar way as for some heavy metals. The main trend however is a strong deposition gradient perpendicularly to the coast line at all latitudes, also in the north where air pollution plays an insignificant role, indicating a natural source. A predominantly natural origin of the excess Se deposition in coastal districts is supported by analysis of peat cores from ombrotrophic bogs (STEINNES 1997). Whereas the surface peat, reflecting the deposition in recent years, exhibits a geographical distribution of Se very similar to that of the terrestrial moss, the peat at 50 cm depth which was formed before the industrial revolution consistently shows 5-10 times higher values near the coast than at sites with a typical continental climate.

3. Surface Soils

A study of humus horizon samples of forest soils from some selected districts in Norway revealed that Se showed a consistent coast-inland gradient, which led to the hypothesis referred to above (LÅG and STEINNES 1974). Additional analyses of similar surface soils from a later, nationwide collection (STEINNES et al. 1997) made it possible to construct the isopleth shown in Figure 2, which appears quite similar to the deposition maps from the moss analysis. A higher Se concentration in coastal districts was shown also for agricultural soils (WU and LÅG 1988).

4. Plant Availability

Very few data exist on the Se content of vascular plants in Norway. Analyses of cereal grains grown at different sites in Norway (LÅG and STEINNES 1978) indicate that the high Se content in coastal surface soils may not be readily available for root uptake in plants. An attempt to study its speciation in organic surface soil and ombrotrophic peat (TORSVIK 1992) led to the understanding that Se is very strongly bound in these humic materials. The exchangeable fraction was always less than 5% of the total Se concentration, and a considerable fraction was

not soluble even in concentrated nitric acid. It is therefore conceivable that the supply of Se from the marine environment to the coastal ecosystems is not paralleled by a higher uptake in the terrestrial food chains in coastal areas.

5. Discussion

Discussions in the scientific literature on the behaviour of Se in the terrestrial environment have so far mainly been concentrated on regions where soils of sedimentary origin naturally contain exessively high concentrations of this element. The opposite problem related to possible Se deficiency problems has received much less attention. In Norway a majority of soils are developed on a granitic or gneissic bedrock. The few data that exist for Se in such rocks indicate that the concentrations are very low, possibly 0.01 ppm or less (BRUNFELT and STEINNES 1967). Thus a major part of the selenum in Norwegian surface soils, at least in coastal areas, is probably cyclic Se from the ocean. The biogeochemical cycling of Se induced by marine organisms is therefore a very significant process, and deserves to be studied further both in Norway and elsewhere.

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THE APPROACHES TO AMELIORATION OF SE ECOCYCLE IN SE-DEFICIENT BIOGEOCHEMICAL AREA AND ITS EFFECTS ON SE DIETARY INTAKE AND HEALTH IN CHINA

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1. Introduction

We have studied the geographical distribution of selenium in the geo-ecosystem (rock-soil-plant-animal-man) in China. Low selenium environments occur mainly in and near temperate forest and forest-steppe landscapes, and form a wide belt of low selenium eco-environment running from North-East to South-West, which coincides well with the belt of Keshan disease (KSD an endemic cardiomyopathy related to low Se biogeochemical area) and Kashin-Beck disease (KDB an endemic osteoarthropathy also linked to selenium deficiency)[1-3]. It has been fully proven that selenium supplementation to people living in Se-deficient areas can prevent both Kashin-Beck and Keshan diseases. Usually sodium selenite and organic selenium such as yeast selenium are directly used for controlling the two diseases in China. However, a better control is to elevate dietary selenium intake of people in affected areas by ecological-economical measures, namely developing the economy and improving low Se eco-environment which will improve living standards, elevate Se intakes levels, to eradicate the diseases, without resorting to frequent Se-supplementation to the residents. We have carried out a five year research project (1991-1995) based on the above ideas. It involves the measures for economic development, environmental improvement, and Se ecocycle amelioration. As a ppart of the project present paper is mainly to study the foliar spray of sodium selinite on wheat, the selenium status in the diet and the Kashin-Beck disease cases in the area.

2. Materials and Methods

The study areas Xialiang and Fuyou villages and control village Baidian are located in Bin County, Shaanxi Province in Loess Plateau of low Se belt. Sodium selenite was applied as foliar spray on plants during grow season at rate of 6-14 g/ha for wheat and 1.5 g/ha for vegetables. Wheat and mixed diet samples were collected before and after the application of the Se. The diet samples were obtained from ten randomly selected families in each studied village. The Se concentrations of all the samples were determined by DAN fluorescence spectrophotometry after wet acid digestion.

3. Results and Discussion

The research results are shown in table 1 and table 2. Table 1 shows that the measures adopted in examined villages were efficient to improve Se dietary intake and hair Se level, which indicates Se metabolism level in human body, and to promote Se ecocycle in environment-life system. Foliar spray of sodium selenite increased selenium concentration in wheat from 0.009mg/kg to 0.081mg/kg ($p<0.01$) (Table 1). As a result, the mixed diet in local residents also increased significantly ($p<0.01$). Accordingly, the selenium dietary intake for adult increased from 12 μg/day before the Se supplementation to 47μg/day after the Se spray. Notice that in our previous study the daily dietary intake of Keshan disease and Kashin-Beck disease areas in China was classified as below 20μg/day, while the US government recommended daily intake of

Se for human was 50-200μg/day [4]. The Se concentration in the hair of the children in the area was 0.014mg/kg, below the Se deficiency threshold of 0.200mg/kg, increased to 0.275mg/kg after the Se application ($p<0.05$).

Table 1. Se levels before and after the measures adopted in village Xialiang (mg/kg)

Samples	Before Measures	After measures	P
wheat grain	0.009□0.002(10)	0.081□0.003(10)	<0.01
Mixed diet	0.014□0.006(6)	0.078 □0.024(8)	<0.001
Se dietary intake(□g□d)	12	47	
Hair	0.014□0.070(8)	0.275□0.015(11)	<0.05

From table 2 you can see that Kashin-Beck disease can be controlled by ameliorating Se ecocycle and elevating Se intake. The foliar spray of Se to plants improved human Se nutrition states, the x-ray detectable cases of Kashin-Beck disease among children of 5-13 years in Xialiang and Fuyou villages deceased dramatically, from more than 40% before the Se treatment, to less than 7% (Table 2). While in the control village of Baidian, the abnormal children detected in the same period were 28.7 and 21.6%, respectively. Last year 1997, we conducted the third examination of Kashin-Beck disease in above villages, almost no new patient can be find there.

Table 2. KDB cases detected by x-ray in children aged 5-13 before and after measures adopted

time tested	Village	Tested	cases by x-ray test		Abnormal metaphysis		Abnormal distal end	
			cases	rate	cases	rate	cases	rate
1992	Xialiang	31	13	43.3	10	33.3	10	33.3
Before M.	Fuyou	82	42	51.2	28	34.2	24	29.3
	Baidian*	108	31	28.7	24	22.2	18	16.7
1995	Xialiang	31	2	6.5	0	0	2	6.5
After M.	Fuyou	96	6	6.3	1	1.1	6	6.5
	Baidian*	102	22	22.0	17	16.7	13	12.8

* Village Baidian without any ameliorative measures

4. Conclusions

This study have proved that the comprehensive measure aiming at amelioration of living standard and elevation of Se dietary intake is very effective and feasible ways to improve human Se nutrition in Se deficiency and to control the diseases associated with Se-Deficiency in low Se biogeochemical regions.

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BACKGROUND LEVELS OF TRACE AND ULTRA-TRACE ELEMENTS IN SOILS OF JAPAN

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1. Introduction

For studies on the behavior of elements in the soil-plant systems, knowledge about background levels, or natural abundance, of elements in soils is required as basic information. In recent years, there has been ever increasing concern about the concentration levels of various trace and ultra-trace elements in environmental samples. In spite of this growing interest, the efforts of most soil scientists appeared to be still centered around rather limited numbers of beneficial elements (B, Mn, Fe, Cu, Zn and Mo) and/or typical pollutants (Cr, Cd, As, Hg and Pb). Phenomena occurring in the "real world" are mostly so complicated that usually no clear cut answer can be reached so far as the research works are limited to the element in question alone. Because the elements within the same group and/or subgroup of the Periodic Table possess similar chemical as well as physical properties, it can be expected that a more comprehensive understanding will be obtained when all the elements are comparatively studied in relation to their position in the Periodic Table. In addition to this, various kinds of elements, which were not commonly used in the past, are now increasingly consumed by modern industries for the production of numerous new materials. There is a strong possibility that the waste disposal of these industrial products will cause new types of environmental pollution. In order to cope with the situation mentioned above, we have attempted to determine as many elements as possible in a large number of soil samples by using the most advanced analytical techniques.

2. Materials and Methods

A quadrupole type ICP-MS (inductively coupled plasma - mass spectrometer), HP-4500 (Hewlett Packard, USA) was used for most of trace elements (> 0.1 mg kg^{-1} soil). For several ultra-trace elements (< 0.1 mg kg^{-1}) and/or trace elements susceptible to interferences due to spectral overlaps, however, it was necessary to use a high resolution ICP-MS, ELEMENT (Finnigan MAT, Germany). This type of ICP-MS is equipped with a double focusing mass spectrometer to separate the analyte ions from the overlapping polyatomic ions originating from gaseous components and/or matrix elements. The commonly used quadrupole type ICP-MS are incapable of separating chemically different ions at the same nominal mass values and are therefore more prone to inaccurate results especially when the concentrations of analytes become low (Yamasaki, 1996). For the latter instrument, a special attachment (capacitive decoupling device) was installed to enhance the sensitivity. Inductively coupled plasma atomic emission spectrometry (ICP-AES) as well as atomic absorption spectrometry (AAS) were also used for elements of relatively higher concentrations in soil samples (> 100 mg kg^{-1}). All soils analyzed were taken at the time of sampling of the soil monoliths which are being displayed at the exhibition hall of the National Institute of Agro-Environmental Sciences, Tsukuba, Japan. More than 100 sampling sites were chosen on a nationwide scale in such a way as to cover a wide range of soil types commonly observed in Japan. The total number of soil samples was about 500.

The dissolution procedure for soil samples was essentially similar to that developed for analysis by AAS (Yamasaki, 1978). One (1.000) gram of finely ground sample was treated with 10 mL of $HClO_4$-HNO_3 (1:1 mixture) to decompose the organic matter in soils and then with 15 mL of $HClO_4$-HF (1:2 mixture) twice in a Teflon beaker. The residue was heated with 5 mL of HNO_3 and dissolved by adding 30 - 50 mL of H_2O with gentle boiling and finally made up to 100 mL. The final solutions were stored in plastic bottles until the measurements. After a 10-fold dilution, the acid digests were analyzed by ICP-MS for all the trace and ultra-trace elements. Indium (In) was used as an internal standard element (Yamasaki

et al., 1990). The validity of the proposed methods were examined by analyzing Standard Reference Soil Samples provided by the Canada Center for Mineral and Energy Technology, Mines and Resources, the Institute of Geographical and Geochemical Prospection, China and National Institute of Science and Technology, USA (Govindaraju, 1994).

3. Results and Discussion

It has been shown that some minerals such as chromite ($FeCr_2O_4$), garnet ($R''_3\ R'''_2(SiO_4)_3$, where R" may be Ca, Mg, Fe, and Mn, and R" may be Al, Fe, Cr and Mn), magnetite (Fe_3O_4), and zircon ($ZrSiO_4$) are highly tolerant toward acid attack and remain undissolved even after the repeated treatment. Consequently, there is a high possibility that the maximum values for elements in these minerals (including those contained as "impurities" such as Hf and heavy rare earth elements ($_{65}$Tb to $_{71}$Lu in atomic number) in zircon) might be somewhat underestimated. Although care should be taken, therefore, for the interpretation of the results obtained by this work, the following general trends were clearly observed.

The concentration ranges were so wide that the ratios of the highest values to the lowest values were more than 100 for a considerable number of trace elements. While the concentrations of the first transition elements ($_{21}$Sc to $_{30}$Zn in atomic number) were higher than the other elements, those of lighter and heavier element tended to decrease, in general, with an increase or decrease of atomic number, with the apparent exception of Pb, Th and U.

Concentrations of the elements having an even atomic number were in most cases, higher than those in the previous and/or next element having an odd atomic number. This tendency is especially noticeable among lanthanide series elements ($_{57}$La to $_{71}$Lu in atomic number). Statistical analyses also showed that highly positive correlations ($r > 0.9$) were observed among the elements within the same group of the Periodic Table. Typical examples were, again, for lanthanide series elements. While the ratios of the highest values to the lowest values of Th and U were, for example, respectively as high as 20.6 and 15.9, that of Th/U was only 4.1 because of the existence of a very high correlation coefficient between the concentration of Th and U in soils ($r = 0.998$).

4. Conclusions

About 500 soil samples collected from more than 100 sites in Japan were analyzed for their contents of trace and ultra-trace elements using the most advanced analytical techniques such as ICP-MS. By combining the results of major elements obtained by conventional analytical methods, it was possible to estimate the background levels (natural abundance) of more than 60 elements in soils.

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MOBILIZATION OF HEAVY METALS FROM POLLUTED SOILS AS AFFECTED BY PH AND OTHER FACTORS.

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1. Introduction

In the vicinity of copper smelters Legnica and Glogow (Poland), there are soils strongly polluted with heavy metals. Previous studies showed relatively high contributions of mobilizable metal forms in those soils (KARCZEWSKA 1995). However, actual metal release into solution depends on various factors, i.e. pH, clay and organic matter contents and others, such as complexation and chelation processes (HOGG et al. 1993, HORNBURG and BRÜMMER 1993, MCBRIDE 1991). In this paper, the impact of soil solution composition and, in particular, the presence of complexing agent added to solution, was studied and discussed.

2. Materials and Methods

Metals mobilisation from six different soils sampled from A horizons in the vicinity of copper smelters was examined in batch experiments, at soil: solution ratio of 1:10. The basic properties of soils and total contents of heavy metals, determined by AAS after $HClO_4$ digestion, are shown in Table 1. Extracting solutions contained 0.2M KNO_3 and HCl/NaOH added to adjust pH in the range of 2-10 (series I). The following, potentially complexing, agents were applied as additions to the solution: citric acid, 100 mg/L (series II), fulvic acids extracted from high moor peat, 50 mg/L (series III) and ammonium oxalate, 100 mg/L (series IV). Extracting capabilities of KNO_3 and $CaCl_2$ (series V) were also compared in the study.

Table 1: Basic soil properties and total concentrations of Cu, Pb, Zn, Fe and Mn

Soil	Percent of fraction		Corg	CEC	pH	Total concentrations, mg/kg				
No	<0.02mm	<0.002mm	%	cmol/kg		Cu	Pb	Zn	Fe	Mn
1	4	2	0.25	3.86	7.38	271	116	36	2300	130
2	15	4	0.61	4.86	5.88	1080	383	118	4950	305
3	42	15	1.10	11.3	4.81	286	153	103	13100	420
4	40	22	1.35	19.6	6.74	292	127	80	14700	490
5	40	12	1.06	10.3	7.06	358	170	189	10200	400
6	54	31	2.34	38.2	6.89	248	99	104	21300	780

3. Results and Discussion

Metal release depended strongly on pH and soil properties (figure 1). At low pH values desorption capabilities decreased with increasing pH, reached very low values at slightly acidic pH and usually increased again at pH about 7, 8.0, 8.5, 9.0 and 9.5, for Cu, Fe, Zn, Pb and Mn respectively. Addition of soluble organic substances caused certain changes in the desorption results. The most pronounced effects were observed for Fe and Cu solubilization supported by addition of citric acid at pH 3.5-6.5 and for Cu - by ammonium oxalate at pH 4.5-7.0 (figure 2). As expected, $CaCl_2$ showed much higher capability of extracting the metals from soils than did KNO_3 at the same concentration.

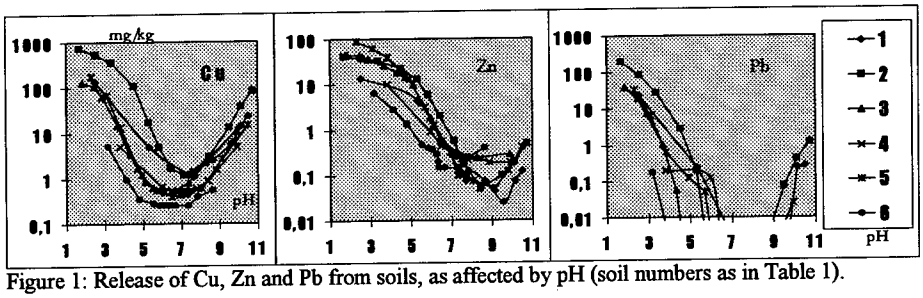

Figure 1: Release of Cu, Zn and Pb from soils, as affected by pH (soil numbers as in Table 1).

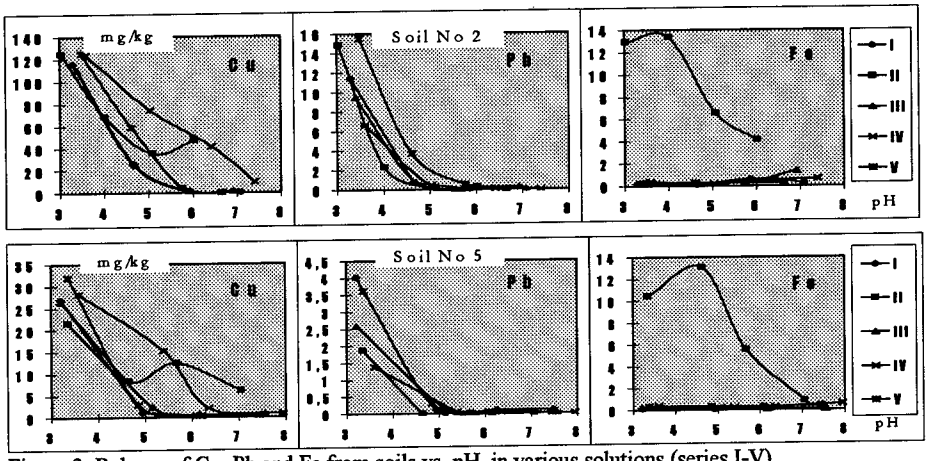

Figure 2: Release of Cu, Pb and Fe from soils vs. pH, in various solutions (series I-V).

4. Conclusions

The results of this study proved that heavy metals desorption from polluted soils may occur not only in acidic conditions but also at neutral or alkaline pH when complexing agents are present. Such possibility was shown for selected organic acids (and salts), however, other compounds may also act in a similar way. Mobilization of metals due to their complexation may play an important role in soils after aforestation, in particular with coniferous species, or after agricultural application of organic fertilisers. That is why this study should be extended and the systematic analysis should be made, focusing on various complexing agents, likely to be present in soils, and their effects on heavy metals release from polluted soils.

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BIOGEOCHEMICAL PATHWAYS OF POLLUTANT ELEMENTS AS AIDS IN ENVIRONMENTAL MITIGATION

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1. Introduction

The Southern Africa Development Community (SADC) is an economic grouping of twelve countries in southern and eastern Africa : Angola, Botswana, Democratic Republic of Congo (former Zaire), Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia, and Zimbabwe. Most of the countries are rich in minerals, and the mineral production in the SADC region is as follows : gold : 536,000 kg. ; diamonds (including industrial diamonds) : 55 million carats ; zinc : 113,000 t ; lead : 106,000 t ; manganese : 3.8 million tonnes (Mt) ; iron ore : 31 Mt ; chromite : 5.7 Mt ; copper :547,000 t; ;coal : 213 Mt (source : *Mining Magazine*, May, 1998). Mineral industry dominates the economies of some countries (for instance, Botswana with a population of about 1.5 million, earns about USD 3 billion from minerals, mostly diamonds). Since the mineral industry is bound to play a large role in the future economic development of the countries of the SADC region, wisdom lies in the countries concerned incorporating the waste minimization and waste recycling strategies into the development frame-work.

2. Methodology

Methodologies based on the biogeochemistry of trace elements could be effectively used in the disposal of mining wastes, in the treatment of industrial discharges arising from the extraction of the metals and in the use of metals and metal-based industries. The probability of risk arising from the toxicity of a given pollutant substance is evaluated. The Total Index of Risk is computed on the basis of the exposure criteria and effects criteria.

3. Case Histories

Two case histories from the SADC region are cited to illustrate the methodology of mitigating the adverse environmental impact due to mineral industries, through the beneficial use of wastes, and the development of non-polluting technologies.

A 250,000 t y^{-1} aluminium plant is coming up at Boane, near Maputo, Mozambique. Waste sludge (red mud) is the primary pollution problem associated with bauxite processing . Other waste streams from bauxite processing are : spent cleaning acid, salts from salting-out evaporator, barometric condenser cooling water, boiler and cooling tower blowdown, water softener sludge, and sanitary waste. Atmospheric emissions of particulates arise from handling of raw material and product, by calcining of the hydrated alumina in a rotary furnace, and by the sintering of the mud when required. In the course of manufacture of prebaked electrodes, atmospheric emissions of fluoride, hydrocarbons and sulphur oxides occur. Incorporation of siliceous materials would make the red mud waste suitable for making building bricks. Red muds may contain 4 - 5 % alkalis, which provide a fluxing action, resulting in good plasticity and bonding of the bricks. Red mud bricks develop a pleasing pale brown, orange or golden yellow colour, depending upon the composition of the red mud and firing temperature. They have therefore good architectural value as facing bricks. Techniques are being developed to find beneficial uses for other liquid and gaseous effluent wastes.

Mercury toxicity arises from artisanal gold mining (say, in Lake Victoria Goldfields of Tanzania). When gold is recovered by the mercury amalgam method, about 1.5 kg of mercury is irretrievably lost to the environment per one kg of gold recovered. This leads to pollution of mercury in soils, sediments, waters and the biota. The bacteria (such as, *Enterobacter aerogenes* and *Escherichia coli*) present in the soil and sediments, and human faeces, convert the metallic mercury to the highly toxic form of methyl mercury. Mercury compounds may enter human body through inhalation (at the time of sublimation), ingestion of food (particularly fish, which is enriched in the highly toxic form of methyl mercury) and water, and transfer through the skin. The intake and uptake of mercury are highly species-sensitive. The critical organs affected by mercury intoxication are the lungs, kidneys and the brain. A knowledge of the biogeochemical cycling of mercury has been useful in designing the mitigation measures (such as, lining the washing ponds with PVC sheet to prevent mercury-contaminated waters from reaching the aquatic systems, and use of gas masks to minimize the inhalation of mercury, etc.) (Aswathanarayana, 1995).

A new kind of " Portable " gold plant developed by Libenberg, Rundle and Storey of San Martin mining company (*Mining Magazine*, July, 97, pp. 8-10) is a veritable godsend for small-scale gold miners. The salient points of the plant are as follows : San Martin 's claims encompasses two dumps around Bonda, Kenya, with 250,000 t of material, grade: 1-3 $g.t^{-1}$. Carbon-in-pulp / carbon-in-leach technique ; Capacity of the plant : 10,000 t / month; production cost : USD 150 / oz. The plant has the following advantages : environmentally-benign as no mercury is used ; can be erected even in remote areas, and shifted and reassembled without much problem ; can be operated with minimal expatriate assistance; economically viable.

4. Conclusions

The case histories suggest that an understanding of the biogeochemical pathways and speciation of the pollutant elements could be effectively used as aids in ecologically-sustainable and economically-viable development of the existing and projected mineral industries in the SADC region.

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TRACE METALS IN THE BERLIN METROPOLITAN AREA

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1. Introduction

Since 1992, research on environmental geochemistry of urban Berlin has been part of the Natural Resources Monitoring Programs. Berlin is the first European megacity to be covered in its entity by a geochemical survey of the topsoil, including large, more or less rural areas.

2. Materials and Methods

About 4000 topsoil samples were taken in suburban areas with little or no contamination as well as industrial areas in and around Berlin. The < 2 mm fraction was analyzed for 11 major elements (XRF: Si, Al, Fe, Mg, Ca, Na, K, P, S; chemical analysis: C_{tot}, TOC) and 41 trace elements (EDXRF: Ag, As, Ba, Bi, Br, Cd, Ce, Co, Cr, Cs, Cu, Ga, Ge, Hg, I, In, La, Mo, Nb, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ta, Te, Th, Tl, U, V, W, Y, Zn, Zr; AAS-HG: As, Sb; AAS-GF: Cd; AAS-ICP: B, Be; flameless AAS: Hg; pyrolysis: F). Electrical conductivity and pH were also determined. Densely - populated areas and industrial areas were sampled at a density of 40 samples per km^2. Field observation provided additional information: geographical situation, geology, morphology, urbanization, land use, vegetation, soil type and horizon, and potential sources of contamination. Survey strategy is nearly always site specific, taking into account the previous history of land use, proximity to existing industrial activities, direction of prevailing winds, etc. and the proposed nature of redevelopment or the kind of present-day land use.

To evaluate and interpretate geochemical data, we are using both single-element and multi-element maps (cluster-Q analysis), maps of geochemical associations (component analysis), and maps showing the geochemical load index for various trace elements.

3. Results and Discussion

The distributions of the elements Al, K, Si, Na, Rb, Zr, Nb, Co, Sc, and Ti are indicating mainly natural origin, i.e. related to the composition of the parent material.

Industrial areas tend to be characterized by contamination of the subsoil with Cu, Cd, Zn, Hg, Pb and Sn. Industrial and commercial areas often display considerably large concentration of Mo, Ni, As, Ag, Cr, Sb, Sr, TOC, Fe, Mn, Mg, P and especially Pb, Hg and electrical conductivity relative to the geogenic background of the area surrounding Berlin.

Wooded areas show no great enrichments except for Cd and Zn. In the area around Berlin, extensive, strong anomalies occur near iron and steel industries and construction materials industries, as well as in the vicinity of sewage farms. There is local heavy-metal pollution (Hg, Cd, Zn) from the sewage farms north and south of Berlin. Studies of Berlin area and other German cities have shown that heavy metals (e.g. Cd, Cu, Cr, Hg, Ni, Pb, Zn and As) are enriched 1.8 to 8.9 times relative to, geogenic concentrations (Table 1).

Especially in the polluted soils of old industrial sites, peak values of 2050 times the geogenic background were measured for Cu, 1780 times for mercury, and 1638 times for cadmium. Assuming a soil density of 1.2 g/cm^3 and the medians of metal concentrations this translate to about 834 t As, 74 t Cd; 5366 t Cr; 6660 t Cu; 40.6 t Hg; 1646 t Ni; 16.377 t Pb and 27.580 t Zn accumulated in the topsoil (0-20 cm) of Berlin (Table 2).

4. Conclusions

For the first time, a comprehensive geochemical data base is available for the urban Berlin environment that permits differentiation between the natural geochemical background and local anthropogenic contamination.

Our studies have shown that heavy-metal concentrations in the topsoil vary considerably in an urban environment. These concentrations primarily reflect land use and the type and volume of industrial production in the areas under consideration.

Multivariate statistical methods allow long-range and local migration of pollutants to be traced and natural and anthropogenic element associations to be distinguished. The regional or local geochemical situation in an urban area can be described only by comparison with the non-industrial surroundings.

A geochemical survey of the urban environment provides a reliable data base for setting concentration limits for urban and other soils.

Table 1: Parameter values for the Berlin topsoils (in mg*kg^{-1})

Parameter	arithmetic mean	geochemical background	x_{max}
As	4.4	2.4	126
B	17.6	13.0	1800
Be	1.14	1.0	53.0
Cd	0.65	0.08	131
Co	1.9	4.0	192
Cr	28.7	12.2	1840
Cu	53.1	6.0	12300
F	280	200	25000
Hg	0.29	0.04	71.2
Ni	8.1	2.7	769
Pb	85.8	21.7	4710
Sb	2.92	2.1	454
Sn	7.6	1.6	498
Zn	166	19.3	25210

N=3746 (sample depth 0 - 20 cm)

Table 2: Approximate metal accumulation in the Berlin topsoils (in t)

Element	Metal content in topsoils (in t)		
	total	geogenic	anthropogenic
As	834	620	214
Cd	74.0	21.8	52.2
Cr	5366	3869	1497
Cu	6660	1753	4907
Hg	40.6	8.5	32.1
Ni	1646	577	1069
Pb	16377	5366	11011
Sn	1283	428	855
Zn	27580	5153	22427
Zr	43829	43188	641

Method of calculation

Metal content (in t) = $A * T * d * X * 10^{-2}$

A = Berlin area in km^2 (890.85 km^2)

T = depth in cm

d = soil density in g*cm^{-3}

X = element concentration in mg*kg^{-1}

BIOGEOCHEMISTRY OF TRACE METALS AT THE CHUNKY GAL MAFIC/ULTRAMAFIC SITE, NORTH CAROLINA, USA

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1. Introduction

The Chunky Gal mafic-ultramafic complex consists of dunite and amphibolite folded into a paragniess (Hadley & Freedman 1949; McElhaney & McSween 1983; Ramaley & Andersen 1998). The three rock types are all found within a 3 km^2 area, allowing biogeochemical comparisons under similar climatic conditions. The climate is temperate to subtropical and humid, with an average temperature of 12.6°C and over 180 cm of rainfall per year. Vegetation is dominated by deciduous forest, but dunite areas bear stunted forests with unusual species composition (Mansberg & Wentworth 1984). The objectives of this study were to characterize the distribution of trace elements in the biogeochemical system and to investigate the roles of micro- and macronutrients in determining forest structure and composition.

2. Materials and Methods

Six rock and soil pairs, two from each rock type, were collected. Soils were collected from the B-horizon when possible, but some of the soils are inceptisols and have poorly developed horizons. The O-horizon was avoided. The chemical composition of the rocks and soils was measured using a Philips PW2400 X-ray fluorescence spectrometer. Mineral composition was determined by petrographic methods and X-ray diffraction. Constant oxide analysis, assuming Al_2O_3 as constant, determined the relative losses and gains of elements. The chemical composition of streams draining each of the lithologies was measured by a Varian ICP-AES.

At sites on each rock type, the four most common woody plant species were sampled. (Hydrologic sampling described above required sites along streams; therefore, plant samples represent primarily riparian species.) Leaf and wood samples were dry-ashed, dissolved in HNO_3, and analyzed using ICP-AES.

3. Results and Discussion

Concentrations of Co, Cr, and Ni for rocks and soils were lowest in the gniess, intermediate in the amphibolite, and highest in the dunite. The absolute concentrations of Co and Cr increase in the soils, but Ni sometimes increases and sometimes decreases. Constant oxide data suggest variable behavior of Cr and Ni during formation of the B-horizon. Cr tends to be lost during the weathering of the gniess and dunite, and accumulates during weathering of the amphibolite. Ni and Co tend to accumulate during weathering of the gneiss, and tend to be lost during the weathering of the dunite. Ni and Co behavior during weathering of amphibolite is variable. Cr and Ni concentrations of water samples were below the detection limit (~50μg/L), suggesting that Co, Cr, and Ni are accumulating elsewhere in the rock-soil-biota system.

Cr and Co were below detection limits (~2μg/g) in all plant samples. Ni concentrations in leaves varied among species and sites (Fig. 1). Overall Ni levels were highest in plants from dunite and

lower in those from amphibolite and gneiss. There was a consistent trend of greater Ni accumulation in deciduous trees (*Betula nigra, Hamamelis virginiana*) and lower accumulation or exclusion in evergreen species (*Rhododendron maximum, Tsuga canadensis, Pinus rigida*). Major element distributions in plants also paralleled those in the edaphic environment, e.g., high Mg and low Ca, K, and P on dunite.

4. Conclusions

Vegetation may represent a major sink for Ni in this system, especially on dunite rocks, and fluxes of Ni may be strongly affected by decomposition of deciduous foliage. Biotic components of the ecosystem appears less important for Cr and Co, at least in terms of the above-ground parts of the dominant woody plants. Vegetational characteristics could be affected by Ni toxicity, but this seems unlikely given the concentrations observed here. Other explanations for the peculiar communities on dunite rocks could include Ca, K, or P deficiencies, interference with cation uptake by high Mg concentrations (Brooks & Yang 1984), or physical properties such as soil depth and drainage.

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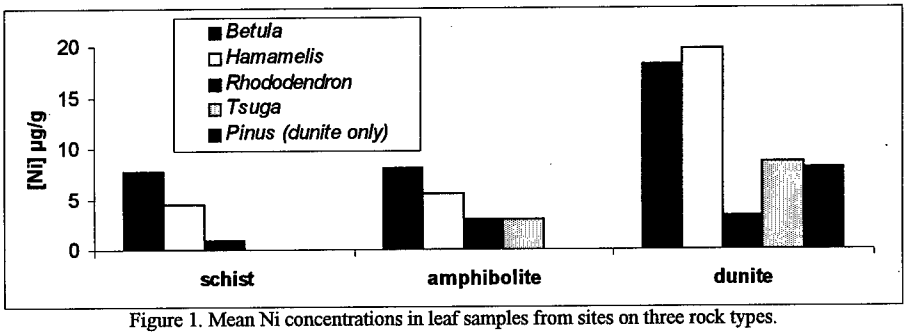

Figure 1. Mean Ni concentrations in leaf samples from sites on three rock types.

CERIUM PRECIPITATES INTO GROUNDWATER NODULAR FERRICRETES UNDER CRETACEOUS SANDSTONE IN SE-BRAZIL

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1. Introduction

Changes in the oxidation state frequently occur associated to variations of the water table in the lower positions of tropical landscapes, where ferruginized horizons (laterites or ferricretes) can be formed due to intense weathering. Positive anomalies of Ce have been described on ferricretes developed on different lithologies: acid, alkaline and basic igneous rocks in tropical Africa and alkaline rocks in Brazil, are some of the examples cited in the literature (Braun et al., 1990), being scarce the information of unusual contents of rare earths in ferricretes formed on soils developed from sedimentary rocks. The observation of rare earth elements (REE) accumulations, especially Ce, has led to open discussion about the conditions of lateritic weathering systems (Beauvais & Colin, 1993; Beauvais & Roquin, 1996). In the landscape of northern State of São Paulo (Brazil), lateritic covers exist in different topographic levels and, although not documented, they are predominantly nodular and constitute a feature that appears frequently on the slopes under Cretaceous sandstone of the Adamantina formation (Ka), Bauru Group. In order to develop an understanding of the genesis and spatial distribution of these features, the objective of this research was to verify the presence and the form of occurrence of REE in nodular ferricretes.

2. Materials and Methods

Submicroscopic studies (SEM JEOL-JSM-T33A) and microanalyses by an EDRX microprobe (Noran TN500) were carried out on undisturbed samples of Fe-Mn nodules. These were previously identified in morphological descriptions (macro and micro) of petroplinthic horizon located on the footslope of a toposequence.

3. Results and Discussion

The studied profile was classified as a Typic Plinthudult with an A, E, Bt, F, Cf and Cr horizon sequence. It presents a sandy texture in the A and E horizons that changes abruptly to sandy clay loam in the Bt. The F1 horizon (60-85cm) is dominated by irregular petroplinthic features of dimensions that vary between 0.3 and 10cm and have dark brown (7,5 YR 5/8) and grayish dark red (10R 3/3) colors. Some nodules located in the base of the F horizon have black (2.5 YR 2.5/0) nucleus, evidencing manganese precipitation. The Cf horizon (85-105cm) is plinthic, made up of nodules that maintain the structure of the parent material. The saprolite (Cr1 and Cr2) dominates under the F horizon, with the parent material appearing at the depth of 164cm.

The scanning electron microscope analysis showed that nodules contained small particles with a glomerular habit on the surface of the Mn-coatings (Fig. 1a). The microanalysis of these particles showed that they were Ce accumulations, probably as cerianite (CeO_2) (Fig. 1b). Since Mn is more soluble and mobile than Fe it requires a higher Eh to precipitate. This probably explains the fact that it could have been deposited during the final stages of the formation of the petroplinthic horizon, during the definitive lowering of the water table, that created higher oxidation

potentials, mainly in the large planar voids and channels that occur in the saprolite. Cerium and Co accompanied the mobilization and segregation of the Mn oxides and hydroxides. This can be a consequence of the ability of these Mn species in concentrating and controlling the distribution of certain metallic ions (Taylor and MacKenzie, 1966). The chemical and mineral composition of the studied nodules, as well as their formation mechanism, agree with the thermodynamic stability of cerianite, which precipitates when oxic conditions dominate (Braun et al., 1990). This supports the idea that the Fe-Mn nodules were formed in more oxidizing conditions than Fe nodules.

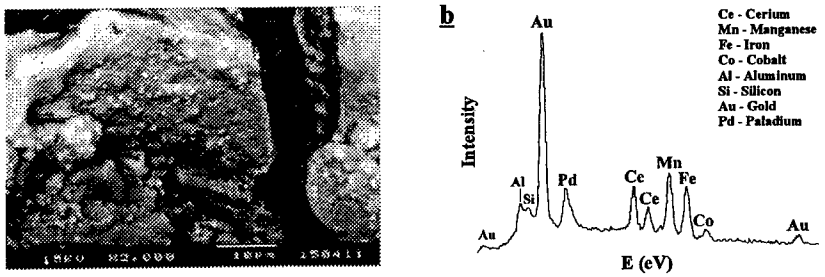

Figure 1. a) SEM micrograph of a petroplinthic nodule showing a Mn-coating on the surface of a quartz grain and small white particles with a glomerular habit (Cerianite–CeO_2). b)Semi-quantitative analysis (EDRXA) of a manganese coating and cerianite particles.

4. Conclusions

1. Localized accumulations of Cerium (Ce) were only found in the petroplinthic nodules that show Mn-oxides coatings, located in the bottom of the petroplinthic horizon.
2. Besides Ce, cobalt (Co) also concentrates inside the Fe-Mn nodules, due to the coprecipitating action of the Mn oxides.
3. The presence of cerianite (CeO2) inside petroplinthic nodules with darkened nucleus is associated to the permanent lowering of the groundwater, when more oxic conditions prevailed in relation to those where ferruginized-only nodules were formed.

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DISTRIBUTION AND MOBILITY OF ZINC AND COPPER IN ARABLE SOILS OF WIELKOPOLSKA LOWLAND, STUDIED BY SEQUENTIAL EXTRACTION

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1. Introduction

The total trace element concentrations in a soil are poor predictors of their bioavailability. A well managed agricultural ecosystem requires knowledge about nutrient stocks and the chemical forms in which macro- and micronutrients occur (MC LAREN and CRAWFORD, 1973; SHUMAN, 1985; KABATA-PENDIAS and PENDIAS, 1992).

The main objectives of this investigation were to characterize mobility and distribution of trace elements (Zn and Cu) in arable soils formed from calcareous glacial till from Wielkopolska Lowland, central Poland. Although data on total soil concentrations of these metals exists, the information available on trace elements mobility and speciation is rather scare. For better understanding the chemistry of metals in these soils, to correct deficiency or to eliminate amounts toxic to plants, the study based on sequential extraction procedure was undertaken.

2. Materials and Methods

Representative soils for Wielkopolska Lowland region were selected. Samples were taken from each soil horizon of eight sites. Basic soil properties were determined by conventional procedures (BLACK, 1965).

Total soil Zn and Cu were determined after acid digestion of soil samples in a mixture of HF and $HClO_4$. For the determination of metal mobility, among fractionation schemes (TESSIER et al., 1979; MILLER et al., 1986; BECKETT, 1989) the extraction according to BCR of European Community has been chosen.

The reagents having increasing dissolution power were sequentially used for the extraction of metals. The following fractions were separated: Fraction 1 (exchangeable and acid soluble metals - extracted with 0,11 M AcOH), Fraction 2 (metals associated with Mn and Fe oxides - extracted with 0,1 M $NH_2OH \cdot HCl$; pH 2) ; Fraction 3 (metals associated with organic matter - extractable with 30% H_2O_2); Fraction 4 - residual - non extractable forms of metals. Metal concentrations were determined by atomic absorption spectrometry (Philips 2900X).

The percentage of zinc and copper in the various fractions was calculated based on the total concentration in soil. Correlation coefficients were calculated for soil properties versus trace elements content in each fraction.

3. Results and Discussion

All soils were neutral (pH: 7,3) to slightly alkaline (pH: 8.1) which coincidences with the presence of free carbonates (MATTIGOLD et al., 1985). The organic matter content was in the range 0,4 - 3,2%, the clay content 14-24% and the total N content 0,01 - 0,17%. Total zinc and copper contents, were typical for agricultural, non contaminated soils of this region.

The extractable metals contents reflected variation in soil characteristics such as pH, texture and contents of organic matter, carbonate and oxides. These results are similar to those found previously (SHUMAN, 1991).

Zinc was found mainly in Fraction 3 (associated with organic matter) and Fraction 4 - the least mobile, residual. About 5% of total zinc was associated with manganese and iron oxides - Fraction 2. Exchangeable and acid soluble zinc - Fraction 1 was very low (1,3 % of total).This fraction represents a small pool of potentially mobile Zn that may become available for plant uptake.
Copper was far less mobile as indicated sequential extraction. The dominant was Fraction 4 - residual (75% of total). About 8 % of total Cu was found associated with soil organic matter. Significant amounts of Cu were extracted by acid (Fraction 1).
Generally, amounts of extractable zinc and copper were 64,6% and 24,5% of total concentration in soils, respectively.

4. Conclusions

Sequential extraction technique gave data on zinc and copper distribution among the various constituents of analyzed soils and on their mobility. Zinc was primarily in the organic and residual fractions, copper was found mainly in the least mobile, residual fraction.

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METAL DISTRIBUTION IN SOILS FROM TWO MARSHES. A STATISTICAL APPROACH

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1. Introduction.

The problem of metal distribution in estuarine environments has been largely studied. This kind of approach arises serious interpretation problems, since frequently a large number of samples have to be collected as well as a large amount of variables are considered. The use of concentration maps has been widespread, but, the maps do not identify relations among variables and no behaviour inference is possible to be interpreted. The use of statistical tools in order to clarify the behaviour of metals as related to other variables is helpful (Vernette, 1970; Waserman et al., 1997). The objective of this work is to apply the statistical analysis with correlation's study, analysis of spatial and temporal variance, multiple range tests, cluster and principal components analysis to interpret the relations among the metal contents and several chemical and environmental factors.

2. Materials and Methods.

Total, and available contents of heavy metals in several soils from two marshes located in the NW of Spain has been studied: the marsh of the Xubia River, and the marsh of the Ferrerías River. The first one is subjected to the action of different contaminating agents such as waste products from industries and towns (Xubia Marsh). However the second one is not affected by contaminating agents (Ferrerías Marsh).

The area was checked monthly during one year. One hundred and ninety four soils samples were taken. The soils were analysed for pH, Organic matter and N content, CEC, clay content, salinity, SO_4^{2-}, the major elements: Al, Fe, Mn, Mg, Ca, K, Na and the trace metals: Pb, Cu, Zn and Ni. The analysis of trace metals was performed by atomic absorption spectrophotometry. Samples were digested with HNO_3, HF and HCl in a microwave oven prior to analysis.

Monitoring as a function of time produce appreciable amounts of data which have difficult presentation and interpretation. Considering the amount of available data in this work, they were statistically analysed by means of correlation's study, analysis of spatial and temporal variance, multiple range tests, cluster and principal components analysis.

3. Results and Discussion

Results (Table 1) indicate that a high positive correlation was established for all the samples between the contents of several metals. A high positive correlation was established for the soils from the Xubia Marsh between the different trace metal contents. For the soils from the Ferrerias marsh, it has been detected a positive correlation solely between Mn and Ni contents.

The analysis of variance and multiple range tests indicate significant differences among the soils from the two marshes in pH, CEC, and O.M., Cu, Ni, Pb, Mn and Zn contents caused by

the contamination of the Xubia Marsh which receives the large quantity of urban and industrial waste.

Principal components analysis indicates that in Xubia Marsh the Zn content is positively related to Mn content, under Factor I (anthropic contamination). This is due to the industrial wastes dumped in the estuary. Factor II associates positively Mg and Si, due to the minerals content in the soils. In Ferrerías Marsh the absence of variability sources indicating the soils from this marsh are uncontaminated.

TABLE 1. SUMMARY OF STATISTICAL ANALYSIS

All the samples		Xubia marsh		Ferrerias marsh	
Correlation Between:	Analisis of variance. tests. Significant differences between soils from two marshes for:	Correlation between:	Principal component analysis	Correlation between:	Principal component analysis
Zn and Ca r= 0,5808**	pH : p= 0.000**	Cu and Fe r= 0,6880**	Factor I (anthropic influence): + Mn and Zn contents	Mn and Ni contents r=0,5999**	absence of variability sources
Zn and Mg r=0.3714**	O.M.: p= 0,012 *	Zn and Ca r= 0,7713**	Factor II (mineral composition): + Mg and Si contents		
Zn and Cu r= 0,4412**	CEC: p= 0,001*	Ni and Cu r=0,7775**			
Zn and Mn R= 0,8230	Ni: p= 0.01*	Ni and Fe r=0,5922 **			
Ni and Cu r=0,5424 **	Pb: p = 0,000**	Pb and Cu r= 0,4960**			
Ni and Fe r=0,4572**	Mn: p=0,000 **	Pb and Fe r= 0,4376 **			
Pb and Cu R= 0,5187**	Zn: p= 0,19*	Zn and Mn r=0,8121**			
Pb and Fe R=0,3992**	Cu: p=0,000*	Zn and Ni r= 0,3567*			
Pb and Mn R= 0,4995**		Zn and Pb r= 0,4378**			
Pb and Zn R= 0,4678**					

(*) and (**):denotes significative differences, or strongly correlation at 0,01 level and 0,001 level respectively

4. Conclusions

The advantage of the statistical approach to the considerable amount of data is indubitable. It takes less time and can moreover provide useful information to decide management purposes to minimize whatever future adverse impact on the environment. The statistical approximation identifies the anthropogenic factors acting on Xubia Marsh easily, and shows that spatial and temporal variations are very irregular.

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IODINE AND BROMINE CONTENTS OF SOME AUSTRIAN SOILS AND RELATIONS TO SOIL CHARACTERISTICS

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1. Introduction

Iodine plays a key role in mammalian physiology as a major constituent of the thyroid-hormone thyroxin. This hormone regulates somatic and cerebral development, in adult organisms it influences basic metabolism (KARLSON, 1980). At present there exists only limited information on the actual iodine and bromine contents of Austrian agricultural soils and crops, respectively.

2. Materials and Methods

Composite soil samples from 0-20 cm depth were collected at 40 locations in Austria from agricultural areas from cereals producing sites. The sites were located in 5 Austrian Federal States, mainly in Lower Austria and Upper Austria. The investigated soils display a significant variation in soil characteristics. The following ranges of parameters were observed: e.g. pH: 5.4 - 7.6, N_t: 0.13 - 0.27%, lime: 0 - 31.7%, C_{org}: 1.31 - 3.42%, clay: 5.5 - 69.6%.

Inductively coupled plasma mass spectrometry (ICP-MS) analysis was used to determine iodine and bromine concentrations in soil samples. The coefficient of variance for a triplicate determination on average was 2.2% and 3.8% for iodine and bromine, respectively, which seems to be very reasonable for the trace amounts measured. Approximate detection limits in solid samples were 0.005 mg I kg^{-1} for iodine and 0.05 mg I kg^{-1} for bromine, indicating that the sensitivities of the method were sufficient for the measurements of these soils (SCHNETGER and MURAMATSU (1996).

3. Results and Discussion

The determined iodine concentrations in topsoils vary between 1.1 and 5.6 mg kg^{-1}, the arithmetic mean amounted to 3.1 mg kg^{-1}, These values are quite low in comparison to literature data, the most common I concentrations in soils range from <0.1 to 10 mg I kg^{-1} (KABATA-PENDIAS and PENDIAS, 1992). Bromine contents are always higher than the corresponding iodine values, varying between 2.4 and 11.9 mg kg^{-1} with an arithmetic mean of 5.7 mg kg^{-1}. Due to their different geological origin non-calcareous soils on average contained significantly less iodine and bromine than calcareous soils, as expected from observations cited in the literature (MURAMATSU and WEDEPOHL, 1998).

Table 1: Correlation (Pearson) between selected soil characteristics and iodine, bromine contents of calcareous and non-calcareous agricultural soils (significant values ($p < 0.05$) printed bold)

non-calcareous soils (n = 21)			calcareous soils (n = 19)		
	Iodine (ppm)	*Bromine (ppm)*		*Iodine (ppm)*	*Bromine (ppm)*
Bromine (ppm)	-0.051	1.000	*Bromine (ppm)*	**0.527**	1.000
N (%)	0.262	0.063	*N (%)*	0.279	**0.435**
C_{org} *(%)*	0.208	-0.033	C_{org} *(%)*	**0.471**	**0.652**
pH	-0.159	0.212	*PH*	0.404	**0.622**
sand (%)	**-0.675**	-0.206	*sand (%)*	-0.256	**-0.464**
clay (%)	**0.620**	0.174	*clay (%)*	**0.596**	**0.691**
$Mg_{exchangeable}$ *(mval)*	0.315	-0.003	$Mg_{exchangeable}$ *(mval)*	0.223	**0.528**
$Ca_{exchangeable}$ *(mval)*	0.412	0.117	$Ca_{exchangeable}$ *(mval)*	**0.535**	**0.817**
$Na_{exchangeable}$ *(mval)*	0.122	0.050	$Na_{exchangeable}$ *(mval)*	0.284	**0.717**
Se (ppm)	0.266	0.273	*Se (ppm)*	0.327	**0.673**

A correlation analysis of results with several soil parameters resulted in positive correlations with clay content and a negative relation to sand content. In the group of calcareous soils I and Br correlated positively with organic carbon contents and exchangeable calcium.

4. Conclusions

In the case of Austrian soils, there are almost no direct contributions for both I and Br from marine sediments, except for carbonaceous rocks (lime). Influence from a marine atmosphere seems to be also not so high. These factors lead to the relatively low concentrations of I and Br in Austrian soils. The low value for Br in Austrian soils indicates that there is no increase of Br in the studied areas due to anthropogenic sources such as soil fumigant (methyl bromide) and automobile exhaust. These data provide information on the background concentrations of I and Br in the central part of Europe.

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DETERMINATION AND REGIONAL DISTRIBUTION OF AU, PD, PT AND RH IN HUMUS AND MOSS SAMPLES IN EUROPEAN ARCTIC

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1. Introduction

The determination of platinum group elements (PGE) in extremely low concentrations in various terrestrial materials has become a major challenge in the environmental sciences after the extensive use autocatalytic converters to treat the exhaust gases from motor vehicle started. Though the benefits of converters in reducing major gaseous pollutants are obvious a new problem has emerged: the introduction of PGE's to the environment by abrasion surfaces of the catalysts. Other substantial sources of precious metal contamination in the environment are mining , roasting and smelting of base metal ores (particularly Ni-ores). The effects of smelting have been studied recently in the surroundings of smelters - but mostly for base metals and sulphur. Reliable data on natural background concentration levels of these elements in various terrestrial materials are still missing mainly due to lack of sensitive analytical methods to provide sufficient data to make these calculations possible. In addition the analytical methods most frequently used (INAA, NiS-fire assay preconcentration / ICP-MS) are mostly very expensive and prone to reagent contamination deteriorating the practical detection limits.

A rapid and cost-effective method has been used to analyze Au, Pd, Pt and Rh from moss- and humus (A0-horizon)-samples collected during the Kola Ecogeochemistry project (Reimann et al., 1998). From 1992-1998 the Geological Surveys of Finland (GTK) and Norway (NGU) and Central Kola Expedition (CKE), Russia, carried out this large, international multi-media, multi-element geochemical mapping project, covering 188,000 km^2 north of the arctic circle.The main aim of the Kola Ecogeochemistry project was the documentation of element levels and variation in different sample media (moss, O-horizon, B- and C-horizon of podzol). The project area is dominated by great contrasts. It encompasses nearly pristine environments (N-Finland, N-Norway) as well as regions receiving some of the highest metal and sulphur depositions in the world. These originate from several point source emitters in the Russian part of the project area (the nickel smelter in Nickel, the ore roasting plant in Zapoljarnij and the nickel refinery in Monchegorsk) . The ores processed in these smelters have been shown to produce an extensive pollution of a large set of elements including PGE's (Boyd et al.1997) which are a component of the ore itself (Monchegorsk) or the bedrock in which the mined ore is situated (Nickel). In addition samples representing natural geologically varying background areas were analyzed. These areas cover bedrock areas varying from granitic rocks to mafic rocks including layered intrusions containing PGE-ores.

2. Materials and Methods

The samples were dried at ambient temperature after sampling. The moss samples were milled with a centrifugal mill to pass a 1 mm sieve (Ti blades and sieves). The humus samples were sieved by rubbing them by hand through a 2mm nylon sieve. A 2g subsample of both sample types was ashed at 600 C, and ash was digested with aqua regia. After dilution the precious metals were preconcentrated using reductive co-precipitation. The metals were reduced to

elements by addition of stannous chloride as reductant and collected to mercury precipitation (Kontas et al. 1990). The precipitate was separated by centrifugation and dissolved to aqua regia. The elements were determined with Perkin Elmer SIMAA 6000 graphite furnace – AAS. Detection limits are for Au 0.1 ppb, Pd 0.1 ppb, Pt 1 ppb and Rh 0.05 ppb. Pt- containing vegetation reference samples (Unversity of Munich) (Wegscheidler et al. 1993) and Finnish humus and moss reference samples were analyzed to assess precision and accuracy. About 5% of samples were analyzed as duplicates. Of the total 700 sampling stations about 300 have been analyzed.

3. Results and Discussion

The analyses of reference materials show good precision and accuracy. The duplicate analyses correlate with each other proving that the subsampling and the used method give sufficiently reproducible results. Extremely high concentrations were noticed in the vicinity of both the smelter in Monchegorks and Nickel in both analyzed materials. The extent of the pollution is comparable to the extent of base metal contamination. The levels of PGE's are however higher in the surrounding of Monchegorsk where the ore that is processed is originating from Norilsk.

4. Conclusions

Background concentration levels can be defined for each bedrock area and the contrast to a large heavily polluted area can be defined. The used method is proven to be reliable and sensitive enough to be used to analyze various types of terrestrial sample materials (topsoils, mineral soils, humus, moss, lichens, needles ,vegetation).

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DETERMINATION OF METHYL- AND INORGANIC MERCURY IN ORGANIC SOILS OF A BOREAL FORESTED CATCHMENT

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1. Introduction

In soils under non-reducing conditions, Hg^{2+} and CH_3Hg^+ are almost exclusively bound to functional groups in soil organic matter. The aim of this study was i) To develop methods to determine organically adsorbed Hg^{2+} and CH_3Hg^+ in soils and ii) To determine the ratio of organically adsorbed CH_3Hg^+ and Hg^{2+} in soils along the major hydrological pathways of a small boreal, forested catchment in northern Sweden.

2. Materials and Methods

Organic rich soils were sampled at varying distance from two streams draining the catchment (see Bishop et al. 1995). Soil solution pH varied between 3.88 and 5.35. Cold vapour atomic absorption spectrometry (CVAAS) was used for Hg-tot determinations after microwave promoted acid (HNO_3) digestion (reference method). CVAAS was also used to determine Hg (II) extracted from soils with a solution containing 0.03 M Cu $(NO_3)_2$, 0.38 M KBr and 4% HNO_3. In order to avoid interference's, organic substances in the extract were oxidised prior to CVAAS determination. For species specific determinations, CH_3Hg^+ was extracted by an aqueous solution containing 0.03 M $CuSO_4$, 0.38 M KBr and 5% H_2SO_4 and subsequently extracted into a toluene phase. After separation, the organic phase was butylated with a Grignard reagent and mercury species were separated by gas chromatography and determined on-line by microwave induced plasma-atomic emission spectrometry.

3. Results and Discussion

Using the extraction method for Hg-tot, the recovery of Hg^{2+} and CH_3Hg^+ added to soil was 90±8% and 103±1%, respectively. A comparison with acid digestion shows that in average 96% of Hg-tot was extracted from the organic soils by the solvent (Table 1). For some soils, four sequential extraction's of two hours duration were needed to give a residue of Hg-tot less than 5% (determined by acid digestion). Further studies are required to show that this extraction method is selective for Hg (II)-organic complexes in a wider range of soils and sediments containing inorganic Hg(II)-sulphides.

Using the species specific method, the recovery of CH_3Hg^+ added to soil was 87±6%. For the certified reference sediment IAEA 356 we obtained a CH_3Hg^+ concentration of 3.22±0.17 ng g^{-1}. This value is in good agreement with that of Hintelmann et al. (1997). These workers suggested that the certified value, 5.46±0.71 ng g^{-1}, is biased by the formation of CH_3Hg^+ during sample work-up. The results obtained here indicate that the extraction method does not create significant amounts of artifactual CH_3Hg^+

Adsorbed CH_3Hg^+ made up 0.4 to 17.2 % of Hg-tot in the soils (Table 1). The highest values were found in the stream bank of stream K. These findings support the hypothesis that the stream bank is an active site for methylation processes within the catchment (Bishop et al. (1995). In addition, the difference in the weighted annual average ratio of CH_3Hg^+ / Hg-tot for stream K

(9%) and stream V (5%), Bishop et al. (1995), can be explained by a generally higher ratio in soils, and mire peat, drained by stream K.

Table 1. Total and methylmercury in soils and certified reference material.

Sampling site and depth, cm	Org-C %	Hg-tot, μg g^{-1} Acid digestion	Hg (II), μg g^{-1} Solvent extraction	CH_3Hg^+,ng g^{-1} Butylation	CH_3Hg^+ % of Hg-tot
Mire peat, 0-5	46.6	0.085±0.006	0.081±0.010	2.86±0.03	3.3
Mire peat, 5-25	47.3	0.027±0.004	0.029±0.006	1.41±0.10	5.2
Mire stream bank, 0-20	49.2	0.055±0.005	0.052±0.010	3.21±0.39	5.8
Stream K, bank, 0-10	33.0	0.078±0.011	0.065±0.011	13.38±0.62	17.2
Stream K, bank, 10-25	30.3	0.113±0.015	0.106 [0.006]*	10.18±0.68	9.0
2m from stream K, 10-20	49.3	0.106±0.003	0.104 [0.003]*	1.73±0.19	1.6
2m from stream K, 30-50	46.9	0.114±0.017	[0.005]*	4.08±0.64	3.6
20m from stream K, 10-20	56.5	0.077±0.007	0.064±0.009	0.60±0.15	0.8
20m from stream K, 30-50	54.9	0.077±0.015	0.065±0.017	0.33±0.04	0.4
Stream V bank, 0-10	13.0	0.041±0.005	0.029±0.003	0.87±0.05	2.1
Stream V bank, 10-25	19.7	0.095±0.008	[0.002]*	1.18±0.17	1.2
2m from stream V, 25-45	33.1	0.101±0.005	0.090±0.005	0.68±0.08	0.7
2m from stream V, 45-60	4.2	0.035±0.001	0.025±0.001	0.58±0.05	1.9
20m from stream V, 35-45	2.4	0.015±0.001	0.020±0.001	0.11±0.01	0.7
Reference IAEA 356		7.449±0.858	6.20±0.70	3.22±0.17	

number of replicates = 2-4; *Sequential extraction 4 times; [ ] denotes Hg-tot in residue

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SPATIAL ANALYSIS OF A BIOGEOCHEMICAL ANOMALY, PARTICULARLY HG, IN A WETLAND ENVIRONMENT, NOVA SCOTIA, CANADA.

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1. Introduction

Kejimkujik Park, Nova Scotia, has the highest Hg concentrations in loon blood in North America (Burgess et al., 1998) and these levels have had negative impacts on loon reproductive rates. Loons, being at the top of the food chain tend to bioaccumulate Hg. However the underlying reason for the high Hg levels in loons and the source of the Hg remains unknown. The overall objective of this project was to determine the relative importance of atmospheric deposition, geological sources, and geochemical and biological processes on contributing to the elevated levels of Hg in aquatic and terrestrial systems. The basic question is why are mercury levels so high in certain parts of the ecosystem at Kejimkujik Park, NS. Fieldwork is based in Kejimkujik Park, NS.; however we consider that results are applicable to other areas particularly areas dominated by wetland conditions. Rasmussen et al (1998) report on a similar study in Ontario. This report will provide some of the basic data already available, including new limnological, biogeochemical and lithogeochemical data acquired by GSC and a preliminary look at relationships between the variables.

2. Materials and Methods

There are a variety of media sampled for trace element analyses (including Hg). Data types include: lake water chemistry, lake sediment chemistry, biogeochemistry (leaf and twig samples of red maple, white birch, white pine and eastern hemlock). Mercury data for yellow perch are included (N. Burgess, pers comm.)
The objective of the study was to determine those environmental factors, which influence the level of Hg in biota, both fish and vegetation. The first step was to construct a database that would permit analyses of relationships between variables. The construction of the database was complicated as the sample points were not always at the same location. To overcome this limitation the data were expressed on a catchment basin basis, with the assumption that the basin is a basis for standardizing the comparisons and the basin generally outlines an area of influence.

3. Results and Discussion

Mercury concentrations in the leaf tissue of red maple showed a 5 fold range in values; which is attributed to site differences as samples were from the same species, same tissue, same height and samples were gathered over a

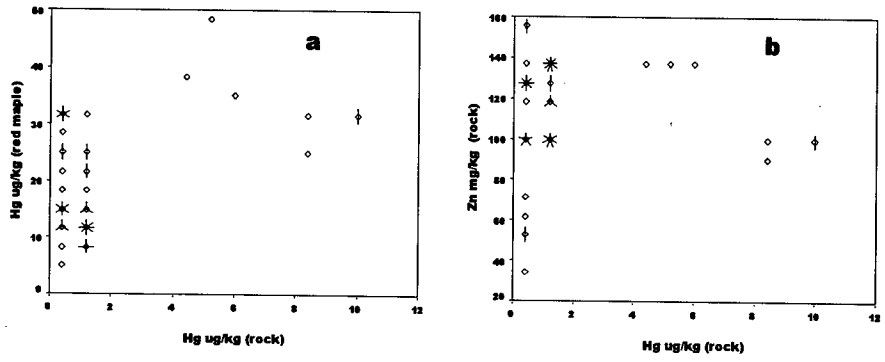

Figure 1: Relationship between: a) Hg in red maple and Hg in rocks and b) Hg in rocks and Zn in rocks for Kejimkujik National Park, Canada

narrow time window (5 days). Similarly mercury (total) levels in lake waters at Kejimkujik were comparable to values in the literature and for other values at Kejimkujik Park (Clair et al., 1998). There is also a five fold range in the Hg values for lake waters which is attributed to site differences as samples were taken using the same protocol at each site and sampling was limited to a two day period. The values in the rocks appear relatively low but are comparable to levels in the literature. There is a wide range in the values and this is directly related to the mineralogy of the rock sampled. Granitic rocks had low Hg values (0.04 $\mu g\ kg^{-1}$); whereas shales had values up to 13 $\mu g\ kg^{-1}$. The pH values in the lake waters varied from 4.1 to 6.2 and dissolved organic carbon (DOC) varied from 2.2 to 13.1 ppm.

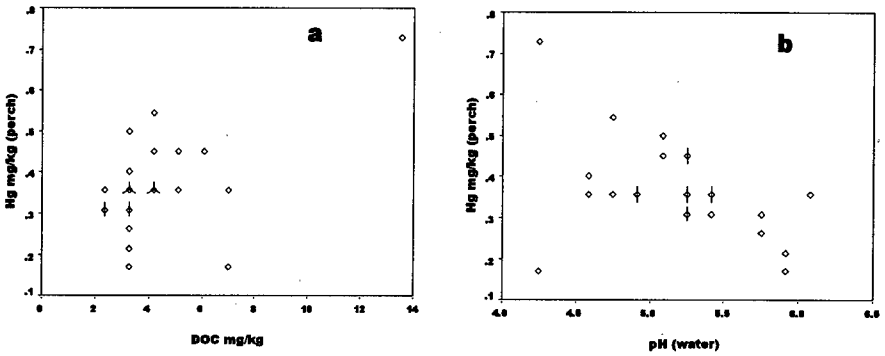

Figure 2. Relationship between: a) Hg in yellow perch and DOC in lake water and b) between Hg in yellow perch and lake water pH for Kejimkujik Park, Canada.

4. Conclusion

The preliminary results from Kejimkujik Park, NS illustrate that the total Hg concentrations in the vegetation, rocks, and lake waters are not considerably higher than elsewhere. However the level of mercury in the fish and the loons are among the highest in North America.

There does not appear to be a strong correlation between any one variable and the level of Hg in yellow perch or red maple. This would suggest that all the variables interact to some degree and undoubtedly that other variables not yet studied will play a major role. The next stages of the research will include other variables and if planned work proceeds then a range of variables including atmospheric input, ground water input and diagenetic processes will be included.

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TRACKING OF SELENIUM IN THE BLACK MESA PIPELINE BIOGEOCHEMICAL CHARACTERIZATION OF COAL SLURRY AND

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1. Introduction

Coal from the Black Mesa Mines in northeastern Arizona is transported through a 300-mile coal slurry pipeline to the Mohave power plant in Laughlin, Nevada. A previous study of the coal slurry pipeline showed that sodium, copper, calcium, magnesium, potassium, lead and iron were extracted from the coal during the transport process, but did not investigate transformations of selenium. This study reports the results of sampling the coal slurry at five stations along the 300-mile distance of the pipeline, and testing the slurry samples for selenium content, particle size distribution, heterotrophic bacteria, nitrate reducing bacteria, dissimilatory sulfate reducing bacteria and *Thiobacillus ferrooxidans*. Coal particles were also examined by transmission electron microscopy (TEM) to determine if bacteria colonies formed on the surfaces of the coal particles.

2. Materials and Methods

Coal slurry samples were collected for chemical and particle size analysis from five stations along the Black Mesa Pipeline. Sixteen samples were taken at each station, and the values were averaged to obtain representative estimates of the selenium concentration at each point sampled. Samples for chemical analysis were centrifuged and then filtered through a 0.45μ filter to separate slurry water from the coal before storing at 4 °C. Selenium concentrations were determined in the coal samples and in the slurry water using ASTM Standard Method D 4606-86. All selenium determinations were made by hydride generation atomic absorption spectroscopy.

Three samples were collected for bacterial analysis at each end of the pipeline. Each sample for bacterial analysis was placed into sterile, 50mL centrifuge tubes. Three milliliters of these samples were pipetted out and mixed with 3 mL 2% glutaraldehyde for storage before processing for TEM analysis. These chemically fixed coal samples were enrobed in 2% (wt/vol) Noble agar, processed through an ethanol dehydration series and embedded in LR white resin. The blocks were trimmed and sections were cut with a Diatome mineral grade diamond knife using a Ultracut E ultramictome. Thin sections (60-70 nm) were picked up on a formvar coated, carbon stabilized copper grids. Thin sections were examined using a JEOL 1200EX II TEM. Heterotrophic bacteria were enumerated through a serial, 10-fold dilution in 0.85% saline solution. Bacteria were spread plated and incubated at room temperature for one week on R2-A medium. Nitrate reducing bacteria (NRB) were enumerated through a serial, 10-fold dilution in 0.85% saline solution. Bacteria were grown in Bacto® Nitrate Broth. The media was dispensed into test tubes containing fermentation vials so that produced nitrogen gas could be detected. Fermentation tubes were examined for gas production after one week. Bacteria were counted using the MPN technique. Sulfate reducing bacteria (SRB) were enumerated by serial 10-fold dilutions processed in 8mL of 0.85% (wt/vol) saline made anaerobic by adding 1 mL of filter sterilized reducing agent supplement. SRB were enumerated using the MPN method and an anaerobic iron sulfate medium. *Thiobacillus ferrooxidans* bacteria were enumerated by a serial 10-fold dilutions processed in).85% (wt/vol) saline solution. Bacteria were incubated at room

temperature for one month in 9K buffer. The positive test for *Thiobacillus ferrooxidans* was taken as a rust colored precipitate on the test tube walls.

3. Results and Discussion

Table 1. Bacterial populations (number of bacteria per mL of coal slurry) at the two stations examined.

Station	Heterotrophic bacteria (cfu/mL)	Nitrate reducing bacteria (MPN/mL)	Sulfate reducing bacteria (MPN/mL)	Thiobacilli (MPN/mL)
Station #1	8.75×10^6	$>1.60 \times 10^4$	2.13×10^3	1.97
Std. Deviation	1.51×10^6	-	1.24×10^3	0.47
Station #5	3.15×10^5	1.58×10^5	3.97×10^4	ND
Std. Deviation	6.36×10^4	2.01×10^4	2.03×10^4	-

Table 2. Selenium concentrations in coal and water at the five stations along the Black Mesa Coal Slurry Pipeline.

Station	Se concentration in coal (μg/g)	Se concentration in water (μg/L)
Station #1	1.81	>0.1
Station #2	1.68	>0.1
Station #3	1.78	1.0
Station #4	1.75	>0.1
Station #5	1.85	0.7

Table 3. Changes in the particle size distribution resulting from transporting coal in the Black Mesa Coal Slurry Pipeline.

Station	Mode particle size	Mean of particle size	Std. Deviation particles
Station #1	1086 nm	768 nm	428 nm
Station #5	790 nm	654 nm	334 nm

4. Conclusions

Transporting coal through a coal-slurry pipeline reduces average particle size of the coal, and results a decreased population of heterotrophic bacteria (96% reduction) and increases nitrate reducing bacteria (10-fold increase) and sulfate reducing bacteria (18.6-fold increase) populations. *Thiobacillus ferrooxidans* bacteria did not survive the transport process in a viable form. Although bacterial populations changed significantly during the coal transport process, selenium concentrations in the coal and in the slurry water were unchanged.

GEOSTATISTICS AND CLUSTER ANALYSIS FOR SOURCE ASSESMENT OF METALS IN SOILS FROM SOUTHEAST MEXICO

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1. Introduction

The petrochemical industry in the states of Tabasco and Chiapas in south east Mexico emits NOx, SO_4, and trace metals (mainly Ni and V). The tephras spread from the volcano, El Chichon, some 300 km further south also contain significant cuantities trace metals, including Cr, Cu, Pb, and Zn in addition to Ni and V (Varenkamp et al., 1984).
We have (i) mapped the spatial distribution of some of the metals in the soil geostatistically, and (ii) attempted to differentiate between anthropogenic (petroleum industry) and geogenic (volcano and soil forming parent material) sources, by univariate anaylsis.

2. Materials and Methods

Topsoil (0 - 15 cm) in a rectangle 10 x 20 km around the industrial facilities was sampled at 1 km intervals by taking 12 cores of soil at each point and bulking them for chemical analysis. Parent material was also sampled in the region. Metals were extracted from bulked samples and parent meterial with aqua regia and measured in the extracts by atomic absorption spectroscopy. The concentrations of metals in the crude oil and volcanic ashes were taken from the literature (Varenkamp et al. 1984, Macías, 1997, Botello, 1996 and IMP, 1977).
The data for each variable were summarized, and their distributions displayed. Those that were strongly skewed were transformed to approximate normality to stabilize their variances for further analysis. Sample variograms were computed, and to them were fitted models by weighted least squares approximation. Each variable was then estimated at close-spaced intervals on a grid by ordinary punctual kriging, from which isarithmic (`contour') maps were made (WEBSTER and OLIVER 1990).

3. Results and Discussion

The maps show how the concentrations of both Ni and V increase from north west to south east (Fig.1). Their general distributions seem to be quite unrelated to the positions of the petrochemical facilities (CPG Cactus and Nuevo Pemex). At a few sampling points close to the facilities, however, the concentrations are unusually large and exceed the `norms'. This suggests that pollution by the industry is very local and not widespread. The longer range dependence captured in the variograms and the general trends in the concentrations apparent in the maps probably arise from other sources, and ash from the distant El Chichon, which erupted massively in 1982 (Carey, 1986), is the most likely.
To corroborate this hypothesis an univariate analysis was performed comparing data sets (metal contents in volcanic ashes, crude oil, soils and parent material) for their normal distribution and Ni/V relations.

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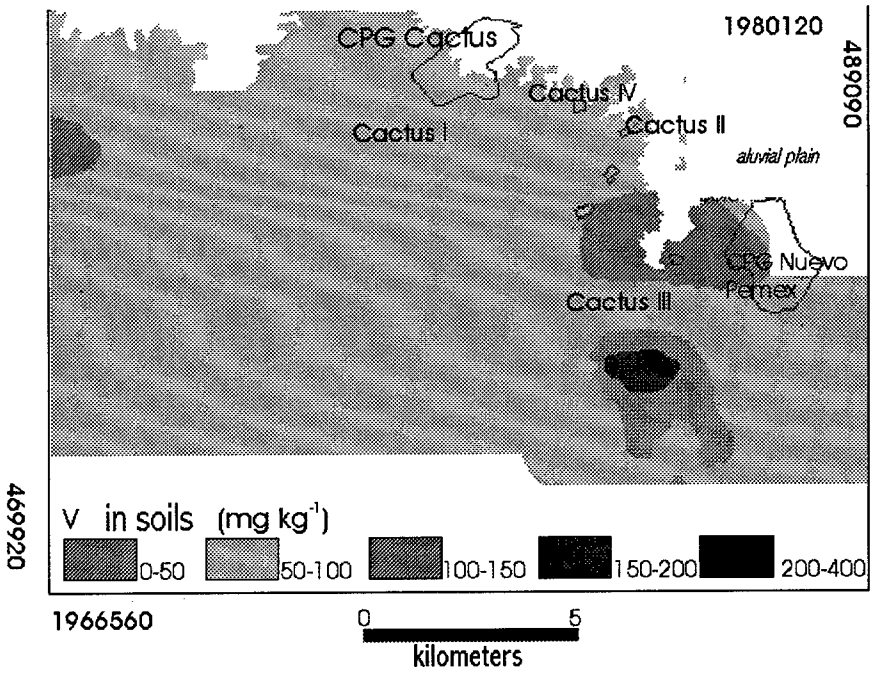

Fig. 1. Isarithmic ('contour') maps of vanadium.

ANTAGONISTIC AND SYNERGISTIC INTERACTIONS BETWEEN AL AND MN UNDER CONDITIONS OF LOW IONIC STRENGTH

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1. Introduction

Concentrations of soluble Al and Mn often reach toxic levels in acid soils. While dose responses for these metals are well documented, the effects of combined exposures have received less attention. Kinraide (1993) hypothesized that ionic interactions in the root apoplasm should lead to a non-specific cation amelioration of cation toxicity. If this is true, it should be possible to detect reciprocal ameliorative effects between Al and Mn. However, Blair and Taylor (1997) and Rees and Sidrak (1961) found no evidence of amelioration in a series of experiments with *Triticum aestivum, Atriplex liastata* and *Hordeum vulgare.* A possible explanation for the lack of ameliorative effects between Al and Mn might be the high ionic strength of nutrient solutions used by various investigators. The objective of this research was to investigate the combined effects of Al and Mn, when both are supplied at low concentrations under conditions of low ionic strength as found in soil solutions of acid soils.

2. Materials and Methods

We examined the combined effect of Al and Mn on growth and metal accumulation in *Vigna unguiculata* grown in solution culture with conductivities < 100 $\mu S\ cm^{-1}$. Nutrient solutions (pH 4.3) initially contained 200 μM nitrogen and all other nutrients in proportions considered optimal for plant growth. Additional nutrients were supplied daily, in increasing quantities to meet the requirements of exponential growth (RGR = 0.22 $g\ g^{-1}\ day^{-1}$). Two reciprocal experiments were performed; (i) a Mn dose response in the presence of 1, 4, or 8 μM Al, and (ii) an Al dose response in the presence of 0.1, 3, or 6 μM Mn. In each experiment, 10 primary metal treatments produced a full dose response, while 3 secondary metal treatments were selected to produce optimal growth and growth reductions arising from toxicity. Monomeric Al (pyrocatechol violet) and total Mn (AAS) were measured prior to planting and periodically during the experiment. Aliquots of $AlCl_3$ or $MnCl_2$ were subsequently added to restore initial values. At the end of each experiment, roots and leaves were harvested separately and concentrations of Al and Mn determined by ICP-AES. A modified Weibull was fit to root mass data to detect potential antagonisms or synergisms (Taylor et al. 1992).

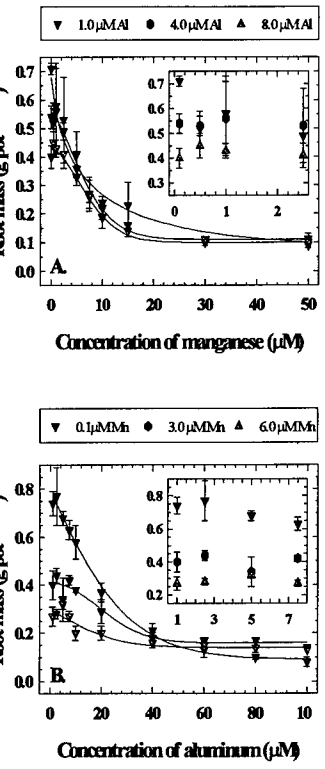
3. Results and Discussion

The nature of interaction between Al and Mn varied with the specific physiological response, the part of the plant investigated, and the relative amount of stress imposed. In the first experiment (Mn x Al), the negative effect of Mn toxicity on root biomass production was evident as Mn concentrations increased up to 30 μM. Further increases had no additional effect (Fig. 1A). Increasing concentrations of Al from 1.0 μM to 8.0 μM further reduced biomass production. The Weibull analysis indicated that Al as a secondary metal altered the

shape of the Mn dose reponse. Data from low concentrations of applied Mn provided insight into the nature of this interaction (Fig. 1, inset). At 1.0 µM Al, root biomass production declined by 31% as Mn increased from 0.1 to 2.5 µM. At 4.0 and 8.0 µM Al, the decline was less than 2%. Thus, elevated concentrations of Al protected plants from Mn toxicity (an antagonistic interaction).

In the reciprocal interaction experiment (Al x Mn), the Weibull analysis indicated no significant effect of Mn on the shape of the Al dose response. While data in the low concentration range (Fig. 1B, inset) suggested a possible reciprocal effect of Mn protecting plants from Al toxicity, the Weibull analysis indicated that the multiplicative model adequately explained the data.

While root mass data failed to provide support for an antagonistic interaction in the reciprocal Al x Mn experiment, visual examination of leaves provided clear evidence of an antagonism. Leaf speckling on unifoliate leaves (a symptom of Mn toxicity) was clearly expressed as Mn increased from 0.1 to 3 and 6 µM (data not shown). The intensity of speckling declined, however, as Al increased from 1 to 100 µM. Thus, Al provided protection from this Mn-induced injury (an antagonistic interaction). In contrast, visual examination of trifoliate leaves demonstrated a synergistic interaction. An Al-induced chlorosis (the most obvious symptom of toxicity in these leaves) became pronounced as Al concentrations increased from 1 to 100 µM. As Mn concentrations increased, the extent of this Al-induced chlorosis increased dramatically. Thus, in the same plants, we have evidence of antagonistic (unifoliate leaves) and synergistic (trifoliate leaves) interactions. Metal content data also provided evidence for antagonistic and synergistic effects, but the effect depended on dose (data not shown). These counteracting effects may account for the apparent lack of an antagonism in the Al x Mn root mass data.



4. Conclusions

In contrast with previous reports, antagonistic and synergistic interactions between Al and Mn were observed under environmentally relevant conditions. Conducting experiments at low ionic strength facilitated the expression of antagonisms and synergisms that are not observed using traditional solution culture techniques. Our data suggest a more detailed analysis of the potential for interactions between metals in the environment may be required.

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CR, NI, CU, CO IN A TROPICAL NATIVE VEGETATION UNDER ULTRAMAFIC (SERPENTINE) SOILS IN SE-BRAZIL

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1. Introduction

Ecological disturbances in areas with the occurrence of serpentinized rocks, particularly those related to the native vegetation, are known in different parts of the world related to several of edaphic factors, such as high contents of Cr and Ni and high Mg/Ca relations of these soils. Tolerant plants and other hyper-accumulators of these metals are frequent in endemisms associated to those ecosystems in different climatic conditions, being the most frequent the disturbances related to the *serpentinic syndrome* in regions of cold and temperate climates (Roberts & Proctor, 1992). In the humid tropical environment, the references of these disturbances are rare although massifs of exposed serpentinized ultramafic exist and, frequently, with similar soils to those described by the ecology researchers of those areas. Various are the hypothesis that can explain this behaviour including the lower bio availability of toxic elements in the tropical environment or the tolerance mechanisms of the different species that compose the ecosystem. To contribute to the knowledge of the ecology of serpentinized areas in the tropical environment, the soils and the native vegetation in two areas of south-western Minas Gerais state-Brazil, were studied.

2. Materials and Methods

Two sampling areas were chosen inside the Greenstone Belt of the Alpinópolis and Fortaleza de Minas regions, in the State of Minas Gerais. The soils are Inceptisols derived from serpentinite, with depths varying between 30 and 80cm, with an abundant stoniness. The native vegetation is a Seasonal Forest, being semi-deciduous in area 1 and transitional to a Forested Savannah in area 2. The vegetation was inventoried and sampled for the leaf chemical analysis in 20 plots in area 1 and 15 plots in area 2, all sized 10 x 5m. The soils were sampled in a complete pedon in each area and in superficial compound samples inside the plots. Total chemical analysis by alkaline fusion and selective dissolutions to obtain the contents of Ni, Cr Cu Co, extractable in citrate-dithionite, acid ammonium oxalate, sodium pyrophosphate, hidroxilamine chloride and lanthanum chloride, were performed on the soil samples.

3. Results and Discussion

The studied soils are morphologically, chemically and mineralogically resembled to many soils where the *serpentinitic syndrome* is described on the planet, having high contents of Ni (2000 to 4000 $mg.Kg^{-1}$) and Cr (2000 to 5000 $mg.Kg^{-1}$), although with a variable Mg/Ca relation in depth and surface. The clay fraction is dominated by 2:1 minerals with vermiculites derived from the alteration of chlorites, interstratified chlorite-vermiculite. Lithoinherited minerals such as chlorite, talc, serpentine and tremolite also occur. With the Cr and Ni contents together with the atypical mineralogy for such a tropical environment, the effects of the *serpentinitic syndrome* such as nanism and component malformation of the vegetation were expected. However, during the floristic inventory no morphologic anomalies were noted in the catalogued individuals, leaving the possibility that the studied plants could present chemical anomalies, especially in the heavy metal contents.

In both areas, 60 species and 27 families were catalogued. The most common families were Euphorbiaceae, Fabaceae, Lauraceae, Myrtaceae, Monimiaceae and Sterculiaceae. Although the content of Ni in the leaves of most species are relatively high, which is normal in serpentinized areas, no sampled

species showed to be hyper-accumulators of Ni, this is with contents of more than 1000ppm of Ni in the leaves (Baker & Brooks, 1989). Even though the studied soils are very similar to those described in the bibliography for serpentinitic environments, this means shallow soils with stoniness and rochosity, and with similar exchangeable Ni contents to those found by Proctor and Baker (1994) for other tropical environments were Ni hyper-accumulator plants exist, such as Zimbabwe, Indonesia, New Caledonia, and also for temperate climate countries such as Scotland and England, all between 10 and 50ppm of exchangeable Ni. This fact also occurs in other parts of the world, where the vegetation on these soils do not develop any type of morphological anomalies (Proctor, 1992). The Cu and Cr contents of the studied plants were similar and between 1 and 10ppm, showing no evidence of plants with high contents of these elements. The total content of Co in the studied soils varies between 100 and 400ppm, this is also occurs in the *Sols brun eutrophé hypermagnesien* of New Caledonia (Proctor, 1992), higher than average contents of Co could be expected in the analysed plants. However, most species presented contents lower than 1ppm although a few bushes of the pasture presented higher contents. These results agree with those found in nearly 60% of the species analysed by Jaffré (1980) in New Caledonia, that were lower than 2.5 ppm. A *Pera glabrata* (euphorbiaceae) specimen presented contents between 130 and 180ppm of Co, in the three collected positions (tree top, base and middle). This same plant showed above average contents of Mn and Ni, although it doesn't reach the necessary levels to be considered as hyper-accumulator of these metals, that are of 10000 and 1000ppm, respectively for Mn and Co (Baker & Brooks, 1989).

4. Conclusions

It was evidenced from the partitioning obtained from the selective dissolutions that most of the soil's non residual Ni and Cr are strongly associated to iron oxides, being Ni more bioavailable than Cr in the other fractions, with high to very high levels in the fractions bound to the organic matter. Comparatively, the partitioning also evidences a higher affinity of Cu for the organic matter, of Cr for iron oxides and a better distribution in the different fractions than those presented by Ni.

60 species and 27 families were catalogued. Although the content of Ni in the leaves of most species are relatively high, which is normal in serpentinized areas, no sampled species showed to be hyper-accumulators of Ni. The little correlation presented between the soil heavy metals contents with the leaf contents does not clearly show which could be the reason for the non occurrence of the *serpentinitic syndrome*.

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PRELIMINARY STUDY ON HEAVY METAL FORMS IN SOILS OF DEGRADED AND NON-DEGRADED ECOSYSTEMS IN THE SUDETY MTS.

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1. Introduction

A process of continuous dying away of the spruce forest and dwarf pine ecosystems has been in progress in some parts of the Sudety Mountains for several decades (DROZD et.al, 1998). The main aim of this study was to determine the forms of 5 heavy metals in soils in the area of ecological disaster and to examine whether the deforestation and grass succession affect the mobility and forms of Fe, Mn, Pb, Cu and Zn.

2. Materials and Methods

The soils examined were located in the Karkonosze Mountains, at the altitude of 1 380 m and represented podzols developed from granite under dwarf mountain pine (*Pinus mughi*) and spruce (*Picea abies*). From each ecosystem 2 soil profiles were analysed, situated under non-degraded and dead forest (or dwarf pine shrubs). Basic soil properties were analysed and total concentrations of metals were determined after $HClO_4$ digestion. In mineral horizons, seven operationally defined metal species: mobile (f-1), exchangeable (f-2), occluded in MnOx (f-3), organic (f-4), occluded in amorphous FeOx (f-5), crystalline FeOx (f-6) and residual (f-7), were determined according to ZEIEN and BRÜMMER (1991). In organic horizons, the easily mobilizable fractions (f-1 and f-2) were determined in a similar way, and remaining amounts of metals were considered as organic residuum.

3. Results and Discussion

In the mountain soils, several problems may occur associated with heavy metals accumulation and potential leaching due to soil acidification (BERGVIST et al.1989, McBRIDE 1989). All the soils examined showed high acidity which decreased in the sequence: non-degraded spruce (pH 1.8-2.4) > non-degraded dwarf pine (2.0-3.0) > dead spruce (2.5-3.0) > dead dwarf pine (2.6-4.0). Profile distribution of heavy metals was influenced primarily by a podzolisation process; eluvial horizons Ees were the poorest and illuvial horizons Bfe and Bhfe the richest in metals (table 1).

Table 1: Fe, Mn, Pb, Cu and Zn contents and their easily mobilizable forms in soils:

Horizon	Total concentrations					Sum of fractions f-1 and f-2				
	Fe	Mn	Pb	Cu	Zn	Fe	Mn	Pb	Cu	Zn
	g/kg	mg/kg				Percent of total				
Ol,Of,Oh	3.9-8.5	24-231	142-315	13-44	16-87	2.0-10.9	6.5-63.5	3.3-50.0	1.7-10.8	4.2-60.8
Ees	3.5-5.2	35-112	8-33	3.8-13.6	29-55	0.4-1.8	1.1-14.2	18.7-45	0	11.6-24
Bh,Bhfe	5.8-18.2	71-309	18-94	5.7-28	36-116	0.2-0.9	0.7-5.8	4.0-28.8	0-2.4	4.0-9.7
C	7.7-13.4	252-413	15-57	17.7-19	131-202	0.2-1.2	1.6-3.6	0-23.4	0-2.9	3.0-7.3

Table 2: Predominating metal species in soil mineral horizons:

Horizon	Fe	Mn	Pb	Cu	Zn
Ees	f-7	f-7	various forms, domination of f-4	f-7, f-4 (also: f-3 - forest)	various forms, domination of f-7
Bh,Bhfe	f-7 and f-5, f-6 (also f-4 in Bhfe)	f-5, f-7 ,f-3 - d.pine f-7 - forest	f-4 (also: f-3, f-5, f-6)	f-7, f-4 (also: f-3 - forest	f-7 also: f-5, f-6
C	f-7, f-5	f-5, f-7	f-5, f-6 also: f-4 and f-3	f-7 also: f-4	f-7 also: f-5, f-6

In Bfe horizons, the substantial amounts of metals were bound to Fe oxides, and in Bhfe also to organic matter (table 2). Organic horizons contained very high amounts of Pb and Cu, much higher than those in mineral parts of soil profiles. Another important feature of the organic horizons, revealed in this study, was high concentrations of easily mobilizable forms (f-1 and f-2) of all metals. This fact indicates the possibility of metals leaching downwards the profiles, both from organic and eluvial horizons. Profile distribution of Fe and Pb and their forms in soil profiles are shown in figure 1. A differentiation in metal species was much better pronounced between different ecosystems (spruce vs. dwarf pine) than between degraded and non-degraded habitats. However, it seems that an increase in soil pH, caused by grass succession, supports the crystallisation of Fe oxides in deeper soil horizons, which goes along with stronger binding of heavy metals.

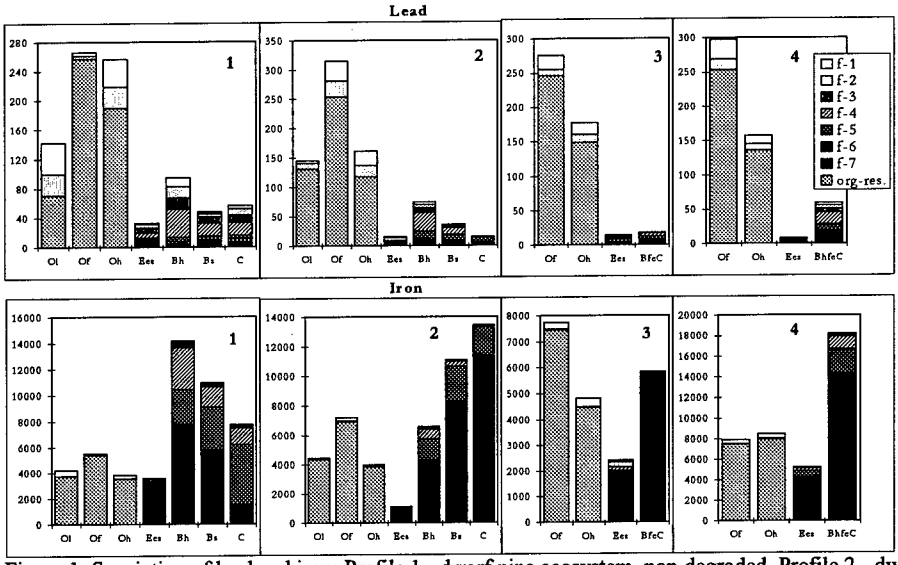

Figure 1: Speciation of lead and iron: Profile 1 - dwarf pine ecosystem, non-degraded, Profile 2 - dwarf pine ecosystem, dead; Profile 3 - spruce forest, non-degraded; Profile 4 - spruce forest, dead

4. Conclusions

All soils examined showed high amounts of easily mobilizable forms of heavy metals, in particular Pb and Mn, in organic horizons. Degradation of forest ecosystems in the Sudety Mountains and grass succession lead to decreasing soil acidity which may affect heavy metal species in soils. The transformation of metal forms in those ecosystems should be examined more thoroughly and further research, based on additional soil profiles, is needed to better describe the fate of metals in those soils.

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SOME TRACE ELEMENTS IN SOILS AND PLANTS OF BIESZCZADY MOUNTAIN MEADOWS ENVIRONMENT

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1. Introduction

The research pertaining to the trace elements content in the environment were carried out by many authors. The content of trace elements in Polish soils and plants was presented by Kabata-Pendias and Pendias [1993] and Kabata-Pendias [1993, 1995].
The Bieszczady Mountains are very specific mountain area in Poland. Human activity on the Bieszczady mountain meadows finished about fifty years ago. All the Bieszczady mountain meadows belong to the Bieszczady National Park. The special role of vegetation consists in locking up scarce elements in the given environment in a biological cycle. The research has also proved that organic and organic-mineral surface horizons of the mountain meadows soils are also the place of anthropogenic pollutants sorption [Wozniak 1996].

2. Materials and Methods

The research referring to the Bieszczady mountain meadows ecosystem began in 1991, but it is still continued. Its main aim is to investigate the interdependences between the chemical composition and other properties of soils and the chemical composition of plants, that is to investigate the trace elements cycle in this unique environment. The total content of the presented elements (Cu, Zn, Mn, Co, Ni, Pb and Cd) was defined by the spectrophotometry of atomic absorption. For the research presented in this paper two plants species dominating on the Bieszczady mountain meadows: *Vaccinium myrtillus* and *Calamagrostis arundinacea* have been selected. It should be added, that the studied plants were collected exactly from soil pits.

3. Results and Discussion

The properties of acid brown soils, dominating on the Bieszczady mountain meadows, were presented in the earlier paper [Wozniak 1996]. The content of some trace elements (selected results) in soils and plants of the Bieszczady mountain meadows are presented in Table 1 and Table 2.
The content of toxic heavy metals (Pb and Cd) in general is lower in the described works than the one accepted by Kabata-Pendias and Pendias [1993], but the accumulation of them in the surface organic horizon of mountain acid brown soils is evident.
The correlation coefficient between the total content of the studied elements in soils and plants turned out to be statistically insignificant (Zn, Pb and Cd), but sometimes were significant (Cu and Mn in soils and in *Calamagrostis arundinacea*; Mn in soils and leaves and stems of *Vaccinium myrtillus*; Ni in soils and in leaves of *Vaccinium myrtillus*; Co in soils and stems of *Vaccinium myrtillus*).

Table 1. The content of Cu, Zn, Mn, Co, Ni, Pb, Cd in soils and plants of the Bieszczady mountain meadows (mg kg^{-1} of d.m.)

Specification	Element (mg · kg^{-1} of d.m.)						
	Cu	Zn	Mn	Ni	Co	Pb	Cd
Soils A	4	20	44	4	0,8	15	0,1
B	42	92	749	44	12,9	70	0,7
C	13	53	202	14	3,4	38	0,3
Calamagrostis arundinacea A	4,2	15	47	0,24	0,005	0,6	0,07
B	9,3	25	524	1,11	0,067	2,5	0,21
C	6,8	20	206	0,52	0,028	1,1	0,14
Vaccinium myrtillus A	4,6	18	503	0,54	0,041	0,5	0,05
B	9,0	48	2556	1,83	0,166	3,2	0,20
C	6,4	27	1555	1,18	0,094	1,4	0,10

A - minimum; B - maximum; C - geometric mean

Table 2. The content of trace elements in one exampled profile of acid brown soils and in plants collected exactly from soil pit

Specification	Element (mg · kg^{-1} of d.m.)						
	Cu	Zn	Mn	Ni	Co	Pb	Cd
Soil Horizons:, Depth [cm]:							
Ofh 0-2	21	55	72	7	1,0	84	0,97
Ah 2-17	13	45	70	14	3,5	45	0,40
Bbr 17-28	13	53	249	34	9,0	42	0,38
BbrC 28-39	17	60	308	27	12,2	36	0,31
Plants:							
Calamagrostis arundinacea	4,6	21	371	0,6	0,007	1,33	0,17
Vaccinium myrtillus							
Leaves	4,9	23	2142	1,2	0,056	1,89	0,14
Stems	5,3	79	1925	0,6	0,038	1,10	0,28
Fruits	6,1	13	414	0,7	0,012	0,16	0,05

4. Conclusions

Soils, particulary their organic surface horizons, and also the plants of the Bieszczady mountain meadows create cycle of trace elements in the environment.

In the mountain areas, which are especially subject to heavy metal pollution, the organic surface horizons of soils become a holder of atmospherical deposit.

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Trace Elements in Forest Ecosystems

(Technical Session 7)

LARGE SCALE BIOGEOCHEMICAL ANOMALIES IN FORESTS OF CANADA: ANTHROPOGENIC OR NATURAL IN ORIGIN?

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1. Introduction

Over the past twenty years many reconnaissance level and detailed biogeochemical sampling programmes have been carried out across Canada by Provincial and Federal Geological Surveys. Multi-element analysis of many thousands of tissue samples from common trees and shrubs has provided a database with well in excess of two million analytical determinations. Extraordinary concentrations of trace elements occur locally, and regional anomalies are identified that extend for up to several thousands of square kilometres in areas of pristine forest.

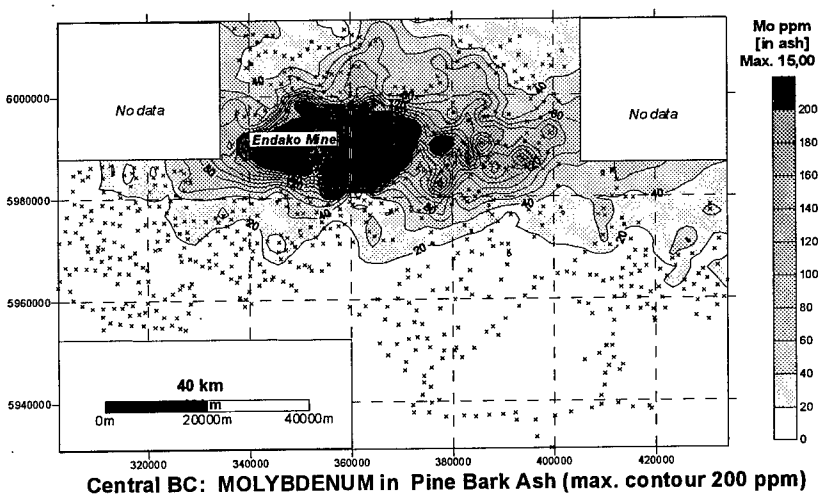
2. Materials and Methods

Most of the systematic surveys have focused on the collection of either twigs or outer bark of conifers, because many chemical elements concentrate in these extremities of the trees. Typically, 50-100 g of bark scales or twigs (less needles) are collected, air-dried, and reduced to ash by controlled ignition at 470°C. Ash samples are analyzed by both instrumental neutron activation (INAA), and ICP-ES after an aqua regia digest. These methods provide a database of more than 50 elements for each sample. Studies show that for the types of tissue being analyzed there is little or no loss of most elements during ignition.

3. Results and Discussion

Uranium: From 1979 to 1982, soon after the discovery of significant uranium mineralization near the margin of the Precambrian Athabasca Sandstone in northern Saskatchewan, biogeochemical surveys using twigs of black spruce (Picea mariana) were undertaken over large tracts of land. Whereas the common background level of uranium in ash of spruce twigs is less that 1 ppm U, it was found that concentrations were at least an order of magnitude greater in all black spruce trees growing within an area of 10,000 km^2. Within this immense anomaly there was an area of 1,000 km^2 where all spruce twigs yielded more than 100 ppm U, attaining a maximum of 2270 ppm U (Dunn, 1983). Air monitoring stations that surrounded the only uranium mine that had ever operated in the area to that time reported low background levels of uranium. Fission track imagery of cross sections of uranium-rich twigs showed that the uranium was concentrated in the bark portion of the twigs, and not in the woody tissue.

Molybdenum: At the Endako mine in central British Columbia molybdenum mineralization is associated with granitic rock. Recent reconnaissance level surveys using the outer bark of lodgepole pine (*Pinus contorta*) have outlined the regional extent of molybdenum enrichment. Unlike uranium, molybdenum is an essential micronutrient yet is commonly present in the ash of plants at concentrations less than 2 ppm Mo. The contour defining 20 ppm Mo extends over an area of 5,000 km^2 and encompasses a region of more than 300 km^2 where bark concentrations exceed 200 ppm Mo, attaining a maximum of 1.5% Mo in ash from trees close to the mine. Although dust from the mining operations contributes to the high levels encountered, historic records indicate similar levels in plants collected from the mine site in the 1960s during the early days of mining operations.



Central BC: MOLYBDENUM in Pine Bark Ash (max. contour 200 ppm)

Base Metals: A multi-species survey of a 25 km^2 area around the large lead-zinc Sullivan mine near Kimberley, British Columbia, has found that twigs of Sitka alder (*Alnus sinuata*) contain up to 5% zinc and 2% lead in ash. In areas remote from base metal mineralization, lead concentrations in lodgepole pine bark are typically about 20 ppm Pb in ash. A maximum of 2.3% Pb is present in pine bark near the Sullivan mine, and because the minimum value in the area surveyed was 345 ppm Pb the areal extent of the lead enrichment has yet to be determined.

Gold: At the Ladner Creek mine in a mountainous region near Hope, BC, local enrichment of gold in bedrock is amplified by gold enrichment in twigs of Pacific silver fir (*Abies amabilis*) extending several km^2 (Dunn and Ray, 1995). Anomalies of similar magnitude are found in lodgepole pine bark near the Nickel Plate mine (Hedley, BC), and around undeveloped mineralization at Mount Washington (Vancouver Island) in twigs of mountain hemlock (*Tsuga mertensiana*) and rhododendron (*Rhododendron albiflorum*). Scanning electron microscopy (SEM) shows that crystalline phases of gold have nucleated in the plant tissues (Dunn, 1995).

4. Conclusions

Mobilization of metals from enriched sources, and subsequent uptake by trees or shrubs can result in biogeochemical anomalies of extraordinary intensity and great areal extent. Some of the enrichment is derived from air-borne particulates associated with mining operations. However, SEM studies show that metal-rich phases form in plant tissues indicating that significant uptake of metals occurs through roots. It appears that natural geochemical systems develop which can give rise to large areas that contain trees and shrubs anomalously rich in metals.

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BINDING OF TRACE METAL CATIONS TO WOOD OF PINE (*PINUS SYLVESTRIS*) AND OAK (*QUERCUS PETRAEA*) TREES

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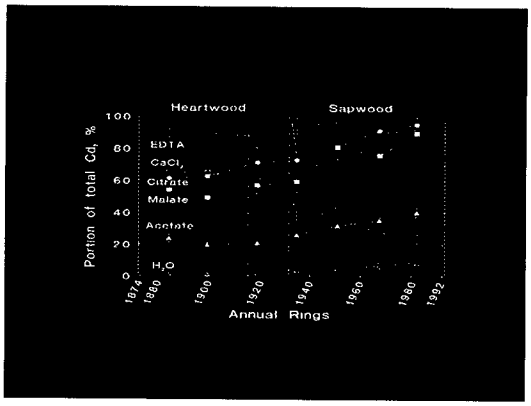
1. Introduction

Radial distribution patterns of trace metals in stems of trees have been used as pollution chronologies (HAGEMEYER 1993, 1997, FORGET and ZAYED 1995). This method of retrospective biomonitoring is known as dendroanalysis or dendrochemistry. A prerequisite for the method, the long-term stability of metal distributions in stem wood was not thoroughly investigated. So far, little is known about the mobility of trace metal cations in tree stems. Therefore, binding forms and binding strength of metal cations in wood tissue were studied.

2. Materials and Methods

Stem wood samples of mature pine (*Pinus sylvestris*) and oak (*Quercus petraea*) trees were obtained from stem discs cut near the stem base. Samples were taken from growth rings of different age. The wood tissue was extracted for 1 h with H_2O or 0.01 M solutions of $CaCl_2$, acetic acid, malic acid, citric acid and EDTA. After centrifugation, concentrations of Cd and Pb were determined in the extracts with graphite furnace atomic absorption spectrophotometry. Total concentrations of the elements in the wood were measured after mineralization with concentrated HNO_3. Extracted quantities of Cd or Pb were expressed as percentage of total concentrations.



3. Results and Discussion

An example of the results is shown in Figure 1. With EDTA, more than 80% of total wood Cd was extractable. Citric and malic acid extracted 50 – 95% of total Cd, Whereas water removed only up to 9.5% of the Cd. In pine stems the Cd-extracting efficiency of organic acids and of water increased from the stem center towards the outer annual rings (Fig. 1). The general order of extractant efficiency for Cd was H_2O < acetic acid < $CaCl_2$ < malic acid < citric acid < EDTA.

The extracting efficiency of the organic acids was correlated with the stability constants of complexes formed by the acid molecules with Cd^{2+} ions. The tested agents had a very low efficiency for Pb extraction except EDTA, which mobilized 55-85% of total Pb in pine wood and 40-70% in oak wood (BALK and HAGEMEYER 1994, HAGEMEYER and SHIN 1995). The results show that Pb is more strongly bound to wood tissue than Cd.
Three different fractions of metal cations in wood tissue were distinguished: (1) A mobile, water-soluble portion which comprised up to 10% of Cd; Pb was not detectable in H_2O extracts. (2) An exchangeable portion, which is reversibly bound to fixed negative charges in the walls of conducting vessels or tracheids. Metal cations of this portion are exchangeable against H^+ or other cations. This portion contained up to 90% of Cd and up to 85% of Pb. (3) An immobile portion which is not extractable with the tested agents. It comprised up to 25% of Cd and up to 60% of Pb, which demonstrates the stronger binding of Pb.
The results show that a considerable percentage of the studied metal cations in wood tissue is only weakly bound. These cations can be mobilized with water or with organic acids (such as malic or citric acid), which occur frequently in xylem sap.

4. Conclusions

Due to the potential mobility of cations, metal binding in tree stems is probably not stable for long time periods. These results are supported by the observation of seasonal fluctuations in element distribution patterns (HAGEMEYER and SCHÄFER 1995). The usefulness of such metal distributions for long-term retrospective biomonitoring of trace metal pollution is questionable (HAGEMEYER 1997).

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FOUR DIFFERENT EXTRACTION METHODS TO ASSESS BIOAVAILABILITY OF SOME HEAVY METALS IN FOREST SOIL FROM NORWAY

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1. Introduction

Various extraction methods have been used to assess the availability of an element to different plants. Most frequently, these methods have been used in the research on agricultural soil and plants. These methods will normally give a rough estimate of the easily available elements for root uptake, even though it has often been difficult to find linear relations between extractable soil concentrations of an element in question and actual concentrations in plants growing on the soil.

The objective of the present study was to find an easy test and a method, which gives the best correlation between heavy metals (Cu, Pb, Zn and Cd) extracted from forest humus soil compared with the total concentration of the element in some forest plants. To test the material, four different single extraction methods were chosen, representing four main groups of extractants. The selected extraction methods for use were HCl (acid extraction), EDTA (chelating agent), NH_4NO_3 (unbuffered salt solutions) and NH_4OAc (buffered salt solution).

2. Materials and Methods

The soil and plant material in the present study were collected from 17 different forest sites in the middle and south of Norway, representing a range of different geological conditions and different loads of heavy metals from atmospheric deposition. The samples were all from pine forests (Pinus sylvestris) and they were taken from the humus layer of podsolic soil only. On each site, five subsamples were collected which were divided into Oe and Oa horizon. Harvested plants were wavy hair grass (Deschampsia flexuosa), bilberry (Vaccinium myrtillus) and mountain cranberry (Vaccinium vitis-idaea) where the last two species were split into leaf and stem before digestion.

Each soil sample was split in two, one half was sieved to pass a 2-mm aluminium mesh while the other was left untreated. The soil was extracted with NH_4OAc (1 M, pH 4.80), NH_4NO_3 (1M), HCl (0.1 M), EDTA (0.05M, pH 7.00) and also digested with concentrated HNO_3. The plant material was digested with HNO_3 in a microwave system.

3. Results and Discussion

Preliminary results indicate a correlation between extractable Zn from the humus layer and the total concentrations in the plant material from the forested areas. Figures 1 and 2 show the relationship between Zn in bilberry stems and ammonium nitrate extractable Zn (Oe horizon) and ammonium acetate extractable Zn (Oa horizon), respectively.

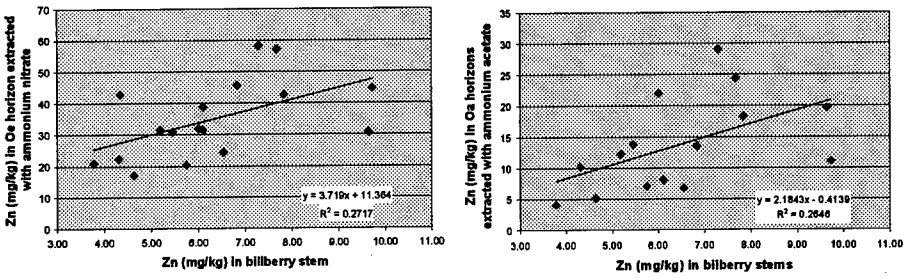

Figure 1. Relationship between extractable ammonium nitrate Zn (mg/kg) from Oe horizons in forest soils and Zn (mg/kg) in bilberry stems.
Figure 2. Correlation between Zn (mg/kg) concentrations extracted from Oa horizons in forest soils and Zn (mg/kg) in bilberry stems.

The results in figure 1 and 2 are consistent with results from other investigations which state that the Zn accumulation in Vaccinium myrtillus increases with the concentrations of the element in the soil (Koski *et al.*, 1988; Løbersli, 1991; Berthelsen *et al.*, 1995). The results in figure 1 and 2 indicates that extractable Zn accounts for respectively 27,2% (ammonium nitrate) and 26,5% (ammonium acetate) of the variation in Zn uptake in bilberry stems.

Statistical analysis and further preparation of the results will be carried out during October-December 1998 and the results from this analysis will be available for the final resubmission of revised abstracts in January.

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ALUMINUM SPECIATION IN ACID FOREST SOIL SOLUTIONS: COMPARISON OF DIFFERENT METHODS

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1. Introduction

High concentrations of aluminum in the soil solution of acid forest soils can be a major stress for trees. The toxic effect depends to a great extent on the chemical nature of the Al species present. A widely used Al speciation scheme is the one proposed by DRISCOLL (1984). Three fractions are determined: acid reactive Al is measured colorimetrically after acidification of the sample to pH 1, mononuclear Al by the same method in the unmodified sample, and ion exchange-labile Al as the difference between mononuclear Al and Al measured after passage of the sample over a strongly acidic cation exchange resin. The method has been automatized using flow injection analysis (FIA) (e.g. 2). A different approach is the determination of complexation-labile Al based on fast reaction with 8-hydroxyquinoline (HQ, e.g. 3). Recently, GÖTTLEIN (1998) has claimed that free Al^{3+} concentrations can be measured by means of capillary electrophoresis (CE). Finally, Aluminum bound by phenolic substances can be detected by fluorescence spectroscopy (e.g. 5). The aim of this paper was to compare the results obtained by these different methods for soil solutions collected from two acid soil profiles typical for two regions of Switzerland. The results are interpreted by comparison with results obtained for synthetic solutions of various model organic ligands which were prepared once by avoiding formation of polynuclear Al species and once by synthesizing polynuclear Al at a defined OH/Al ratio.

2. Materials and Methods

Soil solutions were collected from a haplic alisol located near Ermatingen in north eastern Switzerland (6) and from a haplic podzol located at Copera in southern Switzerland (7). Solutions from just below the litter horizon were collected by tension-free lysimetry, those from mineral horizons by tension lysimetry using ceramic suction cups. In the latter case a vacuum of 60 kPa was applied at the beginning of each sampling interval of 1 or 2 weeks.

Synthetic solutions: „Polynuclear free" solutions of Al complexes with salicylate, oxalate, citrate, chestnut litter extract or spruce litter extract were prepared with a pH of 4 and an ionic strength of 1 mM by avoiding addition of OH^- to Al solutions. The range of total Al concentrations and Al/ligand ratios was selected such as to cover most of the conditions observed in the soil solutions. Solutions with the same Al and ligand concentrations and ionic strength but a defined OH/Al ratio of 3 ("polynuclear conditions") were prepared following the protocols of PARKER and BERTSCH (1992) by first mixing Al and organic ligand and then adding NaOH solution. In addition, "polynuclear" solutions without organic ligands were prepared. All "polynuclear" solutions were aged for 1 week before measuring Al fractions.

Al speciation: Instead of measuring acid reactive Al colorimetrically, total Al concentrations in aliquots of the soil solutions, that were acidified immediately after sampling, were determined by inductively-coupled plasma atomic emission spectrometry (ICP/AES). Mononuclear Al was measured in the original sample solutions by using the FIA method of ROYSET (1992) which is based on the colorimetric determination of Al with eriochrome cyanine R. Carrier solution was 0.01 M NaCl, pH 4. Ion exchange-labile Al was obtained as difference between mononuclear Al and ion-exchange-inert Al. The latter was measured in the same way as mononuclear Al but with a column of strongly acidic cation exchanger installed between injection valve and mixing with the first reagent. Complexation-labile Al was measured in the original sample solutions by using the FIA method of CLARKE et al. (1992) which is based on a 2.3s reaction with 8-hydroxyquinoline. Determination of "free" Al by capillary electrophoresis (CE) followed the method described by GÖTTLEIN (1998). Total luminescence spectra were recorded of soil solutions that were diluted or concentrated to a DOC concentration of 10 mg/L and adjusted to pH 4.

3. Results and Discussion

In "polynuclear free" synthetic solutions mononuclear Al included the Al complexes with all ligands except for chestnut leaf litter extract at high ligand/Al. At "polynuclear conditions" only part of the Al was measured as mononuclear Al with the organic ligands increasing the mononuclear part in the order salicylate, litter extracts, citrate, oxalate. Ion-exchange labile Al was equal to mononuclear Al in most cases, in particular at "polynuclear conditions". The most important exceptions were oxalate and citrate that formed ion-exchange inert complexes at high ligand/Al. Complexation labile Al was always equal or smaller than ion-exchange labile Al. Mononuclear Al complexes of oxalate and citrate were not included in the complexation labile fraction, whereas the complexes of the

litter extracts and of salicylate were partly complexation labile. The Al concentration measured by CE was equal to complexation labile Al in most cases. However, higher concentrations were measured with CE in a few cases at low ligand concentrations.At both soil solution sampling sites the collection period from August 1997 to May 1998 could be subdivided into two parts with respect to Al speciation. For the podzol, the first part from August 1997 to December 1997 was characterized by a higher pH of about 5 at all depths when compared to the second part, during which pH was around 4.5. Dissolved organic carbon (DOC) concentrations were about 20 mg C/L just below the litter layer and 2 mg C/L or less at greater depths year round. There was no difference in the average total Al concentrations between the two periods. The mean annual concentrations were 11±4, 38±7, 29±5, and 28±7 µM at 0, 30, 57 and 110 cm depth, respectively. The Al speciation in the soil solutions was compared with the results obtained for the synthetic solutions, and, based on this comparison, could be interpreted as follows. At all mineral soil depths, complexation by complex dissolved organic matter and simple phenolic ligands played an important role. Just below the litter layer, simple aliphatic ligands like oxalic or citric acid probably acted as the main complexing agents. The lower pH in spring led to a larger mononuclear part at all mineral soil depth when compared to fall. During the latter period, a decrease in the mononuclear part from about 40% at 30 cm depth to about 15% at 110 cm depth could be explained by either a decrease in DOC from 2 mg/L at 30 cm to < 0.5 mg/L at 110 cm or by increasing "age" of the solutions with depth.The pH values in the alisol soil solutions were characterized by larger differences between the different sampling depths but smaller seasonal variations when compared to the podzol. Average values were around 4.5 at 0 and 15 cm, about 5 at 45 cm, and about 5.5 at 95 cm. At all depths, DOC concentrations were higher in the fall period (August 97 to January 98) than in spring. Average concentrations at 0, 15, 45 and 95 cm were 95, 52, 9 and 3 mg/L, respectively, in fall and 58, 43, 4, and 2 mg/L in spring. As for the podzol, the alisol showed no significant differences in total Al concentrations when comparing the two sampling periods. The mean annual concentrations were 21±13, 139±12, 32±8, and 9±4 µM at 0, 15, 45 and 95 cm depth, respectively. Speciation of Al led to very similar results when compared with the podzol. Just below the litter layer there were strong indications for complexation mainly by simple aliphatic compounds, whereas at 15 and 45 cm depth complex dissolved organic matter and simple phenolics probably were the most important complexing agents. The mononuclear part decreased from 85% at 15 cm to less than 60% at 45 cm. At 95 cm the results suggested the presence of long aged polynuclear Al complexes of inorganic or organic nature. At 45 cm a much larger contribution of labile Al fractions was observed in spring. Only a small difference in pH (fall: 5.1; spring: 4.7) seemed to be responsible for this.

4. Conclusions

The comparison of comprehensive Al speciation results for the soil solutions in two different acid soils with results for comparable synthetic solutions allowed a detailed interpretation with respect to the chemical nature of dissolved Al in the two soils. Just below the litter layer, mononuclear Al complexes with simple chelating aliphatic ligands seem to dominate. At greater depths, Al complexes with complex organic matter and simple phenolics are most important. The contribution of polynuclear Al depends mainly on pH with a "critical value" of just below 5, but there are also indications for an influence of DOC concentration and age of the solution.

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MOBILITY OF CU, MN, ZN AND CD IN A PINE FOREST AS AFFECTED BY LIMING AND ACIDIFICATION

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1. Introduction

In 1972 a project named «Acid Rain - effects on Forest and Fish» was initiated by Norwegian scientists. One of the intentions was to examine long-time effects of acid rain and liming on soil properties, tree growth and nutrient status of trees (Abrahamsen *et al.*, 1980). These experiments were carried out during the period 1974-1981 at a site located within the part of Southern Norway which, in addition to being significantly affected by acidic deposition, also is exposed to considerable amounts of airborne deposition of heavy metals such as Zn and Cd from long range atmospheric transport (Solberg and Steinnes, 1983).
The intention of the present study was to investigate how changes in mobility of Cu, Mn, Zn and Cd in soil exposed to artificial acidification and liming would have influenced the bioavailability of these elements to the ground vegetation, represented by the dwarf shrubs *Vaccinium myrtillus* and *Vaccinium uliginosum* and the grass *Deschampsia flexuosa*, fifteen years after the closure of the experiments.

2. Materials and Methods

The experiment was established at Åmli in Southern Norway. The area is stocked with Scots pine (*Pinus sylvestris L.*) planted between 1968 and 1970 after a forest fire in 1968.
The study area is divided into circular plots of 75 m^2 each which were treated during the period 1974-1981 with different combinations of artificial precipitation (pH 3.0, 2.5, 2.0 and not watered) and lime (0, 500, 1500 and 4500 CaO kg ha^{-1}) in a factorial design not fully complete. Each treatment was replicated three times (Tveite *et al.*, 1990/91).
Samples of vegetation and organic surface soil were collected in June 1996. Composite samples of the different vegetation species were obtained from randomized collection within each plot. The plants were cut about 5 cm below the top and samples were manually homogenized after air-drying. Soil samples were collected from the Oe+Oa horizons, air-dried and sieved (< 2 mm). Randomized subsamples (≈ 2 g) of vegetation and soil were digested in nitric acid (65%, 4 ml) in a microwave oven, filtered and analyzed by atomic absorption spectrometry after appropriate dilution with respect to Cu, Mn and Zn (flame) and Cd (graphite furnace). The soil samples were also extracted by ammonium acetate (pH 4.8) for determination of the potenially plant-available fraction of the metals.

3. Results and Discussion

Concentrations of Cu in soil and vegetation were little affected by addition of acid and lime. Only small amounts of Cu were extracted by ammonium acetate.
Manganese is a very mobile element in organic soil under acid conditions and the results showed that conciderably amounts of Mn had been washed out from the organic top soil, especially at pH

2.0 precipitation. Liming reduced these losses, but did not seem to have any positive effects on the mobility of Mn from the organic horizon when exposed to pH 2.0 precipitation.
As with manganese, the concentrations of zinc and cadmium in organic soil are influenced by the precipitation acidity showing increased mobility with increased acidity. Liming decreased the mobility of Zn and Cd in the organic horizon for all the treatments, but the effects were most distinct in plots watered with pH 2.0 precipitation.
The vegetation had no signs of deficiency for any of the essential metals (Cu, Mn, Zn). The results indicated that plants absorbed more trace metals with increased acidity. Generally, liming reduced the concentrations of Mn, Zn and Cd in all the species. Statistical tests, using Spearman correlation, showed little or no correlations between concentrations of the elements in the organic soil and in the plants.
Vaccinium myrtillus accumulated more Mn than the other species, whereas *Vaccinium uliginosum* accumulated more Cu, Zn and Cd. Generally the concentrations were higher in stems than in leaves.

4. Conclusions

The effects of the experiment can still by traced 15 years after its termination. Although the effects are not very clear any longer, it obviously takes long time to re-establish the natural balance in the forest ecosystem.

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CANOPY FILTRATION OF COPPER, NICKEL AND ZINC NEAR THE HARJAVALTA CU-NI SMELTER, SW FINLAND

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1. Introduction

Canopy filtration of dust and aerosols is the major process controlling the throughfall enhancement of most heavy metals. The filtering capacity of vegetation varies considerably: wooded areas are more efficient at intercepting heavy-metal dry deposition than other types of vegetation (Hultberg, 1985). Other crucial factors are the particle size and solubility of heavy metal emissions. Large dust particles have a high settling velocity, and the importance of canopy filtering diminishes rapidly with increasing distance from the source (Parker, 1983). Derome and Lindroos (1998) reported that Ni emissions at Harjavalta were transported over longer distances than Cu, which they assumed to be due to differences in particle size. Although heavy metal emissions from the Harjavalta smelter have been considerably reduced during the past decade (Helmisaari et al., 1995), the Cu, Ni and Zn fluxes in stands 0.5 km from the smelter were still extremely large during 1992-1996 compared to those at 4 and 8 km. This appears to be partly attributable to soil dust from the degraded forest floor and from slag heaps located nearby (Derome and Nieminen, 1998). The aim of this report is to compare the filtering capacity of the canopy for Cu, Ni and Zn at different distances from the smelter.

2. Materials and Methods

Three sample plots were established at distances of 0.5, 4 and 8 km from the Cu-Ni smelter at Harjavalta, SW Finland, in pure Scots pine stands growing on dry mineral sites to the SE of the smelter. The pine stand at 0.5 km was suffering from retarded growth and severe needle loss, and the ground vegetation was almost completely destroyed. In contrast, the stands located at 4 and 8 km appeared to be relatively unaffected. Bulk deposition was collected in open areas using 5 rainfall collectors, and stand throughfall using 20 collectors located systematically inside the stand on 30 x 30 m plots from July 1992 to December 1996. The samples were filtered (0.45 μm membrane filter) and Cu, Ni and Zn were determined by ICP/AES.

3. Results and Discussion

The annual throughfall fluxes for Cu at 0.5, 4 and 8 km were 370, 12.7 and 3.4 mg m^{-2} yr^{-1}, those for Ni 138, 1.6 and 0.7 mg m^{-2} yr^{-1}, and for Zn 45, 5.4 and 3.9 mg m^{-2} yr^{-1} (Derome and Nieminen, 1998). Although the throughfall flux of Cu was the highest of the three metals at 0.5 km, the relative throughfall enrichment of Zn was the highest (Fig. 1). The canopy source of Zn was equal to that from bulk precipitation at 0.5 km. The throughfall enrichment of Zn was relatively high also at 4 and 8 km, suggesting that canopy filtration of Zn also takes place at these distances. Slight Zn enrichment in pine canopy throughfall has also been observed in a background area in eastern Finland by Helmisaari and Mälkönen (1989). The amount of canopy-derived Cu was high at 0.5 km, but it decreased sharply with increasing distance from the smelter. The throughfall enrichment of Ni at 0.5 km was only slightly higher than that at 4 km, suggesting a rather low settling velocity for particles containing Ni. This is in accordance with the long transport distance of Ni, relative to that of Cu, observed in Harjavalta by Derome and Lindroos (1998). Hutchinson and Whitby (1977) also reported differences in transportation

distances of Cu and Ni at the Sudbury mining and smelting area in Canada, and attributed it to differences in particle size.

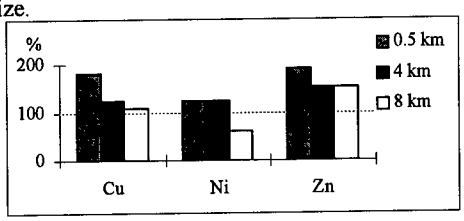

Fig. 1. The throughfall fluxes of Cu, Ni and Zn as a percentage of the corresponding bulk precipitation fluxes at different distances from the Harjavalta smelter.

4. Conclusions

Our results suggest that the canopy-filtrated, dry deposition gradient of Cu was the steepest, that of Ni relatively uniform up to 4 km, while that of Zn remained relatively high up to 8 km. These differences in the transport of the studied metals are probably mainly due to a different size distribution of the metal-containing particles, although chemical interactions and differences in the ability of the canopy to retain the filtered metals may mask to some extent the action of these physical processes.

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TRACE ELEMENTS RETURN THROUGH LEAF LITTER AND THEIR RELATIONS WITH POPLAR (*POPULUS DELTOIDES*) TREE GROWTH UNDER DIFFERENT MANAGEMNET PRACTICES

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1. Introduction

Intensive agriculture, as a result of green revolution, has resulted in a spectacular increase in grain production in Indo-Gangetic plains of India. The over exploitation of land and water resources for achieving high productivity and reduction in forest area to 5.4 per cent has resulted in a considerable loss in their production potential. It is evident that for rehabilitation of these degraded lands and for sustaining agricultural productivity and ecological balance, agroforestry is the best viable alternative land-use system. In central Punjab poplar-wheat system has received the maximum adaptation owing to its winter deciduous nature hence having little adverse affect on inter-crops resulting in higher economic returns. However, scientific information based on long term experiments is lacking for improving the productivity of such systems.

2. Materials and Methods

The experimental site is situated at an elevation of 244 m above mean sea level and lies at 30^0-45'N latitude and 75^0-40'E longitude. The treatments comprised of two levels of irrigation (irrigation according to IW/PAn-E ratio of 0.5 and 1.0), three levels of nitrogen and two cropping patterns [uncropped (UC) and wheat intercropped (C)]. The treatments were laid in a split plot design with all the six combinations of irrigation and N in the main plot and two inter-crop treatments in the sub plot. The leaf (samples collected in September when leaves were fully developed) and leaf litter samples were ground and analysed for trace elements using Inductively Coupled Argon Plasma Spectrometer (ICAP-AES). Regression analysis was used to find out all possible relations for increase in tree volume during current year of growth, trace elements concentration in leaves and in leaf litter, quantity of leaf litter and trace elements return through leaf litter.

3. Results and Discussion

The quantity of leaf litter in various treatment combinations, at the end of fourth year of growth, varied from 3.16 to 7.51 t ha^{-1}. The increase in basal area during fourth year of growth varied from 2.919 to 4.859 m^2 ha^{-1} and corresponding increase in tree volume (over bark) varied from 31.6 to 50.0 m^3 ha^{-1}. The quantity of leaf litter in different treatments was linearly correlated with increase in tree basal area ($r = 0.82$) and with increase in tree volume ($r = 0.86$) during the year. The concentration of all trace elements except As, Cd and Pb decreased in leaf litter as compared with fully developed leaves. However, the magnitude of decrease in concentration differed considerably for different trace elements. The quantity of trace elements returned through leaf litter varied from 25.7 to 760 g ha^{-1} being maximum for Mn and minimum for Ni.
The correlations between tree volume growth during the fourth year of growth and trace elements return through leaf litterfall was significant for Fe, Cu, B, Cd, Ni and Al (Table 1). The concentrations between trace element return in leaf litter and trace element concentration in leaf litter were significant for B, Mo, As, Cd and Na. However, litterfall quantity was linearly and

significantly correlated with nutrient return through litterfall for all trace elements under investigation except Mn, Mo, As, Pb and Na.

Table 1. Correlations coefficients (r) between various parameters for different trace elements.

Trace element	Increase in volume vs Nutrient return	Nutrient return vs Conc. in litter	Nutrient return vs Litter quantity
Zn	0.553	0.553	0.737*
Fe	0.690*	0.117	0.724*
Mn	0.453	0.441	0.561
Cu	0.596*	0.237	0.866*
B	0.764*	0.885*	0.847*
Mo	-0.151	0.878*	0.028
As	0.533	0.834*	0.495
Cd	0.592*	0.781*	0.875*
Hg	0.560	0.310	0.619*
Pb	0.419	0.569	0.440
Cr	0.500	0.380	0.788*
Ni	0.672*	0.033	0.809*
Al	0.640*	0.293	0.732*
Na	0.287	0.819*	0.420

* Significant at 5% level of significance

4. Conclusions

It is revealed from this study that current annual increment in volume is directly related with the litterfall production. The large quantity of different nutrients returned to soil through leaf litter can contribute a substantial amount of the annual nutritional requirements of the plantation which must be kept in mind while devising management and silvicultural practices for improving the biological productivity.

THE STORAGE AND FLUXES OF CADMIUM IN THE SOIL-SALIX SYSTEM

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1. Introduction

In 1996 willow (*Salix*) for biofuel production was grown on 15 000 ha of arable land in southern and central Sweden (NUTEK, 1996). It is well known that *Salix* accumulates high levels of Cd in stems (Brieger et al., 1992). The question has therefore been raised whether the combination of high biomass production and high Cd uptake in *Salix* could be used to remove Cd from moderately contaminated soils. Studies have shown that it is possible to remove 95% of the Cd in the ashes (Åbyhammar, 1992). A study conducted by Eriksson and Ledin (1998) indicates that Cd is taken up from the subsoil. Thus Cd from the subsoil may be redistributed to the topsoil through the leaf litter. If the amount of Cd redistributed in this way exceeds the Cd-amount removed from the topsoil by stems at harvest, an accumulation in the topsoil will be the case. This may be detrimental to succeding shallow rooted agricultural crops. A project was started in 1997 to study storage and fluxes of Cd in energy forests planted with *Salix*. As the project is in its initial stage this paper deals with the experimental design and some preliminary results. The intention is that more results will be presented on the poster at the conference.

2. Materials and Methods

The investigation deals with Cd accumulation in stems and leaves under realistic field conditions and the uptake of Cd from the plough-layer and subsoil respectively. To be able to quantify and trace the fluxes of Cd from different soil levels into the plant this part is conducted in lysimeters. The clone studied is 78 183 of *Salix viminalis*. This clone is a reference clone widely used both commercially and in scientific studies. Among clones studied by Greger and Landberg (1996) it had an intermediate value for the Cd-stem/Cd-soil ratio.

Accumulation in stems and leaves: field study

The aim is to quantify the net removal of Cd via stems at harvest. In order to achieve realistic field data this investigation is conducted in eleven commercial stands. Stands on acid (pH 5 to 5.5), neutral (pH around 7) and calcareous (pH 7.5 to around 8) soils were chosen. The falling leaves are collected throughout the season and for each stand the Cd pool in the soil, together with other soil parameters, will be determined. Stems will be sampled in connection with harvest.

Uptake plough-layer versus subsoil; lysimeter experiments

The aim is to study Cd-uptake from topsoil and subsoil separately under as natural conditions as possible. Two types of lysimeters are used. In one experiment the lysimeters are aluminium frames (area 0.5 m^2), each frame planted with one *Salix* cutting (Fig 1). The upper 25 cm of soil was removed and replaced by labelled (Cd^{109}) plough-layer soil. This design should give a natural root distribution and drainage since the structure of the subsoil remains intact. The treatments are (I) topsoil with "natural" Cd content and (II) polluted topsoil (Cd content doubled compared to treatment I). Treatment I also contains extra replicates so that stems can be harvested every season for three years. Leaves are collected every year in both treatments.

In the other lysimeter experiment the aim is to study how subsoil type affect the proportion of total uptake deriving from the topsoil and subsoil, respectively. This is performed in 2 m deep closed lysimeters with an inner diameter of 60 cm (Fig. 2). This technique means a disturbance of the subsoil structure, but it is the only one practicable if the effect of subsoil properties is to be studied under controlled conditions. In one treatment acid subsoil was placed in the lysimeter and in the other treatment the soil was neutral. The isotope (Cd^{109}) labelling was made in the less voluminous top soil. In one of the treatments the Cd^{109} labelling was instead made to the subsoil to get a subtreatment where we measure the uptake from subsoil directly. This is desirable when there are many uncertainties with the isotope technique, for example to estimate the plant available fractions in the soil. Leaves will be collected every year, whereas the stems will be harvested after two growing seasons.

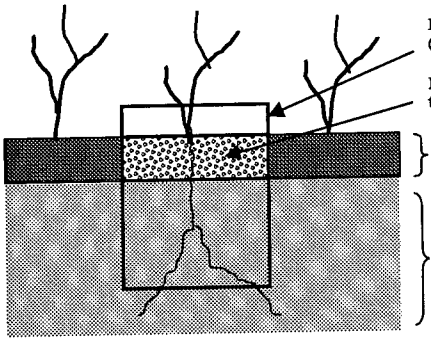

Fig. 1 A descriptrion of the aluminium frames used as open lysimeters.

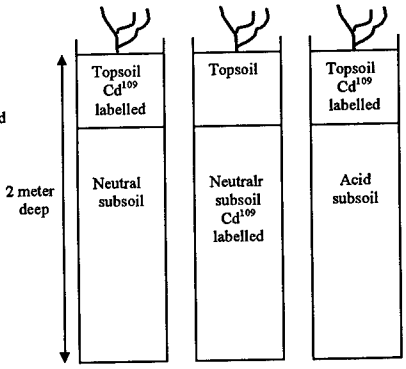

Fig. 2 The experimental design of the closed lysimeters.

3. Preliminary Results

Preliminary results from the first growing season in the field study indicate that the Cd concentration in the leaves of *Salix* is high. Stands on calcareous soils had the lowest leaf concentrations, of around 1 mg Cd kg^{-1}. This means that 1 to 3 g Cd ha^{-1} $year^{-1}$ is recycled to the soil. Stands on neutral soils had leaf concentrations between 3 and 5 mg kg^{-1} corresponding to 4 to 8 g Cd ha^{-1} $year^{-1}$ in recycled amounts. Very high Cd concentrations (around 9 mg kg^{-1}) were found in the leaves from one stand on an acid soil. However, the growth of that stand is not very good, so it is uncertain whether these data are representative for the soil type.

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MICRONUTRIENTS IN SOILS, PRECIPITATION AND SOIL SOLUTION OF NATIVE SAVANNA AND *PINUS* PLANTATIONS IN BRAZIL

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1. Introduction

Thirty years ago, large parts of the Brazilian savanna (the "Cerrados") were cleared and transformed into forest plantations. The plantation of *Pinus* forests often results in increased soil acidification and considerable changes of the soil solution chemistry (PARFITT et al., 1997). This may cause enhanced micronutrient leaching. As micronutrient concentrations in the strongly weathered Oxisols are low and only small percentages of the total concentrations are plant-available (WILCKE et al., 1998), micronutrient leaching possibly results in nutrition deficiencies for plants. The aim of this work was to compare (1) the partitioning of Zn, Mn, Cu, and Fe in soils and (2) the temporal course of micronutrient concentrations in precipitation and soil solution in *Pinus* reforestations with those of native savanna.

2. Materials and Methods

The study area is located 400 km S of Brasilia near the city of Uberlândia (Federal State of Minas Gerais). Three spatially disconnected plots of natural savanna (CE) and *Pinus caribaea* Morelet plantations (PI, 20 years old) were selected and samples from throughfall, litter leachate (PI only) and soil solutions at 0.15, 0.3, 0.8, 1.2, and 2 m depths were sampled with suction cups between Oct. 1997 and Apr. 1998 (rainy season). The solutions were analyzed for pH, Mn, Zn, Cu, and Fe. Additionally, surface soil samples (0-0.15 m) were sequentially extracted in seven steps and analyzed for Mn, Zn, Cu, and Fe (ZEIEN and BRÜMMER, 1989). The fractions (F) are characterized by decreasing plant-availability from F1 to F7.

3. Results and Discussion

Total concentrations of Mn, Zn, Cu, and Fe in the studied surface soils were 39-50 mg Mn kg^{-1}, 11-12 mg Zn kg^{-1}, 17-20 mg Cu kg^{-1}, and 35-39 g Fe kg^{-1}. More than 83 % of total Cu, Fe, Mn, and Zn concentrations are extracted with hardly plant-available fractions (bound to Fe oxides and silicates, F5-F7 in Fig. 1). Less than 6 % of total Cu, Fe, and Zn and ~ 12 % of total Mn are found in the plant-available fractions (extractable with NH_4-acetate at pH 6.0, F1 and F2). Medium-term plant-available fractions (bound to Mn oxides and organically bound, F3 and F4) comprise between 1 and 12 % of the total concentrations. There are no significant differences in soil metal partitioning between PI and CE. Copper was only detected in throughfall (2.7-3.2 µg l^{-1}) and litter leachate (3.8 µg l^{-1}). Iron was not detected in any solution. The small micronutrient concentrations can be explained by their low solubility at solution pH around 5 (BRÜMMER et al., 1986). In both systems, the Zn and Mn concentrations in soil solution are higher at the beginning of the rainy season than during the high rainy season (Fig. 2). This can be explained by enhanced mineralization of easily mineralizable material which accumulated during the dry season and the simultaneous release of micronutrients. In CE, average Mn concentrations are higher in throughfall than at 0.15 m depth (Fig. 3a) and then increase with soil depth. Average Zn concentrations increase from throughfall to 2 m soil depth. In PI, the solution chemistry is considerably different. Average Mn, Zn, and Cu concentrations are larger in throughfall of PI than of CE, because more nutrients are leached from the denser PI canopy or more nutrients are

accumulated by canopy interception. Average Mn concentrations in PI increase with soil depth until 1.2 m and reach concentrations which are up to 70 times higher than those in CE. Between 1.2 and 2 m depth concentrations decrease sharply. The differences in Mn concentrations between CE and PI soil solution can be explained by the more acid soil solution in PI. At 0.15-1.2 m depth, solution pH is 0.5-1 pH units smaller in PI than in CE, at 2 m there are no significant differences in solution pH. There are no significant differences in Zn concentrations of the solutions between CE and PI (Fig. 3b).

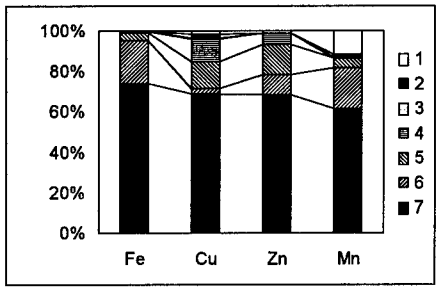

Figure 1: Partitioning of FE, CU, ZN, and Mn in CE soils (0-0.15 m).

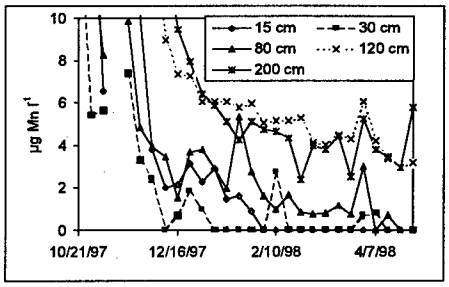

Figure 2: MN Concentrations in the SOIL solution of CE during the rainy season (10/21/97 – 4/28/98).

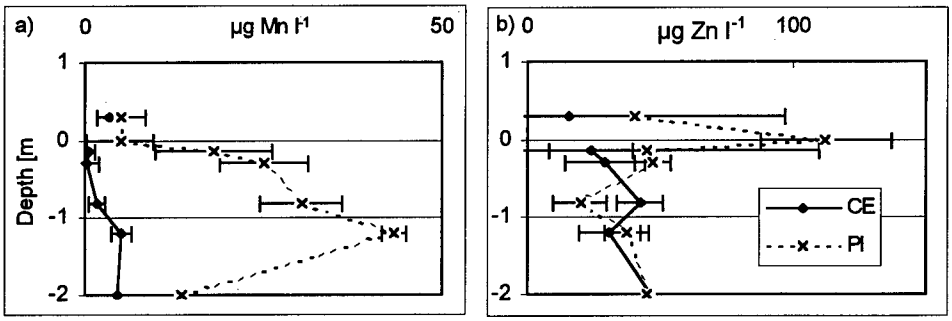

Figure 3: Average concentrations of a) Mn and b) Zn during the high rainy season (12/23/97 - 4/28/98) in CE and PI.

4. Conclusions

Micronutrients are strongly bound in the studied soils and only low concentrations are found in extracts characterizing plant-available fractions and in soil solution. PI reforestation resulted in increased acidification of the soil and thus also in enhanced micronutrient leaching, particularly of Mn. However, this may partly be compensated by the higher micronutrient interception in PI.

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DO ELEVATED LEVELS OF CA AND HEAVY METALS IN SOIL WATER, DUE TO ASH TREATMENT, AFFECT TREE GROWTH?

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1. Introduction

Ash is a waste product formed in pulp and paper industry. Ash contains all mineral elements except nitrogen, and can be used as a vitalizing agent in forests (GREGER et al., 1995). Since ash has a high pH due to a high content of basic cations, e.g Ca, it has also a liming effect on soils. Leakage studies on ash showed that the concentration of Ca, Cu, Zn and Cd can increase in the soil water initially after treatment with 3 tonnes of ash per ha (ERIKSSON, 1996). An increase of plant available Ca was also found in a field studie where 3 tonnes of ash per ha had been spread (FRANSMAN, 1996).

Before spreading ash in the forest one has to know how the very high Ca concentration as well as elevated heavy metal concentration in the ash, affect the forest ecosystem and the wood production. The aim of this studie was therefore to investigate the effect of elevated levels of Ca, Cu, Zn and Cd on wood quality and tree growth.

2. Materials and Methods

Plants of Norway spruce *(Picea abies)*, 2 months old and 2 years old, were used in this investigation. During 3.5 months they were treated with different concentrations of Ca, Cu, Zn, and Cd, in an artificial soil water solution. The concentration of Ca, Cu and Zn was rased 4, 1.5 and 3 times the control, respectively, in accordance with increased levels found in field an leakage studies mentioned above. The Cd treatment was 0.1×10^{-3} measured as a Cd:Ca ratio. The metals and nutrients were added exponentially and and in fixed proportions to each other. For the younger plant material the additions (R_A=0.04) were made daily and evenly distributed over the day via a pump system and for the older plant material (R_A=0.02) every second day by hand. After the treatment time, the plants were harvested and analysed for Ca, Cu, Zn and Cd in the wood using atomic absorption spectrophotometry after wet digestion in $HClO_4$:HNO_3 (3:7). The growth of the plant material was also determined using both fresh and dry (105°C) weight. Stems were also analysed for water content (MQ) and stem diameter.

3. Results and Discussion

The concentration of Ca in the wood increased with the increased addition of Ca. GREGER et al. (1998) showed that plants treated for 18 months with ash got an increased Ca content in the wood but not in the bark. The addition of Cd did not give any higher content of Cd in the wood. The results so far show that the elevated heavy metal concentration does not affect the different parameters tested (table 1 and 2). Calcium, however, increased the plant fresh weight, dry weight of root and stem diameter of the younger plants while this increase was less pronounced in the older plants (table 1 and 2). The water content of the stems did not change with the increased Ca addition (table 1).

Table 1. The fresh weight of root and shoot, the dry weight of root, the diameter and moisture quotient of the stem of 5.5 months old spruce plants treated with increased levels of either Ca, Cu, Zn and Cd for 3.5 months. The addition of Ca, Cu and Zn was given times the control while the Cd treatment is given as a Cd:Ca-ratio. The values represent the mean ± standard error.

Treatment	FW shoot (g)	FW root (g)	DW root (g)	Stem diam. (mm)	MQ (%)
Control	8.4±0.5	7.3±0.7	1.08±0.08	1.90±0.03	61.5±0.8
Ca x 4	10.9±0.5	10.0±0.5	1.37±0.05	2.16±0.03	62.5±0.5
Cu x 1.5	9.1±0.3	8.3±0.5	1.21±0.06	1.92±0.05	62.2±0.6
Zn x 3	8.2±0.3	7.4±0.3	1.07±0.04	1.93±0.04	62.1±0.5
Cd/Ca = 1 x 10^{-4}	9.4±0.4	8.0±0.6	1.21±0.07	1.90±0.05	62.0±0.4

Table 2. The fresh weight of root and shoot, the dry weight of root, the diameter of the stem with and without bark of 2 years old spruce plants treated with increased levels of either Ca, Cu, Zn and Cd for 3 months. The addition of Ca, Cu and Zn was given times the control while the Cd treatment is given as a Cd:Ca-ratio. The values represent the mean ± standard error.

Treatment	FW shoot (g)	FW root (g)	DW root (g)	Stem diam. + bark (mm)	Stem diam. - bark (mm)
Control	40.3±4.6	53.7±6.0	8.1±0.8	8.2±0.4	6.2±0.3
Ca x 4	45.2±2.1	58.7±2.4	8.6±0.4	8.5±0.2	6.5±0.1
Cu x 1.5	38.4±3.3	54.7±4.2	8.2±0.5	8.1±0.3	6.1±0.2
Zn x 3	36.8±2.4	53.9±3.2	8.4±0.5	8.2±0.2	6.0±0.2
Cd/Ca = 1 x 10^{-4}	38.9±6.5	52.2±8.1	8.2±1.0	8.0±0.5	6.0±0.4

4. Conclusions

Our labaratory results so far indicate that the elevated heavy metal concentration that will be found in the soil water after treatment with 3 tonnes of ash per ha is too low to affect the whole plant growth. However, the increased level of Ca seems to give a higher plant growth. The effect caused by Ca is more pronounced in the younger plants. The effect of higher additions of ash will be discussed.

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TRACE METAL BUDGETS FOR A SMALL FORESTED CATCHMENT IN EASTERN FINLAND

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1. Introduction

Input–output budgets are a most useful means of describing the trace element biogeochemistry of a catchment. The difference between input (total deposition) and output (runoff) describes the degree of retention or mobilization within the catchment. Much is known about the levels of trace metals in temperate forest ecosystems (Bergkvist *et al.* 1989), but there is less information available about boreal coniferous ecosystems, particularly in background locations. In a recent paper we described the concentrations of trace metals in the entire water pathway (deposition, throughfall, soil water, ground water, lake and stream runoff) of four small forested catchments in Finland (Ukonmaanaho *et al.* 1998). In this abstract, we present input–output budgets for one of these catchments.

2. Materials and Methods

The data has been collected from the UN-ECE Integrated Monitoring catchment, Hietajärvi. The catchment covers 4.6 km^{-2} and is located in eastern Finland (63° 09'N 30° 40'E) in a forested region with no agriculture nearby and no point sources of trace metal emissions within 100's of km. The mean annual precipitation is 631 mm and temperature 2°C. The bedrock geology is dominated porphyritic granodiorites and is overlain by till and glacifluvial deposits of 5.6 m average thickness. The upland soils are acidic and mainly podzolic with a mor humus layer. Peatland covers c.32% of the catchment, part being sparsely forested. Forest cover (61%) consists of old-growth (>100 years old) stands dominated by Scots pine. The catchment contains a number of lakes and ponds, the largest of which feeds the runoff stream. Runoff waters are clear, having a median *p*H value of 6.2 and alkalinity of 75 μmol l^{-1}. For further details about the catchment, see Bergström *et al.* (1995). For details about sample collection and trace metal analysis, see Ukonmaanaho *et al.* (1998).

3. Results and Discussion

More than 70% of the annual estimated total (wet+dry) deposition of the trace metals were retained within the catchment (Fig. 1). Pb, considered to be totally derived from the atmosphere, showed the highest degree of retention, 96%. Cd had the lowest degree of retention, 74%. The soil, in particular the humus layer, and peatlands were the main sources of retention of trace metal deposition in the terrestrial parts of the catchment. Median groundwater concentrations as a percentage fraction of suction lysimeter soil water (35 cm) concentrations varied from 0.2% (Pb) to 13.8% (Zn) (Ukonmaanaho *et al.* 1998). Bottom sediments are probably the main source of retention in the lakes (Mannio *et al.* 1993). Trace metal concentrations in the soil are presented in Table 1. The concentrations for the humus layer are below reported (Tyler 1992) "lowest effective limit" concentrations: Cu 20, Pb 150, and Zn 300 mg kg^{-1}.

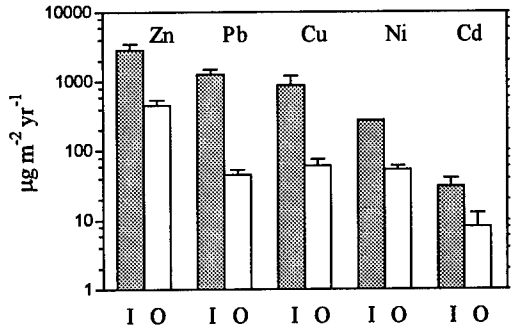

Figure 1: Trace metal input (I, total deposition)–output (O, runoff) mean annual (1994-96) budgets for the Hietajärvi catchment. Error bars are standard deviations.

Table 1: Range in trace metal concentrations (mg kg^{-1}) in upland soils (dry ashing+conc. HCl) at Hietajärvi (Starr 1995).

	Cu		Pb		Zn		Cd
layer	min.	max.	min.	max.	min.	max.	
Humus	5.3	7.1	25.3	37.0	36.5	53.9	<0.5
0–5 cm	1.1	1.9	8.8	12.8	3.2	9.8	<0.5
5–20 cm	1.5	2.4	18.6	24.6	8.8	27.2	<0.5
20–40 cm	2.0	2.9	11.8	13.4	6.2	10.0	<0.5

4. Conclusions

Inputs of trace metals to the Hietajärvi catchment were relatively low and showed a high degree of retention within the catchment. Humus layer concentrations were below toxic levels.

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Trace Elements in Industrial and Municipal Residues

(Technical Session 8)

THE PHYTOAVAILABILITY OF ZINC AND CADMIUM IN LONG-TERM BIOSOLIDS-AMENDED SOILS

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1. Introduction

The majority of studies on metal uptake in long-term biosolids- amended soils have focused on soils amended with high metal biosolids. This was primarily due to 2 factors: a desire to fully understand the potential risks associated with the use of biosolids on agricultural soils, and the occurrence of high metal biosolids before pre-treatment regulations were adopted as part of the US EPA Clean Water Act. Average trace metal concentrations in biosolids in the United States have been steadily decreasing. The current geometric mean concentration of Cd in biosolids is 7.2 mg kg^{-1} (USEPA Statistical Support Document for the 40 CFR, Part 503 11 Nov. 1992). In addition, the Fe concentration in U.S. biosolids ranges from 0.1 to 15% Fe, with a median value of 1.7% (Metcalf and Eddy, 1991). The ability of Fe oxides to complex trace metals has been well documented. This suggests that, for most biosolids currently being land applied, the metal complexing ability may exceed the trace metal concentrations. For this study, plants were grown on median as well as high metal biosolids. An examination of metal uptake in long-term biosolids amended sites that had been treated with median concentration biosolids, may provide a more realistic understanding of the potential risks associated with biosolids application to agricultural soil.

2. Materials and Methods

Two long-term biosolids amended field experiments were used for the current study. The original experiments were established in 1976-8 in Beltsville, MD, one on a Christiana fine sandy loam soil (Typic Paleudult; clayey, kaolinitic, mesic) using a variety of biosolids and another on a Galestown Sand (sandy, siliceous, mesic Psammentic Hapleudult using a single biosolids applied at 2 rates. Properties of the biosolids and amended soils are presented in Table 1. A variety of garden vegetables with different Cd uptake responses were grown in the 1991-2 growing seasons on the Christiana soil. Wheat (*Triticum aestivum* L.) was grown in 1994-5 on both soils.

3. Results and Discussion

Vegetables grown in the high Cd biosolids had significantly higher Cd concentrations than those grown in the median Cd biosolids treatment and the control (Table 2). Cadmium concentrations in all vegetables from the median Cd biosolids treatment were not significantly different from the control. The amount of biosolids applied in this treatment is equivalent to 75 annual biosolids applications. For this study, the use of high rates of a low Cd biosolids was not associated with an increase in plant Cd.

Cadmium concentration in wheat grain and diagnostic leaf grown on median Cd biosolids-treated soils on both sets of plots was not significantly different from the control. Wheat tissue grown on the high Cd biosolids treated plots had significantly higher Cd than that grown on median Cd and control soils. This was the case with application rates up to 448 Mg ha^{-1}. It is

interesting to note that the wheat grown on the biosolids amended (100 Mg ha^{-1}) Galestown sandy loam soil was close to being Zn deficient (11.43 mg kg^{-1} Zn in diagnostic leaf)
For this study, application of high rates of median quality biosolids did not result in increased plant Cd for a range of food crops. This suggests that use of average biosolids may not result in increased plant metal uptake.

Table 1. Characteristics and application rates of biosolids used

Biosolids	Rates Applied	Zn	Cd	Fe
	Mg ha^{-1}	mg kg^{-1}		%
Piscataway Limed Digested	224	639	5.9	2.5
(median quality)	448			
Heat-Treated (median quality)	224	1329	13.4	8.3
Nu-Earth (high metal)	100	4140	210	2.5

Table 2. Cadmium concentration of edible portion of a range of vegetable and grain crops grown on long-term biosolids amended soils.

Biosolids	Rate	Potato	Tomato	Cabbage	Carrot	Lettuce	Wheat
	(Mg ha^{-1})	mg kg^{-1}					
		Christiana soil series					
Control	-	0.35	0.63	0.32	0.42	1.04	0.25
Heat-Treated	224	0.25	0.6	0.53	0.49	0.85	0.2
Piscataway	224						0.14
	448						0.27
Nu-Earth	100	1.63	1.30	1.67	3.22	10.53	1.42
		Galestown soil series					
Piscataway	-						0.11
	224						0.14
	448						0.09

DISTRIBUTION AND TRANSLOCATION OF ARSENIC IN SOILS OF TANNERY INFILTRATION FIELDS

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1. Introduction

Leather industry in central Europe declined in the 1960's and left behind thousands of hectares of infiltration fields contaminated with Cr and As beyond regulatory threshold values. Chromium was the main tanning agent. Arsenic was used as a preservative. Air-dried hides treated with As_2O_3 were imported from overseas. From about 1920 to1940 As_2S_3 was used as a reducing agent in the unhairing and liming step of the tanning process. Arsenic was erroneously believed to have a positive effect on leather quality. In the subsequent neutralization step As was washed off and discharged with the effluent. Part of it precipitated with Ca and can be found in the carbonate-rich waste sedimented in ponds and ditches. The rest remained in solution and was disposed of with the wastewater in infiltration fields. The aim of this research was to investigate the distribution and binding forms of As in soils of former infiltration fields and the potential for As translocation for risk assessment purposes.

2. Materials and Methods

The tannery site under investigation closed down in 1967. For wastewater treatment settling ponds (3 ha) and wastewater infiltration fields (12 ha) were constructed on glaciofluvial sand about 4 m above groundwater. The dominant soil type is cambic podzol. The fields are polder type basins (e.g. 30 by 50 m) arranged at different levels to both sides of a central irrigation ditch in the highest part of the area (elevation: 22 m above sea level). The topography has remained unchanged for 30 years. Most of the area is covered with grass and shrubs. In a separate 1ha section a forest type vegetation (deciduous trees) has developed. The total annual rainfall sum is about 800 mm with an actual evapotranspiration of about 500 mm. Receiving waters are at a distance of 50 m north and 200 m south of the fields. Soil samples were taken from profiles in the irrigation ditch and at different distances from it. The analysis of the <2mm fraction of samples included the determination of C, N, and S by combustion at 1350°C using a CNS analyzer. Different fractions of Cr, As, Fe and Mn were obtained using selected methods of ZEIEN and BRÜMMER (1989) not sequentially but as separate trials (NH_4NO_3, NH_4OAc (pH 6), NH_4-oxalate (pH 3.25), ascorbic acid/oxalate (pH 3.25)). Total contents were assessed by aqua regia extraction according to DIN 38414. Elemental analysis was carried out by flame and graphite furnace AAS. Mineral phases in sedimented waste were identified by X-ray powder diffraction.

3. Results and Discussion

Arsenic is present in different binding forms. In profile 1 (Table 1) it is found in the sedimented carbonate waste with a maximum of 220 mg/kg in the oldest layer (yC2 horizon) which was deposited in the first half of this century. The matrix is mainly calcite with small proportions of

Table 1: Selected soil analytical data of a former irrigation ditch with sedimented carbonate and of two infiltration fields with different vegetation

Horizon/Depth cm		pH_{CaCl2}	C_t[1] %	Fe_{asc}[2] g/kg	As_t[1] mg/kg
Profile 1:		Irrigation ditch			
Ah	-10	7.3	22.8	6.1	31.1
yC1	-47	7.5	17.3	3.1	59.4
yC2	-59	7.5	11.4	3.1	220.8
fBhs	-72	6.9	0.6	2.9	4.0
fBs	-80	6.5	0.2	3.9	2.0
Profile 2:		Infiltration field (shrubs)			
RAh1	-8	5.7	8.2	3.7	51.3
RAh2	-30	6.3	1.9	1.7	52.6
AB	-50	5.9	0.8	4.2	27.9
Bv	-87	4.3	0.3	2.5	70.4
Bb	-160	4.3	0.3	6.5	105.8
C	>160	4.2	0.1	2.0	62.2
Profile 3:		Infiltration field (trees)			
Ah	-8	5.1	2.0	3.0	154.5
Bv	-30	5.4	0.8	2.8	124.1
Bb	-50	4.9	0.3	1.9	30.6
Cb	-87	4.7	0.3	4.6	17.9

[1]total content of carbon and arsenic, respectively
[2]ascorbic acid/oxalate extractable iron

quartz and carbonate hydroxy apatite. Arsenic is probably bound as a calcium arsenate phase which forms under alkaline conditions (SADIQ 1997). This must be very stable because the buried Bs horizon of a former podzol on this place is not contaminated.

Most soils of the infiltration fields are covered with grass and shrubs and are free of carbonate. As in Profile 2 (Table 1) the maximum As concentration is normally found in the subsoil where it correlates with pedogenic iron oxihydroxides. Also typical is an accumulation in the topsoil. Here As is bound to soil organic matter as probably due to soil/plant transfer. This fraction has entered the nutrient cycle. The high C_t value of 8.2 % in the RAh1 horizon is indicative of fine leather particles deposited with the wastewater. Arsenic is not associated with these particles.

The highest As content of >150 mg/kg in the topsoil was found in the section with forest type vegetation (Profile 3). In this soil As correlates with organic matter only. The As distribution is interpreted as being indicative of uptake by plant roots and surface deposition with the leaf litter.

4. Conclusions

Arsenic in contaminated soils can be bound to humic substances, sesquioxides and also to calcium carbonate if available. Trees, in particular, can easily transfer As from the sesquioxide pool in the subsoil to the organic matter pool in the topsoil. Arsenic is accumulated on the surface while the subsoil is depleted. Therefore, vegetation on contaminated sites should always be controlled. The results suggest that the observed effect can be used for remediation purposes. This process has to be investigated further in relation to appropriate plants, possible removal rates and the leaching of As in the vadose zone. A successful treatment would probably take several decades.

5. References

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TRACE ELEMENT MOBILITY FOLLOWING LAND APPLICATION OF PULP MILL RESIDUES TO A FOREST SOIL

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1. Introduction

The US pulp and paper industry generated estimated 4.4 million dry Mg of ash and 5.4 million dry Mg of wastewater treatment sludge in 1995. About 75% of the ash and 50% of the sludge was disposed of in landfills and lagoons; only 9% of the ash and 12% of the sludge was land applied (Unwin, 1997). Limited capacities for landfilling and increasing operational costs are forcing the industry to evaluate alternative management systems (i.e. land application).
Many studies have shown that mill residues can serve as valuable soil amendments and can have beneficial effects on forested sites when properly applied (e.g. Henry et al., 1993). This is mainly due to high concentrations of essential plant nutrients and low concentrations of trace metals (Tab. 1). However, initial research indicated that large amounts of dissolved organic carbon (DOC) originating from secondary pulp mill sludge plays an important role in the mobility of some trace metals (Goldemund et al., 1998). Results of a lysimeter study on small-scale field plots will be presented in the overall context of an operational land application site.

2. Materials and Methods

The land application site is located in the Upper Coastal Plain of Georgia with soils from the Orangeburg series (fine-loamy, kaolinitic, thermic Typic Kandiudult). Small-scale field plots (2 by 3 m) are equipped with ceramic cup and stainless steel lysimeters at two depths (25 cm and 65 cm). The stainless steel lysimeters at 25 cm are zero-tension lysimeters, the other lysimeters (shallow and deep ceramic cup and deep stainless steel) are suction lysimeters. Three treatments (control, a 75% : 25% mix of secondary and primary sludge, and ash), two sites (a regeneration site with incorporation of materials and an established stand where materials are surface applied), two depths, two types of lysimeters and three replicates yield a total of 72 lysimeters. Application rates for small-scale plots are equivalent to 268 Mg ha^{-1} for the mixed sludge and 56 Mg ha^{-1} for the ash. Lysimeters are sampled after each major rainfall during dry periods of the year and every second week during wet periods, and analyzed for major cations, trace metals, nitrogen species, DOC, and pH.

Plots used for the operational field application received two rates of each material: 22 and 134 Mg ha^{-1} of sludge (primary and secondary) and 16 and 28 Mg ha^{-1} of ash. These plots are equipped with ceramic cup suction lysimeters at 65 cm depth for monitoring purposes. They are sampled quarterly, and analyzed for major cations, nitrogen species, and trace elements. Furthermore, soil cores are being taken to a depth of about 1 m, cut up into increments, digested and analyzed for major cations, trace metals, nitrogen species, and pH.

Table 1. Chemical characterization of pulp and paper mill residues

	C	N	pH	P	K	Ca	Mg	Na
	-%[1]-	-%-	(H_2O)	----------	----------	mg kg^{-1}	----------	----------
Ash	15.1	0.04	9.1	1617	3801	52200	6527	6046
Mixed Sludge	34.9	1.36	6.8	790	822	15480	963	330
	Cr	Pb	Mo	Cd	Cu	Ni	Zn	As
	----------	----------	----------	----------	mg kg^{-1}	----------	----------	----------
Ash	18.08	12.34	2.9	0.47	74.5	91.8	2008	3.82
Mixed Sludge	4.50	4.38	1.21	0.22	18.6	5.7	171	2.81

[1]dry mass basis

3. Results and Discussion:

Both lysimeter and soil core samples were very heterogeneous and, therefore, only a few significant differences in metal accumulation or mobility were observed. Sludge significantly increased soil Cu, Ni and Zn concentrations, especially at deeper depths. This can be partly attributed to mobilization through metal-DOC complexes. Soil water samples indicated a similar trend. Samples from plots treated with secondary sludge were higher in Ni, and to a smaller extend, higher in Zn, As, and Cd. Plots treated with ash had increased Zn concentrations in the soil profile. This ash came from a mill that also burned tires; these tires were the source of Zn. Primary sludge and ash significantly increased soil pH and, therefore, overall metal mobility is expected to be decreased. However, during some sampling periods, lysimeter samples associated with these treatments had higher Zn concentrations and, to a lesser extend, higher concentrations in Cu and Ni.

To further investigate the transport of trace elements in these soils, macropore flow collected in zero-tension lysimeters was compared with unsaturated solution collected in tension lysimeters. Results from this comparison are discussed in relation to colloid-facilitated transport and DOC-associated mobilization and transport of trace elements.

4. Conclusions

Field heterogeneity complicates the interpretation of trace element data associated with land application systems of pulp and paper mill residues. However, this research as well as previously published works (e.g. Kookana and Rogers, 1995) indicate that DOC originating from pulp and paper mill residues plays a major role in trace element mobility.

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MOBILITY OF POTENTIALLY TOXIC ELEMENTS IN SOLIDIFIED-STABILIZED WASTES AND CONTAMINATED SOIL MATERIALS : EFFECT OF pH

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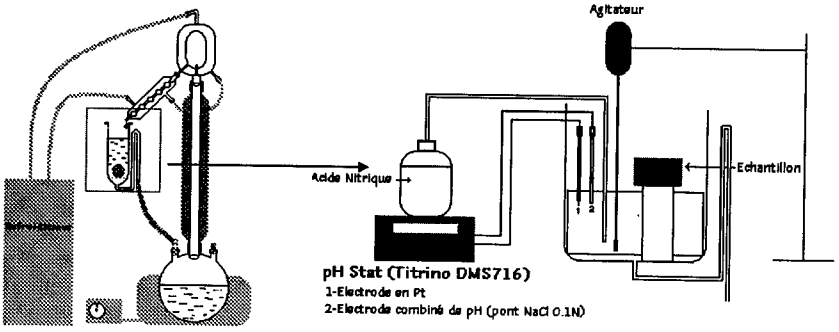
1. Introduction

Mobility of potentially toxic elements included in wastes, sediments or contaminated soils is a major concern to environment. It may be at the origin of the contamination of water tables or of the food chain. The amount of element released is a function of both their physicochemical state (speciation) and their location in the solid phase but also of the pH and redox properties of the pore solution. This paper is dedicated to a better knowledge of the amount of potentially toxic elements which may be released by these contaminated materials as a function of pH.

2. Materials and Methods

The purpose is to get the kinetics of release of elements as a function of the amount of water which flows through the material. Experiments are performed with a water extractor (Fig. 1) which reproduces the water cycle (HUMEZ et al., 1997). The pH of the water solution in contact with the contaminated material is controlled with a pH stat. Samples used as models are solidified-stabilized wastes. It looks like concrete. The equivalent diameter of the sample is about 5 cm. It is a porous solid made of ettringite, gypsum, Na- and Ca chloride, oxyhydroxydes of heavy metals, etc.



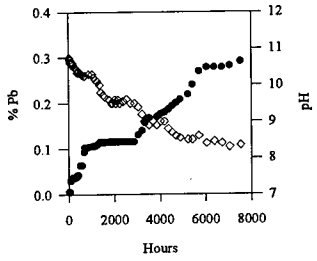
3. Results and Discussion

Results correspond to three different conditions of pH :

a - pH in the extractor was measured but not controlled ($10.5 < pH < 8.5$),
b - pH was controlled at 6.5 and 5.

Figure 2 gives the pH of the solution in contact with the sample and the percentage of Pb released in these conditions as a function of time. Steps on the kinetic of release of Pb and steps on the pH curve are concomitant. The plateau seen on the kinetic of release of Pb corresponds to the formation of Pb oxyhydroxydes. The important release of Pb after the plateau is due to the dissolution of these neoformed compounds.

Figure 3 gives the kinetics of release of SO_4^{--}, Al^{+++}, Pb^{++} and Zn^{++} as a function of pH. Several remarks can be done. The release of all elements increases when pH decreases. Kinetics obtained for pH 6.5 and 5 show no plateau. For SO_4^{--}, Al^{+++} and Zn^{++} the jump in release is between the high values of pH (10.5 > pH > 8.5) and pH 6.5. The kinetics are similar for pH = 6.5 and 5. The release of Pb is increasing for each domain of pH. The same behavior is observed for Fe. The release of Al^{+++} and SO_4^{--} are congruent. The linearity observed between 1000 and 1800 hours is probably related to the weathering of ettringite which is made of both Al^{+++} and SO_4^{--}. The low amount of potentially toxic element released, < 0.3 % for Pb, is probably related to the oxide form of the potentially toxic elements.



Hours $_{Pb\ ;\ pH}$

Fig.2. pH and Pb release versus time

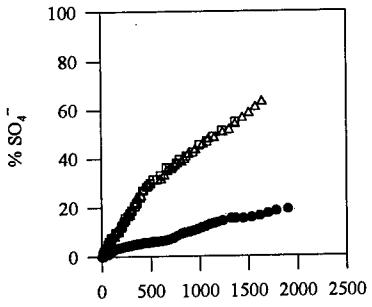


Fig.3.a. Kinetic of release of SO_4^{--}

● 10.5>pH>8.5 ; □ pH=6.5 ; △ pH=5

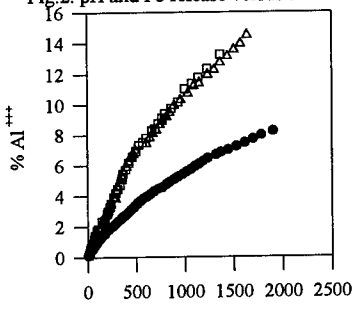


Fig.3.b. Kinetic of release of Al^{+++}

● 10.5>pH>8.5 ; □ pH=6.5 ; △ pH=5

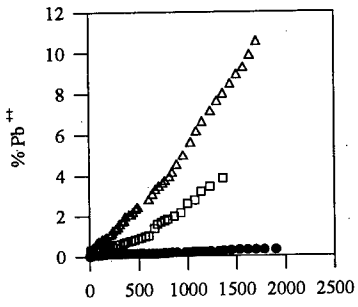


Fig.3.c. Kinetic of release of Pb^{++}

● 10.5>pH>8.5 ; □ pH=6.5 ; △ pH=5

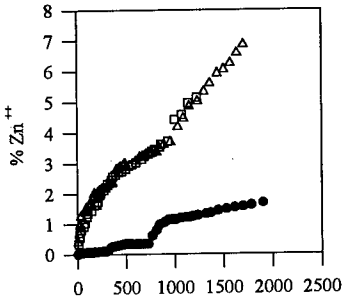


Fig.3.d. Kinetic of release of Zn^{++}

● 10.5>pH>8.5 ; □ pH=6.5 ; △ pH=5

4. Conclusions

pH has an effect on the release of elements. For 10.5 < pH < 8.5 neoformed compounds appear in the pore space which are at the origin of the formation of plateaux and then jumps in the release of elements. These processes do not occur with Pb when pH is lower than 6.5. The low release of potentially toxic elements is related to their physicochemical state: they are included in oxides.

5. References

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SPECIATION OF SOLUBLE AND COLLOIDAL ZN AND CD IN GROUNDWATER BENEATH INDUSTRIAL WASTE LAND SITES

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1. Introduction

In groundwater under industrial wasteland metal concentrations may be in the range of thousands of mg L^{-1}, several hundred times larger than the threshold values for potable water. The latter only consider the total concentrations and do not distinguish between metal species. In groundwater, metals are not only in dissolved forms, but can equally be in colloidal forms in suspension. Metals in the colloidal fraction can be associated in different ways depending on the origin of the colloids. Two types can be distinguished: i) neoformed colloids resulting, for example, from a supersaturated solution and ii) „ inherited " colloids resulting from physical breakdown of metal bearing minerals present in the overlying soils or industrial waste deposit. Depending on the speciation of metals, not only their transport, but also their reactivity in biogeochemical processes is different as well as their toxicity. Our study aims to assess in a qualitative way the speciation of Zn and Cd and to distinguish the soluble from the colloidal forms (Fig.1) in groundwater beneath and around a polluted waste site..

2. Materials and Methods

Zn and Cd pollution originates from a factory of sulphide-acid production which was active in the Northern part of France from 1918-1968, associated to a zinc smelter complex active between 1901 and 1962. For this study, water samples were collected in piezometers placed on the waste site, on agricultural land soil and in a forest soil, according to a Zn concentration gradient in the overlying soils varying from 10 000 to 100 mg Kg^{-1}.
Ultracentrifugation was used on water samples to separate the colloids from the solution according to Mavrocordatos and Perret (1995). Colloids were observed under Transmission Electron Microscope (TEM) and analysed by Energy Dispersive Spectroscopy system (EDS) in order to identify their morphology, chemical composition and to assess their origin. Ultrafiltration was used to size-fractionate the colloids. Evolution of the colloids after sampling in function of time was also studied. When possible, speciation of the dissolved metals was made using cation exchange resins with chelating characteristics (Holm *et al.* 1995).

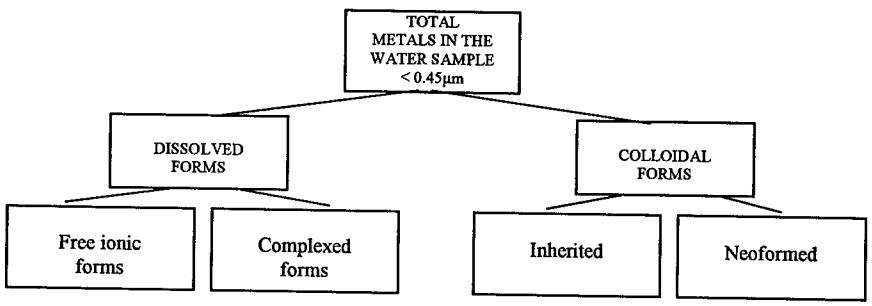

Fig.1: Scheme of the different separation steps

3. Results and Discussion

Groundwater samples may contain up to 1500 mg L^{-1} of Zn and 600 mg L^{-1} of Cd beneath the waste site, and down to 0.1 mg L^{-1} of Zn and 0.001 mg L^{-1} of Cd for the agricultural or the forest sites. On each sample separation of dissolved and colloidal forms of metals were performed.

Tests with a chelating resin showed that a part of Zn and Cd in the dissolved form are forming stable complexes. Zn complexes are more stable beneath the agricultural and forest sites than beneath the waste land. In the latter site TEM–EDS analyses of groundwater showed Zn in the colloidal fraction. Cd could not be evidenced, possibly because of EDS detection limits. The percentage of Zn associated to colloids was always in the order of 5-10% (the EDS data expressed in number of atoms were recalculated in percent). Some colloids resulted of physical breakdown of native minerals of this site but neoformed colloids were also found. The latter consisted of amorphous ferrous particles associated with other elements, mainly Zn, Al, Si, but Pb was also detected. Within the agricultural perimeter, groundwater colloidal fractions mainly consisted of inherited colloids while the presence of neoformed colloids was evidenced depending on the sampling period. Zn was sometimes detected in the colloidal fraction, but as previously Cd could not be evidenced .

Evolution of the colloids was studied in function of time after sampling, and shown to depend on the characteristics of each sample. Main variations were (i) increase of the size of colloids, and (ii) modifications of the chemical composition of the colloids (Fig. 2).

Ultrafiltration was applied directly on the water samples in order to separate colloids depending on their size. Different trends were observed depending on sampling sites. No colloidal fractionation was possible when colloidal agregates were linked together, probably by organic matter.

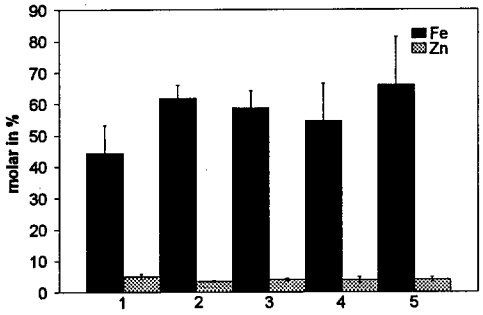

Fig.2: Modifications of the chemical composition of the colloids after sampling, in function of time (1 = 1 day, 2 = 5 days, 3 = 7 days, 4 = 12 days, 5 = 17 days)

4. Conclusions

Zn was found in the dissolved and the colloidal forms in groundwaters in soils at the vicinity of a metal smelter. Cd was only detected in the dissolved form but not in the colloids. Nature and chemical composition of colloids as well as speciation of metals were found to depend on the nature of the overlying substrates.

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MAJOR AND TRACE ELEMENT SOLUBILITY FROM LAND APPLICATION OF FLY ASH AND ORGANIC WASTES: AN INCUBATION STUDY

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1. Introduction

Mixing of coal fly ash with organic wastes to provide balanced soil amendments offers a potential viable utilization of this industrial by-product. When such materials are land-applied to supply nutrients for agronomic crops, potential trace element contaminant solubility needs to be evaluated. In this study, major and trace element soil solution concentrations arising from application of fly ash, organic wastes, and mixtures of the two were compared in a laboratory incubation.

2. Materials and Methods

Two fly ashes, broiler poultry litter, municipal sewage, and mixtures of the fly ashes with either organic waste were mixed with a Cecil sandy loam at application rates of 32.3, 8.1, and 16.1 g kg^{-1} soil for fly ash, poultry litter, and sewage sludge, respectively. The treatments were incubated at 22°C at near field capacity, and soil solution was periodically extracted by centrifugation over 33 days. Major anions were measured by ion chromatography (IC), major cations and trace elements by inductively coupled plasma mass spectrometry (ICP-MS) and As and Se speciation by IC-ICP-MS.

3. Results and Discussion

Initial soluble concentrations of As, Mo, Se, and Cu were much greater in the fly ash/poultry litter treatments than the respective fly ash treatments although the application rate of fly ash was equal in both treatments. For As and Cu increased soluble concentrations were attributable to the presence of these elements in soluble form in the poultry litter. Initial soluble Cu concentrations reached 2000 $\mu g\ L^{-1}$ in the poultry litter and fly ash # 2/poultry litter treatments suggesting that the poultry litter was the main source of soluble Cu. Soluble Cu was highly correlated ($R^2 > 0.96$) to dissolved carbon in all treatments which included poultry litter (Figure 1). Arsenic was highly soluble from poultry litter, indeed, in the individual treatments, poultry litter was a greater source of soluble As than either fly ash, although total As for poultry litter was 35 mg kg^{-1}, compared with 55 and 117 mg kg^{-1} for fly ash 1 and 2, respectively. Using IC-ICP-MS for speciation analysis, As(V) was identified as the major soluble species in the fly ash treatments while dimethylarsinate (DMA) was the major soluble As species in the fly ash/poultry litter treatments. Soluble As (initially 50 $\mu g\ L^{-1}$ for the highest treatment) decreased to near control levels (<5 $\mu g\ L^{-1}$) in all treatments after 20 days incubation.

Soluble Mo and Se were significantly increased in fly ash/organic waste mixtures when compared with either fly ash as an individual treatment, although neither the poultry litter or sewage sludge was a significant source of soluble Mo or Se. For these two elements it is suggested that changes in soil solution chemistry resulting from application of the organic waste, notably initial increased pH, increased soluble SO_4 and soluble organic anions increased the

solubility of Mo and Se from the fly ashes. Speciation analysis of Se by IC-ICP-MS revealed that soluble Se was present as selenate throughout the duration of the incubation study.

4. Conclusions

Land application of fly ash/organic waste mixtures alters trace element solubility when compared to an equivalent application of fly ash alone. Poultry litter can be a significant source of As and Cu, and these trace elements appear to be readily soluble. For Mo and Se, changes in soil solution chemistry as a result of the organic waste increase solubility of these trace elements from the fly ash when fly ash/organic waste mixtures are land applied. Soil solution concentrations of all trace elements approached control levels after thirty days incubation, however soluble Cu was still significantly increased above the control in all poultry litter treatments, while soluble Se was significantly increased in the treatments of fly ash #2 mixed with either poultry litter or sewage sludge.

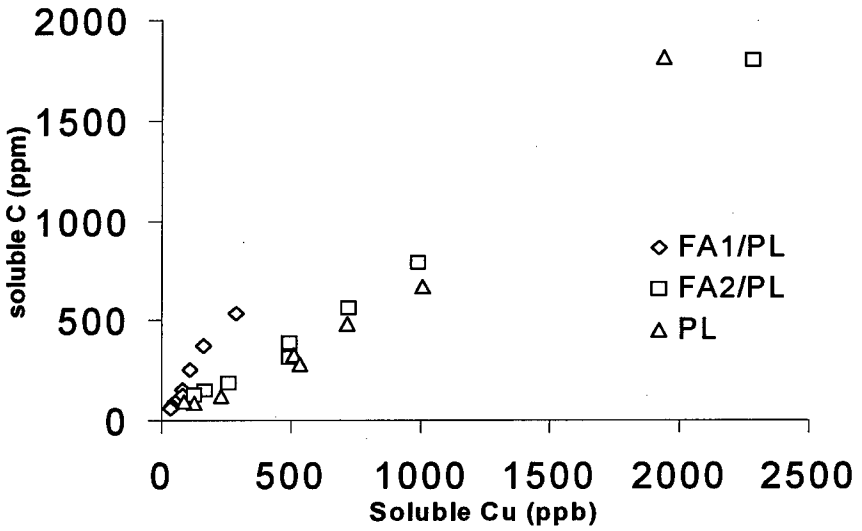

Figure 1: Relationship between soluble Cu and soluble C in soil solutions of poultry litter- and fly ash/poultry litter-amended soils

CADMIUM BINDING BY FRACTIONS OF ORGANIC MATTER EXTRACTED FROM MUNICIPAL SOLID WASTE COMPOST

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1. Introduction

The presence of Cd in municipal solid waste (MSW) is of environmental concern. Bioavailability will depend mostly upon speciation of the metal in the soil solution and binding to dissolved organic matter (DOM). In the present work Cd associations with different fractions of organic matter (OM) extracted from compost were examined.

2. Materials and Methods

An extract of mature MSW compost with 0.1M NaOH was acidified to pH=1, causing humic acid (HA) to precipitate. Fulvic acid (FA) was obtained from the supernatant upon binding to an XAD-8 column and elution with NaOH. FA(>1000) is FA after predialysing against deionized water for six days (MW cutoff=1000Da).

A 1:10 compost-water extract was separated into six fractions as described in CHEFETZ et al. (1998): The six fractions are denominated HoB (hydrophobic bases), HoN (hydrophobic neutrals), HoA (hydrophobic acids), HiB (hydrophilic bases), HiN (hydrophilic neutrals) and HiA (hydrophilic acids) according to the extraction procedure. The fraction HoB constitutes less than 1% of the DOM in mature compost and did not provide sufficient material for metal binding studies.

Measurements of Cd binding with a Cd-specific electrode and subsequent calculations were performed essentially as described by Stevenson and Chen (1991). $Cd(ClO_4)_2$ was titrated into a 10mg C/l solution of the ligand and free divalent Cd was measured at pH=7.0, 25°C and I=0.01. Binding of Cd on FA(>1000) and HA was also examined with the equilibrium dialysis technique at pH=7. The procedure and calculations were similar to those described by RAINVILLE and WEBER (1982). $Cd(ClO_4)_2$ and ligand was added to the outside solution; the solution inside the dialysis bag (MW cutoff=1000) was 0.001M $KClO_4$ initially. Cd concentrations were measured by ICP-AES.

3. Results and Discussion

Results for Cd-complexing capacity obtained by the equilibrium dialysis technique and with the Cd-selective electrode for the FA(>1000) and HA were identical (table 1). FA(>1000) and HA exhibited a similar capacity to complex Cd, exceeding all DOC fractions at least twofold. Among these HoA stood out as the highest Cd-complexing agent; its binding capacity comes close to that of undialysed FA. This is in accordance with findings of other researchers who showed that HoA resembles FA (WERSHAW et al. (1995), CHEFETZ et al. (1998)).

Table 1: Measured Cd-complexing capacities (CC) for various ligands at pH=7. Results for potentiometric measurements are averages of three titrations with standard deviation in parentheses; for dialysis results are obtained by linear regression of data points. *The HiA fraction contains salts due to the extraction procedure.

Ligand	HA	FA(>1000)	FA	HoA	HoN	HiN	HiB	HiA
CC (Cd-selective electrode)	2321	2553	1652	1042	441	546	366	778*
[µmol Cd/ g C of ligand]	(168)	(632)	(363)	(102)	(169)	(84)	(23)	
CC (equilibrium dialysis)	2386	2468						
[µmol Cd/ g C of ligand]	(174)	(724)						

With the Scatchard plot analysis, the stability constants K_{int} for the strongest binding sites (at low Cd concentrations) were calculated (STEVENSON, 1994). Log K_{int} was found to be very similar for FA(>1000), HoA, HoN, HiB (7.67, 7.77, 7.77 and 8.16, respectively). Log K_{int}(HiN) was somewhat lower (6.95) and log K_{int}(HA) higher (8.96). These stability constants are high compared to data from studies on unfractionated FAs and HAs (SPOSITO et al., 1981; STEVENSON, 1977). Besides the chosen calculation method the relatively high pH and low ionic strength serve as contributing factors to the high K_{int} values obtained in this research.

4. Conclusions

Equilibrium dialysis and the Cd-selective electrode yield identical results for complexing of Cd by organic ligands. Among organic ligands extracted from compost, HA, FA and HoA have the highest Cd-complexing capacity. Cd at low concentrations may bind more strongly to organic matter than previously reported. HA forms the strongest associations with Cd, but binding site strength varies over a wide range.

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ACID MINE DRAINAGE ABATEMENT WITH FLUE GAS DESULFURIZATION BYPRODUCTS: WEATHERING STUDY.

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1. Introduction

The Appalachian coal region is faced with a number of environmental issues. As mining has been a crucial component of Appalachian economy since the early 1800's, the region has been affected environmentally by pollution of streams, rivers, lakes, and local drinking-water supplies with acid mine drainage (AMD). AMD originates from active and abandoned mines when pyrite (FeS_2) or other metal sulfides associated with mineral deposits are subjected to oxidizing conditions (Kleinmann et al., 1981). AMD is generally characterized by low pH (< 3.5), high amounts of dissolved Fe, sulfate and dissolved toxic metals (e.g. Al, Mn, Cu, Cr, Zn). In the late 1960's, more than 16,100 km of streams in the eastern half of the U.S. were degraded by coal mining activities (National Academy Press, 1980). Unlike many other industrial water quality problems, AMD pollution can continue for many years after commercial operations have ceased. There have been various approaches to abate AMD, all with limited success. Traditional abatement techniques involve either treatment of surface discharges or sealing the mine to prevent the discharges. Conventional treatment technologies include limestone drains, sediment pond/chemical treatment systems and passive wetlands (USEPA, 1983). These systems are costly to build and require perpetual maintenance without eliminating the problem at the source. Another issue affecting the coal-mining and combustion industries is the Clean Air Act of 1990, which limits sulfur emissions from coal-fired power plants. Coal is primarily composed of C and varying proportions of H, N, O and S. All Ohio coals fall into the medium to high volatile bituminous ranks (USGS., 1996) with high sulfur contents (average sulfur content in Ohio coal is 3.4%; USDOE, 1982). A solution to reduce sulfur dioxide (SO_2) emissions from plants using high sulfur coal is the wet flue gas desulfurization (FGD) process, which can reduce SO_2 emissions by 90%. When mixed with CaO, FGD Byproducts can be transformed into low strength grouts (FGDG) which can potentially be used to seal the openings to underground mines. Thus decreasing the possible contamination of surface waters by AMD. Additionally, FGDG is a highly alkaline material which can directly ameliorate AMD acidity through a series of dissolution reactions. Unfortunatetly, these two functions are somewhat counter to each other. For FGD to act as an effective sealant, it must remain intact and "water tight". However, neutralization of AMD only occurs as a result of dissolution of the alkaline solids in present in the FGD matrix. This same matrix contains a variety of trace elements, which could potentially impact aquatic environments in a deleterious way, if they are released into solution. The present study focussed on the geochemical stability of FGD Byproductss in contact with AMD, and ascertained the potential for release of selected trace elements to aquatic systems.

2.Materials and Methods

FGDG was collected from a power-plant near Coshocton, Ohio. The FGDG was reacted with groundwater collected from the Roberts-Dawson Mine. Effluents discharging from the abandoned mine have low pH, ranging from 2.8 to 3.5, and important precipitates of iron oxide: goethite and schwertmannite. Cylindrical sub-samples of FGDG were prepared for the weathering experiment. Each experimental treatment consisted of a 6-g sample plus 200 ml of AMD. All samples were placed in an incubator and allowed to equilibrate at 25°C for periods of 5 to 168 days. The chemical and mineralogical composition of FGDG was determined before and after contact with AMD. Also, the pH and chemical composition of water were determined before and after contact with FGDG.

3.Results and Discussion

All metals present in FGDG were also present in coal but the high Sr content was due to the lime used in the process. The FGDG was composed of fly ash (FA), filter cake (FC) and lime. Most of contaminants in FGDG were present at high concentrations in FA. As, Pb, Mo, Co, Sc, Mo, La and Zn were present in FC. The FC is composed of hannebachite ($CaSO_3.1/2H_2O$) and bassanite ($CaSO_4.1/2H_2O$). The FA is composed of mullite ($Al_6Si_2O_{13}$), quartz (SiO_2), hematite (Fe_2O_3), magnetite (Fe_3O_4) and glass. The identification of the different mineral phases in FGDG before contact with AMD already showed evidence of weathering. No lime or bassanite was detected but a new mineral phase was identified by XRD as ettringite ($Ca_6Al_2(OH)_{12}(SO_4)_3.26H_2O$). On the XRD patterns of the bulk samples, all original peak intensities decreased over time and new peaks appeared corresponding to the

transformation of the original minerals to new phases. After 5 days contact with AMD, the peaks intensity corresponding to ettringite decreased dramatically and detectable quantities of calcite started to precipitate. By 84 days, XRD reflections corresponding to gypsum were evident. After 168 days of reaction with AMD, the only minerals detected by XRD were quartz, mullite, hematite, magnetite, hannebachite, calcite and gypsum. After only one week, the pH increased from 3.2 to 8.8, then the pH slowly decreased day after day to reach a plateau at 8.0. The initial pH increase was likely due to dissolution of FC components, and the decrease to pH 8.0 was due to a slow carbonation of FGDG by atmospheric CO_2. Arsenic concentrations increased exponentially in less than 14 days from 0 to 15 $\mu g\ L^{-1}$, as pH increased. This was followed by a progressive increase in dissolved As concentration which reached a level of 22.7 $\mu g\ L^{-1}$ by 168 days. Total dissolved Se concentrations also increased with reaction time but soluble Se never exceed 1.5 $\mu g\ L^{-1}$. The concentration of dissolved B increased as a function of time to reach 5.34 $mg\ L^{-1}$ at 168 days. As expected, Fe, Al, Mn, Zn and Cr concentrations decreased as pH increased.

4. Conclusions

The dissolution of ettringite and hannebachite deteriorated water quality by increasing dissolved solids and hardness (120 $mg\ L^{-1}$). Sulfate has no sanitary significance but it is considered by U.S. EPA as a secondary contaminant. At high concentration it is considered undesirable in water because of purgative effect it can have on users, and it can affect the taste of water as well. The USEPA recommends a 250 $mg\ L^{-1}$ limit for drinking water. The AMD examined in this study already contains a large amount of dissolved calcium (80 $mg\ L^{-1}$) and sulfate (150 $mg\ L^{-1}$). The use of FGDG to abate this drainage could potentially increase the amount of these element ions discharged to surface waters. Most trace elements are generally more abundant in coal than in the average composition of the earth's crust (Powell, 1988). In particular, As, Cd, B, and antimony (Sb) are 5 to 8 times enriched and Se is 82 times enriched in coal. In consequence, FGDG also contains high concentrations of many trace elements. For example, the fly ash contained 127 $mg\ As\ kg^{-1}$. This element typically exhibit increased solubility and mobility at alkaline pH. Thus, it was not surprising that the dissolved concentration of As increased as the FGDG reacted with AMD. Indeed, while the concentration of dissolved As (23 □g L^{-1}) did not exceed the drinking water limit of 50 $\mu g\ L^{-1}$. Further solubilization of As could result in solution concentrations in excess of drinking water standards. The long-term solubility of As in these systems is not known and warrants further study.

The objective of this study was to demonstrate the technical feasibility of recycling FGD Byproducts to reduce and mitigate acid mine drainage. The laboratory experiments showed that FGD Byproducts effectively neutralized acid mine water. Neutralization was obtained because of the complete dissolution of some minerals in the FGD Byproducts, which increased the hardness of the water, and raised the concentrations of sulfate, boron and most importantly arsenic. Both the solution data and the solid phase mineralogy indicate that this system was far from equilibrium and will likely continue to change over time. The relevance of this laboratory study to field sites must be carefully considered. In the present study, the massive blocks of FGD solids were reacted with the AMD, in static reactor vessels. For each sample the acid solution was allowed to react with all sides of the solid. One might argue that this contrasts with the mine environment in which the intent of FGD Byproducts injection was to seal the mine and prevent fluid bypass around the grout seal. Thus in the field environment, it is expected that a smaller percentage of the FGD Byproducts was actually in physical contact with the ground water present in the mine, resulting in less potential for reaction with the FGD Byproducts over short time interval. However, during the actual field injection phase the amount of direct contact between the slurried FGD and the AMD was quite high, resulting in potentially greater dissolution than was observed in the laboratory conditions. Thus, it is likely that one would see transient, elevated concentrations of dissolved As and B in the field samples. Once the initial disturbance due to field injection decreases it is likely that the use of FGD Byproducts as mine sealant will not produce concentrations of dissolved As and B as great as were observed in the laboratory study. Nevertheless, this contention must be compared to real field data. Clearly, long term data on surface and ground water geochemistry as well as on the solid phase composition and mineralogy of FGD Byproducts, in the field, is needed before we can fully anticipate its utility and impact in field environments.

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UTILIZATION OF STEEL MILL LIME CAKE ON ACIDIC SUGARCANE LAND

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1. Introduction

Lime cake, the object of this study, is a steel mill by-product. It is produced by the lime-making process. Since the limestone shares a transporting process with coke and the iron ore, together with other ore materials, lime cake contains some undesirable substances. The objectives of this study were to: (1) assess the feasibility of lime cake as an acidic soil conditioner for resource reuse, and (2) determine the effects on soil properties and sugarcane growth of applying lime cake to sugarcane land.

2. Materials and Methods

A lime cake from China Steel Co. was analyzed for its total and leachable heavy-metal content. Soil samples were taken from the studied field before and after applying lime cake. The soil pH and cation exchange capacity (CEC) were measured. The total and available quantities of heavy metals in the soils were determined (CHANG et al., 1984). At harvest, the sugarcane was weighed; plant tissues, including leaves, bagasse, and juice, were sampled and digested with $HClO_4$ and HNO_3 mixtures to analyze the heavy-metal content.

3. Results and Discussion

Calcium, Mg, and Fe were the major and Mn, Cr, Zn, Cd, Pb, Ni, and Cu were the minor components of the lime cake (Table 1). Comparing the concentrations of Cr, Zn, Cd, Pb, Ni, and Cu in the lime cake with those in a sludge that were appropriate for land application as suggested by CHANEY (1973), the heavy-metal level in the lime cake was within the safe range. The heavy-metal leaching quantities of the lime cake were low; therefore the potential harm of the heavy metals to plants was low. The soil pH rose from 5.66 to 6.13, and CEC increased from 8.93 to 9.35 when 27 t ha^{-1} of lime cake was applied. Comparing concentrations of heavy metals in soils on which lime cake was applied with critical total soil concentrations above which phytotoxicity is possible (ALLOWAY, 1990), total concentrations of Cd, Ni, Cr, Cu, Pb, and Zn were within safe ranges. Application of lime cake to acidic Ultisols increased the effective stool by 7%, stalk height by 19%, and cane yield and sugar yield by 25%. A comparison between the heavy-metal concentrations in the sugarcane tissue and the critical concentrations in plants - above the levels likely to cause toxicity (ALLOWAY, 1990) – showed that concentrations of these metals were all below the critical levels. Most heavy metals taken up by the sugarcane usually accumulated in the roots (Table 2). Application of lime cake to sugarcane fields seems to have no adverse effect on the growth and quality of sugarcane.

4. Conclusions

Lime cake is a by-product of steel mills; it contains Ca, Mg, and some heavy metals. Application of lime cake to acidic sugarcane land increases the yields of cane and sugar and does not deteriorate the soil properties or sugar quality. Lime cake could be used as an agricultural liming material.

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Table 1. Total and leaching quantities of metals for lime cake from China Steel Co.

Metal	CaO	MgO	Fe	Mn	Cr	Zn	Cd	Pb	Ni	Cu
						mg kg^{-1}				
Total	47.6	1.20	0.37	93.8	38.95	38.45	11.1	5.95	46.2	8.25
Leaching	-	-	ND	0.52	0.04	0.16	0.02	ND	0.01	ND

Table 2. The heavy-metal contents in sugarcane tissue from the experimental field

Cane tissue	Lime cake	Heavy-metal content in tissue					
		Cd	Ni	Cr	Cu	Pb	Zn
		mg kg^{-1}					
Leaf	With	0.06±0.00	1.68±0.18	1.96±0.03	5.72±0.22	2.56±0.31	28.67±1.75
	Without	0.09±0.03	1.38±0.07	2.05±0.00	4.65±0.03	2.56±0.31	30.38±3.03
Bagasse	With	0.06±0.00	1.21±0.34	1.74±0.06	3.53±0.09	ND	14.76±1.09
	Without	0.03±0.03	0.28±0.28	1.71±0.03	3.50±0.19	0.53±0.53	15.51±0.78
Juice	With	trace	ND	ND	0.72±0.28	ND	3.22±1.45
	Without	trace	ND	Trace	0.70±0.12	ND	4.14±1.36

STABILIZATION OF APC-RESIDUES WITH $FESO_4$

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1. Introduction

Air pollution control (APC) residues from municipal solid waste incineration (MSWI) are difficult to dispose of due to the leaching of high concentrations of salts and trace metals, especially Pb. Pretreatment of the APC-residue before disposal may be needed.
A treatment process is suggested involving mixing of the APC-residue with a ferrosulphate-solution and subsequent oxidation of the suspension. This treatment washes out salts and contains the trace metals in the solids, thereby leading to less leaching of salts and metals from the treated APC-residue.
The treatment process builds on well-established geochemical knowledge that many iron oxides are stable and able to bind substantial amounts of trace metals. The wastewater separated from the solids should have a high content of salts and a low content of trace metals.

2. Materials and Methods

The experiments involved two different APC-residues from MSWI: a semidry gas cleaning residue containing fly ash and lime (AF) and a fly ash from an electrostatic precipitator (VF). APC residue (800 g) was mixed with a ferrosulphate-solution (4 l) corresponding to 50g Fe^{2+}/kg semidry APC and 25g Fe^{2+}/kg fly ash. The amount of iron added was limited by the alkalinity of the materials, in order to allow ferro-iron to precipitate as ferrohydroxides at high pH. After adding the APC-residue the suspension was oxidized for 24 h with atm. air. The solids were separated from the wastewater by filtration and afterwards the wet product was dried at room temperature.
The treated residues were characterized by a leaching procedure (CEN-test, two-stage batch test (L/S 2 and 10 l/kg), European Committee for Standardization, 1996) and a pH-static-test (L/S 10 l/kg, 24 h, computer controlled pH). Likewise untreated residues were characterized. The samples as well as the wastewater generated by the treatment were analyzed for pH, conductivity, Cl^-, Ca, Na, K, Mg, Al, Fe, S, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb and Zn.

3. Results and Discussion

The concentrations of components in the wastewater are shown in Table 1. The wastewater contains relatively low concentrations of trace metals. As intended, large amounts of salts were transferred to the wastewater, e.g. more than >80 % of Na^+ and K^+ were washed out from the APC-residues during the process.
Table 2 and Fig. 1 show the reduction of the leaching for selected components. The leaching of Pb was reduced with two orders of magnitude at fixed pH and up to 4 orders of magnitude at the actual pH-values of the test (CEN-test) due to the amphoteric behaviour of Pb. Likewise, Cd leaching was significantly reduced according to the pH-static-test. At the natural pH, the Cd release from the untreated material was limited, due to precipitation as hydroxide, and thus the effect of the treatment is less significant. Similar results were observed for Zn, Ni and Cu.

Table 1. Concentrations of selected components in wastewater.

	AF	VF
pH	10.65	10.68
Cond. (mS/cm)	73.6	62.3
Cl (g/l)	27.0	17.5
Ca (g/l)	10.7	2.1
K (g/l)	4.1	8.7
Na (g/l)	4.0	6.8
Fe (mg/l)	0.15	< 0.1
As (µg/l)	< 700	< 700
Cd (µg/l)	3.3	7.5
Co (µg/l)	< 26	3,0
Cr (µg/l)	< 200	320
Cu (µg/l)	75	54
Hg (µg/l)	3.0	2.5
Ni (µg/l)	< 300	39
Pb (µg/l)	162	29
Zn (µg/l)	148	37

Table 2. Concentration in first stage of CEN-test (L/S 2 l/kg).

	AF		VF	
	Untreated	Treated	Untreated	Treated
pH	11.4	9.7	11.6	8.9
Cond. mS/cm	150	51	130	23
K (mg/l)	9300	2000	21200	2230
Cd (µg/l)	18	3.8	26	5.4
Cr (µg/l)	50	400	25	1410
Hg (µg/l)	130	50	0.36	12
Pb (µg/l)	388000	36	1470	16

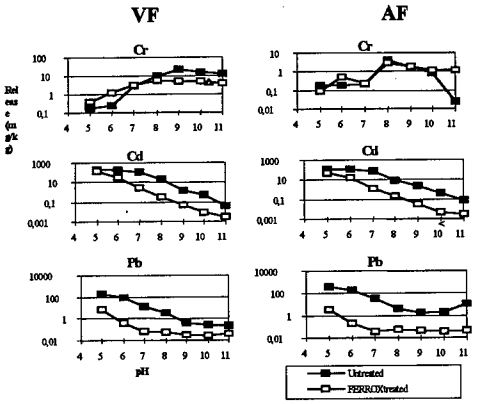

Figure 1: Release vs pH for selected metals.

As seen from Table 2 and Fig. 1 the effect on the leaching of Cr was limited. This is due to the presence of chromium as oxyanions with little affinity for the formed iron oxides. The leaching of Hg was not reduced but in some cases even increased. This may have been caused by reduced concentrations of Cl^- which affects a possible equilibrium with calomel ($HgCl_2$).
Overall the process shows positive results. However, the testmethods used are short-term tests, and as the process is based on the formation of a iron oxide phase, the stability and nature of the formed ironoxide must be addressed in future research.

4. Conclusion

Treatment of semidry-APC and fly ash with $FeSO_4$ improved the leaching properties of the materials with respect to Pb, Cd and other trace metals. The effect on metals which forms oxyanions is more uncertain and there is no reduction in the release of Hg. The wastewater from the process does not contain unacceptably large amounts of trace metals.

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EFFECT OF ZN ON THE MICROBIAL BIOMASS CONTENT OF SEWAGE SLUDGE-TREATED SOIL

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1. Introduction

Heavy metals accumulate in agricultural soil from recycling sewage sludge as well as from other sources including farm and industrial wastes, fertilisers and atmospheric deposition, with potential consequences for long-term soil fertility. In contrast to the other inputs, however, land-spreading of sewage sludge is monitored and controlled and maximum soil limit values for heavy metals are enforced to avoid environmental problems. Ecotoxicological indicators are amongst the most sensitive to soil damage by heavy metals and continued vigilance and research is necessary to demonstrate that recycling sludge on farmland is safe and sustainable. This paper presents the results of a field trial to assess the significance of Zn accumulation from long-term sludge application on the soil microbial biomass.

2. Materials and Methods

Loamy sand soil (pH 6.9) from a long-term sewage sludge-treated field site near Worksop, Nottinghamshire was transferred to ADAS Gleadthorpe in 1995 and lime or elemental sulphur were added to generate a gradient of 7 pH treatments in the range: pH 5 to 8. Soil at the experimental site had similar physical properties and was used as the untreated control with corresponding pH manipulations. Ryegrass was established and the trial was equilibrated for 2 years before sampling for soil microbial properties in spring 1997. Total soil microbial biomass was measured by soil-fumigation-extraction and CO_2 efflux was determined with an automatic gas-switching manifold attached to an infrared gas analyser.

3. Results and Discussion

The total Zn content (Table 1) of the sludge-treated soil exceeded UK guidance (200 mg Zn kg^{-1}, pH 5-7) and statutory (300 mg Zn kg^{-1}, pH 6-7) limits. The ammonium nitrate (AN) extractable Zn content was increased by 100 times compared with the control at equivalent pH values, indicating a large potentially bioactive fraction in sludge-treated soil (Figure 1). The other elements were present in small amounts compared to their respective limits and were unlikely to be environmentally significant (Table 1), providing a unique opportunity to examine specifically the effects of Zn on soil fertility responses. This is important because UK and European soil limits for Zn are identified as having the greatest potential to damage microbial processes compared to the maximum permissible concentrations of other elements in sludge-treated soil (MAFF/DoE, 1993). The pH adjustments to sludge-treated soil were representative of UK statutory pH requirements for agricultural recycling, since sludge application to farmland is not permitted below pH 5.

Table 1 Soil Physico-chemical properties

Soil property	Control	Sludge-treated
Total Zn (mg kg^{-1})	45	450
Total Cu (mg kg^{-1})	9.9	23.8
Total Ni (mg kg^{-1})	6.0	6.0
Total Cd (mg kg^{-1})	0.13	0.56
Clay (%)	7	5
Silt (%)	18	13
Sand (%)	75	82
Organic C (%)	1.2	2.1
pH	6.8	6.9

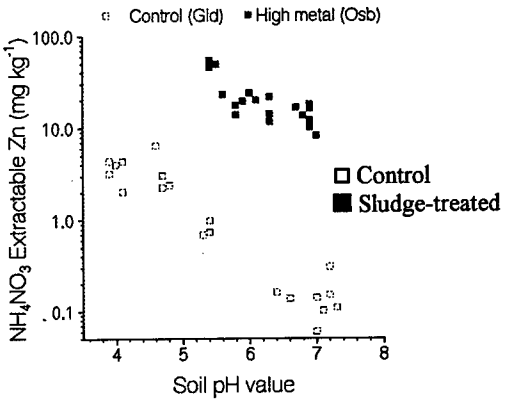

Figure 1 Soil pH and AN extractable Zn

Despite significant Zn enrichment, no toxic effects on the microbial biomass were detected in sludge-treated soil compared to the unamended control (Figure 2). The biomass C:organic C ratio was comparable in both soils and the metabolic quotient, which is indicative of microbial stress, was smaller with sludge application suggesting lower stress exposure compared with the control. By contrast, the ratio decreased and quotient increased as soil pH declined, but this was independent of metal content. The soil types are amongst the most vulnerable to metal contamination so this is valuable evidence supporting current UK and EU maximum limits for Zn, which protect soil from adverse effects measured in terms of microbial biomass.

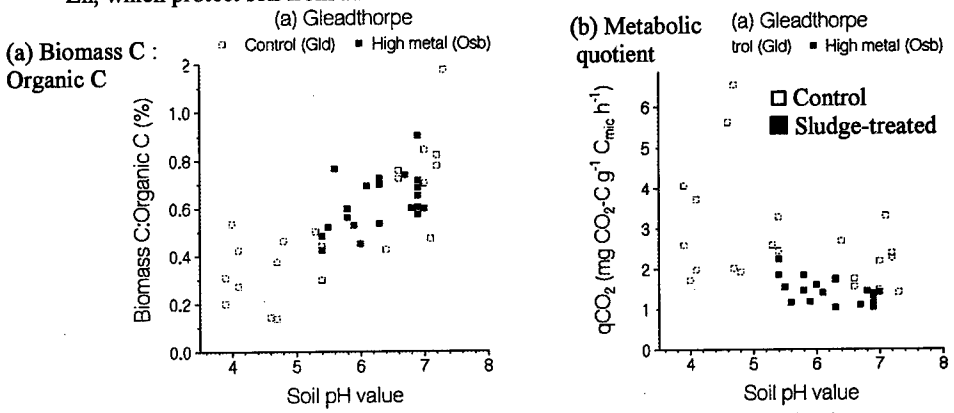

Figure 2 Soil microbial properties in relation to pH value and sludge application

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MAFF/DoE (1993) Review of the Rules for Sewage Sludge Application to Agricultural Land: Soil Fertility Aspects of Potentially Toxic Elements. PB 1561. MAFF Publications, London.

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BINDING OF TRACE METALS TO IRON OXIDES- STABILIZATION OF INCINERATION RESIDUES

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1. Introduction

From geochemistry it is well known that iron oxides and hydroxides bind trace metals, either as adsorbed species or as coprecipitates where metals substitute in crystal lattice sites. In this way, iron oxides help to control the distribution and mobility of trace metals in the natural environment. This natural behaviour of iron has served as a model for the design of a new treatment process where air pollution control (APC) residues from municipal solid waste incineration (MSWI) (e.g. fly ash) are mixed together with a ferrosulfate solution, leading to a new Fe-rich phase in the treated product. Much of the trace components released by dissolution of salts present in the residue are then retained in the new Fe-rich phase, resulting in much lower metal concentrations in leachate from the treated residues.

The extent of trace component immobilisation depends to a large extent on the properties of the particular iron oxide or hydroxide that forms, the solution and the component itself. In order to understand the long-term effects of the treatment, it is necessary to identify the solid phases that form and to determine which uptake processes play the leading roles for the metals. The classical methods for bulk solid analysis such as X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) use the macroscopic approach, so they are unable to provide the molecular level information that is necessary to answer these questions. We have supplemented our investigations with complementary data from several high resolution techniques.

2. Materials and Methods

Iron oxides and hydroxides were formed by mixing APC residue with a ferrosulfate solution. The resulting suspension was oxidized for 24 h by bubbling with air. The solids were collected on a filter and dried at 60°C untill complete dry (approx. four days). Dried samples were analysed using XRD, SEM with and without energy dispersive spectroscopy (SEM-EDS), as well as Transmission Electron Microscopy (TEM), X-ray Photoelectron Spectroscopy (XPS) and Time-of-Flight Secondary Ion Mass Spectroscopy (TOF-SIMS). By use of these methods it was possible to analyse the iron structure as well as the binding of metals. Leaching tests were performed on the residues before and after treatment.

Table 1: Typical trace metal content in untreated APC residues from Danish MSWI determined by total acid digestion (7N acetic acid) and ICPMS (mg/kg).

As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
<7,0	100	15	150	1200	10	50	6000	17000

3. Results and Discussion

During Fe-treatment of the APC-residues, a new iron oxide or hydroxide solid phase formed, in which most trace metals were retained. Consequently, these metals showed a decrease in leachate concentration of 1-3 orders of magnitude due to the Fe-treatment.

XRD showed peaks typical of gypsum ($CaSO_4 2H_2O$) and anhydrite ($CaSO_4$), which are expected precipitates resulting from dissolution of lime (CaO) from the original fly ash and SO_4 from the ferrous sulphate solution. Although the residue definitely had the red-orange colour diagnostic of Fe-oxides and hydroxides, there was no detectable XRD signal for any Fe mineral; there was only a hint of a broad background typical of a poorly crystallised ferrihydrite. We could learn nothing about the location of the heavy metals because concentrations were too low (Table 1). SEM showed only a host of very fine particles; mineral identification was not possible. From SEM-EDS it was possible to obtain an elemental identification of the upper layers of a polished sample. These analyses showed the presence of very few coated particles and indications of trace metals associated with the Fe-rich phase. The trace metals were, however, hardly detectable due to their low concentration. Characteristic cube formed crystals of alkali-chlorides were observed, as were gypsum, quartz and various Ca-Al-silicates.

XPS, which gives information about chemical identity and bonding in the surface-most layers, proved that the iron compounds were not simply present as surface coatings on pre-existing particles, but that they had formed a new, separate solid phase. TOF-SIMS can make chemical maps of the top-most atomic layers; images show an affinity of calcium for the iron-rich areas. TEM proved that the iron phase is fibrous and almost exclusively an Pb-rich ferrihydrite. Lead is not seen to form $Pb(OH)_3$ on its own, but is intimately mixed as $Fe_xPb_{(1-x)}(OH)_3$. The two-line form dominates, indicating a poor crystallinity, but the four-line variety is also present. Various elements are also associated with this phase, particularly Zn and Sn, which has been identified as separate, tiny crystallites of $ZnSnO_3 \cdot 4H_2O$; this material is insoluble through treatment. Cadmium did not form a separate phase that we could observe with any technique for measuring solids; Cd is known to form solid-solution with calcite, but in these samples, it is probably below our detection limits.

Although this study demonstrates the effectiveness of the Fe-treatment process over the short-term, we are still concerned about the long-term retention capacity of the treatment product. Over time, under some geochemical conditions, ferrihydrite is expected to recrystallise, which means that it could transform into other more crystalline minerals. The fate of Pb and other cations from ferrihydrite during such a process is not known at present and is the subject of further research.

4. Conclusion

By use of various analytical techniques it has been proven that a ferrihydrite structure is formed when APC residues are treated in a $FeSO_4$-solution. Lead has been shown to be trapped within this iron structure, whereas the uptake sites of other trace elements have not yet been identified. Further work is needed to explore the longer term stability of the treatment products.

EFFECT OF BIOSOLID TREATMENT ON METAL TRANSPORT FOLLOWING APPLICATION TO AGRICULTURAL SOILS.

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1. Introduction

EC Directive 91/271/EEC (CEC, 1991) bans the disposal of sewage sludge at sea from December 31st 1998, and application to agricultural land is seen as an economic and beneficial alternative. In Britain, 550,000 tonnes of sludge produced this year will be spread on farmland, and by 2002, this will have increased to ca. 915,000 tonnes (MacKenzie, 1998).

Multiple sludge applications coupled with low mobility and low solubility in water, can lead to heavy metal accumulation in soils. The EU has set limits for soil concentrations of individual heavy metals (CEC, 1986), however maximum limits vary widely within different member states. In the UK, heavy metal application rate limits are relatively high, and at the Code of Practice application rate (250 kg N/ha/yr) they will not usually be a limiting factor.

To quantify potential accumulation of heavy metals in soils, the mobility of heavy metals within biosolid amended soils needs to be identified and an estimate made of the quantities lost through leaching. In this study, steady state column experiments were conducted on sewage sludge amended soils at field capacity with controlled rainfall, under a mass balance approach, collecting leachates for analysis. Mobility of heavy metals was assessed and kinetic information on the rate of leaching obtained. Hydrological profiles were determined using conservative tracers.

2. Materials and Methods

Two soils representative of those in agricultural use were selected on the basis of their different physical characteristics, namely : a red loamy sand (G) and a typical argillic brown earth (W). Three sewage sludges commonly applied to agricultural land were used, viz : fresh mesophilically anaerobically digested dewatered sludge cake, the same sludge cake dried and ground, and composted sludge cake. Sludges were applied at the UK Code of Practice application rate of 250 kg N/ha/yr. Triplicate mixtures of each soil and sludge were placed along with triplicate controls in polythene columns, to simulate a cultivated horizon of 20 cm depth and 10 cm diameter.

The columns were leached with deionised water at a rate equivalent to 10 mm rainfall/hour, for three 24 hour periods. For the first two periods leachate samples were collected for analysis of metals after 1, 2, 4, 8, 16 and 24 hrs; in the third period one sample was collected after 12 hrs. Leachate samples were evaporated to dryness and redissolved in 2M HCl, as a pre-concentration step prior to analysis using a Pye-Unicam SP9 FAAS. $SrCl_2$ releasing agent was used for Ni. Conservative tracers (Cl^- for the first period, Br^- for the second and none for the third, at 1000 ppm of their respective K salts) were detected by ion specific electrodes. Water volumes applied and leached were recorded each hour during the first two periods, and at the end of the final

period. All soil/sludge mixtures were digested to determine total metal concentrations on mixing, after equilibration and post-leaching. The digestion method used was a HCl-HNO_3 digestion, with analysis by FAAS (HMSO, 1987).

3. Results and Discussion

The conservative tracers showed excellent uniform flow thorough the cultivated horizon in the sandy soil and very good flow through the brown earth.

Table 1 shows the mean and maximum leachate concentrations observed during the first leaching period in both soils. Preliminary analysis indicated that Zn, Cu, Pb an Ni were leached in all experimental periods. Cd was either absent from leachates or only present at very low levels. There was little difference in leachate metal concentrations between sludge treatments or from the controls. Total metal analysis in the soil/sludge mixtures post-leaching is currently being conducted to complete the mass-balance.

Table 1: Mean and maximum leachate concentrations observed in the first period.

	Zn	Cu	Cd	Pb	Ni
G soil mean (ppb)	81.2	51.7	2.4	70.6	39.9
G soil max (ppb)	263.5	113.8	14.7	277.8	125.8
W soil mean (ppb)	90.9	29	5.8	77.9	61.0
W soil max (ppb)	755.1	54.3	13.1	157.5	179.8

4. Conclusions

Concentrations of Cd and Cu in the leachates were lower than of Zn, Pb and Ni, reflecting the lower soil concentrations. Leachate concentrations were similar to those found by other researchers (e.g. Brown & Thomas, 1983). Although at present there appears to be little difference between the sludge treatments, the mass balance results may allow treatment differences to be identified. Results from this study will allow estimates to be made of heavy metal leaching rates following land application of different types of sewage sludge.

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REDUCTION OF URBAN RESIDENTIAL LEAD EXPOSURE: BALTIMORE'S EXPERIENCE, USA

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1. Introduction

Lead poisoning is a worldwide environmental problem. In the U.S., lead-containing paint and dust remain the major ead sources of exposure. The U.S. did not sign the 1923 international treaty to ban the manufacture, sale, and use of residential white-lead paint (>2% lead). Only after 1978 did the U.S. limit the lead content of residential paints to 0.06%. Consequently, 83% (75 million) of occupied residential units built before 1980 contain lead-based paint. Nearly 6 million U.S. children 1 to 2 years old live in lead-painted houses. At highest risk are poor children living in older (pre-1946) urban houses; 16.4% of such children aged 1 to 5 years have blood lead concentrations (PbB)>10μg/dL. As a scientific basis for prevention, field studies in Baltimore summarized below have documented the short and long-term effectiveness and costs of alternative methods of controlling residential lead-based paint and dust hazards.
In the past, untrained workers commonly burned and sanded lead-based paint in an effort to reduce lead hazards in the home. This work, often done without safety precautions to protect occupants, greatly increased the amount of lead in house dust which in turn caused children to be lead poisoned or to have worse lead poisoning, FARFEL and CHISOLM (1990). In the early 1990s, we found that major residential lead-based paint abatement by trained workers using safety precautions greatly reduced the amount of lead in dust, FARFEL (1994). Unfortunately, the major renovations cost more than many families and property owners could afford. For this reason, in two more recent studies we tried to find ways to repair and maintain older houses that could reduce lead in dust and paint and also be affordable to more families and owners.
The ongoing „Lead-Based Paint Abatement and Repair and Maintenance (R&M) Study" sponsored by U.S. EPA and U.S. HUD is an investigation of the short- and long-term (up to 5 years) effectiveness of three types of repair interventions (n=75 houses) designed to reduce children's exposure to lead in paint and settled dust (Table 1), FARFEL (1997). The ongoing „Treatment of Lead-Exposed Children (TLC) - Clinical Trial" sponsored by NIEHS implemented the R&M types of lead hazard control interventions, as well as professional cleaning interventions without repairs, in the homes of more than 220 children prior the start of their clinical treatment, TLC (1998). Both studies provide data on changes in dust lead before and after intervention.

2. Materials and Methods

Cyclone dust samples (R&M Study) and wipe dust samples (TLC Trial) were collected from floors, window surfaces, and entryways before intervention and periodically post intervention. The cyclone provided estimates of dust lead loadings (μg Pb/m^2) and lead concentrations (μg Pb/g). Wipes provided estimates of lead loadings. Cyclone dust was decomposed using a closed vessel microwave nitric acid digestion method (modified US EPA SW 846 Methods 3015 and 3051) and analyzed by ICP-AES (SW 846 Method 6010). Wipes were prepared using a modified US EPA Method 3050 for hotplate acid digestion and analyzed by Flame-AAS. In the R&M Study, children's venous blood was periodically tested for lead using Graphite Furnace AAS. Longitudinal data analysis was used to assess changes in lead in dust and children's blood.

Table 1: R&M Study Interventions

ELEMENT	LEVEL I	LEVEL II	LEVEL III
Cost ($)	1,650	3,500	6,000
Time (days)	within 1	within 3	within 7
Safe Practices	✓	✓	✓
Paint Stabilization	✓	✓	✓
Windows	well caps	sash guides + well caps	new windows + ext. trim cover
Floors & Stairs	not treated	sealed	sealed/covered
Entryway Mat	✓	✓	✓
Professional Clean-up	✓	✓	✓

3. Results and Discussion

On average, all three R&M interventions reduced dust lead loadings on window surfaces by 90% and on floors by 70% or more immediately post-intervention. The average dust lead level during the first two years after all three R&M interventions remained lower than the amount of lead in dust before the repairs. R&M III was associated with the lowest dust lead levels over time compared to R&M I and R&M II. The TLC Trial documented reductions in dust lead loadings after repairs and after professional cleaning interventions. Houses built after lead was banned from house paint had the lowest dust lead levels. Also important was the finding that children's PbBs did not get worse after the R&M study repairs. The greatest reductions in PbB (35%) during two years of follow-up were in children with PbB>15μg/dL at the start. Lastly, houses in neighborhoods without lead in paint were associated with the lowest PbB in children (<10μg/dL).

4. Conclusions

Both studies show that significant reductions in dust lead levels and children's blood lead concentrations can be achieved and sustained over a period of years in older lead-painted houses through the implementation of various risk reduction interventions by trained workers. Such interventions are an important elements of lead poisoning control and prevention efforts.

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HEAVY METAL CONTENTS IN URBAN AND INDUSTRIAL SOILS

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1. Introduction

Industrialisation and urbanisation have transformed a large proportion of natural soils into new soil types with specific characteristics due to their high degree of anthropisation (Bullock and Gregory, 1991). Industrial derelict land appeared in the sixties and since then, their number has been increasing in relation with the decline of the traditional steel and coal industrial activities. On the other hand, rapidly expanding urban agglomerations have a sustained influence on soils. The diversification of the tilled technological substrates and their possible contamination related with the degradation of natural soil functions is one of the major problems presented by urban soils (Burghardt, 1996). This work was undertaken to determine the value and adaptability of traditional approaches to describe anthropogenic soil profiles and especially determine their degree of metal pollution.

2. Materials and Methods

Study of historic records of each site was necessary to choose the most appropriate locations for representative soil profiles. Profiles were described and soil samples were collected according to horizons, and analysed for their physico-chemical characteristics and content in total and available metals. The industrial sites were representative of three main former industrial activities, *i.e.* steel industry, smelting and coking. In the urban area, the German-French Park (Saarbrücken, Germany) was selected due to its important social function for the citizens and the changing land use and construction history. Two profiles were considered : an extensive waste disposal site from domestic refuse and ashes and a second profile where soil was replaced after transport from another location. The prediction of the risk of transport of metals within the profiles by chemical extractions, toxicity tests and isotopic methods are under investigation.

3. Results and Discussion

Urban and industrial soils showed various degrees of anthropisation from park soils to industrial soils developed on technological materials. The soil profiles revealed a high horizontal and vertical variability of the distribution of heavy metals (Figure 1), which was due to the successive depositions of various raw materials before and during the urban and industrial activities. We can classify between industrial by-products, materials coming from the demolition of buildings, wastes and earthy materials. Their organisation varied among the sites depending on the way they were brought and added to the soil.

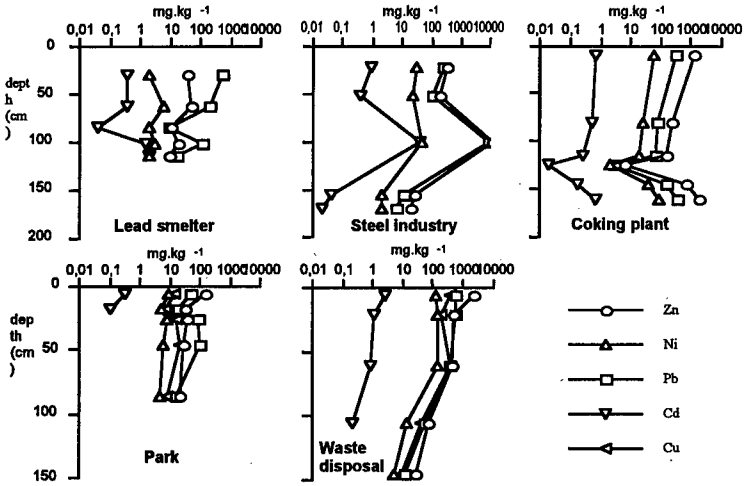

Figure 1: Distribution of total contents of metals in 3 industrial and 2 urban soil profiles

4. Conclusions

Traditional methods of pedology, which are widely known, may serve as a support for the study of urban and industrial soils. However, these environments do show a certain number of specific characteristics (heterogeneity, pollution). Characterization of modal and extreme soil profiles

HEAVY METAL PHYTOAVAILABILITY ESTIMATED BY DTPA EXTRACTION IN AN OXISOL INCUBATED WITH MUNICIPAL REFUSE COMPOST

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1. Introduction

Urban garbage usually contains around 50% organic matter that can be recycled on agricultural soils after being composted. The compost produced is not only rich in organic matter but also contains reasonable amounts of some plant nutrients such as N, P, Zn and Cu. The municipal refuse compost can be further digested by earthworms, producing earthworm cast which is also used as an organic fertilizer. However, both organic materials may contain heavy metals such as Ni, Pb and Cd in its composition. This would restrict their agronomic use because these metals can be absorbed by plants, and hence enter the food chain. The DTPA method was developed to micronutrients, Fe, Mn, Cu and Zn (LINDSAY and NORVELL, 1978) but it can also be used for others elements such as Cd, Pb, Ni and Cr with good correlation to plant availability.
This paper is aimed at studying plant availability of heavy metals (Cu, Cd, Cr, Ni, Pb and Zn), through extraction with DTPA, after the soil has received increasing amounts of municipal refuse compost and the casts produced by earthworms after being fed with this organic material.

2. Materials and Methods

Soil samples from the top layer (0-20 cm) of a Hapludox were air dried, sieved to pass a 2 mm screen and presented the following properties: organic matter 15g kg^{-1}, pH 4.0, exchangeable K, Ca and Mg (in $mmol_c$ dm^{-3}) 2.4, 5.0, 2.0, respectively, H+Al: 4.3 $mmol_c$ dm^{-3} and resin P: 2.0 mg dm^{-3}. Municipal refuse compost (MRC) and earthworm casts resulted from the digestion of MRC (EC) were collected at the garbage recycling plant in Novo Horizonte, State of São Paulo, Brazil. The organic materials were air dried, sieved to pass a 2 mm screen and analyzed for total carbon and heavy metal content (table1). Treatments consisted of mixing the equivalent of 0, 20, 40 and 80 t/ha (dry weight) of MRC, EC and dairy manure (DM) in 500 mL plastic containers, in a complete randomized block design with four replications Samples were collected at 0, 7, 15, 30, 60 and 90 days after the organic materials were added to the soil, air dried and stored in plastic containers. DTPA extraction for Cu, Cd, Cr, Ni, Pb and Zn was performed in each sample and analyzed by ICP-AES. Analysis of variance was used to determine significant differences due to treatments. Means for the organic sources were separated using the Dunnett test (P=0.05).

Table1: Organic matter and heavy metal contents of the organic wastes.

Material	O.M.	Cd	Cr	Cu	Ni	Pb	Zn
	g kg^{-1}	mg kg^{-1}					
MRC	1.3	9.0	85	89	25	212	332
EC	1.5	8.0	90	103	15	137	361
D	2.5	ND	82	28	5.0	6.6	72

3. Results and Discussion

After 90 days of incubation (Table 2), the amounts of heavy metals extracted by DTPA at the rates of 40 and 80 t ha^{-1} were higher for the EC treatment, followed by MRC and DM. The addition of 20 t ha^{-1} of MRC or EC change the soil Zn content from low to high, according to Zn interpretation dosages for the State of São Paulo (Brazil). The EC increased the soil Cu content no matter the amended dosage, while the MCR only increased for the 80 t ha^{-1} rate.

Regarding the toxic heavy metals (Cd, Cr, Ni and Pb), the addition of any organic materials did not alter DTPA extracted Cr in any rate applied. Amounts of Cd were significantly different from control (0 t ha^{-1}) only at the highest applied rates of MRC and DM, and at the rates of 40 and 80 t ha^{-1} of EC. All organic materials increased DTPA extractable Ni in the soil, except at the rate of 20 t ha^{-1} for the treatments MRC and EC. .DTPA extractable Pb was significantly higher than the control treatment only at the rates of 40 and 80 t ha^{-1} for MRC and EC. Hence, except for Cr, a tendency of increasing municipal refuse compost, digested or not by earthworms, was found, and it may become significant if the soil continues to receive successive applications of these organic materials.

Table 2: Heavy metal concentration in soil, extracted by DTPA, after an incubation period of 90 days with increasing amounts of organic materials.

Organic Materials	Treatment	Cd	Cr	Cu	Ni	Pb	Zn
	t ha^{-1}	mg dm^{-3}					
Control	0	0.02	0.02	2.53	0.12	2.13	0.73
Municipal Refuse (MRC) Compost	20	0.02	0.05	2.87	0.18	2.53	1.77*
	40	0.04	0.04	2.87	0.29*	2.67*	2.47*
	80	0.05*	0.06	3.27*	0.34*	3.05*	4.60*
Earthworm Cast (EC)	20	0.03	0.04	2.97*	0.20	2.46	2.03*
	40	0.05*	0.08	3.13*	0.34*	2.85*	3.17*
	80	0.06*	0.07	3.37*	0.42*	3.38*	5.00*
Dairy Manure (DM)	20	0.03	0.06	2.63	0.21*	2.29	0.97
	40	0.04	0.06	2.67	0.21*	2.34	1.10
	80	0.05*	0.09	2.73	0.29*	2.27	1.53*

*average values differ significantly from the control by Dunnett test (P=0.05)

4. Conclusions

- Additions of 40 t ha^{-1} of MRC or EC should be sufficient to supply adequate amounts of Cu and Zn to the crop.
- DTPA extractable Cd, Ni and Pb increased with the amounts of municipal refuse compost, digested or not by earthworms, added to the soil. Though the increases were small, more research is needed to study heavy metal phytoavailability when these organic materials are continuously applied to the soil.

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EFFECT OF SOLID URBAN WASTE COMPOST APPLIED TO SEVERAL SOILS AND THEIR CHEMICAL AND PHYSICAL PROPERTIES ON SOME METAL EXTRACTABILITY[1]

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1. Introduction

The use of urban waste compost in agriculture has brought up the risk of soil and plant contamination (Petruzzelli, 1989). The main concern on the application of urban waste compost to agricultural soils is the presence of Cd, Cr, Cu, Ni, Pb and Zn at a considerable concentrations, which may be accumulated in the soil upper layer and become toxic to plants, where they take part of the food chain, and they become harmful to animals and humans. It is therefore important to investigate which and how soil properties affect the bioavailability of these metals. In this study the interaction between soil metal extractabilities and chemical and physical properties of several soils, affected by solid urban waste compost application, combined or not with lime and fertilizers, was evaluated.

2. Materials and Methods

The solid urban waste compost, collected in the city of São Paulo, Brazil, was air dried and passed through a 4 mm mesh sieve. Some of the compost characteristics were: 271.5 g kg^{-1} total C, 12.8 g kg^{-1} total N, 21.2 C/N ratio, 7.9 pH, and 6.1 dS m^{-1} electric conductivity. The experiment was carried out in greenhouse conditions with twenty one acid and five alkaline soils collected from the surface horizon of different States of Brazil. Compost (30 g dm^{-3}) was applied sole, with mineral fertilizers (N, P, K, S, B, Cu, Fe, Mn, Mo, and Zn), with lime, or with lime plus fertilizers. Details of the soils and of the experiment conduction are described in ABREU JUNIOR et al. (1998). The soil samples were taken one month after compost application. Metals in soil samples were extracted by Mehlich-3 and analyzed by ICP-AES for B, Cu, Fe, Mn, Mo, Zn, Ba, Cd, Co, Cr, Ni, Pb, Sr, Ti, and V determinations. Soil pH (1:2.5) was measured in 0.01 mol L^{-1} $CaCl_2$, H+Al by the SMP (SHOEMAKER et al., 1961) buffer approach, electric conductivity (EC) in a 1:1 soil/water solution, N by the Kjeldahl method, and organic C by wet digestion. Particle size analysis was made by the pipette method. The interaction analysis between soil metal extractabilities and chemical and physical properties was made by means of correlation analysis.

3. Results and Discussion

The effect of compost application on the correlations between concentration of metals extracted by Mehlich-3 and pH, EC, N, C, C/N ratio, H+Al, base saturation (BS), and sand content are shown in Table 1. The concentrations of these metals are shown in ABREU JUNIOR et al. (1998). Evaluation of Cr, Cd, Ni and Pb in 31 non-contaminated soils of São Paulo, Brazil, by Mehlich-3 (ABREU et al., 1995) showed comparable values to those obtained in this work, but wider ranges were observed. Although significant correlations with metal uptake by rice plants and soil metal contents were observed by ABREU JUNIOR et al. (1998), ABREU et al. (1995) found that Mehlich-3 was ineffective to evaluate Cd, Cr, Ni, and Pb availability for wheat, and Cd and Pb for beans, because of the poor correlation with metal extractability and plant uptake,

probably due to the low metal concentration in those soils. Among the soil properties, pH was significantly correlated to the concentration of all metals. A large number of significant correlations were also observed between soil metal contents and soil EC, N, C/N ratio, H+Al, BS and sand content, except for Cu which presented correlation only with soil pH, H+Al and sand. These correlations were consistent with the known agronomic importance of these soil properties on metal availability. Although soil organic matter plays an important role on soil fertility and metal adsorption, the correlations between soil organic C and metal concentrations were low, except for Fe, Mo, and Ti. In this experiment, soil C/N ratio had higher effect on metal availability than organic C. For acid soils, on the average, the treatment with lime + fertilizers increased soil pH from 4.6 to 5.2, compost + fertilizers to 5.6, sole compost to 5.8, compost + fertilizers + lime to 6.1, and compost + lime to 6.4. Metal extractabilities (B, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) for each soil and for the compost treatments decreased as pH increased.

4. Conclusions

The solid urban waste compost application to soil did not increase only metal concentrations, but also pH, CE, N, and base saturation. However these effects did not differ from those caused by lime + fertilizers application. Soil pH was the most important property controlling metal extractabilities.

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Table 1. Correlation between metal extractabilities and pH, EC, total N, organic C, C/N ratio, H+Al, base saturation (BS), and sand content in soils treated with solid urban waste compost. (N = 156, * $p < 0.05$ and ** $p < 0.01$)

Soil property	B	Cu	Fe	Mn	Mo	Ni	Zn	Ba	Cd	Co	Cr	Pb	Sr	Ti	V
pH	0.66**	0.26**	-0.18*	0.35**	-0.40**	0.66**	0.47**	0.16*	0.40**	0.42**	0.56**	0.51**	0.35**	0.30**	0.64**
EC	0.62**	0.12	0.21**	0.03	-0.18*	0.29**	0.64**	-0.11	0.13	0.05	0.49**	0.22**	0.79**	0.52**	0.38**
N	0.33**	0.06	-0.21**	0.26**	0.23**	0.35**	0.17*	0.28**	0.27**	0.35**	0.40**	0.38**	0.08	-0.39**	0.23**
C	-0.07	0.05	-0.21**	-0.09	0.46**	-0.09	0.07	-0.01	-0.15	0.01	0.08	0.01	-0.05	-0.40**	-0.05
C/N	-0.47**	-0.02	-0.02	-0.48**	0.32**	-0.53**	-0.16	-0.37**	-0.58**	-0.43**	-0.43**	-0.44**	-0.20*	-0.02	-0.34**
H+Al	-0.52**	-0.24**	-0.04	-0.25**	0.48**	-0.48**	-0.46**	-0.10	-0.33**	-0.28**	-0.46**	-0.30**	-0.31**	-0.32**	-0.47**
BS	0.70**	0.15	-0.16*	0.40**	-0.15	0.79**	0.26**	0.45**	0.54**	0.56**	0.67**	0.59**	0.43**	0.08	0.67**
Sand	0.04	-0.18*	0.40**	-0.29**	-0.39**	-0.33**	0.17*	-0.42**	-0.36**	-0.36**	-0.32**	-0.23**	-0.09	0.42**	-0.31**

THE ASSESSMENT OF ECONOMIC AND ENVIRONMENTAL IMPACTS IN NORTHERN ITALY OF A NEW WATER PURIFICATION SYSTEM

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1. Introduction

An EC funded consortium is studying a new technology in which the binding properties of dead bacteria, elution and electrolysis are used to separate metals from water. Clean water, biomass and metals for reuse are the final products without waste production. To assess the influence of this system in Northern Italy urban and industrial sludge production and related economical aspects, environmental contamination and laboratory experiments on contaminated water were considered.

2. Materials and Methods

Urban sludge The main plant's managers were asked about sludge destination. The amount was calculated summing up the inhabitants equivalent (IE) of the plants. Cost of transport, reuse and dumping were considered as well as sludge fertilizing value. Some plants producing contaminated sludge were chosen and the relationship between kind of industrial discharges and sludge composition assessed.
Industrial sludge In five provinces waste production was investigated considering solutions and sludge contaminated by heavy metals. Treatment costs determined using market prices and an industrial case-study are reported.

Environmental contamination Discharged water quality and sediments were investigated in Reggio Emilia and Cremona to individuate the activities particularly involved.The soil background level was used to estimate the contamination.

Laboratory experiments Samples with known concentration of metals were tested in short scale experiment and in a little pilot plant. In short scale experiments solutions were prepared and mixed with dead bacteria. Samples were drawn (a) each 30" or 1'up to 10' and (b) after 30'. Filtration and analysis followed. In a single stage process, the biomass was pumped with poly-elettrolite in the water flux in the pilot plant. When recovered a sample of water was analysed. In a double stages process the biomass was reused after treatment of low contaminated for higher contaminated water.

3. Results and Discussion

The over cost for dumping is 42.5 ecu/t equivalent to L 32.5 Milion ecu per year plus a waste of about L 3.5 Milion ecu in fertilisers. In many cases the sludge is dumped because of its metals content (Tab.1). Small plants are more involved than bigger. Electroplating industry and potteries can explain high Pb and Zn content while Cu should come from wine makers or pig farms. Less then 1% industrial water can deteriorate the sludge of a plant (Tabb. 2-3).
Considering 60 ecu/t and 135 ecu/t respectively for solutions and sludge the expenditure is L 18 milion ecu/year (including transport). The cost changes among the industries.

Table 1. Urban sludge destination and related costs (1 ECU=2000 £)(dati Federgasacqua 1997).

Region	Dumping (I.E.)	Sludge (t 20% dm)	Agronomic reuse (I.E.)	Sludge (t 20% dm)
Lombardia	1577000	129314	485000	39770
E. Romagna	2055000	168510	1950000	159900
Piemonte	3063000	251166	736000	60352
Veneto	2750000	225500	958000	78556
Total	9445000	771490	4129000	338578
Cost (ecu/t)		62.5		20

Table 2. Incidence of industrial discharges on sludge composition in small town plants(dati AGAC)

Plant location	Pb	Cu	Zn	Plant capacity m3/d	Industrial water m3/d	% on total
Limits (mg/Kg dm) for agronomic reuse in Italy	750	1000	2500			
S.Giovanni di Q.	224	1219	970	203	not available	-
Le Forche	85	1292,3	1446	3850	12,83wm	0,33
Sorbolo a levante	283	513	34000	493	not available	-
Cadelbosco	126	546	7282	2303	15,65g	0,68
Meletole	801	574	1666,5	4712	18,46p + 37,97g	0,4c+0,80g=1,2
Novellara	101	415,7	4102	4208	33,38g	0,79

Note: (wm) wine maker, (g) electroplating (p) pottery. For Sorbolo a levante it is for sure an industrial source not reported

Table 3. Amount of contaminated waste produced in different provinces (ARPA Bologna 1996)

Provinces	Solutions + sludge (t)	Dumping leachate (t)	Number of firms involved	Number of employees	Population density (I/Km2)	Population
Reggio Emilia	24711	23225	144	9018	114	261389
Modena	27296	17672	299	14571	226	607646
Bologna	15923	72470	199	11559	245	907043
Piacenza	612	12219	64	6239	103	266667
Cremona	1505	12047	23	1515	186	330406
Total	70047	137633	729	42902		2373152
Treatment (ecu/t)	50-350	17.5				

In Cremona 6 industries discharged contaminated by Cu, Zn and Ni water in 1996-97. Five of them were electroplating or metal's industries. In Reggio Emilia some sediments contain combined high Zn, As and Cr while others Cd and Pb. Electroplanting and pottery industries are tought to be responsible. When contamination is slighter agriculture is the source for Zn and Cu in lot of streams in the area.
In industrial and artificially contaminated waters in small scale experiments biosorption was fast and removed metals alone and mixed. The pilot plant experiments shown similar results of both single and counter current processes.

Table 4. Amount of contaminated waste produced in different provinces (ARPA Bologna 1996)

Stage	Cu initial	Cu final	Zn initial	Zn final	Ni initial	Ni final
Single	9.32	1.99				
Single (mix)	10.1	1.74	50.3	3.52	1.83	0.6
Double	0.73	0.16				
	8.81	0.39				
Double	1.08	0.13	14.78	1.22	0.86	1.84
(mix)	7.16	0.44	45.04	23.04	0.11	1.47

4. Conclusions

Solutions and sludge contaminated by HM represent an environmental and economical problem in part of the area investigated. High treatment costs are creating problems to the producers with increasing risks of illegal management. Electroplating, pottery and wine industries are some examples of firms dealing with this problem and creating pollution. The first results obtained with the new purification system encourage to follow this pattern with further research. The main figures involved should be the indutries, the urban plants manager and the pubblic administration in the attempt to solve jointly this problem.

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INFLUENCE OF COMPOST FROM MUNICIPAL WASTES (MSWC) ON THE CONTENTS OF SOME HEAVY METALS IN SOIL AND PLANT

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1. Introduction

Rapid growth of civilization has been observed in the last decades. As a results an increasing of municipal wastes and their storing can be a serious danger for the environment. Municipal refuses content about 40-60% organic matter and lots of mineral components, which after composting process can be used to improve soil fertility (Mackowiak and Orzechowska 1993). Municipal wastes can also contain various heavy metals as well as other harmfull substances which inhibiting influenced on the growth and development of plants (Gallardo-Lara and Nogales 1987). High concentration of the heavy metals can also be the reason of decreasing the utilization value of plants consumed by humans and animals. Because of that researches on utilization of composts produced from municipal wastes in agriculture and horticulture, are a very serious problem (Iglesias and Perez 1989).
The aim of this research was to determine the influence of different doses of composts produced from municipal wastes (MSWC) on the contents of some heavy metals in the soil and uptake by plant.

2. Materials and Methods

MSWC from a big industrial center, produced according Dano technology, was used. Material leaving the technological line was composted during 9 months and after that was applied to the soil. In the compost contents of some macro and microelements was determined (Tab.1).

Table 1: Characteristics of the compost from municipal wastes (MSWC)

Ct	Nt	P	K	Cd	Cr	Cu	Ni	Pb	Zn
g kg^{-1}		mg kg^{-1}							
144.29	12.85	2600	5200	11.2	52.4	171	29.5	530	2425

The field experiment was conducted on Mollic Gleysol (developed from loam, pH $_{KCl}$ 6.2) as a random blocks in three replications and plots were treated as following: control (without fertilization), 30t, 60t, 120t MSWC ha^{-1}, 70 kg Nmin ha^{-1} and 30t MSWC + Nmin .
The tested plant was lettuce.

3. Results and Discussion

After harvesting the plants, contents of microelements were determined in lettuce and in the soil samples (taken from the depth 0-20 cm), which are presented in Table 2. Results of the analysis show that applying doses of MSW compost as well as N min, significantly influenced on the increasing of total forms of Cu, Pb and Zn in the soil. The effect was not visible for Cu and Pb when mixed fertilization was used (object 6).

Table 2: Content of microelements in the soil and lettuce after applying municipal waste compost (MSWC)

		Soil (0-20 cm)						Lettuce					
No	Treatment	Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cr	Cu	Ni	Pb	Zn
		mg kg^{-1}											
1	Control	0.98	15.35	33.12	20.75	41.50	99.70	0.40	1.50	5.60	1.10	4.00	33.70
2	Nmin 70 kg ha^{-1}	0.92	15.98	36.12	20.00	46.57	115.25	0.47	1.70	5.50	1.50	4.20	40.90
3	30t MSWC ha^{-1}	1.08	16.62	35.50	20.87	53.45	118.40	0.43	1.70	5.40	1.40	4.30	41.20
4	60t MSWC ha^{-1}	1.08	15.92	38.37	20.00	57.12	150.40	0.40	1.60	5.50	1.20	3.90	43.10
5	120t MSWC ha^{-1}	1.02	16.12	41.00	20.75	59.66	182.10	0.47	1.57	6.40	1.4	5.70	54.30
6	30t MSWC + Nmin ha^{-1}	0.95	15.88	34.66	20.20	43.40	118.85	0.40	1.70	6.70	1.37	5.70	46.20
	LSD α			2.23		3.83	7.22				0.19		3.58

In the lettuce samples significant increase of the content of Ni and Zn on the objects fertilized by MSW compost (excluding dose 60 t ha^{-1}) and N min, was observed.
Results of the works point, that some heavy metals (Cu, Pb) introduced with MSW compost to the soil can be present in the forms non available for plants. Because of that, despite of increasing their content in the soil, they were not overtaken by lettuce, while Ni and especially Zn after applying to the soil were accumulated in higher amount by the plant. It is also worth to mention, that Nmin fertilization significantly influenced higher uptake of Ni and Zn.

4. Conclusions

1. MSW compost added in doses 30, 60, 120 and 30 + N T/ha in the field experiment significantly influenced on increase of Zn, Pb and Cu in the soil and Ni and Zn in the plant.
2. Nitrogen fertlization influences on higher uptaking of Ni and Zn by lettuce.

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UPPER ARKANSAS RIVER ECOLOGICAL RESTORATION: BIOSOLIDS DEMONSTRATION PROJECT, LEADVILLE, CO.

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1. Introduction

In the Upper Arkansas River floodplain near Leadville, Colorado, USA, erosion of mine tailings left alluvial deposits up to and exceeding 0.7 m. These deposits contain high levels of trace metals and sulfur, leading to acidic conditions that are toxic to riparian vegetation. The bare contaminated soil is highly susceptible to continued erosion by the river.

This reach of the Arkansas River is primarily used for irrigated hay production and cattle grazing. The Lake County Soil Conservation District, local landowners, private industry and federal and state agencies are cooperating to restore a 15-km reach of this floodplain to a healthy condition. The overall objective of this project was to demonstrate the potential for using residuals as soil amendments to restore soils to a condition that can support and sustain a desirable plant community along the Upper Arkansas River. Specific objectives include: (1) to reduce riverbank erosion and channel degradation, and (2) to conduct an evaluation of the effectiveness of restoration practices.

2. Basis for Amendment

The alluvial tailings deposits on the Upper Arkansas are characterized by high concentrations of Zn, Pb, and Mn. In addition, the tailings are rich in pyrite, which oxidizes, generating sulfuric acid. The acidity, in combination with high metal concentrations, are sufficient to inhibit plant growth. Potential soil amendments must correct this acidity as well as reduce metal availability. Lime application (or application of a material with a high calcium carbonate equivalent) must be sufficient to correct existing and potential acidity. The alluvial tailings also have poor soil physical characteristics and low nutrients. Biosolids, municipal wastewater residuals, consist of organic matter, inert solids and nutrients. They can increase the nutritional status of the soil and improve soil physical characteristics. Biosolids have also been shown to reduce metal availability in soils with high metal concentrations.

3. Demonstration Project Installation

Several areas along the river, totaling 4 ha, were selected for demonstrating the potential for residuals to restore vegetation. Soil samples to depth from each site were analyzed for the lime requirement to estimate potential as well as actual acidity; an example as shown in Table 1. Preparation for amendment application included bank stabilization, some road building and fencing.

Table 1. Soil properties of one of the amended areas.

	Depth (cm)	pH	Lime Req'd (Mg ha^{-1})	Zinc	Lead
				--- mg kg^{-1} ---	
Site 1	0–15	3.8	43	2800	2200
	15-30	2.7	54	2100	5300
	30-45	2.5	184	2400	8000
Total Lime Req'd			281		

Biosolids from Denver Metro were applied at 224 dry Mg ha^{-1} during the summer of 1998. Lime was mixed with the biosolids prior to application. Amendments were tilled into the soil to a depth of 30 cm. Plots were seeded with a mixture of native seed and ryegrass in September 1998.

4. Research Plot Installation

A small plot study is being conducted to help design future operational sites. This study tests different mixtures of soil amendments. Treatments include: 1) control soil, 2) biosolids only, 3) lime only, 4) biosolids at 180 Mg ha^{-1} with lime, 5) biosolids at 90 Mg ha^{-1} with lime, 6) pelletized biosolids at 180 Mg ha^{-1} with lime, and 7) pelletized biosolids at 90 Mg ha^{-1} with lime. Changes in soil characteristics will be monitored as well as plant growth and metal uptake.

FRACTIONATION OF CD, ZN, AND PB APPLIED BY SMELTER FLUE-DUST TO A SOIL

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1. Introduction

Soil contamination in vicinities of Zn smelters has been a serious issue in several countries. In Poland, zinc smelter in Bukowno, Upper Silesia, is one of the oldest factory, and has been active since early XIX century. Environmental changes in this area are related not only to pollution due to smelter emissions. The surface ground layer is build up mainly of calcareous and dolomite rocks of the Muschelkalk period (Middle Triassic). These deposits contain unregular (nest type) admixture, mainly of Zn and Pb minerals, in carbonate and/or sulfide forms. Thus, the transformation of anthropogenic metal species can not be studied in local soils containing a relatively high levels of lithogenic metals.

2. Materials and Methods

The experiment was located far away from the pollution source, in another region of the country. The Zn-Pb smelter flue-dust was applied (at rates from 0 to 67 kg per 10 m^2) to the plough layer of a common kind of soils in Poland, Podsoluvisol, light loamy sand. Basic characteristic of the soil is: granulometric fraction of < 0.002 mm 5%, SOM 0.7 %, pH (HCl) 6.8. Total contents of trace metals were measured in air-dried soil samples, after digestion in concentrated HCl/HNO_3 acids, by AAS spectroscopy. Sequential extraction of metals were done using Tessier et al. method (*fide* Kabata-Pednias and Piotrowska 1998). Analytical errors estimated using reference material and multiplied analyses varied from 5 to 20% depending upon the metals. The soil (Table 1) was fortified with the flue-dust of the Pb-Zn smelter at four different levels of metals, the highest one was as follows (in mg kg^{-1}): Cd 100, Zn 15,000, and Pb 6,000. Flue-dust contained (in %): Cd 0.4, Zn 45, Pb 20, and dominated forms were oxides operational species (% of total content): Cd 47, Zn 90, and Pb 81.

Table 1. Total and species contents of Cd, Zn and Pb of soil used for the experiment (mg kg^{-1} air dried)

Species	Cd	Zn	Pb
Total content (measured)	0.70	60	20
EX exchangeable	0.24	4	2
CB carbonates	0.19	10	5
OX oxides (Fe, Mn)	0.28	19	11
OR organic	0.10	6	3
RS residual	0.25	14	10
All species	1.06	53	31

3. Results and Discussion

The proportion in contents of metal species in blank soil did not change during the experiment in spite of growing various crop plants: cereals, legumes, grass, and potatoes. This indicate a relatively small impact of plants on metal speciation in the soil. The addition of metals with flue-dust, however, affected pronounced changes in metal species as compared to the blank soil (Table 2). Although the predominating metal forms in flue-dust were so called "oxides" OX.

After third and fourth years of the experiment, a significant increase in "carbonates" CB form was observed. RIS values for CB spieces at the third year of the experiment varied from 400 (Cd) to 1466 (Zn), and at fourth year from 56 (Pb) to 747 (Zn). Degree of changes in species ratios was different for each metal being the highest for Zn. The greatest mobility, expressed in a relative increase of EX fraction (with one exception of Pb in the fourth year) was observed for Cd. Very small changes (within analytical errors) were noticed for the residual RS fraction.

Table 2. Ratio of increase of species contents (RIS)[a] in soil after flue-dust addition

Species	Cd		Zn		Pb	
	3Y	4Y	3Y	4Y	3Y	4Y
EX[b]	96	104	44	48	65	290
CB	400	500	1466	747	1302	56
OX	133	63	70	37	130	121
OR	60	90	27	24	114	96
RS	10	2	7	5	5	5

[a] RIS value is calculated as a ratio of metal species contents of soil at the highest rate of flue-dust application (average of four replicates) to control soil, after the third (3Y) and fourth (4Y) years of the experiment. [b] Symbols, see Table 1.

4. Conclusions

Metals, Cd, Zn and Pb, applied to soil in slightly mobile OX species were transformed mainly to more mobile CB form, and in case of Cd and Pb, also to exchangeable EX fraction. The increase of more mobile (bioavailable) fraction of metals in soil, has not affected significantly their contents of crop plants, partly due to a relatively high pH of the soil (Kabata-Pendias and Piotrowska 1998). More affective parameters that controlled metal contents of plants were total metal concentrations in soils, and growing seasons, e.g. climatic impact. Therefore, elevated contents of metals in crop plants grown in the vicinity of the smelter in Bukowno is affected mainly by atmospheric metal depositions (Piotrowska 1997). Metal contents of soil are secondary factors that influence metal status of plants in that area.

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AGRICULTURAL USE OF IRRADIATED SEWAGE SLUDGE: EFFECTS ON CLOVER AND GRASS DRY MATTER PRODUCTION

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1. Introduction

Land application of sewage sludge is practiced throughout the world and is an economical means of waste disposal. Beneficial effects can include increases in crop yield, soil organic matter, cation exchange capacity, water holding capacity and soil fertility in general (EPSTEIN *et al.* 1976). However, it can contain contaminants potentially toxic, such as heavy metals, salts, and organic pollutants (HUANG *et al.* 1974). To assess the effects in clover and grass dry matter production as a result of application of sewage sludge, a pot experiment using different rates of an irradiated sewage sludge as a fertilizer was conducted.

2. Materials and Methods

A pot experiment was carried out under glasshouse conditions at Estação Agronómica Nacional (Oeiras), using a composite soil sample (Table 1), from a calcaric cambissol, and an irradiated (6.17 kGy), anaerobically-digested sewage sludge (Table 1).

To the pots containing 1600g of soil, sludge at the rates: S1 (4.49 g pot^{-1}), S2 (8.98 g pot^{-1}), S3 (17.96 g pot^{-1}), S4 (44.90 g pot^{-1}), and S5 (71.12 g pot^{-1}), equivalent to 5, 10, 20, 50 and 80 t ha^{-1} (values based on the soil weight) was applied. Two controls were also included, one without sludge application but with the addition of a standard mineral fertilization (PK), and a second one without addition of sludge or fertilizer (zero).

Four weeks after mixing the soil with the sludge or fertilizer, half of the pots were sown with grass (*Lolium multiflorum* L.) and half with subterranean clover (*Trifolium subterraneum* L.). Five clover seeds were sown in each pot, inoculated with an indigenous *Rhizobium leguminosarum* bv. *trifolii* strain. To the pots, eight replicates of each treatment, deionized water was added, when necessary, to maintain the soil at 50-75% WHC.

The plants were cut 3 times during the growth cycle, and the total dry matter production (g/pot), from all cuts is presented in Table 2. Chemical analyses were made for the treatments PK, S2, S4 and S5. Due to the small dry weights of the shoots, data from all replicates, in each treatment, were bulked. The results (chemical analyses) presented in Table 3 are the average of all growth cycles.

3. Results and Discussion

Dry weights of clover shoots at all rates of sludge, except S1, were significantly lower than the controls. In contrast to clover, the dry weights of grass plants at all rates of sludge were significantly higher than in the controls.

For clover plants, the harmful effects of the highest rates of sludge tended to disappear as a function of growth cycles. Grass plants were less sensitive than clover to these negative effects.

Macro and micronutrient (trace elements) contents of shoots of both types of plants seemed to be adequate and toxic effects of heavy metals were not found (CHANEY *et al.* 1977; BERGMANN 1992). However, some nutritional disorder (high Fe/Mn ratio) could occur (BERGMANN 1992), but it appears to be not enough reason for the strong decrease in clover plant production. Additional studies are being done to determine the reasons for this decrease and it seems that soil electrical conductivity could be involved.

Table 1. Some characteristics of the soil and sludge used for experiments

Soil				Sludge			
pH (H_2O)	7.2	Fe (g kg^{-1})	37.6	pH (H_2O)	6.25	Fe (g kg^{-1})	106.6
O.M. (%)	1.655	K (g kg^{-1})	23.0	O.M. (%)	47.9	K (g kg^{-1})	1.01
Total N (g kg^{-1})	1.13	Mg (g kg^{-1})	6.4	Total N (g kg^{-1})	26.7	Mg (g kg^{-1})	6.6
P_2O_5 (mg kg^{-1})	384.5	Mn (mg kg^{-1})	181.0	P_2O_5 (mg kg^{-1})	81295	Mn (mg kg^{-1})	110.0
Al (g kg^{-1})	56.8	Pb (mg kg^{-1})	46.5	Al (g kg^{-1})	13.5	Pb (mg kg^{-1})	132
Cd (mg kg^{-1})	0.2	Na (g kg^{-1})	7.6	Cd (mg kg^{-1})	1.8	Na (g kg^{-1})	2
Ca (g kg^{-1})	24.1	Ni (mg kg^{-1})	21.5	Ca (g kg^{-1})	80	Ni (mg kg^{-1})	36.0
Cr (mg kg^{-1})	55.0	Zn (mg kg^{-1})	69.5	Cr (mg kg^{-1})	64.0	Zn (mg kg^{-1})	1780.0
Cu (mg kg^{-1})	15.0			Cu (mg kg^{-1})	302		

* All metals are totals recovered from HNO3+HClO4+HF digests (soil).
**All metals are totals recovered from aqua regia digests (sludge).

Table 2. Total clover and grass dry matter production (g/pot).

Treatment	clover	grass
Control	8.03 c	2.09 a
PK	9.71 d	2.20 a
S1	7.81 c	4.40 b
S2	6.41 b	6.07 c
S3	4.81 a	8.38 d
S4	4.81 a	15.50 e
S5	4.66 a	14.92 f

Values followed by the same letter in the columns are not significantly different (at the 5% level, one way anova).

Table 3. Analysis of clover and grass plants (average of all growth cycles)

	clover				grass			
	PK	S2	S4	S5	PK	S2	S4	S5
N (%)	1.96	2.38	3.25	3.70	1.18	1.52	2.32	4.04
P (%)	0.17	0.24	0.23	0.26	0.34	0.27	0.30	0.34
Fe (mg kg^{-1})	1903.52	1758.75	1704.21	1690.24	103.51	98.73	126.65	169.30
Mn (mg kg^{-1})	29.97	23.11	18.46	15.82	40.95	25.47	18.73	14.66
Zn (mg kg^{-1})	22.01	58.59	65.57	83.87	21.48	31.77	59.59	81.24
Cu (mg kg^{-1})	4.33	7.37	9.35	12.04	5.39	4.51	7.81	11.96
Pb (mg kg^{-1})	5.10	4.52	6.50	8.29	5.93	3.50	4.41	6.11
Cr (mg kg^{-1})	4.99	5.70	4.29	5.98	2.16	1.83	1.74	2.51
Cd (mg kg^{-1})	0.55	0.31	0.40	0.80	0.25	0.25	0.35	0.50
Ni (mg kg^{-1})	5.29	9.60	8.36	8.25	8.08	7.82	6.74	7.76

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AGRICULTURAL UTILISATION OF URBAN SEWAGE SLUDGE : METAL TRACE ELEMENTS IN SOILS AND PLANTS

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1. Introduction

Urban waste must be managed through a sustainable and reliable processing system which requires an expertise on the different possibilities of elimination, assessing the environmental impact and predicting control mesures. This research on the agricultural utilisation of sewage sludge will be to assess and to control the impact on to-be-preserved natural resources. An analysis of metal trace elements (MTE) in soils and plants was conducted. It took into account the agricultural soil practices so as to specify the medium and long term innocuousness of the agricultural utilisation of sewage sludge to local communities, to farmers, and to plant production organisations.

2. Materials and Methods

Experimental design (Table 1)

Six study sites were selected in Puy-de-Dôme, Auvergne (France). Each site has a control parcel (without sewage sludge) and a parcel spread with sewage sludge (1 or 2 applications). The crop management sequence of these parcels varies according to the farmer. Limed sludge was spread at the rate of 7.5 t of dry matter/ha. MTE contents in sewage sludge were within Frenh regulations (J.O. : décret n°97-1133 and arrêté 8/1/98).

Table 1 : Description of experimental design

	Maize	Wheat	Sugar Beet	Grassland
Study Sites	S1(N1, 6.3, 3.4, 0) S9(N1et2, 7.2, 1.7, 0)	S2(N1, 7.2, 3.07, 2) S4(N1, 7.6, 2.11, 30)	S5(N2, 7.5, 4, 32)	S7(N1, 6.2, 5.8, 0)

(N:number of sludge applications; pH ; organic matter rate; % total limestone)

Measured parameters

MTE contents in both the control parcels (early in the sequence) and the spread parcels (at the end of the sequence) were analysed. At the same time, MTE total contents in wheat and maize grains, sugar beet roots, and grassland aerial parts from the spread parcels were measured.

3. Results and Discussion

A-MTE contents in control parcels

MTE analysis of the control parcels provides data of the initial status of the parcels before sewage sludge spreading. Some differences were noticed.

+Soils S1,S2,S4 and S5 are best suited for field crops (wheat, maize, and sugar beet). On the whole, they have normal MTE contents (Cu:27.3ppm, Zn:87.7, Cr:67.7, Pb:45.2, Cd:0.365, Hg:0.02, Se:0.335ppm) except Ni for which contents were close to standards (41.3/50 ppm).We can notice that contents in Cr
(67.7/150ppm) and Pb (45.2/100ppm) are about half of regulation standards. There is a MTE content variability among these soils, especially in Zn (70.8-131.3ppm) and in Se (0.12-0.50ppm).

+Soil S9 has a low-agricultural capability, with a small proportion of organic matter and more extensive cultural practices. MTE contents are very small.
+Soil S7 has a volcanic geochemical deposit, frequently found in Auvergne. This soil contained in Ni and Cr concentrations higher than regulations (Cr 226 ppm/150 ppm ; Ni 130 ppm/50 ppm). The contents in other MTE are similar to those in soils with a good agricultural capability.

B-MTE contents in parcels spread with sewage sludge

Urban sewage sludge application on soils with good agricultural capabilities shows a small increase of contents in Zn (97.5/87.7 ppm), Cr (79.1/67.7 ppm) and Ni (45.9/41.3 ppm) according to the soils. A small rise of contents in Cu (37.9/31.9 ppm), Cd (0.30/0.22 ppm), Hg (0.05/<0.02 ppm) was observed in pH6 soils. Element Se has variable contents according to the soils, though the contents remain low after application (0.225/0.335 ppm). On some soils, there was a second sewage sludge application. Their MTE contents do register any difference from the first application contents.
On the low agricultural capability soils, MTE contents after sewage sludge applications remain inferior to those of good agricultural capability soils (Cu : 7.9 ppm, Zn : 43.1, Cr : 26.7, Ni : 11.2, Pb : 43.1, Cd : 0.14, Hg : <0.02, Se : 0.15 ppm).

C-MTE contents in plants

On the whole, sewage sludge application shows :
+Wheat and maize grain contents are weak in relation with literature data (Gomez, 1985 ; Kabata-Pendias et al., 1992) except Se for which quite high contents are noted in grains reaching 2 ppm although soil contents in Se are very low.
+Sugar beet roots contents in Cr, Pb, and Cd are normal according to Sauerbeck et al. (1988). However, contents in Cu and Zn are higher in our study (Cu : 2.7-3.9 ppm / 1-2 ppm ; Zn 7-9 ppm /1-2 ppm). Contents in Hg and Se seem very low, despite the lack of references.
+Aerial parts of herbaceous plants have normal Cr and Ni contents (Cr : 0.523 ppm, Ni : 2.31 ppm) according to Chaney (1990) though the soil contents are high. The high Se content in plants cannot be explained by sewage sludge applications. The differences that were noted in MTE contents (except for Se) in soils S1 and S9 do not crop up in maize grains from parcels with sewage sludge except for Ni and Pb. Thus, the level of total MTE contents in plants is not necessarily linked to the soils'level. For the maize, it would depend on the chosen element.

4. Conclusions

The control parcel study shows the variability that could exit among the soils from a same area according to the activities practised on these soils. That is why agricultural utilisation of sewage sludge must take into account the land uses (animal farming, field cropping) and the cultural practices (intensive or integrated management) as well as the geochemical deposit and the possible diffused contamination (hunting, highways, industries,...) Soil enrichment does not clearly show up after sewage sludge application as far as MTE and soils are concerned. Besides, the relation between MTE contents in soils and in plants is complex : it could depend on the chosen MTE for one given species.

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THE EFFECT OF SEWAGE SLUDGE FERTILISATION ON THE HEAVY METALS BEHAVIOUR IN SOIL-PLANT SYSTEM: A LONG TERM EXPERIENCE.

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1. Introduction

The use of sewage sludge in agriculture, is one of the possible alternatives to landfill or incineration. Sewage sludge is a potential source of nutrients for crops, but its heavy metal content is one of the main hazards to the environment (accumulation of heavy metals in soil, uptake by crops and/or migration to the water table). On long term, applications of sewage sludge might give rise, in soil, both to a change in the bonds between the metals and various organic and inorganic soil components and to an increase of heavy metal concentration.
The aims of this research were:
- to investigate the accumulation and, by means of chemical speciation, the distribution of the various Zn, Cu, Ni and Pb forms in soil and sewage sludge, after six years of sludge administration.
- to evaluate the uptake by plants through the analysis of metal content in corn (Zea mais L.) shoots.

2. Materials and Methods

Maize (*Zea mais* L), wheat (*Triticum aestivum* L.) and sugarbeet (*Beta vulgaris* L.) were cultivated in a three course rotation. Since 1988, anaerobically digested de-watered, liquid and composted sludge were applied to soil every year at two different rates.
Soil samples were collected in 1990 and 1996, once the maize harvested. Heavy metals fractionation was performed according to the Sposito et al. (1982) procedure. Soil total heavy metal contents were determined by wet digestion in a nitric-percloric acid mixture, whereas available forms of trace elements were measured by a DTPA extracting solution according to Lindsay and Norvell (1978) procedure. The plant uptake was determined by mineralization of maize tissues in a HNO_3 mixture. Metals in the extracts were determined by Inductively Coupled Plasma (ICP).

3. Results and Discussion

After six years, no significant differences among heavy metals sequentially extracted were observed except for Zn (Fig. 1), for which, both a significant increase of the potentially available fractions (Na_2-EDTA and NaOH-extractable) and a decrease of the residual form (HNO_3-extractable), were recorded for treated plots. These results confirm the hypothesis that the application of sewage sludge to soil generally causes a shift of the metals contained in the solid phase of soil from those more resistant (residual/sulfidrilic) towards those extractable with milder reagents NaOH, Na_2-EDTA (Sims & Kline, 1991)

Total Cu and Pb, did not differ from control (T) in any treatment. Ni and Zn instead showed a different behaviour. Available Cu and Pb concentrations did not differ significantly in any treatment. For Ni and Zn there was an overall increase with respect to control (T). The effect of sewage sludge on Zn e Ni accumulation in the soil appear primarily related to the doses applied

and in some cases seems to be influenced by the type of matrix used (dehydrated, liquid or composted sludge).
The plant uptake essentially occur for Cu and Zn. No significant differences in the amount of heavy metals detected in cob and in grains were observed. A significant reduction for Zn was observed in the adsorption of this metal in the stalk of the treated plots with respect to the control.

4. Conclusions

Results suggest that a six years sludge administration did not result in soil pollution. In fact, the final amount of total and available heavy metals in the soil did not exceed the legal threshold established by the Italian law for unpolluted soils. Rather, a shift towards more available forms was observed (Lake, 1984). Nevertheless, the increased availability of metals in soil has not been matched by a greater uptake in maize.

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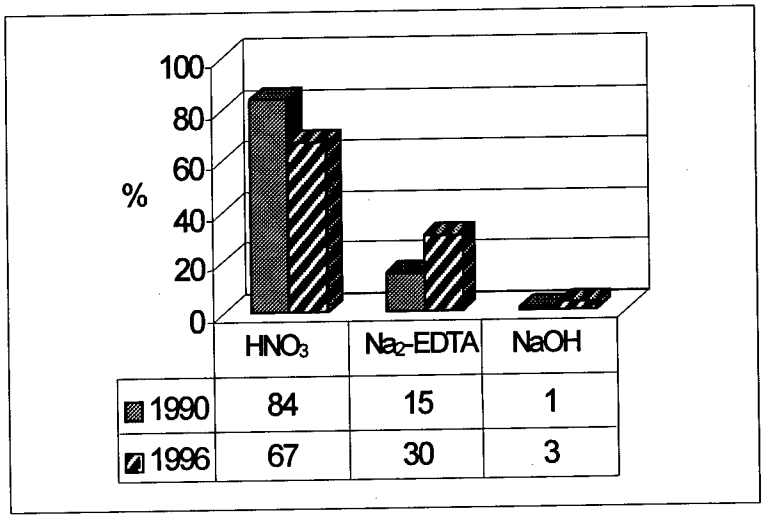

Fig. 1. Percentages of available (Na_2-EDTA-extr. and NaOH-extr.) and residual (HNO_3-extr.) Zn forms in the soil (average values of treated plots).

HEAVY METALS UPTAKE BY SUBTERRANEUM CLOVER AND THEIR EXTRACTABILITY IN SOILS TREATED WITH AN ALKALINE INDUSTRIAL RESIDUE

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1. Introduction

Previous studies on an alkaline residue derived from the acetylene industry indicated that trace elements concentrations were quite low and that after incubation of the residue with several soils, soil acidity was reduced and subterraneum clover yields increased at some residue rates (1). This work aims to evaluate the effect of the residue on the total uptake of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn by clover in a pot experiment. Soil AAAc-EDTA extractable concentrations of these elements in different soils after the residue application and plant growth, and the relationship between soil pH and crop uptake were examined too.

2. Materials and Methods

Seven topsoil samples belonging to the soil units Aric Antrosol (ATa), Dystric Cambisol (CMd), Dystric Leptosol (LPd), Gleyic Podzol (PZg), Humic Alisol (ALu), Humic Cambisol (CMu), and Umbric Fluvisol (FLu) were studied. Some soil properties are listed in Table 1. AAAc-EDTA extractable Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined using a 0.5M NH_4OAc + 0.5M HOAc + 0.02 M EDTA solution at pH 4.65 (2). Aqua regia was also used as extractant.

Table 1 Some initial properties of the soil samples

Characteristics	Soil unit						
	PZg	CMd	ATa	CMu	ALu	LPd	FLu
Texture	S	LS	SiL	SL	L	SiL	L
pH (H_2O)	4.60	4.65	4.70	4.35	4.10	4.65	4.60
O.M. (g kg^{-1})	7.37	7.76	16.8	31.6	36.2	41.9	60.5
AAAc-EDTA extractable Cd (mg kg^{-1})	nd	nd	nd	nd	0.11	nd	0.51
AAAc-EDTA extractable Cr (mg kg^{-1})	0.10	0.10	0.10	0.20	0.10	0.15	0.20
AAAc-EDTA extractable Cu (mg kg^{-1})	0.20	0.25	3.35	0.50	7.30	0.35	39.5
AAAc-EDTA extractable Fe (mg kg^{-1})	62.0	28.0	43.8	210	430	192	1050
AAAc-EDTA extractable Mn (mg kg^{-1})	0.30	3.90	54.5	0.35	25.6	11.0	44.0
AAAc-EDTA extractable Ni (mg kg^{-1})	0.10	nd	nd	0.60	0.20	0.20	1.45
AAAc-EDTA extractable Pb (mg kg^{-1})	1.00	0.60	2.60	4.50	7.70	3.70	85.5
AAAc-EDTA extractable Zn (mg kg^{-1})	0.30	0.10	0.60	0.45	2.60	0.60	12.0

S - Sand; LS - Loamy Sand; SiL - Silt Loam; SL - Sandy Loam; L - Loam; nd - not detected;

The experiment was previously described in detail (1). It was a completely randomized block design with four residue application rates for each soil (L_0, L_1, L_2, and L_3), and three replications. Rate L_0 was the "nil" rate. The rate L_2 was the amount required to bring the soil pH (H_2O) to 6.0. The rate L_1 was half L_2, and the rate L_3 was 1.5 L_2. After incubation of the soils with the residue

for a month, N, P, K, S, Mg, B, Mo, Co, Cu, and Zn were applied using a nutrient solution. Two harvests of the tops of subterraneum clover cv. Seaton Park were taken and the pH and AAAc-EDTA extractable Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analysed again in the soil samples. Plant material was analysed for total concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, by digestion with HNO_3 and $HClO_4$ acids and atomic absorption spectrophotometry.

3. Results and Discussion

The soils were quite acid (Table 1). The pH increased with the rate of residue, except in the FLu soil, in which pH values were similar at L_2 and L_3 rates. The value 6.0 was only reached in the ALu, CMu, and ATa soils at the rate L_3. Initial AAAc-EDTA extractable Cu and Zn in PZg, CMd, CMu, and LPd soils were lower than the critical limits of deficiency (3). AAAc-EDTA extractable Zn was also low for plant growth in the ATa soil. On the contrary, AAAc-EDTA extractable Cu was high in the FLu soil (3). Only the FLu soil presented aqua regia extractable Cu, Pb, and Zn concentrations exceeding the limit values of the portuguese legislation. Comparisons between total uptake by clover of the various heavy metals at the L_0 residue rate and the other residue application rates ($P \leq 0.05$) indicated that there was not an uniform trend for the studied soils. Total Cd uptake slightly increased in the ATa soil at the L_1 and L_2 rates while in the ALu soil it decreased at the higher L_2 and L_3 rates. Total Cr uptake increased at the L_3 rate in CMd soil. Total Cu uptake increased at L_1, L_2, and L_3 rates in ATa and LPd soils. Total Fe uptake also increased at the L_3 rate in CMd soil and at the L_1 rate in the ATa soil. Total Mn uptake decreased in most of the soils, excepting the PZg and CMu soils. The highest decrease was observed in the ALu soil. Total Ni uptake decreased in the CMd and CMu soils, and total Pb uptake increased in the ATa soil. Sharp decreases in total Zn uptake were observed in the CMd and ALu soils. For each heavy metal, total uptake by clover did not differ in the other soils. Significant and positive simple correlations were found between soil final pH and total Ni uptake and total Pb uptake by clover, whereas significant and negative correlations were also observed between soil final pH and total Mn uptake, and total Zn uptake. After harvesting, soil AAAc-EDTA extractable heavy metals concentrations result from heavy metals initial contents, their fate in soil during plant growth, related to soil factors as the pH, and plant uptake. As expected, for the elements Cu and Zn, which had been included in the basal dressing, soil AAAc-EDTA extractable concentrations increased at the "nil" residue rate when compared to the initial ones. For most of the soils, AAAc-EDTA extractable Cu, Fe, Mn, and Zn tended to decrease with the residue application. Regarding the other elements, the concentrations varied among soils.

4. Conclusions

Total uptake of each heavy metal by clover clearly differed among soils with the residue application, and concomitant change in soil pH. Also, the soil AAAc-EDTA extractable heavy metals concentrations obtained at the different soil pH values did not show uniform trends for the soils under study. In the FLu soil, with relatively high amounts of extractable Cu, Pb, and Zn, according to the portuguese legislation, the decreases in AAAc-EDTA extractable Pb and Zn can be particularly beneficial, but extractable Cu was not reduced by the residue application.

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MANGANESE ELEVATION IN LANDFILL COVER SOILS: PRELIMINARY INVESTIGATION

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1. Introduction

The revegetation of landfills throughout the world has met with many difficulties, due to the harsh environmental conditions commonly found (Chan *et al* 1996; Ettala *et al* 1988; Moffat & Houston, 1991). One of the causes of revegetation failures is poor soil quality and structure, however, even with the use of good quality cover soils, landfill gas and leachate infiltration can detrimentally change the soil conditions (Dobson & Moffat, 1994). In this investigation significant changes in extractable manganese (Mn) concentrations raised questions about the interactions between cover soils and the underlying waste.

2. Materials and Methods

The investigation was conducted on an operational municipal landfill in Durban, South Africa. On a temporarily complete section of the landfill two 25m x 25m plots were established. One was left with the original 0.5m waste cover soil and one received an extra 1m of good quality topsoil. A control plot situated off of the landfill also received 1m of topsoil from the same stockpile. The plots were planted with 70 indigenous trees of 10 species. After a period of 8 months extractable Mn, Zn, P, K, Ca, Mg (with ammonium bicarbonate), soil moisture, stone content, pH, carbon content, clay content, extractable acidity, conductivity, soil atmosphere methane, carbon dioxide, oxygen, and the number of tree surviving were measured.

3. Results and Discussion

The variables which had a significant ($p<0.01$) difference between the topsoil on the control plot and topsoil on the landfill are shown in Table 1. Considering the topsoil came from one well-mixed stockpile, the results suggest that the underlying landfill influenced these specific soil variables. The infiltration of landfill gases, from anaerobic decomposition of underlying waste, can explain the significant changes in the topsoil gas concentrations. These are common changes and are probably the main cause of poor vegetation growth on landfills (Dobson and Moffat, 1994) therefore, the higher tree mortality on the landfill in this investigation can be expected (Table 1). Lower soil moisture levels are explained by the poor structure of underlying waste and cover material, thus, reducing upward migration of moisture (Dobson and Moffat, 1994). However, the six fold higher Mn levels in the topsoil on the landfill has no clear explanation. Leachate contamination of landfill soils significantly increases soil total and extractable Mn concentrations (Winnant *et al* 1981). However, in this investigation drainage lines were installed to prevent leachate causing surface contamination of the experimental plots and there was no visual evidence of leachate contamination. The upward migration, by capillary action, of moisture, carrying Mn in solution, was also unlikely due to the high compaction and poor soil structure of the underlying waste and cover material. It may be possible that the upward migration of warm landfill gas carried a Mn condensate which was deposited in the topsoil layer as the gas cooled towards the soil surface. A further explanation could be a change in the proportion of extractable Mn due to biogas-induced shifts in redox potential.

Table 1: Selected variables in soils from three experimental plots (control topsoil; landfill topsoil and landfill waste cover soil.

Parameter	Control Plot	Std. Error	Topsoil Plot	Std. Error	Landfill cover material	Std. Error
Tree mortality (%)	0	---	15	---	32	---
Mn (mg/kg)	4.76a [1]	0.32	31.02b	3.1	22.49b	2.6
% moisture (by weight)	11.80a	0.43	10.32b	0.26	8.70c	0.23
Methane (%)	0a	0	22.3b	1.3	41.9c	0.4
Carbon dioxide (%)	3.9a	0.2	25.6b	0.7	48.4c	0.4
Oxygen (%)	16.0a	0.3	3:2b	0.3	0.6b	0.07

[1] a,b,c: denotes significantly different means with a Sheffe multiple range test ($p<0.01$)

4. Conclusions

If the carrying of condensate in landfill gas was responsible for the six fold increase in Mn levels in the topsoil layer over a 8 month period, it would be indicative of an important waste / topsoil interaction. It is unlikely that Mn is the only chemical transported in this condensate, therefore this interaction may have an important role in the poor establishment of vegetation on landfills and demands further investigation.

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FORMATION AND DISSOLUTION OF LEAD PHOSPHATE IN HOUSEHOLD DUST

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1. Introduction

Direct ingestion of lead (Pb) contaminated house dust, garden soil and road dust is the primary health risk posed by these materials to children. Ever since Nriagu (1974) suggested that Pb in waste waters could be removed by precipitation with phosphate (P) as pyromorphite, particularly chloropyromorphite ($Pb_5(PO_4)_3Cl$), many authors have demonstrated the *in situ* immobilisation of Pb species in soils by the addition of P (Ma et al. 1993). This study presents the results of the *in situ* immobilisation of Pb in household dust using KH_2PO_4 and assesses techniques to measure Pb solubility in P-treated dusts.

2. Materials and Methods

The household dust was sampled by vacuum filtration from a private residence in Port Pirie, South Australia, the site of a primary Pb smelter. The ability of P to immobilise Pb in the dust was established by reacting 0.2 g dust (<53 μm) with 1 mL of 0, 1, 10, 50, 150, 300, 500, 1000 and 1500 mM P using reagent grade KH_2PO_4 for 0.5, 1, 2, 4, 6, 24 and 48 h at 20°C.

The degree of Pb bioavailability was assessed by extraction of the P-reacted dust with 0.05 M HCl (pH 1.4) at a dust:solution ratio of 1:100 for 2 h, which approximates the human stomach pH and residence time of a fasting child (Brown and Chaney 1997). A dust:solution ratio of 1:500 was also used to simulate dust ingestion less than maximal. Solubility of Pb was also assessed by extraction of the P-reacted dust with a dilute salt solution viz. 0.01 M NH_4NO_3 (pH 5.5) at the above ratios. All extracts had pH determined, were filtered (<0.45 μm) and acidified (pH 1) prior to the determination of Pb using either inductively-coupled plasma atomic emission or furnace atomic absorption spectroscopy.

Dust was also extracted with 500 and 1500 mM P solutions adjusted to pH 1 to 8 using HCl at a dust:solution ratio of 1:100.

3. Results and Discussion

Results for Pb solubility in the NH_4NO_3 extract at both dust:solution ratios suggested Pb hydroxy- or chloropyromorphite species had formed and reduced readily-soluble Pb concentrations in the dusts as P concentrations increased (Fig. 1). Lead immobilisation was found to be independent of reaction time (data not shown).

Concentration of Pb extracted with HCl at a dust:solution ratio of 1:100 also declined as a function of P concentration, again suggesting formation of Pb phosphate phases (Fig. 2). However, reduced Pb solubility in these treatments was also due to the differences in extractant pH (Fig. 2). This variable pH in the 1:100 extractions can be attributed to the buffering capacity of the dust (carbonate content = 11.2%) and the increasing P concentration buffering the solution pH.

Figure 1. Extraction of dust Pb using NH_4NO_3 after reaction for 2 hours with increasing concentrations of P

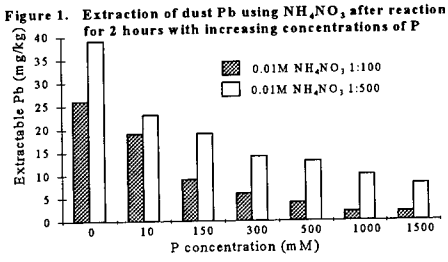


Figure 2. Extraction of dust Pb using HCl after reaction for 2 hours with increasing concentrations of P

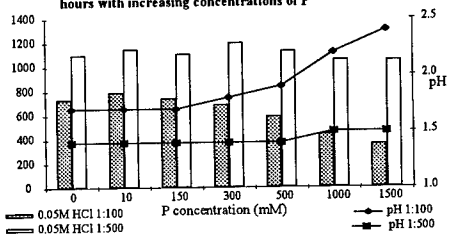
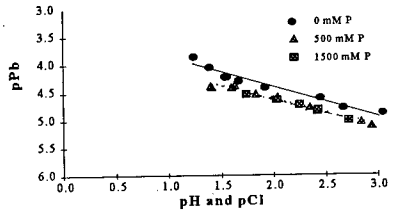


Figure 3. Solubility of dust Pb in relation to P treatment and solution pH and Cl concentrations



In the 1:500 extractions these buffering effects were overcome by the wide dust:solution ratio. This indicates that the *in vitro* determination of Pb bioavailability is extremely sensitive to the buffering capacity of the soil, and added P. Immobilisation of Pb by P may be erroneously quantified if extractant pH is not carefully controlled.

Examination of Pb solubility in dust (Fig. 3) and pure chloropyromorphite (data not shown) in relation to P addition in the pH and pCl concentration range relevant to gastric conditions, suggests that insoluble intrinsic dust Pb and Pb phases formed *ex vivo* are more easily dissolved in acidic conditions than pure

CPM and are therefore likely to be dissolved *in vivo* in the gut. Formation of Pb phosphate phases under alkaline conditions simulating the small intestine should be the focus of further studies.

4. Conclusions

Soluble Pb in dusts decreased with increasing P levels suggesting the formation of Pb phosphate phases. Determination of Pb bioavailability is sensitive to the buffering capacity of the soil and added P, and therefore may be erroneously quantified if extractant pH is not controlled. To our knowledge, no studies have investigated the dissolution of pyromorphite in the gut but our studies would suggest that lead phosphates formed *ex vivo* are likely to be substantially dissolved *in vivo* in the gut.

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Microbial and Enzymatic Interactions

(Technical Session 9)

FIELD-MEASURED OXIDATION RATES OF BIOLOGICALLY REDUCED SELENIUM IN SLUDGE

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1. Introduction

Sludge generated during surface-water transport or biological treatment of selenium-laden agricultural drainage water contains high concentrations (20-100 mg/kg) of selenium. These sludges contain reduced forms of selenium [Se(0) and organically associated forms] that are nearly insoluble under anoxic conditions. When these sludges are removed from their anoxic subaqueous depositional environment and placed in an oxidizing environment, re-oxidation of is expected to occur. Oxidized forms of selenium Se(IV) and Se(VI) are relatively soluble and mobile, and at high enough concentrations are toxic to wildlife and humans. Leaching and biological uptake of selenium reoxidized from these sludges may lead to unacceptable environmental impacts. Therefore, finding safe and economical sludge disposal methods requires an understanding of the rate of re-oxidation of selenium and subsequent transport into groundwater and biota.

Laboratory experiments have shown that reduced forms of selenium can be re-oxidized in oxic soils and bacterial cultures with rate constants on the order of 10^{-2} to 10^{-4} day^{-1} (Zawislanski and Zaverin, 1996; Dowdle and Oremland, 1998). Field-measured selenium re-oxidation rates in Kesterson Reservoir soils, California ,are at the low end of this range (Benson et al, 1992; Wahl and Benson, 1996).

2. Materials and Methods

The sludge used for this experiment was obtained from the San Luis Drain. It is dominated by mineral matter but contains from 1 to 5% organic carbon. Samples of sludge have been observed to accumulate over 100 mg/kg (dry-weight) of selenium. Speciation studies of these sludges using both synchrotron X-ray spectroscopic methods and sequential extraction techniques indicate that selenium is in reduced forms, primarily as Se(0) and organically associated Se. Reduced forms of selenium are believed to be concentrated in these sludges by a combination of dissimilatory bacterial reduction of selenium in the anoxic sludges and deposition of decaying biomass (e.g. algae and rooted aquatic vegetation). The relative contribution of each of these processes is unknown but it is likely that the contribution from decaying biomass is comparatively large.

Three treatments for sludge disposal were compared and experiments have resulted in data on changes in selenium concentrations and speciation spanning an 8-year period (1990-1998). The experiments were conducted at Kesterson Reservoir, California. Treatments included application to upland soils with tillage to depths of 30 cm (Test Plot 1), 15 cm (Test Plot 2), and direct application with no tillage (Test Plot 3). Sludge contained 59.3 ± 15.8 (n=5) mg/kg total selenium. The average water-extractable concentration (5:1 water to soil extract) was 3.4 ± 2.5 (n=5) mg/kg, approximately 5% of the total selenium. Soil cores and groundwater monitoring were used to track changes in selenium speciation and transport of re-oxidized forms of selenium over an 8-year period.

3. Results and Discussion

Soil monitoring data comparing the concentration of selenium in 1990 to that measured in 1998 are shown in Figures 1 and 2 for total and water extractable selenium [Se(VI) & Se(IV)] from Test Plot 2. Similar data were obtained from the other test plots. No significant decreases in total selenium were observed over the 8-year period in any of the test plots. Significant decreases averaging 62% (±10% n=3) in the water extractable selenium concentration are observed in all test plots in the top 15 cm interval. Water extractable selenium concentrations increased (4 times) at 45 to 60 cm in in Test Plot 1 and decreased (0.5 times) in Test Plot 2 deeper in the profile (15 to 45 cm). No significant changes were observed in Test Plot 3. Water extractable selenium concentrations are dominated by Se(VI) (81% ±12% n=12). No significant changes in the ratio Se(IV)/Se(VI) were observed over the 8-year period.

These data demonstrate that re-oxidation of selenium from these sludges is very slow and, most likely, lower than the lowest value reported in the above studies of 10^{-4} day^{-1}. Three lines of evidence support this conclusion. First, changes in total selenium concentrations in the treatment interval are not significant. While spatial variability will confound detection of small temporal changes, even with the observed spatial variability, declines associated with an oxidation rate of 10^{-4} day^{-1} should have been significant over the eight-year period (e.g., 25% decline would be expected). Second, water-extractable selenium concentrations declined in the treatment interval. If oxidation occurred at rates of greater than 10^{-4} day^{-1}, higher concentrations of Se(VI) and perhaps Se(IV) would be observed in the treatment interval (e.g., 5% of the total, rather than the 1 to 3% observed). Finally, large increases in the water extractable inventory of selenium would be expected deeper in the profile if oxidation rates were higher than 10^{-4} day^{-1}. Re-oxidation, followed by leaching due to winter rains (300 mm/year average) would be expected to lead to increases in water extractable selenium deeper in the profile, as observed by Tokunaga et al (1991).

4. Conclusions

The rates of selenium re-oxidation observed in this experiment are slow (<10^{-4} day^{-1}) and appear to be slower than those measured in the laboratory and field studies cited above. Additional mechanistic studies that include the effect of depositional processes (assimilative vs. dissimilative reduction), presence and nature of soil organic matter, allotropes of Se(0) and mineral composition are needed to increase our understanding of the biogeochemical cycling of selenium.

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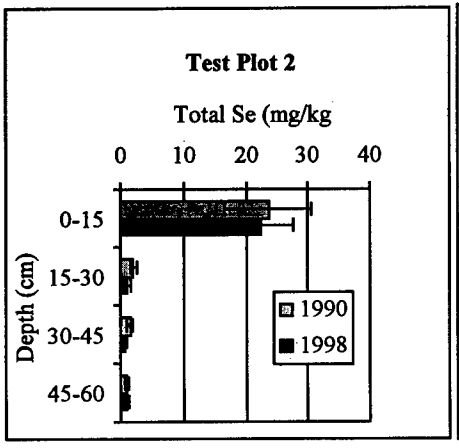

Figure 1. Total selenium (±1 std n=3).

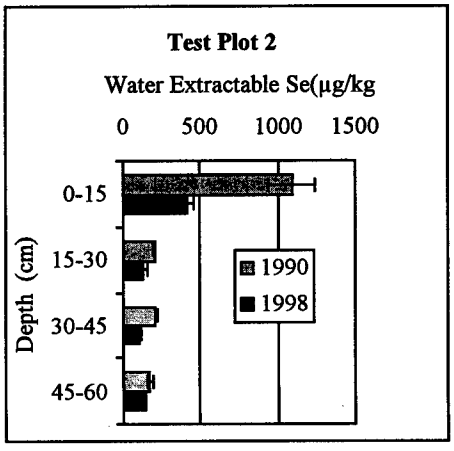

Figure 2. Extractable selenium (±1 std n=3).

PYROGALLOL INHIBITION OF AL_{13} TRIDECAMER FORMATION AND THE SYNTHESIS OF HUMIC SUBSTANCES

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1. Introduction

The Al_{13} polycation is the dominant hydrolyzed species formed from the hydrolysis of Al in aqueous systems. The existence of this Al species with the idealized structure of $[AlO_4Al_{12}\cdot(OH)_{(24+n)}H_2O_{(12-n)}]^{(7-n)+}$ was confirmed by ^{27}Al and ^{17}O NMR spectroscopy (Akitt, 1989). The Al_{13} ion was reported to be 10-fold more toxic to plants than the hexaquo Al ion (Parker et al., 1989) indicating the importance of the study on the formation and transformation of Al_{13} polycation. Direct ^{27}Al NMR evidence of the existence of the Al_{13} polycation in an acid forest soil was recently obtained (Hunter and Ross, 1991). Subsequent investigations at the same site failed to prove the existence of Al_{13} polycation, although it is possible that the presence of Al_{13} polycation is masked by other paramagnetic metals (e.g., Fe, Mn) in these soils (Bertsch and Parker, 1996). Polyphenols are common in the decomposition products of plant and animal materials and microbial metabolites in soils (Wang et al., 1986). The objective of this study was to investigate the influence of pyrogallol on the perturbation of the formation of Al_{13} tridecamer species and the concurrent abiotic formation of humic substances.

2. Materials and Methods

Solutions with OH/Al molar ratio of 2.2 were prepared by titrating 100 ml of 0.1 M $AlCl_3$ solution to pH 4.53 with 0.1 M NaOH and the solution was diluted to 500 ml. The final concentration of Al in the solution was 0.02 M. Hydroxy-Al solutions titrated to pH 4.53, at pyrogallol/Al molar ratios (R) of 0.01, 0.05, 0.10 and 0.50 were also prepared as described above. Fifty milliliters of 0.5 M sodium sulfate was mixed with 200 ml of the OH-Al solution formed both in absence and presence of pyrogallol, pre-aged for 5 days, to make the final concentration of 0.016 M Al and 0.1 M sulfate. The precipitate formed from the solution aged for 12 days was collected by ultrafiltration (0.01 μm pore size) and then examined by ^{27}Al CPMAS NMR and ESR spectroscopies, X-ray diffraction, and scanning electron microscopy.

3. Results and Discussion

The ^{27}Al NMR spectrum of the sulfate precipitates formed in the absence of pyrogallol (R=0) shows only one strong resonance peak at 62.5 ppm (Fig.1a) indicating that the precipitates were dominant in Al_{13} tridecamer. The presence of pyrogallol perturbed the formation of Al_{13} tridecamer species as indicated by the decease in the height of the resonance peak at 62.5 ppm and the presence of a broad peak around 6.8-8.2 ppm (octahedral Al-pyrogallol complex) in the ^{27}Al NMR spectra (Fig. 1). The crystallization of the precipitated Al sulfates was also perturbed by pyrogallol, leading to the formation of X-ray noncrystalline products. The morphology of the precipitates was also modified from the tetrahedral particles (R=0) to flat-like particles (R=0.01) to irregularly shaped microparticles (R=0.50).

The ESR spectrum of the precipitates formed at R=0.50 shows a single symmetrical line devoid of any fine splitting, which indicates the presence of free radicals. The free radicals have a g-value of 2.0031 with the line width of 7.2 G, indicative of semiquinones, the major free radicals normally observed in the humic acid fraction of soil organic matter (Schnitzer, 1978). Further confirmation of the formation of humic substances was obtained from the ^{13}C CPMAS NMR spectrum of the samples which shows aliphatic C signals containing OH groups at 61 and 74 ppm, strong resonances at 106, 120, 138 and 149 ppm indicative of aromatic C, and that of phenolic C at 164 ppm, and that of carboxyl C at 173 and 193 ppm (Fig. 2). The high aliphaticity

of natural humic substances (Hatcher et al., 1981) may be partially accounted for by the abiotic formation of humic substances from polyphenols such as pyrogallol catalyzed by Al species.

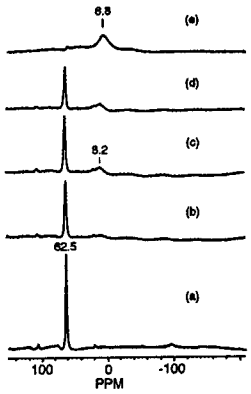

Fig. 1. ^{27}Al CPMAS NMR spectra of the precipitates after 12- day aging, from 5-day aged OH-Al solutions formed at pH 4.53 and different pyrogallol / Al molar ratios (R). (a) Control at R = 0; (b) Sample at R = 0.01; (c) Sample at R = 0.05; (d) Sample at R = 0.10; and (e) Sample at R = 0.50.

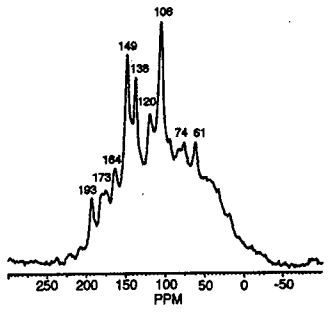

Fig. 2. ^{13}C CPMAS NMR spectra of the precipitates after 12-day aging, from 5-day aged OH-Al solutions formed at pH 4.53 at pyrogallol/Al molr ratio (R) = 0.05.

4. Conclusions

The present report highlights the perturbation of pyrogallol on the formation of Al_{13} tridecamer species and the concurrent abiotic formation of humic substances. The results have a significant implication in the failure to detect Al_{13} tridecamer species and in the abiotic formation of humic substances in the terrestrial environment.

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BIOLOGICAL ACTIVITY OF SOILS IN METAL CONTAMINATED ECOSYSTEMS

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1. Introduction

Concerns about the influence of heavy metals on soil quality has prompted work assessing influence of metal contaminants on biological function in soil. Various bioassays have been used in assessing the impact of metals on soil biology with potential use as tools in screening this aspect of soil quality. An expected outcome would be development of general guidelines for assessing status of metal toxicity in soils and thereby permit assessment of the changes in soil quality over regions containing diverse soils. This work explores use of current applications in assessing metal toxicity in soil.

2. Materials and Methods

Soils were sampled from a long-term field experiment in Poland containing plots treated with different amounts of zinc smelter fly ash. Analysis of these soils permitted determination of the influence of heavy metals on biological activity in relatively uniform soil containing a single source of contaminating metals (principally Zn, Pb, and Cd). To determine whether results obtained from this field experiment can be extrapolated to assessments in natural ecosystems containing diverse soils with varied source and amount of contaminant metal, soil samples were also collected from the Silesia region of Poland. This highly industrialized region contains soils with large variation in metal content (e.g., 38 - 4500 mg Zn kg^{-1} soil) as well as other soil properties such as pH (4.0 - 7.6) and organic C (0.6 - 6.4 %).

Various biological activities (respiration, biomass, nitrification, urease, phosphatases, and arylsulfatase) were determined using standard protocols for assay. The soil samples were also analyzed for extractable (DTPA and $CaCl_2$) and total amounts of Zn, Pb, and Cd.

3. Results and Discussion

Results obtained from the field experiment showed strong relationships between $CaCl_2$ and DTPA extractable metals in soil and near-linear relationships between extractable Zn content and inhibition of the assayed biological activities in soil. These results demonstrated that with largely homogeneous soil and a single source of contaminating metals, relationships between Zn content and inhibition can be very strong and generally consistent. In contrast, assessment of the diverse soils collected within the Silesia region showed no apparent relationship between the indigenous extractable metals and biological activity. Multivariate analysis of these data using relevant soil properties could not detect metal content as being a statistically significant parameter in estimation of biological activities, despite the very wide range in Zn content of these soils. Addition of Zn salt to a set of diverse soils caused inhibition of biological activity to varying degree but the extent of inhibition showed very little relationship to amounts of extractable Zn in the amended soils. Normalized parameters such as specific respiration also showed little ability to detect the influence of Zn on biological activity in these diverse soils.

4. Conclusions

This study shows a sharp contrast between the results which can be obtained in controlled field experiments involving relatively homogeneous soils and results obtained in a survey of the diverse soils found in real-world ecosystems. The consistent relationships which were observed in controlled experiments had little apparent reflection on results obtained with the varied soils of natural systems. This study indicates the difficulty in developing a generalized basis for assessing metal toxicity in soil.

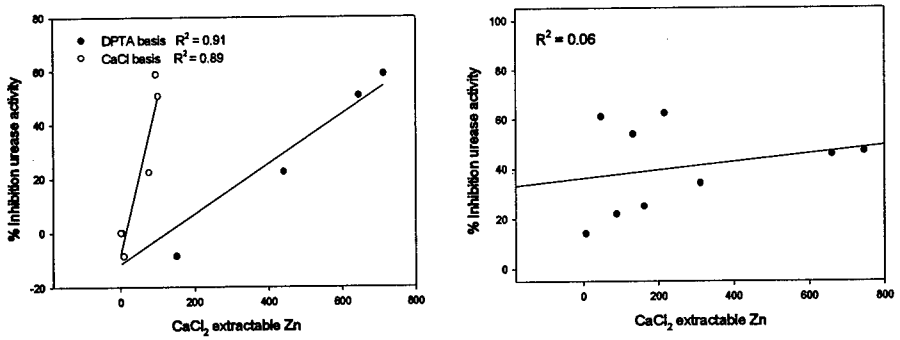

Fig. 1 Comparison of relationships between inhibition of urease activity and extractable Zn in the field experiment soils and in 9 diverse soils treated with Zn.

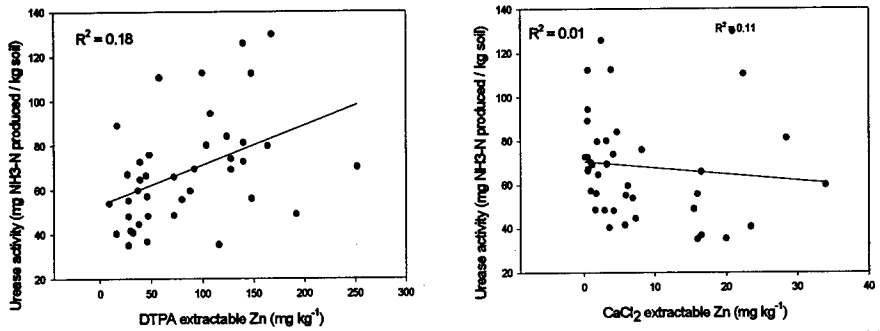

Fig. 2 Relationships between urease activity and extractable Zn in 40 diverse soils collected within the Silesia region.

ROLE OF FERRI-REDUCING BACTERIA IN TRACE ELEMENT MOBILITY IN SOIL AND RIVER SEDIMENT

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1. Introduction

Ferric oxihydroxides are present in most soils and sediments and are very often associated with trace elements. Otherwise, some cations other than Al or trace elements such as Ni, Cr, Co, Pb, may be adsorbed or substituted in iron oxides and their behaviour can be linked with iron. The iron oxides are generally stable but can be solubilised by biological and chemical processes like reduction.

Several studies show that reduction processes of ferric oxides influence metal mobility and availability. A major influence of bacterial reduction in both waterlogged or temporary waterlogged environments can be suspected. Bacterial reduction involves anaerobic bacteria which use organic matter as an energy source and ferric iron as a direct or indirect electron acceptor (Berthelin, 1982, 1988). So the iron cycle is partially linked with the carbon one and has a drastic influence on the solubility and mobility of trace elements.

The aims of this study are i) to determine the role of ferri-reducing bacteria in iron and associated metal solubilisation, ii) to relate the ferri-reducing activity to the organic matter biodegradation.

2. Materials and Methods

Two types of material were studied (Table 1) : a surface soil sample of a Gerric(?) Ferralsol from the Ouenarou station (South of New Caledonia, Becquer et al, 1995) and a river sediment from Lorraine (France). The soil sample is originated from an oxisol in an alluvio-colluvial position with temporary waterlogged phases. It is very rich in iron (up to 50 % Fe_2O_3), with high contents of Ni and other transition metals like Cr, Co, Mn... and about 5 % of organic matter. The river sediment was collected down-river from a petrochemical complex. This sediment is rich in natural and anthropogenic organic matter and contains high levels of trace elements like Zn, Pb, Cr, Cu, Cd.

Both samples were characterised by elemental mineral analysis after diacid digestion. The organic content (C, N, FA, HA and humin) was determined. Total, cellulolytic and ferri-reducing microflora were enumerated using specific media and agar plates methods (Bromfield, 1954 ; Alef and Nannipieri, 1995).

Batch experiments were performed as reactor studies to determine the rates of carbon mineralisation, ferric oxides dissolution and the concomittant metal release in solution. The CO_2 evolved from organic matter decomposition was determined using an IR Gas analyser and several parameters like pH, Eh, dissolved organic carbon and metals in solution were followed .

Table 1.- Characteristics of Ouenarou soil sample and river sediment

	orgC %	orgC/N	Fe_2O_3 %	Ni mg/kg	Cr mg/kg	Co mg/kg	Zn mg/kg	Pb mg/kg	Cd mg/kg	Cu mg/kg
Ouenarou	2.60	21.7	56.8	7750	10850	720	580	156	81	47
Sediment	4.97	27.6	3.3	21	30	7	525	100	6	74

3. Results and Discussion

The organic matter decomposition rates for both samples are relatively high. The mineralisation is greater under aerobic conditions than anaerobic one. The supply of an easily-biodegradable organic matter (cellulose) stimulates the biodegradation activity mainly for the river sediment. This stimulation does not appear as well for the oxisol sample although cellulolytic microflora is present too.

During experiments, a lower Eh is observed in biotic anaerobic conditions for both samples combined with Fe^{2+} solubilisation. Comparison with abiotic experiments shows that this reduction of Fe(III) to Fe(II) is mainly due to bacterial activity. Metal mobilisation is observed but some precipitation and coprecipitation phenomenons occured. The metal mobilisation is greater in conditions stimulated by cellulose addition. The data for Fe(III) to Fe(II) reduction along with trace element concentrations are illustrated as a function of incubation time, redox status and in fact bacterial activity.

Such mobilisation of trace elements in soils and river sediments is of major importance with regard to their bioavailability to fauna and flora, their transfer in river and soils, the risks of toxicity, the potential for leaching down the soil profile to the groundwater and their removal from polluted sites.

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MECHANISMS OF MEMBRANE PERMEATION BY TRACE METALS

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1. Introduction

Very little is known about the mechanism by which trace metals enter plant cells. It is generally presumed that there are specific transporters for each metal, or at least a reasonable number of transporters with affinities for various groups of metals. However, trace metals often exist in solution as a number of different species with different chemical properties (e.g. charge, hydrated radius, hydrophobicity) and it is therefore unsatisfactory to consider that there will be only one mechanism for membrane transport for each metal. We have been investigating the mechanism of membrane permeation of trace metals and we present here data from two series of experiments which have been designed to answer the question - Do metals complexed by inorganic or organic ligands cross biological membranes?

2. Materials and Methods

One of the main problems in measuring uni-directional fluxes of trace metals in plant cells is to distinguish between the rapid and extensive binding of metals in plant cell walls and actual uptake across the membrane into the cell. There have been considerable doubts expressed over the effectiveness of desorption protocols used in radiotracer studies to remove extracellular bound metals following the uptake periods. To avoid this problem we have adopted a giant freshwater algal system in which it is possible to distinguish easily between cell wall binding and uptake across membranes. *Charophyte* internodal cells are sufficiently large that the cell contents can be simply and rapidly washed out of the cell wall (for details see Reid and Smith 1992). Influx measurements were conducted using ^{109}Cd over 4 h in a simple solution buffered with MES to pH 6, followed by desorption for 30 min in 5 mM $CaCl_2$ to remove most of the extracellular metal. The cell contents were then separated from the cell wall in order to measure membrane influx.

In the organic ligand studies, the activity of Zn was kept constant at 1 μM by varying the total Zn concentration and nitrilotriacetic acid (NTA), hydroxyethyl-imino-diacetate (HEIDA) and ethylenediamine-N,N,N',N'-tetraacetate (EDTA) concentration varied (0-30 μM) according to calculations made with GEOCHEM-PC. Short term influx measurements were performed using *Chara* cells while longer term (48 h) uptake measurements were made on mung bean seedlings.

3. Results and Discussion

In an experiment in which relative concentrations of Cd^{2+}, $CdCl^{+}$ and $CdCl_2$ were varied by mixing the proportions of 50 mM NaCl and 50 mM $NaNO_3$ solutions containing 50 nM Cd, influx decreased as the NaCl concentration increased (i.e. as the concentration of the chloro-complexes increased) (Fig. 1) but less than would have been predicted if Cd influx was a linear function of the free Cd^{2+} ion activity. Influx was only well correlated with the concentration of Cd^{2+} in solution (Fig. 2). We conclude from this experiment that the main species transported is Cd^{2+} but we cannot rule out the possibility that one or more of the chloro-complexes has significant, but lower, membrane permeability.

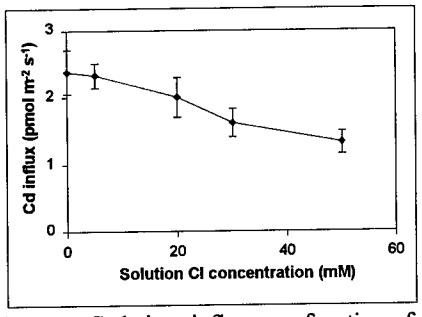

Figure 1. Cadmium influx as a function of the Cl^- concentration.

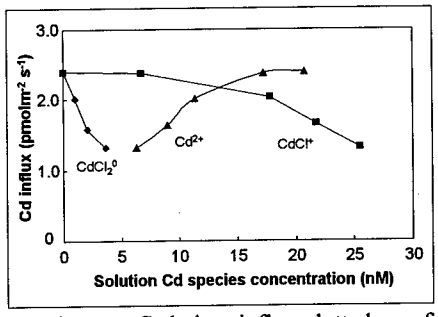

Figure 2. Cadmium influx plotted as a function of the concentration of ionic species in solution

In the second series of experiments, the effect of chelates on the uptake of Zn was studied. Influx of Zn into *Chara* was strongly enhanced by 30 µM NTA and HEIDA but was inhibited by EDTA (Fig. 3). The effect of NTA on the uptake of Zn was also measured in mung beans. Uptake of Zn over 48 h was significantly higher in solutions containing 10 and 30 µM NTA (Fig. 4), despite Zn^{2+} activity being constant across NTA treatments.

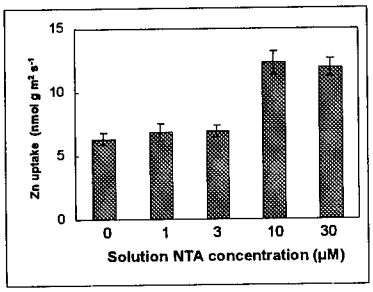

Figure 3. Effect of chelates on influx of Zn in *Chara* (Zn^{2+} constant)

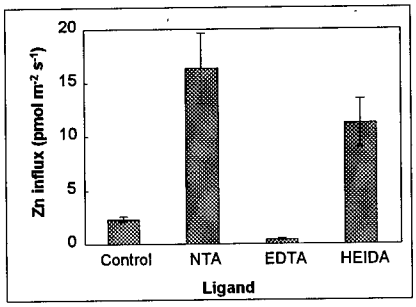

Figure 4. Effect of NTA on Zn uptake in 12 day old mung bean seedlings (Zn^{2+} constant)

4.Conclusions

We conclude from these latter experiments that metal-chelate complexes are able to enter plant cells but that the membrane permeability is strongly influenced by the physical properties of the chelate, most probably the overall charge on the metal-chelate complex.

5.References

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MICROBIAL COMMUNITY DIVERSITY AND RESILIENCE IN RELATION TO HEAVY METAL CONTAMINATION OF SOILS

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1. Introduction

Recently, there has been concern over the impact of heavy metals that may cause lowered microbial diversity and impair critical soil biological functions. Various parameters have been suggested as indicators of ecosystem damage from heavy metals (Brookes, 1995), including measurements of specific respiration rates, nitrogen fixation (Brookes et al., 1986), and shifts in microbial community composition to metal tolerant species (van Beelen and Doelman, 1997). One problem has been that almost all biological parameters are subject to large spatial and temporal variation as a result of normal disturbances that occur in the field. Consequently, specific biological processes can only considered to be impaired when a long recovery time is required for the process to resume following a disturbance.

This research was conducted to examine the impact of metal contamination on microbial community diversity and the relationship between microbial diversity and functional resilience. Experiments were conducted with intact soil cores taken from field plots that had received sewage sludge amendments for the past 20 years, and which differed significantly in heavy metal content.. To measure resilience, half of the cores from each plot were subjected to an artificial disturbance caused by heat at 55 C, and were then monitored and sampled over a 3-month recovery period.

2. Materials and Methods

Intact soil cores were collected from field plots located in Fulton County, Illinois that had a 20 year history of sludge application. The soil was originally a mine tailing that had been amended with sludge, and which is now being used for corn production. Soil cores were taken from 4 plots based on differences in acid extractable metal contents, particularly cadmium (1, 5, 15, and 52 ppm Cd). Half of the soil cores were subjected to a disturbance caused by partial heat sterilization at 55C for 24 h. The cores were then placed onto a manifold to monitor respiration rates during recovery. Every 10 days, replicate cores were destructively sampled for analysis of microbial community structure by fatty acid methyl ester (FAME) analysis, and for genetic fingerprinting using 16S rDNA banding patterns. Banding patterns were generated using PCR of total soil DNA using universal primers for a 200 bp segment of the 16S rDNA sequences in the bulk DNA from each soil sample. PCR-amplified DNA was separated into discrete bands using denaturing gradient gel electrophoresis (DGGE). The banding patterns were then subjected to image analysis to estimate species evenness (lack of predominant species) and richness (total number of species).

Microbial community fingerprints based on FAME analysis were analyzed by principal components analysis to determine the similarity between communities over time following the disturbance. FAME fingerprints were subjected to principal components analysis for comparison of the microbial community structure over time. Functional resilience of the communities was determined using BIOLOG ecoplates containing 23 different substrates. Data for substrate diversity were compared to determine shifts in rates of substrate utilization as a result of the disturbance, and were further analyzed by principal components analysis to determine whether there was a relationship between substrate usage patterns and soil metal contents.

3. Results and Discussion

Soil cores containing high levels of heavy metals had elevated respiration rates and lower diversity, but did not differ significantly in functional resilience or in substrate use patterns. This suggests that soil communities that develop after long exposure to heavy metal contamination have similar functional properties with respect to the substrates tested with the Biolog plates, but are less efficient in converting these substrates to biomass than are communities associated with non-contaminated soils.

Analysis of microbial diversity using 16S rDNA banding patterns showed that diversity decreased with increasing metal content. Decreased diversity was associated primarily with lowered species richness rather than differences in species evenness. Previously, it has been hypothesized that decreased species diversity may lead to lowered substrate diversity. This was not observed in this research. However, the degree of functional redundancy for broadly used substrates such as those provided in the Biolog assay plates may not fully test the relative abilities of the metal exposed and nonexposed microbial communities to use more rare substrates. Such rare substrates are of concern since some of these may be toxic to both plants and microbes if they accumulate in soils. The relationship between microbial diversity and substrate diversity requires further investigation before broad conclusions can be drawn, but could be a possible assay method for determining the impact of heavy metals on soil communities.

Microbial community structure, as determined by FAME analysis was highly variable in soils containing 4, 14, and 49 ppm Cd as compared to soil containing 1 ppm Cd, which clustered tightly at all time points. Soil microbial communities are likely to vary over time with normal disturbance, but overall community structure is still constrained by environmental factors that define the niche and niche size for individual species. With fewer species, it is reasonable to speculate that there should be fewer possible community assemblage patterns and that the FAME fingerprints should thus cluster more tightly for species depauperate communities. In this case, we observed the opposite result. This possibly suggests that succession was more chaotic in the metal contaminated soils and led to greater variation in community structure over time.

4. Conclusions

New tools for microbial community analysis are available for investigating the impact of metals on the biological and functional properties of soil. Heavy metals have been speculated to have a significant impact on microbial diversity and resilience, but many of these ideas remain untested. Research reported here, using culture independent methods for community analysis, suggested that heavy metal contaminated soils have lower species richness, but did not demonstrate any relationship between species diversity and substrate diversity for commonly used substrates.

SOIL MICROBIAL BIOMASS AND AMILASE ACTIVITY IN A SOIL TREATED WITH SEWAGE SLUDGE ENRICHED WITH CR

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1. Introduction

In the recent years, waste water treatment has increased, accumulating large rates of sewage sludge, generating a new great problem to be solved. As this residue presents in its composition organic matter and nutrients for plant growing, its use in agriculture would resolve two great problems: a way of sewage sludge disposal and a decreasing in the use of mineral fertilizers, another mechanism of environment pollution.

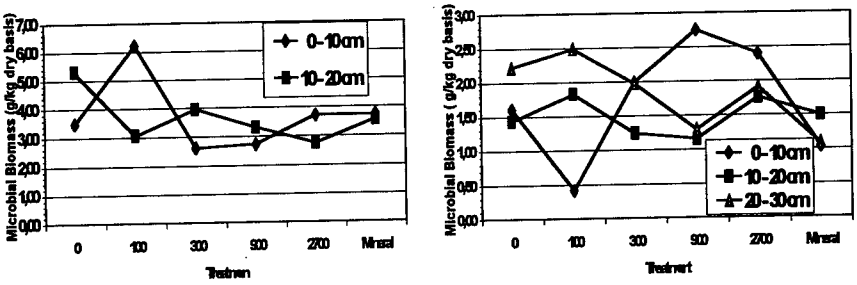
But, sewage sludge contains heavy metals in its composition, among them the chromium, which also are causes of pollution and of toxicity to living organims. Thus, the objective of this work was to estimate the effect of sewage sludge, when enriched with increasing rates of chromium, on soil microbial biomass and amylase activity.

2. Materials and Methods

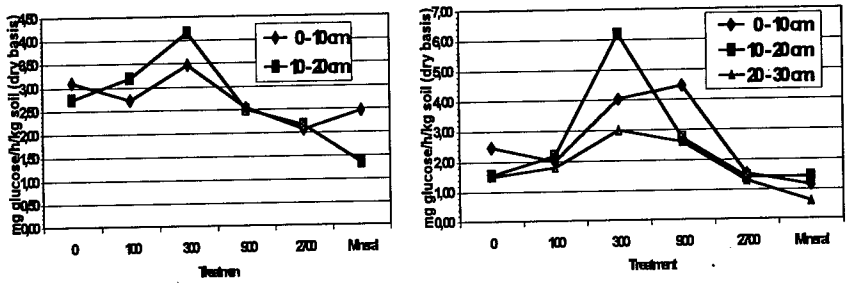
The trial was carried out in Jaboticabal county, State of São Paulo, Brazil, in a Typic Haplorthox type soil (OM= 26 g kg^{-1}, pH ($CaCl_2$ 0.01 mol L^{-1})= 4.7, K= 3.2, Ca= 14, Mg= 4, H+Al= 38 $mmol_c$ dm^{-3}, sand= 640, silt= 60, clay= 300 g kg^{-1}) under field conditions. Sewage sludge (K= 108.33, Ca= 150.00, Mg= 216.67, Cu= 9.33, Fe= 3,941.67, Mn= 17.42, Zn= 8.42, Cr= 8.58, Cd= 0.33, Ni= 6.25, Pb= 9.58 mg kg^{-1}), supplied by SABESP (Suzano, SP) was air-dried, ground to 40 mesh and enriched with chromium ($CrCl_3.6H_2O$) in the rates 0 (without addition of Cr), 100, 300, 900 and 2700 mg kg^{-1} sewage sludge. A liming was made 148 days before sowing to elevate the V% value to 70% (3 Mg ha^{-1} dolomitic limestone). Sewage sludge, so prepared, was applied to the soil, in the furrow, in the rate of 40 Mg ha^{-1}. A treatment, including no sewage sludge and a mineral fertilization, was also used. Sorghum was then cropped in a density of 140,000 plants ha^{-1}. The experimental design was a randomized blocks with four replications, and the data were analyzed as a split plot design. Soil was sampled at 64 and 104 days after sorghum sowing at the depths 0-10, 10-20 and 20-30 cm and analyzed for carbon microbial biomass (fumigation-extraction method) and for amylase activity.

3. Results and Discussion

Soil microbial biomass was affected by the treatments end by the sampling depth (Figure 1). In the first sampling, the highest soil microbial biomass was observed in the depth 0-10 cm and for the rate 100 mg Cr kg^{-1} sewage sludge, which differed from the other treatments. In the second sampling the highest value for carbon in the biomass also occurred in the depth 0-10 cm, but when 900 mg Cr was added to kg of sewage sludge. At this time, the lowest value for soil microbial biomass was observed in the depth 0-10 cm and for the rate 100 mg Cr kg^{-1} sewage sludge. The data suggest that little rates of Cr increases soil organisms development and that the highest soil microbial biomass in the higher rates in the second sampling was due to Cr imobilization in the soil.



Soil amylase activity was also affected by the treatments and by the depth of sampling (Figure 2). In the first sampling, the highest enzyme activity was observed in the rate 300 mg Cr kg^{-1} sewage sludge and in the depth 10-20 cm, decreasing for both the sampling depth for the higher Cr rates. In the second sampling, the highest amylase activity also occurred in that treatment, followed by those observed in the depths 0-10 and 10-20 cm.



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EFFECT OF ZINK AND LEAD ON FERMENTATIVE ACTIVITY OF CALCAREOUS SOILS

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1. Introduction

Biological processes are a base of circulation of materials and energies, making in soil and defining its fertility and production. Pollution TM can emerge in dug ecotoxicology factor, defining direction of biological processes.

2. Materials And Methods

The influence of heavy metals (HM)-Zn,Pb on fermentation activity of calcareous chernozem and chestnut soils of the south of Russia, its nutrient regime were studied in vegetation experiments. .The contents of humus in chernozem -3,9% , carbonates - CaCO3 -1,44% and in chestnut soil 2,6 and 0,4 accordingly. Pollutant elements were introduced in the form of acetates in the following doses:Zn-24, 45, 75, 105, 300 mg/kg for chernozem and chestnut soils, Pb-22, 33, 55, 110 mg/kg for chernozem; 20,30, 50, 100 mg/kg for chestnut soils. Potential reserve of HM was determined in 1n. HCl-extract, mobile forms were extracted by ammonium-acetate buffer solution with pH-4.8, fermentative activity – according to Khasiyev method (1). Sample Selection conducted packed ripeness of barley in phase of. Ground was analysed on the second year after a contributing the elements-pollutants.

3. Results and Discussion

It was installed that Pb reduces processes of decomposition of hydrogen peroxide in calcareous chernozem and in chestnut soil (tabl.1). The difference between control and first variant was not essential (not reliable). Zn oppresses catalase activity of chestnut soil more than chernozem. Probably more high contents of humus and carbonates allow reducing toxicity of Zn in chernozem, though concentration of soil Zn inheres on identical level practically.

Reduce influenced TM in high concentrations on fermentative activity probably connected with possibility TM to precipitate a protein. In relatively low concentrations ions of these metals are connected with amides and carbonite groups being included in molecules of ferments and reduce their activity (2) Process of decomposition of urea in all variants of experience is greatly actuated. But degree enrichment soil of urease under soiling Zn and Pb does not changing and is characterized as poor on the scale Zvygenzev (3).

It was revealed that phoshotase activity by a contributing greatly falled. It is herewith-noted positive action Zn and Pb on the mobility of calcium phosphates in calcareous chernozem.

Installed that Zn and Pb oppress a carbohydrate transformation process in chernozem; in chestnut soil negative influence on the invertase activity renders only Pb. High physio-biochemical activity Zn stimulates hydrolysis of complex sugars.

4. Conclusions

1 Characteristics of soil, concentration and nature of elements-pollutants stipulate intensity of fermentative processes in soil under pollution.

2 Degrees of reduce actions of Zn and Pb in the second year of experiment after their a contributing on the activity of catalase, urease, phoshotase calcareous soils greatly does not differ.

3 Invertase is the most sensitive indicator soil of pollution Pb.

5. References

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TABLE 1. ACTIVITY OF PHOSPHOTASE AND INVERTASE OF CALCAREOUS CHERNOZEM AND CHESTNUT SOILS

VARIANT	**INVERTASE** mg GLUCOSE 1g\24 hour	KATALASE ml O_2 1g/1min	UREASE mg NH_3 1g/24 hour	PHOSPHOTASE mg P_2O_5 10g/24 hour
chernozem				
control	**43.25**	**7.0**	**1.07**	**2.70**
Pb 22 mg/kg	**31.31**	**6.4**	**3.5**	**2.94**
Pb 33 mg/kg	**27.56**	**6.0**	**3.0**	**2.59**
Pb 55 mg/kg	**16.56**	**6.0**	**3.0**	**1.87**
Pb 110 mg/kg	**6.56**	**5.8**	**2.5**	**1.89**
Zn 24 mg/kg	**28.00**	**7.4**	**4.3**	**1.98**
Zn 45 mg/kg	**24.20**	**6.4**	**3.6**	**1.99**
Zn 75 mg/kg	**21.38**	**5.2**	**3.0**	**1.84**
Zn 105 mg/kg	**14.25**	**5.0**	**2.4**	**1.69**
Zn 300 mg/kg	**0.13**	**2.2**	**1.9**	**1.57**
chestnut				
control	**31.75**	**5.4**	**0.98**	**2.37**
Pb 20 mg/kg	**35.00**	**5.8**	**2.5**	**2.50**
Pb 30 mg/kg	**32.44**	**6.0**	**2.6**	**2.12**
Pb 50 mg/kg	**30.50**	**5.0**	**2.4**	**1.96**
Pb 100 mg/kg	**7.69**	**4.8**	**2.2**	**1.80**
Zn 24 mg/kg	**95.94**	**4.0**	**3.3**	**3.37**
Zn 45 mg/kg	**71.50**	**3.2**	**2.6**	**2.11**
Zn 75 mg/kg	**66.50**	**2.6**	**2.1**	**2.06**
Zn 105 mg/kg	**80.81**	**2.6**	**1.8**	**1.86**
Zn 300 mg/kg	**43.44**	**2.2**	**1.4**	**1.59**

TABL .2. THE TRANSFORMATION OF Zn IN CHERNOZEM AND CHESTNUT SOIL

EXTRACT	VARIANT, mg/kg	CONCENTRATION, mg/kg **chernozem**	**chestnut**
NH_4OAc pH=4,8	**control**	**4.43**	**4.57**
	24 mg/kg	**9.70**	**11.23**
	45 mg/kg	**14.28**	**14.68**
	75 mg/kg	**29.66**	**31.00**
	105 mg/kg	**32.17**	**34.17**
1N HCL	**control**	**6.97**	**8.87**
	24 mg/kg	**30.48**	**32.46**
	45 mg/kg	**43.20**	**43.52**
	75 mg/kg	**85.82**	**84.75**
	105 mg/kg	**95.70**	**95.37**

ROLE OF SOIL MICROORGANISMS IN BIOGEOCHEMICAL FLUORINE CYCLE IN ECOSYSTEMS UNDER CONTAMINATION

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1. Introduction

The role of microorganisms in biogeochemical cycles of important elements for agricultural plant nutrition and heavy metals is wellknown. In soils contaminated with heavy metals the quantitative microorganism composition is changed, any strains and species resistant much more to the enhanced toxicant concentrations appear. The study of changed microorganism composition in soils is interesting in the case of contamination with fluorine compounds, which have been involved by any tones per year in the mineral and ore processing technologies (1). Changes in microbiocenoses of soils contaminated with fluorine have received a little investigation, and identification of the most resistant strain to be able to accumulate and transform fluorine compounds is not known (2). That is why we examined the basic changes in microbiocenoses strucrure in industry polluted soils, which indicate an intesity of biogeochemical fluorine cycle processes in altered ecosystems.

2. Materials and Methods

Fluorine forms with various mobility were detected in soil samples by potentiometric method using fluorine-selective electrodes "Crytur" (Czechia). The water-soluble fluorine forms amounts were determinated in standard water extract with volume aliquots of buffer, consisting of
(in g/l): 61.5, CH_3COONa; 58.5, NaCl; 0.348, $C_6H_5O_7Na$; and 14 ml CH_3COOH. The acid-soluble fluorine forms were extracted with 20 ml $HClO_4$ (pH=1.0), and 3 ml 30% $C_6H_5O_7Na$. The soil microorganism amounts were measured by widely used methods after growing them on dense nutritive media, as follows: the meat-peptone agar (MPA) or the starchy-ammoniac agar (SAA) for bacteria, and Chapeck's medium for microfungi.

3. Results and Discussion

Experimental data showed that in soils near a source of gaseous emission, concentrations of acid-soluble fluorine forms as well as water-soluble ones were increased by 150 times above to the background means (Table 1). That significant increase in fluorine content has led to changes in the quantitative composition of bacteria and microfungi in soils, which are responsible for fluorine involvement into biogeochemical cycles. In Table 1, data are presented to show reduced amounts of bacteria using organic and inorganic nitrogen forms, respectively MPA-grown and SAA-grown strains, as a basic source of nutrition. Amounts of the MPA-grown bacteria from soils with strong, middle and weak contamination levels were reduced from 21.06 to 2.9 millions/g dry soil. Results of correlation assay indicated an obvious dependence between increased fluorine concentration and reduced amount of bacteria in soil (r_s=-0.87-0.96). Thus, when fluorine contents were enhanced from 1.1 to 5.2, 86.2, 120.2, and 169.4 mg/kg in top soil layer, numbers of MPA-grown bacteria were decreased accordingly by 6.1, 25.4, 64.5, and 86.2%. In the case of SAA-grown bacteria we observed a great inhibition effect of soil contamination upon bacteria. The same increased fluorine content led to reduced numbers of SAA-grown microorganisms by 6.3, 36.1, 76.4, and 90.3% respectively.

Table 1. Changes in microbial groups in relation to increasing fluorine content in soil samples (0-10 cm layer); $p<0.05$, n=5.

Sites of sample selection	Fluorine content, mg/kg	Bacteria amounts, million/g				Microfungi amounts, thousand/g	
		MPA		SAA			
		1	2	1	2	1	2
Zone of strong contamination	169.4 ± 2.8	2.90 ± 0.30	5.9	2.73 ± 0.08	6.3	7.16 ± 0.12	35.5
Zone of middle contamination	120.2 ± 2.9	7.48 ± 0.63	11.3	5.80 ± 0.20	5.2	9.34 ± 0.08	35.3
Zone of weak contamination	86.2 ± 1.2	15.72 ± 1.52	9.0	17.95 ± 0.22	13.1	14.29 ± 0.13	15.2
Sanitary-protection zone	5.2 ± 1.0	19.79 ± 2.61	6.3	26.35 ± 0.18	9.9	18.71 ± 0.15	3.3
Background	1.1 ± 0.01	21.06 ± 1.92		28.11 ± 0.31		20.06 ± 0.11	

In column 1 - M ± m; in column 2 - ratio of deviation between paired data means to standard Student's "t"-coefficient.

In soil from the zone of weak contamination and the sanitary-protection zone decreasing index of measured microfungial numbers exceeded that for MPA-grown bacteria, but it was less that relative index calculated for SAA-grown bacteria. In soils from the zones of both strong and middle contamination microfungi were reduced by 53.4 and 64.3%, that was less than decreasing index of MPA-grown bacteria. It would be concluded that in the top soil layer (0-10 cm) MPA-grown bacteria and microfungi play the more significant role in processes of fluorine involvement into biogeochemical cycles when fluorine concentration in soil was
enhanced, because their amounts were reduced by a small order comparing to a number of bacteria using inorganic nitrogen forms as a basic source of nutrition.
Examination of samples from deeper soil layers (15-30 cm) resulted in fluorine content exceeding that in the non-contaminated soil samples. Concentrations of water-soluble fluorine forms were from 3.0 to 42.3 mg/kg in soils from the zones with various contamination levels. Increased fluorine content in soil layer of 15-30 cm reduced numbers of microorganisms grown on MPA, SAA, and Chapeck's medium by 3.2, 7.1, and 1.7 times. Whilest fluorine concentration in soil increased from 45.3 to 142.3 mg/kg the amounts of bacteria were reduced by, as follows: SAA-grown - from 28.2% to 85.8%, MPA-grown - from 18.2% to 68.8%, and microfungi - from 14.8% to 41.4% (Table 2). Thus, the number of soil microfungi and MPA-grown bacteria were less reduced in comparison to SAA-grown ones, as well as in the case of top soil layer.

Table 2. Changes in microbial groups in relation to increasing fluorine content in soil samples (15-30 cm layer); $p<0.05$, $n=5$.

Sites of sample selection	Fluorine content, mg/kg	Bacteria amounts, million/g				Microfungi amounts, thousand/g	
		MPA		SAA			
		1	2	1	2	1	2
Zone of strong contamination	142.3 ± 12.1	4.15 ± 0.40	9.2	3.12 ± 0.08	11.9	9.28 ± 0.11	22.3
Zone of middle contamination	80.5 ± 8.3	8.62 ± 0.56	15.3	6.51 ± 0.05	18.6	10.94 ± 0.10	18.1
Zone of weak contamination	45.3 ± 0.5	10.86 ± 0.79	12.8	15.82 ± 0.10	9.1	13.52 ± 0.06	11.4
Sanitary-protection zone	3.0 ± 0.3	10.91 ± 1.16	6.5	23.62 ± 0.26	8.7	14.03 ± 0.10	6.7
Background	1.0 ± 0.05	13.31 ± 1.53		22.03 ± 0.28		15.86 ± 0.07	

In column 1 - M ± m; in column 2 - ratio of deviation between paired data means to standard Student's "t"-coefficient.

Interesting results were obtained after comparison of data on changes in amounts of examined microorganism groups from non-contaminated and strongly contaminated soil samples selected with various deepness. Thus, in 15-30 cm layer of non-conteminated soil the numbers of all MPA-grown and SAA-grown bacteria and microfungi were less by 63.2, 78.3, and 79.0% accordingly those in 0-10 cm layer. Whereas in soil samples from the zone of strong contamination level these indeces exceeded the top layer parameters by 43.1, 14.1, and 29.6% respectively. Presented data could be explained with reduction of fluorine concentration in lower-lied soil layers. But in this case both MPA-grown bacteria and microfungial numbers were less reduced in comparison to the SAA-grown bacterial numbers.

Analyzing obtained experimental data it would be concluded that soil contamination with fluorine showed the smallest inhibition effect upon bacteria using organic nitrogen forms for nurition, and microfungi. Therefore, these soil microorganism group play the most significant role in processes of fluorine involvement in biogeochemical cycles, than bacteria using inorganic nitrogen forms as a basic source of nutrition.

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METABOLIC QUOTIENT, ENZYME ACTIVITIES, L/D-AMINO ACID RESPIRATION RATIO AS AFFECTED BY CADMIUM IN A FOREST SOIL

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1. Introduction

High levels of heavy metals usually cause reductions in microbial biomass and activity in soil (NANNIPIERI et al., 1990). Several authors reported an increase in the metabolic quotient (respiration per unit biomass) of contamined soils (LEITA et al., 1995). It was supposed that soil microorganisms under polluted conditions respire more CO_2 with less incorporation of organic C into the biomass. On the contrary, other authors observed a decrease in the metabolic quotient (qCO_2) in heavy metals contamined soils (HATTORI, 1992). Recently HOPKINS et al. (1997) proposed as an indice of microbial stress the L/D amino acids respiration ratio because microorganisms, under polluted conditions, probably did not discriminate between the two stereoisomers. Other parameters, such as enzymatic activities have often been measured to study the effect of heavy metals on specific microbial reactions (TYLER, 1976).
Our purpose was to determine the effect of high concentrations of Cd on qCO_2, enzyme activities and the L/D glutamic acid respiration ratio.

2. Materials and Methods

A forest soil (pH(H2O) 4.8; 2.27% organic C; 0.09% total N; 87.4% sand, 8% silt 4.6% clay) was sampled from 0-10 horizon, sieved (< 2mm), stored moist at 4°C and pre-cubated for 4 d at 25°C before incubation. Thereafter, it was treated with distillated water (control) or a $CdSO_4$ solution to give 0, 50 and 500 mg Cd Kg^{-1} soil and reach 50% of WHC. Soil was incubated at 25 °C for 28 d into jars containing water and 1M NaOH to trap evolved CO_2. At every incubation time, soil respiration was measured and subsample was analysed for soluble Cd, microbial biomass C (VANCE et al., 1987), dehydrogenase and phosphatase activities. Another subsample was amended with D or L-glutamic acid (2 mg g^{-1}), incubated at 25°C for 6 h and analysed for respiration induced by isomeric forms of the glutamic acid.

3. Results and Discussion

In the 0-4 d incubation period the water-extractable Cd content was about 20% and 2% of the total; for 500 and 50 mg Cd Kg^{-1}, respectively. Thereafter both levels decreased to 1-2% and remained constants throughout incubation period (Fig. 1). A significant reduction in the activities of both enzymes and in the ratio of these activities with microbial biomass C was generally observed in the 0-4 d incubation period in contamined soil. The major effect was found at the highest Cd concentration (Fig 1). These results suggest that the inhibitor effect depends on the amount of soluble Cd. No significant differences were found for qCO_2 and microbial biomass throughout incubation, between Cd contamined and uncontamined soil, whereas the addition of Cd decreased soil respiration in the 0-4 d incubation period (data not shown). Also the L/D respiration ratio was reduced in contamined soil during the 0-4 d incubation period, indicating that this parameter could be a sensitive index of microbial stress (Fig. 1).

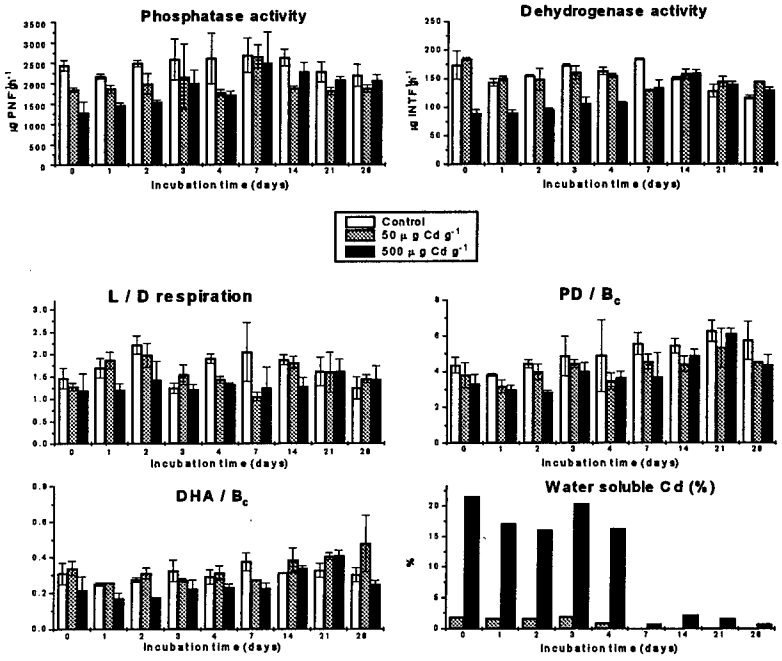

Fig. 1- Change of phosphatase activity (PD), dehydrogenase activity (DHA), L/D respiration, PD/microbial biomass C (Bc), DHA/Bc and water soluble-Cd during incubation. Bars indicate SD values.

4. Conclusions

In conclusion the L/D respiration ratio for glutamic acid, the enzymatic activities and their ratios with microbial biomass C seem to be sensitive indexes of Cd pollution. Nevertheless, future researches are needed to investigate if changes in biodiversity occurred during the highest levels of the water-soluble fraction of Cd.

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HEXAVALENT CHROMIUM REDUCTION BY BACTERIA ISOLATED FROM A TANNERY CONTAMINATED SOIL

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1. Introduction

Several microorganisms have the exceptional ability to adapt and colonise the noxious metal polluted environments that are uninhabitable by higher organisms. These microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms such as adsorption, uptake, methylation, oxidation and reduction etc. Dissimilatory reduction of metal oxyanions (such as AsO_4^{2-} and CrO_4^{2-} by bacteria is considered to be an important mechanism of metal detoxification. Thus, the reduction of Cr (Vl) to Cr (Ill) is an important step in the remediation of Cr contaminated environments. The traditional treatment methodologies for soils and groundwater contaminated with Cr (Vl) are based on excavation and pumping of contaminated material followed by the addition of chemical reductant resulting in the precipitation and sedimentation of reduced Cr (Nyer, 1992). These processes are expensive and energy intensive. Bioremediation is emerging as a safe and cost effective technology alternative to the traditional physico-chemical methods. However, the availability of effective Cr (Vl) reducing organisms is an essential prerequisite for bioreduction based bioremediation of Cr (Vl) contaminated water/soil. Hence the present study was aimed at the isolation and characterisation of Cr (Vl) reducing bacteria from Cr contaminated soil.

2. Materials and Methods

Hexavalent chromium resistant bacteria were isolated from a tannery contaminated soil using M9 minimal mineral medium agar supplemented with 100 μg ml^{-1} Cr (Vl) as $K_2Cr_2O_7$ and 0.5 % glucose as a carbon source. Bacterial isolates were identified by FAME analysis. M9 minimal medium was used for growth of the bacteria throughout the experiment. Cell free extract was prepared by harvesting the exponentially growing bacteria in 10 mM Tris-HCl buffer (pH, 7.2) (Megharaj et al., 1997). For permeabilisation of cells overnight grown cultures were harvested and washed with Tris-HCl buffer (pH, 7.2) and suspended in the same buffer at an OD600 of 1.6. Toluene (1 %) and Triton X100 (2 %) were added to the cell suspension and vortexed to permeabilise the cells. The Cr (Vl) in the supernatant medium was estimated by modified 1,5-diphenyl carbazide (DPC) method. Total Cr concentration in the medium and acid-digested cell pellets was measured by Atomic Absorption Spectrophotometer using air acetylene flame.

3. Results and Discussion

The two Cr(VI) resistant bacterial isolates were identified as *Arthrobacter* sp. and *Bacillus* sp. The growth response of the two bacteria towards different concentrations of Cr (Vl) differed greatly (results not shown). The growth of *Artrobacter* sp. was not affected up to 50 μg ml^{-1} Cr (Vl) while 10μg ml^{-} Cr (Vl) was toxic to *Bacillus* sp. eventhough growth proceeded at a lower rate than the control. The time for total reduction of Cr (Vl) increased with increasing concentration of Cr (Vl). The Cr (Vl) reduction ability of the bacteria were growth dependent and *Arthrobacter* sp. was more efficient Cr reducer than the *Bacillus* sp. *Arthrobacter* sp. reduced nearly 30 μg ml^{-1} of Cr (Vl) during 46 h incubation time where as *Bacillus* sp. could reduce only up to 10 μg ml^{-1} Cr (Vl) during

the same period. Thus, significant differences were observed in the bioreduction abilities of the two bacteria (table 1).

Table 1. Cr(VI) (10 μg ml^{-1}) reduction (% Cr (VI) recovery) by bacteria

Treatment	*Arthrobacter* sp.		*Bacillus* sp.	
	10 min	6 h	10 min	6 h
Control	100	100	100	100
Resting cells	33	0	80	0
Permeabilised cells				
1% Toluene	15	0	74	0
2% Triton X100	21	0	73	0
Cell free extract	12	0	84	41

The reduction of Cr (Vl) to Cr(lll) in Cr (Vl) reducing bacteria is carried out by either cell membrane or soluble proteins (Bopp and Ehrlich, 1988; Wang et al., 1990). Partial characterisation of the Cr (Vl) reduction ability of these bacteria (Table 1) as revealed by the Cr (Vl) reduction assay with permeabilised cells and cell-free extracts showed that Cr reductase is mainly associated with the soluble fraction of the enzyme. Almost all the reduced Cr was found in the culture supernatants. Also this study shows that Cr (Vl) reduction efficiency can vary considerably among the different Cr (Vl) reducing bacteria. Given the efficient Cr (Vl) reducing ability of Arthrobacter sp., this bacterium has great potential for use in detoxification of Cr(VI) in contaminated soil and water.

4. Conclusions

Two species of bacteria resistant to Cr(VI) were isolated from a tannery contaminated soil and identified as Arthrobactor sp. and Bacillus sp. The ability of these bacteria to reduce Cr(III) to Cr(III) was studied by using cell suspensions and cell extracts. Both the tested bacteria exhibited considerable difference in their bioreduction abilities of Cr (Vl). *Arthrobacter* sp. was found to be superior to the *Bacillus* sp. in terms of their Cr (Vl) reducing abilities. Further, assays with permeabilised cells and cell-free extracts demonstrated that the Cr-reducing ability in these bacteria was mainly associated with the soluble protein fraction of the cell. Arthrobacter sp. has potential for bioremediation of Cr(VI) containing waste.

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THE EFFECT OF SOIL FUNGI ON COPPER, NICKEL AND ZINC AVAILABILITY IN POLLUTED PODZOLS

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1. Introdution

The ability of chemical elements to exert the toxic influence on living organisms depends on the form of their presence in the environment. Among compounds present in soil, the compounds containing in liquid phase and in solid phase that are capable to the exchange with soil solution are of the most ecological danger. The availability of trace elements compounds in soil depends on many factors, which separate influence is difficult for estimating. One of the factors is the effect of microorganisms on trace elements availability in soil. This phenomenon is especially important in polluted soils, where the risk of secondary mobilization of trace elements under the effect of natural biological processes exists. In the northern ecosystems conditions soil fungi are prevailling and the most resistant to pollution among soil microorganisms. Therefore microbial transformation of trace elements compounds in northern soils is mainly connected with soil fungal communities activity.

2. Materials and Methods

The samples of the A_O horizon of background and polluted podzols of pine forests of Kola peninsula were studied.
Two approaches were used to study the effect of soil fungi on copper, nickel and zinc availability:

a) analysis of the correlation between copper, nikel and zinc availability and the growth of fungal mycelium in the soil samples;
b) analysis of the differences in the contents of copper and zinc available compounds dynamics in native and steril samples of polluted podzols.

Soil fungi community initiation was carried out by wetting samples up to 60%. Soil samples were incubated at the temperature 18-20^0□. Available trace elements compounds were extracted by 0,05 N $CaCl_2$ and 1 N CH_3COONH_4. Trace elements content in extracts was determined by AAS method. Mycelium was separated by means of membranous filters. Soil samples were sterilized by gamma radiation. The irradiation doze was 2,5 □rad.

3. Results and Discussion

Initially the availability of trace elements in background and polluted podzols differs greatly (table 1). The difference in dynamics of their mobility also is observed. The correlation between the available trace elements compounds content and the length of fungi mycelium in the soil is noted.

Table 1: The content of available trace elements in background and polluted podsols

	Cu, ppm		Ni, ppm		Zn, ppm	
	0,05 N $CaCl_2$	1 N CH_3COONH_4	0,05 N $CaCl_2$	1 N CH_3COONH_4	0,05 N $CaCl_2$	1 N CH_3COONH_4
Background podzol	4,83	11,91	—	2,60	14,87	20,36
Polluted podzol	201,11	401,00	170,22	225,51	10,63	13,88

The period of active increase of mycelium length coincides with a period of trace elements availability increase both in background and polluted soils. At the stage of mycelium growth delay the stabilization of copper and nickel concentration in soil solution occurs in polluted podzols. In background podzols this stage is characterized by the decrease of available trace elements amount in soil solution.

Dynamics of available trace elements content in the sterile samples are characterized by the small fluctuations. The tendency to increase the amount of available compounds is observed as a result of wetting.

4. Conclusion

The difference in trace elements mobility dynamics in native and sterile soil samples testifies the effect of soil fungi on trace elements availability in podzols.

The directions of microbiological transformation of trace elements compounds in the investigated soils can be different. The effect depends on an element, its concentration in soil solution and a developmental stage of fungal community. The initial stage of fungal community development is characterized by increase of trace elements availability. The subsequent period of fungal community development is characterized by decrease of availability of trace elements, which concentration in soil solution is near to background content. For the trace elements, which contain in soil solution in high amounts this period is characterized by the stabilization of available compounds content.

MICROBIAL BIOMASS IN A SOIL CONTAMINATED WITH INCREASING RATES OF PB, CULTIVATED WITH BLACK OAT (*AVENA SPP*)

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1. Introduction

The microbial biomass, defined as the living part of the organic matter of the soil, excluded the roots of plants, constitutes the first stadium of the carbon of the residues in decomposition in the soil. It represents from 1 to 4% of the total carbon of the soil, and could reach tons per ha.It is important under three aspects. First, because it is capable of promote important alterations in the soil system. Second due to the great amount and to the fact of being the largest component of the organic matter of the soil, it becomes potentially an important reservoir of nutrients for the plants. And, last, it represents an indicator of great sensibility to evaluate changes in soil, being influenced by fertilization, cultivation methods and contamination by heavy metals.

Then, the present work had for objective to evaluate the effect of the addition of increasing rates of Pb on the C and N of the soil microbial biomass .

2. Materials and Methods

The experiment was conduced under greenhouse condition. The used soil was a Tipic Eutrorthox, medium texture, collected in the layer 0-20cm and presenting the following characteristics: pH $CaCl_2$ = 5.6; OM = 21g dm^{-3}; P = 51mg dm^{-3}; K = 2.7; Ca = 30; Mg = 13; H+Al = 22; S = 45.7 and T = 67.7 ($mmol_c$ dm^{-3}); V = 68%; 340 g kg^{-1} of clay; 20 g kg^{-1} of silt; 330 g kg^{-1} of fine sand and 310 g kg^{-1} of thick sand.

The used plant was black oat (*Avena* spp). The used experimental design was completely randomized, with 4 treatments (0, 200, 400 and 600 mg Pb kg^{-1} soil as Pb Cl_2) and 4 replications. Each pot received 8 kg soil. The pots received water periodically in order to maintain the WHC, and after 44 days (incubation period) 15 seeds of black oat were sowed, being kept 4 plants by pot, when they presented 5 cm of height. All the treatments received a plantation fertilization with: 5mL solution Fe.EDTA, 10g of triple superfosfate and 15 mL of 1mol L^{-1} potassium chloride. Two covering fertilization with nitrogen and potassium were accomplished every 15 days after the sowing by adding 150mg kg^{-1} of N (ammonium nitrate) and 100 mg kg^{-1} of K (KCl).

At 104 days installation soil was sampled and analyzed for the content C and N in the soil microbial biomass and for Pb in the Melich-1 extract.

3. Results and Discussion

The lead rates affected the content of carbon of the microbial biomass ($y = 0{,}2993 + 0{,}0643x$, where y is the content of carbon and x is the rate of Pb) as shown in Table 1. The content of N of the soil microbial biomass was not affected by the contamination of the soil with lead.

Correlation was not observed between the content of Pb with the N of the soil microbial biomass, but there was correlation between Pb and C, evidencing the affinity of the metal for the organic matter of the soil.

Table 1 - Correlation coefficient between C and N of the soil microbial biomass with extractable Pb in a Typic Eutrorthox treated with of different rates of Pb.

Parâmeters	F	R^2	equation
soil C biomass	10,75**	0,7801	y=29,9930 + 0,0643x
soil N biomass	4,14ns	-0,6439	y=30,2534 + 0,0189x
Pb Melich-1	172,71**	0,7067	y=0,8300 + 0,0534x
Pb x C	5,06*	0,5151	y=30,543 + 0,7763x
Pb x N	0,87ns	-0,2387	y=28,1707 - 0,1460x

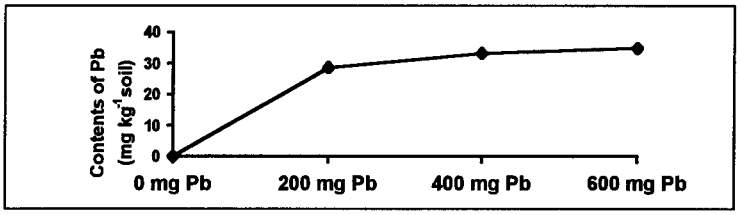

Figure 1 - Contents of extractable Pb of the soil contaminated with Pb and cultivated with black oat using the extractor Melich-1.

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Mobility and Transport of Trace Elements

(Technical Session 10)

MOBILIZATION AND IMMOBILIZATION OF AL AND MN IN ACID SOIL

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1. Introduction

Aluminium in soil can appear in an immobile, highly bound to organic ligands form, as well as in a mobile form in soil solution, if pH drops down below 4,5 (Robson, 1989; McBride, 1994). Mn, unlike aluminium, is an element which is necessery for normal growth and development of plants (Kamiska, 1981; Robson, 1989). In highly acid soils, manganese appears mostly in the double value form.

The factors which significantly influence mobilization and immobilization of Al and Mn in soil are as follow: soil pH, presence of organic matter, liming and mineral fertilization (Tiller, 1983; Badora and Filipek, 1998). Accumulating surplus amounts of active manganese and exchangeable aluminium in soil results from acid degradation of soil.

2. Materials and Methods

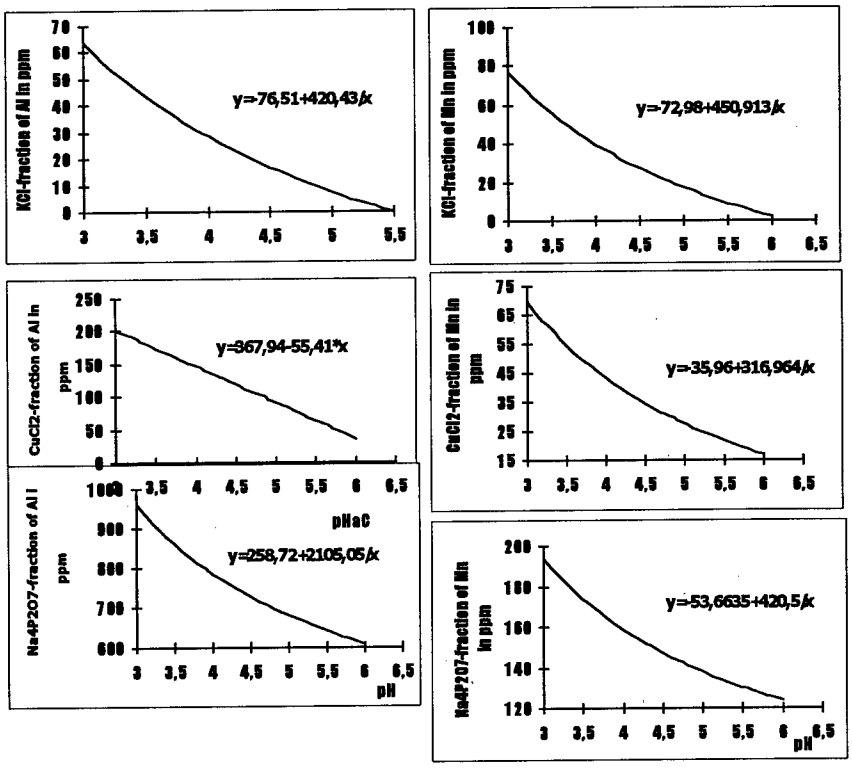
Two year research on the listed problems were carried out in a vegetation hall in years 1995-1996, on podzolic soil derived from light loamy sand. The investigated soil came from an agricultural plantation and showed high acidity, pH<4. The experimental unit was a pot containing 5.5 kg of dry soil. The tested plants were barley and spring rape.

Ecologically active forms of Al and Mn were investigated in extracts: 1M of KCl (the reagent extracted the exchangeable forms of Al and Mn); 1M of $CuCl_2$ (the reagent extracted the exchangeable forms of Al and Mn, which had been weakly bound to the organic matter); 0.1M of $Na_4P_2O_7$ (the reagent extracted the exchangeable forms of Al and Mn, which had been strongly bound to the organic matter (Por□bska and Mulder, 1994).

3. Results and Discussion

As carried experiments shown, fractions of Al weakly and strongly bonded to the organic matter clearly exceeded the organic fractions of Mn at the same pH conditions, but the increase of soil pH, so the decrease of ecologically active Al and Mn fractions, displayed more slowly decrease of Al fraction content than that of Mn strongly bonded to organic matter (Fig. 1). Easy release of Mn ions into the soil solution from the organic matter may be also proven by the fact of higher concentration of soluble Mn fraction where lower concentration of Al fraction existed at a given pH. Strong complexation of Al ions, and hydrated Al ions, both its monomeric, and polymeric forms, was found by many authors (Robson, 1989; McBride, 1994; Filipek et al., 1998). In a range of pH 5.5 - 6.0 one can observe the presence of free Mn^{2+} ions, while free aluminium ions are absent. Tiller (1983) found that Mn^{2+}, in opposition to Al^{3+}, had low affinity to mineral fraction of the soil at the acidic conditions, because it was not able to hydrolize in such conditions.

Figure 1. Al and Mn fractions and their dependence of pH in soil



4. Conclusions

Aluminium was more strongly complexed by organic matter than manganese that was easily released into the soil solution even from the fraction accepted as strongly bonded to organic matter at the decrease of soil pH.

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TURSKI R., BARAN S. 1995. DEGRADACJA, OCHRONA I REKULTYWACJA GLEB. LUBLIN, WYD. AR.MOBILITY OF COPPER, ZINC AND CADMIUM IN A LOAMY SOIL

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1. Introduction

The more and more frequent use of sludges in agriculture has given rise to a new series of environmental problems such as the accumulation of undesired substances in the soil, their transfer to crops, their mobility in the soil with the consequent contamination of ground waters. For this purpose, a study has been carried out for three years now on a field and territorial scale in order to monitor the effects of heavy metals in the long run deriving from the application of sludges in compliance with the present law (Capri et al., 1998).

2. Materials and Methods

The experimental field has been chosen inside an area characterized by an intense farming activity of corn growing and zootechnics and where sludges have been used as fertilizers and soil conditioners (Boccelli et al., 1997). Inside this area two neighbouring fields have been chosen as experimental, having a surface > 1 ha, having similar chemical-physical and hydrological properties. On average they present a fine texture, high permeability and surface ground water. The two fields differ only in the history of treatments that is to say wether sludges had been applied (treated field- TF) or not (never treated soils-NTF) in the latest 5 years. In each field 4 experimental sampling sub-units have been defined. In order to evaluate the accumulation of heavy metals in the soil deriving from sewage sludges and the influence of the crop, a sampling of the soil and shallow ground water has been carried out. The profiles of the soil have been sampled before the annual sludge application and in preparation of the ground for sowing maize and sugarbeet though deep core boring (6 per sub-unit) of the unsaturated and saturated areas of the soil (0-240 cm). The sampling of the soil has been repeated at the harvest. Ground waters have been sampled on a monthly basis through piezometres. All samples have been analyzed following standard methods and ICP determination.

3. Results and Discussion

The investigated soil presents contents in metals which are far from the limits set by the law after the application of 14.4 tonnes/ha sewage sludges and the differences between NTF and TF are not significant. This holds true both for the total fraction of metals and for the fraction of assimilable metals.

Results indicate that these metals do not accumulate in the soil in significant concentrations and if they increase year after year, it is within the analytical variability.

All metals are not so mobile if we only consider laboratory results but we can not rule out that small quantities may reach the saturated layer of the soil as it has been measured on the field in ground waters.

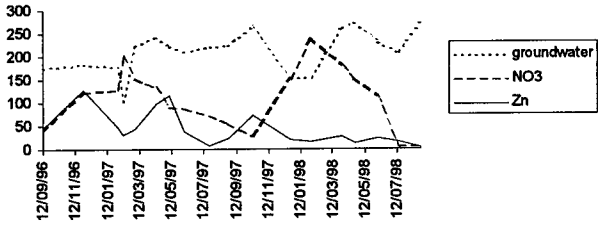

Figure 1 – Trend of zinc (µg/l) and nitrate (mg/l) in shallow groundwater (cm) after soil application of sludge (2/96 and 1/97).

This may be due to the presence of preferencial flows inside the profile of the soil and/or a poor adsorption balance soil/solution to be observed in the field. This assumption is partially confirmed in the concentrations of heavy metals measured in shallow ground waters which have a rythmic progress near precipitations whereas nitrates, which are highly soluble, increase immediately after the application and then decrease over time. Metal concentrations in ground water decrease with the passing of time starting from the day of the first sludge application (Figure 1 and 2).

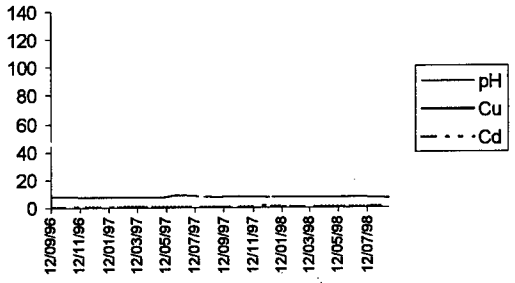

Figure 2 – Trend of cadmium (µg/l), copper (µg/l) and pH in shallow groundwater after soil application of sludge (2/96 and 1/97).

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MOBILIZATION OF TRACE METALS IN ACID TREATED CONTAMINATED SOILS

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1. Introduction

Mobilization of potentially toxic metals in the soil, i.e. their transfer from solid pools to the liquid phase, may pose a serious environmental risk since water soluble forms are essential both for migration and plant availability of metals. Soil acidification may induce and promote metal release to the soil solution. The objective of this research was to model the release of Cd, Cr, Ni, Pb, and Zn from excessive soil contamination into the soil liquid phase (which was obtained at known, well-defined energy status) and to estimate the impact of different acid loads on metal mobilization.

2. Materials and Methods

Experiments were carried out on samples of "A" horizons of a slightly acidic sandy soil (No.1) and a medium textured, acidic brown forest soil (No.2) (pH[H_2O] 5.6 and 4.3, CEC 6.7 and 20.7 $cmol_c$/kg soil, clay fraction 12.7 and 27.8%, resp). Metals were applied in aqueous multicomponent solutions of nitrate salts at concentrations equivalent to (1*L*) or 10 and 100 times higher (10*L* and 100*L*) than those corresponding to the allowed limits in sewage sludges used in agriculture; (1*L* loading rate = 0.125 mg Cd, 1.67 mg Ni, 8.33 mg Cr and Pb, 25 mg Zn /kg soil). Effect of soil acidification was studied on previously contaminated then dried soil samples, which were rewetted with distilled water or with 0.001, 0.1 or 1.5 mol/L HNO_3 solutions. Soil solution was centrifuged (CSILLAG et al., 1998) after one week equilibration from uncontaminated, contaminated and contaminated+dried+acidified wet soil samples which had water potentials equal to field capacity (-20 kPa). Since the rotor speed corresponded to -1500 kPa (conventional wilting point of plants), composition of directly plant available liquid phase of contaminated and acidified soils could be modelled in the experiment.

3. Results and Discussion

Cd, Cr, Ni, Pb, and Zn concentrations in the soil solution were negligible or low when the officially allowed loading rate (1*L*) was applied. At 10*L*, only Ni and Zn entered the liquid phase in considerable amounts (≅10 and 200 mg/L, resp.), but at the extreme overloading (100*L*) all elements were present in very high concentration in the soil solution. Metal concentrations expressed as percentage of the applied amounts showed the order of mobilities (Cr<Pb<Cd≅Ni≅Zn, Fig. 1), rather than contamination ratios (Cd<Ni<Cr=Pb<Zn).

Similar order of pH dependent mobilization was observed in the contaminated then dried and acidified soil samples. While element concentrations generally matched the contamination ratios, especially at higher acidic loads (except for the less mobile Cr), recoveries were closely related to the mobility order of the metals (Fig. 2). At 10*L* and lower acid loads, only

Zn was present in the solutions in appreciable amount. With increasing acidity, recovery increased considerably only for the mobile Cd, Ni, and Zn. Lead and Cr were able to enter the liquid phase in significant amounts only after extreme acidic treatment (Fig. 2a, c). At 100*L* a great part of the added metals were released into the soil solution, also at weaker acidic treatments, again with the exception of the strongly adsorbed Cr (Fig. 2b). Comparison of the two soils at 10*L* showed that, at the highest acid load, mobilization of elements was stronger in the sandy soil. In contrast, at smaller acid loads, somewhat higher or similar recovery values were found in the brown forest soil, probably because the effect of its more acidic character compensated the impacts of texture and cation exchange capacity on element retention.

4. Conclusions

Although the extremely high pollution levels and/or strong acid loads applied in this study are rare, such situations may occur locally because of accidents or improper/illegal waste deposition. In such cases the release of trace metals to the soil solution might substantially increase, in accordance with the mobility of the metals.

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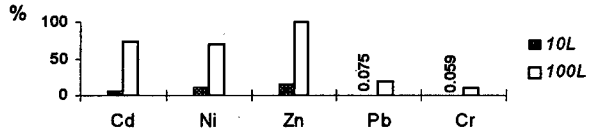

Figure 1: Release of metals into the soil solution in soil No.1 (% = $100c_S/c_m$, where c_S and c_m are concentrations in soil solution and in contaminating metal salt solution)

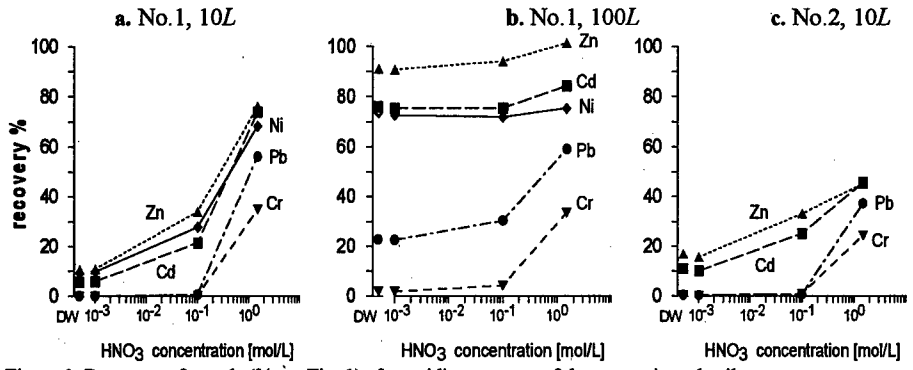

Figure 2: Recovery of metals (% see Fig. 1) after acidic treatment of the contaminated soils

LEACHING OF CHROMIUM (VI) FROM SOIL PROFILES CONTAMINATED WITH TANNERY WASTES

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1. Introduction

Extensive contamination of land due to disposal of tannery waste has been reported in several countries including India and Australia. In India alone, chromium (Cr) and salts from tannery wastes have contaminated some 55000 ha of productive agricultural land. Since hexavalent Cr [Cr(VI)] is both toxic and mutagenic, it is imperative that the release behaviour of Cr is properly understood and the techniques of remediating both Cr-contaminated water and soils are developed. Therefore, we investigated the release and migration behaviour of Cr under flow conditions from a soil historically contaminated with leather tannery wastes.

2. Materials and Methods

Large size intact cores (30 cm internal diameter and up to 1 meter length) were collected and used for transport studies. To avoid any preferential flow along the walls of the core, vaseline sealant was used. Salt-free water was fed to the column via a pump-driven delivery-head fitted with hundreds of hypodermic syringes /needles. Water samples were collected through ports fitted with pretreated fiberglass wicks at different depths in column. The effluent samples obtained through the wick samplers under suction and from the drainage exiting the column, were immediately analysed for pH, EC and Cr(VI) and the solution was then stored at -14°C for the analysis of total Cr and other elements with ICP. Eh was measured on-line at several points in column, using polished platinum electrodes. Moisture was monitored frequently through TDR probes. Total Cr in soil solution was measured by flame AAS and Cr (VI) was analysed using colorimeteric method based on diphenylcarbazide (DPC). Other elements were analysed by ICP spectrometry.

3. Results and Discussion

The soil profile was highly alkaline in the surface 10 cm and highly acidic at depth below 40 cm. In fact during the study period, the mean pH values observed at different depths were: 8.1 (±0.43) at 10 cm, 4.2 (±0.2) at 40 cm decreasing down to 3.5 (±0.2) at 90 cm depth.

The concentration of Cr obtained at 10 cm, and at 1m depth (effluent exiting at the bottom) in the core has been plotted in Figure 1A and B for both total Cr and Cr (VI). Initially, relatively higher concentrations of Cr (> 1 mg/l) were present in the solution at 10 cm depth, most (> 90%) of which was in Cr (VI) form. In contrast, however, much lower concentrations of Cr eluted out of the system at 1 m depth, and essentially all of the Cr was in Cr (III) form. The concentrations of Cr at 40 cm depths were comparable to those at 1m depth and also consisted of Cr (III) species. The Cr (VI) concentrations at 10 cm depth decreased with time and essentially all Cr (VI) had been washed out of the column by day 70. However, after a no flow and drying phase, Cr (VI) reappeared at the depth. Even after 120 days of leaching > 0.5 mg/L Cr(VI) was present in the solution at 10 cm depth.

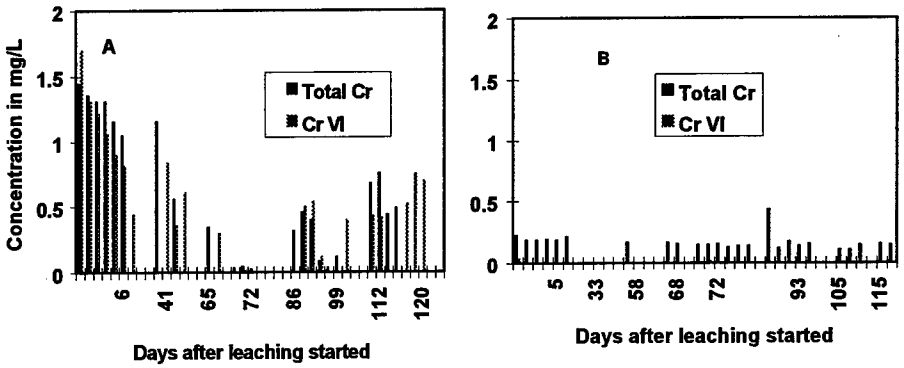

Figure 1. Concentrations of total Cr and Cr(VI) observed at 10 cm depth (A) and at 100 cm depth (B) during the leaching study on an intact core.

The redox potential values in combination with pH values measured at different depths in the column showed that while in the surface layer of the profile the conditions were favourable for the stability of Cr (VI), in the subsurface layers (> 40 cm) the converse was true. The highly acidic pH of the soil at depths > 40 cm in the core was conducive for Cr (VI) reduction. Hence, consistently no Cr (VI) was recorded at depths >40 cm in the soil profile. It is highly likely that Cr (VI) anions were reduced by either organic matter or ferrous iron to Cr(III) cations in the strongly acidic subsurface environment. This transformation of Cr(VI) to Cr(III) has been reported by several other workers (Eary and Rai, 1991). Since at low pH Cr (III) sorption is likely to be small, some Cr (III) eluted out of the column. When the experiments were carried out on intact cores containing alkaline soils throughout the profile, small concentrations of Cr (VI) migrated through the column and eluted at 1 meter depth. This shows much smaller potential of reduction of Cr(VI) under alkaline conditions in the soil.

4. Conclusions

In soils heavily contaminated with Cr, arising from land disposal of leather-tannery waste, significant concentrations of Cr(VI) can be available for offsite migration. In such soils, pH has a major effect on Cr speciation, release and transport of Cr(VI).

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LEACHING OF LEAD FROM SOILS CONTAMINATED WITH LEAD SHOT

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1. Introduction

Despite the decrease in emissions from various sources, lead continues to be one of the toxic metals commonly found as environmental pollutants. A source of lead (Pb) contamination which has been identified recently is the oxidation of lead shot deposited on land at clay target shooting sites (Jorgensen and Willems, 1987; Murray *et. al.*, 1997). Soil Pb concentrations greater than 10,000 mg kg^{-1} are consistently reported at such sites. The migration of lead though the soil caused by water infiltration is of particular concern, as contamination of water supplies may occur. The objective of this study was to determine the potential for leaching of lead from soils contaminated with lead shot.

2. Materials and Methods

Three clay target shooting sites with differing soil textures were selected for the study. Intact lysimeter cores (68 mm diameter, 100 mm deep) were collected from two areas at each of the sites so that background and approximate maximum soil lead concentrations were obtained. Lysimeter casings and method of sampling were as described by Cameron *et. al.* (1992). The annular gap between the casing and soil core was sealed with warm liquid petrolatum to prevent edge-flow effects once leaching commenced in the laboratory (Cameron *et. al.*, 1992). The lysimeters were brought into the laboratory and a cellulose acetate/ acetone mixture was allowed to harden on the base of the cores before being removed in order to open any pores blocked due to smearing during core collection. The bottom of the cores were covered with acid-washed silica sand and a polyester mesh cover secured to the lysimeter base to prevent soil loss. The cores were wet up to field capacity and stored at 4°C until required.

The leaching experiment consisted of leaching one pore volume of distilled water every second day in order to allow for a re-equilibration period. The leachate was collected manually in fractions of one-tenth of a pore volume for ten leaching days. The leachates were analysed for pH, Pb (flame or graphite furnace AAS), cations (FAAS), anions (IEC) and soluble carbon (TOC analyser).

After leaching, the cores were removed from their casings and the soil cores cut into 20 mm depth sections. The soil was air-dried and sieved (2 mm), and any lead shot > 2 mm was removed and weighed. Total and EDTA-extractable Pb concentrations in the < 2 mm soil were determined.

3. Results and Discussion

Soil A, a sandy loam overlain by 30 mm of peat, contained approximately 50,000 mg Pb kg^{-1} soil (< 2 mm) and about 40% Pb as particulate Pb shot in the most contaminated area. The amount of Pb leached from the contaminated soil cores was notable. Up to 3.4 μg Pb mL^{-1} was measured in the leachate, as shown in Fig. 1. The concentration of Pb leached from soil B, a silt loam containing approximately 2,000 mg Pb kg^{-1} soil (< 2 mm) and approximately 5% particulate Pb, reached 540 ng Pb mL^{-1}. In comparison, there was little or no Pb in leachates collected from soil containing background concentrations of soil Pb.

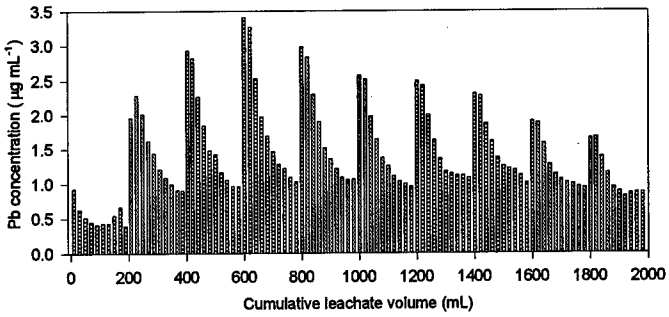

Figure 1. Leaching of Pb from Soil A.

The Pb concentration of the leachate from contaminated soil cores exceeded the 1992 EEC directive for Pb in drinking water of 50 ng mL^{-1}. The leaching of Pb from a recent, sandy soil will also be examined. The speciation of Pb in the leachate and methods of reducing Pb leaching will be discussed.

4. Conclusions

The results clearly show that substantial amounts of Pb are currently being leached from some clay target shooting sites. The potential exists for much greater concentrations of Pb to be leached due to the large amounts of Pb shot which have yet to oxidise and dissolve in the soil. Thus, there are serious implications where such large amounts of Pb are contained within the soil.

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LEACHING BEHAVIOR OF HEAVY METALS IN SURFACE SOILS DERIVED FROM DREDGED SEDIMENTS

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1. Introduction

In Flanders, about 4 million m^3 of dredged materials must be disposed off on land yearly (DEMOEN, 1989). Changes in redox conditions, pH and organic matter content of dredged sediments will affect the mobility of contained metals (GAMBRELL, 1994). Leachability data are needed to estimate potential effects of dredged sediment disposal on nearby ground and surface water quality. Potential and actual leachability of heavy metals in oxidized surface soils from abandoned upland dredged sediment disposal sites were assessed.

2. Materials and Methods

Surface soils were sampled from an uncontaminated (LG) and a relatively contaminated (MG) confined disposal site in the neighbourhood of the city of Gent (Belgium) (Table 1). According to the U.S.D.A. texture triangle, the sediments are classified as silt loam and silt clay, respectively. They were characterized by high level of carbonates (10 and 7.8%, respectively). Soil LG contained 0.5% of organic C, soil MG 5.5%. Leaching behaviour was assessed using a standard leaching method (VAN DER SLOOT et al., 1996). Potential leachability is defined as the amount of metals that is released at constant pH 4. Actual leachability is assessed by a column test to assess leaching at liquid to solid ratios (L/S ratio) varying from 0.1 to 10 and by a cascade shaking test for L/S ratios from 20 to 100.

3. Results and Discussion

3.1 *Total Metal Contents.* Total metal contents (Table 1) of soil LG were, except for Ni, within the ranges for unpolluted Flemish soils with a similar content of organic carbon and clay (TACK et al., 1996). Total contents for soil MG, in contrast, strongly exceeded these ranges by a factor 5 to 10.

Table 1. Total metal contents and potentially leachable amounts (mg/kg DM)

Soil		Cd	Cu	Pb	Ni	Zn
LG	Total	1.3	7.7	18	14	38
	Pot. Leach.	0.7	2.3	4.8	0.6	18.2
MG	Total	17	210	102	274	1450
	Pot. Leach.	7.5	16.4	22.6	6.3	713

3.2 Potential Leachability. The potentially leachable heavy metal contents (Table 1) represent a fraction that ever may become available for leaching (VAN DER SLOOT et al., 1996). Even in the contaminated sediment, potentially leachable amounts of Pb and Cu remained lower than baseline metal levels in Flemish soils. In contrast, leaching of Cd, Zn and Ni of the contaminated sediment MG potentially could result in a significant metal transfer to the surrounding environment.

3.3 *Actual Leachability.* The actual leachability test provides insight in the dynamics of metal release. The concentration levels in the cascade leaching test suggests that a release of metals in

the field can be expected to occur slowly, but steadily (Table 2). Metal concentrations in the leachates both from the contaminated and the non-contaminated soil were in the µg/l range. Concentrations in the leachates from the contaminated soil were significantly higher than these from the non-contaminated soil, but remained below maximum permissible levels in water intended for human consumption (SMEETS and AMAVIS, 1981).

Table 2. Trace metal concentrations (µg/L) in the fractions of the cascade shaking test

L/S ratio	Cd	Cu	Pb	Ni	Zn	Cd	Cu	Pb	Ni	Zn
	Soil LG					Soil LG				
20	0.5	9	0.7	3.6	49	3.3	39	0.6	45	129
40	0.1	5	0.2	2.5	13	0.7	37	1.1	12	33
60	0.1	4	0.4	2.5	13	0.5	22	1.2	10	29
80	0.2	3	0.2	2	12	0.6	20	2.4	9	27
100	0.2	4	0.2	2.1	15	0.6	13	1.2	7	38

4. Conclusions

Although the potential leachability test revealed the presence of a large mobile pool of Cd, Zn and Ni in the contaminated sediment, the actual leachability of all elements was very low. This is caused by the high amount of carbonates, that effectively buffered any acidity present in the leachate. The pH in the soil solution was thus maintained at neutral to slightly alkaline conditions, ensuring a low mobility of all heavy metals.

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SOLUBILITY OF NATIVE AND APPLIED MOLYBDENUM IN SURFACE COAL MINE DISTURBED SOILS

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1. Introduction

Molybdenum (Mo) is important in ecosystems as a micronutrient for both plants and animals. It can also accumulate in the environment in toxic concentrations. Certain land use activities may result in accumulation of Mo by plants in soils. As an example, during surface coal mining, which is an important land use in western United States, reduced soil material overlying the coal bed is removed and exposed to the atmosphere. This process will oxidize organic compounds and sulfides containing Mo and increase dissolved Mo in soil solutions. Plants grown on such soils may accumulate Mo and cause molybdenosis in animals. The objective of this study was to examine the potential solid phases controlling dissolved Mo in soil solutions under native and applied molybdenum conditions.

2. Materials and Methods

Two surface coal mine disturbed soil samples were air dried and passed through a 2 mm sieve. Sieved samples were used for subsequent experiments. Solubility study was conducted with the following treatments: (1) Control soil, (2) Hoagland's nutrient solution, (3) 1 mg Mo kg^{-1} of soil plus nutrient solution, (4) 3 mg Mo kg^{-1} of soil plus nutrient solution, and (5) 5 mg Mo kg^{-1} of soil plus nutrient solution. The Mo solubility study consisted of extraction and geochemical modeling of soil solutions. Fifty grams of each treated and untreated samples (1:3, solid to water ratio), in duplicate, were reacted with 150 ml distilled-deionized H_2O in 250 ml plastic bottles. After reacting for 21 days, soil suspensions were centrifuged and filtered using 0.45-ΦM millipore filters. Each extract was analyzed for pH, cations, and anions. GEOCHEM speciation model was used to predict the potential solid phases controlling dissolved Mo in soil solutions.

3. Results and Discussion

The saturated paste pH of soils ranged between 7.0 to 8.4 . The organic matter content was between 3.9 and 9.5 g kg^{-1}. Lindsay (1979) predicted that $PbMoO_4$ and $CaMoO_4$ as the most stable Mo solid phases in soils. The calculated ion activity products (IAPs) for $PbMoO_4$ and $CaMoO_4$ solid phases are plotted in Figure 1. The IAPs for control and nutrient applied samples were very close to the $PbMoO_4$ solid phase *Ksp* value of $10^{-16.0}$. These results suggest that IAPs for these samples approached saturation with respect to $PbMoO_4$ solid phase. Several studies have shown that $PbMoO_4$ solid phase could occur in semi-arid environments of western United States (Rosemeyer, 1990; Bideaux, 1990). For soil samples treated with 3 and 5 mg Mo kg^{-1} Mo, the IAPs suggested a very high supersaturation with respect to $PbMoO_4$. These results suggest that $PbMoO_4$ solid phase was not controlling the dissolved Mo in these samples. Next to $PbMoO_4$, $CaMoO_4$ is predicted as the most stable solid phase in soils. The calculated IAPs for $CaMoO_4$ solid phase suggested that samples treated with 3 and 5 mg Mo kg^{-1} Mo were near saturation with $CaMoO_4$ solid phase. A possible explanation is that additions of Mo to soils increased dissolved Mo concentrations in soil solutions above the saturation of $CaMoO_4$ solid phase, which probably resulted in the precipitation of $CaMoO_4$ solid phase.

The results in this study suggest that $PbMoO_4$ solid phase may control the dissolved Mo concentration in native and nutrients applied soils. When 3 and 5 mg kg^{-1} of Mo were added to the soil samples, the IAPs probably reach saturation with respect to $CaMoO_4$ solid phase. Eventually, dissolved Mo in alkaline soils may approach saturation with respect to $PbMoO_4$ solid phase, because this solid phase is most stable when compared with other Mo solid phases.

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DOWNWARD MIGRATION RATE IN SOILS OF HEAVY METALS AND ITS CONTROL BY SOIL PROPERTIES

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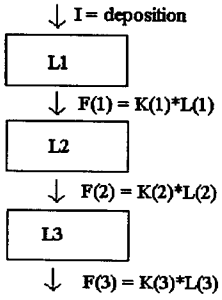
1. Introduction

The biogeochemical fluxes of heavy metals are strongly altered by human activities. A significant anthropogenic enrichement of several heavy metals is observed in topsoils of the Northern Hemisphere. The Smelter plant emissions are important sources of these metals.. This study is aimed at evaluating the downyard migration rate of Pb, Cd, and Cr emitted by a smelter plant and its control by soil properties.

2. Materials and Methods

The investigated area is near the town of Villadossola, NW Italy. Soils are developed on till and floodplain and are Entisoils and Inceptisoils. In eleven sites downwind from the emission, 5-cm-thick sample layers were separately collected to a depth of 20 cm. Main soil properties and concentrations of pseudototal Pb, Cd, and Cr were determined. The lithogenic concentration of the pollutants was estimated by average concentration in the rocks upstream the area, weighted by the surface of outcropping. The lithogenic portion of the metal content was subtracted to the total, in order to perform the calculation of the downward migration rates only on the anthropogenic one. The concentration data are expressed in mass/volume ($\mu g \ cm^{-3}$).
A box model was adopted to describe the vertical distribution and the migration of the metals in the profiles: each 5-cm-layer is represented by a compartment **L**, **I** is the input flux to the first layer (deposition rate) and a linear flux **F** from each layer to the underlying one is assumed. A schematic diagram of the model and the corresponding set of differential equations are:



$$\frac{dL_1}{dt} = -k_1 \cdot L_1 + I$$

$$\frac{dL_2}{dt} = -k_2 \cdot L_2 + k_1 \cdot L_1$$

$$\frac{dL_3}{dt} = -k_3 \cdot L_3 + k_2 \cdot L_2$$

When data on the time elapsed since the pollution event, or on the deposition, are available, and when a plausible assumption on the initial distribution is made, it is possible to calculate the values of k, that is a parameter related to the rate of migration and representing the inverse of the residence time of the element in the box (soil layer). The downward migration rate (V) within each layer can be easily calculated as:

$$V = (\text{thickness of layer n}) \bullet (k_n \text{ value})$$

In our case, concerning the emissions from a smelter, the athmospheric deposition occurred in a few months in the year 1990, therefore a simplified assumption of an instantaneous input was made, with total amount of deposited metal present in the first layer at $t = 0$. The samples were collected 7.5 years after the deposition.

Calculation was performed taking into consideration only the flux $F_{1,2}$, because of low values and high relative errors in the concentration data of deeper layers. The k_1 values obtained are therfore related to the migration rate of metal in the first layer.

The soil properties which affect the downward migration rates have been investigated by regression analysis.

3. Results and Discussion

The downward migration rates calculated in the soils ranged from 0.2 to 2.0 cm $year^{-1}$ for the three metals, the mean values are 0.83 ± 0.72 cm $year^{-1}$ for Pb, 0.60 ± 0.36 cm $year^{-1}$ for Cd, and 0.75 ± 0.56 cm $year^{-1}$ for Cr. These values show a high standard deviation and no significant difference between the downward migration rates of each element is observed. For the three metals, the downward migration rates were positively correlated to the fine sand content, suggesting that mobility of these metals is enhanced by high soil macroporosity. An inverse correlation was also found between the migration rate and the soil organic matter content. This indicates that organic matter is highly effective in retaining metals in the top horizons.

METALS SOLUBILITY IN TWO CONTAMINATED SOILS TREATED WITH LIME OR COMPOST.

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1. Introduction

Revegetation of soils contaminated with zinc, cadmium and lead as a result of former zinc mining and smelting industry activities is an environmental challenge. Revegetation must reduce water and wind erosion extending affected areas and minimize risk related to metals transfer to food chain. A successful method using a mixture of lime amendment and biosolids application for alleviation of metals phytotoxicity has been widely illustrated (Brown et al., 1998, Stuczynski et al, 1998).
This laboratory study was conducted to evaluate lime and biosolids compost effects on Zn, Cd and Pb solubility in two highly contaminated soils with different properties from near a zinc smelter at Palmerton, PA, and a mine waste contaminated area at Leadville, CO. Two questions were addressed: 1) Does high Zn inhibit reaction of limestone?; 2) Do differences in metal adsorbent levels in amended soils affect success of remediation?

2. Materials and Methods

Zn, Pb and Cd contaminated soils from Palmerton, PA, and Leadville, CO, were treated with a series of rates of fine limestone or lime- and iron-rich "Compro" biosolids compost to test the effect on metal sorption and solubility. Amended soils (150 g) were adjusted to approx. 60 % of water capacity and incubated for 21 days. Then soils were dried and analyzed for pH (1:2 soil:water ratio) and metals solubility (0.01 M strontium nitrate extraction, 10g/20 mL, 2 hr). Additionally, amorphous iron and manganese oxides were determined using acid ammonium oxalate extraction in darkness.

3. Results and Discussion

Leadville soil was severely acidic due to sulfide oxidation (initial pH=3.5) while Palmerton soil was slightly acidic (pH=6.5). Soils were similar in total Zn and Cd. Pb level was much higher in Leadville soil (Table 1). Both soils had little or no vegetative cover.

Table 1. Selected properties of studied soils.

Soil	Total metals concentration (mg kg^{-1})			Amorphous oxides (mg kg^{-1})		C (%)
	Zn	Pb	Cd	Fe	Mn	
Leadville	12167	5800	144	12360	108	2.6
Palmerton	13867	647	138	6720	2208	8.8

In both soils, extractable Pb was < 0.1 mg/kg even in unamended acidic soils. Increasing pH due to $CaCO_3$ application caused remarkable reduction of Zn and Cd extractability; however the data showed substantially different relationship between pH and Zn and Cd solubility for Leadville vs. Palmerton soil (Fig.1, 2) at similar pH. Metals sorption related to a higher level of "amorphous" iron oxides in Leadville soil (Table 1) might have caused these differences. Adsorption and/or occlusion in Fe oxides may be important factors limiting availability of heavy metals in remediated soils at higher pH levels. Biosolids compost treatment elevated amorphous Fe oxides concentration in

Palmerton soil (65% increase) that was reflected in lower concentrations of extracted Zn in Compro amended vs. unamended soils at comparable pH (Fig. 3).

4. Conclusions.

Liming and application of high Fe limed biosolids compost dramatically lowered Zn and Cd extractability in both soils. Higher amorphous Fe oxides caused strong reduction of metals solubility at higher pH levels. Such "Tailor Made" biosolids and composts are effective and economic supplements or substitutes for commonly used agricultural limestone to remediate soil Zn phytotoxicity of highly contaminated soils.

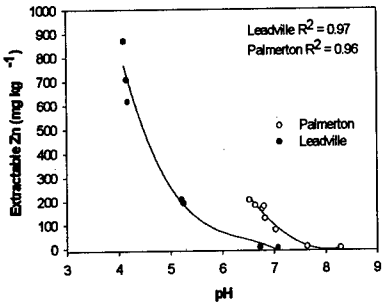

Fig. 1. Effect of pH and Zn extractability in soils in soils treated with increasing $CaCO_3$ amounts. amounts.

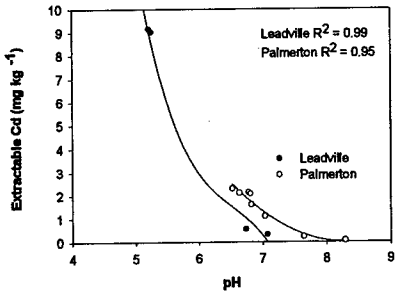

Fig. 2. Effect of pH on Cd extractability in soils treated with increasing $CaCO_3$

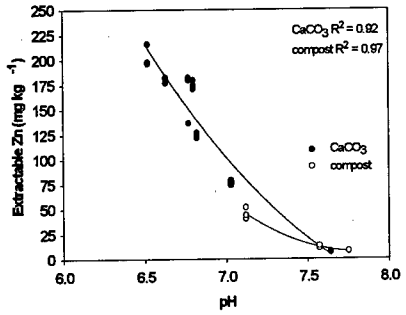

Fig. 3. Comparison of lime and compost effects on Zn solubility in Palmerton soil.

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LEAD DESORPTION AND REMOBILIZATION IN CONTAMINATED SITES INDUCED BY SOIL COLLOIDS

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1. Introduction

This study presents the results of intact soil column leaching experiments, which evaluated the potential of suspended ex-situ colloidal particles passing through macropores of contaminated soils to desorb Pb retained by the soil matrix and transport it to the groundwater.

2. Materials and Methods

The undisturbed soil columns represented upper soil horizons of a Typic Paleudalf and a Typic Argiudoll with contrasting macroporosity and organic carbon content. The ex-situ soil colloids were fractionated from low ionic strength Bt soil horizons with montmorillonitic, mixed, and illitic mineralogy, and variable physicochemical- and surface charge properties. The columns were contaminated with Pb by applying 300 pore volumes of 100 mg/L Pb solution to a breakthrough eluent Pb concentration of about 5 mg/L (30-40% saturation), and subsequently flushed with 300 mg/L of ex-situ colloid suspensions and D-H_2O (control). Eluted soluble and colloid-bound Pb (following HCl + HNO_3 extraction) was determined by AA spectrometry.

3. Results and Discussion

The results indicated a sharp decrease, to near zero, of Pb desorbed by deionized water-flushing solutions after 3 pore volumes of leaching, but a continuous desorption and transport of Pb in the presence of colloids up to fifty times greater than D-H_2O solutions (Fig.1). The colloid-induced desorption and remobilization of Pb was in the range of 40-60% of the initial eluent Pb concentration for the montmorillonitic, 20-30% for the illitic, and 15-20% for the mixed mineralogy colloids (Fig. 2). Colloids with high surface charge and soils with greater macroporosity (Paleudalf) contributed more to Pb desorption and transport. Mineral colloids with high surface charge were also more effective desorbers than organically-enriched colloids due to their higher binding energy. However, organically-enriched colloids caused greater Pb mobilization in the soluble fraction due to formation of organometallic stable complexes. The increase in Pb desorption and remobilization in the presence of colloids was observed in both, the soluble and the colloid eluted phase, suggesting involvement of ion exchange, complexation and physical exclusion mechanisms.

4. Conclusions

The findings of this study suggest that the presence of suspended ex-situ colloidal particles in flushing solutions may enhance Pb desorption and remobilization in contaminated soils. While this enhancement may increase the risk for groundwater contamination in some sites, it may also serve as a better remediation alternative in others, where the use of acids or chelating agents is not feasible. Therefore, a better understanding of the role of colloids in contaminant transport processes is essential in developing more effective groundwater contamination and remediation strategies.

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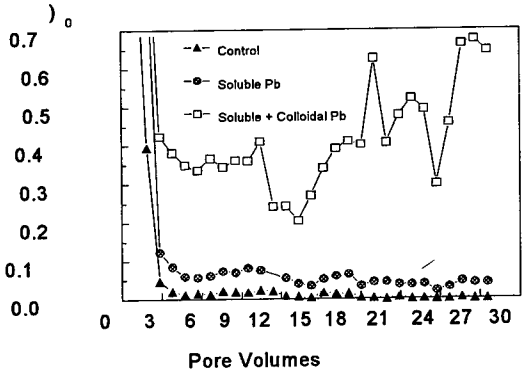

Fig. 1. Lead desorbed and transported by deionized water (control) and Beasley soil colloids (montmorillonitic) through undisturbed Maury (Paleudalf) soil columns.

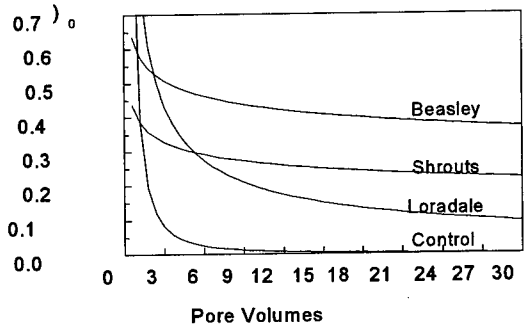

Fig. 2. Power function-fitted BTC's of soluble + colloidal Pb desorbed and remobilized from Maury soil columns by Beasley (montmorillonitic), Shrouts (illitic), and Loradale (mixed) colloids and by deionized water (control).

MOBILIZATION OF ZN AND CD IN THREE SWISS SOILS BY USE OF ELEMENTAL SULPHUR

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1. Introduction

A major limitation for phytoextraction of heavy metals is their limited availability to plants in soils high in pH and/or rich in metal adsorbing properties. It has therefore been suggested to increase the mobility of heavy metals by either adding chelates (Blaylock et al., 1997) or lowering soil pH (Wasay et al., 1998). Both methods, however, may be subject to both practical and legal restrictions. In the case of pH, large amounts of acidifying agents may be needed. Gaur et al. (1971) proposed the use of elemental sulphur to treat iron chlorosis in plants grown on calcareous soils. Elemental sulphur is oxidized by soil microorganisms, releasing protons to the soil solution. This process is commercially applied to leach low grade ores (Bosecker, 1994) and has recently been suggested to mobilize Cd in soils (Tichy et al., 1997). The aim of our study was to investigate the effect of an S_8 application on pH and mobility of Zn and Cd in 3 Swiss soils in batch and pot experiments.

2. Materials and Methods

In the batch studies, 10 g soil (<2mm) was mixed with S_8 (see Table 2), placed in Erlenmeyer beakers containing 100 ml H_2O_{deion} and shaken at 120 rpm at 25°C. In the pot experiments, soil was sieved to <1cm and was filled into 1.5 l pots. Pots were kept in a climatize chamber at 20°C/16°C,16 h/8 h per day, respectively. *Brassica juncea cv* 426308 was used as a test plant.

Table 1: Selected soil properties

Site	Soil type	pH	$CaCO_3$	C_{org}	Clay (%)	Silt	Sand	CEC_{pot} meq·100g^{-1}	Cd_{tot}	Zn_{tot}	Cd_{sol} mg·kg^{-1}	Zn_{sol}
Ziefen	Cambisol	7.2	6.4	3.3	27.2	53.6	19.2	58.2	7.8	130	0.004	n.d.
Dornach	Anthrosol	7.4	13.4	4.2	31.8	50.0	18.2	51.9	2.8	695	0.003	0.08
Rafz	Luvisol	6.8	0.6	1.6	18.4	27.6	54.0	16.9	0.9	813	0.007	5.8

3. Results and Discussion

Oxidation of S_8 and subsequent acid production in the batch experiments started after a short lag-period of approximately 48 hours. A decrease in pH was observed in the Rafz and Ziefen soil (Fig.1). In the S_8 treated Dornach soil, pH remained more or less constant. An increase in the control pH was also observed for the Rafz soil, but not in the vessels containing the Ziefen soil. Subsequent to the lower pH in the Ziefen and Rafz soil, Zn and Cd were released from the soil matrix, as can be seen in Fig. 2. An almost linear correlation was observed between proton concentrations and the two metals (data not shown). In the pot experiment, the decrease in pH observed was even more pronounced if the same amount of S_8 was applied than in the suspensions study (Tab. 2). Consequently, $NaNO_3$ extractable Cd increased almost 10 fold. Cd uptake by *Brassica juncea* also increased from 3.1 to 83.9 mg·kg^{-1} in the dry matter. However, growth limitations were observed in the 0.4 mmol·kg^{-1} treatment. The results indicate that elemental sulphur application is capable of lowering soil pH, as had previously been shown by Tichy et al. (1997). Surprisingly this was observed not only in weakly buffered soils, but also in the Ziefen soil, which has a higher pH and contains up to 7% $CaCO_3$. In contrast, pH in the Dornach soil was not affected by the S_8 treatment. Plant uptake of Cd could be increased 10fold compared to the control and to the results from a field experiment at the Ziefen site described by Felix (1997).

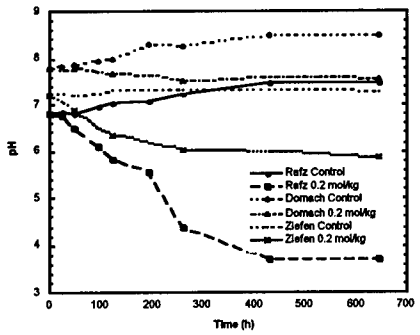

Figure 2: Zn and Cd (mg/l) in soil suspensions after elemental sulphur application

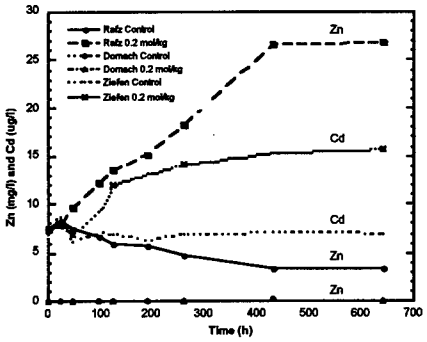

Figure 1: pH in three soil suspensions after elemental sulphur application

Table 2: pH and Cd concentrations in Ziefen soil and Cd contents in *Brassica juncea*

Treatment	pH(670 h)	Cd_{soil} µg·kg^{-1}	Cd_{plant} mg·kg^{-1}
Control	7.24	3.2	3.07
100 mmol·kg^{-1}	5.9	12.9	21.8
200 mmol·kg^{-1}	4.9	19.9	72.8
400 mmol·kg^{-1}	3.6	28.9	83.9

4. Conclusions

Elemental sulphur can be used to lower pH and subsequently enhance Zn and Cd bioavailability in soils with low buffer capacities. Practical limitations apply when S_8 is to be used on well buffered soils. Further research is needed to assess the processes involved in the release of Cd from the carbonatic Ziefen soil, which could not be ascribed to a low buffer capacity. In addition, the effects of S_8 applications on soil fertility and plant growth need further attention.

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CHROMIUM MOBILITY IN TANNERY CONTAMINATED SOILS: EFFECT OF SOLUTION COMPOSITION

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1. Introduction

In Australia, tannery wastes were commonly disposed onto agricultural land prior to the introduction of regulations prohibiting such disposals. This has resulted in extensive contamination of localised areas of agriculturally productive land. The presence of toxic hexavalent chromium [Cr(VI)] in runoff and subsurface water samples at the contaminated sites prompted us to investigate the soil and solution factors that influence mobilisation and transport of Cr in the contaminated soils. Since, tannery wastes contain elevated concentrations of P, S, Cl^-, Na^+ and Ca^{2+}, we investigated the role of these ionic species on the desorption of Cr in surface and subsurface soils from the contaminated site.

2. Materials and Methods

Soil samples were collected from the hot spots at the site, air dried and passed through a stainless steel 2-mm sieve and stored in plastic containers for further analysis. The effect of solution composition on desorption of Cr was investigated using background electrolytes containing either H_2O, Na, Ca, P or Cl ions. While the ionic strength of the solutions was maintained at approximately 0.03 mol dm^{-3}, the concentrations of P (as KH_2PO_4) was varied from 0 to 0.03M. The soils were successively extracted with electrolyte solutions for every 2 hours in an end-over-end shaker, centrifuged at 15000 rpm (26,000 rcf) for 15min and passed through a 0.45μm millipore filters for further analysis. Total Cr in soil solution was estimated using either Flame AA or Graphite furnace and Cr(VI) by modified 1,5-diphenylcarbazide (DPC) method (Bartlett and James,1979).

3. Results and Discussion

The amount of Cr desorbed varied substantially with the duration of equilibration and the depth of soil samples. The surface samples with pH values exceeding 7 showed an initial rapid release in Cr followed by a slow and continuous desorption. Although, the soils contain over 6% Cr, < 0.001% was desorbed in water. Nonetheless, the amounts desorbed exceeded the USEPA (1991) and NHMRC (1992) permissible concentrations for clean water by 5 to 20 times. The continued slow release of Cr in water extracts indicate the potential for slow but continued loading of subsurface soils with Cr in percolating water at the contaminated sites.

The strongly acidic subsurface soils released <10% of the total Cr desorbed from surface soils. This is not surprising given that the total Cr content of the alkaline surface soils was 30 times greater than the acidic subsurface soils. Speciation of the Cr in the soil water extracts shows that over 90% of the Cr in the surface soils is present as Cr(VI) while all the Cr in the subsurface soils was present as Cr(III). The amount of Cr desorbed varied with both the cationic charge and with depth of soil samples. In the surface soils, the desorbed Cr decreased with increasing cationic charge (water >Na^+ >Ca^{2+}) while the reverse trend (Ca^{2+} >Na^+ >water) was observed in the subsurface soils.

There was a strong effect of P (Na^+ + 0.003M P > Ca^{2+} + 0.003M P > water in surface soils and Ca^{2+} + 0.003M > Na^+ + 0.003M > water in the subsurface soil) on the cumulative amount of Cr desorbed compared to water and index cations Na^+ and Ca^{2+}. This was particularly true for P concentrations exceeding 10mg L^{-1}. The increase in Cr(VI) release in the presence of P in the alkaline surface soils is not surprising given the similarity in the sorption mechanism of the two ions. However, increased Cr(III) desorption by P in subsurface acidic soils is not clear and further work is being conducted to understand the nature of interaction between P and soil colloids in the subsurface soil environment.

4. Conclusions

Repacked column studies revealed significant mobilisation and leaching of Cr in long-term tannery waste contaminated soils using widely varying solution composition indicating potential for subsurface Cr contamination of soils at the tannery waste disposal sites. Highest Cr was released in the presence of phosphate solutions and least when Cl solution was used as the leaching electrolyte.

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EFFECT OF SALINITY ON THE HEAVY METALS MOBILITY IN CALCARIC FLUVISOLS.

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1. Introduction

In this paper a study on the space-temporal variability of chemical species in a calcaric Fluvisols zone, is carried out. The zone studied (about 60 Km^2) is mainly devoted to the cultivation of citrics. The soils located at the Vega del Segura (Region de Murcia, SE. Spain) have been cultivated during many centuries, the crop yields being so high that this zone has been named as Europe Garden. The traditional cultivation systems including culture rotations, made that, in spite the fact that the soils have been extensively cultivated for so long periods of time, no degradation processes or fertility losses have been noted for many years. However, during the last years, errors in the irrigation procedures, including the use of waste waters or waters with a high salinity, have produced a serious chemical degradation of the soil.

2. Materials and Methods

To carry out this study 17 samples were collected and, for comparison purposes, these were obtained from the same soils that were previously studied three years ago. The general characteristics of the arable soil layer were determined by the methods used to draw up a soil map of the area in the LUCDEME project (Alias et al, 1998; SOIL SURVEY STAFF, 1994) .

The total metal content (Zn, Pb, Cr and Cd) was determined by electrothermal atomization atomic absorption spectrometry (ETAAS) or flame atomic absorption spectrometry (FAAS) (Bautista et al, 1994) . To this purpose, the samples were slurried in a dilute hydrofluoric acid solution and the suspensions were directly introduced into the flame or the electrothermal atomizer.

The selective extractants used for the soil samples were: sodium dithionite + sodium citrate, M-J, (Mehra and Jackson, 1960), DTPA (Lindsay and Norvell,1978), HNO_3 0.1 M and $NaHCO_3$ 0.1 N (Olsen and Sommers, 1982).

3. Results and Discussion.-

Table 1.- Analytical data of soil samples

	% Ca CO_3 equiv.	% O.M 1993	% O.M 1996	% Sand	%Silt	%Clay
Average	39,8	1,8	1,6	9,0	56,3	34,8

Our data indicate that errors in the cultivation procedures are the responsible of the chemical degradation of the soils. The use of high salinity waters has produced a high salinization velocity and even a high alkalinization which is specially severe in some sampling points (Figure 1) (FAO, 1984; FAO, 1988).

In addition, there is a loss in the soil fertility, since the levels of some assimilable elements (Fe, Mn, Zn and Cu) has decreased considerably, the plant assimilation of heavy metals such as Pb and Cd being increased. The heavy metals mobilization is especially severe for the more degradated soils.

Table 2.- Variation of Bioavailability Metal ($mg*Kg^{-1}$)

	Fe		Mn		Cu	
	1993	1996	1993	1996	1993	1996
Average	2,0	0,8	3,7	3,3	1,9	1,6

Table 3. Mean values obtained from soil samples (soluble extracted x 100/total)

	Total $mg*Kg^{-1}$		HNO_3		DTPA		HCO_3^-		M-J	
	1993	1996	1993	1996	1993	1996	1993	1996	1993	1996
Zn	130	135	33	35	3	2.6	2	2	25	28
Pb	54	60	9	9	20	25	<0.1	<0.1	8.5	9
Cr	55	54	6	8	<0.1	<0.1	<0.1	<0.1	5	7
Cd	0.5	0.7	80	80	10	12	<1	<1	50	60

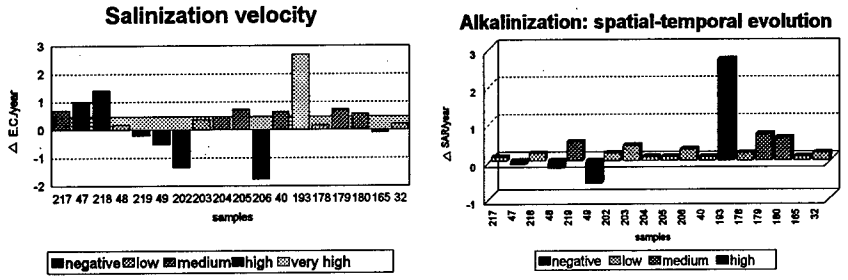

FIGURE 1

4. Conclusions

The prolongued use of high salinity waters for irrigation and subsequent salt accumulation affects the heavy metal availability, the effect being more pronounced for lead and cadmium. A possible explanation lies in the formation of chloro-complexes of these metals, the mobility being in this way increased.

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MOBILITY AND SPECIATION OF METALS IN THE STEPPE-ZONE SOILS

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1. Introduction

Transformation of heavy metals in soils depends on the edaphic characteristics, the chemical characteristics of metal introduced and its concentration in soil. This research is aimed at examining Zn, Cu, and Pb mobility and speciation in steppe-zone soils exposed to different stages of pollution.

2. Materials and Methods

Calcareous chernozem and chestnut soil were the objects of the research, whose properties are presented below (Table 1).

Table 1. Physico-chemical properties of the soils studied

Soil	Humus, %	□□□O_3, %	Exchangeable cations, mmol/kg			pH	Clay, %
			Ca	Mg	Na		
Calcareous chernozem	3.9	1.44	560	200	1	7.6	61.3
Chestnut soil	2.6	0.10	520	140	24	7.2	50.9

The model-based experiment was carried out over two years. The experiment dealt with spiking the chernozem and chestnut soils with Zn, Cu, and Pb-acetates in a variety of doses applied both separately and cumulatively taking into account the metal background level in the soils. Barley was the culture being examined. Metal forms were determined according to the methods offered by Solovyov (1) (Table 2) and Tessier et al (2).

Table 2. The Solovyov's method

Reagent	Extractive forms	Extraction time	Soil to reagent
NH_4OAc, □□ 4.8	Exchangeable and dissoluble by weak acids (actual stock of HM)	18 h	1:5
NH_4OAc+ 1%EDTA, □□ 4.8	Bound to organic complexes + extracted by NH_4OAc	18 h	1:5
1M HCl	Bound to amorphous compounds and carbonates (potential stock of HM)	1 h agitation	1:10

Defined fractions by Tessier procedure were marked as: exchangeable (exch), bound to carbonates (carb), bound to Fe and Mn oxides ((Fe+Mn)ox), bound to organic matter (org) and residual (res). Total metals contents were determined.
Analysis of metals in extracts was made using an AAS.

3. Results and Discussion

The model experiment proved that there was a decrease in the actual Pb stock, Pb amount dramatically increased in EDTA in the second experimental year. This may be explained by the

increase in the reactive potential of Pb to interact with the humus and high adsorption by the soil. In comparison with the control sample, at contamination, a relative amount of Pb forms increased more dramatically in chestnut soil, than in chernozem, which is due to their physico-chemical properties.

It was noted that very low contents of the most mobile forms of Cu occured. Cu was extracted by EDTA and HCl mainly, at maximum dose – by EDTA mainly. It is accounted for by the great affinity of Cu to organic matter.

NH_4OAc and HCl extracts contain large amounts of Zn. This may be due to Zn-adsorption by the soil where the main part is played by mechanisms facilitating storage of Zn forms, both exchangeable and carbonate-relating.

At cumulative metal application, some specific features of Zn, Cu, and Pb only clearly manifested themselves during the second year. Now Cu content and, especially, Zn content prevailed in HCl extracts, while Pb prevailed in EDTA. This may be due to Pb ability to form complexes that results in Cu and Zn displacement out of its complexation with organic substances, which finally occupied other adsorption positions. In polymetal contamination, the contents of some more mobile forms of Zn, Cu, and Pb increased in NH_4OAc. According to their ability to be adsorbed by organic substances, cations may be distributed as follows: Pb > Cu > Zn. Carbonates and Fe and Al hydroxides adsorbed the metals as follows: Zn > Cu > Pb.

To determine the Zn and Pb fraction content in soils (2), we discovered in the same model-based experiment that Zn and Pb forms in the soil may depend on their chemical nature and concentration in the soil. The Pb pattern was as follows: on control variant $Pb_{res}(83,5\%) >> Pb_{org}(8,8\%) > Pb_{(Fe\text{-}Mn)ox}(4,6\%) > Pb_{carb}(2,0\%) > Pb_{exch}(1,1\%)$; under contamination $Pb_{res}(68,9\%) >> Pb_{org}(13,9\%) > Pb_{(Fe\text{-}Mn)ox}(9,5\%) > Pb_{carb}(4,8\%) > Pb_{exch}(2,9\%)$.

The Zn pattern was different: on control variant $Zn_{res}(76,4\%) >> Zn_{(Fe+Mn)ox}(9,3\%) > Zn_{carb}(7,2\%) > Zn_{org}(5,1\%) > Zn_{exch}(2,0\%)$; under contamination $Zn_{res}(49,1\%) > Zn_{(Fe+Mn)ox}(27,0\%) > Zn_{carb}(13,5\%) > Zn_{org}(7,2\%) > Zn_{exch}(3,2\%)$.

4. Conclusions

1. Some specific features of Zn, Cu, and Pb clearly manifested themselves during the experimental second year in chernozem and chestnuts soils.
2. There was higher extracting ability of EDTA for Cu and Pb, and HCl for Zn.
3. In polymetal contamination, the contents of more mobile forms of Zn, Cu, and Pb increased.
4. Zn tendet to accumulate in the exchangeable forms, Cu and Pb formed stronger complexes with organic substances.

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MOBILITY OF CU IN SOIL COLUMNS AS AFFECTED BY THE PRESENCE OF THE PESTICIDE GLYPHOSATE

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1. Introduction

Glyphosate (N-[phosphonomethyl]glycine) (GPS) is a non-selective, postemergence herbicide with a very broad spectrum, being widely used in agronomic and vegetable crops, and in orchards.
GPS has three groups (amine, carboxylate, phosphonatė) that can form strong coordination bonds with metal ions, particularly with the hard transition metal ions (MORILLO et al., 1997). This ability places GPS in an almost unique class of strongly chelating herbicides. The effect of chelation of Cu and other metals on controlling solubility and biological activity of GPS may have some significance in the soil environment. Furthermore, the results may be of interest on the possible role of GPS in altering the solubility of trace metals in soil.
Therefore, the aim of this work is to study whether the mobility of Cu in soil columns can be affected by the presence of the pesticide glyphosate.
Cu has been selected due to its extensive use for agricultural purposes as a fungicide, being frequently added together with the pesticide GPS, and also due to its capacity for complex formation.

2. Materials and Methods

The soil used was a sandy soil from Huelva (SW Spain), before (SR) and after having been amended with urban waste compost (RSU). Column experiments have been performed to study the mobility through these soils. Experiments in handpacked soil columns have been carried out under saturated flow conditions. Columns were constructed from PVC pipe and were 15 cm long x 5 cm inner diameter. They were packed with 150 g of dry soil. Columns were conditioned passing through them about 200 mL of 0.01 N $Ca(NO_3)_2$ solution, using a flow rate of 1 ml/min controlled by a peristaltic pump. A solution (60 mL) containing 60 ppm of Cu alone or 60 ppm of Cu and 100 ppm of GPS (simultaneous addition) was added to the top of the column and, immediately, 0.01 N $Ca(NO_3)_2$ solution was employed as eluent at a flow rate of 1 ml/min. Other experiments have been carried out treating previously the soil with GPS solution and later with Cu (successive addition). Column leachate was collected using a fraction collector and Cu was determined. In all cases, a GPS solution was passed through the soil column after each experiment in order to desorb the residual Cu remaining in the column.

3. Results and Discussion

Both soils, SR and RSU, retained completely the Cu when applied alone, and 1 L of 0.01 N $Ca(NO_3)_2$ solution was unable to mobilize any significant portion of the Cu retained. After that, more than 85% of the Cu retained was removed from both soils by passing through them a GPS solution (figures a and b).
When Cu was added simultaneously with GPS, part of the Cu applied to the column was eluted when $Ca(NO_3)_2$ solution was passed through the soils (about 35.5% for soil SR (figure c) and 18.8% for soil RSU (figure d)), indicating that the soil which has received urban waste compost retains Cu more strongly than the untreated soil.
If the soils are previously treated with GPS solution before Cu application, the percentage of Cu desorbed when 1L of 0.01 N $Ca(NO_3)_2$ was passed through the soils was 25.7% for soil SR and 4.23% for soil RSU (figures e and f, respectively), showing again the higher tendency of soil RSU for retaining the metal. The difference of percentages of Cu desorbed in this experiment is also due

to the higher facility of GPS to be desorbed from the soil SR than from RSU, as it has been previously observed in batch adsorption experiments, so there is more GPS in solution to form complexes with Cu and therefore it is less adsorbed to the soil. In both soils, 1.5L of 100 ppm GPS solution was passed through the columns (second part of figures e and f), extracting 86.7% of GPS from soil SR and 89.3% from soil RSU.

4. Conclusions

The addition of urban waste compost to the soil increased the Cu adsorption capacity. The Cu retained by the soils can not be removed passing an electrolite solution through the soil column, but it is removed to a great extent by passing a GPS solution. This is probably due to the formation of very strong Cu-GPS complexes in solution that have lower tendency to be adsorbed on the soils than free Cu. It indicates the high extracting power of this pesticide, showing it's potential in remediation of metal contamination of soils.

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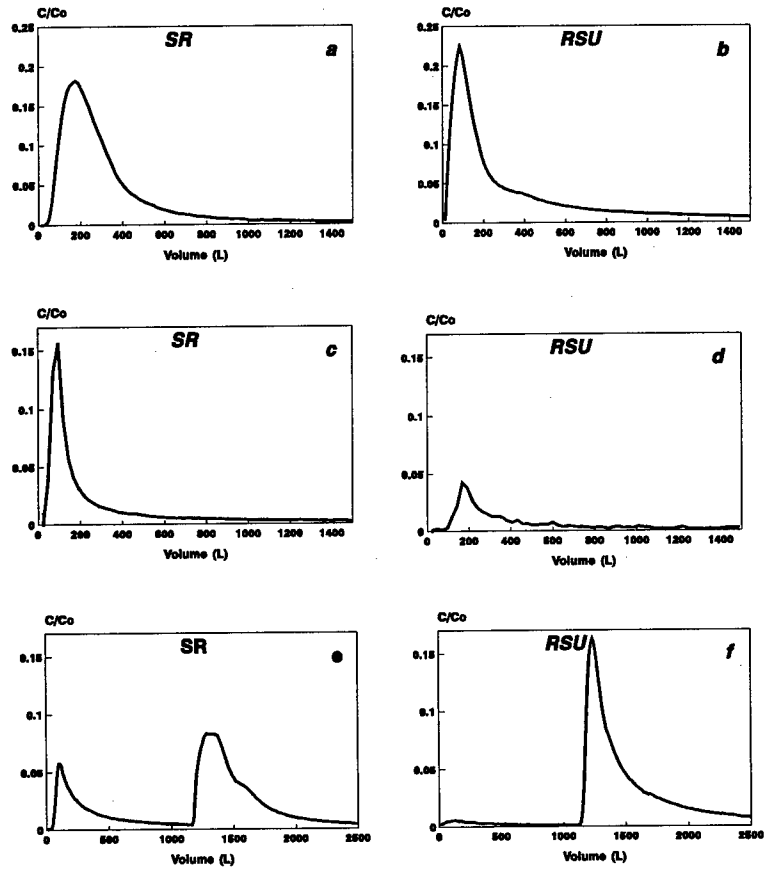

MOBILITY AND AVAILABILITY OF MICROPOLLUTANTS IN CALCAREOUS SOILS

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1. Introduction

In order to monitor the *dynamics of micropollutants*, their transformation into different forms, retention within an ecological system, and transfer through biological pathways, a food chain study has been initiated based on long-term field trials with toxic elements on calcareous loamy chernozem and calcareous sandy soil. (KÁDÁR, 1994, 1995). The *AAAc-EDTA-extraction* is widely used *to assume available (mobile) fraction* of trace elements. In spite of the observed phytotoxic effects and significant plant uptake of heavy metals, weaker extractants, i.e. distilled water and 0.01 mol/L $CaCl_2$, was not effective to indicate their available fraction on calcareous soils, beacuse they dissolved the heavy metals only in an amount under the detection limit from the soils of our experiments (KONCZ et. al., 1994). So, the purpose of this paper is to study the behaviour of potentially toxic elements in calcareous soils, to assess their availability and mobility, as well as the change in their retention through years.

2. Materials and Methods

Long-term field trials was set up at Nagyhörcsök Experimental Station on calcareous loamy chernozem soil in 1991 and at Őrbottyán Experimental Station on calcareous sandy soil in 1994. Four levels (doses: 0, 90, 270, 810 kg ha^{-1} on chernozem, 0, 30, 90, 210 kg ha^{-1} on sandy soil) of the selected water soluble salts of potentially toxic elements were added to plots and mixed into the ploughed layer. The treatments were arranged in a split-plot design with replications. The experimental plots were cultivated with commonly used agrotechnics, chemical fertilizers were added yearly to ensure sufficient macronutrient supply, and different crops were grown each year. Composite soil samples consisting of 20 subsamples were collected yearly from the ploughed layer of each plot. To check the vertical movement of these pollutants, samples were also taken from the subsoil of plots with the highest level of added metal salts in 1996. The "total" amount of the elements in homogenized soil samples were measured after microwave digestion with cc. HNO_3+H_2O_2 in some years, and the so called "mobile" fraction extracted with AAAc-EDTA (LAKANEN AND ERVIÖ, 1971) was also determined yearly. The composition of extract was measured using inductively coupled plasma spectrometry (ICP- AES) detecting 25 elements.

3. Results and Discussion

The values of "total" amount determined using microwave digestion with the mixture of concentrated nitric acid and peroxide reflected more or less the expected amount of added elements to experimental plots. Since the applied digestion totally destroys organic material in samples, high recovery of examined elements was assumed. In spite of the uncertainities in the obtained "total" parameters, it became clear that this method solute different fractions of different heavy metals and its effectiveness can be changed even with rising loads.The value of AAAc-EDTA extracted per "total" amount of heavy metals clearly indicates the rate of this "available" fraction. Chromium showed rather small available fraction rate. (2-3%), while nearly

the whole amount of Cd in soil was found to be available (80-100%). This ratio found to be low in case of Hg, Ba (10-20%), moderate in case of As, Ni, Se and Zn (below 50%), higher in case of Cu, Pb, Sr (up to 80-100%) with very wide range. Assessing the "available" fraction of an applied toxic element, it was learned that the extractability rate increased significantly with higher treatment levels. This was observed in case of each examined micropollutant except Mo.
The field trial on calcareous chernozem was initiated nearly a decade ago in 1991 and the AAAc-EDTA extractable fraction was determined in most years.This fraction is proved statistically to decrease through years in case of Cd, Cr, Hg and Mo. The rest of the examined toxic elements showed neither rising nor lowering trend. It must be mentioned that the observed reduction in extractability may have been caused partly by leaching, but checking the movement of applied heavy metals into subsoil, the concentration of only Se, Sr, Mo and slightly Hg were found to raise over the background concentration in lower soil layers. In case of sandy soil similar conclusions could be drawn.

4. Conclusions

The soil tests of two field trials with potentially toxic heavy metals on calcareous chernozem and sandy soil gave meaningful results to characterise the behaviour of these elements in soil of croplands through years. The observed change in AAAc-EDTA extractability means that presumably there is difference not just between the different applied micropollutants, but even between the different loads of the same heavy metal in distribution of their total amount among its possible fractions in soil. The decrease of this fraction with time in case of some heavy metals may have been caused by their continuous redistribution of added amount among fractions with different chemical characteristic and resulted in their increasing retention. . Under the slightly alkaline condition of both calcareous soils leaching could effect only the two toxic elements added to soil in anionic form.

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STRONTIUM, CESIUM, AND CHROMATE TRANSPORT IN ZEOLITIZED TUFFS FROM THE NEVADA TEST SITE, NEVADA, U.S.A.

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1. Introduction

During the period of nuclear weapon testing, the U.S. conducted a series of tests at several sites. These tests resulted in contamination from radionuclides and other toxic metals and organic compounds. Assessment of migration potential for these contaminants from controlled areas to the accessible environment is based on flow and transport models. These models must account for any contaminant retardation relative to the groundwater by incorporating geochemical interactions of contaminants with aquifer materials. Sorbate-sorbent interactions have been frequently incorporated in transport models through the use of equilibrium distribution coefficients, K_d. Nonequilibrium sorption, however, has been reported in a number of studies, both in the field and in the laboratory (BRUSSEAU et al., 1991; MA and SELIM, 1997). The scope of this work was to determine retardation factors from sorption of cations and anions in columns of zeolitized tuffs from the Nevada Test Site (NTS) as a function of geochemical parameters, specifically, pH and ionic strength. Two model cations, strontium (Sr) and cesium (Cs), and one anion, chromate (Cr^{VI}), were used to model the behavior of cations and anions with different sorption affinities for different types of sorption sites. The obtained breakthrough curves were used to determine the degree of nonequilibrium.

2. Materials and Methods

Experiments were conducted with zeolitized tuff particles having diameters between 2.85 and 4.00 mm. The true density of the material was 2.32 g/cm^3. The intraparticle porosity was 0.58. Bulk densities and interstitial porosities were determined specifically for each column. The specific surface area of the tuff was 8.13 m^2/g, suggesting that the majority of the surface area was internal and accessible. Clinoptilolite and feldspars were the predominant mineral phases, based on x-ray diffraction (XRD). All experiments were conducted in 15-cm glass chromatographic columns with inner diameter 1.5 cm. The wet packing method was used for packing all columns. The metal concentration was either 10^{-5} or 10^{-4} M. The velocity was regulated by a constant head reservoir. All reagents used were of ACS reagent grade quality. The samples were analyzed by atomic absorption spectroscopy.

3. Results and Discussions

Experiments were conducted with solutions of different ionic strengths ranging from 0.001 to 1.0 M at different pH values. The breakthrough curves (BTC) obtained were analyzed using CXTFIT, a CDE model including equilibrium and nonequilibrium sorption (PARKER and VAN GENUCHTEN, 1984). For the anion, the retardation factors obtained were approximately 1.2, indicating very low retardation, regardless of pH and ionic strength, consistent with batch equilibrium studies showing very little anion sorption on this material.
The BTC of the cations, Cs and Sr, were strongly ionic strength dependent. At low ionic strength, breakthrough did not occur even after 2000 pore volumes. At higher ionic strength, Cs

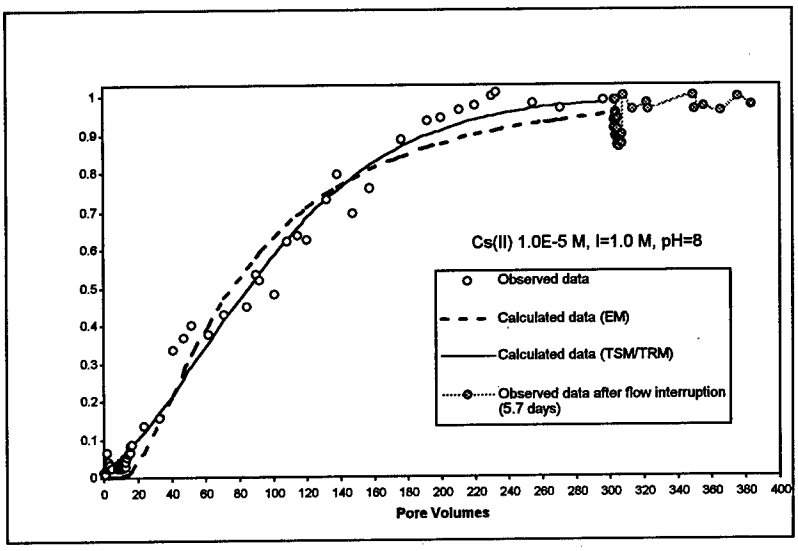

Figure 1. Breakthrough curve of Cs; I = 1.0 M; pH = 8.1

and Sr cannot compete with Na for the internal cation exchange sites of the zeolites and breakthrough occurs faster. Under these conditions, the Cs retardation factor obtained was approximately 80 (Fig. 1). Under similar ionic strength conditions, (I=1.0 M) Sr showed essentially no retardation, consistent with results obtained from batch equilibrium experiments. To test for nonequilibrium sorption, the flow was interrupted after breakthrough occurred. The data shown in Fig. 1 are consistent with nonequilibrium Cs sorption under these flow conditions. Qualitatively similar results were obtained with Sr.

4. Conclusions

Column experiments were conducted to study the sorption of two cations, strontium, and cesium, and one anion, chromate, on zeolitized tuffs from the NTS. Experiments were conducted as a function of pH and ionic strength. Breakthrough curves were obtained and the data were modeled using the CXTFIT model. The retardation of cations was substantially higher than the retardation of anions, in agreement with reported cation and anion exchange capacities for zeolites. Retardation factors were obtained using both the equilibrium and two-site/two-region model. The retardation factors ranged from 1.13 for Cr to more than 2000 for Sr, depending on geochemical conditions. Cesium was more strongly retarded than Sr. Interpretation of data obtained using the flow interruption technique suggests that sorption nonequilibrium was not a significant factor in anion sorption but it affected cation sorption.

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EVIDENCE CONCERNING THE RECYCLING OF PARTICULATE MATTER DUE TO RAIN AND VEHICLE CIRCULATION

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1. Introduction

Santiago, Chile, is a highly polluted city mainly due to their particular meteorological and geographical characteristics and to an explosive increasing number of motor vehicles, buildings, and industries. According to epidemiological studies, gases and particulate matter <10 μm (PM10) and its chemical composition are the principal pollutants especially affecting children, old people and sufferers of respiratory diseases. Previous studies have shown that a great percentage of the PM10 comes from the soil. Using the criteria of Enrichment Factor (EF criteria) against local soil [1] to determine origin of the elements, this research intends to show some evidence about the effect of the resuspended soil, especially after a rain event.

2. Materials and Methods

A monitor for particulate matter PM10 in real time with a system for chemical analysis (TEOM/ACCU system) was used [2]. Samples of 24-h, 12-h and 6-h over Teflon filters of 47 mm were taken. Sampling was carried out from May to July 1996 at the top of Calán Hill (864 m above sea level) at Santiago, in the direction of the winds coming from downtown during the day and returning to downtown during the night. The period includes one rain event during a pollution episode (PM10 values over standards).

Fifteen elements were analyzed using AAS (Mg, Fe, Cu, Zn and Pb) and ICP/MS (Na, Ca, Al, Cr, Mn, As, Sn, V, Ba and Ni) after acid digestion in a microwave oven. Also some secondary ions produced by transformation of the gases NH_3, SO_2 and NO_x using IC (NH_4^+) and CE (NO_3^- and $SO_4^=$) were quantified

3. Results and Discussion

A rain event is classically considered as an effective method to wash the atmosphere (the effectiveness depends on many factors). Normally, when rain begins the authority responsible for the control of atmospheric pollution in Santiago stops vehicle restriction during the episode of pollution. Figures 1-2 (concentrations for selected chemical species sampled every 6 hours: before, during and after the rain) show that this statement is true for compounds related to visibility ($(NH_4)_2SO_4$ and/or NH_4NO_3) which rapidly decrease, but it is not so for some elements which do not always decrease or do not do so in the same proportion. A rapid increase of some elements is also observed soon after the rain stops falling. Using the EF criteria against terrestrial crust and against local soil[1] the origin of the elements was determined as follows: Na, Ca, Al, Mg, Mn and Fe, of natural origin; Ni, V, Ba, As, Sn, Cu and Pb, anthropogenic; Cr and Zn, with a mixed origin.

Figures 3-4 show much greater values for Zn, Cu and Pb when the rain period and some hours after are included which in turn means an increase in motor vehicle circulation traffic.

4. Conclusions

Rain it is not a good solution for decreasing atmospheric pollution. The soil dust and/or the PM10 removed and re-injected to the atmosphere by motor vehicles containing much more anthropogenic elements and species derived from the washing of the atmosphere can increase the concentrations of some dangerous elements, bringing about more problems to human health immediately after the rain.

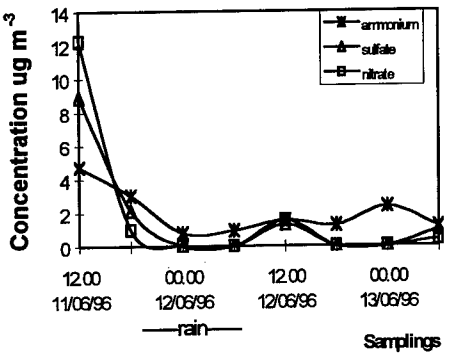

Figure 1. Ions in atmospheric wintertime aerosols

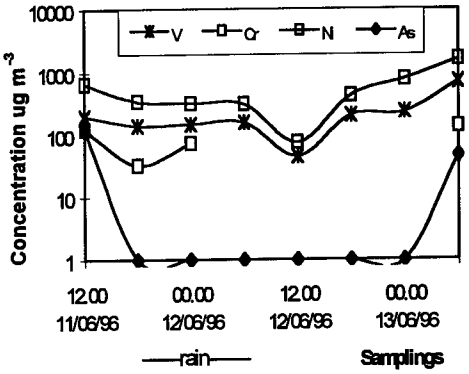

Figure 2. Anthropogenic elements in aerosols

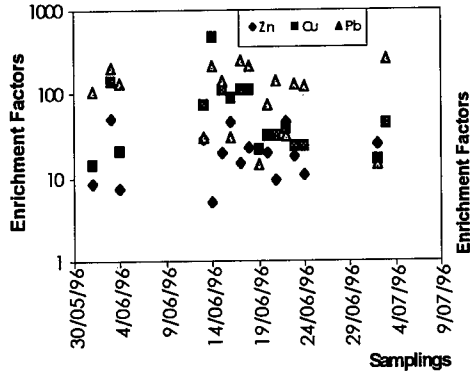

Figure 3. Afternoon samples of wintertime aerosols

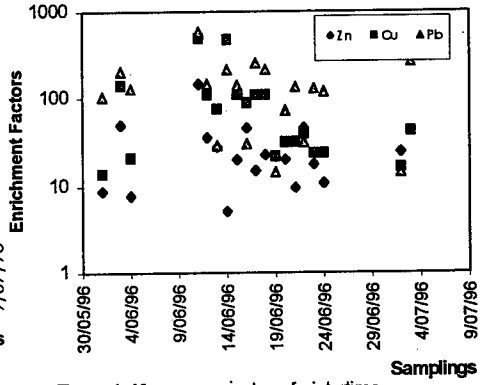

Figure 4. Afternoon samples of wintertime aerosols, including a rain period

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CHARACTERIZATION OF ARSENIC IN SOIL FROM THE LÖCKNITZ SITE USING SYNCHROTRON X-RAY ABSORPTION SPECTROSCOPY

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1. Introduction

Chemical speciation is one of the principal factors affecting the mobility, bioavailability, and treatability of potentially toxic soil contaminants at hazardous waste sites. Preliminary results are presented which used x-ray absorption spectroscopy (XAS) to characterize different forms of organic and inorganic arsenic species in soil from the Löcknitz site in eastern Germany, which is contaminated with an arsenic-based chemical warfare agent - Clark I. Analyses were conducted on contaminated soil samples from the site, and on samples of uncontaminated soil that were reacted with various forms of arsenic (e.g., phenyl arsines or m-arsenite). Shifts in the x-ray edge energy were used to determine changes in oxidation state of the arsenic.

2. Materials and Methods

Two soil samples (BR1D3 and BR1E4) containing high levels of As (196 and 440 mmol/kg, respectively) were collected in one region of the site. A soil sample uncontaminated by As but typical of the soil in the rest of the site was also collected for the spiked soils studies. Standard compounds used included sodium m-arsenite, triphenylarsine, and triphenylarsineoxide. These samples were diluted using BN to give arsenic concentrations of 50 mmol/kg for the XAS measurements. Spiked soils samples were prepared by adding known amounts of the latter three standard compounds to the uncontaminated soils from Löcknitz. The concentration ranges for the spiked samples were 2-4 mmol/kg of As.) XAS measurements were carried out on beam line X-11A at the National Synchrotron Light Source at Brookhaven National Laboratory. Measurements were made at the As K edge in the fluorescence mode using a 13 element Ge detector.

3. Results and Discussion

All of the spiked samples showed some changes as evidenced by changes in the near edge structure (XANES) as shown in figures 1 and 2. In the case of the sodium arsenite and triphenylarsine, there appeared to be some oxidation as evidenced by a shift of the main absorption edge to higher binding energy. It can not be ruled out, particularly for the triphenylarsine, that this oxidation is induced by the x-ray beam. In all of the spiked samples an enhanced absorption appears at the edge. For arsenic compounds, this has been found to be due to an adsorbed complex being formed. The two contaminated soil samples also show large white lines and an edge position consistent with As(V) (fig. 2) . They are generally similar in appearance to the sodium arsenite spiked soil. Some differences in the height and width of the white line are apparent between the two samples.

Fig. 1 The As K edge XANES for (A) triphenylarsine, (B) triphenylarsineoxide, (C) triphenylarsine spiked soil, and (D) triphenylarsineoxide spiked soil.

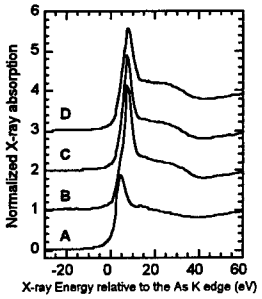
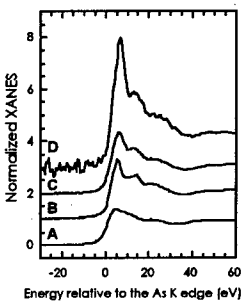


Fig. 2 The As K edge XANES for (A) sodium m-arsenite, (B) arsenite spiked soil, (C) Soil BR1D3, and (D) Soil BR1E4



4. Conclusions

Preliminary studies of arsenic contaminated soils from the Löcknitz site along with soils spiked with standard arsenic compounds have shown that arsenic compounds react when in contact with the soil, generally forming an adsorbed complex. In the two soils samples studied, the arsenic was present as As (V) and appeared to be part of an adsorbed complex. These results suggest that oxidation-reduction reactions in the contaminated soil may transform arsenic into less mobile or less hazardous forms.

Modelling and Prediction of TraceElements

(Technical Session 11)

DELINEATION OF SELENIUM CYCLING AND ECOSYSTEM EFFECTS WITH FIELD RESEARCH AND COMPUTER MODELING

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1. Introduction

Selenium (Se) is a naturally occurring trace element that is essential for a variety of microbial, plant, and animal life forms. In animals Se can be toxic at tissue concentrations <2 orders of magnitude greater than the essential minimum. In aquatic ecosystems Se is bioaccumulated by primary producers and passed to higher trophic levels RIEDEL et al. (1996). Toxic effects for fish include lethality, decreased reproduction, and teratogenesis GILLESPIE and BAUMANN (1986). Severe declines in fish numbers and fish community diversity have occurred in reservoirs and lakes receiving industrial effluents containing elevated Se concentrations LEMLY (1985). The study reported here was a comprehensive attempt to identify and model the pathways and fates of Se discharged to a reservoir receiving effluent from a fly ash settling pond serving a coal-fired power plant. The Selenium Aquatic Toxicity Model (SeATM) has served as a framework to help focus experimental research and integrate results with data from the literature. The model framework consists of five interactive modules that simulate biogeochemistry, pharmacokinetics, trophic transfer, toxic effects, and ecosystem effects of Se in lentic sytems. The pools and pathways identified and examined in the research program are depicted in Figure 1.

2. Materials and Methods

The experimental approach was developed in concert with model development. The mix of field and laboratory experiments elucidated Se speciation through biogeochemical processes in sediments and the water column CUTTER 1992. Bacterial and algal uptake rates and transfer to primary consumers and higher trophic levels were evaluated for each of the dominant Se species [Se(IV), Se(VI), Se(0), Se(-II), Se-organic] using radiotracer techniques. The model was field tested in a case study of a hydrologically complex, Se-contaminated reservoir in North Carolina, USA.

3. Results and Discussion

Field experiments revealed that Se(IV) dominated speciation in the water column except during periods of hypolimnetic anoxia when fast reduction reactions converted Se(IV) and Se(VI) to insoluble elemental Se(0), which accumulated in sediments. During a 5-year period, >50% of industrial Se loadings (mostly selenite) were deposited in sediments, with ca. 45% flushed through with outflows, and 5% lost via volatilization BOWIE et al. (1996). Decomposition of deposited algal detritus was the major Se removal pathway from sediments. The model favorably predicted Se dynamics in the food web, speciation in the water column and sediments, and the long-term fate of Se loadings consistent with monitoring data.

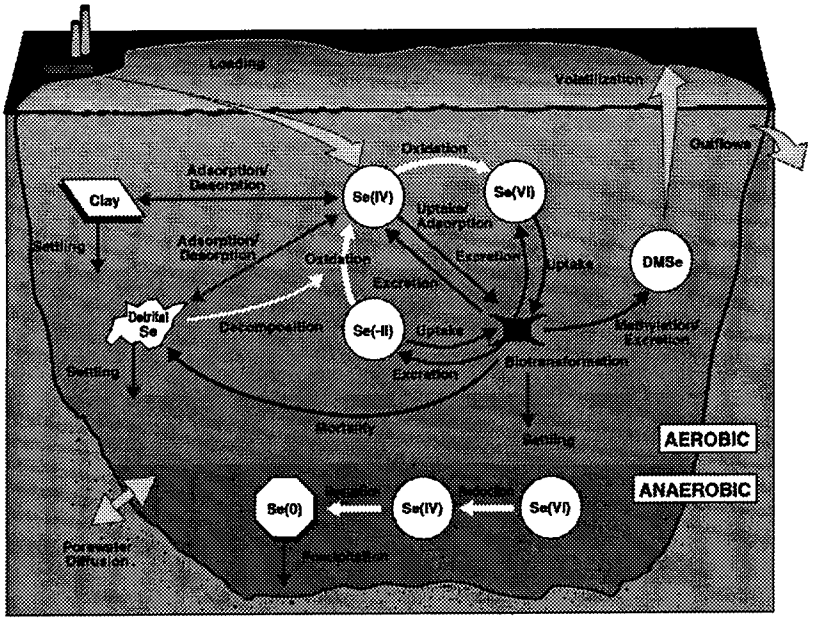

Figure 1. Selenium pools and pathways included in the research and modeling effort.

4. Conclusions

Microbes (algae and bacteria) bioconcentrated Se several orders of magnitude above the concentration in water RIEDEL et al. (1996). This increased Se bioavailability for higher trophic levels, such as zooplankton and fish, which obtained most of their Se from food. Microbial processes in sediments both immobilized Se in its elemental form and regenerated inorganic Se from detritus. Sediments served not only as an important long-term sink for Se, but also as a source to fish via the benthic food web, a process that continued long after industrial sources had ceased.

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NEW AND SIMPLE MODEL OF THE ORAL UPTAKE OF TRACE ELEMENTS FROM SOIL BY SNAILS

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1. Introduction -

A novel and simple three-compartment (soil, foot, viscera) model has been developed with snails *Helix aspersa* to predict bioavailability of soil trace elements. As snails are phytophagous and saprophagous terrestrial invertebrates and as they also ingest soil (Seifert and Shutov, 1981; Gomot *et al.*, 1989), an experimental model was developed in which snails are fed for 4 weeks with a diet composed of a specific flour (Helixal) and the contaminated soil sample to be tested.

2. Materials and Methods

Two soils were used : soil sample S0 which is the reference soil (a Luvisol, sampled from the "Ferme expérimentale de la Bouzule" 54, in France). Surface soil (<20 cm) was oven dried at 40°C and sieved through a 2 mm stainless steel mesh. S1 is the same soil spiked with 4 trace elements (Cd, Cr, Pb, Zn). Metal solutions with : $Cd(NO_3)_2$, $K_2Cr_2O_7$, $C_4H_6O_4Pb$, $C_4H_6O_4Zn$ were prepared in distilled water and added to the soil to give the required nominal concentrations (NC). The soil was then thoroughly mixed by stirring. Heavy metal levels (MC : measured concentrations) were estimated by AAS after soil sample digestion in hot HNO_3 (Table 1).

Table 1.

Soils	Metals ($\mu g.g^{-1}$) dry weight							
	Cd		Cr .		Pb		Zn	
	NC	MC	NC	MC	NC	MC	NC	MC
SO	-	0.377 ± 0.01	-	88.6	-	49.5	-	131
S1	20	10 ± 4.1	800	826 ± 21	800	840 ± 54	2000	2109 ± 115

To study the effects of metal bioaccumulation on snails, three diets were used corresponding to different concentrations of soil in the flour (50, 75 and 85%). Snails used for the test were 1 month old juveniles, mean fresh weight approximately 1 g. Each diet was replicated with 5 snails. Breeding conditions were those described by Gomot (1997). At the end of the experiment, snail tissues (foot and viscera) were prepared for chemical analysis as previously described (Gomot and Pihan, 1997).

3. Results and Discussion

Measured concentrations are presented in Table 2. For every metal, the concentrations in the viscera were higher than those found in the foot. These results confirm that the two tissues should be analysed separately. Metal concentrations were always higher in the viscera of snails exposed to the spiked soil sample. But for Cd and Zn, concentration factors (CF) were >2 (macroconcentration) whereas Cr and Pb were excluded (CF <1). Generally metal concentrations increase with the quantity of spiked soil in the diet ; however regulation occurred for higher concentrations of soil S1 in the diet for Cd, Cr and Zn. For Zn the uptake increased in viscera

with the proportion of soil whilst metal concentrations remained very similar in food, probably under the influence of other constituent(s) of soils (such as organic matter, Fe and Mn oxides, etc.). The CF of Zn remained in the same range (1.7 to 3.3) with control and treated soils likely because of the physiological parameters of this essential metal.

Table 2.

Metals	Cd (µg.g^{-1}dry weight)			Cr (µg.g^{-1}dry weight)			Pb (µg.g^{-1}dry weight)			Zn (µg.g^{-1}dry weight)		
Batches	Food	Foot	Viscera	Food	Foot	Viscera	Food	Foot	Viscera	Food	Foot	Viscera
Control	0.16	0.48	0.57 ± 0.1	9.3	1.02	5.34 ± 0.80	0.94	1.56	2.45 ± 0.9	137	75.63	241.50 ± 55
SO 50%	0.26	0.87 ± 0.17	1.97 ± 0.33	48.9	1.27 ± 0.78	2.06 ± 0.80	25.22	0.16 ± 0.10	0.59 ± 0.24	134	64.26 ± 8.7	232.66 ± 19
SO 75%	0.32	1.66 ± 0.34	4.33 ± 0.66	68.7	1.34 ± 0.95	4.09 ± 1.41	37.59	0.96 ± 0.84	1.89 ± 0.91	132.50	83.76 ± 29	365.29 ± 26
SO 85%	0.34	1.84 ± 0.70	5.68 ± 0.47	76.7	2.35 ± 2.18	3.30 ± 2.45	42.87	1.12 ± 0.59	2.67 ± 1.91	131.90	56.54 ± 5.2	426.9 ± 41
S1 50%	5.08	4.32 ± 0.82	93.25 ± 5.08	417.6	12.88 ± 3.33	173.5 ± 20.6	420.47	2.25 ± 0.37	29.21 ± 7.9	1123	42.81 ± 14.3	2012 ±576
S1 75%	7.58	10.95 ± 4.28	171.72 ± 46	621.8	41.07 ± 18	432.9 ± 91	630.23	2.91 ± 1.64	65.57 ± 13.5	1615.75	41.46 ± 21.6	5295 ± 745
S1 85%	8.58	4.14 ± 0.74	101.9 ± 39	703.5	43.59 ± 6.3	412.8 ± 147	714.14	2.84 ± 1.68	154.37 ± 68	1813.40	115.64 ± 80	5028 ± 808

4. Conclusions

These results are in agreement with the ability of snails to accumulate metal trace elements as found in snails collected near polluted areas (Hopkin, 1989 ; Dallinger, 1993). In addition, the use of standards animals in homogeneous conditions allows the bioavailability of metals to be evaluated in soils. This experimental approach is a means to explain differences of metal concentrations detected in snails from different natural habitats (Bártošová *et al.*, 1995) and to complement investigations into the impact of heavy metals on invertebrates. The estimation of metal accumulation in different tissues allows the prediction of risks linked to the transfer of metals in the terrestrial food chain (risks for snail predators). This approach, developed on a laboratory scale, allows the control of sludge or waste disposal in agricultural or forest soils. Furthermore, the same protocol may be applied to measure the influence of contaminants on snail growth and to study the relationship between bioaccumulation and toxic effects.

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PREDICTING CONCENTRATIONS OF CD, CU, PB AND ZN IN THE SOLUBLE AND EXCHANGEABLE PHASES OF SOILS

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1. Introduction

The critical loads concept is currently being evaluated as an appropriate method for assessing the impacts of long-range transport of several toxic metals on terrestrial and freshwater ecology. The work described here is part of a project, focussed on UK uplands, that investigates metals in terms of deposition processes, soil bioavailability and pathways into freshwaters. A critical load of a substance is the level of deposition of that substance below which damage to sensitive elements in the environment will not occur according to current knowledge. The effects of metals on sensitive ecosystem receptors, such as micro-organisms, depend to a large extent on their concentrations in soil solution. For this reason, data on total metal concentrations in soils are likely to be inappropriate for risk assessment, since such data do not always reflect metal levels in the soil solution. However, in the scientific literature and in national soil metal inventories, metal concentrations are generally expressed as total values.

2. Materials and Methods

To predict 'bioavailable' metal concentrations in soils, we are developing simple empirical models which use generally available input data on total metal concentrations together with those soil properties thought to influence the partition of metals between the solid and solution phases (K_d). We have collected data on soil properties and 'bioavailable' and total metal concentrations from >100 soils covering 6 soil series in North Wales. Standard methods were used for determination of pH, organic matter content (loss-on-ignition), CEC, exchangeable base cations, dissolved organic carbon (DOC) and dissolved anions. Total metals were determined by a standard nitric/perchloric acid digestion. 'Bioavailable' metals were extracted in two ways: (i) field moist soils equilibrated at field capacity with purified water were ultra-centrifuged; (ii) air-dried soils were shaken with 0.01 M $CaCl_2$ for 2 hours and centrifuged. The extracts were analysed by Inductively Coupled Plasma-Mass Spectrometry.

3. Results and Discussion

The results of the water extractions indicate that K_d values increase for Cd and Zn with increasing pH (R^2 = 0.73 and 0.62, respectively). This can be explained by processes such as the ready adsorption of these elements onto cation exchange sites and their replacement by H^+ ions with increasing acidity and the preferential adsorption of metal hydroxo complexes at higher pH. K_d values for Pb and Cu do not correlate with pH. For these elements, DOC is the strongest predictor (R^2 = 0.23 and 0.19, respectively) suggesting that soluble organo-Cu and -Pb complexes are formed increasingly as the DOC content rises.

For the $CaCl_2$ extractions, significant relationships were recorded between Cd, Pb and Zn concentrations and several soil parameters. However, the strongest correlations for extractability

of all three elements was observed with pH. The strong influence of pH on $CaCl_2$ extractability was evident for the whole dataset (R^2 values of 56%, 68% and 69% were recorded for Cd, Pb and Zn, respectively) as well as for subsets such as topsoils (Fig.1), peaty and brown-earth soils and individual soil series. R^2 values were generally low for $CaCl_2$-extractable Cu (eg. 11% for pH).

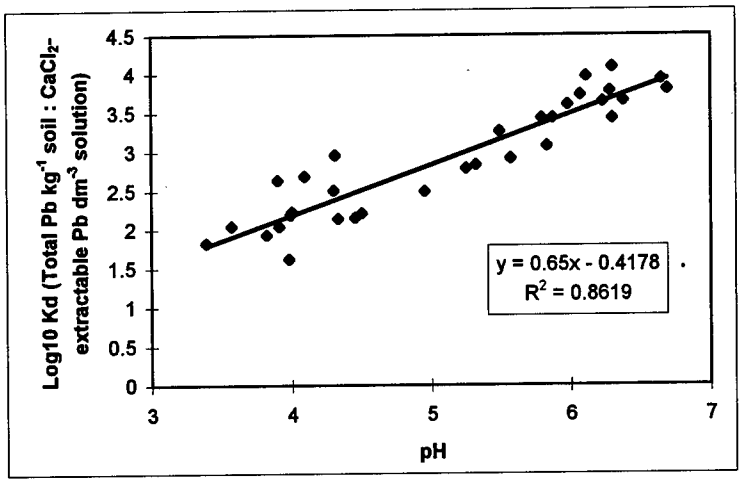

Figure 1: Influence of pH on K_d of Pb in topsoils (0-15cm)

4. Conclusions

The results emphasise the role of pH as a predictor of metal bioavailability and mobility but also highlight the potential importance of dissolved organic matter. The models derived for each of the metals are currently being integrated into a GIS framework enabling the development of preliminary critical loads maps for England and Wales.

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MODELLING OF A REDOX-ACTIVATED EXTRACTION OF MERCURY POLLUTED SOIL FOR A PROCESS INTEGRATION

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1. Introduction

One of the most important challenges in site remediation is related to mercury because of its high vapour pressure and the high toxicity of some of its species. Although thermal decomposition techniques are available there are two weighty drawbacks of such retorting processes: Because of a large thermodynamic sèparation factor the costs are extremely high. Moreover, air pollution problems have to be avoided. Here a hydrometallurgical process is favourable. But a wet extraction raises the problem how to overcome the low solubility of several mercury species and the high affinity to organic matter (Xu and Allard, 1991). A solution is given by the electroleaching process (Thöming et al., 1998) that combines a complexing extraction at a high redox level under weak acidic conditions with an integrated electrolytical preparation of the leachate. To meet the requirements of scaling up this process the leaching has to be modelled.

2. Materials and Methods

The mercury species within the used soil samples were distinguished into „elemental" (Hg^0) and „non-elemental" ($Hg_{n.e.}$) by a thermal release analysis with reference to Windmöller et al. (1996). Soil samples were taken from three sites (soil 1: $[Hg_{n.e.}] = 220$ mg/kg; soil 2: ($[Hg_{n.e.}] = 6$ mg/kg; soil 3: $[Hg^0)] = 990$ mg/kg). In a stirred tank with a varying solid/liquid ratio σ the soil samples as well as pure elemental mercury droplets were leached in a sodium chloride solution containing anodically prepared hypochlorous acid of varying concentrations. The leaching kinetics were monitored measuring the dissolved mercury amounts. Due to the electrolytical preparation of the spent leachate the solution could be kept in the circuit process to be reused afterwards.

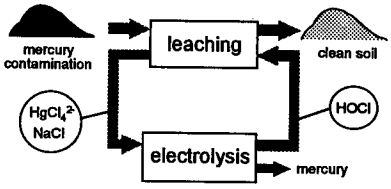


Figure 1: Electroleaching process for a clean-up of mercury polluted soils.

3. Results and Discussion

To value the mercury oxidation separately the leaching kinetics of the Hg^0-droplets were analysed in dependence of pH, σ, [HOCl] and [NaCl]. For pH ≤ 6 and [NaCl] ≥ 1 mol/L the kinetics can be sufficiently described only by σ and [HOCl] in an exponential way (Fig. 2). This leads to an expression that allows to calculate the residence time needed for a complete oxidation of the elemental mercury in soil 3. But in a solid containing solution the success of the clean-up, the extraction yield, is controlled by solid/liquid interactions. These re-adsorption processes (Fig. 3) are of three magnitudes faster than the oxidation of Hg^0. For the soil samples 1 and 2 with low $[Hg^0]$ the re-adsorption was modelled assuming a surface-complex model. To explain also the effect of chloride the model has to be extended (Tiffreau et al., 1995). Residual concentrations in the treated soil lower than 1 mg kg^{-1} can be achieved. Up to 99.6 % of the mercury can be extracted from the industrial samples bearing higher mercury concentrations (sample 1). At lower initial concentrations (sample 2) the removal rate is about 80 to 90 %.

4. Conclusions

Mercury contaminated soil can be sufficiently cleaned by a redox-activated extraction even if it contains a large fraction of elemental mercury. The two regimes of this leaching process, the oxidation and the re-adsorption, can both be simulated by means of models which were derived from theoretical approaches. This is the basement for a integration of the extraction into the whole process as well as a scale-up.

Figure 2: Extraction kinetics of Hg^0 with varying σ and [HOCl]; s = stand. deviation of the simulation.

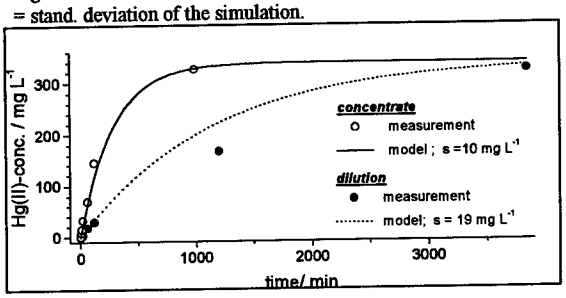
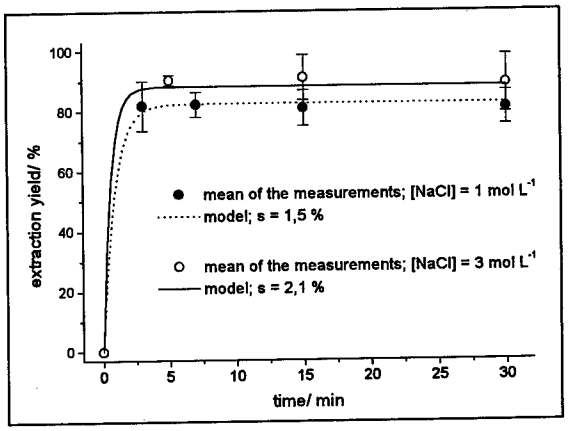


Figure 3: Model calculations for soil containing non-elemental mercury.



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PREDICTING CADMIUM TRANSPORT IN A NON-CALCAREOUS SOIL FROM COMPETITIVE SORPTION ISOTHERMS

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1. Introduction

Contamination of soils with heavy metals as a result of industrial emissions, sludge application, and waste disposal is a wide-spread problem. Due to its toxicity, soil contamination with cadmium can pose a serious threat to ecosystem health. Therefore, understanding the factors controlling the sorption and mobility of cadmium in soils is of great importance. The objectives of the present study were to (i) investigate the sorption of Cd^{2+} to a non-calcareous soil material as influenced by major cations (Ca^{2+}, Mg^{2+}), (ii) to predict the transport behavior of Cd as a function of electrolyte composition, (iii) to validate model predictions by comparison with column transport experiments.

2. Materials and Methods

Adsorption of Cd^{2+} to a non-calcareous soil (B-horizon, Riedhof soil, Switzerland) in different electrolytes (at pH 4.5 ± 0.2) was studied using a flow-through reactor technique described by GROLIMUND et al.(1995). Single- and multi-site adsorption models based on cation exchange or Langmuir-type equations were used to quantitatively describe the entire set of competitive sorption data. A non-linear least squares procedure was applied to obtain the best-fit parameters for each model (CERNIK et al., 1995).

For predicting Cd transport, the calibrated sorption models were coupled with the mixing-cell transport code Impact (JAUZEIN et al., 1989). Transport experiments were performed in glass chromatography columns packed with the soil material. Column pore volume and dispersivity were determined from tracer breakthrough experiments. Cadmium pulses (10^{-5} M $CdCl_2$) were passed through the soil columns. Effluent concentrations were monitored by atomic absorption spectroscopy.

3. Results and Discussion

The adsorption of Cd^{2+} displayed a strong dependence on the concentrations of major cations (Ca^{2+}, Mg^{2+}) in solution. This adsorption behavior was well described by multi-site models which account for cation exchange reactions and specific adsorption of Cd^{2+} to the soil material. An example for the transport behavior of Cd in a soil column is shown in Figure 1. In the presence of low electrolyte concentrations (10^{-4} M $CaCl_2$+$MgCl_2$, Ca:Mg = 1:1), Cd exhibited strong retention and Cd breakthrough occurred after ~350 pore volumes. Increasing the electrolyte concentration resulted in a drastic decrease in Cd retention. In the presence of 10^{-2} M $CaCl_2$ + $MgCl_2$ (Ca:Mg = 1:1), Cd breakthrough occurred after ~15 pore volumes. The solid lines in Fig. 1 represent predictions of Cd transport based on a combined cation exchange and specific adsorption model. In both cases, the breakthrough of Cd was accurately predicted. The performance of various other single and multi-site models will be discussed.

The results of this study show that Cd transport in non-calcareous soils strongly depends on the chemical composition of the soil solution. The effects of varying electrolyte concentration can be predicted, if high-quality competitive adsorption data spanning over wide concentration ranges are available and if the data are described by adequate adsorption models.

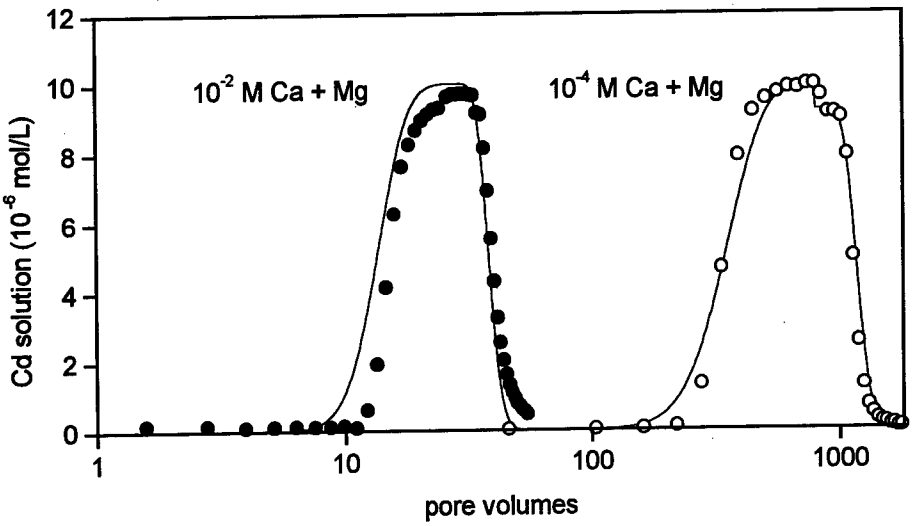

Figure 1: Breakthrough of cadmium (10^{-5} M) at two different electrolyte concentrations (10^{-2} M or 10^{-4} M $CaCl_2$ + $MgCl_2$, Ca:Mg = 1:1). Solid lines represent predictions based on a combined cation exchange - specific adsorption model.

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MODELING SELENIUM TRANSFER IN A LABORATORY SOIL-PLANT SYSTEM

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1. Introduction

Selenium (Se) can be either beneficial or toxic to humans and certain animals and plants depending on the concentration at which it is present. The difference between essential levels and toxic levels is very small thus it is important to understand the processes controlling the distribution of Se in the environment. The development of a research model for Se behavior in a soil-plant system may help to increase basic knowledge of its distribution in the environment. The aim of this study was to develop an empirical model to simulate Se transfer in a soil-plant system, using experimental data obtained in a previous study (Camps Arbestain, 1998).

2. Model Description

The model shown below is based on a simplified system (Fig. 1) that was used as a preliminary approach in developing a more comprehensive one.

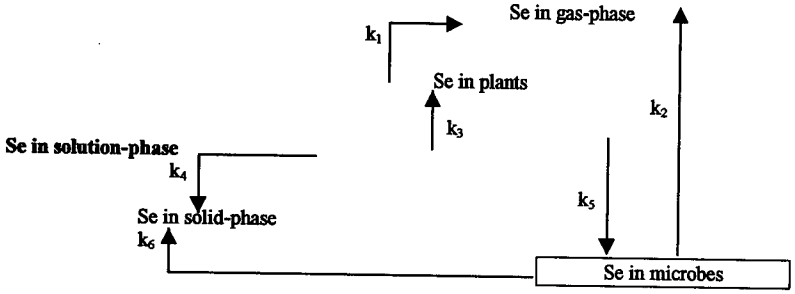

Fig. 1. Flow diagram of the simplified system.

The main assumptions and considerations made were that: 1) all reactions follow first order kinetics, 2) all Se species were computed on a soil concentration basis for computing convenience, 3) only total Se was considered, 4) k_4 represents the rate of microbial reduction of Se (mainly as SeO_4^{2-}) to more insoluble Se forms, which is assumed to take place only when both microbial biomass and activity are high, 5) Se in the solid phase includes both organic and inorganic forms of Se; the net outgoing fluxes of these forms to other phases are not considered in this short term experiment, 6) Se (as SeO_3^{2-}) adsorption/desorption fluxes, if occurring, counterbalance each other (based on experimental data), 7) microbial volatilization of Se is represented as a two-step process: (i) microbial assimilatory reduction of Se (mainly as SeO_4^{2-}) to the level of selenide (k_5), and (ii) the subsequent microbial release of alkyl selenides (k_2), 8) significant Se uptake by plant roots at 13 cm depth takes place after 30 days of root growth. Based on the diagram and on the above assumptions and considerations, the numerical model includes the following differential equations:

$$d(Se)_{solution}/dt = -(k_3+k_4+k_5)(Se)_{solution}$$
$$d(Se)_{plant}/dt = k_3(Se)_{solution} - k_1(Se)_{plant}$$
$$d(Se)_{soil}/dt = k_4(Se)_{solution} + k_6(Se)_{microbes}$$
$$d(Se)_{gas}/dt = k_1(Se)_{plant} + k_2(Se)_{microbes}$$
$$d(Se)_{microbes}/dt = k_5(Se)_{solution} - (k_2+k_6)(Se)_{microbes}$$

The k values utilized in the model were as follows: (i) for $t \geq 0$, $k_1=0.00001$, $k_2=0.9$, $k_5=0.0033$, and $k_6=0.0001$; (ii) for $t \geq 30$, $k_3=0.0024$, otherwise $k_3=0$; and (iii) for $6 \leq t \leq 30$, $k_4=0.13$, otherwise $k_4=0$ (t units in d and k_i units in d^{-1}). Rate values were contrasted with others estimated from data reported in similar studies (Karlson and Frankenberger, 1989; Tokunaga et al., 1996). The differential equations described above were solved using the 4-step Adams-Bashforth-Moulton predictor-corrector method and starting by means of the 4th order Runge-Kutta method.

3. Results and Discussion

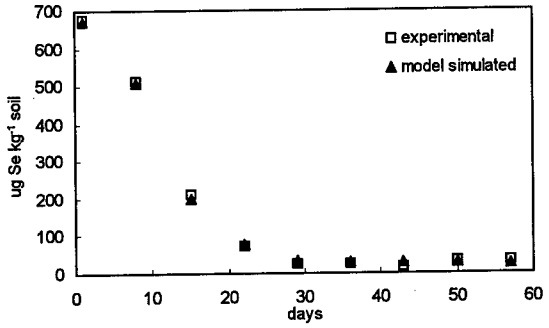

Fig 2. Experimental and model simulated data of total Se in soil solution corresponding to PISt treatment (barley plants + straw amendment) as described by Camps Arbestain (1998).

The results obtained demonstrated a good fit between experimental and simulated data (Fig. 2). Moreover, the Se distribution in the different phases at the end of the 57 days matched the values obtained experimentally. We are presently working on modeling of the linkage existing between the Se cycle and the N and C cycles, as demonstrated by the experimental results obtained by Camps Arbestain (1998).

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MODELLING ADSORPTION KINETICS OF ZINC IN SOILS OF TAMIL NADU, INDIA

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1. Introduction

It is vital to understand the mechanism of Zn adsorption on soil material as plants respond primarily to Zn activity in soil solution. Adsorption of Zn, one of the most important factors governing the release and fixation of applied Zn determines the efficiency of Zn fertilization. Nearly 75% of Zn was adsorbed on application. Knowledge on the kinetics of adsorption – desorption reaction may be essential for the sound prediction of nutrient availability to plants. A number of models have been used to describe the kinetics of adsorption process of trace elements. The work on this line in Indian soil is very limited. Hence, studies have been conducted to assess the kinetics of Zn adsorption and for providing information on the behavior of Zn in Indian soils.

2. Materials and Methods

Batch test was conducted to investigate the adsorption behavior of Zn in thirteen Indian surface (0-25 cm) soils. Background electrolyte (20 ml 0.01 M $CaCl_2$) containing 40 gm^{-3} Zn as Zn SO_4 $7H_2O$ was added to soil (1 g) in 50 ml centrifuge tubes in triplicate. Shaken the contents for 10 min and allowed to equilibrate at 27±1°C in a water bath for different time intervals ranging from 0.25 to 96 h. At the end of the reaction time, centrifuged the suspension. The Zn concentration in the equilibrium solution was determined with atomic absorption spectrometer. The amount of Zn adsorbed at various time intervals were fitted to different kinetics models. The adsorption experiments were also conducted using the same soils. $CaCl_2$ solution (0.01 M; 20 mL) containing varying concentration of Zn (2.5 to 200 mg kg^{-1}) as Zn SO_4 $7H_2O$ was added to soil (1 g). Shaken the contents and allowed to equilibrate to a predetermined equilibrium time (24 h) and centrifuged. Adsorbate concentration in the equilibrium solution was then determined as described above. Zinc adsorption was modeled with a Langmuir adsorption isotherm. Simple correlation, multiple linear regression analysis and path analysis were made to establish whether any relationship existed between soil properties and Zn adsorption parameters.

3. Results and Discussion

Zinc adsorption pattern was characterized in all soils by an initial quick reaction followed by a slow reaction (Fig.1); probably due to slow diffusion of Zn in the soils. Zinc adsorption was almost complete after 24 h of equilibrium. The concentration of Zn in equilibrium solution reduced with fineness in texture. The parabolic diffusion described Zn adsorption over a limited part of isotherm (0.25 to 4 h) showing discontinuity beyond 4 h reaction time. This suggested that two different mechanisms are operating in respect of rate of Zn adsorption (3). Zinc adsorption was best described by Elovichian kinetics (Table 1) based on high R^2 and low SE (1).

Table 1. Mean and range of coefficient of determination (R^2) and standard error of estimate (SE) of various kinetic models used for Zn adsorption

Models	R^2		SE	
	Range	Mean	Range	Mean
Zero order	0.203 – 0.497	0.378	5.3 – 85.0	44.0
First order	0.196 – 0.459	0.355	5.3 – 89.6	44.1
Second order	0.188 – 0.417	0.310	5.3 – 97.7	45.7
Third order	0.181 – 0.375	0.311	6.0 – 114.3	48.8
Parabolic diffusion	0.343 – 0.739	0.588	5.3 – 86.9	43.6
Elovich	0.601 – 0.962	0.860**	2.1 – 30.7	17.7

** P=<0.01

Zinc adsorption on soils conformed to Langmuir isotherm (Figure 2). The adsorption maxima (b) and simple correlation values (r) ranged from 1.95 to 4.00 mg g^{-1} soil and 0.90 to 0.98 respectively. The soil properties *viz.*, clay, silt, $CaCO_3$, ex. Ca and CEC correlated well with 'b' values. To relate the soil properties, the adsorption maxima could be used with confidence (2). The correlation established between clay and CEC with 'k' values indicated that zinc was held with greater bonding energy as the content of clay and CEC of the soil increased (r=0.610** and 0.681*).

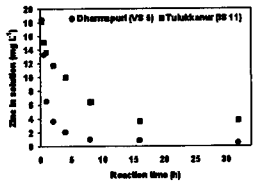

Fig. 1. Zinc in solution Vs reaction time

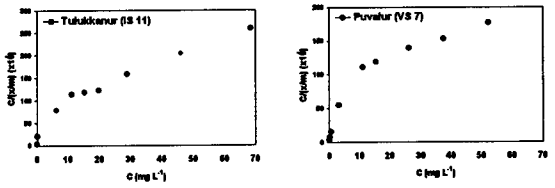

Fig. 2. Langmuir isotherms for zinc in soils

Thus the Elovichian model was best suited to describe the kinetics of Zn adsorption by soils. The Zn adsorption data clearly shows that the capacity of the soil to retain Zn and the energy with which they adsorb it, influences the soil to release and maintain the concentration of Zn in the soil solution.

Table 2. Langmuir constants for Zn

Soil No.	b mg g^{-1}	k mL μg^{-1}	Soil No.	b mg g^{-1}	k mL μg^{-1}	Soil No.	b mg g^{-1}	k mL μg^{-1}
VS 1	3.46	0.39	VS 6	3.82	0.48	IS 11	2.93	0.08
VS 2	4.01	0.08	VS 7	3.08	0.14	IS 12	3.52	0.11
VS 3	3.93	0.10	**VS 8**	3.17	0.19	VS 13	3.70	0.48
VS 4	3.64	0.19	VS 9	3.26	0.33			
VS 5	3.84	0.24	VS 10	3.87	0.78			

b – Adsorption maxima k – bonding energy coefficient VS – Vertisol IS–Inceptisol

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MODELLING AL^{3+} ACTIVITY IN NUTRIENT MEDIA FOR SCREENING GENOTYPES OF ALUMINIUM TOLERANT *CYNODON DACTYLON*

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1. Introduction

In South Africa the waste product of the gold extraction process are deposited in slimes dams. These slimes are characterised by low pH, high aluminium and a very fine particle size that is easily eroded by wind and water. One of the most effective stabilisation strategies is to rehabilitate these areas by establishing a grass cover, mainly C. dactylon, on the slimes dams. However, this approach is costly because it currently involves leaching the slimes with overhead sprays in order to increase the pH. An alternative method is to vegetate the slimes with genotypes of *C. dactylon* that are tolerant to high aluminium and low pH. Aluminium tolerant genotypes of this grass species can be selected by screening whole plants in nutrient solutions or parts of plants (explants) in an *in vitro* culture medium. The MINTEQA2 program (Allison *et al.*, 1991) was used to evaluate present selection protocols with respect to Al^{3+} activity. The Al^{3+} was selected for study because it is the most toxic form of aluminium (Kinraide, 1997). However, this toxic ion interacts strongly with other ionic components in the nutrient media producing precipitates and thus reducing its availability.

2. Materials and Methods

In this study we investigated the chemical interactions with aluminium ions in various published nutrient media and media modified in this study, using MINTEQA2 program. The program was set to run at pH 4, using the mode that allows for the identification and quantification of precipitates and maximising the chemical iterations (200).

3. Results and Discussion

The work of several authors who have established protocols for screening aluminium tolerant plants were evaluated. Aluminium was supplied to the nutrient medium either as $AlCl_3$ or $Al_2(SO_4)_3$. The MINTEQA2 simulations showed that nutrient media which received $Al_2(SO_4)_3$ as the aluminium source resulted in decreasing Al^{3+} activity with increasing aluminium concentration whereas when $AlCl_3$ was used, the Al^{3+} activity increased or remained constant at the maximum value (Table 1). Of all the media formulations simulated (including the modified media established in this study), the highest Al^{3+} activity obtained was 7.5 µM. The observed Al^{3+} activity differs considerably from the initial aluminium concentration because this ion interacts with other components in the media and forms precipitates, in particular alunite (Al^{3+}, K^+, SO_4^{2-}, H^+, H_2O) and diaspore (Al^{3+}, H^+, H_2O). The aluminium precipitate diaspore is only formed at an Al^{3+} activity of 7.5 µM and the amount of diaspore formed increases with increasing aluminium supply while the amount of alunite produced remains constant. Phosphate is known to have strong binding properties in the soil environment and is usually reduced or eliminated from nutrient screening media. However, MINTEQA2 simulations of the standard MS (Murashige & Skoog, 1962) nutrient medium showed that only a very low level of PO_4^{2-} (0.015 pM) is available for interaction with other components in the medium. Thus no interaction between Al^{3+} and PO_4^{2-} was detected. Since it has been established in this study that

phosphate is an essential component of the *in vitro* callus induction medium, this ion was not eliminated or reduced. However, Al^{3+} interacts strongly with SO_4^{2-} in the chemical environment producing the precipitate alunite. Therefore, MS nutrient media were modified by reducing this ion from 1.7 to 1 mM. Further modifications included removing EDTA from the medium and setting the pH level at 4. This modified MS nutrient medium was simulated using MINTEQA2 and resulted in the highest Al^{3+} activities (3.1 – 7.5 µM) over the 0.5 – 3 mM $AlCl_3$ concentration range.

Table 1 Media used for screening aluminium tolerant plants were assessed using MINTEQA2. A range of aluminium concentrations (0.5 – 3 mM) was used at pH 4.

type of culture	Al source 0.5-3 mM	Al^{3+} activity µM	Alunite MM	diaspore mM	reference
nutrient solutions	$Al_2(SO_4)_3$	1.5 – 1.1	0.2 - 2	-	Lukaszewski & Blevins, 1996
	$AlCl_3$	7.5 – 7.5	-	0.2 - 3	Horst *et al.*, 1997
in vitro media	$Al_2(SO_4)_3$	3.4 – 1.7	0.1 - 2	-	Van Sint Jan *et al.*, 1997
	$AlCl_3$	2.4 – 7.5	0.2 – 0.7	0.3 – 0.7	Yamamoto *et al.*, 1996

4. Conclusions

The MINTEQA2 chemical speciation program can be used to determine the Al^{3+} activity in culture media used to screen for aluminium tolerance. This study has shown that $AlCl_3$ is a more suitable aluminium source than $Al_2(SO_4)_3$ since the former supports higher Al^{3+} activities than the latter. Further research has shown that, without aluminium, the modified MS nutrient media described here is suitable for callus growth and, in fact, supported significantly higher growth rates than the standard (unmodified) growth medium. The effects of a range of Al^{3+} activities on growth of and Al^{3+} uptake by calli from different *C. dactylon* genotypes will be presented.

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DYNAMIC MODEL FOR THE ELECTRODIALYTIC SOIL COPPER REMEDIATION

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[2]Departamento de Matemática, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal.

1. Introduction

The electrodialytic process is a promising remediation technique for removal of heavy metals from polluted sites. Due to the electric field present when a direct current is passed between a pair of electrodes, placed in a contaminated soil, the pollutant species are driven towards one of the electrodes, from where they may be removed. Two main mechanisms are responsible for this movement: electromigration and electroosmosis. On inert electrodes (titanium, for example) the electrode reactions will produce H^+ ions at the anode and OH^- ions at the cathode, which means that if pH is not controlled, an acid front will be propagated into the soil pores from the anode, and a base front will move out from the cathode. • Sequential extraction procedures are designed to remove metals selectively from various fractions of the substrate with which they might be associated. • This work reports results from the application of a sequential extraction scheme to the study of the chemical associations of Cu in a Portuguese contaminated soil during electrodialytic treatment of the soil. The main goals are: Establishment of a model for the removal of Cu by electrodialytic remediation; assessment of the main soil fraction(s) responsible for metal release; and identification of other key parameters that might be relevant to the process.

2. Materials and Methods

Soil: From a Portuguese wood preservation site, at Famalicão, in the north of the country (polluted by Cu in addition to As and Cr). The 'total' Cu was extracted by HNO_3-$HClO_4$-HF. By means of a six-step sequential extraction scheme, the 'total' Cu content was sub-divided into 6 fractions: a) 'soluble and exchangeable', b) attached to 'Mn-oxides', c) 'organic matter', d) attached to 'amorphous Fe-oxides', e) attached to 'crystalline Fe-oxides', f) 'strongly bound' [described in (1)]. Laboratory cell: Five experiments differing in time were carried out in a cell recently developed at the Technical University of Denmark [Fig.1 described in (1)]. The contaminated soil is placed in the central compartment of the cell (L=15 cm, $\varnothing_{int}$=8 cm) (Fig. 1). Experimental conditions: Current density = 0.2 mA/cm^2 and duration of treatment =18, 35, 65, 85 and 125 days for experiments A, C, H, N and F, respectively. Determinations: At the end of each experiment, the soil from the central compartment of the cell was cut vertically into 5 slices of thickness 3 cm each. $pH_{(H_2O)}$, 'total' Cu content and the sequential extraction were made with each slice. By comparing the amounts of metal in the soil before and after the passage of the current, the fractions where Cu had been removed where identified. Copper was determined by Atomic Absorption Spectrophotometry (Perkin Elmer 5000-AAS).

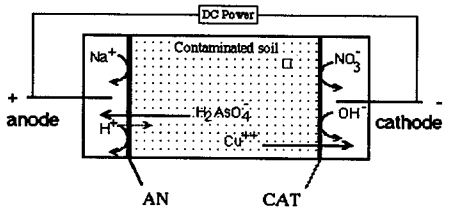

Figure 1. Schematic principle of the electrodialytic process. AN=Anion exchange membrane; CAT=Cation exchange membrane.

3. Results and Discussion

Model. The dynamic model presented is based on a biregressional design. In these designs the treatments are given by level combinations of quantitative factors. A first set of regressions is adjusted, one per treatment. We now have, as controlled (independent) variables, the levels of the factors for the treatment and, as dependent variables, the adjusted coefficients. A second set of regressions is then adjusted, in order to study the effects of the factor levels on the coefficients of the initial regressions. The assumptions behind the adjustment of the regressions in the second set

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The model proposed for the electrodialytic removal of Cu is illustrated in Fig. 2, where:

- **Zj** represents the removal of Cu from the soil and is directly estimated by the sum of the quantities of Cu obtained in sequential extraction steps b), c), d), e) and f), before and after passage of the current ($\mathbf{Zj = \Sigma\ Cu_{initial} - \Sigma\ Cu_{final\ in\ slice\ j}}$);
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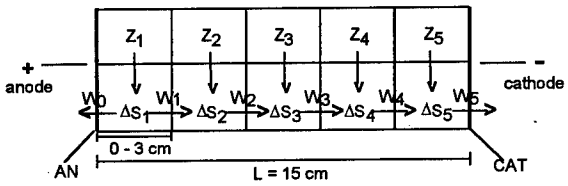

Figure 2. Summary of the electrodialytic system used. AN=anion exchange membrane; CAT=cation exchange membrane (2).

4. Conclusions

The authors have studied the removal of copper from an industrially heavy metal contaminated soil, using the application of electric current. Three aspects of the study contribute to its originality: (i) the use of a actual contaminated soil, rather than a spiked soil sample; (ii) the use of a sequential extraction procedure to monitor changes in the chemical associations of copper during the electrodialytic treatment of the soil; and (iii) the presentation of a dynamic model based on a biregressional design which enables a joint analysis of experiments which were carried out in a laboratory cell. Although there is some discussion about possible uncertainties with sequential chemical extraction procedures, this technique is believed to be one of the most reliable ones available for predicting metal leaching rates as well as transformations induced in the soil by the application of electric current.
The dynamic model proposed enables us to draw the following statistically significant conclusions: the electrodialytic removal of Cu from the soil is time dependent, local (depends on the distance from AN), and depends on the release of metal by different soil fractions (defined by the steps of sequential extraction). The process is also very much a result of the interaction between time (duration of treatment) and distance from AN. In the process of releasing Cu from the soil, the 'organic matter' and 'amorphous Fe-oxides' fractions [respectively steps c) and d) of the sequential extraction] are the most relevant, although it is not possible to distinguish between them in terms of importance.

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Acknowledgments. A. B. Ribeiro is grateful to Departamento de Pedologia, Estação Agronómica Nacional and to the Technical University of Denmark for support

DYNAMIC MODEL FOR THE ELECTRODIALYTIC SOIL COPPER REMEDIATION

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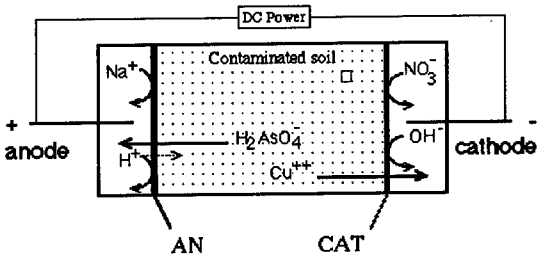

Figure 1. Schematic principle of the electrodialytic process. AN=Anion exchange membrane; CAT=Cation exchange membrane;

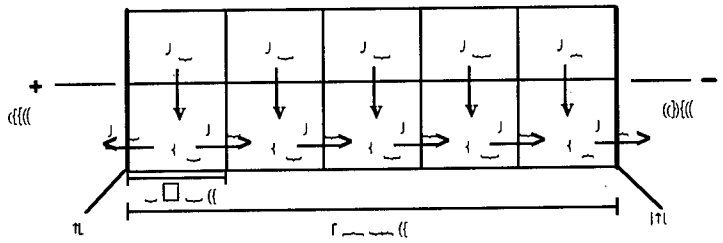

Figure 2. Summary of the electrodialytic system used. AN=anion exchange membrane; CAT=cation exchange membrane (2).

4.Conclusions.

The authors have studied the removal of copper from an industrially heavy metal contaminated soil, using the application of electric current. Three aspects of the study contribute to its originality: (i) the use of a actual contaminated soil, rather than a spiked soil sample; (ii) the use of a sequential extraction procedure to monitor changes in the chemical associations of copper during the electrodialytic treatment of the soil; and (iii) the presentation of a dynamic model based on a biregressional design which enables a joint analysis of experiments which were carried out in a laboratory cell. Although there is some discussion about possible uncertainties with sequential chemical extraction procedures, this technique is believed to be one of the most reliable ones available for predicting metal leaching rates as well as transformations induced in the soil by the application of electric current.

The dynamic model proposed enables us to draw the following statistically significant conclusions: the electrodialytic removal of Cu from the soil is time dependent, local (depends on the distance from AN), and depends on the release of metal by different soil fractions (defined by the steps of sequential extraction). The process is also very much a result of the interaction between time (duration of treatment) and distance from AN. In the process of releasing Cu from the soil, the 'organic matter' and 'amorphous Fe-oxides' fractions [respectively steps c) and d) of the sequential extraction] are the most relevant, although it is not possible to distinguish between them in terms of importance.

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Biomonitoring and Risk Assessment

(Technical Session 12)

EVALUATION OF POLY(TETRAFLOUROETHENE) POROUS CUP SOIL SOLUTION SAMPLERS FOR HEAVY METAL MONITORING

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1. Introduction

Heavy metal mobility in unpolluted soils is an area of growing interest. To estimate the mobility of the heavy metals in soils several methods have been used. Single and sequential extraction procedures have been used to estimate more or less mobile fractions. The analysis of soil solution is used to estimate the most mobile fraction of heavy metals in the soils that is readily available for plant uptake or leaching. The concentrations of heavy metals in soil solution from slightly and unpolluted soil is generally low which means that the sampling method can have a proportionately significant effect on the results.
Sampling of soil water has been debated, since it is difficult to sample soil water without changing the composition of the soil solution. The use of ceramic cups has been questioned e.g. (HANSEN and HARRIS (1975); RAULUND-RASMUSSEN (1989)) but other soil samplers and methods have been criticised as well e.g.(McGUIRE et al.(1992)). To avoid the problems caused by the use of ceramic cups the use of polytetraflouroethene (PTFE) suction cups has increased. The effect of using PTFE suction cups has only been poorly investigated and the use of PTFE suction cups for sampling soil water for heavy metal analysis has not been subject for investigation.

2. Materials and Methods

Two new PTFE suction cups (PRENART SUPER QUARTZ, PRENART Equipment, Frederiksberg, Denmark) with 0.5 m of PTFE tubing were connected to a fraction collector via a peristaltic pump. The PTFE suction cups were lowered into a suprapur 0.01 M HNO_3 solution and the acid solution was pumped through the cups until the concentration of heavy metals in the effluent could not be detected. The cups were lowered into a solution of heavy metals containing 5 ppb of Cd and Cu and 10 ppb of Zn and Ni, at pH 5.3. The solution was slowly pumped through the cups at a rate of approximately 3.0 ml $hour^{-1}$ which resembles the pumping rate at field conditions. The same experiment was conducted using a solution containing 5 ppb of Cd and Cu and 10 ppb of Zn and Ni and 10 mM DOC (Dissolved Organic Carbon).
The DOC was isolated by centrifugation from O-horizon material under Norway Spruce, passed through a 0.45µm Millipore filter and through a column with cation exchange resin three times to remove metal ions and bring the DOC into its protonated form.

3. Results and Discussion

It was possible to clean the PTFE suction cups with 0.01 M HNO_3 to a level below the detection limits of the heavy metals except for copper. The cleaning was quite fast, only 250 ml of acid solution were necessary to clean the cups. Creasey and Dreiss (1988) also showed that acid cleansing of PTFE cups was a fast process. They used 1 N HCl which is a standard method for cleaning soil water samplers. We have avoided to use such strong acid solution to prevent the creation of too many adsorption sites on the cup surface.

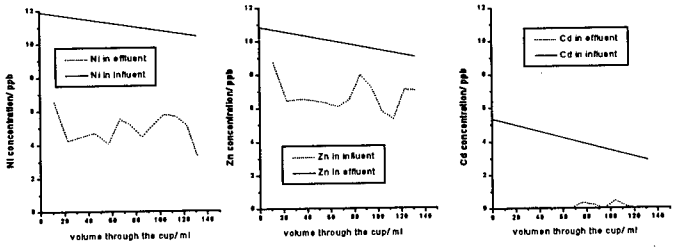

Figure 1: The difference between the influent and effluent concentrations of heavy metals in the solution pumped through the cups.

The concentrations of most of the heavy metal cations were lowered by passing through the PTFE suction cup. Especially, Cd and Ni concentrations were affected, as none of the Cd initially present in the solution passed through the cup and the Ni concentration was almost halved. The effect on Zn was less. Addition of 10 mM DOC to the solution had no effect on the heavy metal adsorption to the PTFE suction cup, and the DOC itself was not adsorbed to the PTFE suction cup. The adsorption of metals by the PTFE suction cup was not distributed even between the metals which suggests the sorption mechanism is not ion exchange but specific sorption.

Maitre et al. (1991) tested the same brand of PTFE suction cup for contamination. They found a significant release of Ca, Mg and Fe from the PTFE suction cups. This suggests that the adsorption sites occupied by Ca, Mg and Fe can be accessible for specific adsorption of heavy metals, especially Cd and Ni.

4. Conclusions

The tested new PTFE suction cups are not contaminated with heavy metals but they adsorb heavy metal cations, especially Cd and Ni. This means that a long period of time is required to reach equilibrium between the PTFE suction cup and the soil solution. The samples taken will not contain the same concentration of heavy metals as the soil solution, since it will be levelled by the adsorption or desorption from the PTFE suction cup.

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IN-VITRO GASTRO-INTESTINAL METHOD TO ESTIMATE BIOAVAILABLE ARSENIC IN CONTAMINATED SOILS

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1. Introduction.

Soil ingestion from incidental hand-to-mouth activity by children is an important issue in assessing public health risks associated with exposure to As-contaminated soils and media. Risk from enteric bioavailability of As is difficult to assess because As exists in many geochemical forms (e.g. oxides, sulfides) and physical forms (flue dust, slag, tailings, waste ore) at hazardous waste sites contaminated by mining or smelting of ore. The purpose of this research project is to determine the ability of chemical methods (chemical speciation, *in-vitro* gastrointestinal) to provide an estimate of As bioavailability in contaminated media.

2. Materials and Methods.

Fifteen contaminated soils and solid media ranging from 401 to 17,456 mg As kg^{-1} were analyzed by an *in-vitro* gastrointestinal (IVG) method. In this *in-vitro* gastrointestinal (IVG) method, As is sequentially extracted from contaminated soil with simulated gastric and intestinal solutions. Arsenic measured by IVG methods was compared with *in-vivo* bioavailable As determined from dosing trials using immature swine.

3. Results and Discussion.

Both IVG stomach and intestinal methods extracted similar amounts of As as in-vivo methods and provided estimates of bioavailable As across all contaminated media (Table 1). The IVG methods were not accurate for calcine materials. Work is underway to determine the geochemical basis for the poor agreement As bioavailability measured by IVG and in-vivo methods for calcine media. There was excellent agreement between arsenic bioavailability measured by IVG and in-vivo methods for non-calcine media (Table 1). Arsenic extracted by the IVG stomach and intestinal phases was linearly correlated ($r = 0.83$ and 0.82, respectively) with *in-vivo* arsenic ($P < 0.01$) (Figure 1).

4. Conclusions.

All IVG methods extracted similar amounts of arsenic and provided estimates of bioavailable As in contaminated media. The IVG method may may be a useful tools to evaluate the effectiveness of remediation technologies and aid in the design of remedial strategies of arsenic-contaminated sites.

Table 1. Comparison of Methods Used to Measure Bioavailable Arsenic in Contaminated Soils and Solid Media

Samples	IVG stomach	IVG intestinal	*In-Vivo*	LSD
All Media	16.7 ab	14.8 b	21.0 a	5.3
Calcine	3.66 b	3.52 b	13.5 a	5.1
Iron Slag	24.8 a	22.7 a	25.4 a	7.4
Not calcine	24.8 a	21.9 ab	25.9 a	6.6

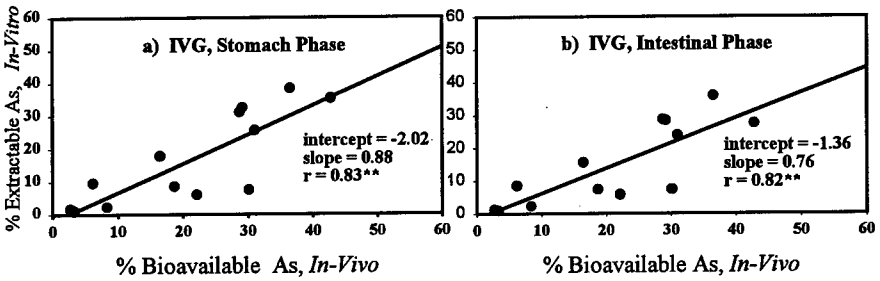

Figure 1. Relationships between bioavailable arsenic measured by (a) IVG stomach phase or (b) IVG intestinal phase *in-vitro* methods and *in-vivo* bioavailable arsenic.

RISKS OF METAL CONTAMINATION IN VIEW OF SOIL PROPERTIES.

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1. Introduction.

Elevated total contents of heavy metals (Cd, Cu, Zn, Pb) can be found in soils as a result of anthropogenic inputs. This does however, not provide good information on ecotoxicological risks since effects are in general poorly correlated with total metal contents in soil. Critical contents for metals in soils as set in various countries in general lack an ecotoxicological basis (De Vries and Bakker, 1998). Interpretation of laboratory tests for ecotoxicity are hampered because of differences in (bio) availability of the metal under laboratory and field conditions. The free ion concentration or activity in soil solution gives in many cases much better relationships with effects than total metal contents in the soil solid phase. The free metal ion activity or concentration not only depends upon the degree of metal contamination of the soil but also on soil properties. Soil properties such as organic matter content, CEC, pH and the concentration of Ca in soil solution influence metal mobility and bio-availability.

2. Materials and Methods

From different sites in the Netherlands, which differ in degree of contamination, soils were sampled for soil and soil solution measurements. Soils were analysed for total contents and "reactive contents" of metals in soil and organic matter content. In soil solution, concentrations of the metals and major cat- and anions including DOC were measured.
According to the method described in an accompanying paper (Bril and Groenenberg, 1999) activities in soil solution were calculated from "reactive" metal contents and soil properties with the use of transfer functions (Bril, 1995). Activities were also calculated from soil solution concentrations using a simple chemical speciation model that accounts for metal complexation with DOC.
From available ecotox data critical limits in soil solution were derived using data for aquatic organisms and by reinterpretation of ecotox experiments for soils for which concentrations in soil solution were estimated from total contents using the transfer functions mentioned above.

3. Results and Discussion

Table 1 shows "total" metal contents as extracted with aqua regia. The two forested sites have the lowest contents of heavy metals. The plots of agricultural land show somewhat higher contents and the plots of the former wastewater infiltration fields Zandleij are clearly the most contaminated plots. Table 1 also shows that higher metal contents not necessarily result in (proportional) higher concentrations in soil solution due to differences in soil properties It is thus not possible to assess the ecotoxicological risk from total contents only.

Table 1 Total metal contents, soil properties and metal concentrations in soil solution

Plot	Pb	Cd	Cu	Zn	OM	pH	Ca	DOC	Pb	Cd	Cu	Zn
		$mg.kg^{-1}$			(%)	-	$mg.l^{-1}$	$mg.l^{-1}$		$\mu g.l^{-1}$		
young forest	12	0.19	12.5	13.1	7.7	5.5	41	111	4.3	0.43	34	383
old forest	20	0.20	0.9	5.8	9.6	3.4	25	121	12.8	2.4	322	690
agric. land 1	11	0.24	14.7	31.1	2.9	6.8	37	363	8.6	4.0	370	350
agric. land 2	20	0.27	20.3	29.4	3.6	4.9	9.2	59	2.9	0.6	154	266
Zandleij 1	169	2.86	101	529	16	5.5	66	75	46	27	367	2416
Zandleij 2	180	3.01	115	610	12	5.7	70	57	41	14	201	1720

Free ionic concentrations were calculated from both "reactive" metal contents and soil solution data according to Bril and Groenenberg (1999) and gave comparable results. Calculated free ionic concentrations and measured concentrations were compared with critical limits for the soil solution. Figure 1 shows a concentration gradient for free metal ion concentrations of Zn in a wastewater

infiltration field (Zandleij). These concentrations were compared with a critical limit for Zn based on free metal ion concentrations for Zn (Environment Canada, 1998) (critical limit 1) and a critical limit based on data for aquatic organisms (critical limit 2). Critical limits however include uncertainty due to assumptions on the transferability of ecotox data for aquatic organisms to soil organisms and uncertainty in the calculated free ionic activity that may be relatively large for low contents.

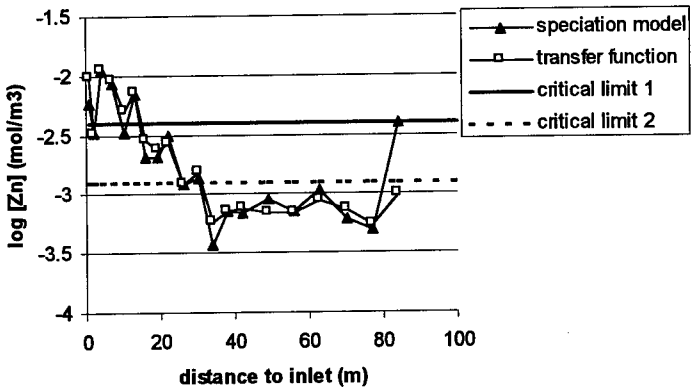

Figure 1. Comparison free metal ion concentrations Zn with critical limits.

4. Conclusions

The method to calculate free metal concentrations in soil solution from measured contents in the solid phase is a practical method to be used for risk assessment. Labour intensive measurements to determine soil solution concentrations of the metals of interest together with the measurement of the macro chemical composition of the soil solution can be left out. Furthermore the use of transfer functions in combination with critical limits for the soil solution can give insight in changes in ecotoxicological risks due to changes in soil properties which is of interest in the case of land use changes (i.e. acidification due to afforestation) or in case of active soil protection when measures are to be taken to decrease harmful effects as for instance liming of acidified soils.

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REGIONAL-SCALE ASSESSMENT OF HEAVY-METAL ACCUMULATION IN THE NETHERLANDS

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1. Introduction

A simple process-oriented model SOACAS (Tiktak et al., 1998) was used to assess the magnitude of the accumulation of cadmium, zinc, copper and lead in soil due to non-point sources on a regional-scale. Clay-content, organic matter content and present values of pH were obtained from the 1:50,000 digital soil map of the Netherlands, all other model inputs were derived from pedotransfer functions, or were obtained from the literature (generic data). The objectives of this study are (i) to evaluate whether a general purpose model of metal behavior in combination with generic process parameters can simulate the currently observed heavy-metal contents, and (ii) to predict future soil contents of heavy-metals.

2. Materials and Methods

Figure 1 summarizes the general approach for the current study. In an earlier study for cadmium, we used 2544 recent point observations to create maps of the currently observed heavy-metal contents (left side of figure 1). A Generalized Additive model was used for this purpose. We then tested whether it was possible to reconstruct these maps, using the SOACAS model and independent estimates of past heavy-metal loads (right side of diagram).

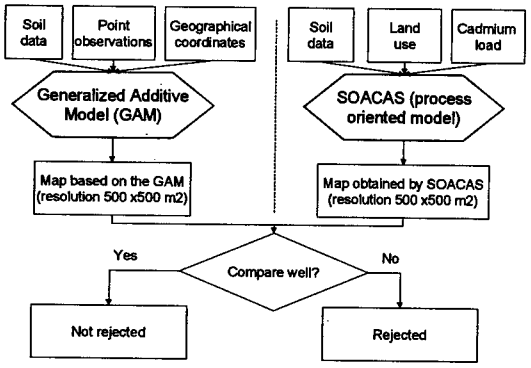

Figure 1: General approach for the current model validation study

SOACAS describes the fate of a metal in one completely mixed soil compartment. Partitioning of metals is described by a Freundlich equilibrium isotherm. The leaching and uptake of metals are described by first-order rate processes. The model also considers inorganic complexation and complexation of metals by dissolved organic matter (DOC). In a first approach for cadmium (Tiktak et al., 1998), we have used a generic sorption isotherm, which is based on batch-experiments published in the literature (Elzinga et al., 1999):

$$\log(Q) = \beta_0 + \beta_1 \log(c) + \beta_2 \log(clay) + \beta_3 \log(org) + \beta_4 \mathrm{pH} \qquad (1)$$

where Q (mg kg^{-1}) is sorbed metal content, c (mg L^{-1}) is heavy-metal activity, *clay* (g g^{-1}) is clay-content, *org* (g g^{-1}) is organic matter content, and $\beta_0 \ldots \beta_4$ are regression coefficients.

3. Results and Discussion

Despite large uncertainties about past cadmium loads, the simulations for cadmium showed that the model predictions were within a factor of 2 from the observed total cadmium contents at 90% of the total area (figure 2). In forest soils, total cadmium contents were systematically underestimated due to not considering biocycling. Also, in the south-eastern part of the country past cadmium loads were underestimated. Simulations for zinc, copper and lead showed that the observed total metal contents were seriously underestimated. The most important reason is that a considerable fraction of these metals is stored in non-reactive fractions, such as organic matter and oxides. These fractions have recently been quantified by combining the batch data with field-partition data, which also consider metals in non-reactive pools. Results of this exercise will be presented at the conference.

4. Conclusions

The SOACAS model could well be parameterized on the basis of commonly available soil characteristics. Comparison of the map based on the observed total heavy-metal contents and the map obtained by hind-cast simulation shows good correspondence for cadmium. For the other metals, however, the model underestimated the currently observed total metal contents. An important reason for this underestimation was that batch sorption isotherms do not consider non-reactive metal fractions.

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MAPPING SOIL METALS IN NEW ORLEANS: PRELIMINARY COMPARISON OF TWO SURVEYS

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1. Introduction

Soil surveys underway in New Orleans elucidate the environmental health impact of urban accumulations of metals, especially lead (Pb) (Mielke 1991). This report describes preliminary comparisons of two surveys. Survey I was conducted in 1989-91. The project showed that 10 to 15 samples, collected from the top 2.5 cm soil surface, stratified by census tract and organized by sample type, were sufficient to map a city (Mielke 1994). The data confirm fundamental differences (p-value $<10^{-80}$) between the inner-city and other areas of the city (Mielke et al. 1983; Mielke et al. 1993). Further research demonstrates the link between the soil pathway of exposure to humans by showing that children picked up more lead on their hands outdoors than indoors and that the quantity of lead on hands was directly related to the amount of lead in the soil (Viverette et al. 1996). Finally, community measures of soil lead were shown to be strongly associated with community measures of children's blood lead, and the association between soil lead and blood lead is over 10 orders of magnitude stronger than the association between old housing and blood lead (Mielke et al. 1997). Survey II (1998-) expands the urban research by replicating Survey I and adding Mn and Ni to the database. Survey II assists to determine the efficacy of the collection protocol and to evaluate possible changes in the quantity of Pb, Zn, Cd, Mn, and Ni between 1989-1991 and 1998-2000. This report evaluates similarities and differences between Survey I (1989-1991) and Survey II (1998-) of New Orleans.

2. Materials and Methods

2a. Soil Sampling. Soil samples for Survey I (1989-91) and Survey II (1998-) were collected with a density of 15 per census tract. Three types of soil samples were collected in each census tract, streetside (9 samples), open space (3 samples) and foundation (3 samples). Streetside samples were collected from residential streets, at least one block away from the busiest road(s). Documentation for each sample includes, census tract number, address of site, sample type, site characteristics, distance from the nearest building, name of sampler, and date. All samples were collected from the top 2.5 cm of the surface by scraping the soil and placing the soil in a labeled plastic bag. This report compares data of Survey II (to date 55 or 19% of 286 census tracts) with data of Survey I.

2b. Soil Extraction. All soil samples are air-dried, sieved (2 mm screen), shaker extracted 5:1 (volume to weight) with 1 mol L^{-1} nitric acid for 2 hours at room temperature, centrifuged (1000 x g for 15 min.), and filtered (Fisher Qualitative P4 Filter Paper) (Mielke et al. 1983) and analyzed for Pb, Zn, Cd, Mn, and Ni using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

2c. Statistical Analysis. First, the general results for the two databases are described using percentiles, See Table 1. Second, the median results of the soil metal analyses for the two surveys were analyzed for differences and similarities for Pb, Zn, Cd, Mn, and Ni using the Wilcoxon Signed Rank Test (SigmaStat, 1995). The reported P-values are also given in Table 1. The P-value is the probability of a test statistic being as or more extreme than the observed statistic under the null hypothesis.

3. Results and Discussion

Comparable data of Survey I and Survey II currently available for Metropolitan New Orleans are given in Table 1. Note that each metal is given (along with Roman Numeral in parentheses). All soil data is given in mg/kg. The P-values are the results for paired results for Survey I and II. Pb, Zn and Mn are not significantly different between Survey I and Survey II. This is expected because these metals are generally immobile and relatively stable in soils. These data also show that there are significant differences for Cd and Ni between Survey I and Survey II. Specifically, a reduction for Cd and Ni occurs between 1989-1991 and 1998. The apparent reductions of both Cd and Ni suggest that a previous source of these metals has been curtailed and that these metals are now undergoing mobilization and removal from urban soils.

TABLE 1: Percentiles of median soil data for Pb, Zn, Cd, Mn and Ni of Survey I (1989-91) compared with Survey II (1998-). Preliminary results are for 55 Census Tracts of Metropolitan New Orleans. (mg/kg). P-values for paired data as determined by the Wilcoxon Signed Rank Test.

Metal (SN)	0	10	25	50	75	90	100	N	P-value
Pb (I)	12	51	120	297	482	777	875	55	0.137
Pb (II)	*17*	*57*	*144*	*339*	*544*	*866*	*1344*	*55*	
Zn (I)	45	70	109	249	374	569	908	55	0.059
Zn (II)	*26*	*87*	*164*	*257*	*425*	*602*	*885*	*55*	
Cd (I)	1.2	2.1	3.0	4.2	5.5	6.4	7.6	55	<0.001
Cd (II)	*0.1*	*1.3*	*1.7*	*2.6*	*4.2*	*4.7*	*5.3*	*55*	
Mn (I)	77	110	127	141	163	178	207	55	0.166
Mn (II)	*109*	*115*	*129*	*149*	*164*	*190*	*246*	*55*	
Ni (I)	5.4	8.0	9.0	10.6	11.8	13.3	15.5	55	<0.001
Ni (II)	*2.1*	*5.1*	*6.0*	*7.4*	*9.1*	*10.7*	*12.9*	*55*	

4. Conclusions

An implication of this finding is that Survey I accurately describes the distribution of Pb, Zn and Mn in residential communities of Metropolitan New Orleans. Further research is being conducted to evaluate the differences between Cd and Ni identified in these preliminary findings.

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FACTORS INFLUENCING METAL ACCUMULATION IN DIFFERENT AGE SEGMENTS OF *HYLOCOMIUM SPLENDENS* (HEDW.)B.S.G.

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1. Introduction

Lichens and mosses are good heavy metal bioaccumulators and for this reason they have been used for many years for evaluating the rates of atmospheric heavy metal deposition. But some aspects of the accumulation and location mechanisms and the influences of environmental factors on the metal concentration rates have not been clearly explained yet. The purpose of this paper is to quantify the concentration of As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V and Zn obtained from three different age parts of the moss species *Hylocomium splendens* (Hedw.) B.S.G..

2. Materials and Methods

The moss samples were collected in 14 stations in the Alpine and pre-Alpine belts of the Friuli - Venezia Giulia Region (N-E Italy) according to the sampling procedures described by Rühling (1994). All the gametophytes were divided into three portions of different age: the youngest part, developed in the last growing season, the intermediate one, developed in the previous growing season and the oldest part including all the other portions of the moss. Each moss sample (2 gr) was digested and the metal concentration of the solution obtained was evaluated by using an ICP - Mass Spectrometer. Significant differences between the different age portions of *H. splendens* were tested by one-way ANOVA. The metal concentration of the youngest and the oldest portions was submitted to a clustering analysis in order to verify the similarity pattern of different elements in the same age part of the moss; complete linkage was used as calculating algorithm and Euclidean distance was used as similarity measure.

3. Results and Discussion

As maintained by other authors (Brown, 1982) metal concentration generally increases from the youngest towards the oldest moss portions (Tab. 1). The ANOVA analysis shows that there is a significant ($p < 0.01$) difference in metal concentration between the different age segments for all metals except Cu ($p < 0.05$) and Cd for which there is no significant statistical difference.
The reduction of metal concentration from the oldest to the intermediate segment ranges between 96 and 72 per cent of the maximum value of the oldest portion, except for As (67 %) and Hg (64%). In the youngest portion, V, Fe, As, Hg and Pb show a total concentration less than 40% of the maximum value; the other metals have a lesser reduction, with a total concentration higher than 50%, except Ni which has an intermediate value of about 46 %.
The cluster analysis (Fig. 1) shows that three principal groups of metals can be identified in the youngest part of *H. splendens*. Cadmium alone characterises one group (C). The second group (A) is composed of Pb, Fe, As and Zn. These elements could be considered metals of soil origin. The third group (B) includes Hg, Cu, Ni and V. All these elements can be sent out into the atmosphere in high amounts by human activities (Rühling, 1994). Also in the oldest portion three groups can be identified: the C group is composed of Cd alone, the B group is formed by Hg and Cu and the A group includes all the other metals. In this portion of the moss a high influence of

the soil matter can be assumed and it is more difficult give a clear explanation for the deposition pattern of different metals.

4. Conclusions

The soil influence on heavy metal concentration in the youngest parts seems to be lower than in the oldest one and so the amount of elements in the younger moss portions could be proportional to the atmospheric deposition levels, according to all studies about mosses used as bioaccumulators.

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Tab. 1 – Mean values, standard errors and percentage of maximum value of metal concentration in different age segments of *H. splendens.*

Moss portion		V	Cr	Fe	Ni	Cu	Zn	As	Cd	Hg	Pb
Youngest	Mean	3.26	5.77	492	4.46	24.9	120	0.37	1.41	0.22	28.3
	St. Error	0.392	0.578	62.67	0.348	2.003	11.06	0.058	0.223	2.315	3.599
	% of max	*25.5*	*53.9*	*21.9*	*46.6*	*73.9*	*56.9*	*19.4*	*83.9*	*31*	*31.4*
Intermediate	Mean	9.94	8.38	1637	7.39	32.4	165	1.29	1.39	0.46	75.6
	St. Error	0.934	0.646	330.5	0.5	3.095	15.59	0.238	0.214	4.28	8.576
	% of max	*77.7*	*78.3*	*72.8*	*77.1*	*96.1*	*78.2*	*67.5*	*82.7*	*64.8*	*84*
Oldest	Mean	12.8	10.7	2249	9.58	33.7	211	1.91	1.68	0.71	90
	St. Error	1.332	0.725	263.5	0.981	2.691	18.99	0.179	0.323	5.025	7.651

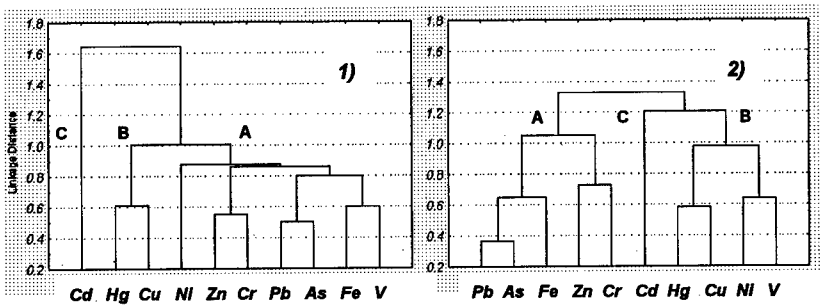

Fig . 1 – Dendrograms of metal concentrations in the oldest (1) and youngest (2) portions of *H. splendens.*

POTENTIAL RISK ESTIMATIONS OF HEAVY METAL POLLUTED SITES BY EXTRACTION SCHEMES

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1. Introduction

The importance of distinguishing between the acute and the potential risk of heavy metal polluted sites becomes obvious as the latest legislative activities in Germany show. The new Federal Soil Conservation Act focuses on the endangering of the groundwater. In proposed regulations (LAWA/LABO/LAGA, 1997) extraction tests are assigned for assessing the acute risk only.

The acute risk may be estimated by analysing the heavy metal concentrations of the seepage water taken from the boundary of the saturated and unsaturated zone. But the potential risk which takes the long term hazard into account is not properly defined yet. Moreover, there is a significant lack in estimating long term effects by means of short term extraction tests. Such tests may base on the circumstance that species with lower bound strength are more easily extractable than those with a higher bond strength. Following the idea of sequential extractions it should be possible to distinguish between these operational defined group of species. The question is whether an extraction sequence that represents the potentially mobilizable amounts can be substituted by a single extraction.

2. Materials and Methods

Three soils highly contaminated with Pb, Cu and Zn were investigated for a statistical comparison of the following extraction schemes: (1) the first four steps of a sequential extraction (SE) described by Thöming and Calmano (1998) and (2) five single extractions (a) dest. water, (b) 1 M NH_4NO_3, (c) 0.1 M NH_2OH-HCl, (d) pH_{stat} = 4 (HNO_3) and (e) aqua regia (pseudo total content), all in duplicate. To ensure the comparability of the schemes the significance of the results has to be shown by a variance analysis using an F test.

The sum of the amounts extracted by the SE scheme was defined to be the potentially extractable part ρ_{pm} of the soil's metal content. For a comparison of the extraction tests the amount $\rho_{j,\,i}$ of the soil j yielded by means of procedure i was set into a ratio r with the potentially mobile amount $\rho_{j,\,pm}$ (Eq. 1). The gained matrix was tested statistically by variance analysis using a two-way classification with no replicates.

$r_{1,1}$	$r_{2,1}$	$r_{3,1}$	← RSD
$r_{1,2}$	$r_{2,2}$	$r_{3,2}$	·
$r_{1,3}$	$r_{2,3}$	$r_{3,3}$	·
$r_{1,4}$	$r_{2,4}$	$r_{3,4}$	·
$r_{1,5}$	$r_{2,5}$	$r_{3,5}$	·
$r_{1,6}$	$r_{2,6}$	$r_{3,6}$	·

Figure 1: RSD calculation for the rows of the matrix r

$$r_{\text{(soil j, scheme i)}} = \frac{\rho_{j,i}}{\rho_{j,pm}} \qquad (1)$$

3. Results and Discussion

The F-tests show that a comparison of the extraction tests is statistically allowed. This is since the types of single extractions have a significant influence on the ratio $r = \rho_{j,i} / \rho_{j,pm}$ while, on the other hand side, the types of soils tested do not influence this ratio with high certainty.

As Figure 2 indicates, the amount of lead extracted by a single EDTA extraction differs in two of three cases significantly from the SE-extraction that uses the same EDTA solution in the fourth step. This does not depend on the total lead concentration in the soil. From the single extractions investigated only the aqua regia extraction correlates with the sum of the SE for Pb, Cu and Zn simultaneously.

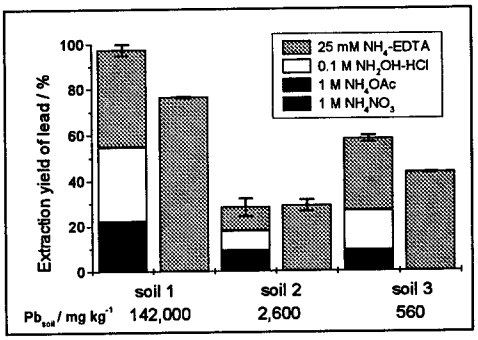

Figure 2: Pb-results of the fourfold sequence compared with an EDTA single extraction. the error bars illustrate the 80 % confidence interval (t test) referring to the total process variance of the scheme. A 100 % extraction yield is equal to the total amount of lead.

able 1: Testing for correlation (corr.) of a scheme i with the SE-scheme.

1.Schemes i	Zinc RSD of r	Zinc corr.	Copper RSD of r	Copper corr.
H_2O	1.25		1.11	
NH_4NO_3	0.89		0.32	
NH_2OH	0.39		0.80	
HNO_3 pH 4	0.05	yes	0.89	
Aqua regia	0.10	yes	0.15	yes

4. Conclusions

Potential risk means that also long term percolation of weak extractants with changing composition are capable to mobilise heavy metals from stronger bound portions. An estimation of the potentially mobilizable fraction by short term extraction with weak agents tests suggests "stable" residual fractions. "Stable" as this term is used here means that these fractions do not deliver any metal amounts into the mobilizable fractions. The results give hints that even the non-mobilizable fractions will release metal amounts in long term percolation. Thus it seems advisable to use an aqua regia extraction for estimating the potential risk of a heavy metal contaminated soil.

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ECOLOGICAL ASPECTS OF REMADIATED POST-MINING AREAS AFTER ZINC AND LEAD EXPLORATION IN SOUTHERN POLAND

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1. Introduction

Mining and processing of zinc and lead ores from Triassic formation in southern Poland are located in the northern part of Katowice Province on a line Piekary - Bytom - Bukowno - Olkusz. The zinc and lead ores occurs in sulphide and carbonate forms. The carbonate forms are separated from other ores during a flotation process and as a wastes they are transported by fluming to settling tanks. The area of the individual settling tank is between 20 and 40 ha. The total area covered by settling tanks in Katowice Province is 350 ha. The grain size composition (prevailing of silt fraction) of flotation wastes as well as high content of Zn (3.5%), Pb (1.5%) and Cd (> 100 mg/kg) are not an obstacle to soil-less reclamation of settling tanks. The reclamation of these areas using grasses mixtures as well as shrub and tree species were carried on in 1975 - 1985 (Strzyszcz 1975, Strzyszcz 1980, Patrzalek Strzyszcz 1980).

2. Materials and Methods

During the reclamation in 1970's the following tree species were planted on the settling tank surface: poplar - 5 clones *(Populus Hybrida 275, 194, Populus Italica 214, Populus robusta, Populus Serotina)*, birch *(Betula verrucosa)*, maple *(Acer platanoides)*, red oak *(Quercus rubra)*, common oak *(Quercus robur)* linden *(Tilia cordata)*, alder *(Alnus glutinosa)*, robinia *(Robinia pseudoacacia)*, pine *(Pinus sylvestris)*, larch *(Larix eurolepis)*. Additionally grass mixtures were sowed. A heavy metals concentration in grass and in leaves or needles were analysed 20 -25 years after reclamation. The grass samples were taken in June from 6 places with area $1m^2$, located on settling tank surface. Leaves and needles from 5 trees of each species were taken in second decade of September. Leaves and needles were washed 2 times in distilled water, then dried and dry-combusted in temperature 450°C. Ash were dissoluted in 10% HCl. Each heavy metal was determined using AAS (1100 Perkin Elmer), (Ostrowska et al. 1991). The grass was not washed before the treatment. It was combusted after initial drying.

3. Results and Discussion

The highest heavy metal uptake from a substratum was found in poplar clones (Tab. 1). A different Zn, Pb and Cd content which was observed in poplar leaves is result of different dose of phosphorus fertilisers on a studied areas (0 - 200 kg/ha P). The high content of heavy metals was observed only in leaves taken from surfaces where phosphorous fertilisation was not applied. The other tree species used for reclamation were not fertilised. A high content of Zn and Pb was also observed in birch, alder, robinia and maple leaves. For the biological reclamation processes the first three species serves as pioneer species. A considerable lover content of Zn and Pb was observed in leaves of linden and two oak species. In the case of coniferous trees the larch needles contained considerably lower amount of heavy metals in relation to pine needles (Tab. 1). A high content of heavy metals is observed in grass samples. It is probably caused by fact that grass samples were not washed before treatment.

From several years a population of hares and roe deer are observed on the reclamated settling tanks. They utilise a grass and papilionaceous plants (white clover) as a feeding stuff.

4. Conclusions

From the study it appears that, in spite of high content of zinc, lead and cadmium the settling tanks remained after zinc and lead ore flotation may be soil-less reclamated. However, because of environmental criterions, the surface of settling tanks must be covered by at least 30 cm of soil layer in case of short revegetation or 150 - 200 cm in a case of shrub and abrorescent revegetation. For this purpose any other waste materials with a low content of heavy metals could be also utilised. In Upper Silesia a many kinds of mining wastes connected with hard coal exploration seems to be a suitable material for reclamation of lead and zinc wastes.

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Tab.1.Content of heavy metals in leaves and needles of some tree species and grass.

Tree Species	Fe	Mn	Zn	Pb	Cd
	mg/kg				
Poplar H 275	100-400	50-150	1150-1950	10-100	2-12
Poplar H 194	75-100	50-70	1250-1800	10-90	4-6
Poplar I 214	75-130	60-80	1350-1900	10-50	2-8
Poplar Robusta	75-95	60-90	1300-1750	40-110	2-6
Poplar Serotina	60-105	60-70	1450-2050	70-90	4-12
Birch	115	70	1250	110	2
Maple	105	20	455	80	2
Red oak	90	20	265	60	2
Common oak	70	120	200	10	2
Linden	110	70	185	50	2
Alder	205	90	800	80	2
Robinia	115	40	550	60	2
Pine	210	30	225	70	2
Larch	275	80	155	5	0.5
Grass mixture	-	91	694	454	19

GEOPHAGY: A DIRECT SOIL-ANIMAL GEOCHEMICAL PATHWAY

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1. Introduction

The deliberate consumption of soil (geophagy) is very common in the Animal Kingdom. For example, although often previously described as eccentric, perverted, odd or bizarre certain human societies, especially those of the world's poorer or more tribally oriented people, indulge in deliberate soil ingestion. Geophagy, however, remains a largely unknown, ignored or misunderstood practice by the research fraternity of developed countries. This paper summarises 2 recent studies investigating geophagy (one studying geophagy by humans in Uganda, the other looking at soil licks utilised by animals such as elephant in the Mkomazi Game Reserve, Tanzania), and highlights the potentially important soil-animal pathway of elements which results because of this practice.

2. Materials and Methods

Following collection and appropriate preparation, the geophagical samples were variously analysed for their mineralogy and % clay content, pH, organic matter content, salinity, carbonate content, cation exchange capacity and total and bioavailable concentrations of 12 elements using standard methodologies, equipment and analytical quality control procedures.

3. Results and Discussion

The geophagical samples from Uganda vary from neutral to strongly acidic in terms of their reaction, are generally low in organic matter and, relative to known typical soil concentrations, the samples are not anomalous in a geochemical sense. Although deliberate soil consumption by humans is known to be caused by a number of factors, it has often been tempting to explain this geophagy as being a response to a mineral nutrient deficiency. Iron is the element that is frequently implicated in this hypothesis, yet the evidence is conflicting and not wholly convincing. Nevertheless soils do have the potential to supply mineral nutrients to humans even if they are not being consumed for this purpose. The ability of soils to supply elements to humans can be assessed by subjecting samples to a 0.1 M HCl extraction. In this study, the bioavailability of Fe is very low (median = 1.3%; range = 0.16-5.6%) relative to the total concentrations of this element in the 12 samples investigated. Despite this, Table 1 shows the average intake of various elements including Fe that would be supplied in a bioavailable form for every 5 g of soil consumed by the geophagist. These values are compared to Reference Nutrient Intake (RNI) values (i.e. the daily dietary values of mineral nutrients above which the amounts will almost certainly be adequate for everybody). Table 1 presents the RNI values for the 15-18 year age group, those humans who generally have the highest requirement for mineral nutrients, and shows the potential of the geophagical samples in supplying significant amounts of Fe (and Mn) to such individuals. This potential is greater than Table 1 fully indicates, bearing in mind that a majority of people will have nutrient requirements lower than the stated RNI values.

Table 1. The average (median) intake of bioavailable elements for every 5 g of soil consumed, and a comparison with the Reference Nutrient Intake (RNI) and safe intake values for 15-18 year olds. °n = 12; *safe intake for adults; RNI and safe intake values (mg/d) from UK Department of Health (1991).

Element	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
Average element intake per 5 g soil supplement (mg)°	6.71	0.01	2.5	3.82	2.29	0.29	0.93	0.03
RNI for 15-18 yr.:								
males	1000	1.2	11.3	3500	300	1.4*	1600	9.5
females	800	1.2	14.8	3500	300	1.4*	1600	7.0

For the remainder of the Animal Kingdom, the practice of geophagy is well documented. Among terrestrial vertebrates, many herbivore and omnivore animals undertake soil eating. Often the soil intake is seasonal and selective, concerning the exploitation of specific sites and sometimes even particular soil horizons. These sites are frequently referred to as mineral licks, natural licks or salt licks, and such terminology suggests that the animals are indulging in geophagy to satisfy a mineral nutrient imbalance. Whilst sodium is believed to be an important constituent causing the exploitation of licks, the possible causes of this geophagy are manifold. To investigate further, during a geochemical soil survey of the ecologically important Mkomazi Game Reserve in Tanzania, three lick sites were visited and sampled for mineralogical and geochemical analysis. The lick soils are distinct from topsoils sampled elsewhere in the Reserve, and there are notable differences amongst the lick soils themselves. One lick soil is enriched in kaolinite; a clay mineral that can act as a pharmaceutical agent by preventing or treating gastrointestinal upsets. Two lick soils are enriched in sodium, which may attract wildlife, although additionally both soils contain constituents such as $CaCO_3$ and smectite that can overcome a disorder called lactic acid acidosis. One of these soils is particularly distinctive, being strongly alkaline, highly calcareous and saline-sodic. This soil has the potential to provide a range of benefits to animals if consumed in appropriate amounts.

4. Conclusions

Overall, the practice of geophagy is widespread in the Animal Kingdom. Such soil ingestion can either be beneficial or detrimental to the geophagist in a number of ways. As far as environmental geochemistry is concerned, geophagy provides a direct link between the chemistry of soils and animal (including human) health and, for example, ingested soils have the potential to supplement important mineral nutrients such as iron and sodium to the geophagist. Despite this and other important consequences of geophagy however, collaborative and integrated clinical trials linking soil and food analysis with detailed investigations on the health and nutrition of geophagists remain limited.

LEAD ISOTOPE COMPOSITION IN LICHENS FROM URBAN, RURAL AND VOLCANIC SITES IN EASTERN SICILY.

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1. Introduction

This short note is part of a comprehensive multi-element biogeochemical study carried out in eastern Sicily with the aim of defining the source and pathway of metals in the atmospheric environment. It reports the results on the lead isotope composition measured in lichens from urban and rural sites located near the two most active sicilian volcanoes (Mt. Etna and Volcano island). Applications of lead isotopes in lichens are quite scanty in environmental studies. Two main reasons suggested us to follow such a technique: a) lichens reflect long term deposition, as they are constantly exposed to the air environment, entrapping solid particles that impinge on their whole surface and being capable of accumulating metals to concentrations that exceed their needs, b) stable lead isotopes may be used to pinpoint the origin of atmospheric Pb and, therefore, to apportion the relative contribution.

2. Materials and Methods

Mt Etna (3300 m. above sea level) is the largest active volcano in Europe and its activity is mostly effusive with frequent eruptions. The island of Vulcano belongs to the volcanic arc of the Aeolian islands. Its last eruption occurred in 1888-1890. The present-day volcanic activity consists of intense hydrothermal activity concentrated round the crater and in the area surrounding the cone. A total of 25 lichens samples of the species *Parmelia conspersa* (Ehrh) were collected exclusively from rock surfaces. Each sample was dried at 40 °C, carefully separated from the substrate particles and then finely powdered. Isotopic analysis were performed by thermal ionization mass spectrometry and by ICP-MS. The isotopic signature of italian leaded gasoline was determined by analysing samples directly collected from the main suppliers (AGIP, ESSO, IP). To evaluate the industrial Pb isotope ratios, samples of airborne particulate matter were collected near important industrial sites. Data for natural (crustal and volcanic) lead were compiled from the literature (Carter and Civetta, 1977; Ferrara et al., 1995).

3. Results and Discussion

Lead isotope composition in the analyzed lichen samples is plotted in Fig. 1 as $^{206}Pb/^{207}Pb$ vs. $^{208}Pb/^{206}Pb$ scatter diagram. The isotopic signatures of the main potential lead sources are also shown for comparison. The sample points on the figure display an excellent linear correlation, where anthropogenic (gasoline+industrial) and natural sources represent the end members. With respect to the anthropogenic lead signature, the observed isotopic range for lichen samples (1.156-1.208) is shifted towards the compositional field of radiogenic sources. Such a natural input is more evident for the lichens collected at Vulcano island than for those from Mt. Etna, where the anthropogenic activities are considerably more effective. The natural source consists of crustal lead and volcanic emissions, too. However, the amount of soil-derived lead, estimated by considering total Sc (or Al) concentrations in lichens and the average Pb/Sc (or Pb/Al) ratio in the local rocks, results less than 5% in all the samples. Therefore, we can argue that the observed lead isotope composition in lichens may be explained only if the volcanic source (characterized

by higher Pb/Sc and Pb/Al ratios) is taken into account. A rough estimation of the volcanic contribution can be made considering two different binary mixings:

1. gasoline ($^{206}Pb/^{207}Pb$=1.080) - volcanic ($^{206}Pb/^{207}Pb$=1.230-1.260)
2. industrial ($^{206}Pb/^{207}Pb$=1.155) - volcanic ($^{206}Pb/^{207}Pb$=1.230-1.260).

Such computation yields an estimated volcanic lead ranging between 20-60%, with the highest values regarding the island of Vulcano.

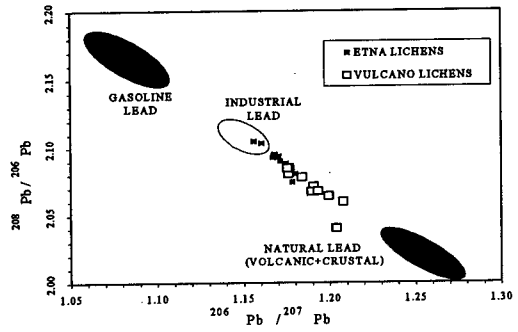

Fig.1. $^{206}Pb/^{207}Pb$ vs. $^{208}Pb/^{206}Pb$ scatter diagram.

4. Conclusions

These results corroborate the previous conclusions (Dongarrà and Varrica, 1998) that volcanic activity should be also considered when studying the atmospheric budget of Pb. Volcanoes, being a significant source of release of trace elements to the atmosphere, even during normal fumarolic activity, tends to increase the background levels of some metals in air and soils.

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PHENOLIC COMPOUND BUILD-UP IN PINE AS BIOINDICATOR OF POLLUTION

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1. Introduction

Sclerosis and phenolic compound synthesis are included in the responses of plants to abiotic stress (ultraviolet rays, ozone and other pollutants, poor nutrition, drought etc.). An accumulation of flavonols, precursors of tannin, has been observed in forests in a state of decline, and it has been demonstrated that sclerosis is often associated with phenol accumulation (5).

This study describes the structural and biochemical alterations in leaves of *Pinus pinea* L. located in various sites in and around the city of Palermo, Italy, with different, ecological gradients and with different levels of pollution. Histochemical tests reveale a considerable increase in phenols throughout the year. Found in the leaves, they increase under stressful conditions, and accumulate in the vacuoles of the cells of the upper epidermis and in the mesophyll cells. They are initially found in the distal portions of the leaves and later in the proximal portions.

At a later stage the phenols appear solubilized in the cytoplasm and, probably, re-translocated; they end up also permeating the outer walls of the epidermic cells, between the walls and the cuticle, mainly in the distal portions of the leaves. An examination of the epidermic cells also reveals an increase in the thickness of their external walls (1;4).

The question, therefore, is whether the role of secondary metabolites in the pine, including polyhydric phenols, is to detoxify and protect. Moreover, this study aims to determine if the accumulation of these polyhydric phenols can be considered a viable bioindicator of pollution or abiotic stress in general.

Leaves pine are sensitive to a variety of pollutants, which can increase the permeability of the cuticles and have been used in many studies as bioindicators.

2. Materials and Methods

Sampling has been performed in two different urban (with strong road traffic) and periurban areas of Palermo. Leaf and pollen samples were taken from the tip and from middle-base leaves.

Histochemical analyses were carried out on cryosections for phenolic compound reaction. The solutions used were those recommended by Gahan (2), (1974), with FeCl3 (2% in 95% ethanol) and fast blue BB (0,008% in acetate buffer, at pH 6,5). Leaves and inflorescences with pollen were gathered randomly, taking note of exposure and location.

Samples were taken from the lower foliage from around the whole perimeter of the trees in order to obtain representative samples, and included both healthy and green as well as partially or completely necrotised leaves, and both healthy and brown, viscous inflorescences.

The leaves were dried and mineralized by digestion and lead was mesuared using atomic absorption spectrophotometer with graphite furnace. Concentrations are expressed in mg for gram of dry weight of vegetal structures.

Morphological analyses were carried out in the field, and microanatomical, cytochemical and chemical analyses were performed in the laboratory.

3. Results and Discussion

Phenols are absent in the controls, while they permeate the vacuoles in the leaves taken from the sites with the highest levels of pollution. The leaves adapt to stress caused by pollutants and the structural modification are accompanied by an increased production of phenols and by accumulation of lead in the cells.

Data differ considerably for the control plant leaves, the leaves taken from the peripheral areas, and the leaves from plants located in the city in polluted areas with heavy traffic. These differences concern the

size of the epidermic cells, which are more coarctated initially in the leaves from plants in the more polluted city areas.
The thickness of the mesophyll, inferior in leaves from polluted sites, demonstrates a coarctation of cells in leaves from the more polluted stations, while the width of the mesophyll cells indicates the accumulation of phenols, higher in leaves from the more polluted sites. There is a greater accumulation of phenols in the distal portions, therefore both the size of the epidermic cells and the width of the mesophyll cells increase in the samples from the more polluted stations and the mesophyll cells are seen to be deformed, dysplasic, with wide gaps between the cells.

4. Conclusions

Lead is taken up only in small measure by plant roots. Leaves almost everywhere in the inner city have higher levels which can be traced back to higher lead inputs from the air. Mostly this contamination can clearly be traced back to motor vehicle traffic emission.
Phenol synthesis is regulated by a series of key enzymes which may be formed by adverse environmental conditions.
Ozone also plays a part in the formation of key enzymes which are precursors of flavonoid and non-flavonoid phenolic compounds. They carry out an antioxidant action and are therefore de-toxifiers.
The key enzymes are located in the mesophyll, while the flavonoids also accumulate in the epidermis, in particular in the upper epidermis. A translocation probably takes place: it is uncertain if this can be related to the increase in the thickness of the epidermal cell walls.
Histochemical testing for phenolic compounds revealed modifications in mesophyll cells before discolouration of *Pinus Pinea* L. stressed leaves could be macroscopically discerned. This was followed by degradation of the cytoplasm, disappearance of organelles and a release of vacuolar contents and accumulation of damaged membranes precipitated by the phenolic compounds which finally led to the death of the mesophyll cells. Similar responses were visible after incubation in NaCl culture medium (3;6).
Further experiments are required in order to establish quantitative differences between controls and stressed samples, and to demonstrate that increased phenol production is localised and can be considered a bioindicator of pollution. Specific reactions towards other atmospheric pollutants must also be investigated.

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PAH AND HEAVY-METAL BIOMONITORING PROGRAM ALONG THE A32 MOTORWAY (NORTH WEST ITALY)

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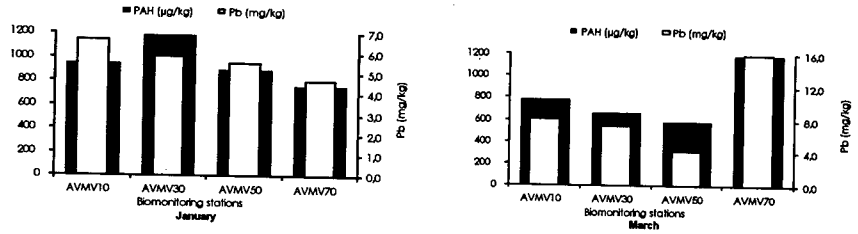
1. Introduction

From 1995 different plants species were exposed to traffic pollution in pots located at different distances from the A32 Rivoli-Bardonecchia motorway to measure the vehicular air pollutant dispersion. Since 1997, the biomonitoring programme was implemented also by the utilization of aeroponic cultivation techniques to free the vegetal material from any contact with soil pollutant. The project was ordered by SITAF to "CENTRO", a no-profit consortium made up of the Turin, Basilicata and Sheffield Universities, Turin Osservatorio di Genetica Animale and Consulagri Srl.

2. Materials and Methods

The program comprise bio-monitoring stations equipped with aeroponic modules: five located close to regional meteorological-survey stations along the motorway edge; two located near the Frejus Mountain tunnel (area T4); four located at increasing distances from the motorway edge in the pilot area of Avigliana - Messa Vecchia and one located into the urban area of Avigliana (Turin) to obtain comparable data from an urban environment. Different plants species were also exposed in pots (T4a,b,c,d and MV10,30,50,70) in corrispondance of the aeroponic modules in the pilot area of Messa Vecchia and in T4 area. Plant species considered were: *Brassica oleracea, Holcus lanatus* and *Taraxacum officinale. Brassica oleracea* was exposed in November 1997 and sampled in January and March 1998. PAHs (PHE, ANT, FLU, PYR, BαA, Bαp, PER, INP, BPE, DBA, COR, CHR, NAF, ACE, AFT, FOR) and Heavy Metals (Pb, Zn, Cd, Cr, Cu, Ni, Al, Fe, Mn) concentration has been determined. *Holcus lanatus* and *Taraxacum officinale* were exposed in July 1998 and sampled during the month of September. Heavy Metal concentrations was considered.

3. Results and Discussion



Figures 1 reports the trend of lead and PAH concentrations in *Brassica oleracea* leaves exposed in pots at different distances from the motorway (10, 20, 50, 70 m) in Avigliana Messa Vecchia area (AVMV) and sampled in January and March respectively.

Data show a decreasing trend of lead and PAH concentrations starting from plants exposed near the motor way border to those far away, except for the 70 m March sample showing a peak probably due to the presence in the area of an intensive agriculture activity.

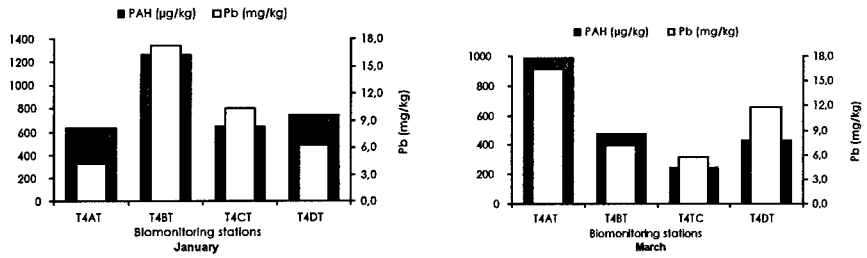
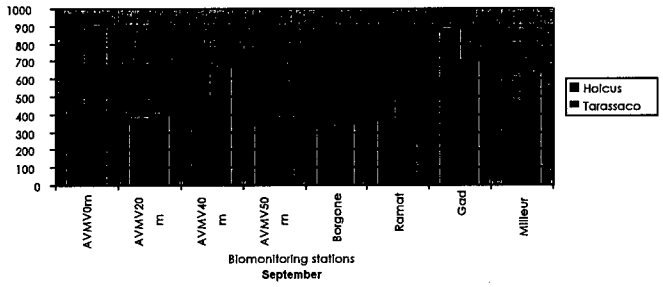


Figure 2 shows the trend of lead and PAH concentrations in Brassica oleracea leaves exposed in pots near the Frejus tunnel access area (T4AT and T4BT) and the slip road area (T4CT and T4DT), sampled in January and March respectevely.
Results show a relation between seasonal meteorological changes and lead and PAHs trend. In particular in the two stations closer to the Tunnel, the PAH concentrations are inverted from January to March while in the slip road area we have a comparable trend. Lead trend confirm the PAH's trend in the Tunnel area.

Table 1 shows data obtained considering all toghether PAH concentrations values determined in *Brassica oleracea* exposed in pots and sampled in January and March along the motorway and in Avigliana downtown.

LOCATION	□ PAHs CONCENTRATIONS (ppb)			
	MEANS	SD	MIN	MAX
A32	772,58	278	245	1185
URBAN	1901	396	1901	2135

Highest values are found in the urban environment.



AVMV: Avigliana Messa Vecchia area

0, 20 40, 50 m: distances from the motorway edge

Borgone, Ramat Gad , Milleur: module located close to meteorological survey stations

Figure 3 compares total Heavy Metals concentration, in *Holcus lanatus* and *Taraxacum officinale* exposed in aeroponic module sampled during the month of September 1998 .
The comparison shows in *Holcus lanatus* and *Taraxacum officinale* similar trends as well as good performances as Heavy Metal biomonitors.

4. Conclusions

Our data provided a detailed evaluation of pollutants diffusion in the valley environment.
The concentration of metals and PAHs in the exposed plants shows a trend related to the distance of exposure from the motorway edge as well as a seasonal variations of PAH's foliar concentrations due to the degradation caused by the solar exposure during spring months despite of the invernal ones. The above mentioned conclusion was also confirmed by the detection of a significant seasonal increase in metals concentration. By collected data we can deduce that the area along the motorway is less polluted than urban site even if we can identify along the motorway tracing critical points with high concentration of contaminants. We can also say that inspite of the use of green petrol, lead is still one of the most indicative tracer of motor vehicle emissions.Moreover, the use of *Brassica oleracea* and local plants, *Holcus lanatus* and *Taraxacum officinale* , unable us to point out them as the most suitable biomonitors for the detection of contaminants considered.

TOXIC ELEMENTS INPUTS TO AGRICULTURAL SOILS – AN EVALUATION AT A NATIONAL AND LOCAL SCALE

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1. Introduction

Application of a sustainable management principles in agriculture implies controlling soil quality parameters including inputs of toxic elements in soils. The sources of the inputs are either anthropogenic or natural. In natural conditions, the contents of their elements stays at the same level for a long time but under the anthropogenic impact, they can increase rapidly in a relatively short period of time and threaten the production and ecological functions of such soils. It can be misleading to count critical doses and critical levels in a global scale since some anthropogenic sources can vary in space very much. In order to assess the potential risks, the balances of risk elements for individual areas (watersheds, fields, parcels) should be counted.

2. Materials and Methods

For the area of agricultural land areas the sources of inputs of toxic elements into soil were assessed and recorded

Atmospheric deposition

In the period of 1995 – 1998 the deposition was measured by the bulk method over a network of 160 localities on agricultural soil and 40 localities in protected areas. Contents of the main nutrients and toxic elements were analysed and inputs in $g.ha^{-1}.y^{-1}$ were recorded

Animal manure and industrial fertilisers

The average contents of toxic elements in manure and fertilisers and their application rates were recorded .

Sewage sludge

In each district 1 – 3 water treating plants were tested three times a year (1996 – 1997) for the toxic element contents in sewage sludge, analysing about 1000 samples. At the same time, the amount of sludge being applied to agricultural soil was estimated. Based on these data, inputs of toxic elements in agricultural soils in $g.ha^{-1}.y^{-1}$ were recorded. On the other hand an inventory on about 50 fields with sewage sludge application was made in co-operation with farmers. The following parameters were assessed and analysed: applications of sludge ($t.ha^{-1}$), number of applications, contents of toxic elements in sludge dry matter
($mg.kg^{-1}$), contents of toxic elements in soil – plough layer). Inputs of toxic elements to soil in individual fields could be counted as well as the absolute and relative increase in the soil.
The importance of individual sources was evaluated on a global scale and compared with balances made on a local scale for fields with sewage sludge application.

3. Results and Discussion

Average inputs of toxic elements to agricultural soil are shown in table 1.

TABLE 1. INPUTS OF TOXIC ELEMENTS TO AGRICULTURAL SOIL FROM DIFFERENT SOURCES

source of input	Cd	Cr	Pb	Hg
	$g.ha^{-1}.y^{-1}$			
Fertilisers	0.639	7.610	1.624	0.004
Atmospheric deposition	1.312	7.132	30.790	0.200
Sewage sludge application	0.058	4.250	1.790	0.060

These were used to calculate the relative contribution of individual sources both in the averages for the whole country and for balances on the fields with sewage sludge application. Figure 1 shows the relative proportion of individual sources of toxic elements in soils for the whole area of agricultural soil In figure 2, similar data is presented for fields with sewage sludge application.

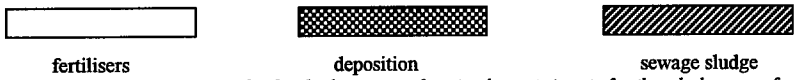
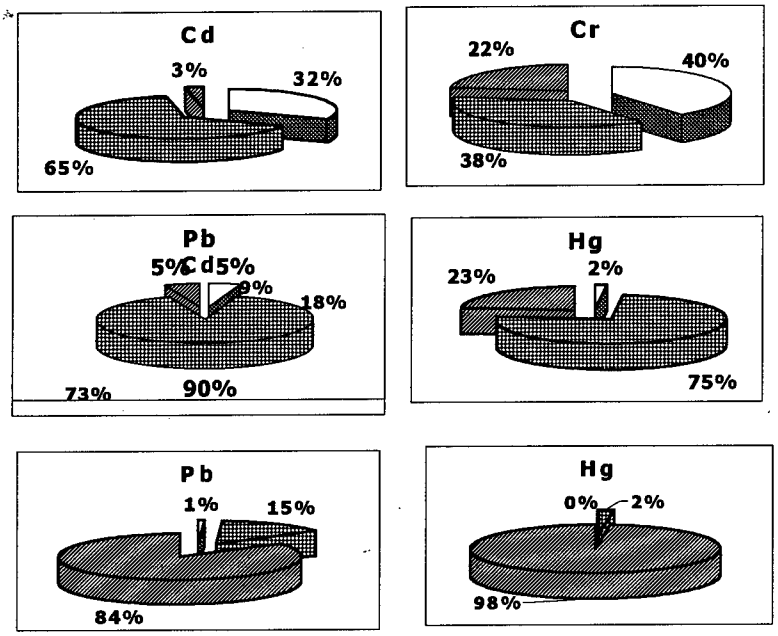


Figure 1. *Relative proportion of individual sources of toxic elements inputs for the whole area of agricultural soil*

Figure 2. *Relative proportion of individual sources of risk elements inputs for the fields with sludge application*



4. Conclusions

At the global scale, atmospheric deposition is the most important source of toxic elements in soils, while at a local scale, other sources may have higher importance from the point of view of soil pollution. This is especially true for localities with sewage sludge application.

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RISK ASSESSMENT OF SOIL FLUORIDE INGESTION BY CATTLE

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1. Introduction

With increasing amounts of F added to soils through pollution there are two major pathways which could increase dietary intake of F in cattle: Increases in F concentrations of pasture plants and ingestion of soil with increased concentrations F. The ingestion of soil F by cattle grazing to closely, or by forage contaminated by dust or rain splash, is potentially a significant source of F (ANON 1974).

The aim of this paper was to determine the risk of fluorosis (chronic fluoride toxicity) in cattle due to increases in F intake through ingestion of soil.

2. Materials and Methods

Absorption of fluoride across the stomach wall from ingested soil, water or plant material was calculated assuming: Fluoride is absorbed rapidly once dissolved in the stomach (ANON 1974). Soil labile F (LARSEN and WIDDOWSON 1971) was 10% of total soil F (STEWART et al. 1974) and soluble soil F was approximately half of labile F (BRAEN and WEINSTEIN 1985). Digestible F (F dissolved from soil or plant and absorbed across the stomach) was estimated to be 30% of total soil F (STEWART et al. 1974) and 70% of plant F (STEVENS et al. 1995). Given a concentration of total F in soil, F concentrations in plants grown in this soil were calculated from the relationship of total soil F with soluble soil F described above, and soluble soil F was used to calculated plant F. As F is reactive in soil, total F in soil refers to the top 5 cm of the profile, pasture plants roots are predominantly in the top 15 cm of the profile, consequently total soil F concentrations used to calculate plant F were divided by 3. For all calculations, F intake through water was equivalent to less than 2 mg/kg dietary intake.

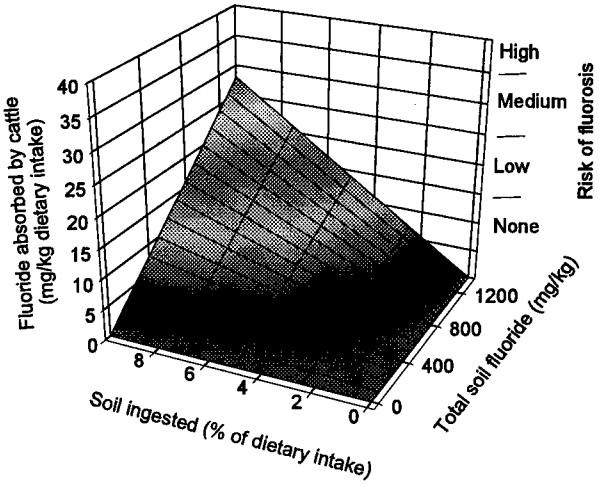

Figure 1. Effect of total fluoride concentrations in soil and quantity of soil ingested on fluoride absorbed by cattle through ingestion of soil and risk of fluorosis.

Annual loading of F applied to soil have been calculated assuming a soil bulk density of 1500 kg/m^3 and F remains in the top 5 cm of soil.

Fertiliser application rates were assumed to be 50 kg P/ha/yr using single superphosphate (9% P) containing 2% F as an impurity. Annual F losses from soil through plants was calculated assuming 10 tonne of dry matter per hectare produced annually with a F concentration of 20 mg/kg.
A range of total F concentrations in soils (0-1400 mg/kg) and a range of possible soil ingestion values by cattle, expressed as a percentage of dietary intake (0-10%), were used to assess dietary ingestion of F by cattle. Risk of developing symptoms of fluorosis was defined as none, low, medium and high when mg bioavailable F/kg dietary intake was < 15, 15 - 25, 25 - 35 and > 35, respectively (ANON 1974).

3. Results and Discussion

Through ingestion of soil alone, calculations show that high concentrations of total F in soil (1400 mg/kg) combined with increased soil ingestion could result in a medium risk of fluorosis developing in cattle (Figure 1). Fluoride concentrations of agricultural soils normally average 150-350 mg/kg (McLAUGHLIN et al. 1996), however, application of high rates of single superphosphate for 100 years could increase total concentrations of F in soil to 1400 mg/kg. If cattle graze on soils contaminated through F additions in phosphatic fertilisers, pastures may also contain raised concentrations of F, increasing total dietary intake of F. In this situation, dietary intake of F due to grazing could range from 10 - 50 mg bioavailable F/kg dried pasture.
Fluoride absorbed through ingestion of soil was calculated to be 0-50 % of that through ingestion of herbage (data not shown). These data suggest that the major source of F intake by cattle is through plants, but that soil fluoride concentrations could add significantly to the intake of F, thus increasing the risk of fluorosis. Because of the small margins between risk categories it is also possible that small increases in F intake by cattle (i.e. soil ingestion) may induce fluorosis.

4. Conclusions

In situations where total soil F concentrations have been increased 3 or 4 times the original concentrations through long-term addition of soil additives, ingestion of soil F could be a significant sources of dietary F for cattle.

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IMPACT OF FLY-ASH ON SOME PHOTOSYNTHETIC ASPECTS IN *VICIA FABA*

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1. Introduction

Fly-ash (FA) is a coal combustion waste generated in large quantities through coal based thermal power plants. Disposal of FA has significant impacts on terrestrial and aquatic ecosystems due to leaching of toxic substances from the ash into soil and ground water, as well as reduction in plant establishment and growth (Wong & Wong, 1990; Gupta et al., 1999). The FA is not suitable for agriculture due to its high pH, low soil microbial activity and deficient supply of essential nutrients nitrogen and phosphorus. Since, there is no consistent trend of effective utilization, the FA becomes a significant waste disposal problem. It is desirable to establish vegetation on these areas for both stabilization and aesthetic reasons. The use of amenders also suitable for the better management of crops. The present study planned to evaluate the effects of FA on the photosynthetic pigments and related parameters on *Vicia faba* with and without amendment.

2. Materials and Methods

Fly-ash was directly obtained from the pipelines of Feroz Gandhi Unchahar Thermal Power Project, Raibarelli, India. 45 days old seedlings were planted in earthen pots containing 100% soil (I^{st} set), 100% FA (II^{nd} set) and FA amended with 50% press mud (sugar industry waste, III^{rd} set). Three replicates were used for the each set. The pots were watered daily. Plants were sampled after 60 days for further analysis. The following parameters were determined; pH, electrical conductivity, total organic carbon, nitrogen and phosphorus. Metal content was estimated using AAS, Perkin Elmer 2380. Chlorophyll was estimated following Arnon (1949). Carotenoid was estimated according to Duxbury & Yentsch (1956). δ- aminolevulinic acid dehydratase (ALAD) was assayed following the procedure of Schneider (1970). Photosynthetic rate was measured using Li-cor model 6200 portable photosynthesis system. Protein was analyzed using BSA as standard according to Lowry et al., (1951).

3. Results and Discussion

Table 1 shows the general properties of FA, soil and press mud. pH and EC was high in FA as compared to soil and press mud . Uptake of metals were more in FA treated plants and the order of accumulation was Fe>Zn>Cu>Mn. All the photosynthetic pigments decreased in 100% FA, however, plants grown on amended ash showed higher contents although they were lower than those from soil. The activity of ALAD was more in amended ash as compared to FA and soil treated plants.(Table 2).

Fly-ash contains many beneficial plant nutrients but their availability to plants seems restricted. In 100% FA non-essential elements eg., Cu, Al, Ni, Se, Cr, Pb and Cd might impair various metabolic processes which are reflected by delayed or inhibited responses (Wong & Bradshaw, 1981; Singh et al., 1994). In the present study also all the contents inhibited in 100% FA, may be due to decreased level of chlorophyll or the metals restricting the level of precursors or through targeting -SH groups of ALAD (Van Assche & Clijsters, 1990).

4. Conclusions

The study indicates that amendment of FA with press mud is beneficial for the growth and development of plants, although the plants are able to survive in FA. However, trials should be carried out to find more suitable amenders for the for growing plants in these solid waste.

Table 1 General properties of FA, press mud and soil

Parameter	FA	Soil	Press mud
pH	9.6	7.6	7.2
EC (m mhos cm^{-1})	8.8	0.015	4.2
CEC $(meq(100g)^{-1}$	1.28	10.40	4.4
C (%)	1.172	1.40	1.248
N (%)	0.02	0.08	0.95
P(%)	0.02	0.03	6.8
Metals (µg/g)			
Fe	870	540	1750
Zn	148.24	108.42	220
Mn	74.42	14.42	63.20
Cu	86.78	27.42	139.50

Table 2 Changes in photosynthetic aspects in *Vicia faba*

Treatments	Chlorophyll (mg/gFW)	Carotenoid (mg/gFW)	ALAD nmolPBG/mg protein/2.5h)	Photosyn.rate (µmol m-2s-1)	protein (mg/gFW)
Soil	1.55±0.07	0.58±0.02	71.66±3.5	10.32±0.62	8.87±0.5
100%FA	0.82±0.04	0.38±0.01	54.66±2.6	7.43±0.41	4.90±0.29
Press mud	1.17±0.06	0.45±0.02	83.00±4.1	9.93±0.5	7.00±0.33

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Phytoremediation and Metal-Accumulation in Plants

(Technical Session 13)

DISPOSAL OPTION FOR PLANTS USED IN THE PHYTOREMEDIATION OF SE-LADEN SOILS

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1. Introduction

Selenium's bioconcentrations and apparent toxicity in the wetland food chain at Kesterson National Wildlife Refuge (Ohlendorf et al., 1990) compelled some researchers to evaluate using phytoremediation as a technology to lower Se concentrations in the soil (Bañuelos et al., 1997; Parker and Page, 1994). Bañuelos and Meek (1990) demonstrated that plant species which require high concentrations of sulfur (S), such as *Brassica*, will indiscriminately accumulate high concentrations of Se when grown in Se rich soils. They hypothesized that plants could be cultivated, harvested, and removed as Se-enriched plant material, resulting in lower Se concentrations in the Se-rich soils.

Selenium, while not required by plants, is an essential trace element for adequate nutrition and health for mammals (Mayland, 1994). Selenium deficiencies are generally a far greater problem than Se toxicities in animals in the US (Mayland, 1994). In this regard, Bañuelos and colleagues have suggested plants that were used for phytoremediation of Se, may be harvested, carefully blended with other animal forage (depending on the tissue Se concentration), and fed to animals in Se-deficient areas. This strategy may be considered as a potential disposal option for plants used for phytoremediation of Se, pending approval by regulating agencies (Bañuelos et al., 1997).

The objectives of this study were to: (I) determine the feasibility of feeding lambs with canola (used for phytoremediation of Se); (ii) evaluate the excretion of Se; and (iii) evaluate the absorption of Se into different organs of the animal.

2. Materials and Methods

Selenium accumulation and Se excretion by lambs fed with *Brassica napus* (canola) was investigated in Fresno, CA between January 1998 and April 1998. Purebred white-face „Southdawn" lamb were randomly housed in individual pens (2.5 m x 5 m). There were a total of 10 lambs used in the study. Treatments consisted of feeding two types of canola; control (containing no Se) and Se-containing canola. Se-containing canola was grown on a 5 ha field site on the west side of the central valley in California, where Se-laden effluent containing 75-100 μg Se L^{-1} had been used as a source of irrigation water, while control canola was grown at an adjacent 2 ha site, where better quality water (<3 μg Se L^{-1}) was used as a source of irrigation water. Both sites were planted in November of 1997.

Plant material was cut from each respective growing site and fed daily to the lambs. Leaf subsamples were collected from each feeding and analyzed later for Se and free seleno-amino acids (Janak et al., 1994). During the study, urine and feces samples were collected weekly from each lamb, frozen and analyzed later for Se.

After sixty days the study was terminated. Blood samples were taken prior to feeding trial and taken again at end of study for changes in Se content. The following organs were collected and

lyophilized from each lamb: heart, liver, kidney, spleen, loin, and toe nail. Plant and lyophilized organ samples were wet-acid digested as described by Bañuelos and Meek (1990) and analyzed for total Se by atomic absorption spectrophotometry with an automatic vapor accessory, while EDTA-Se levels in blood were wet-acid digested and analyzed by ICP atomic emission using hydride generation.

3. Results and Discussion

Lambs readily ate the canola plant material from both treatments throughout the study. Among the identified free seleno-amino acids (Se-methyl-selenocysteine, selenocysteine, and selenomethionine), only selenomethionine was found in Se-containing canola and not in control canola (data not shown). Significant differences in total weight gain for lambs between each treatment were not observed (data not reported).

Selenium concentrations increased in the collected urine and fecal samples, and in all tested organs, including blood, from lambs fed Se-enriched canola (Table 2). Among the tested organs, Se concentrations were greatest in the kidney and lowest in the loin and toenail.

The lambs were able to absorb Se that was fed to them as Se-enriched canola. Some Se compounds in the canola were likely insoluble and/or have a low digestibility. These forms of Se pass through the digestive tract and were excreted in the feces and urine. We could not deduce from this study whether higher Se concentrations in canola would have resulted in higher Se concentrations for organs and blood. Because most animals develop a chronic selenosis when the Se concentration of diet increases to levels 3-15 mg Se kg^{-1} DM (Mayland, 1994), it is of the utmost importance to monitor the Se concentration of the plant material that is to be disposed of as animal feed.

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Table 1. Concentrations of Se in canola fed to lambs and in feces, urine, and blood of lambs

Canola	Canola†	Feces	Urine	Blood
Treatment	(μg kg^{-1} DM)	(μg kg^{-1} DM)	(μg L^{-1})	(μg L^{-1})
+Se	3250(140)‡	530(61)	187(42)	278(10)
no Se	35(2)	32(7)	<1(0)	83(8)

†Canola fed to lambs during the study

‡Values are the means from five replications followed by the standard error in parenthesis.

Table 2. Mean Se concentrations in different tissues of lambs fed seleniferous and non-seleniferous canola

Canola	Heart	Liver	Kidney	Spleen	Loin	toenail
Treatment	(μg kg^{-1} DM)	(μg kg^{-1} DM)	(μg kg^{-1} DM)	(μg kg^{-1} DM)	(μg kg^{-1} DM)	(μg kg^{-1} DM)
+Se	682(14)†	809(42)	2100(99)	525(66)	213(11)	227(27)
no Se	360(11)	438(25)	1507(59)	216(10)	68(4)	98(20)

†Values are the means from replications followed by the standard error in parenthesis.

ESTABLISHMENT OF A VEGETATIVE CAP ON A ZINC SMELTER SLAG PILE: GREENHOUSE STUDIES

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1. Introduction

The Spelter Smelter located in Spelter, WV, USA, is a former Zn smelter that operated from 1911 to 1974. The site is 45 ha in size, about 20 ha of which is waste materials, mainly slag. One option under consideration to prevent erosion into the adjacent river and to restore the ecological productivity of the site is the establishment of a vegetative cap. Soil amendments would be incorporated directly into the slag material and plants established as a method to vegetate the material (HENRY and BROWN, 1997; SOPPER, 1993).

2. Materials and Methods

Slag material was collected from the tailings pile to a depth of 30 cm for laboratory and greenhouse studies. Total concentrations of trace elements in soil was determined by a inductively coupled plasma-atomic emission spectrometer (ICP-AES) after $HNO_3/HClO_4$ digestion with microwave heating on < 9.5-mm material that was finely crushed. Material characterization of the <2mm size particles included soil pH (1:1 water to soil), SMP buffer pH, Bray-1 phosphorus, extractable Ca, Mg, K, and Na using a modified ammonium acetate method, and organic matter by loss-on-ignition. Treatments included dolomitic limestone and fertilizer (N, P, K) according to soil test recommendations, dolomitic limestone and phosphorous (P) fertilizer (as CaHPO4) at application rates greater than soil test recommendations, iron filings, and composted (high carbon to nitrogen ratio - C/N > 20) and non-composted biosolids (low C/N=10 to 12). Treatments were well mixed into the material, which was then put into plastic greenhouse pots (10-cm top diameter). A grass species, tall fescue (*Festuca arundinacea*), was planted as seed. Plants were harvested after about 6 weeks of growth. Dry weights (dried at 65°C) of plant tops at harvest were measured.

3. Results and Discussion

The slag does not support plant growth in the field. Soil pH of the slag material ranged from 4.4 to 5.6 and had low to deficient levels of N, P, K, Mg, and Ca. Constituents of concern (COC) included Zn, Cu, Pb, As, Ni, and Cd. The elemental concentrations of two separate slag samples collected from the field are shown in Figure 1. Amendments (dolomitic limestone, biosolids, and plant nutrients) were effective in dramatically improving the chemical and physical characteristics of the slag so that tall fescue germinated and grew well (Figure 2). Dolomitic limestone added at rates well above soil test recommendations and non-composted biosolids had the greatest effect on the ability of the slag to positively support plant growth. Composted biosolids had a negative impact on plant grow, probably because of induced N deficiency even with the addition of N fertilizer (as NH_4NO_3). Iron filings and P fertilizer mixed into the slag at 1% and 10 times the soil test recommendation for P (about 1000 kg ha^{-1}), respectively, had little effect, positive or negative, on plant growth.

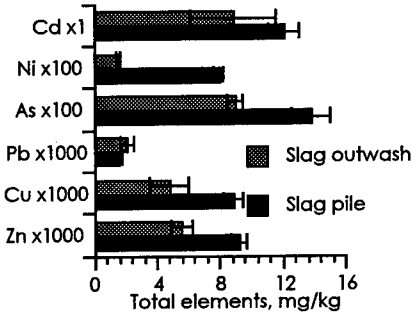

Fig. 1. Total elemental concentrations (times multiplier) for individual elements of material from top of zinc smelter slag pile and outwash. Error bars denote standard deviations, n≥2.

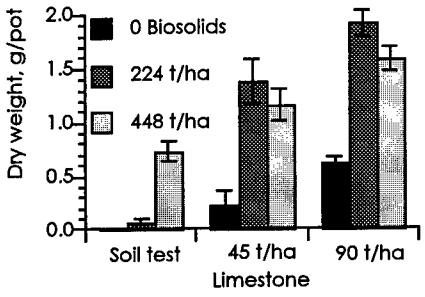

Fig. 2. Dry weight of tall fescue shoots grown in zinc smelter slag to which soil amendments were applied. Error bars denote standard error, n=3.

4. Conclusions

High application rates of limestone and biosolids may be an effective method of establishing a natural vegetative cap on a Zn smelter slag pile. Longevity of the treatment and public acceptance of this remediation option, however, still need to be addressed.

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REMEDIATION OF THE JALES MINESPOIL BY INACTIVATION AND PHYTOSTABILISATION: ON SITE EXPERIMENTS

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1. Introduction

On large bare industrial sites the extremely high concentrations of trace elements can result in the absence or disappearance of natural vegetation. Dispersion of the contamination by wind and water erosion is potentially dangerous to surrounding ecosystems. The establishment of a closed vegetation cover can be, a relatively low-cost, solution to prevent this dispersal. The present abstract reports on the current revegetation experiments on the spoils of the former Jales gold mine, Portugal, an As-Zn contaminated spoil, as a part of the PHYTOREHAB project. On site, the spoil was treated with Organic Matter, Beringite, and/or Steel Shots to inactivate the contaminants and ameliorate conditions for plant growth and revegetation. Growth of the grass *Holcus lanatus* L. is used as an indicator of the effects of the spoil treatments.

2. Materials and Methods

The testfield, created on one of the terraces of the spoils, consists of 7 plots subdivided into 30 subplots of $1m^2$. In Spring 1997 Organic Matter (commercially available compost), Beringite (a modified Al-silicate from the fluidized-bed burning of coal refuse from the former coal mine in Beringen, Belgium, Vangronsveld *et al.* (1995)), and/or Steel Shots (Fe-bearing material.Mench *et al.*, 1994 from England) were incorporated into the upper 30cm of the spoil (Figure 1).

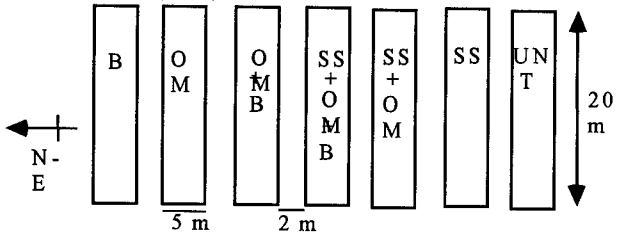


Figure 1. Untreated spoil (UNT) and Organic Matter (OM, 5%), Beringite (B, 5%) and/or Steel Shots (SS, 1%) incorporation in the Jales testfield.

One year after incorporation of the additives, spoil samples consisting of an upper part (0-12.5cm) and a deeper part (12.5-25cm) were taken from each plot. PH-H_2O was measured and water soluble As concentrations of the samples were determined by AAS-methods. Simultaneously 1.5g of *Holcus lanatus* L. seeds, obtained from a populationp of plants colonizing the Jales spoils, was sown in 3 subplots per treatment, in presence and absence of P-fertilizer (100g containing 18% P_2O_5 per subplot). The average height of the grasses in each plot was measured once a week. 84 Days after sowing the surface cover (%) was measured.

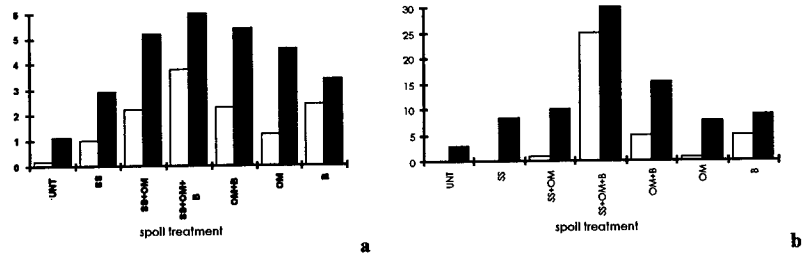
3. Results and Discussion

One year after incorporation of the additives into the spoil, the additives appear to affect the pH (Table 1). The values measured differ considerably though from results obtained when equal tests are performed under controlled conditions (Verkleij, 1998). Addition of OM in the field did not increase the pH, contrary to results reported by Verkleij (1998). This is possibly due to a different composition of the OM.

Table 1. As-H_2O (mg.kg^{-1}) and pH-H_2O at the Jales testfield in untreated plots (UNT) and plots treated with Steel shots (SS), Organic Matter (OM) and/or Beringite (B). (Means of upper and lower samples, n=10).

	UNT	SS	SSOM	SSOMB	OMB	OM	B
pH-H_2O	3.6	4.0	3.4	5.8	4.2	3.2	4.8
As-H_2O (mg.kg^{-1})	0.47	0.43	0.54	2.80	0.49	0.52	0.85

Best results in the field in increasing spoil pH is obtained by adding the full combination of additives (SSOMB), although this combination resulted in a 6-fold higher As-H_2O concentration compared to the untreated spoil (Table 1). All other treatments influence As-H_2O in a minor way. Addition of B in the field leads to an increase in As-H_2O, although a much lower increase than under controlled conditions (Verkleij, 1998). Above a pH of 4.5 a positive correlation between pH and As-H_2O was determined (R=0,86), at lower pH this correlation does not exist.



a b

Figure 2. (a) Average height (cm) and **(b)** average cover (%), 84 days after sowing *Holcus lanatus* in squares treated with Steel Shots (SS), Organic Matter (OM) and/or Beringite (B). (Open bars: without P addition, closed bars: with P addition).

In all treatments height of *Holcus lanatus* is higher in the presence of P-fertilizer in the spoil. Compared to the untreated spoil, all additives induce better growth. Best growth is found in the squares with SSOMB, OMB and SSOM (Figure 2a). Cover percentage in the absence of P-fertilizer is negligible in untreated spoil and SS, SSOM and OM treatments. Better results are found after OMB and B addition (5% cover each). Highest cover is observed in the squares containing a combination of all additives (25%). In the presence of P-fertilizer higher cover percentages are observed. Again the subplots containing SSOMB give the best results (30%) (Figure 2b).

4. Conclusions

Using *Holcus lanatus* as an indicator of the effect of the spoil treatments it is concluded that best results are obtained with addition of a full combination of additives (SSOMB) with P-fertilizer. This treatment appears to ameliorate the conditions at the Jales spoils where especially an increase in pH seems to be of importance for successful growth of *H. lanatus*, although this treatment does not appear to inactivate the contaminant, since the As availability did not diminish. Apparently *H. lanatus* on this site exhibits a sufficient level of As-tolerance.

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SELECTION OF GRASS SPECIES AND AMENDMENTS FOR REVEGETATION OF PB/ZN SMELTER WASTES

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1. Introduction

The Katowice District in Upper Silesia, Poland, is littered with thousands of open-dumped solid waste piles (Ochrona, 1993), many of which are immediately adjacent to homes, schools, and businesses. Solid wastes (furnace slag) from Welz and Doerschel process Pb/Zn smelting are particularly phytotoxic due to salinity and high soluble metals (Table 1). The overall objective of this research program was to develop cost-effective strategies for stabilizing these piles against fugitive dust and runoff loss via revegetation, along with minimization of plant metal uptake. In this paper we will report results from greenhouse species selection trials, soil amendment experiments, and subsequent field demonstrations.

Table 1. Average total and water soluble metal content in Upper Silesia Pb/Zn waste materials.

Waste	Zinc		Cadmium		Lead		EC
	Total (g/kg)	H_2O Sol. Total (mg/kg)	H_2O Sol. Total (g/kg)	(mg/kg)	H_2O Sol. dS/m (g/kg)	(mg/kg)	
Welz	30.9	343	0.54	17.6	7.9	1.8	7.3
Doerschel	75.1	1670	2.31	108	23.8	5.4	16

2. Materials and Methods

The smelter waste area selected for study in 1994 was covered by Doerschel furnace and Welz process smelting waste (furnace slag) between 1950 and 1985. The area was barren. Over 160 point samples were initially collected from the area and analyzed for total and water soluble metals and electrical conductance (EC). An initial greenhouse pot experiment with Welz material was performed to characterize the growth response of 20 grass and legume cultivars grown in a high metal and high salinity environment. Germination and biomass production were observed over a 90 day period. One subset of pots was spiked with 2% Na_2SO_4 to simulate observed field conditions. A second pot experiment was subsequently carried out with *Lolium multiflorum* (*Telga*) grown on Welz and Doerschel waste treated with municipal sewage biosolids (10% w:w), two forms of lime (CaO and $CaCO_3$), two rates of super-P (600 and 1200 kg/ha P_2O_5) and two rates of NH_4NO_3 (O and 200 kg/ha N). Previous work by the authors and others (Sopper, 1993) had indicated that biosolids could be used effectively to suppress metal solubility in similar wastes and greatly enhance revegetation potential. Phosphate and lime were also assumed to be important factors suppressing the solubility of heavy metals. The experiment was conducted over a 90-day period. Field revegetation demonstrations were installed in 1994 and 1995 on both waste materials (Welz and Doerschel) to test the resultant revegetation strategies as detailed by Daniels et al. (1998).

3. Results and Discussion

These waste materials pose unique challenges to plant materials in that they are simultaneously very high in soluble metals and salinity (Table 1). Of the 20 commercially available species/varieties screened for combined metals/salinity tolerance, the following exhibited marked tolerance: *Lolium perenne (Solen), Lolium perenne (Argona), Lolium multiflorum (Telga), Lolium multiflorum (Koga) and Lolium x boucheanum Kunth. (Mega).* A number of species exhibited metal tolerance *(Poa pratensis (Alicja),Festuca rubra (Atra), Festuca arundinacea (SZD 492),* and *Festuca ovina (Sima),* but only limited salt tolerance. A mixture of these species was used for the field revegetation trials and *Lolium multiflorum (Telga)* was selected for use in further detailed greenhouse trials.

In the second greenhouse trial, P additions at 600 kg/ha resulted in a 90% increase in grass yield. Strong interactions among P rate and lime type (CaO vs. $CaCO_3$) were also noted. Fertilizer N applications had no effect on grass biomass, most likely due to N mineralized from sewage sludge. Amendment with biosolids and CaO has been shown to reduce the water solubility of heavy metals in these wastes in the lab (Stuczynski et al., 1997), and those results were reconfirmed. However, it was not clear originally whether this reduced solubility would have significant impact on the transfer of heavy metals into plant tissue. Surprisingly, we saw no differences between different lime forms on grass Zn uptake. It seems that Zn uptake is controlled not only by Zn solubility, but there are other physiological mechanisms involved. Tissue concentrations of Cu and Ni were acceptable, even though their level in the waste was quite high. Lead was also present in the wastes at very high levels (up to 6%), but acceptable thresholds for plants were only exceeded by 70%. Cadmium levels in grass greatly exceeded recommended levels regardless of lime form, N, or P treatments. Further detail on complete treatment effects and interactions with biomass yield and metal uptake will be presented in the final paper.

A large (3 ha) field experimental area was established in Upper Silesia on Welz and Doerschel wastes utilizing biosolids, waste lime ($CaCO_3$ + CaO), P fertilizer, and the tolerant grass species as specified above. Persistent grass vegetation has been maintained over three growing seasons. Revegetation prescriptions and effect on plant establishment and metal uptake will be discussed in the final paper.

4. Conclusions

Mixed Pb/Zn smelter wastes pose a serious fugitive dust and runoff risk in Upper Silesia and the toxicity of the materials varies considerably by waste type. Effective revegetation strategies must rely on soil amendments to suppress heavy metal solubility, particularly Zn, coupled with utilization of locally adapted metal- and salt-tolerant grasses. Combined waste treatments of biosolids+lime+P appear to be effective at limiting plant uptake of Zn, Cu, Ni and Pb, but not Cd. Sewage biosolids and CaO containing limes can be used to successfully suppress metal solubility and ameliorate these smelter wastes for direct revegetation without expensive topsoil covers.

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HEAVY METAL ACCUMULATION IN METALLICOLOUS AND NON-METALLICOLOUS POPULATIONS OF *ARRHENATHERUM ELATIUS*

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1. Introduction

The study of metal accumulation capacity of plants is experiencing a revival due to the interest in phytoremediation technology. Some authors, like CHANEY (1997), recommend the use of hyperaccumulator species. Others prefer plants with a lower accumulation rate but high biomass. The aim in both cases, is to enhance heavy metal extraction (BLAYLOCK *et al.*, 1997). Whichever approach is chosen, it seems necessary to fully understand the plant-metal interactions in order to optimize heavy metal accumulation.
This study examines the differential accumulation of heavy metals by a perennial grass: *Arrhenatherum elatius*. The purpose is to evaluate inter-population variations to estimate potential range of metal uptake and to direct the selection of populations of the species to ensure optimal accumulation.

2. Materials and Methods

Seeds of *Arrhenatherum elatius* sourced from 3 calamine (Zn-, Pb- and Cd-rich) soils and 3 non-contaminated soils of northern France, were chosen to study their differential potential for accumulation. In the greenhouse, different concentrations of artificially contaminated soil were prepared, as shown in table 1.

Table 1: Soil concentration for each metal (mg/Kg). (Each metal is studied separately).

Cd	0	4	200	300
Pb	0	250	3 000	15 000
Zn	0	1 000	3 000	10 000

For each metal and concentration, 10 seeds from each site were sown per pot (4 replicates per concentration). Six weeks after germination, shoots and soils were assayed for digestion. A hot perchloric and nitric acid mixture (1V/4V) was used to digest plant materials. 4 M HCl and 1 M ammonium acetate, both shaken overnight, were used to estimate the total and extractable metal fractions of the soil.

3. Results and Discussion

The extractable fractions are different for each of the studied metals. Cadmium is more extractable (29% to 65%) than zinc (13% to 59%) and lead (1.85% to 16%), as often observed in literature, ROBINSON (1997).

Due to both its high biomass and cadmium accumulation close to the cadmium hyperaccumulation threshold (100 mg/kg), *Arrhenatherum elatius* is considered a good candidate for soil decontamination (Deram, 1998).

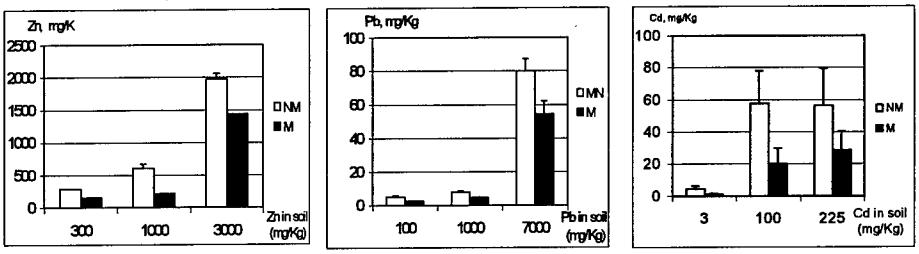

Figure 1: Accumulation of zinc (a), lead (b) and cadmium (c) in shoots of metallicolous (M) and non-metallicolous (NM) populations versus metal concentrations in soil (mg/Kg). (n=12).

We can observed significant differences ($p>0.001$) between accumulation of the two kind of populations, whatever the metal and the concentration (figure 1 a, b, c). This study shows that non-metalliferous plants accumulate higher concentration of metals than plants from heavily polluted soils. It is also important to note that the differences between the two population types are more important when the soil metal concentration is low. These results could be used for phytoextraction technology which is generally applied to weakly contaminated soil.

It seems likely that metallicolous plants prevent penetration and /or translocation of heavy metals into the above ground material. This proves that heavy metal accumulation is not a mechanism of tolerance for this species and that accumulation and tolerance are negatively correlated. Such a hypothesis is in agreement with conclusions reached for hyperaccumulators species such as *Thlaspi caerulescens,* MEERTS and VAN ISACKER (1998).

4. Conclusions

Our data showed that non-metallicolous plants are potentially a better agent for phytoextraction. Future work will examine the evolution of these results in relation with time. We will also work on intra-population selection.

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SALIX AS PHYTOEXTRACTOR

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1. Introduction

Remediation of contaminated soils using non-food crop plants to clean soil from toxic metals, so called phytoextraction, has been paid a great deal of interest during the last years (McGrath et al 1993, Salt et al. 1995, Felix 1997). Conventional remediation methods (soil washing, acid extraction, electrokinetic remediation) are destructive to soil fertility and structure, while phytoextraction is not. Another advantage with phytoextraction is that the method is not expensive. The disadvantage is that the plants based method needs a long time period to decrease the metal level in the soil, the length, however, depends on the initial metal concentration and bioavailability. Phytoextraction is best suited for low and medium contaminated (e.g. agricultural soils and not for high contaminated e.g. mining wastes.

A good phytoextractor should have 1) high accumulation and 2) high transport to the shoot for the metals, 3) the properties should be stable and 4) the high uptake should be metal specific. It also needs to have 5) high biomass production and 6) be of economical interest. In addition, 7) it is an advantage if it is unattractive to animals minimizing the risk of importing metals into the food chain.

All these properties can be found in different Salix clones/genotypes and we have found Salix to be a good phytoextractor (GREGER 1997). Salix is used in bioenergy production and there are different clones with different accumulation properties (LANDBERG and GREGER 1994). The aim of this paper is to present data on Salix as phytoextractor and some suggestions how to increase the phytoremediation efficiency.

2. Materials and Methods

Different clones of Salix viminalis were screened for uptake and accumulation into root and shoot of Cu, Cd and Zn. The screening was performed both on material grown under field and controlled indoor conditions.

Salix clones with high accumulation in shoots of Cd were compared with hyperaccumulators for Cd (Thlaspi caerulescens and Alyssum murale) on the phytoextraction efficiency. This cultivation was performed during one season in the field in soil containing 8 mg Cd/kg. The shoots of all three plant species were harvested and analysed for Cd. Additionally, the biomass production was calculated.

The removal of Cd from soil was investigated in a pot experiment running for 90 days. The total and bioavailable contents of Cd in the soil were analysed in the beginning and at the end of the growth period.

The influence of the biomass production on Cd uptake and transport as well as the effect of water (mirroring the effect of irrigation) on Cd uptake and transport were studied in a controlled experiment in a climate chamber.

Since Salix is often harvested after leaf fall, and the Cd in leaves then returns to the soil, the removal of Cd then is promoted by a low transport of Cd to the leaves compared to the stem. Therefore, the ratio of Cdleaf:Cdstem was investigated in 70 different Salix genotypes, collected

in the field. The aim was to find out if it is possible to have a high Cd concentration in the stem but a very low Cd concentration in the leaves.
Heavy metals were analysed by atomic absorption spectrophotometry using the method of standard addition.

Table 1. Removal of cadmium by 3 different plant species.

Plant species	Cd removal g/ha year
Thlaspi caerulescens	35 ± 11
Alyssum murale	43 ± 15
Salix viminalis	216 ± 21

3. Results and Discussion
The screening shows that the Cd uptake capacity could differ by as much as 43 times between clones with the lowest and the highest uptake value. The results so far show that Salix, due to high biomass production and high Cd uptake and transport to the shoot, has a high capacity to remove Cd from the soil. The capacity was about 5 times higher for Salix than for T. caerulescens and A. murale. This was shown with the most high accumulating clone of those in the screening study. The soil analyses showed that after 90 days 20 % of the total Cd and about 30% of the bioavailable Cd was removed. Thus both the concentration of Cd in Salix and the lowered Cd concentration in the soil did show that Salix removed Cd from the soil.
To increase the removal of Cd it is not worth increasing the biomass production since there is a dilution effect, decreasing the Cd concentration in the stems at increased biomass growth. The number of plants area showing the optimum value in Cd removal was 9 plants per square meter. Irrigation could be a tool to increase the uptake since water releases Cd in the soil.
The possibility of clones with low Cd content in leaves but high in stems is still under investigation. However, if there is too high Cd concentrations in the leaves the whole shoots must be harvested.

4. Conclusions
A Salix clone with all above mentioned properties can be suitable as phytoextractor on Cd contaminated soil. One can optimize the remediation by right density of plants per area in a well watered soil, however, probably not by increasing the biomass production.

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BIOTECHNOLOGICAL IMPROVED TOBACCO FOR PHYTOEXTRACTION OF HEAVY METALS IN SOIL

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1. Introduction

Soils contaminated with heavy metals are recognized as a growing problem in many parts of the world, and phytoremediation may offer an alternative to destructive clean-up technologies. About 400 metal-accumulating wild plants that accumulate high concentrations of heavy metals in their shoots have been reported [1]; however, their slow growth rate and limited yields impose limitations on the rate of heavy metal removal and make phytoremediation a time-consuming process. By contrast, high-yield crops generally have low accumulation capabilities and exhibit lower tolerance of heavy metals. To resolve this problem, we selected two powerful cadmium-accumulating tobacco varieties after screening a large number of crop species [2]. With the help of specific in vitro breeding and selection techniques [3], we are improving their accumulation capabilities for future use in phytoremediation.

2. Materials and Methods

Callus cultures of two tobacco cultivars (N. tabacum) grown in vitro were induced from leaf disks on a solid proliferation medium. Callus fragments of about 50 mg were then exposed for 18 months to a selection medium containing different heavy metals (Cd, Zn, Cu), in which the heavy metal concentration was gradually increased. Callus crumbs that survived and showed growth on the selection medium were subsequently cultivated, either to fresh heavy metal-spiked media or to a special medium for regeneration into plantlets (variants). Regenerated plantlets from selected cell lines were then micropropagated. Clones obtained were tested for heavy metal accumulation in the shoots and compared with the parent variety. The screening tests were performed on nutrient solutions containing heavy metals, in mini-pot trials in the greenhouse, and also on a zinc/copper contaminated test field at Dornach (CH).

3. Results and Discussion

Tissue culture responses on heavy metal-spiked media showed different reactions. At an exposure of 300 µM, copper tobacco calli showed total growth inhibition and death. For cadmium, the tissues turned brownish and became necrotic at 700 µM. By contrast, tobacco tissue grew quite undisturbed at an exposure of 5000 µM zinc. Approximately 40,000 tobacco calli were cultivated in four different heavy metal-spiked selection media, and from this batch 812 variants were regenerated. A rapid hydroponic testing system was developed in order to identify heavy metal-accumulating variants. Under these conditions, we found tobacco variants with shoot accumulation 5-7 times as great for copper, 2-5 times as great for cadmium, and 0.5 times as great for zinc, in comparison with the parent plants. Based on this accumulation screening, 16 potentially improved plants were chosen for further study and field evaluation.

4. Results and Discussion

The complex genetic regulation mechanism that triggers heavy metal tolerance and accumulation can limit success in conventional breeding programmes, as polygenic regulation mechanisms are very difficult to select and incorporate in the plant genome. In vitro breeding and selection techniques may be an appropriate procedure for dealing with the problems under consideration. Results obtained from the identification of heavy metal-accumulating variants suggest that our strategies based on somaclonal variation [4, 5] (the genetic changes that occur spontaneously during tissue culturing cycles) have been successful. We are currently testing the remaining variants and re-testing the improved clones on nutrient solution. At the same time, mini-pot and field experiments with 16 heavy metal-accumulating variants are in progress, with and without mobilizing agents, in order to confirm accumulation capacity and study biomass performance. After having proved their genetic stability in the next generations, these selected tobacco variants will provide an appropriate tool for future phytoextraction engineering, especially when combined with a targeted and optimised metal-mobilizing technique in soil [6]. The goal of this integrated optimization is to significantly shorten the restoration time from decades to a period of a few years, which will be a practical benefit for future phytoextraction engineering.

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PHYTOEXTRACTION: THE USE OF INDIAN MUSTARD AND RAPE TO REMOVE TL, CD AND ZN FROM CONTAMINATED SOILS

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1. Introduction

Phytoextraction is a soil-preserving solution for the remediation of contaminated soils. Laboratory studies show that *Brassica juncea* (Indian Mustard) accumulates high amounts of Pb, Cd, Cu, Ni and Zn in shoots (BLAYLOCK et al. 1997). Metal uptake and accumulation in shoots of *B. juncea* is shown to be enhanced by application of chelators BLAYLOCK et al. 1997; HUANG et al. 1997). Little is known of Tl-extraction by plants from contaminated soils. Field pot experiments show that *Brassica napus* (Rape) accumulates high amounts of Tl (LEHN 1986). In the present study field pot experiments were conducted to extract Tl, Cd and Zn by *B. juncea* and *B. napus* from a Tl-, Cd- and Zn-contaminted. EDTA and citric acid were applied to increase heavy metal uptake by plants.

2. Materials and Methods

Soil samples were taken from an agricultural field south of Heidelberg, Germany. Contamination of Tl, Cd and Zn are caused by industrial emissions of a cement roaster. The plants *Brassica juncea* L. cultivar Vitasso and *Brassica napus* L. cultivar Napus were cultivated in pots for four weeks. Chelators (EDTA and citric acid) were applied. One week later the plants were harvested. Two additional cultivations in the same pots followed.
Soil and plant material were digested with concentrated nitric acid. Measurements of heavy metals were conducted by means of flame and graphit furnace atomic absorption spectroscopy.

3. Results and Discussion

B. napus removes 15 % of the total Tl content of the soil (2.5 µg / g D.W.) within the first cultivation on the untreated soil (Fig. 2). This result is attributed to a high Tl accumulation in leaves of *B. napus* (585 ± 102 µg / g D.W., Fig.1). Tl extraction decreases to 1-2 % in the two following cultivations. Amendments with chelator decreased the total amount of Tl extracted by *B. napus*. The extraction of Cd and Zn by both plants and the extraction of Tl by *B. juncea* remains lower than 0.4 % of the total Tl-, Cd- and Zn-content in the soil within three cultivations. This is also due to small biomass production. Other field experiments show similar results (BAKER et al. 1994). Application of EDTA increased Cd- and Zn-accumulation in shoots of *B. juncea* 3-fold compared to untreated soil. The effect of citric acid amendments was even lower. In laboratory studies (BLAYLOCK et al. 1997), Pb uptake in shoots of *B. juncea* could be increased 25 fold or 160 fold by amendment with of citric acid or EDTA.

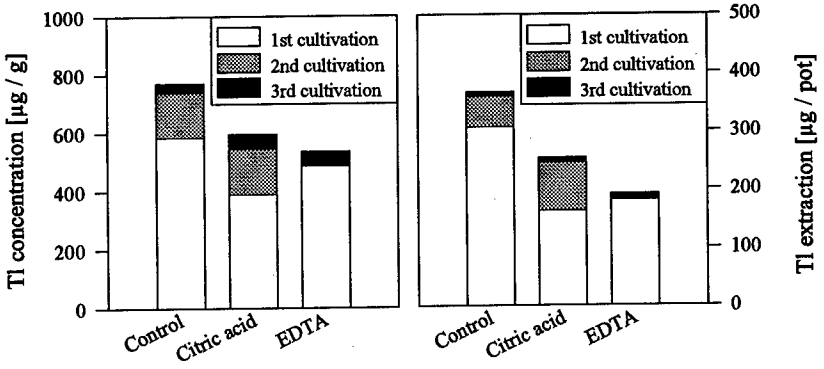

Figure 1: Changes in Tl concentration [µg / g D.W.] in leaves of *B. napus* within three cultivations and different soil treatments.

Figure 2: Changes in Tl extraction [µg / pot] in shoots of *B. napus* within three cultivations and different soil treatments. (pot = 800g)

Both figures: There was no plant growth on EDTA-treated soil for the second cultivation. Data are expressed as means of 3 parallels.

4. Conclusions

Tl extraction by *B. napus* was successful within the first cultivation. Reasons for the decline in Tl uptake for the following cultivations are not known. Extraction of Cd and Zn by both plants and Tl extraction by *B. juncea* remains low. The effect of chelator application is very limited, except in the case of Tl, where it causes a lower extraction. Results of this and other field studies indicate that heavy metal binding forms in grown soils are different and less plant available than amended heavy metal binding forms in laboratory studies. Therefore it is necesscary to find means to improve the metal uptake from contaminated soils or to modify plants genetically to improve metal extraction and biomass production.

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ROLE OF SELENIUM VOLATILIZATION IN THE MANAGEMENT OF SELENIUM-LADEN AGRICULTURAL DRAINAGE WATER

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1. Introduction

Intensive irrigation of the agricultural soils in the western side of the San Joaquin Valley of California results in a large amount of Se-laden subsurface drainage water. Management and treatment of Se-contaminated drainage water is a critical issue for California agriculture due to its potential toxicity to wildlife. Recent studies have demonstrated that volatilization of Se by plants and microorganisms represents a novel approach for the remediation of Se-contaminated drainage water (Losi and Frankenberger, 1997; Hansen et al., 1998). Biomethylation of Se converts inorganic soluble Se into organic volatile Se, which is released harmlessly into the atmosphere (Cahill and Eldred, 1998). Volatilization removes Se completely from the water-soil-plant system, thus reducing the problem of disposing of large quantities of Se-laden plant biomass produced by phytoextraction (Zayed et al., 1998). The objectives of this study were: 1) to evaluate the potential of Se volatilization in removing Se from Se-contaminated water by *Salicornia bigelovii* under field conditions, and 2) to demonstrate the significance of Se volatilization versus phytoextraction in removing Se from irrigation water in the field.

2. Materials and Methods

The research site investigated was located at Five Points, California, and formed part of the *Salicornia* treatment component of an Integrated on-Farm Drainage Management System established for managing agricultural saline drainage water (Lin et al., 1999). The soil was classified as ciervo clay and contained 4.04 ± 0.91 mg Se kg^{-1}. The field was irrigated with agricultural drainage water containing 1.106 ± 0.224 mg L^{-1}, which resulted in a Se-loading rate of 958 mg Se m^{-2} y^{-1}. Volatile Se was collected using an open-flow sampling chamber system (Lin et al., 1999). Rates of Se volatilization were determined in the field biweekly during the *Salicornia* growing season from February to August 1997, and monthly from September 1997 to January 1998. Soil moisture inside each chamber was monitored by a LIC soil moisture meter immediately after the volatilization measurement.

3. Results and Discussion

Accumulation of Se in plant tissues: Selenium, dominantly as Se^{6+} in agricultural drainage water, was accumulated mainly in the above-ground parts of *Salicornia*. The Se concentration in shoots was 12.88 ± 2.015 mg kg^{-1}, which is three-fold higher than in roots (i.e., 3.445 ± 0.368 mg kg^{-1}). The annual biomass production of *Salicornia* was 0.755 ± 0.086 kg m^{-2} y^{-1} for shoots, and 0.057 ± 0.023 kg m^{-2} y^{-1} for roots. The total Se accumulation in shoots was 10.5 ± 1.5 mg m^{-2} y^{-1}, which was about 35-fold more than in roots (i.e., 0.2 ± 0.1 mg m^{-2} y^{-1}).

Selenium volatilization rate: *Salicornia* exhibited the highest daily rate of Se volatilization ever published, e.g., 484 and 576 μg Se m^{-2} d^{-1} observed in July and January, respectively (Fig. 1). The mean rate of Se volatilization over *Salicornia* varied from 420 ± 91 μg Se m^{-2} d^{-1} in July to a minimum rate of 22 ± 9 μg Se m^{-2} d^{-1} in March. The overall mean of all volatilization

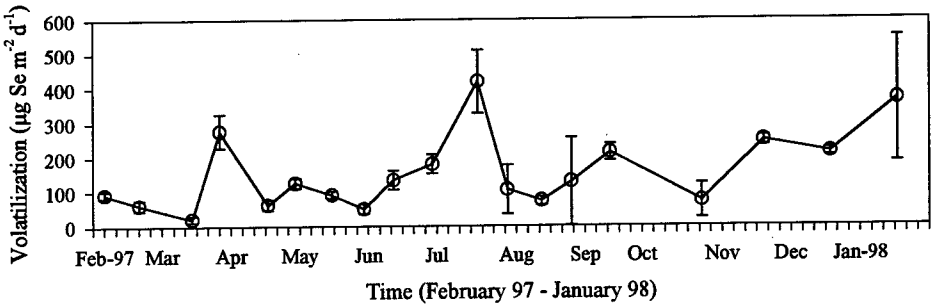

Figure 1. Changes in Se volatilization rate with time (meam and standard deviation, n=3)

measurements conducted during a 12-month study period and the associated standard error (n=57) was 155 ± 25 µg Se m^{-2} d^{-1}. High rates of Se volatilization (e.g., >150 µg Se m^{-2} d^{-1}) from the treatment system were associated with high levels of soil moisture. Irrigation management is therefore likely to be an important factor in maximizing the use of volatilization for the remediation of Se contamination.

Selenium removal by volatilization versus phytoextraction: Volatilization from the *Salicornia* system removed 62 mg Se m^{-2} y^{-1} and accounted for ~5.5-fold more Se than was removed by phytoextraction. During the *Salicornia* growing season from February to September, phytoextraction removed 10.7 mg Se m^{-2} and volatilization removed 34.6 mg Se m^{-2}. If we assume that the rate of Se volatilization could be increased from the annual average of 155 µg Se m^{-2} d^{-1} to the observed maximum rate of 576 µg Se m^{-2} d^{-1}, then annual Se removal by volatilization would account for as much as 21.9% of the total annual Se input (i.e., 958 mg Se m^{-2} y^{-1}) in the *Salicornia* system.

4. Conclusions

Salicornia achieved rates of volatilization of up to 576 µg Se m^{-2} d^{-1}, the highest rates so far obtained by any plant/soil system. The total annual Se removed by volatilization was 5.5 times more than by phytoextraction. Volatilization of Se represents a potentially effective and environmentally-friendly technology for the remediation of Se-contaminated agricultural drainage water.

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HETEROLOGOUS EXPRESSION OF HEAVY METAL RESISTANCE GENES IN ENDOPHYTIC BACTERIA AND COLONIZATION OF THEIR HOST PLANT IN THE PERSPECTIVE OF PHYTOREMEDIATION.

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1. Introduction

Although the use of plants for the decontamination of heavy metal polluted soils offers some important advantages when compared with traditional cleaning techniques, it is still economically not interesting. For an economically efficient phytoextraction process, in which the pollutant is accumulated in the above ground parts, the plant should have a high metal accumulating biomass that is easy to harvest and a high tolerance for the accumulated heavy metals.

In this study we report the use of metal resistant endophytic bacteria and their possible contribution to the heavy metal accumulation and tolerance of their host plant. Since endophytic bacteria are able to colonize and interact with plants without causing diseases, they could possibly be able to reduce the toxicity of heavy metals due to expression of heavy metal resistance systems.

Therefore, some endophytic bacteria belonging to the group of β-proteobacteria were selected to test heterologous expression of metal resistance genes from bacteria of environmental polluted areas.

Burkholderia cepacia (W.S.9.1 and L.S.2.4) which was isolated from wheat roots and lupin shoots and *Herbaspirillum seropedicae* (HS2284 and HS6513), isolated from grasses, were used in these experiments. To evaluate the colonization capacity of *B.cepacia* and *H.seropedicae* in their host plant, these strains were marked with reporter genes.

The heterologous expression of heavy metal resistance mechanisms by the endophytes, as well as their effect on the host plant, is currently under investigation.

2. Materials and Methods

2.1. Heterologous expression of heavy metal resistance genes.

In order to test the heterologous expression in the endophytic strains, cloned metal resistance genes from *Ralstonia eutropha* CH34 (*czc, cop, pbr*), *Pseudomonas aeroginosa* CMG103 (*czr*), and *Ralstonia* sp. 31A (*ncc-nre*) were used in triparental matings. The expression of heavy metal resistance was evaluated on solid Tris minimal agar plates.

2.2. Colonization experiments.

The ability of the endophytic strains to colonize their host plant was done by inoculating surface sterilized seeds in a bacterial suspension for 30'. Germination and growing experiments were carried out in sterilized pots under controlled conditions.

3. Results and Discussion

3.1. Heterologous expression of heavy metal resistance genes.

Various plasmid encoded heavy metal resistance genes were cloned in broad host range Inc P and Inc Q plasmids, and transferred to endophytic hosts (table1). The best expression was observed for the *ncc-nre* gene cluster in both *B.cepacia* and *H.seropedicae*. The *ncc-nre* region is in *Ralstonia* sp. 31A responsible for a high resistance to both nickel, cobalt and cadmium. Tn5 insertion mutagenesis showed that *ncc* and *nre* are distinct nickel resistance operons (Schmidt & Schlegel, 1994). As in our strains only an increased nickel resistance was observed, this prompted us to study this resistance system in more detail. Due to the instability of the plasmid in the endophytic strains, miniTn5 transposons were used to clone the *ncc*

and *nre* genes in order to have the resistance determinants stabily inserted in the chromosome.

Table 1: Heterologous expression of the different plasmid encoded heavy metal resistance operons in *B.cepacia* and *H.seropedicae*.

Marker resistance to:	*pbr* (Pb)	*Czr* (Zn, Cd)	*cop* (Cu)	*ncc-nre* (Cd, Co, Ni)
B.cepacia	-	-	-	Expression of Ni resistance
H.seropedicae	-	Expression of Zn resistance	Expression of Cu resistance	Expression of Ni resistance

3.2. Colonization experiments.

Mini-Tn5*lux*AB transposons were transferred to the *B.cepacia* and *H.seropedicae* strains. Mutants were obtained with stabely inserted copies of mini-Tn5*lux*AB. Due to transcriptional fusions, some colonies produced light after incubation with n-decanal. This *lux* marker, as well as the tetracycline (Tc) resistance were used as identification criteria in the follow up of inoculation experiments. Both strains seemed to be able to colonize their host plant. Transmission electron microscopy in presently under investigation to determine the localisation of the endophytic bacteria in the plant tissue.

The next step will be the inoculation of Ni resistant strains in the host plant and the evaluation of Ni tolerance and accumulation due to the colonization of the plant by the bacteria. Therefore, some tests were done to evaluate the natural tolerance of Ni in the plant.

4. Conclusions

- Among the tested heavy metal resistance markers (*czr, cop, pbr, ncc-nre*), only the *ncc-nre* resistance system was efficiently expressed in both *B .cepacia* as *H. seropedicae*. Due to the instability of the pKT240 plasmid carying the *ncc-nre* genes, miniTn5 transposons were constructed and used to clone the Ni resistance determinants genes and to introduce them into the genome of the endophytic bacteria.
- Colonization experiments with mini-Tn5 *lux*AB marked *B. cepacia* and *H. seropedicae* suggested a good colonization of the upper plant parts.
- Presently we are studying the effect of Ni tolerance and accumulation in the host plant after inoculation of the plant with Ni resistant *B. cepacia* and *H. seropedicae* strains.

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METAL UPTAKE BY *THLASPI CAERULESCENS* AND METAL SOLUBILITY IN A ZN/CD CONTAMINATED SOIL AFTER ADDITION OF EDTA

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1. Introduction

Large areas of agricultural land are contaminated with heavy metals due to industrialization and agricultural activities. Clean-up of heavy metal-contaminated soils is difficult. Bioremediation for example phytoextraction by using hyperaccumulator plants may provide an effective and in situ way of removing heavy metals from contaminated soils (BAKER et al., 1994). *Thlaspi caerulescens* has been recorded as a Zn and Cd hyperaccumulator plant (BROWN et al., 1994; REEVES and BROOKS, 1983). KNIGHT et al. (1997) showed that about 99% of the Zn taken up by the plant were from exchangeable and insoluble pools of Zn. HUANG et al. (1997) indicated that EDTA was the best chelating agent for mobilizing lead from the soil particles. The objective of this study was to compare metal uptake by *Thlaspi caerulescens* and metal solubility in a Zn- and Cd-contaminated soil after addition of EDTA.

2. Materials and Methods

The soil (0-15 cm depth) was a slightly acidic sandy loam (pH 6.2) derived from Silurian shale and Triassic sandstone in Northern Ireland. Its *aqua-regia* soluble concentrations of Zn, Cd and Cu were 43.5, 1.6 and 16.4 mg kg^{-1}, respectively. Prior to the pot experiment soil subsample (<2-mm) was mixed with appropriate amounts of $ZnCl_2$ and $Cd(NO_3)_2$ (as solid particles) in order to add 500 mg Zn and 20 mg Cd per kg of dry soil. Basal fertilizers applied were 100 mg N kg^{-1} dry soil as ammonium nitrate, and 80 mg P kg^{-1} and 100 mg K kg^{-1} as KH_2PO_4. After thorough mixing, aliquots (1.0 kg oven dry weight) were transferred to acid washed plastic plant pots. Soil moisture sampler (Rhizon SMS) was installed in the center of each pot to allow a sample of the soil solution to be extracted. Eight seedlings of *Thlaspi caerulescens* (population from Prayon, Belgium) were transplanted into each of the pots and grown in the glasshouse. There were four replicates of each treatment. The pots were adjusted daily to 70% WHC with distilled water. EDTA (5 mmol) was added as a liquid solution into each pot 70 days after transplanting. The plants were harvested two weeks later and digested in a mixture of nitric and perchloric acids. Zn, Cd and Cu in the solutions were determined by AAS. Analysis of variance was performed on all data sets.

3. Results and Discussion

Table 1 shows that *Thlaspi caerulescens* accumulated about 0.5% Zn and 200 mg Cd kg^{-1} under conditions of combination pollution of Zn and Cd in a sandy loam with pH 6.2, in a general agreement with previous reports (BROWN et al., 1994; KNIGHT et al., 1997). Addition of EDTA increased greatly Zn concentration to 1% in the hyperaccumulator after two weeks. This was in accordance with a substantial increase in soil solution Zn concentration (Fig. 1) after

treatment with EDTA. EDTA also proved to be mobilizing Cd and Cu from the soil particles (Fig. 1). However, plant Cd uptake did not increase compared to the control without addition of EDTA, perhaps due to the interaction of Zn to Cd and also the formation of chelated Cd.

4. Conclusions

Thlaspi caerulescens (Prayon, Belgium) can grow well in soil freshly-polluted with 500 mg Zn kg^{-1} and 20 mg Cd kg^{-1} and has a high potential in removing Zn and Cd from the soil by phytoextraction. EDTA proved to be an effective chelating compound for mobilizing the metals from the soil and enhanced Zn uptake by the hyperaccumulator.

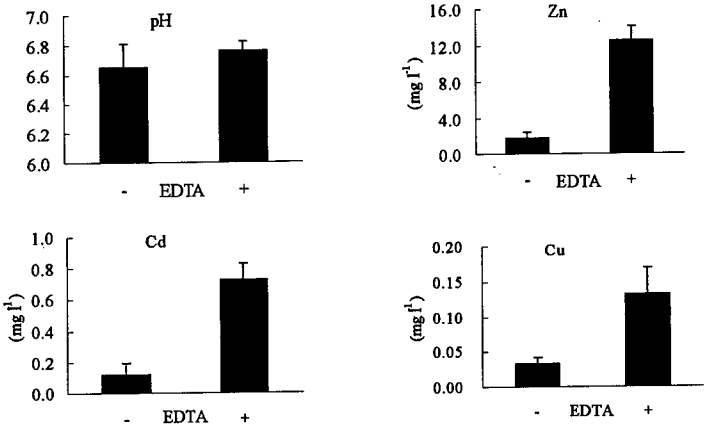

Fig. 1 Differences in soil solution pH, Zn, Cd and Cu between EDTA addition (+) and the control (-)

Table.1 Hyperaccumulator dry matter (DM), concentration and uptake of Zn, Cd and Cu after addition of EDTA (mean values±SEs)

EDTA Addition	DM	Plant Concentration			Plant Uptake (□g pot^{-1})		
	(g)	Zn (%)	Cd mg l^{-1}	Cu mg l^{-1}	Zn	Cd	Cu
-	1.50±0.17	0.58±0.20	191±21	7.4±0.8	8765±3190	289±61	11±1.9
+	1.47±0.19	1.07±0.54	167±54	8.6±1.0	15196±5839	253±116	12±2.5

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THE COMPLEXES OF CADMIUM WITH PHYTOCHELATINS. A QUANTUM MECHANICS STUDY

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1. Introduction

Plant synthetized phytochelatins are gamma-glutamyl-cysteinyl peptides of the general formula PCn with n = 2-11 (Fig.1). They have the ability of forming stable complexes with cadmium. It is generally assumed that the phytochelatin-metal complexes are less toxic to cellular plants metabolism than the free metal ions so that these substances have a considerable interest from a biochemical and environmental point of view. The present study is addressed to determine the structure of the complexes of divalent cadmium with PC_{2-4}. The results well agree with existing EXAFS data.

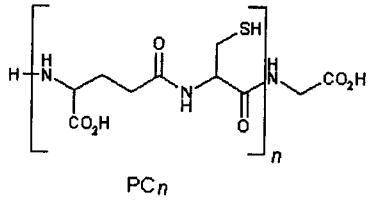

Figure 1. Structure of phytochelatins

2. Materials and Methods

Table 1. Optimized Cd-S distances (Å) for the Cd(PC1)2 and Cd(PC2)2 complexes. Sulphur atoms are numbered according to Fig. 4.

	rCd-S1 (Å)	rCd-S2 (Å)	rCd-S3 (Å)	rCd-S4 (Å)
Cd(PC1)2	2.41	2.42	-	-
Cd(PC2)2	2.59	2.61	2.61	2.61
Cd(PC2)2-9H2O	2.49	2.56	2.65	2.77

Calculations were performed on $Cd(PC_1)_2$ and $Cd(PC_2)_2$ complexes in vacuum as well as on the $Cd(PC_2)_2$-$9H_2O$ complex to evaluate the effect of the solvent on the geometry of the complexes. Theoretical calculations were carried out at the restricted Hartree-Fock level (RHF) using the PM3 semi-empirical SCF-MO method, as implemented in the MOPAC6 program. The MMOK correction to the energy of peptide linkages was applied. For every structure, a previous molecular

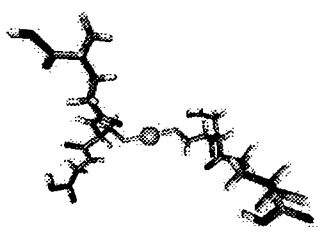

Figure 2. Optimized structure of the $Cd(PC_1)_2$ complex.

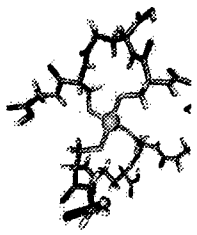

Figure 3. Optimized structure of the $Cd(PC_2)_2$ complex.

mechanics optimization of the geometry was carried out using the MM3 force field.

3. Results and Discussion

The optimized geometries of the $Cd(PC_1)_2$ and $Cd(PC_2)_2$ complexes are shown in Figs. 2 and 3. The optimized Cd-S distances and S-Cd-S angles are reported in Table 1 and 2, respectively.

Cd(PC1)2 complex

Cd exhibits the linear coordination geometry shown in Fig. 2, only S atoms enter its coordination shell. The optimized value of the S-Cd-S angle in the $Cd(PC_1)_2$ complex is 175.7 deg. The Cd-S distance is 2.42 Å

Cd(PC2)2 and Cd(PC2)2-9H2O complexes

The values of the S-Cd-S angles reported in Table 2 show that cadmium in the $Cd(PC_2)_2$ complexes exhibits the distorted tetrahedrical coordination shown in Fig. 3. The mean Cd-S distance is about 2.6 Å. Only the sulphur atoms enter the coordination sphere of cadmium. These findings well agree with EXAFS data which exclude the participation of atoms different than sulphur to the coordination of cadmium. The computed rCD-S values (Table 1) also well agree with reported Cd-S bond length of 2.52±0.02 Å from EXAFS data. The water molecules do not enter the coordination sphere of Cd but rather forms hydrogen bonds with the sulphur atoms.

Table 2. Optimized S-Cd-S angles (deg) for the Cd(PC2)2 *in vacuum* complex. Sulphur atoms are numbered according Fig. 4.

	S2	S3	S4
S1	107.9	114.4	116.6
S2	-	116.9	102.0
S3	-	-	98.6

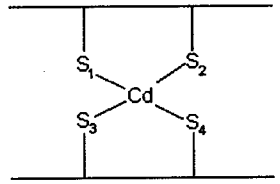

Figure 4. Numbering of the sulphur atoms in the Cd(PC2)2 complex

4. Conclusions

Semiempirical calculations on the complexes of Cd with PC's have been performed with satisfactory agreement with structural data from literature. These results integrate EXAFS information on the coordination of Cd by PC's and constitute a valid starting point for further investigations on the biochemical mechanism of the plants response to the stress from toxic metals.

5. References

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EFFECT OF ZINC AND COPPER ON CADMIUM UPTAKE BY *THLASPI CAERULESENS* AND *CARDAMINOPSIS HALLERI*

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1. Introduction

The use of metal-hyperaccumulating plants for decontamination of metal-polluted soils is now recognised as a feasible remediation technology. *Thlaspi caerulescens* and *Cadaminopsis halleri* are two plant species known to hyperaccumulate metals such as Cd and Zn. In many soils affected by mining, smelting and industrial processes, pollution by a single metal is most uncommon, with several metals usually occurring at high concentrations. Copper is often found in high concentrations in polluted soils with Cd and Zn, and the sensitivity of highly Cd/Zn tolerant hyperaccumulators to Cu has not been documented. Furthermore, competition between metals for uptake by non-hyperaccumulating plants has been observed (Cataldo *et al.* 1983), but the efficiency of metal uptake by hyperaccumulating species is poorly defined in situations where several metals are present at high concentrations. The aim of this series of experiments was to determine the effect of varying Cu^{2+} and Zn^{2+} activities in solution on growth of, and metal uptake by, two hyperaccumulator plant species.

2. Materials and Methods

Thlaspi caerulescens (ecotype "Ganges") and *Cadaminopsis halleri* (ecotype "Hartz") seeds were germinated in vermiculite and transferred (6 individuals of each species) to 8.3 L buckets containing nutrient solution with composition as follows - Ca (3.45 mM); Mg (1.45 mM); Na (85 μM); K(1.2 mM); SO_4 (1.45 mM); NO_3 (8.3 mM); H_2PO_4 (75 μM); Fe as FeHBED (75 μM); Cl (30 μM); H_3BO_3(30 μM); Mn (10 μM); Cu (0.2 μM); Zn (1 μM); MoO_4 (0.2 μM) and Cd (10 nM). Treatments imposed were 2 concentrations of Cu and Zn to give final Cu^{2+} activities of 1.0 and 2.5 μM, and Zn^{2+} activities of 25 and 50 μM with 3-fold replication.Plants were grown in a climate controlled growth chamber at 20°C day/15°C night on a 14 h day/10 h night cycle with photosynthetically-active radiation of 350 μmol quanta m^{-2} s^{-1}. Metal concentrations in solution were regularly monitored using inductively-coupled plasma atomic emission spectroscopy (ICP-AES) and graphite furnace atomic absorption spectroscopy (GFAAS) and solutions replenished when depletion exceeded 15% of nominal concentrations. Both species were harvested after 35 days of growth and separated into roots and shoots. Extracellular metal on roots was desorbed using a 1 mM $LaCl_3$/ 5 mM 0.01 M $CaCl_2$ solution.(Reference) After oven drying (70°C for 48 h), dry weights were recorded and metal concentrations determined by ICP-AES and GFAAS after digestion of dried ground plant material by concentrated HNO_3 acid.

3. Results and Discussion

In the 35-day growth period *Cardaminopsis halleri* produced almost double the shoot biomass of *Thlaspi caerulescens* with root growth being similar for the two species (Fig. 1). There was no significant difference ($P>0.05$) in root or shoot growth of both species when Zn^{2+} activities in solution were increased from 0.5μM (Control) through 25 to 50μM. However, increasing Cu^{2+} activities from 0.1 μM (Control) through 1 to 2.5 μM significantly ($P<0.001$) reduced root and shoot growth of both species, particularly *Thlaspi caerulescens* (>90% growth reduction). In the Cu treatments, roots of both species were stunted and leaves were necrotic and showed a purple discoloration.

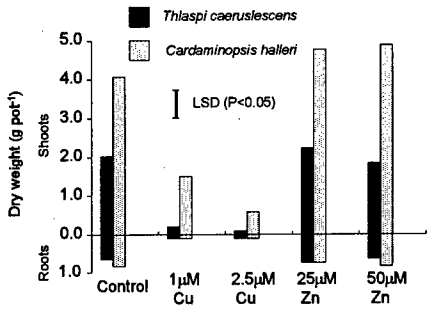

Figure 1. Plant dry weight as affected by varying Cu^{2+} and Zn^{2+} activities in solution.

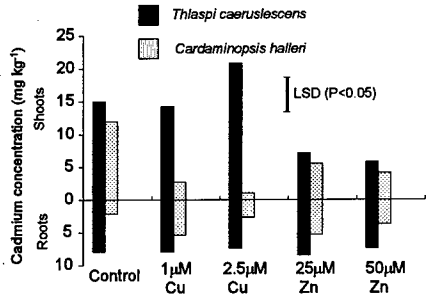

Figure 2. Plant Cd concentrations as affected by Cu^{2+} and Zn^{2+} activities in solution.

Cadmium concentrations were similar in the shoots of both species, but *Thlaspi caerulescens* tended to have a greater retention of Cd in roots. Shoot Cd concentrations were significantly ($P<0.05$) decreased when Zn^{2+} activities in solution were increased from 0.5 μM through 25 to 50 μM (Fig. 2). Total Cd uptake (μg pot^{-1}) into shoots was similarly affected (data not shown). Increasing Cu^{2+} activities in solution reduced Cd concentrations in shoots of *Cardaminopsis halleri* but did not affect those in *Thlaspi caerulescens*.Neither species accumulated high concentrations of Cu in shoots (< 80 mg kg^{-1}), but Cu concentrations in roots were high (approx. 1000 mg kg^{-1}) at a solution Cu^{2+} activity of 2.5 μM (data not shown). Zinc concentrations in shoots of both species were similar (approx. 700 mg kg^{-1}) when solution Zn^{2+} activity was 0.5 μM, but at 25 and 50 μM *Cardaminopsis halleri* had almost twice the concentration of Zn in shoots (>12,000 mg kg^{-1}) compared to *Thlaspi caerulescens*. Accumulation of Zn was reduced by increasing Cu^{2+} activities in solution (data not shown).

4. Conclusions

While *Cardaminopsis halleri* and *Thlaspi caerulescens* were tolerant of high Zn concentrations in solution, in agreement with previous studies, these species were not tolerant of high Cu^{2+} activities in solution. Where Cu is a co-contaminant with Cd or Zn, these plant species may be susceptible to Cu toxicity. A further complication of mixed metal pollution is that extraction efficiencies of Cd and Zn by these hyperaccumulator species may be significantly reduced due to competition between metals for uptake by the roots.

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INCREASED SELENATE UPTAKE, REDUCTION AND TOLERANCE IN TRANSGENIC *BRASSICA JUNCEA* PLANTS

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1. Introduction

Selenium (Se) is an essential trace element, but is also toxic at higher concentrations. Due to irrigation of Se-containing soils and the use of Se-containing oil and coal, Se has become a serious environmental pollutant in the Western U.S.A. and other areas worldwide, even causing death and deformities of wildlife. A promising new technology for the remediation of Se-polluted water and soil is through the use of plants, i.e., phytoremediation. Plants can accumulate Se in their tissues and volatilize it as relatively non-toxic dimethylselenide (Terry *et al.*, 1992). The reduction of selenate was proposed to be rate-limiting for the selenate assimilation pathway (de Souza *et al.*, 1998). *In vitro* studies have proposed that ATP sulfurylase mediates the reduction of selenate as well as sulfate in plants (Shaw and Anderson, 1972). Thus, ATP sulfurylase may be rate-limiting for Se assimilation, and overexpression of this enzyme may increase the flux of the pathway. To test this hypothesis, the *APS1* gene from *Arabidopsis thaliana* , encoding a plastid-localized ATP sulfurylase (Leustek *et al.*, 1994), was overexpressed in Indian mustard (*Brassica juncea*.). The transgenic APS plants were compared with untransformed plants with respect to selenate reduction, Se accumulation, and Se tolerance.

2. Materials and Methods

(Pilon-Smits *et al.*, 1999)

Hypocotyls from 3d old Indian mustard seedlings were transformed using *Agrobacterium tumefaciens*, with a DNA construct containing the *A. thaliana APS1* cDNA including its own chloroplast transit sequence, under the control of the CaMV 35S promoter. For Se tolerance and accumulation experiments, hydroponically grown Indian mustard plants were exposed to 20µM selenate for 7 days, after which growth and S and Se accumulation were measured. Sulfur was analyzed by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) after acid digestion, and Se concentrations in the acid digests were analyzed using atomic absorption spectroscopy with hydride generation. X-ray absorption spectroscopy (XAS) analysis was performed on Se-treated Indian mustard plants (7d, 20µM selenate) at the Stanford Synchrotron Radiation Laboratory.

3. Results and Discussion

The transgenic APS plants showed 2-2.5 fold higher ATP sulfurylase activities in their shoots and roots than untransformed plants. This increase in ATP sulfurylase activity had a dramatic effect on selenate reduction: x-ray absorption spectroscopy showed that root and shoot tissues of mature APS plants contained mostly organic Se (resembling selenomethionine), while wildtype plants accumulated selenate (Table 1). The APS plants were not able to reduce selenate when shoots were removed immediately before selenate was supplied, indicating that the shoot is the site of selenate reduction. The transgenic APS plants also showed increased Se accumulation: Se concentrations in APS plants were 2- to 3-fold higher than in untransformed plants (Table 1). Furthermore, Se tolerance was higher in both seedlings and mature APS plants, compared to wild type. The APS plants also showed altered sulfur metabolism (Table 1): they had 1.5-fold higher shoot sulfur concentrations compared to untransformed plants, and 2-fold higher glutathione and total thiol concentrations in roots and shoots.

4. Conclusions

These studies are the first to show that ATP sulfurylase mediates selenate reduction *in vivo*, and that ATP sulfurylase is rate-limiting for selenate uptake and assimilation. Furthermore, the results show that ATP sulfurylase is a rate-limiting enzyme for sulfur assimilation and for glutathione biosynthesis.

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Table 1: Se and S accumulation in shoots of wildtype and APS transgenic Indian mustard plants.

	Se-form accumulated (XAS Se spectra)	Se conc. ($\mu g.g^{-1}$ DW)	S conc. ($mg.g^{-1}$ DW)	GSH conc ($nmol.g^{-1}$ DW)
Wildtype	100% selenate	877 ± 144	9.3 ± 1.7	67.7 ± 18.4
APS 8	95% organic Se	1434 ± 360	13.9 ± 3.6	138.6 ± 8.8
APS 9	80% organic Se	2046 ± 246	15.0 ± 1.0	151.3 ± 12.2

OVEREXPRESSION OF GLUTATHIONE SYNTHESIZING ENZYMES ENHANCES CADMIUM ACCUMULATION IN *Brassica juncea*

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1. Introduction

Heavy metal pollution of soils and waters, mainly due to mining and the burning of fossil fuels, is a major environmental problem. An attractive biological approach to deal with this problem is phytoremediation, i.e. the use of plants to clean up polluted waters and soils. The goal of this study was to use genetic engineering to increase heavy metal tolerance and accumulation in plants. The chosen strategy was to overproduce the heavy metal binding peptides glutathione and phytochelatins. Glutathione (γ-Glu-Cys-Gly, GSH) plays several important roles in the defense of plants against environmental stresses, and is the precursor for phytochelatins (PCs): heavy metal-binding peptides involved in heavy metal tolerance and sequestration. Glutathione is synthesized from its constituent amino acids in two enzymatic reactions, catalyzed by γ-glutamyl-cysteine synthetase (γ-ECS) and glutathione synthetase (GS), respectively. To obtain plants with superior Cd accumulation and tolerance, we overexpressed the *E. coli* ECS and GS enzymes in *Brassica juncea* (Indian mustard), a particularly suitable species for trace element remediation. The transgenic ECS and GS plants were compared with untransformed Indian mustard plants with respect to their Cd accumulation and tolerance, as well as their levels of heavy metal binding peptides.

2. Materials and Methods (Zhu et al., 1999)

Hypocotyls from 3d old Indian mustard seedlings were transformed using *Agrobacterium tumefaciens*, with DNA constructs containing the *Escherichia coli gshI (ECS)* or *gshII* (GS) gene, under the control of the CaMV 35S promoter (Noctor et al. 1996, Strohm et al., 1995). For Cd tolerance and accumulation experiments, hydroponically grown Indian mustard plants were exposed to 100μM CdSO4 for 14 days, after which growth and Cd accumulation were measured. Cadmium was analyzed by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) after acid digestion. Glutathione and phytochelatins were measured by HPLC, on a C18 reverse phase column.

3. Results and Discussion

The transgenic ECS and GS plants both accumulated significantly more cadmium in their shoots than wildtype plants (Table 1): Cd concentrations were up to 2-fold higher, and total cadmium accumulation per shoot was up to 3-fold higher. Moreover, ECS and GS plants showed

enhanced tolerance to cadmium, both at the seedling and mature plant stage. Cadmium accumulation and tolerance were correlated with the expression levels of the bacterial genes introduced. The concentrations of glutathione, phytochelatin, and total non-protein thiol, were higher in ECS and GS plants compared to wildtype plants (Table 1); they were higher under all conditions for the ECS plants, but only in the presence of Cd for the GS plants.

4. Conclusions

In the absence of Cd, the ECS enzyme appears to be rate-limiting for the biosynthesis of GSH and PCs, while in the presence of Cd the GS enzyme becomes rate-limiting as well.
Since the Cd tolerance and accumulation capacity of Indian mustard was correlated with GSH and PC levels, these compounds appear to play an important role in Cd tolerance.
Overexpression of ECS and GS offers a promising strategy for the production of plants with superior heavy metal phytoremediation capacity.

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Table 1: Accumulation of Cd and S-compounds in wildtype, ECS or GS Indian mustard plants (average of 10 plants ± s.e.). GSH: glutathione; NPT: non-protein thiol.

	Shoot Cd conc. ($\mu g.g^{-1}$ DW)	NPT conc. +Cd ($nmol.g^{-1}$ DW)	GSH conc. –Cd ($nmol.g^{-1}$ DW)	GSH conc. +Cd ($nmol.g^{-1}$ DW)
Wildtype485 ± 97	5500 ± 300	280 ± 50	93 ± 40	
ECS 4	775 ± 110	7500 ± 400	380 ± 20	170 ± 20
GS 7	698 + 91	9100 + 300	360 + 40	456 + 80

HEAVY METAL PHYTOEXTRACTION CAPACITY OF SEVERAL AGRICULTURAL CROP PLANT SPECIES

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1. Introduction

Phytoremediation is a novel environmental technology to clean-up heavy metal or radionuclide contaminated soils or waters with the help of metal accumulating or hyperaccumulating plants. During phytoextraction, metal-accumulating plants are used to transport and concentrate metals from the soils into the harvestable parts of roots and above-ground shoots. Metal hyperaccumulation is not known among agricultural plants, species of *Brassicaceae* and *Compositae* family are capable, however, to accumulate and tolerate high levels of heavy metals in their above-ground plant organs (Baker et al., 1994; Raskin et al., 1997). The aim of our study was to investigate the heavy metal accumulation and distribution in several agricultural crop species grown in a galvanic mud contaminated soil.

2. Materials and Methods

The uncontaminated (control) soil (pH_{KCl}: 6.6, OM: 1.3 %, CEC: 18.1 meq/100 g, Cd 1.3 mg/kg, Cr 19.2 mg/kg, Cu 14.7 mg/kg, Ni 9.3 mg/kg, Zn 51.0 mg/kg, 0-25 cm) used in this experiment originated from the demonstration garden of College of Agriculture, Nyíregyháza. Soil contaminated with heavy metals (pH_{KCl}: 6.8, OM: 1.1 %, CEC: 8.2 meq/100 g, Cd 29.9 mg/kg, Cr 246.7 mg/kg, Cu 100.3 mg/kg, Ni 66.7 mg/kg, Zn 117.3 mg/kg, 0-25 cm) was collected in a kitchen garden located near a former galvanization plant (Nyíregyháza, Vasgyár street). Both soils were slightly acidic loamy sands and had brown forest soil character. Greenhouse pot experiments were conducted with Indian mustard (*Brassica juncea* L. Czern., cv. Negro Caballo), white mustard (*Sinapis alba* L., cv. Budakalászi sárga), fodder radish (*Raphanus sativus* L. convar. *oleiformis* Pers., cv. Leveles olajretek), turnip (*Beta vulgaris* L. var. *Rapa*, cv. Horpácsi lila), hemp (*Cannabis sativa* L., cv. Kompolti hibrid TC), rape (*Brassica napus* L. ssp. *oleifera* Metzg. ap. Sinsk., Mécses) and amaranth (*Amaranthus hypochondriacus* L., cv. Edit). Three plants were grown in plastic pots with 1500 g of uncontaminated (control) or heavy metal contaminated soil with 3 replications. After 8 weeks of growth the plants were harvested and separated into roots and shoots. The elemental composition of soil and plant samples was determined by ICAP technique in triplicate at the Central Chemical Laboratory, Debrecen University of Agricultural Sciences, Hungary.

3. Results and Discussion

Cadmium appeared equally in roots and shoots of investigated plants, the highest value (Cd 81 μg/g) was found in the shoots of turnip. Roots of all plants accumulated high levels of chromium from the contaminated soil, but the transfer of this element into the shoots was negligible. The highest shoot Cr concentration was detected in fodder radish (Cr 13 μg/g). The highest Cu and Zn concentrations were measured in the beet roots of turnip (Cu 75 μg/g and Zn 191 μg/g). The highest shoot Cu and Zn concentrations were detected in Indian mustard (Cu 52 μg/g) and white mustard (Zn 160 μg/g). Nickel accumulation in plants and its translocation from roots to shoots was low, and did not correlate with nickel contamination of soil (data not shown). Phytoextracting capacity of the test plants was also evaluated by calculating the total

concentration of heavy metals (sum of Cd, Cr, Cu, Ni and Zn concentrations) in plants. This value in roots was the highest in turnip, rape and amaranth (data not shown). In shoots lower values were found; Indian mustard, white mustard, fodder radish, turnip and amaranth accumulated 200-250 μg of heavy metals in one gram of shoot dry matter (Figure 1). Considering the whole plants the total concentration of accumulated heavy metals was the highest in turnip (data not shown).

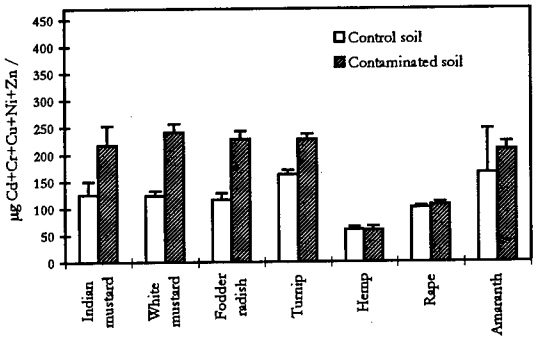

Figure 1. Total concentration of the accumulated heavy metals in shoots of test plants grown in an uncontaminated or galvanic mud contaminated soil.

Multiplying the dry matter content of plants (accumulated during the experiment) with heavy metal concentrations, the total amount of extracted heavy metals (μg/plant) was calculated. The total amount of heavy metals in roots was the highest in turnip, rape and amaranth. In shoots this tendency has been changed, turnip, Indian mustard and white mustard were the most effective in heavy metal extraction from contaminated soil. In whole plants the total amount of heavy metals was the highest in turnip and amaranth (data not shown).

4. Conclusions

Considering that during the phytoremediation the most easily harvestable plant parts are shoots, the above data suggests that *Brassicacae* (e.g. turnip, Indian mustard or white mustard) could be the most effective in heavy metal (Cd, Cr, Cu, Ni and Zn) phytoextraction of contaminated soils. This tendency could be changed, however, under open-field conditions, regarding the higher biomass of hemp or amaranth than that of *Brassicacae*. For effective phytoremediation under open-field conditions the amount of heavy metals accumulated in our test plants should be enhanced.

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CHANGED METAL UPTAKE IN TOBACCOS TRANSFORMED WITH *ESCHERICHIA COLI* COPPER RESISTANCE GENE *PCOA*

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1. Introduction

Heavy metals, like Cu, Zn, Mn, Fe, Ni and Mo are essential elements for biological systems. At increased levels, both essential and non-essential metals (Cd, Pb, Hg) are toxic. Local metal increases are caused by human activities: smelting, mining, processing, agricultural and waste disposal technologies. Due to technological advancements, metal concentrations in the air have significantly decreased. Metal concentrations in soils are increasing, leading to potential increases in leaching to water, uptake by plants and intake by human population. Contamination affects growth and survival of plants and microbes. However, many species have adapted to increased metal concentrations and have even developed a heritable tolerance to heavy metals. The mechanisms of metal tolerance have been explored mainly in microbes, but in plants they are still poorly understood. Microbial genes conferring heavy metal tolerance might be transferred into plants to increase the metal tolerance and to influence the uptake and distribution of metals, to produce plants optimised for decontamination of agricultural fields and remediation of soils around industrial emission sites, and by that way to improve the quality of the environment. Tolerant plants can be used for revegetation of metal contaminated areas, which also helps immobilising the retaining metals in the soil.

In order to improve plant heavy metal tolerance and to increase uptake of metals we decided to transfer *pco*A gene encoding periplasmic metal-binding protein from copper-resistance determinant of *Escherichia coli* plasmid pRJ1004 (BROWN et al. 1995, ROUCH and BROWN 1997) into a model tobacco plant.

2.Materials and Methods

Construct

*pco*A gene was PCR-amplified with gene-specific primers containing artificial *Xba*I and *Bgl*III sites for cloning in the binary vector pBI121. The construct was designed to express *pco*A as a transcriptional fusion with ß-glucuronidase reporter gene *gus* (polycistronic mRNA). Recombinant plasmids were first transformed into *E. coli*, and then transformed into *Agrobacterium tumefaciens* LBA4404.

Agrobacterium-mediated transformation and regeneration of plants

Greenhouse-grown tobacco leaves (SR1, Little Havanna) were sterilized, cut into 5 mm pieces, and inoculated with recombinant *Agrobacterium* cells in MS medium for 30 min and cocultivated on solid MS medium for 3 days. Selection was made on MS agar containing 250 µg/ml kanamycin, 500 µg/ml cefotaxime, 1.0 µg/ml 6-benzylaminopurine and 0.1 µg/ml I-naphthaleneacetic acid for about three weeks, and the shoots were transferred on 1/2 MS medium containing 250 µg/ml kanamycin and 500 µg/ml cefotaxime.

Verification of transformants

Two months from regeneration, the transformants were checked with PCR using *pco*A gene specific primers. The plants showing *pco*A amplification were further studied by Southern blot analysis with *pco*A specific and *gus* specific DIG-labeled probes made by PCR.

Expression of *pco*A in the transgenic plant

The expression at mRNA level was studied by Northern blot hybridization with *pco*A and *gus* specific probes. To study ß-glucuronidase (GUS) expression, a histological staining was made from pieces of leaves using X-Gluc as a substrate. To study PcoA protein expression, antibodies have been raised in a rabbit against a fusion protein.

Metal exposure

Micropropagated *pco*A tobacco clones (PCO-14 and -21) and SR1 parent tobacco were planted into soil for F1 seeds.The F1 seeds were sown in soils containing Cd (0, 10, 100 and 500 mg/kg) or Cu (0, 100, 500 and 1000 mg/kg) and grown for three months. Leaf samples were taken for metal analysis (atomic absorption spectrometer).

3. Results and Discussion

Twenty-four plants survived in kanamycin selection, nine of them showing *pco*A-specific amplification by PCR. Seven independent tobacco clones were studied by Southern analysis, and five of those showed integration of the *pco*A gene (PCO-14, -15, -20, -21 and -23).Transcription of the *gus* reporter gene and *pco*A was low: the *gus* probe gave weak hybridization of mRNA size ~2kb; one clone (PCO-23) showed a larger transcript of 4 kb, probably indicating a *gus* and *pco*A fused transcript. The *pco*A probe showed even weaker hybridization giving a smear-like pattern. No GUS activity was seen in transgenic tobacco leaves in histochemical staining. Although the transcription of *pco*A was faint in transgenic tobacco clones, one of them apparently expressed an active PcoA protein: compared to SR1 tobacco and to another transgenic tobacco clone (PCO-21), PCO-14 clone showed increased metal uptake: uptake of Cu was increased maximally by four-fold and Cu uptake was doubled (Table 1). Increased metal uptake did not markedly affect the growth and appearance of the plants.

Table1. Cu content of SR1 and transgenic tobacco leaves exposed to Cu in soil.

Cu in soil (mg/kg)	Cu-content in tobacco leaves (µg/g DW)		
	SR1	PCO14	PCO21
0	11.3 8.5	12.8 14.1	9.4 10.1
100	24.7 22.3	25.2 21.3	34.3 21.6
500	28.8 24.8	97.4 42.6	79.3 69.1
1000	49.7 51.6	27.1 35.6	39.1 61.7

4. Conclusions

- Microbial *pco*A gene could be transferred into a tobacco.
- The gene is expressed was plant and increased the uptake of metals: Cd uptake maximally by 2-fold and Cu uptake maximally by 4-fold compared with the control.
- The *pco*A gene alone, in the absence of other genes of the copper-resistance determinant, can influence metal uptake in the plant, while in *E. coli* all the genes in the operon are needed.

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METALLOTHIONEIN GENES IN COPPER SENSITIVE AND COPPER TOLERANT *SILENE VULGARIS*. DO THEY PLAY A ROLE IN COPPER TOLERANCE?

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1. Introduction

Copper is a heavy metal that, in excessive amounts, causes damage to plants. Within minutes it induces depolarization of the plasmamembrane and leakage of solutes, such as potassium, from the rootcells (Kennedy and Gonsalves, 1987; De Vos *et al*, 1989). A few plant species such as *Silene vulgaris* have evolved ecotypes with large differences in copper tolerance level, such as that growing at the Imsbach copper mine, which is 50-fold more tolerant than the normal soil ecotype.
There are indications that metallothioneins (MT) could be responsible for the copper tolerance. MT's are low-moleculair-weight, cysteine-rich proteins that can bind metals. They are thought to play a role in controlling the cellular concentrations of free ions of certain heavy metals such as copper and zinc. MT2gene expression seems to be the primary determinant of ecotypic differences in the copper tolerance of nonpretreated seedlings of *Arabidopsis thaliana* (Murphy and Taiz,1995).

2. Materials and Methods

We made pair crosses between plants from the copper tolerant population Imsbach and the copper sensitive population Amsterdam. The highly copper tolerant and copper sensitive plants were selected from the F2 families. These plants were used to produce tolerant and sensitive F3 lines, which were used for further research.
mRNA was isolated from these F3 lines and from Imsbach andAmsterdam and cDNA was made. We tried to find the MT's using primers based on MT specific parts of the MT DNA sequence of *Arabidopsis thaliana* in a PCR reaction. Positive products were sequenced.

3. Results and Discussion

PCR gave positive results with the MT2b primers. The product was sequenced and the sequence of the MT2b-like cDNA shows 73% homology with the *Arabidopsis* MT2b and is expressed in the copper sensitive and copper tolerant population of *Silene vulgaris*, both at low (0.1 µMCuSO4 for Amsterdam and 1 µM CuSO4 for Imsbach) and high copper concentrations (3.2 µM CuSO4 for Amsterdam and 170 µM CuSO4 for Imsbach, corresponding with the copper concentration that causes an inhibition of the root growth of 50%). The complete sequence was found in the cDNA bank of Imsbach by A.Tervahauta from the group of Finland. The sequence of the sensitive population has at least five mutations compared to the tolerant population (Table 1). The cDNA of some tolerant and sensitive F3 families will be sequenced to see if the mutations in the sequence will co-seggregate with copper tolerance. Difference in expression will

be measured by using Northern blotting and radioactively labeled *Silene vulgaris* MTprobe. These measurements will be carried out with copper tolerant F3 families and Imsbach and with copper sensitive F3 families and Amsterdam. mRNA will be isolated from roots and shoots of plants on 0.1 µM CuSO4 (t=0) and 50 µM CuSO4 (t=4, 24 and 48 hours after exposure) and the expression level of the gene will be measured.

Table 1: cDNA sequence of the MT2b like gene from Imsbach: cDNA Amsterdam population (only difference with sequence of Imsbach shown): underlined Primers indicated by "|"
Cysteines: **bold**

ATG	TCG	TGC	TGT	AAT	GGA	AAC	TGT	GGT	TGT	GGA	
met	ser	**cys**	**cys**	aspar	glyc	aspar	**cys**	glyc	**cys**	glyc	
								|primer -->			
TCT	GCC	TGC	AAG	TGC	GGC	AGC	GGC	TGT	GGA	GGA	
ser	alan	**cys**	lysin	**cys**	glyc	ser	glyc	**cys**	glyc	glyc	
	|				AAC						
						aspar					
TGC	AAG	ATG	TTC	CCT	GAC	TTT	GCC	GAG	GGA	AGT	
cys	lysin	met	fenyl	prol	asp a	fenyl	alan	glut	glyc	ser	
								GCA			
									alan		
TCC	GGC	TCA	GCA	AGC	CTT	GTC	CTC	GGG	GTA	GCA	
ser	glyc	ser	alan	ser	leuc	val	leuc	glyc	val	alan	
				CTC							
					leuc						
CCC	ATG	GCC	TCA	TAC	TTT	GAC	GCA	GAG	ATG	GAA	
Prol	met	alan	ser	tyros	fenyl	asp a	alan	glut	met	glut	AAG
			TTC					GAG			
	lys				fenyl					glut	
ATG	GGT	GTC	GCA	ACT	GAG	AAT	GGT	TGC	AAG	TGC	
met	glyc	val	alan	threo	glut	aspar	glyc	**cys**	lys	**cys**	
GGT	GAC	AAC	TGC	CAG	TGC	AAC	CCT	TGC	ACT	TGC	
Glyc	asp a	aspar	**cys**	glut	**cys**	aspar	prol	**cys**	threo	**cys**	
						|			<-- primer		
AAA	TGA										
Lys	stop										
	|										

4. Conclusions

-A MT2b-like gene is expressed in copper tolerant and copper sensitive plants of *Silene vulgaris*. Expression levels of this gene will be measured using Northern blotting of tolerant and sensitive plants.

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PHYTOSTABILIZATION OF MIMICKED CADMIUM CONTAMINATED SOIL WITH LIME AMENDMENT

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1. Introduction

Cadmium is one of the most problematic soil contaminants and exhibits toxic properties at very low concentration. In China, nearly 15,000 ha of arable land are contaminated with Cd due to residues from metalliferous mining and the smelting industry, and the use of wastewater, sewage sludge and fertilizer (Chen, 1985). Phytoremediation, as a more cost-effective technology, would likely be a more feasible technology to be adopted for remediation of such a large area of contaminated soils (Baker et al., 1994). It is therefore the aim of the present study to evaluate the uptake of Cd from a mimicked lime stabilized Cd contaminated acidic loamy soil by *Agropyron elongatum* and *Brassica juncea*.

2. Materials and Methods

An acidic loamy soil was spiked with three levels of Cd, i.e. 0, 5 and 20 mg Cd kg^{-1} soil in the form of carbonate salt and then stabilized with lime to pH 4 $\pm$ 0.4 and 6 $\pm$ 0.4. Selected physicochemical properties of the loamy soil were: pH 4.05, electrical conductivity 0.1 dS m^{-1}, organic carbon 1.4%, N, 0.093%, P 0.031%, K 0.96%, and Cd <0.01 mg kg^{-1}. 100 seeds of *A. elongatum* and 8 seeds of *B. juncea* were sown into each pot containing 1.5 kg of soil and. all pots received a complete fertilizer treatment. Plants were harvested after 8 weeks of growth and the dry weight yields were recorded. Oven-dried plant tissues were digested using 65% HNO_3 followed by Cd determination using atomic absorption spectrometry. Soil samples were also collected from each pot before plant growth for the determination of pH and DTPA extractable Cd contents.

3. Results and Discussion

pH of soil deviated slightly from the expected pH following lime adjustment and decreased with an increase in Cd concentration. Soil DTPA-extractable Cd contents increased while the percentage of extractable Cd of total Cd added decreased with an increase in spiked Cd concentration. With an increase in pH, there was a significant reduction in DTPA-extractable Cd content and the percentage of reduction at each Cd spiked level ranged from 22-26%.

Dry weight yields of both *A. elongatum* and *B. juncea* decreased according to the concentration of Cd in soil especially at low pH. *A. elongatum* demonstrated a better growth than that of *B. juncea* especially at lower pH condition as revealed by the higher relative dry weight yield percentage. This indicated that *A. elongatum* exhibited a higher resistance than *B. juncea* towards Cd toxicity. As expècted, both root and shoot tissue Cd contents increased with an increase in soil Cd contents. Both species accumulated higher concentrations of Cd in the roots than in the shoot. The accumulator factor, defined as the ratio of Cd concentration of shoot to that in soil (Baker et al., 1994), ranged from 4.1 to 7.9 for *A. elongatum*, and from 6.9 to 7.5 for *B. juncea*. However,

the two plant species demonstrated different trends of accumulation with *A. elongatum* having higher Cd accumulated in root than in shoot tissue while *B. juncea* exhibited an opposite trend. The shoot to root ratio of *B. juncea* was higher than that of *A. elongatum* at each Cd level. This indicated *that B. juncea* was more efficient in translocating Cd from the roots to the shoots than *A. elongatum*.

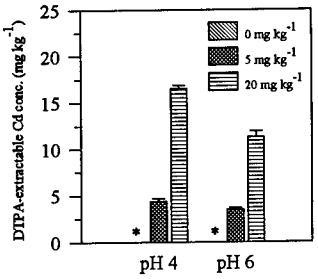

Figure 1. Concentration of DTPA extractable Cd in contaminated soil. (* conc. <0.05 mg kg^{-1})

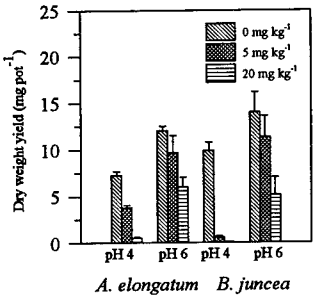

Figure 2. Dry weight yields of *A. elongatum* and *B. juncea* grown in cadmium contaminated soil with lime stabilization.

Table 1. Cadmium concentrations in shoot and root tissues of *A. elongatum* and *B. juncea* grown in cadmium contaminated soil with lime stabilization (mg Cd kg^{-1}).

Cd level (mg kg^{-1})	*A. elongatum*			*B. juncea*		
	Shoot	Root	Shoot/ root	Shoot	Root	Shoot/ root
0	0.85 c*	1.40 c	#	1.52 c	1.03 c	#
5	39.5 b	384 b	0.10	34.7 b	81.6 b	0.43
20	82.6 a	1584	0.05	150 a	538 a	0.28

* Means followed by the same letter within the same column do not differ significantly according to the Least Significance test.

4. Conclusions

The present study demonstrated that lime stabilization was effective in facilitate the establishment of both *B. juncea* and *A. elongatum*. *B. juncea* was more effective in translocating Cd from root to shoot than *A. elongatum* and hence had a higher potential for extracting Cd from soil. The vigorous growth of *A. elongatum* in soil with high Cd contamination in soil revealed its ability in avoiding the uptake of Cd to shoot which would warrant further investigation.

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WILL THE CD UPTAKE BY *SALIX* RESULT IN REDISTRIBUTION OF CD BETWEEN SUBSOIL AND TOP-SOIL?

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1. Introduction

The high toxicity and bioavailability of Cd is well established (DAS et al., 1997). As a result of human activities, such as mining, fossil fuel combustion and the use of commercial fertilizers, the soil concentrations of Cd in many parts of the world are increasing (NICHOLSON and JONES, 1994). To reduce the content of Cd in soils, phytoextraction, i.e. the cleaning of soils from Cd by use of high-accumulating plants, is one alternative. The main object of phytoextraction is to clean the top-soil, the part of the soil used by most crops. Of higher plant species, *Salix* has been shown to have a high Cd uptake capacity (BRIEGER et al., 1992). In *Salix* there are two apparently different root systems, one growing in the top-soil and one penetrating into the subsoil. Cd taken up by deeper growing roots, lost to the top-soil through leakage from top-soil roots would counteract the purpose of the phytoextraction. Therefore, besides the total uptake of Cd by the plants used for photoextraction, differences in uptake from different soil layers are of importance. The aim of this study was to find out if there is a risk that Cd from the subsoil is redistributed to the top-soil.

2. Materials and Methods

Cuttings from four clones of willow (*Salix viminalis*), with different properties regarding accumulation and transport of Cd were planted in plastic containers. The containers were filled with 30 cm of top-soil superposed on 60 cm of subsoil. After three months the intact plants were released from the containers and the roots washed in water. Each plant was then transferred to a split-root system, consisting of two containers filled with a concentrated nutrient solution. Top-soil roots were placed in one of the containers and subsoil roots in the other. ^{109}Cd in a low, non-toxic concentration was then added to one of the containers. During 24 hours, solution samples were taken, at intervals, from both of the containers. The uptake studies were performed in a climate chamber at 23/19°C, 75-80% relative humidity and 16/8 h (light/dark) with a photon flux density of about 200 $\mu E\ m^{-2}\ s^{-1}$. After harvest each plant was separated into stem, shoots, top-soil roots and subsoil roots. The fresh weights of the plant material were registered, after which it was dried at 105°C for 48 hours. Dry weights were then determined and thereafter the plant material was wet digested in HNO_3:$HClO_4$ (7:3, v/v). The Cd content of the plant material and the solution samples was analysed with atomic absorption spectrophotometry (SpectrAA-100, Varian, Springvale, Australia) using standard addition.

3. Results

The root mass was about the same in top-soil and in subsoil. Cadmium is taken up by both root systems. At an initial phase of uptake, the subsoil roots from all clones showed a higher net uptake capacity per gram dry weight than the top-soil roots. After 24 hours, subsoil roots from two of the clones still showed a higher net uptake capacity than the top-soil roots. In the remaining two clones, the net uptake capacity was about the same in top-soil roots and subsoil roots. Uptake was about 3-6% of the amount of ^{109}Cd added. Leakage from roots in the non-

treated container was, for all clones and for both top-soil roots and subsoil roots, about 10% of the amount of ^{109}Cd taken up.

4. Conclusions

The risk that Cd will be redistributed from the subsoil to the top-soil is dependent on which clone is used. If the subsoil roots have a higher net uptake capacity than the top-soil roots, a small redistribution of Cd may be the result. Even in these clones, however, the amount of Cd redistributed will only be a fraction of the total amount of Cd taken up.

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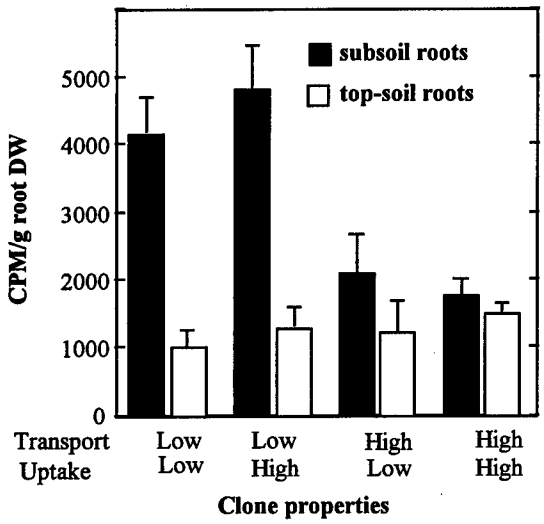

Figure 1. Cadmium net uptake in subsoil and top-soil roots after 24 hours, shown as CPM per g root DW. n=3-4, ±SE.

HEAVY METAL ACCUMULATION IN HIGHER PLANTS FOR USE IN URBAN STORMWATER TREATMENT.

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1. Introduction

As the amount of stormwater is increasing with the amount of areas covered with roads and roofs, it is also becoming more polluted. The content of heavy metals, in stormwater, causes problems in the recipients, as heavy metals are toxic to many forms of life.
Sometimes wetlands are used to improve the stormwater quality before it is released. Plants used as vegetation filters in stormwater treatment, to decrease the heavy metal concentration of the water, should have a high uptake of many metals (high accumulation factor). Furthermore, they could either accumulate the metals in the roots or in the shoot, the latter gives the possibility to remove the metals by harvest. It is known that some plant species hyperaccumulate metals, some are high or medium accumulators and some plants hardly accumulate heavy metals at all.
The aim of this study was to investigate if there are plants with high metal accumulation and distribution to the shoot which can be used in phytoremediation of metal contaminated stormwater.

2. Materials and Methods

Samples of plants were taken at three different wetlands for stormwater treatment, a stormwater ditch and a sewage treatment wetland on a mining waste area. The plant species were; *Alisma plantago-aqaticaa, Balsamin impatiens, Carex pseudocyperus, C. rostrata, Elodea canadensis, Eriophorum angusifolium, Equisetum palustre, Filipendula ulmaria, Juncus effusus, Lemna trisulca, L. gibba, L. minor, Lythrum salicaria, Persicaria amphiba, Phlaris arundinacea, Phragmites australis, Potamogeton natans, Salix spp., Scirpus sylvaticus, Sparganium erectumi, Typha latifolia* and *Urtica dioica*. Samples were taken in triplicates for each species, together with soil samples. The plants were divided in roots and shoots. They were thoroughly washed in redistilled water and then dried for 48 hours at 105 °C. Dry weight were determined and the samples were wet digested ($HClO_4$:HNO_3, 3:7) before analysing metal content on atomic absorption spectrophotometer (Varian spectrAA 100). The pH, total carbon content and dry weight of the soil samples were measured. Metal content of soil was analysed on atomic absorption spectrophotometer after wet digestion in 7 M HNO_3. The metals analysed were Zn, Cu, Cd and Pb as they are common in stormwater. The concentrations of metals were then used to calculate the accumulation factor, a measure of metal uptake efficiency by the different plants.

3. Results and Discussion

Results so far indicate that there are both high and low accumulators among the plants. *Phragmites australis, Phlaris arundinacea, Scirpus sylvaticus, Sparganium erectum* and *Typha latifolia* showed a low accumulation factor for all metals compared to high accumulating Salix, which have an accumulation factor near 2 (GREGER and LANDBERG 1995). This is shown for some plants in Fig. 1. The higher the metal concentration in the soil the lower is the accumulation factor. Zinc showed the highest accumulation factor.

The distribution of the metals to the shoot of the plants differed in different plant species. More data and a discussion, on plant species suitable for stormwater treatment, will be presented.

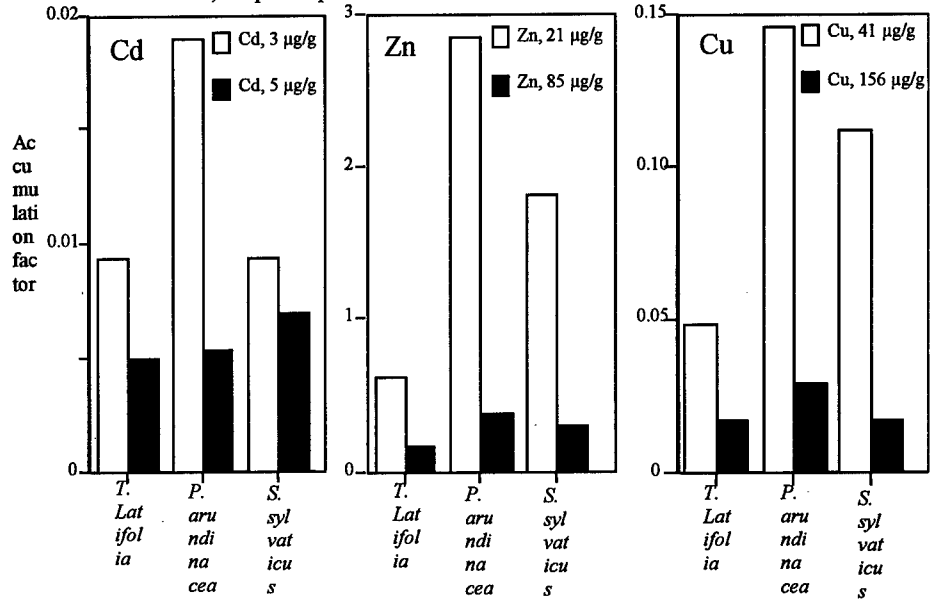

Figure 1. Shoot accumulation factors of 3 plant species at 2 sites with different metal levels in the sediment.

4. Conclusions

Different plant species have different accumulation factors, depending on metal and level of metal. Therefore, a combination of plant species is probably necessary to be able to remove most of the metals from stormwater. *P. arundinacea* does though seem best in this test, yet, on all metals. It is also worth notice that the accumulation factor is higher at sites with low contamination. This suggests a continous cleaning of stormwater wetlands, to keep the contamination level low.

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CHEMICAL ELUTION AND PHYTOREMEDIATION OF SOILS CONTAINING HEAVY METALS

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1. Introduction

The efficiency of chemical elution and phytoremediation to reduce heavy metal concentrations in soil is compared and discussed. The results of a two years lasting cultivation period for different crops (*Reynoutria sachalinensis, Helianthus anuus, Brassica napus*) grown on a contaminated field are presented. The concentration of lead is about 1800 mg/kg and of cadmium about 12 mg/kg in the upper 0,25 m of the soil. The field is located in the vicinity of a former lead works near to Freiberg/Germany.

2. Materials and Methods

Chemical elution: Experiments in a laboratory scale using citric acid and different mechanical treatments have been carried out /4/. The influence of the parameters pH, ultrasound, liquid-solid-ratio and reaction time has been investigated. Further experiments in a small technical scale (batch and continuous process) including recycling and regeneration of the eluate using heavy-metal-selective ion-exchangers are planned.

Phytoremediation: Experiments have been realised using sequential extraction of soil to characterize the mobility of the different heavy metals /2; 3/. *Brassica napus* and *Helianthus anuus* have been tested for the extraction of heavy metals from soil in greenhouse experiments. The accumulation of heavy metals by the plants has been investigated for different concentrations in the soil. In additional experiments chemical substances in varying concentrations have been added to influence the mobility of heavy metals in the soil thus increasing the metal uptake of the plants. Field experiments to prove the results of the greenhouse experiments under realistic conditions have been carried out /2; 3; 4/.

3. Results and Discussion

The results of the chemical elution of heavy metals by citric acid are dependent on the composition of the soil. In this context the experiments offer interesting perspectives. The results are significantly influenced by the nature of the metal, pH of the soil and the eluate, the reaction time and the liquid-solid-ratio. Cadmium which occurs in harmful concentrations can successfully be removed. Lead is much stronger bond to the material and can only be removed in a moderate degree.

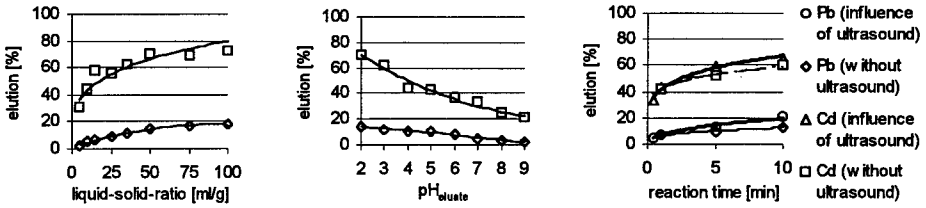

Fig. 1: Results of chemical elution with citric acid in the laboratory scale depending on the liquid-solid-ratio, pH of the eluate and the reaction time

The results of phytoremediation given in Fig. 2 refer to the second cultivation period of the field in Freiberg.

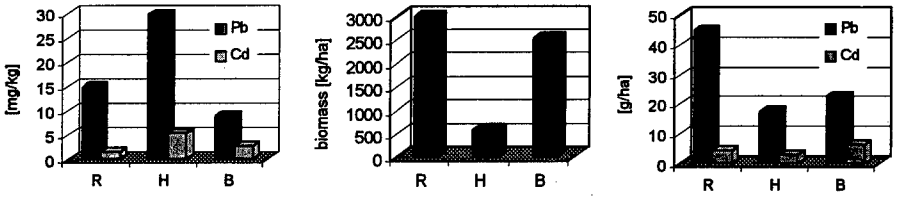

Fig. 2: Results of phytoremediation without fertilising or mobilisation additives in the second year (R: *R. sachalinensis*/ H: *H. anuus*/ B: *B. napus*)

The greenhouse experiments to improve the heavy metal uptake show that the accumulation can be increased in a wide range. Investigations to optimise the heavy metal uptake of plants using biotechnological or genetic methods have not been started yet. Further it would be interesting to increase the biomass.

4. Conclusions

Both methods used for the decontamination of soils polluted with heavy metals are suitable for special applications. The chemical elution is a high cost method which requires a considerable amount of energy. It could be applied to *smaller amounts* of soil containing *high concentrations* of heavy metals. The phytoremediation is a low cost method which could be applied to *larger amounts* of soil containing *lower concentrations* of heavy metals. This method offers the possibility of using the plants as renewable resources.

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ASSESSMENT OF ECOLOGICAL RISK AT INTENSIVE PHYTOEXTRACTION OF SOIL CONTAMINATED BY HEAVY METALS

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1. Introduction

Phytoextraction is an agricultural technology utilizing the continuous growing of a particular plant species on contaminated land in order to remove heavy metals from the soil by the root system of the plants and to concentrate the heavy metals in the overground biomass for subsequent processing. It is known that the availability of metals in the soils for plants is one of the factors limiting the efficiency of phytoextraction. Therefore, the key to the phytoextraction of heavy metals is an increase and sustention of the metal concentration in the soil solution, e.g., by synthetic chelating agents. However, little has been reported regarding the effect of chelating agents, which are applied in the remediation practice at levels from $n \sim 10^2$ to $n \sim 10^3$ mg/kg of soil to enhance phytoextraction, on the biological activity of soil and, particularly, on its enzymatic activity. Therefore, the main goal of this work was to assess the effect of different concentrations of a chelating agent, ethylenediaminetetraacetic acid (EDTA) on the enzymatic activity of the soil contaminated by heavy metals from the Katowice province of Poland.

2. Materials and Methods

The calcareous rendzina (h = 0-15 cm) from the experimental plot in the territory of the Przyszlosc collective farm, Katowice province, Poland, was studied. We researched the dynamics of the catalase, dehydrogenase, and cellulase activities in the soil under the effect of the potassium salt of EDTA (1, 5, 10, and 20 mmol/kg). Soil samples (m = 50 g) were treated with aqueous solutions of EDTA in Petri dishes up to a moisture content in the soil corresponding to 70% of the field capacity. Next, the soil was incubated in a thermostat at 30°C. On separate dates (the 3rd and 63rd days), the contents of Zn, Pb, and Cd in aqueous extracts at the soil: water ratio of 1 : 5 were also determined for different experimental treatments on a Varian "Spectr AA 300" flame atomic-absorption spectrometer.

3. Results and Discussion

The treatment of soil with EDTA at the lowest rates (1 and 5 mmol/kg) resulted in no significant changes in the catalase activity. A statistically reliable stimulation of the activity (by 20%, as compared to the control) was observed for the 10-mmol/kg rate at the end of the experiment (on the 14th day). The steady stimulation of the enzymatic activity by 14.4-58.3% was observed during two weeks upon a the further increase in the EDTA rate (up to 20 mmol/kg). The effect of the lowest EDTA rates on the dehydrogenase activity in the soil was also minor. The steady stimulation of the enzymatic activity by 8.6-48.5% for two weeks was observed at 10 mmol/kg EDTA. The treatment of the soil with the highest EDTA rate (20 mmol/kg) resulted in an ambiguous response of the dehydrogenase: the stimulation (by 5.5-13.6%) was interrupted by a

sharp drop in the enzyme activity by 15.3% on the 7th day; then the stimulation recommenced and run up to 6.3%.The different responses of two the enzymes, especially to the highest EDTA rate (i.e., the more stable trend in the catalase activity and the less stable shifts in the dehydrogenase activity), can be explained by the major role of catalase (extracellular soil enzyme, which occurs in the immobilized form) in the transformation of hydrogen peroxide in the top soil. It is known that the dehydrogenase activity in soil, related to the catalysis of the splitting off hydrogen reaction, is 95 percent controlled by enzymes of living microflora and, hence, is more responsive to the negative effect of high rates of EDTA, which is known to be an oxidation inhibitor. The cellulase activity as a whole was stimulated by 1.5-12.8, 5.9-13.9, and 34.4-59.2% when the soil was treated with EDTA at the rates of 1, 5, and 10 mmol/kg, respectively. It was found that the peak stimulation of cellulase activity took place on the 35th day for all treatments. These results agree with the dynamics of cellulose-decomposing fungi during succession (successive and regular changes in the complex of soil microorganisms and in microbiological processes). The amount of cellulose-decomposing fungi increased from the first day and peaked to about 16-35 days; then, the fungal population decreased [Zvyagintsev, 1987]. In addition, it was found that the cellulase activity in the soil correlated well with the total amount of cellulose-decomposing fungi [Aleksandrova a. Shmurova, 1974]. As the soil under study contained high concentrations of Zn, Pb, and Cd, it was of interest to evaluate the content of water-soluble chelated forms of heavy metals in the soil under treatments with the increased EDTA concentrations. It was also important to realize the hazard of ground-water contamination when phytoextraction effector were applied at high concentrations. As can be seen from Table, the content of heavy metals in an aqueous extract from soil increased with increasing the rate of applied EDTA. The sharpest increase was observed in going from 1 to 5 mmol/kg EDTA: the concentrations of Zn, Pb, and Cd increased by 3.8, 5.6, and 6.0 times, respectively.

Table. The content of heavy metals in aqueous extracts from the soils undergoing different experimental treatments on the third day of soil incubation, mg/l

Treatments	Zn	Pb	Cd
Control	1.6±0.3	0.23±0.06	0.014±0.008
EDTA, 1 mmol/kg	15.8±3.7	0.74±0.07	0.105±0.010
EDTA, 5 mmol/kg	59.4±11.6	4.17±0.15	0.628±0.002
EDTA, 10 mmol/kg	111.2±13.0	15.03±0.37	1.551±0.071
EDTA, 20 mmol/kg	180.9±29.5	54.73±3.45	2.313±0.130

4. Conclusions

The investigations performed revealed that EDTA, applied at rates from 1 to 20 mmol/kg, induced no irreversible and profound negative changes in the activity of some enzymatic factors of the soil at the early diagnostic stages (during two weeks). At the same time, it should be borne in mind that the treatment of soil with EDTA, even at rates of 1 and 5 mmol/kg, results in a significant increase in the content of water-soluble Zn, Pb, and Cd compounds, which remains for two months. This is evidence for a certain hazard of contamination of the ground water, because of the intensive phytoextraction (using chelating agents) of the soils contaminated by heavy metals, which can aggravate the ecological situation in the regions undergoing phytoremediating management.

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USE OF THE HYPERACCUMULATOR *THLASPI CAERULESCENS* FOR BIOAVAILABLE CONTAMINANT STRIPPING

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1. Introduction

Phytoextraction, or the use of plants to extract contaminants from soils, is an emerging remediation technology. A potential contamination issue confronting many countries is the increase in metal contents of agricultural soils due to land disposal of biosolids, which can contain high concentrations of metals including Cd and Zn. In biosolid-amended soils, the bioavailable fraction of metals can be high enough to lead to unacceptable concentrations of Cd in crops for human consumption or, in the case of Zn, to phytotoxic effects. A remediation strategy to improve crop quality may not necessitate a significant reduction in the total concentration of metals, but simply requires a decrease in the fraction available to plants. Such a remediation strategy, which may be appropriate for both inorganic and organic contaminants, we have termed 'Bioavailable Contaminant Stripping' (BCS). In this study we tested the efficacy of removal of the fraction of Cd and Zn that is available to crop plants growing in biosolid-amended soils by the metal hyperaccumulator *Thlaspi caerulescens*.

2. Materials and Methods

An isotopic dilution method (for details of the experimental methods see Hamon *et al.* 1998) was used to examine the pools of Cd and Zn in contaminated soils which were accessed and removed from soil by *Thlaspi caerulescens* and by a non-hyperaccumulating species, wheat (*Triticum aestivum*). Two soils, a Mollic palexeralf (M) and a Calcic rhodoxeralf (C), which had been contaminated with metals through the addition of different biosolids three years previously (Table 1), were spiked with ^{109}Cd and ^{65}Zn.

Table 1. Soil treatments.

Treatment	Soil	Soil metal concn. (mg kg^{-1})		
		Cd	Cu	Zn
1	M	0.1	7	18
2	C	0.1	11	21
3	M	1.2	306	280
4	C	1.2	306	280
5	M	5.8	260	529
6	C	5.8	260	529
7	M	19.5	759	1451
8	C	19.5	759	1451

Thlaspi caerulescens (Tc) and wheat were grown in pots in a growth chamber as outlined by Hamon *et al.* (1997) (Experiment 1). Following harvest of the plants and determination of metal and isotope composition, all of the soils were then replanted with wheat (Experiment 2). Isotopic and metal composition of this crop was used to determine which pools of metal had been depleted by the two species in Experiment 1.

3. Results and Discussion

Plant yields were found to vary with biosolid treatment possibly, in the case of Tc, as a result of Cu toxicity (see McLaughlin and Henderson, this conference). Concentrations of Cd in Tc plants were an order of magnitude higher than Cd in the wheat plants (data not shown). However, a linear relationship was observed between concentration of Cd in the soil and in Tc plants. This is consistent with the Tc population used in this study acting as an indicator species rather than a hyperaccumulator of Cd (Baker, 1981). By contrast, the concentration of Zn in Tc was two orders of magnitude higher than in wheat at the lower Zn concentrations in the soil, and was independent of soil Zn concentrations, which is diagnostic of Zn hyperaccumulation.

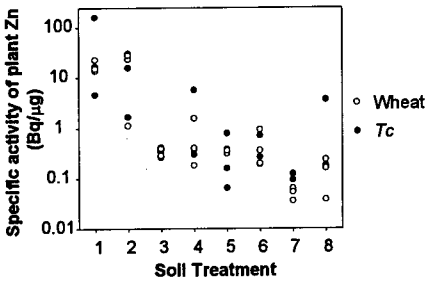

Fig. 1. Specific activity of Zn in shoots of Tc and wheat (Experiment 1).

There was no difference in specific activity of Cd or Zn taken up by Tc or wheat (Zn data shown in Fig. 1) for plants growing in the same soil treatment. This indicates that Tc was accessing the same pools of metals that were available to the wheat plants. However, large decreases in Zn specific activity with increasing Zn concentrations in soil indicate that the Zn added in biosolids was highly labile. Pregrowth of either Tc or wheat did not affect concentrations of Cd or Zn in the wheat grown in Experiment 2, with the exception of the soil treatment which had the highest yields of Tc (data not shown).

For this treatment, subsequent uptake of both Cd and Zn by wheat were lower following growth of Tc than in Experiment 1. This occurred despite the fact that pregrowth with Tc did not significantly lower the total soil content of metals.

4. Conclusions

It is concluded that Tc extracts the same pools of metals that are available to other crop species (Hamon *et al.* 1997) and, when yields are not marginal, can significantly reduce metal availability to subsequent crop species. Hence, providing yields can be optimised, phytoextraction with Tc may be a useful tool for the implementation of BCS.

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APPLICATION OF PHYTOREMEDIATION PROCESS TO CHROMIUM-CONTAMINATED SEDIMENT

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1. Introduction

Today, the phytoremediation of the soil and sediment in areas contaminated with heavy metals constitutes one of the most exciting challenges for environmental researches and problem solving. Among the phytoremediation processes, it is the in-situ inactivation technique that has special significance, since it reduces the dangers caused by the potential toxic impacts of heavy metals (Berti and Cunnigham, 1997). Due to its providing green surfaces, the phytoremediation process is environmentally friendly and inexpensive.

The most novel and modern form of phytoextractive processes is the so-called induced phytoextraction, in the course of which the heavy metal uptake is enhanced by the addition of chelates (Salt et al., 1998). For denoting the traditional procedure, the term continuous phytoextraction is used, which means that the ionic release of the bound metals, as well as the uptake and storage of the metal ions by the roots, and their transport take place in the stem and the leaves.

This paper presents the results of the floristical examinations performed in the units of a former secondary sedimentation pond system and the ecological tolerance state of the plant species adapted to the high-chrome sediment. The outcome of analyses on the chromium content of the plants will also be discussed. Beside the chemical analyses, observations on the plants, the ecophysiological and stressbiological factors, as well as on the peroxidase enzyme group will also be made.

2. Materials and Methods

The sampling sites were selected from dried units of the chromium-contaminated, oxidation-wastewater, sedimentation pond system. In accordance with the vegetation period, sampling was performed on four occasions, including the test sampling at the end of the winter. From the spring sampling on, floristical measurements were made in order to determine the plant species existing in the pond units. The mass stocks of the reed, bulrush and sea club-rush were always subjected to above ground (stem) and underground (roots) sampling from a pre-defined sediment volume. From these sites, sediment samples were also collected for further mass, chemical and toxicological analyses. For further observations, leaf and rhizome fractions were taken from the in-situ collected reed (*Phragmites australis*), bulrush (*Typha angustifolia*) and sea club-rush (*Bolboschoenus maritimus*) samples, they were frozen into liquid nitrogen, and later the examinations were accomplished in laboratory.

3. Results and Discussion

According to the observations, the units of the pond system can be floristically characterized by a relative diversity of species (42 species). The reed, sea club-rush, bulrush, orach (*Atriplex* ssp)

and aster (*Aster punctatus*) can be considered as being existent in large masses, and the weeds also need to be mentioned. The green plant covering varies from unit to unit, ranges between 85 and 100 %, however, regarding the previous years there occurs a favourable tendency of increasing green covering.

Considering the results of the chromium analyses, none of the plants composing the natural flora - in the present case of natural, extensive, continuous phytoextraction - can be described as hyperaccumulator species. Nevertheless, categorizing the analyzed plants on the basis of their "accumulative features" as regarding their ash and chromium contents seemed to be expedient and necessary.

The total chromium results of the sediment fall into the ten-thousand (12,000 – 43,000 ppm) range. This extremely high degree of the chromium concentration multiplies the time demand of the phytoremediation processes. Fortunately, it does not endanger the vegetation with lethal phytotoxicity, and the green plant covering is significant. The green covering is given by the presence of the vegetation, while the absorption and reduction of chromium(VI) are realized in the form of chromium(III), consequently the elimination of the toxic form can be evaluated as a more important process than the possibility of the present phytoextractive procedure.

The results of the ecophysiological examinations on the plants indicate that the plants living in the high-chrome sediment have a special pigment composition that provides them with an effective mode of defense against the potentially appearing damages in the photosynthetic apparatus due to the metallic loading.

In laboratory, the preliminary test-culture experiments have brought promising results as considering the intensive chromium uptake of the komatsuna (*Brassica campestris* L. subsp. *napus* f. et THOMS. var. *komatsuna* MAKINO) and *Raphanus sativus* L. convar. *oleiformis* Pers., cv.

4. Conclusions

The shaping of the phytoremediation techniques is currently in the R+D phase, and in Europe, in the framework of COST Action, it forms the theme of a research task (COST Action, No: 837). In the future, it will certainly have outstanding importance. It can be safely claimed that plants play an essential role in the absorption of the toxic, mobile, easily admitted chromium(VI) ions, and through their enzymatic reduction, the process leads to the less toxic chromium(III) as the end result. The outcome of the phytotechnical researches having been launched all over the world is very hopeful.

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REVEGETATION OF PB/ZN MINE TAILINGS (CHAT) IN THE TRI-STATE MINING REGION, U.S.A.

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1. Introduction

Mine tailings (or *chat*) present a widespread risk of Pb, Zn, and Cd contamination at former mining sites in and around the town of Galena in southeastern Kansas, USA. Revegetation of these sites would reduce the off-site transport of the contamination by wind and flowing water. Phytoxicity, plant nutrient deficiencies, and poor soil physical properties can limit plant growth in the mine wastes. In this study, test plots were established in an area of graded chat in order to determine the effectiveness of various treatments in the growth of tall fescue grass, their effectiveness in the containment of the heavy metals, and to evaluate chemical changes in the chat caused by the vegetation and soil amendments. The objectives were to determine the benefits of mycorrhizae inoculation on the growth, survival and composition of tall fescue and to determine the effects of manure amendments and plant growth on the distribution of metals in a high-Zn mine spoil.

2. Materials and Methods

Six treatments with four replications were imposed on 24 plots in the chat material. The chat material contained total concentrations of Cd, Zn, and Pb of 53 mg/kg, 22700 mg/kg, and 2050 mg/kg, respectively. The treatments were an unamended and unseeded control; a (cattle) manure amended unseeded control; a manure amended and seeded control; manure amended, seeded and periodically treated with benomyl fungicide; and two treatments that were amended, seeded, and inoculated with mycorrhizae. The mycorrhizae inoculum contained *Glomus clarum* and *G. etunicatum*. After satisfactory stands of fescue were obtained following seeding in February 1996, vegetative cover was measured twice each year and samples of chat, fescue roots, and fescue tissue were collected periodically. Root samples were assessed for mycorrhizae infection with Tryptan staining. Plant tissue samples were digested in $HNO_3/HClO_4$ and analyzed for Zn, Cd, and Pb. Chat samples were extracted by the sequential extraction procedure of Tessier et al. (1979).

3. Results and Discussion

Tryptan staining and molecular marker techniques failed to show successful inoculation of the fescue by the mycorrhizae. Accordingly, there were no significant differences between treatments for biomass production, vegetative cover, or Zn, Cd or Pb content of fescue tissue. Average Zn, Cd, and Pb concentrations in fescue tissue taken in September 1996 were 482, 5.2, and 10.6 mg/kg, respectively. Vegetative cover increased after seeding and has been steadily declining since (Figure 1). The high Zn concentrations in the fescue tissue suggest the cause of this decline may be Zn phytotoxicity. The addition of manure significantly reduced

exchangeable Cd, Pb, and Zn (Figure 2) and significantly increased organically bound Cd, Pb, and Zn (data not shown). The presence of plants significantly reduced exchangeable Cd with no such effects for Pb or Zn (data not shown). Complete fractionation results for Pb are shown in Table 1. The manure amendment moved Pb from the exchangeable and carbonate fractions to the organic fraction. There was significantly higher Pb in the exchangeable and carbonate fractions of the (unmanured) unseeded control (167 and 776 mg/kg) than for the same fractions of the other treatments (an average 57.7 and 502 mg/kg, respectively), and significantly lower Pb in the organic fraction of the (unmanured) unseeded control(<10 mg/kg) than in the other fractions (which averaged 17.6 mg/kg).

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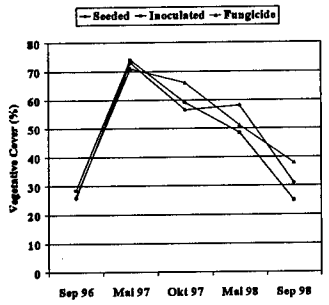

Figure 1. Vegetative cover over time

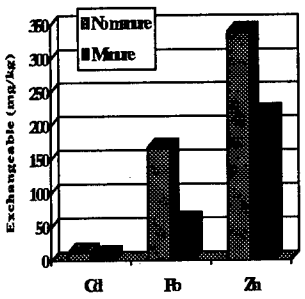

Figure 2. Manure effects on exchangeable metals.

Table 1. Fractionation of chat Pb into exchangeable, carbonate-bound, Fe/Mn oxide-bound, organic and residual fractions (mg/kg). Numbers within a column followed by the same letters are not significantly different at P=0.05.

Treatment	exch.	carb.	Fe/Mn	organic	residual
seeded control	70.7b	593b	344a	16.9a	1174a
inoculated-1	67.4b	432c	257b	12.8a	778b
inoculated-2	52.8b	438c	252b	17.9a	875b
fungicide	49.4b	531bc	296ab	20.9a	932b
unseeded control	167a	776a	329ab	<10b	1172a
unseeded manured	48.1b	516bc	313ab	19.4a	980ab

REMEDIATION OF THE JALES MINE SPOIL BY INACTIVATION AND PHYTOSTABILISATION: STUDY IN LYSIMETERS

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1. Introduction

Many large bare industrial sites display a high trace element availability in the soil. This debased state can lead to the contamination of nearby ecosystems *via* water and wind erosion. As soil remediation by cost-effective technologies becomes an evident need, this may be more or less solved by several ones. Strategies designed to optimize inactivation and phytostabilisation have raised. Long-term and semi-field trials began to emerge. The remediation of an As/Zn-contaminated spoil (Jales gold mine, Portugal) is investigated under the Phytorehab project. A field trial has been established on site whereas a semi-field trial is carried out at the INRA Bordeaux Centre. This study reports As, Cd, Pb, and Zn concentrations in percolates, plant growth and trace element taken up in lysimeters filled with Jales spoil, following its treatment by compost application combined with or without steel shots and beringite. The additive choice was based on previous studies (MENCH et al, 1998).

2. Materials and Methods

Spoil was collected in January 98. This very sandy material derived from the toplayer of the gold mine wastes. It is mainly contaminated by As and Zn, and by Pb and Cd in a lesser extent (table 1). The material was homogenised before treatment. A French sandy soil (0-0.3 m soil layer) was used as non contaminated control.

Table 1: Characteristics of the Jales spoil and of amendments

	Control soil	Jales spoil	Compost	Steel shots	Beringite
Organic C (g/kg)	25.7	0.45			
Organic N (g/kg)	0.95	0.05			
CEC (cmol/kg)	7.1	0.9			
pH (water)	4.5	4.1		8.5	
As(mg/kg DW)	1.1	1325	12.7		
Cd (mg/kg DW)	0.08	3.8	0.9	0.03	9
Cu (mg/kg DW)	2.8	15.2	40.2	1010	120
Ni (mg/kg DW)	<2	<2	14.1	739	123
Pb (mg/kg DW)	9.9	170	70.4	20	203
Zn (mg/kg DW)	12.2	165	215.7	104	630
Fe (%)				97	

Former plant tests had indicated the potential effectiveness of the amendments (see table 1 for composition). Besides the untreated samples (UNT), subsamples (340 kg DW) were mixed based on DW with 5% compost (C) and with one of the following minerals: 1% steel shots (an iron-bearing material, SS); 5% beringite (a modified aluminosilicate, B), 1% SS and 5% B (SSB). Samples were put into 0.125m^3 vats, equipped with a 20 cm compensation zone, and placed outside. Percolates were collected in teflon flasks rinsed with HNO_3 and distilled water. After a 18-day reaction period, raygrass (*Lolium multiflorum Ital.* Balmutra) was sown in the compensation zone and common velvet grass (CVG) (*Holcus lanatus* L.) in the vat middle. Growth of dwarf bean (*Phaseolus vulgaris* L. vroege Limburgs) was also tested for 3 weeks. Plant material was wet digested in HNO_3/H_2O_2 and mineral composition was investigated by ICP-AES and GFAAS, reference material being included in each series.

3. Results and Discussion

The total volume of percolates collected is similar for all Jales treatments (table 2). Better plant growth in the control soil led to greater evapotranspiration. Beringite led to the highest pH increase in percolates. Ratio between total amount of element collected in UNT vs. Control percolates was 12.7 (As), 4 (Pb), 156 (Zn), and 530 (Cd). Zinc and Cd leaching was reduced in all treated spoils. The highest decrease in Zn and

Cd percolation was for CSSB, followed by CSS and CB. All treatments increased total As amount collected likely due to compost dressing (*i.e.* exchange with phosphates and organic complexes, raise in pH), however this was less effective in CSSB and greatest for C. Lead was also increased by C amendment, possibly by complexation. This was limited by combining C with B and SS. Figure 1 illustrates the pattern of As and Zn concentrations in UNT and CSSB treatments. Dwarf bean showed visible symptoms of Zn toxicity on primary leaves. Shoot yield in UNT was 4 times reduced compared with control (table 3). CSS and CSSB restored bean growth up to 50% whereas C and CB were less effective. Ryegrass germinated but did not grow in UNT. Amendments increased its growth in the following order: CSSB>CB>C, CSS. This sequence was different compared with that of bean except CSSB was again the most effective to increase plant growth. Common velvet grass germinated in all treated spoils but again did not grow in UNT. Shoot yield of CVG was similar in all treated spoils. Plant materials are currently under analysis.

Table 2: Volume and total amount of element collected, and pH in percolates (after a 124-day period, mean of 2 replicates).

	Control	**UNT**	**C**	**CSS**	**CB**	**CSSB**
Volume (L)	28.9	37.4	40.9	39.3	42.6	39.8
pH	6.4	4.6	7.4	7.5	8	8.0
As (mg)	0.13	1.6	210	24.7	24	7.6
Pb (mg)	0.05	0.2	17.7	1.1	0.07	0.03
Zn (mg)	5.6	872	36.5	5.4	5	2.2
Cd (mg)	0.04	12.2	0.4	0.15	0.05	0.03

Table 3: Shoot yield of plant species (in % compared with yield on control soil)

	Replicates	**Control**	**UNT**	**C**	**CSS**	**CB**	**CSSB**
Ryegrass	2	100 a	1.1 d	21.5 c	20.5 c	24.3 c	39.7 b
Dwarf bean	10	100 a	24.8 d	29.9 cd	48.2 b	31.5 bc	42.1 b
Common velvet grass	6	100 a	0 c	14.3 b	14.4 b	13.2 b	9.2 b

Within a row, mean values followed with the same letter are not different according to standard deviation.

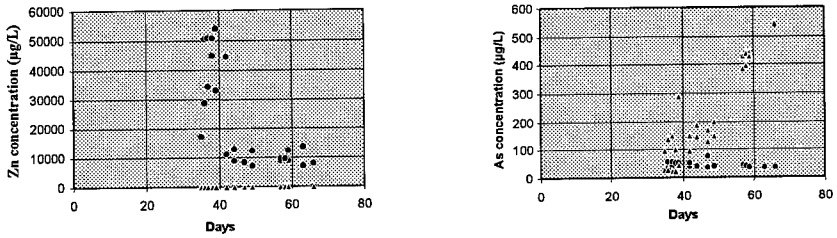

Figure 1 : Zinc and arsenic concentrations in percolates of untreated (UNT, ●) Jales spoil and of compost and steel shots plus beringite (CSSB, ▲) amended Jales spoil.

4. Conclusions

CSSB treatment showed reduced Zn, Pb, and Cd leaching and resulted in the lowest increase of As in the percolates. Compost dressing increased plant growth but led to the highest As and Pb leaching. Additives restored plant growth for both monocots (ryegrass, common velvet grass) and dicots (bean). This is likely due to decreased Zn availability and nutrient addition. Restoration of monocots growth was maintained on a 124-day period and is continuing. Biota in the lysimeters will be survey. Several spontaneous plant species (*e.g. Salix*) appear now in CSSB whereas fungi (*i.e. Coprinus*) were observed in CSSB and CB.

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RHIZOFILTRATION METHOD FOR THE CLEANING OF RADIOACTIVE, CONTAMINATED AQUATIC SYSTEMS

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1.Introduction

The problem of clearing the environment polluted with radionuclides is important for many countries. For example, only in the Ukraine more than 4.6 billions hectares of agricultural land have undergone to radioactive pollution as a result of failure at the Chernobyl Nuclear Power Station (ChNPS) at 1986. There are unsolved problems of environmental cleaning from the long-living radionuclides like Cs-137 and Sr-90. These isotopes are the main source of irradiation for the people and animals in the polluted territories. A plant-based technologies rhizofiltration seem to be the way to solve of the some environmental clean-up problems (Dushenkov et.al., 1995).

The largest part of Cs-137 (more than 90 %) and significant part of Sr-90 (not less than 60-70 %) exist in soil-fixed forms and they are practically inaccessible for plants. However, phytotechnologies can be rather effective in the case of aquatic systems, clearing where the radionuclides exist primarily in the dissolved state. The transfer factors (TF) of radionuclides from water to plants can reach several thousands units. It is essential that such high values of TF are necessery for water-grown plants, but also for terrestrial plants when they grow at the conditions of water culture (Mikheev et al., 1994)

2.Materials and Methods

The experiments have been carried out both in the laboratory and field during the seasons of 1995 and 1996. The laboratory investigations took place with sunflower plants (*Helianthus annus*), indian mustard (*Brassica juncea*) and pea (*Pisum sativum*). The plants were previously cultivated as the hydroponic culture at the greenhouse followed by transfer on plastic containers with contaminated water. The field trials were carried out at small natural pound located in the 10-km exclusion zone of ChNPS.

In the laboratory experiments on rhizofiltration the plants were cultivated in the 50 l plastic containers which were filled with water samples collected from the same natural ponds where the field trial took place. Plants were incubated during 8 days in these containers. Another approach means a periodically replacements of plants growing at capacities with radioactive water were carried out. The stems and roots of investigated plants were cut off at the end of experiments. A number of terrestrial plant species were tested during field experiments on rhizofiltration. Plants were cultivated directly at the water surface by using the special floated constructions.

Definition of the specific activity of vegetative samples was made after their air-drying. The Cs-137 activity was measurement at gamma-spectrometer equipped with germanium-lithium

detector. Sr-90 definition was carried out by classical oxalate method when affiliated Y is determined.

3. Results and Discussion

The maximal Cs-137 TF was determined in the roots of mustard and was equal to 2350. The maximal TF for Sr-90 was equal to 374. The large plants' sorption capacity allowed considerably reduction of the specific and total activity of water despite the short time of rhizophiltration. The activity of Cs-137 was reduced from 90 Bq/l up to 9 Bq/l. The efficiency of water clearing from Sr-90 was not so effective. The another approach was also tested besides the experiments with continuous plants incubation. New adult plants were replaced each 48 hours in the same containers with contaminated water. This was effective for Sr-90 removal from water.

In field experiments non-uniformity of Cs-137 and Sr-90 distributions over plants parts was also recorded. Sr-90 was located mainly in plants stem whereas Cs-137 was concentrated in their root system. Using of plant species capable of producing large biomass seems to be a determining part in rhizofiltration technology. Sunflower plants and poa plants are most preferable from this point of view. The plants have taken out from a reservoir more than 380 kBq Cs-137 and near 1100 kBq Sr-90. It means accordingly 74% and 14% from initial amount of these isotopes.

4. Conclusions

Our results allow us to make a conclusion about the high degree of purifing of environmental water polluted with the soluble forms of radionuclides. Collected vegetative biomass with high activity must be ashed and burned as high activity waste. This solves the problem of concentration of a radioactivity and reduction of quantity of such waste. In addition, the low radioactive phytomass may be used for the production of biogas, cellulose etc.

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PHYTOAVAILABILITY OF CADMIUM IN LEAF OF THE HYPERACCUMULATOR *THLASPI CAERULESCENS* INCORPORATED INTO SOIL

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1. Introduction

Hyperaccumulator plants have the ability to take up large amounts of trace elements from soil and to transfer them to their aerial parts. *Thlaspi caerulescens*, which can contain more than 1% Zn and 0.01% Cd in shoots, is one of the best candidates for phytoremediation of metal-contaminated soils (Baker *et al.*, 1994; McGrath, 1998; Schwartz, 1997). In the phytoextraction scenario shoots are harvested, incinerated, and the ashes disposed in landfill or reused in smelters. However, contaminated above ground parts may inadvertently return to the soil, becoming a secondary source of pollution for agricultural crops. Therefore, a thorough knowledge of the fate of metals associated with hyperaccumulator plant material is required to assess the risk connected with this emerging technology.

This work was undertaken to determine whether the Zn-Cd-hyperaccumulator, *T. caerulescens*, accelerates or reduces the mobility of the toxic elements in the soil-plant system. *Thlaspi* leaves collected from plants grown on a ^{109}Cd-labelled soil were incorporated into a non contaminated soil cultivated with ryegrass and uptake of Cd was measured.

2. Materials and Methods

Soil material was collected from the Ap horizon of an agricultural brown leached soil (Luvisol-FAO). It was amended with Cd at a rate of 0.4 mg kg^{-1}. The metal was supplied as $CdCl_2$ solution, Cd-contaminated soil (176 mg Cd kg^{-1}, and 1371 mg Zn kg^{-1}), or Cd-bearing leaves of *Thlaspi caerulescens* (100 mg Cd kg^{-1}, and 2441 mg Zn kg^{-1} DW). Each form of Cd was labelled with ^{109}Cd. Amended soils were thoroughly mixed, and rye grass (*Lolium perenne*) was immediately sown (0.4 g per pot of 500 g soil). A control without any addition of Cd was prepared under the same conditions. Pots were placed in a growth chamber, and soil humidity was maintained daily at 80% of the water holding capacity. Each treatment had four replicates. Shoots were harvested 25 d and 61 d after sowing. Dry biomass was determined after drying for 48h at 70°C. Total content of Cd in shoots was measured by ICP after acid digestion, and radioactivity in ryegrass leaves was measured directly on the ground plant material by γ counting.

3. Results

Average biomass produced by ryegrass was 1.70 g DW per pot for the first cut and 1.90 for the second. No phytotoxicity symptoms were recorded.

Plants harvested on the control soil (0.1 mg Cd kg^{-1}) contained less than 0.5 mg Cd kg^{-1}. However, Cd content in ryegrass increased when soil was amended. The highest concentration was observed when Cd was added to soil as *T. caerulescens* leaves (2.65 for the first cut and 4.53 mg Cd kg^{-1} for the second cut), whereas the increase was much lower with $CdCl_2$ (1.70 and 1.94 mg Cd kg^{-1}), and with Cd added as contaminated soil (1.17 and 1.34 mg Cd kg^{-1}). Hence, Cd in

the soil/*T. caerulescens* leaves system appeared more mobile than Cd in soil/$CdCl_2$ or soil/Cd contaminated soil systems.

Measurement of the radioactivity in plants allowed the calculation of percentage of ^{109}Cd taken up by ryegrass. On average, values were lower at the first cut (1.41%) than at the second (3.03%). Cd added as soluble salt gave the highest values (2.2% and 4.1%). *Thlaspi* leaves, on the contrary, produced the lowest percentage of transfer (0.8 and 2%). Values were intermediate (1.4 and 2.7%) when Cd was incorporated as contaminated soil.

Specific activity of Cd in ryegrass and in the different forms of Cd allowed to determine the contribution of the inputs to the Cd total content in ryegrass. Only 31% of Cd measured in ryegrass originated from *T. caerulescens* leaves, the remaining being the soil contribution. Values reached 75% with Cd solution, and 67% with Cd-contaminated soil.

4. Discussion and Conclusions

This work has shown that the incorporation of *T. caerulescens* leaves induced a larger uptake of total Cd by ryegrass than when Cd was added as a soluble salt or contaminated soil. If only the total Cd content in ryegrass was considered, it could be concluded that phytoavailability of Cd was much higher as *T. caerulescens* leaves than as soluble salt or Cd associated with soil material. However, the use of ^{109}Cd allowed to separate soil Cd and incorporated Cd. Hence it was possible to determine the exact fraction of the input which was actually taken up by ryegrass. Phytoavailability of Cd associated with *T. caerulescens* appeared rather low when compared to that of other forms of Cd. Calculations of the contribution of the soil and of each Cd amendments showed that Cd was mainly derived from the soil in presence of *T. caerulescens* leaves. This result could be explained by a higher mobilisation of Cd in the soil-amendment system or possibly by a non uniform labelling of the leaves used in this experiment. Work is underway to determine whether *T. caerulescens* leaves have increased the availability of initial soil Cd, or if Cd came in fact from the leaves themselves.

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NICKEL UPTAKE BY HYBRID POPLAR: ASSESSING PHYTOREMEDIATION POTENTIAL

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1. Introduction

Successful field-scale phytoremediation is dependent on several factors; site-specific research which targets the contaminant mixture on-site, and a consideration for the potential end-products of the remediation effort. The research project of which this screening study forms an initial part aims to develop a core technology for the phytoremediation of mixed-waste contaminated plumes and soils, with a planned demonstration on a former waste-disposal area at the Savannah River Site. The site chosen for this work has a contaminant profile consisting of elevated concentrations of nickel and uranium in the soil sediments and trichloroethylene (TCE) in the groundwater. Recent research efforts in phytoremediation have produced a database of plant species, which have the ability to either extract or degrade single inorganic or organic contaminants, or to stabilize sediments contaminated with radionuclides. This database was the starting point for the present study. Work on hybrid poplar suggests that they can degrade organic contaminants such as trinitrotoluene (Thompson *et al* 1988), atrazine (Burken and Schnoor, 1997), 1,4 dioxane (Schnoor et al 1997) and TCE (Newman et al 1997; Gordon et al 1997). Due to the high concentration and phytotoxicity of nickel at Steed pond, hybrid poplars were screened in Ni-amended nutrient solutions for their ability to survive and accumulate Ni. In this way, the suitability of specific cultivars of *Populus* for phytoremediating this specific site are assessed.

2. Materials and Methods

Hybrid poplar cuttings DN5 (*deltoides* x *nigra*) and NM6 (*nigra* x *maximowiczii*) were obtained in May 1997 and maintained in distilled water until adventitious root primordia and shoot initials were observed. Cuttings were then planted in a 45 liter-capacity hydroponic bioreactor, containing half strength Hoagland's solution. Treatments consisted of a control, 0.5, 1.0, 2.5 and 5.0 mg Ni L^{-1}, with Ni supplied as the sulfate salt. Metal levels were based on earlier dose-response experiments carried out on *Salix* cuttings (Punshon, 1996). Nutrient solutions were prepared with reagent-grade chemicals, constantly aerated throughout the experiment and were replaced every 10 days, maintaining the nutrient and contaminant concentration within the solutions at or near target levels. Each hydroponic unit contained 16 replicate cuttings of each poplar hybrid and the experimental design allowed for three duplicate blocks arranged randomly in an air-conditioned greenhouse. On a daily basis 20 ml solution samples were collected in triplicate from each unit, acidified with HNO_3 and analyzed for Ni content on a Perkin Elmer 5100 PC Atomic Absorption Spectrophotometer (AAS). On a weekly basis a destructive harvest removed 4 plants from each treatment unit, and gravimetric and AAS analysis was carried out to determine biomass production and Ni uptake. Data was analyzed using the SAS statistical package, and all significant data is at the $p>0.05$ level unless stated otherwise.

3. Results

Data collected through sequential destructive harvests revealed that Ni uptake characteristics differed markedly both between the cultivars tested, and between leaf and root material. Cultivar NM6 appeared to accumulate more Ni in the leaf tissue, with a greater uptake rate at 1.0 and 2.5

mg Ni L^{-1} overall than that observed at 5 mg Ni L^{-1}. Ni accumulated preferentially in the roots as expected. Growth and survivorship of the cultivars tested showed severe phytotoxicity above 1.0 mg Ni L^{-1}.

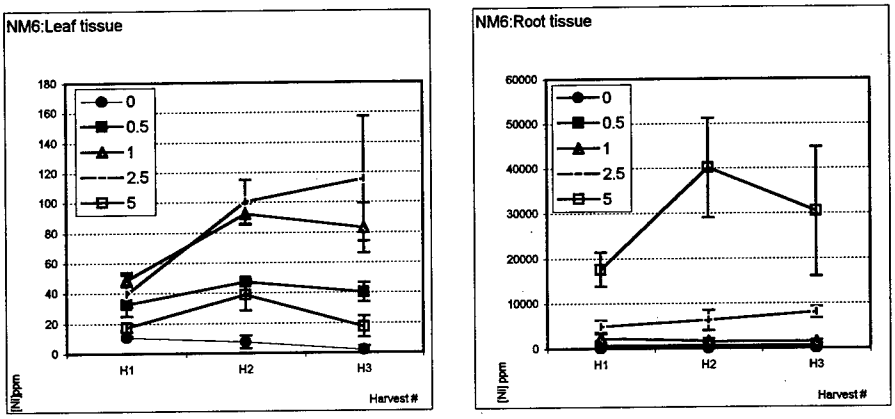

Figure 1. Uptake of Ni by poplar cultivar NM6 leaf and root tissues over three sequential destructive harvests (harvests carried out at 14 day intervals). Means ± standard deviation, where n=9.

4. Discussion

The hybrid poplars used in this study showed on a slight ability to accumulate significant concentrations of Ni from the growth medium, while maintaining healthy growth. It is thought that less-adapted species such as these could only be included in a phytoremediation program if accompanied by the use of metal-binding amendments and fertilizers, adding to the total cost of the programme. The data suggests that greater overall removal of Ni from a growth medium is achieved in these cultivars when the plant available concentration is in the range of 1-2.5 mg Ni L^{-1}. This study is a forerunner to further site-specific poplar screening studies using metals, and mixtures of metals and organic contaminants.

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ROOT ARCHITECTURE OF A ZN-HYPERACCUMULATOR PLANT AS AFFECTED BY METAL CONTENT AND LOCALISATION IN SOIL

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1. Introduction

Hyperaccumulator plants have proved potentially useful in soil clean-up as they can remove significant amounts of toxic metals from contaminated soils (Baker *et al.*, 1991, Schwartz, 1997). However, the efficiency of this new developing technology is dependent upon many factors including the capacity of the root system to take up and transfer metals to aerial plant parts. In general, anthropogenic contamination of soils is characterised by a large vertical and horizontal heterogeneity in the soil profile which attenuates the accessibility of pollutants to plants. Therefore, the efficacy of phytoextraction will be related to the development of the root system in contaminated soil and to its ability to explore the contaminated zones. The root system of metallophytes have been little studied outside hydroponic cultures. The work reported in this paper was undertaken to study the behaviour of the root system of the hyperaccumulator *Thlaspi caerulescens* compared to the non-hyperaccumulator *Lupinus albus* under various conditions of soil contamination including type, content, chemical form, and localisation of metal in soil.

2. Materials and Methods

Soils samples were collected from an agricultural site and from a former Zn smelter site. Rhizoboxes were constructed, and filled with several contaminated materials to produce homogeneous soil profile, superposition of three different soil layers separated with sand, inclusion of contaminated soil into uncontaminated soil or inclusion of uncontaminated soil into uniformly contaminated soil. Four seedlings of *T. caerulescens* and *L. albus* were transplanted on each rhizobox and the length of each root was recorded periodically. The pH in the rhizosphere was also recorded at the end of the experiment, and metal content in shoots was determined. Treatments were prepared in triplicate.

3. Results and Discussion

Unlike the roots of non-hyperaccumulating cultivated plants which concentrate in uncontaminated soil zones and penetrate only to a limited extent contaminated soil, roots of *T. caerulescens* explored predominantly Zn and Cd polluted soil areas. Moreover, the quantity of available Zn in soil was a primary factor controlling the development of roots. Root formation and organisation of *T. caerulescens* seemed to be a specific response to metal concentration, form and localisation in soil. Changes in the rhizosphere pH occurred according to the presence of metal in soil. Plants took up large amounts of Zn and Cd from homogeneous and spots of metal-bearing materials.

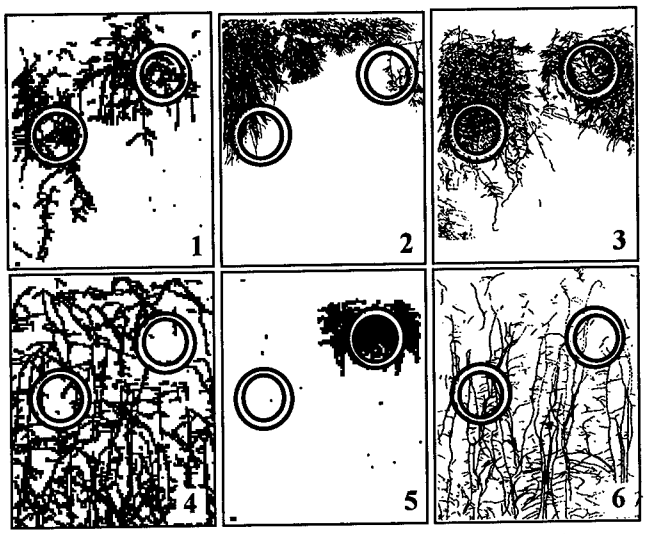

Figure 1: Root system of *T. caerulescens* (1,2,3) and *L. albus* (4,5,6) in the presence of inclusions of Cd shots in agricultural soil (1,4), inclusions of agricultural soil in Zn smelter soil (2,5) and inclusions of Zn smelter soil in agricultural soil

4. Conclusions

The development of roots of *T. caerulescens* can be interpreted in terms of soil exploration and exploitation in respect of phytoremediation. The architecture of the root system has a significance in determining exploitation of metals, *i.e.* tolerance and uptake under the variety of heavy metal soil situations. In heterogeneous industrial and urban polluted soils, the main part of the root system would explore the metal "hot-spots", and the rest would develop in lower contamination areas ensuring a higher rate of phytoextraction of metals.

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RESPONSE OF ANTI-OXIDATIVE ENZYMES TO METAL STRESS IN NON- AND HYPER ACCUMULATOR PLANTS

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Introduction

The high amount of metals in the hyperaccumulator plant's tissues suggests the existence of defense mechanisms to avoid the harmful effects caused by the metal. One possible mechanism via which elevated concentrations of heavy metals may damage plant tissues is the stimulation of free radical production, by imposing oxidative stress (Foyer et al., 1997). We examined whether a sequence of enzymes activities controlling oxidative stress [superoxide dismutase (SOD), ascorbate peroxidae (APX) and glutathione reductase (GR)] plays a role in the tolerance mechanisms towards Ni^{2+} and Cd^{2+}. We compared *Alyssum argenteum*, a known Ni^{2+}-hyperaccumulator plant to *Lubularia maritima*, whose response to theses metals is unknown. We also examined the enzymes' activities in both plants in the presence of Cd^{2+}.

Materials and Methods

Seeds (purchased commercially) were germinated on cheesecloth soaked in 1 mM $Ca(NO_3)_2$ in closed plastic boxes for 7 days. After germination, plants were transferred to a sterile polycarbonate vessel covered with a membrane-vented polycarbonate lid containing 300 ml of 1/10 Hoagland's No. 2. The boxes were placed in a growth chamber at day/night temperatures of 25-33°C/14-18°C, 65-90% relative humidity. After a week, the growth-culture solution was replaced with 1/10 Hoagland's supplemented with 0.01 and 0.1mM Ni^{2+} (as $NiSO_4$) or 0.02 and 0.05 mM Cd^{2+} (as $CdSO_4$); 1/10 Hoagland's solution with no metal served as the control. Plants were grown in the presence of the metal for 24 h or 14 days (with one change of growth culture solutions after 7 days). Plants were harvested without roots, washed in distilled water, grounded in a reaction mixture composed of 50 mM phosphate buffer pH 7.0, 1.0 mM EDTA, 0.05% (v/v) Triton X-100, 2% (w/v) polyvinylpolypyrrolidone and 1 mM ascorbic acid, and then homogenized. The homogenate was centrifuged at 16,000 g for 15 min, after which the supernatant was transferred to a new tube. This crude protein preparation was used for all enzyme assays. SOD activity assay was as described by Donahue et al. (1997). Measurement of APX activity was based on its ability to prevent the accumulation of formazan in the presence of H_2O_2 (Maccarrone et al. 1990). GR activity was measured by following the decrease in absorbance at 340 nm due to NADPH oxidation.

Results and Discussion

At these concentration range used , no response of the antioxidative enzyme system was detected after 24h. After two weeks, SOD activity in plants of *A. argenteum* and *l. maritima* grown in the presence of 0.05 mM Cd^{2+} increased significantly whereas that of APX was not induced by elevated concentrations of Cd^{2+} in the growth culture. Low levels of Cd^{2+} induced GR activity; however, this activity was reduced at higher Cd^{2+} concentrations. At all Cd^{2+} concentrations, higher GR activity was measured for *A. argenteum* than *L. maritimum.* These results suggest a complex mode of action for Cd^{2+}-imposed oxidative stress in both species. Significant induction

of SOD activity was measured in *L. maritima* at a Ni^{2+} concentration of 0.1 mM. This increase was concomitant with an increase in APX and GR activities (Figs 1A, 2A, 3A, respectively), displaying a typical antioxidative defense mechanism (Foyer et al. 1997). *A. argenteum* exhibited a different enzyme response pattern, with a significant reduction in SOD activity (Fig. 1A), and increased APX and GR activities only at a Ni^{2+} concentration of 0.1 mM, which nevertheless remained lower than in *L. maritima* (Figs 2A, 3A, respectively). This activity pattern of the antioxidative enzymes may imply that the tolerance mechanism of the Ni^{2+}-hyperaccumulator *A. argenteum* involves a system that either reduces the formation of or removes free radicals, preventing the production of $O_2^{\bullet -}$, and therefore reducing the requirement to activate the antioxidative enzymes.

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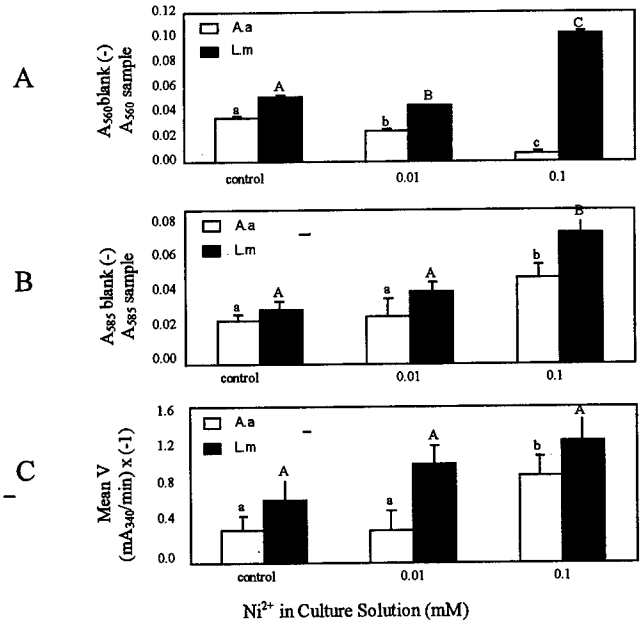

Figure 1: SOD (A), APX (B) and GR (C) activity in the presence of Ni^{2+}

RELATIVE EFFICIENCY OF *BRASSICA* GENOTYPES FOR PHYTOREMEDIATION OF LEAD CONTAMINATED SOILS

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1.Introduction

Recent studies reported that heavy metals were accumulated in soil at hazardous levels which may be toxic to plants and animals due to the continuous application of metal contaminated sewage sludges and industrial effluents (Zhao *et al.* 1997, Adhikari *et al.* 1998). The ability to hyperaccumulate heavy metals from soils is one of the most important criteria in the selection of plant species for phytoremediation purposes (Mc Grath *et al.*, 1997). The decontamination of soils polluted with heavy metals presents one of the most intractable problems of soil clean up (Baker *et al.* 1994). Plants indentified that could be of potential value in phytoremediation include *Brassica juncea* which takes up lead and other heavy metals (Kumar *et al.* 1994). In the present study four genotypes of *B. juncea* and two of *B. campestris* were evaluated for their ability to decontaminate soil polluted with lead.

2. Materials and Methods

The experiment was carried out in a screenhouse using earthenware pots. A soil was obtained from a field near one of the biggest cycle factory in India having history of environmental pollution. The soil had 7.31 pH, 1.35 % organic carbon, 0.42 dSm^{-1} EC, traces of $CaCO_3$ av. P 62.6 mg kg^{-1} and 18.1 mg Pb kg^{-1}. The air dry soil was sieved (<2mm) and 4 kg soil was placed in each pot. Basal fertilizers were applied in solution form alongwith Pb in the form of Pb $(No_3)_2$ at the rate of 0, 100, 200 and 400 mg Pb kg^{-1}. The treatments were replicated three times, mixed throughly with the soil and were imposed 15 days before sowing ten seeds each of four genotypes of Indian mustard *B.juncea* and two of *B. campestris* were sown. Four seedlings per pot were grown to maturity. Matured plants after harvest were partitioned into grain, stem, leaves and root. The metal concentrations in the plant parts were determined on AAS after digestion in nitric-perchloric acid and expressed on a dry matter basis.

3. Results and Discussion

All tested genotypes grew well under all the Pb levels tried, however *B. campestris* produced less biomass and almost half leaf yield than *B. juncea* genotypes. The differences between four genotypes of *B. juncea* in the dry matter yield of various plant parts were generally very small. Beneficial effect of Pb was observed at lower levels and harmful at highest level of Pb addition. Lead @ 100 and 200 mg kg^{-1} significantly increased the grain, leaf and stem yields than the control Table 1. But no significant difference in yield of various plant parts was noted between these two treatments. There were generally small significant reduction of about three per cent in yield of different plant parts at 400 mg Pb kg-1 soil. The differences in yield due to genotypes were also significant. Similarly interaction between genotype and Pb levels was significant. Highest dry matter yields of different plant parts among all the *Brassica* tested were for Vardan genotype which is presently being grown in large areas as an important oilseed crop in India and may be used for soil remediation of Pb contaminated soil.

The Pb concentration at 0 Pb level in grain, leaf, stem and root ranged from 35.7 to 38.5, 44.5 to 55.8, 44.2 to 50.2 and 54.0 to 68.4 mg kg^{-1} respectively, in different genotypes. The variability in Pb concentration of the most of the genotypes decreased after Pb addition. The Pb concentration increased significantly in different plant parts in all the genotypes due to increased level of Pb application. (Table 1) but there existed no relationship with their yield. This indicated that the accumulation of Pb in large quantities takes place without affecting crop yields. It is interesting to note that root contained higher Pb concentration than other plant parts and genotypes differed significantly in their capacity to accumulate Pb in root. Much of the Pb accumulated in plants is held in roots (Huang and Cunnigham, 1996). The *B. juncea* genotypes had the higher capacity to concentrate Pb in different plant parts than *B. campestris* and the values obtained were still far below the toxic concentration found in solution culture experiments (Kumar *et al.* 1994) suggesting that

B. juncea could respond even further to increasing soil Pb levels. Amongst *B. juncea*, Vardan genotype had higher Pb concentration in different plant parts indicating that it has better Pb absorbing ability than others. Shoots of most plants do not accumulate more than 100 mg Pb kg^{-1} weight (Flathman and Lana 1998).

Table 1: Effect of levels of Pb on dry matter yield and Pb concentration in various parts of *Brassica* results are the mean values of six genotypes

Parameter	Pb levels (mg kg^{-1}) 0	100	200	400	C.D. at 5%
	Av. Dry matter yield (g pot^{-1})				
Grain	11.26	11.71	11.66	10.93	0.28
Leaf	10.63	10.94	10.96	10.30	0.21
Stem	30.34	31.96	31.84	29..29	0.48
Root	5.99	6.10	6.07	5.83	0.10
	Av. Pb Conc. (mg kg^{-1} DW)				
Grain	37.05	47.37	58.34	64.61	1.80
Leaf	50.15	62.44	69.97	81.03	2.19
Stem	47.13	63.02	68.96	74.94	1.65
Root	61.63	92.19	104.31	115.28	2.77

Results suggest that *B. juncea* genotype Vardan has higher potential for removing Pb from moderately to higher contaminated soils.

4. Conclusion

This experiment will be further studied for the residual fraction of bioavailable and fixed forms of Pb in soils following phytoextraction and the kinetics of their re-equilibrium. *B. juncea* genotype Vardan has higher biomass and potential for removing Pb from moderately to highly contaminated soils.

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QUANTITATIVE EVIDENCE FOR ACTIVE FORAGING FOR ZINC BY THE ROOTS OF *THLASPI CAERULESCENS*

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1. Introduction

The Zn-hyperaccumulator *Thlaspi caerulescens* typically contains in excess of 10,000 mg kg^{-1} Zn in its shoot dry matter even when grown on soil of low Zn status. There is high spatial heterogeneity in the distribution of Zn in the soil at the native sites of *T. caerulescens*. The ability of *T. caerulescens* to obtain Zn from these Zn patches may be one of the major factors controlling the expression of Zn hyperaccumulation. Little is known about the root development of hyperaccumulator plants in response to heterogeneous Zn concentrations in soil (e.g. Schwartz *et al.*, 1998; Whiting, 1998). This study examined the root allocation patterns of *T. caerulescens* and the related non-accumulator *Thlaspi arvense* when grown in juxtaposed control and Zn-enriched soil.

2. Materials and Methods

A sandy-loam soil (14 mg Zn kg^{-1}) from Woburn (UK) was used as the control soil (**C**). The Zn-soil (**Z**) was prepared by enrichment of control soil with 500 mg Zn kg^{-1} supplied as ZnO. Root chambers (10 x 10 x 2 cm) were filled with the soils to give three treatments (Figure 1). The soils were not separated by a barrier therefore permitting roots to grow freely within the chamber. One seedling of either *T. caerulescens* or *T. arvense* was transplanted into each root chamber. Five replicate chambers were prepared for each soil/species treatment. After 42 days the roots were removed separately from each side of the chamber and washed until free from soil. Root length was measured using the line intercept method (Tennant, 1975) before drying and weighing. The shoots were analysed for Zn content.

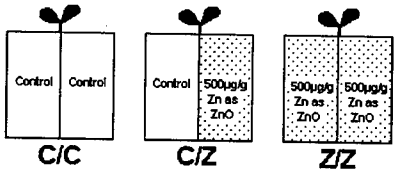

Figure 1.
The three levels of soil treatment used in the root chambers

3. Results and Discussion

T. caerulescens had significantly greater root length (Figure 2a) and mass (data not shown) in the ZnO-enriched soil of the heterogeneous treatment **C/Z** than the two homogeneous soil treatments **C/C** and **Z/Z** which had even (50%) allocation. In contrast, *T. arvense* had lower root length in the ZnO-enriched side of the heterogeneous treatment (Figure 2b). The concentration of Zn in the shoots of both species (Table 1) demonstrates that *T. caerulescens* plants in the **C/Z** treatment are able to accumulate Zn to a similar concentration to those in the **Z/Z** treatment. Conversely, Zn uptake by *T. arvense* in the **C/Z** treatment is similar to that in the **C/C** treatment.

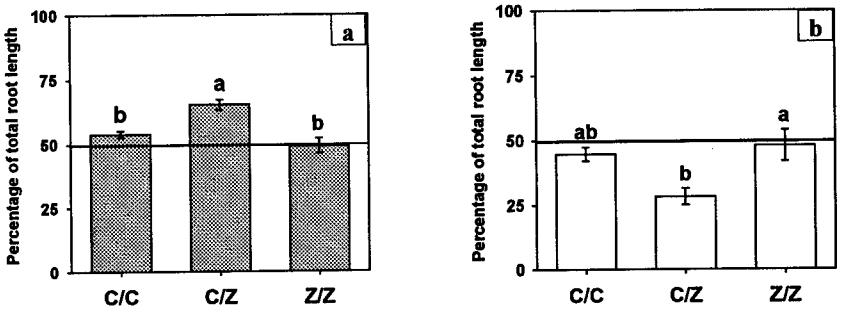

Figure 2. Root length of *T. caerulescens* (filled bars) and *T. arvense* (open bars) in the right side of the chambers expressed as a percentage of the total root length. Bars showing the same letter are not significantly different ($P>0.05$, Tukey multiple comparison test).

Table 1. Zn concentration (mg kg^{-1}) in the shoots of both species. Numbers showing the same letter are not significantly different ($P>0.05$, Tukey multiple comparison test).

	C/C	C/Z	Z/Z
T. caerulescens	1225 **a**	5632 **b**	5868 **b**
T. arvense	26 **a**	37 **a**	59 **b**

4. Conclusions

The increased allocation of root length and biomass by *T. caerulescens* into the Zn patch, with a concomitant decrease in roots allocated into the control patch, was successful in maintaining a high Zn uptake. This positive response, the opposite of the non-accumulator response, supports arguments which favour the active acquisition of zinc by *T. caerulescens*. Whiting (1998) demonstrated that acquisition of Zn from the insoluble phase was enhanced by higher root density. The 'zincophilic' root response described here may act synergistically with the passive mobilisation mechanism involving depletion of soil solution Zn which he proposed.

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A MULTIDIMENSIONAL METHOD FOR EVALUATING PHYTOREMEDIATION ON HEAVY METAL POLLUTED SOIL

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1. Introduction

Benefits and drawbacks of phytoremediation techniques have been often discussed in an intuition-based way, experimental results have been commonly presented in specific and individual terms. Only few authors have already tried to overcome these limitations, integrating information derived from past experiences (e.g., van Deuren, 1997). This paper is designed to discuss possibilities to enhance these approaches. In particular a multidimensional methodology for evaluating both preliminary alternative plans and feasible projects is presented.

2. Materials and Methods

A general flowchart of the decision-making process for a cleanup problem is shown in Figure 1. A comparison between heavy metal polluted soil (C_o) and threshold (C_T) concentrations must be done. This determines whether the decontamination task is compulsory or not. In the former case, a filter matrix, correlating the C_o/C_T ratio with the expected cleanup duration, identifies a set of alternative preliminary plans. Once one of these has been selected, alternative feasible projects are being derived and evaluated. The evaluation of preliminary plans can be omitted when the cleanup is not compulsory. The evaluation to be performed in both paths of Figure 1 can be based on a Multicriteria Evaluation Approach (MEA), as presented here. It requires to built an Impact Matrix (criteria x alternatives). The list of criteria is based upon all main factors that influence the decision process. For each alternative, the impact factor is derived from the related preliminary or feasible projects. A weight value is *a priori* assigned to each criterion. It represents its relative importance within the decision process. Weight-values' distribution reflects the decision-maker profile. MEA provides a set of ranked preferences among the alternatives based upon a concise score calculated considering simultaneously positive and negative aspects of one project with respect to the others. The stability of such a ranking order must be evaluated performing a sensitivity analysis on the weight vector. The method requires a preliminary classification of the criteria into 2 or 3 homogeneous groups. The weight of each group is given

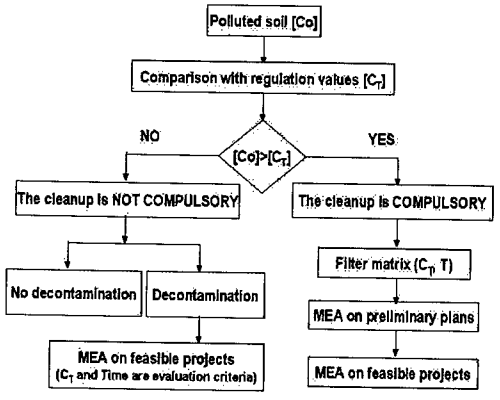

Figure 1: Cleanup decision process schema.

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by adding the weight of each criterion included into the group. MEA calculations are performed by a program (Mazzetto and Bonera, 1998) running on Windows platform, that provides graphical outputs of the ranked preferences. When 3 groups are used, the output can be "mapped" on triangular diagrams.

3. Results and Discussion

MEA has been tested for the selection of a preliminary cleanup plan to be applied to the Arnoldstein area (Carinthia, Austria). This area displays large concentrations of Zn, Cd, Pb and Cu (Kasperowski, 1993), thus requiring a compulsory cleanup. A 15-criteria x 3-projects matrix was constructed. The following alternatives were considered: removal (REM), *in situ* phytoremediation (PIN), *ex situ* phytoremediation (PEX). The criteria were then divided into the following homogeneous groups:

- ***Technically-related factors***: referring to the resources needs of each project;
- ***Economically-related factors***: referring to the monetary and financial aspects of each project;
- ***Socially- and Environmentally-related factors***: referring to the impact of each project on the society and the environment.

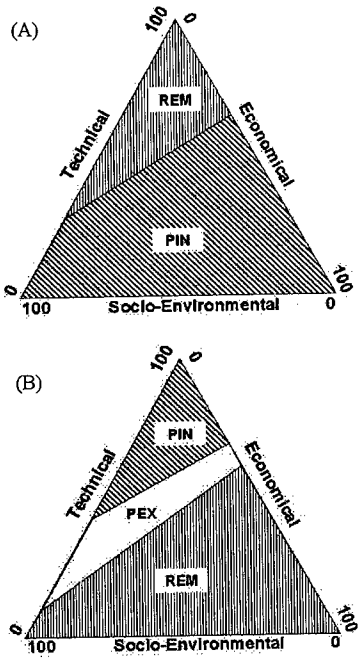

Figure 2: Sensitivity analysis for the "best" (A) and "worst" (B)

The results presented in Figure 2 (A: "best" ranked results, B: "worst" ranked results) show that PIN has great chances of being ranked as "best solution". In particular this occurs when economic and social-environmental factors tend to prevail. On the contrary, when technical factors becomes prevalent, PIN is ranked as "worst solution", REM becoming the "best" one. PEX does not perform well in any case. Among technical factors, the negative performance of PIN is related to the duration of the treatment. REM is negatively affected by large investment costs.

4. Conclusions

MEA final rank is highly influenced by the chosen criteria and the distribution of their weight within each homogeneous group. To get more reliable results iterative runs must be performed. Further efforts are required to improve the list of criteria used in the selection process, in particular for the evaluation of feasible projects.

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ARESENIC AND HEAVY METAL CONTAMINATION AND UPTAKE BY RICE CROPS AROUND AN ABANDONED AU-AG MINE IN KOREA

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1. Introduction

Rice plant is one of the most common crops cultivated as diet cereals in Korea. The varieties of natural and anthropogenic contaminants have polluted even the paddy field where the rice crop is growing. Kubong Au-Ag mine which was one of the largest Au-producing mines in Korea ceased operation in 1970 due to worse economic condition and has been abandoned since then. Tailings solid and effluent were discharged directly to nearby stream without treatment or control. Previous survey by Chon et al. (1997) for the Kubong mine area revealed them to be dominant pollution sources in the nearby agricultural field. In the present study, the effects of the old mining activities and waste materials on rice crop growing were assessed, and the characteristics of toxic element uptake by rice plant were also investigated.

2. Materials and Methods

Surface paddy soils (0-15cm in depth) and corresponding rice plants were sampled from the presumed contaminated paddy field and control area, considering the stream flow path and topography in October and November 1997 which is the harvest season for rice crop. Stream sediments and water samples from tailings effluent, nearby stream and shallow dug wells were also collected. On-site analysis for water samples comprised temperature, conductivity, total dissolved solids (TDS), pH and Eh. The multielement concentrations in all samples were analyzed with an ICP-AES and an AAS. Basic properties of soil samples were also measured such as pH, CEC, LOI and particle size distribution. Sequential extraction procedure (Davidson et al., 1994) was applied to selected paddy soil samples to examine the chemical forms of heavy metals.

3. Results and Discussion

High concentrations of As, Cd, Pb and Zn in tailings were found from the chemical analysis as 5,000 mg/kg, 54 mg/kg, 2,800 mg/kg and 1,560 mg/kg, respectively. Paddy soil samples collected from the contaminated site contained 59.4 mg/kg of As, 1.4 mg/kg of Cd and 67 mg/kg of Pb in average values. The samples near the mine and along the stream path had highly elevated As, Cd and Pb concentrations compared to those from the control site and normal soil (Bowen, 1979). Stream sediments sampled downstream from the abandoned mine contained as much as 4,400 mg/kg As, 32.8 mg/kg Cd, 2,000 mg/kg Pb and 1,000 mg/kg Zn indicating that stream is contaminated directly by tailings solid input. The elevated element concentrations in sediment were found about 4km downstream from the tailings. Element concentrations in the water samples from the stream and shallow dug wells were not high as expected except for As. However, effluent samples collected on tailings contained up to 1,890 ug/l of As and 25 ug/l of Cd, showing that As and Cd in tailings as well as polluted sediment can be easily mobilized into the stream and irrigation water. Arsenic, Cd, Cu, Pb and Zn concentrations in rice crop samples are summarized in Table 1. In comparison to natural contents in brown rice from non-polluted

area in Korea (Rhu et al., 1988), concentrations of As, Cd and Pb are elevated in rice grain samples. Their concentration factors (conc. in plant versus conc. in soil) were high in the order, Cd > As > Pb, and translocation of As and Pb in stalk and leaves was limited to rice grains. Contents of As, Cd and Pb were high at slightly acidic (pH 5.3), and lower CEC and organic matter conditions, although their ranges were narrow. Geographic distributions and chemical forms of As, Cd and Pb in paddy soils suggested that stream water, continuously affected by tailings leachate and contaminated sediment, can be an important pathway for accumulation in rice.

4. Conclusions

The concentrations and distribution of As and other heavy metals have been investigated in agricultural area around the Kubong Au-Ag mine. Elevated levels of As, Cd and Pb were found in stream sediment, nearby paddy soils and rice crops along the path of the stream around the abandoned mine. It is suggested that stream water used for irrigation can be the most likely source of the contamination in paddy soils and rice crops.

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Table 1: Means and ranges of As and heavy metal concentrations in rice crops around the Kubong Au-Ag mine (unit in mg/kg).

		As	Cd	Cu	Pb	Zn
Contamin-ated area	Rice stalk and leaves (N=36)	5.24[a] (2.1-9.1)	2.8 (0.1-18.9)	4.3 (2.2-8.0)	3.3 (1.1-9.4)	36.9 (18.4-103)
	Rice grain (N=50)	0.3[a] (0.2-0.6)	0.4 (0.02-3.1)	4.1 (1.6-27.6)	0.6 (0.2-2.2)	16.3 (4.7-28.7)
Control area	Rice stalk and leaves (N=7)	2.46[b] (0.7-3.6)	0.16 (0.1-0.2)	2.8 (1.8-6.1)	2.5 (1.8-3.8)	25.9 (18.5-42.5)
	Rice grain (N=12)	0.26[b] (0.23-0.31)	0.04 (0.01-0.07)	1.9 (0.7-3.0)	0.5 (0.1-1.0)	15.0 (12.5-17.5)
Normal rice grain in Korea (Rhu et al., 1988)		0.088	0.064	2.306	0.438	16.559

N : number of samples, [a]N = 17, [b]N = 3

CONTAMINATION OF SOILS AND PLANTS BY TELLURIUM

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1. Introduction

Tellurium(Te)is toxic for organisms including human beings. About 80% of the world's supply of Te is derived from electrolytic Cu refining. The remainder is recovered from slimes and slugs produced during Pb refining, and from pyrite and pyrrhotite burned in pulp and paper mills and sulfuric acid plants(Fishbein, 1991). The use of Te and its compounds are principally in the areas of solid state thermoelectric and electronic applications as well as microalloying in ferrous and non-ferrous metallurgy(Fishbein, 1991). Since Te is produced during refining non-ferrous heavy metals such as Cu and Pb, we determined Te in the soils and plants near non-ferrous mines and/or smelters. The effects of Te on some crops were also investigated by a water-culture experiment.

2. Materials and Methods

Soil sampling was conducted near the Hitachi Factory of Nippon Mining & Smelting Co. Ltd. and the Osaka Factory of Mitsubishi Materials Corporation which have been producing Te, the Kunitomi Factory of Sumitomo Metal Mine Co Ltd. which produced Cu, Pb, and Bi, and the Hosokawa Mine and Smelter of Mitsubishi Metal Mining Co. Ltd. which produced Ag, Pb, Zn and Cd. Sediments were taken from the rivers near the Kunitomi Factory and the Hosokawa Mine and Smelter. Some plants were also sampled near the Hitachi Factory. Soils(5g) were digested by 6M-HCl. The amounts of Te in the digested solutions were determined by the continuous hydride generation-AAS with standard addition method. Plants(2g) were digested by H_2SO_4-HNO_3 and the amounts of Te in the digested solutions were determined by the above mentioned method.

3. Results and Discussion

For determining the concentration of Te in uncontaminated soils, 25 surface soils were taken from Hokkaido, Fukushima, Nagano, Shizuoka and Saga prefectures. Geometric and arithmetic means(range) of Te in the uncontaminated soils were 0.041 and 0.047(0.010-0.109)mg kg^{-1}DW. These figures are nearly the same as the results of Chinese soils(AM 0.029(0.007-0.113)mg kg^{-1}) determined by Qi et al.(1992). Mean and maximum concentrations of Te in these soils are 3-12 and 46-164 times higher than the natural abundance(Table1). Te is observed to have high correlation coefficients with Cd, Zn, Pb, Cu, Se, Sb, and Bi in the soils near the Hitachi Factory, the Kunitomi Factory and the Hosokura Mine and Smelter. On the other hand, correlations are not observed between Te and the other metals in the soils near the Osaka Factory.

The concentrations in the river sediments were high, and GM(range) of the sediments near the Kunitomi Factory(n=4) were 5.86(2.60-11.5)mg kg^{-1}DW and those near the Hosokura Mine and Smelter(n=10) were 7.55(0.12-102)mg kg^{-1}DW.

Yomogi(mugwort)(*Artemisia princeps* Pamp) and hebinonekoza(*Athyrium yokoscense* Christ) were taken near Hitachi Factory. GM(range) of Te in the plants(n=11) were 4.21(1.79-9.18)mg kg^{-1}DW, and those in the plants(n=4) in the uncontaminated areas were 0.023(0.018-0.033)mg kg^{-1}DW.

Table 1 The concentrations of Te and the related heavy metals in the soils near mines and/or Smelter (mg kg^{-1}DW)

	Te	Cd	Zn	Pb	Cu	Se	Sb	Bi
Hitachi Factory(n=18)								
Geometric mean(GM)	0.106	0.946	158	55.9	104	0.949	---	---
Minimum	0.011	0.331	75.0	21.0	34.0	0.460	---	---
Maximum	6.55	56.0	1834	2250	3141	8.95	---	---
Osaka Factory(n=10)								
Geometric mean	0.501	0.713	347	126	371	---	---	---
Minimum	0.043	0.333	145	88.2	87.9	---	---	---
Maximum	6.17	3.44	1859	232	1941	---	---	---
Kunitomi Factory(n=24)								
Geometric mean	0.324	1.06	327	84.6	94.1	---	3.19	4.16
Minimum	0.079	0.189	103	8.2	22.0	---	0.612	0.448
Maximum	6.74	9.80	3310	710	1820	---	37.3	122
Hosokura Mine and Smelter(n=19)								
Geometric mean	0.19	2.85	472	263	48.4	---	4.03	0.65
Minimum	0.05	0.59	117	31	18.4	---	1.00	0.18
Maximum	1.88	13.6	1760	2630	204	---	25.2	7.10
Natural abundance (GM)	0.041	0.295	59.9	17.2	19.0	0.47	0.37	0.34

Natural abundance: Te; this work,Se;Kubota et al.(unpublished), and the others; Asami et al.(1988).

A water-culture experiment was conducted using soybean(*Glycine max* L.), corn (*Zea mays* L.) and komatsuna(*Brassica campestris* L.) for 14 or 16 days in a growth chamber. Te(as $TeCl_4$) was added to be 0, 10, 20, 50, 100□M. Growth of 3 crops was retarded from 10□M plots, and the weight of crops decreased considerably according to the increase of Te concentration in culture solution. Te concentrations(mg kg^{-1}DW) in the shoots of 10, 20, 50, and 100□M plots were as follows: soybean; 5.95, 9.04, 11.8 and 133, corn; 8.02, 11.6, 75.0 and 22.3, komatsuna; 13.2, 25.9, 105 and 114. About 93-99% of absorbed Te was accumulated in the roots.

4. Conclusions

Te contamination of soils and plants was observed near mines and/or smelters of non-ferrous metals. Since Te is toxic for all organisms including human beings, investigation on the environmental contamination with Te is necessary.

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CADMIUM AND ZINC IN PASTURE PLANTS EXPOSED TO ATMOSPHERIC DEPOSITION FROM DISTANT SOURCES

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1. Introduction

The southern parts of Norway are exposed to considerable heavy metal deposition originating from distant sources out of the country. Although deposition has decreased significantly during the last 20 years (STEINNES et al., 1994), the metal load in surface soil still remains high (BERTHELSEN et al., 1995), representing a continous source for uptake into vegetation and further accumulation in the food chains. Both the highly toxic Cd and the chemically similar but much less toxic Zn are strongly enriched in this atmospheric deposition (BERG et al., 1994). This study focuses on the uptake of Cd and Zn in pasture plants grazed by sheep and big game in this area.

2. Materials and Methods

The study is carried out in a birch forest ecosystem at Lund in Southern Norway. The area is located about 25 km from the coastline, in the zone receiving the highest amounts of precipitation and atmospheric deposition of heavy metals (STEINNES et al., 1989). Six plots considered to be representative for the area were chosen for plant and soil sampling. Plant species known or suspected to be part of sheep's or big game's diet were selected for sampling. The plant sampling was conducted in spring, mid-summer and autumn. Organic top soil samples were collected in spring. The material was dried and digested in conc. hot nitric acid, and metal concentrations were determined by AAS flame (Zn) and AAS HGA (Cd).

3. Results and Discussion

Cd and Zn concentrations in some of the plant species are summarised in table 1. Cd concentrations in plants show great variation between different plant species collected from the same soil. Among the trees, aspen (*Populus tremula*) takes up 60 times more Cd than rowan (*Sorbus aucuparia*) in the leaves. Similar large differences are also observed among dwarf shrubs and grasses. This implies that the Cd exposure to animals strongly depends on the diet composition. Zn concentrations vary less, except for a very high uptake in aspen and birch (*Betula pubescens*). Cd and Zn concentrations in the plant samples correlate significantly, the Zn to Cd ratio however varies by a factor of 20.

Cd and Zn concentrations in plant material decrease significantly from spring to summer. There are also differences in Cd concentrations between the adjacent sampling sites, which cannot be explained by total or extractable soil Cd. Both Cd and Zn concentrations are significantly higher in this material than reported values in corresponding species from areas in Norway less exposed to atmospheric deposition (BERTHELSEN et al., 1995).

Table 1: Mean dry weight concentrations and standard deviations of Cd and Zn in plants.

Species		*n*	Cd (ng/g)	Zn (µg/g)	Zn/Cd
Trees					
Birch (*Betula pubescens*)	leaf	18	280 ± 109	334 ± 53	1195
Rowan (*Sorbus aucuparia*)	leaf	15	26 ± 17	16 ± 6	684
Aspen (*Populus tremula*)	leaf	3	1524 ± 377	321 ± 82	210
Dwarf shrubs					
Northern Bilberry (*Vaccinium uliginosum*)	leaf	3	578 ± 173	91 ± 11	158
Bilberry (*Vaccinium myrtillus*)	leaf	18	24 ± 22	22 ± 8	618
Heather (*Calluna vulgaris*)	shoot	9	24 ± 12	27 ± 6	1161
Grasses and sedges					
Scirpus caespitosus	shoot	6	1253 ± 803	73 ± 18	58
Deschampsia flexuosa	shoot	16	31 ± 15	34 ± 6	1084
Anthoxanthum odoratum	shoot	6	564 ± 213	46 ± 9	81
Agrostis tenuis	shoot	6	180 ± 226	79 ± 46	437
Molinia caerulea	shoot	10	638 ± 739	52 ± 14	82

4. Conclusions

The plant uptake of Cd varies greatly between different species, making diet composition an important factor in the animal exposure to Cd. There are also significant differences in concentrations during the growing season (Cd and Zn) and within the site (Cd).

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ZN, PB, AND CD TRANSFER IN POLLUTED SOILS SITUATED IN THE PROXIMITY OF METALLURGIC PLANTS

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1. Introduction.

Large areas in the northern part of France are contaminated by trace metal pollutants as a result of former or contemporary metallurgic industrial activities (GODIN et al., 1985). In the proximity of zinc smelters, concentrations of Zn, Pb, and Cd frequently reach values as high as 2000, 700 and 20 mg kg^{-1} respectively (van OORT et al., 1998). Depending on the soil's physico-chemical and hydraulic properties, metal transfer from the soil to groundwater tables may present a major environmental risk. In this work, we studied the *in situ* vertical transfer of metal elements, soluble or associated to colloids, and we assessed metal fluxes in soils with different pH.

Sites description: Two sites were chosen close to zinc smelters (M7, UM4). The soils which have high Zn, Pb, and Cd contents differ in pH and, to a lesser extent, in texture (Table 1).

Table 1: General characteristics of soils studied

Site	Horizons	Depth cm	Texture	pH	Organic matter %	Zn content	Pb content	Cd content
						mg kg^{-1}		
M7	LA_{2g}	10-25	L	8.3	2.9	915	670	12.2
	BT_{1g}	30-40	LS	8.1	-	59.4	23.6	0.357
	BT_{2g}	45-55	LS	8.3	-	49.5	19.8	0.2
UM4	A_{1g}	9-36	LSA	5.6	2.8	1819	383	15.5
	A_{3g}	36-50	LAS	6.3	0.8	767	21.7	4.3
	S_{1g}	50-70	LAS	6.5	-	612	18.2	2.65

2. Materials and Methods

Zero-tension lysimeters were introduced at the lower limit of pedological horizons. Soil solutions were collected after each major rainy period and filtered through a 5 µm diameter filter. Ultracentrifugation was performed in order to separate the colloidal and soluble fractions (MAVROCORDATOS and PERRET, 1995). Total carbon, pH and trace metal elements contents were analysed, both in filtered bulk samples and in ultracentrifugated supernatants.

3. Results and Discussion

In acidic soil conditions (UM4), vertical transfer of Zn, Cd and Pb is evidenced, particularly in the surface horizon (figures 1, 2, 3). Soil solutions collected at the M7 site evidenced very low concentrations of Zn, Pb, and Cd (<100, <0.1, and 1-2 µg l^{-1} respectively) (an example for Cd is shown on figure 1). The high pH explained these results. In both soils, Cd concentrations in solution are higher than Pb concentrations, although the Pb content in soil is far higher than the Cd content.

In acidic soil colloidal species are responsible for about 5% of Zn and Cd fluxes in soils but about 40% of Pb transfer in the upper horizons (figures 2 and 3).

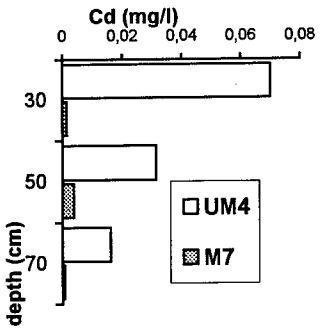

Figure 1: Mean total Cd transfer in UM4 and M7

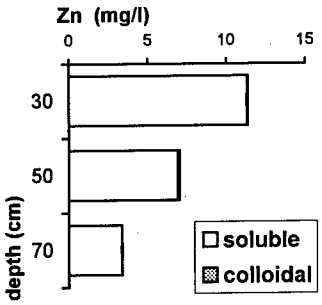

Figure 2: Mean colloidal and soluble transfer of Zn in UM4

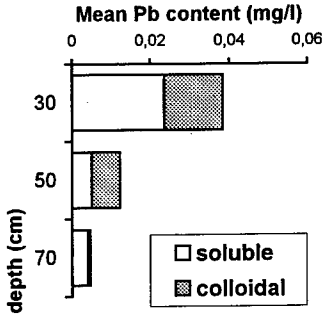

Figure 3: Mean colloidal and soluble transfer of Pb in UM4

Two kinds of behaviour were observed during the year, depending on the soil moisture and the rainfall intensity. In moist periods, trace element contents and the proportion of colloids in the soil solutions decreased with increasing depth. On the contrary, after severe rewetting following a dry period a general increase of trace metal contents was observed with depth. In case of Pb, the colloidal content also increased with increasing depth (figure 4).

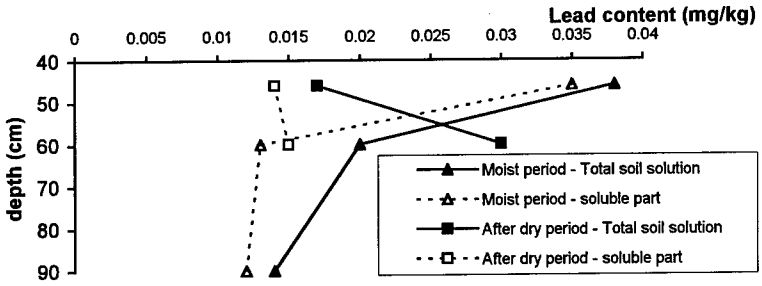

Figure 4: Examples of Pb transfer in the UM4 soil profile after two different climatic events.

4. Conclusions

These results highlight rather high values of metal fluxes in metal contaminated soils. Zn and Cd transfer is important in acidic soils. An estimation of annual fluxes indicates that about 2‰ of total Cd and Zn but only 0.03‰ of total Pb are transferred from the A_{1g} to the A_{2g}. Pb is largely transferred in association with colloids. In alkaline soil, the mobility of trace elements is low and the role of colloids insignificant.

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ZINC MOBILISATION IN FOREST SOIL NEAR THE HARJAVALTA CU-NI SMELTER, SW FINLAND

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1. Introduction

Zinc is a micronutrient that is essential for the metabolic functioning of higher plants and animals, but in high concentrations it is one of the trace metals potentially most hazardous to the biosphere (Kiekens, 1995). A number of studies have been carried out on the detrimental effects of Zn emissions from Zn smelters on soils and surface waters, but relatively little attention has been paid to the fact that many other nonferrous smelters (especially Cu-Ni smelters) also emit considerable amounts of Zn into the environment. For instance, up until 1991 the smelter at Harjavalta, SW Finland, emitted more Zn than Cu or Ni, even though it is generally referred to as a Cu-Ni smelter. Although heavy metal emissions from the smelter have been considerably reduced during the past decade (Helmisaari et al. 1995), in 1995 there were signs of an increase in the Cu, Ni and Zn concentrations in percolation water (20 cm depth) in the immediate vicinity of the smelter. The aim of this report is to present long-term (1992-98) trends in percolation water Zn concentrations close to the smelter, to assess whether Zn leaching is likely to affect ground water quality, and to determine whether liming agents could be used to counteract this potential hazard.

2. Materials and Methods

Percolation water samples were collected during 1992-1998 at a depth of 20 cm on one control and two treated plots in a severely damage Scots pine stand located 0.5 km from the Harjavalta smelter. The experimental layout and the pre-treatment and analysis of the water samples are described in detail in Derome and Nieminen (1998). Mg-rich limestone (2 t/ha) was applied to one of the plots, and a correction fertiliser (1 t/ha) consisting of slow-release powdered minerals (including 20% calcium and magnesium carbonate) and a mixture of fast-release, water-soluble salts (including Cu and Zn, 0.8 kg/ha) to the other.

3. Results and Discussion

The uppermost soil layers at the site are severely contaminated with a range of heavy metals including Zn (Derome and Lindroos, 1998). Despite the strong reduction in Zn emissions from the smelter, during 1995-98 there was a clear increase in the Zn concentrations in percolation water on the control plot (Fig. 1) in 1996. The deposition of Mg, K, SO_4 and Ni within the stand increased strongly during 1995-96, but there was no increase in Zn deposition. In addition, during 1992-96 there was a net loss of Zn from the 0-40 cm soil layer down into deeper layers, i.e. the output of Zn in percolation water (40 cm depth) was greater than the input to the soil in throughfall (Derome and Nieminen, 1998). The corresponding fluxes for Cu and Ni were negative, i.e. the pools of Cu and Ni are increasing in the 0-40 cm soil layer. Zinc is more mobile than Cu or Ni in the soil: it forms soluble complexes with chloride, phosphate, nitrate and sulphate, and soluble complexes and chelates with organic matter (Geering and Hodgson, 1969), and has a lower affinity for cation exchange sites than Cu and Ni. The increased deposition of Mg, K, SO_4 and Ni in 1995-96 appears to have increased Zn mobilisation from the organic layer in 1996. The liming and correction fertiliser treatments reduced the Zn concentrations to about

half the level on the control plot. The initial increase in 1992 was presumably due to the displacement of Zn in the overlying soil layers by the added cations (primarily Ca^{2+}, Mg^{2+}, K^{+}), and to the small addition of Zn in the CF treatment. The subsequent decrease in the Zn concentrations was presumably due to the immobilisation of Zn caused by the increase in soil pH values resulting from liming (Kiekens, 1995).

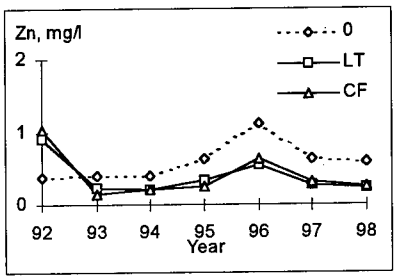

Fig. 1. Effect of liming (LT) and correction fertilisation (CF) on the Zn concentrations in percolation water (depth 20 cm) at a distance of 0.5 km from the Harjavalta smelter during 1992-1998. O = control. The bars indicate the standard error of the mean.

4. Conclusions

Despite the considerable reduction in emissions from the Harjavalta Cu-Ni smelter, there is strong evidence to suggest that the large amounts of Zn that have accumulated in the surface soil of the forest ecosystems surrounding the smelter during the past 50 years are susceptible to mobilisation and may be carried deeper down into the soil. This poses a potential threat to groundwater quality in the area. Liming at suitable doses will help to prevent this problem.

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ACCUMULATION OF CU AND NI IN SOIL AND VEGETATION NEAR THE HARJAVALTA CU-NI SMELTER, SW FINLAND

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1. Introduction

A Cu-Ni smelter has been operating in Harjavalta, south-western Finland, since 1945. Heavy metals emitted from the plant have accumulated in the forest soil (Derome and Lindroos 1998). Elevated heavy metal concentrations in the soil have toxic effects on soil micro-organisms, the understorey vegetation and the tree stand (Helmisaari et al. 1995, Nieminen and Helmisaari 1996). During the 1990's, dust emissions and deposition have been drastically decreased owing to changes in the process technology, the installation of new filters, and the construction of a taller smoke stack (Nieminen et al. 1999). The aim of this study was to determine the distribution of Cu and Ni in the soil and vegetation of Scots pine stands at different distances from the Harjavalta smelter.

2. Materials and Methods

Three study sites were established in Scots pine stands at distances of 0.5, 4 and 8 km to the SE of the Cu-Ni smelter in 1991. The pine stand at 0.5 km was severely damaged by heavy metals, but the stands at 4 and 8 km were relatively unaffected. Organic layer and mineral soil (Derome and Lindroos 1998), fine-root, understorey, tree needle, litter and stemwood (Helmisaari et al. 1995) samples were collected, and precipitation and percolation water (Derome and Nieminen 1998) were monitored in all the stands during 1992-1994.

3. Results and Discussion

Both exchangeable and total heavy metal concentrations increased in the soil on moving towards the smelter. The Cu gradient was steeper than that of Ni. Total Cu concentrations were 40 times higher at 0.5 km than at 8 km (Fig. 1), and the Ni concentrations correspondingly 12 times higher (Derome and Lindroos 1998). Heavy metal concentrations in all understorey species also increased with decreasing distance to the smelter. Mosses had higher Cu and Ni concentrations than lichens or dwarf shrubs. *Empetrum nigrum* is a species that is resistant to very high heavy metal concentrations. *Empetrum* accumulated heavy metals in stems especially, and restricted their transport to the leaves. Older plant parts had higher heavy metal concentrations than younger ones. Needle Ni and Cu concentrations were 9 and 15 times higher in the stands at 0.5 than at 8 km. At distances of 8 and 4 km, the Cu concentrations in the fine roots were 7-9 times higher than in the needles, but in the stand at 0.5 km they were only three times higher. Cu concentrations in the needles and fine roots were 40-70-times higher, in the litter components 165-270-times higher, and in *Empetrum nigrum* 150 times higher in the stand at 0.5 km compared to an unpolluted stand located 60 km away (Helmisaari et al. 1995). Concentrations in the litter increased with age and degree of decomposition. The increase in concentrations in the litter is due to the fact that litter (especially bark) has accumulated heavy metals over a long period. In relation to other tree compartments, Cu and Ni concentrations in stemwood were very small.

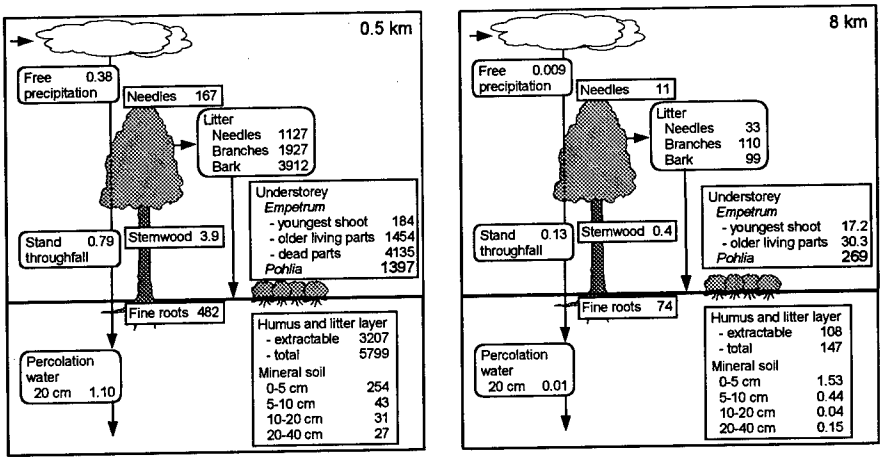

Fig. 1. Cu concentrations (mg/kg) in Scots pine stands at 0.5 (left) and 8 km (right) from the smelter.

4. Conclusions

Due to the relatively low stacks during the 1940's - 1980's when the emissions were very high, most of the emitted Cu and Ni was deposited close to the smelter (< 2 km). The deposited Cu and Ni have accumulated in the soil, resulting in a steep concentration gradient in the vegetation that is still visible. Emission reduction has a direct positive effect on air quality and deposition near smelters but a minimal effect on the amount of heavy metals accumulated in the forest soil for decades. Without any soil restoration measures, the accumulated heavy metals continue to affect the vegetation for a very long period.

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HEAVY METAL CONTAMINATION OF SOILS AND PLANTS AROUND THE DALSUNG COPPER-TUNGSTEN MINE, KOREA

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1. Introduction

There are over 1,000 metalliferous mines in Korea and most of the surrounding areas have been contaminated by heavy metals mining. However, limited studies of heavy metal contamination of soils and plants around the mining sites have been undertaken (JUNG and THORNTON, 1996; JUNG and THORNTON, 1997). Thus, this present study focuses on an investigation of metal mining and associated waste materials on the heavy metal contamination of soils and crop plants around the Dalsung Cu-W mine in Korea.

2. Materials and Methods

Surface soil samples (0-15cm depth) were taken by hand auger, from the mine dump sites, uncultivated upland and alluvial soils, household gardens and a nearby control area. Random samples of plants were also taken from each site within the mine dump areas and household gardens including corn grain *(Zea mays)*, jujube grain *(Zizyphus jujuba)*, perilla leaves (P. *frutescens var. japonica)*, red pepper *(Capsicum annuum)*, soybean leaves *(Glycine max)* and spring onions *(Allium cepa)*.

After appropriate preparation, soils and plants were digested using HNO_3 and $HClO_4$ and then leached with 5M HCl. The solutions were analyzed for Cd, Cu, Pb and Zn by ICP-AES. The measurements for soil pH, loss-on-ignition and cation exchange capacity were also carried out.

3. Results and Discussion

Heavy metal concentrations in surface soils and plants

Elevated levels of heavy metals were found in the mine dump soils with an average of 4.4, 1,935, 1,028 and 419 $\mu g\ g^{-1}$ of Cd, Cu, Pb and Zn, respectively. Soils from the alluvial and high land and household garden areas were also contaminated. This may be due to continuous dispersion of heavy metals from the mine dumps by water and wind.

Metal concentrations in plants grown on the soils in and around the mine varied with each plant type. It is generally agreed that metal concentration in leaves are usually much higher than those in grains (ALLOWAY, 1995). This study also confirmed that the leaves of soybean leaves and perilla contained much higher metal concentrations than corn and jujube grain. Thus, metal concentrations in plants taken in the study area decreased in the order of spring onions > soybean leaves > perilla leaves > red pepper > corn grain ≈ jujube grain.

Factors affecting metal uptake by plants

Metal uptake by plants can be affected by several factors including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants and plant age (ADRIANO, 1986). Because there is a combination of those factors affecting metal uptake by plants, a stepwise multiple regression method was applied to find the dominant factors

influencing metal uptake by plants and to predict metal concentrations in plants under those soils and climatic conditions. According to the results, total metal concentration in soil is the main factor, and is correlated positively with metals in plants. In addition, soil pH, correlated negatively with metals in plants, plays an important role in governing metal uptake by plants. Other factors such as cation exchange capacity, loss-on-ignition and soil texture are also good parameters for predicting metal concentrations in plants in some cases.

Dispersion of heavy metals from the mine dump sites

Texture is an important characteristics of soils, mine dump soils are sandy; therefore, they have a low sorption capacity for metal ions, a low pH and low organic matter contents. Thus, heavy metals derived from the mine dump sites are washed into downstream areas used for agriculture.

4. Conclusions

The highest levels of metals were found in the mine dump soils with averages of 4.4, 1,953, 1,028 and 419 $\mu g\ g^{-1}$ for Cd, Cu, Pb and Zn, respectively. Significant levels of the metals were also found in soils of household gardens and uncultivated areas. Metal concentrations in plants varied between plant species. Spring onions and soybean leaves contained high levels of the metals, for example, over 200 $\mu g\ g^{-1}$ (dry weight) and 1 $\mu g\ g^{-1}$ (dry weight) for Zn and Cd, respectively. These levels may adversely influence human health in this area if locally grown vegetables are continuously consumed. Metal concentrations in plants taken in the control area, however, were within normal ranges. The use of a stepwise linear multiple regression method as a technique for finding the dominant factors affecting metal uptake by plants, and for predicting metal concentrations in plants. This showed total metal content in surface soils to be a dominant factor influencing metal uptake by plants; soil pH was also a major factor. In addition, cation exchange capacity, loss-on-ignition and soil texture are also good parameters for predicting metal concentrations in plants in some cases.

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REACTION OF THE SOIL GAS-PHASE TO ENVIRONMENTAL POLLUTANTS

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1. Introduction

Experiments were carried out on large, undisturbed, cropped, unsaturated soil monoliths, with the aim to follow the fate of selected toxic elements (Cd, Cr, Ni, Pb, and Zn) in the soil-plant system. Possible stress effects of the metals, as indicated by changes in the amounts of biologically important gases such as O_2, CO_2, N_2, and water vapor were followed by quadrupole mass spectrometry technique (BRIDGER and WRIGHT 1988). In this paper only reactions of the soil gas-phase are discussed.

2. Materials and Methods

Large undisturbed soil monoliths (40-cm diameter, 100-cm long) were excavated at selected field sites and the cylindrical surfaces were coated with fiberglass cloth impregnated with a synthetic resin. The soils were: strongly leached typical Ramann brown forest soil from Gödöll□, Hungary and a slightly acidic sandy soil from Somogysárd, Hungary. The main properties of the soils in the upper 5-15 cm layer were: pH(H_2O): 6.3 and 5.6; $CaCO_3$[%]: 0 and 0; organic matter[%]: 1.2 and 1.3; CEC[me/100g soil]: 8.5 and 6.7; <0.02mm fraction[%]: 18.9 and 20.2 respectively (PÁRTAY et al. 1994).

Sensors were inserted into the monoliths through holes drilled in the coatings to follow changes in temperature, soil moisture content (TDR method) and gas composition along the soil profile. Sampling the gas phase in the soil was done by special microsensors inserted at 10, 20, 30, 50, and 80 cm depths into each monolith, and coupled to the 20-channel inlet system of a computer-controlled quadrupole mass spectrometer (QMS).

Automatic collection and analysis of QMS data operated continually during the experiments. Soil moisture contents were regulated along the soil profile by saturating the columns from the bottom through a special built-in valve, using a hanging water-column; or by sprinkler irrigation at the soil surface. Water was added on the basis of TDR measurements and/or weighting.. Aqueous multicomponent solutions of Cd, Cr, Ni, Pb, and Zn nitrates were added to samples of compressed communal sewage sludge (dry matter content 20.6 %, inorganic matter content 48.2 %). This metal-spiked sludge was mixed into the upper 10 cm of the soil after saturation of the monoliths with deionized water and subsequent drainage. Original concentrations of the metals in the sludge were comparable to or less than the limits in the HUNGARIAN STANDARD (1990). Additions of metal nitrates to this sludge were calculated to give metal loadings in the soil equivalent to 0x(control), 10x, 30x and 100x the permitted loading levels resulting from an average sludge application practice. Identical amounts of the metal-spiked sludges were applied in the various treatments in order to obtain uniform sludge (organic matter) loading levels. Maize and tomato were used as test plants.

3. Results and Discussion

The quadrupole mass spectrometer proved to be suitable for continuous (uninterrupted) measurement of the changes in the soil gas-phase. During the five months long experiments carbon dioxide levels showed the highest sensitivity to the changes in the chemical and physical properties in the soil profile, nitrogen and oxygen had less response. After saturation and especially after sludge addition an abrupt elevation of CO_2 concentration occurred along the whole soil profile (Figure 1). The duration of this process was longer in the brown forest soil than in the sandy soil. In both soils the $[CO_2/O_2]$ concentration ratio was continuously increasing towards the deeper layers in the monoliths. The tendency of the changes of N_2 concentration was similar to CO_2 but its extent was much smaller. After application of metal-enriched sewage sludges, the concentrations of these biologically important gases altered in the whole profile as compared to the initial status before sludge application. However, no changes in the gas concentrations of the gas-phase of the soils could *directly* be attributed to the heavy metal loadings. The observed changes were different from those observed after water saturation or after drainage. Composition of soil gas phase in the root zone was clearly different from gas composition inside the plants (also measured).

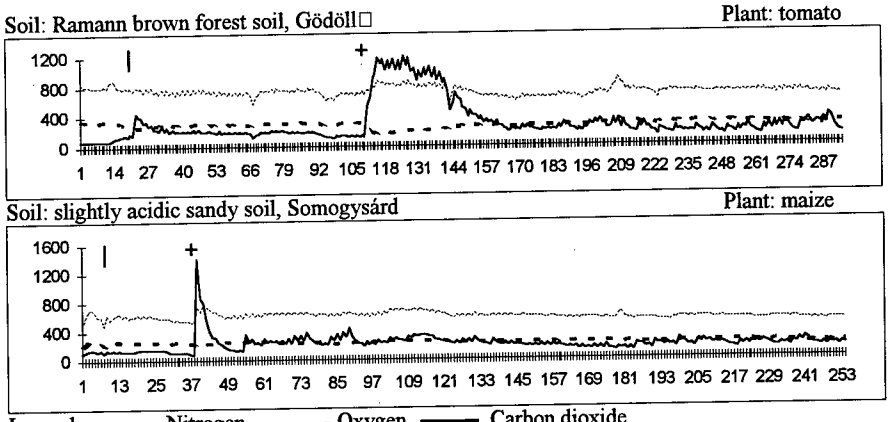

Figure 1. Relative gas concentrations in the soil monoliths at 10cm depth and at 30x loading.

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CHARACTERIZATION OF HEAVY METALS IN SOILS FROM AN IRON AND STEEL WORKS IN NAPLES, ITALY

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1. Introduction

A number of single- and multi-step extraction methods based on the use of specific reagents have been applied for estimating the status of heavy metals in highly polluted soils (Krishnamurti et al., 1995). Nondestructive physicochemical fractionation schemes have been proved to facilitate heavy metal associations characterisation by chemical, X-ray diffraction (XRD) and electron microscopy (Essington and Mattigod, 1991). The aim of this investigation is to study the mobility and mineralogical forms of heavy metals in contaminated soils from a recently dismantled iron and steel production plant. NH_4-oxalate extractions have been utilised to obtain indications of the "active" fractions of heavy metals in bulk materials from the various soil horizons. Mineralogy has been determined by XRD.

2. Materials and Methods

The investigation has been conducted at Bagnoli, on the west of coastal plain of Naples (South Italy) where abundant falls of pyroclastic material from the Phlegrean Fields have occurred. The study area is the former site of the ILVA iron and steel production plant which occupied, before its closure in 1991, an area of 300 ha. The soils not directly sealed by the plant works are strongly disturbed by the stratification of materials used in (Fe pellets, Fe minerals) and derived from (coke, iron products, slag) the industrial process (Buondonno et al., 1998).
For this study a number of profiles in zones of the plant set up for stocking raw materials and intermediate products and in zones apparently undisturbed were excavated. Bulk samples from the horizons were sieved (<2.0 mm) and kept field moist at 4 °C. Metals (Sn, Mo, Ni, Mn, Cr, Zn, Cu, Pb, Co, Cd, Fe, Al) and Si were extracted by NH_4-oxalate (0.2 mol L-1 pH 3) at a ratio sample:extractant of 1:100 with shaking for 4 h in the dark and determined by AAS.

3. Results and Discussion

All the soils of the study site have an ustic water regime and mesic temperature regime. The morphological features of a representative contaminated soil are given in Table 1. The profile is characterised by a very complex morphology induced by the heavy impact of the human activities on the site. The top of the profile is characterised by horizons produced by the human activities. The surface horizons (Cm) are strongly indurated, their morphology is highly variable as colour and mottling is concerned. This confirms the heterogeneity of the sediments due to the human activities. The 2C5 subsoil horizon at 68 cm is finer and has a platy structure with fine laminations characteristic of an alluvial deposition environment. The two 3CB horizons are mostly made by volcanic ash and pumice layers. The lowest one shows presence of few silt and clay coatings indicating illuviation process of fine sediments downward the profile.

Table 1. Morphological features of a representative contaminated soil

Horizon	Depth (cm)	Main soil forming factor	Colour (dry)	Industrial rock fragments	Structure	Mottling	Coatings
C1m	0-20	anthropic	10YR 4/2	few	massive	none	none
C2m	20-28	anthropic	7.5YR 4/4	few	massive	few	none
C3m	28-37	anthropic	5Y 5/3	few	massive	few	none
C4m	37-42	anthropic	7.5YR 5/4	common	massive	none	none
CB1	42-68	anthropic	5Y 6/3	few	subangular blocky and massive	few	none
2C5	68-72	anthropic	7.5YR 4/4	none	platy	few	none
3CB2	72-80	natural	5Y 6/3	none	massive and single grain	none	(none)
3CB3t	80-120	natural	5Y 6/3	none	massive and single grain	none	few

NH_4-oxalate extractable amounts of metals (Table 2) are greatest in the surface horizons and tend to decrease regularly with depth. For Mn, Cr, Zn, Cu, Fe, Al and Si the largest concentration were measured in the C1m horizon. For Pb and Co in the C3m horizon. The thin layers of stratified clayey materials C4m and 2C5 show accumulation of metals compared with the layers above and below. With the exception of Pb and Co the amounts of all metals extracted by NH_4-oxalate correlates well ($R^2 \geq 0.6$) with NH_4-oxalate extractableFe , suggesting heavy metal associations with iron phases.

Table 2. Heavy metals, Al and Si (mg kg^{-1}) removed by NH_4-oxalate from horizon samples of a representative contaminated soil

Horizon	Depth	Mn	Cr	Zn	Cu	Pb	Co	Fe	Al	Si
C1m	0-20	1542.	5.	210.	90.	36.	18.	9456.	958.	529.
C2m	20-28	1478.	1.	140.	53.	59.	46.	2310.	478.	170.
C3m	28-37	543.	0.	41.	13.	18.	5.	873.	679.	117.
C4m	37-42	690.	1.	141.	33.	71.	6.	1704.	1373.	549.
CB1	42-68	411.	1.	37.	10.	19.	5.	605.	495.	44.
2C5	68-72	892.	2.	91.	28.	34.	10.	1350.	882.	216.
3CB2	72-80	95.	1.	7.	2.	7.	1.	133.	219.	29.
3CB3t	80-120	208.	1.	6.	1.	10.	1.	233.	323.	27.

The nature of the Fe minerals was identified by XRD. Magnetite, goethite and hematite are present in all horizons down to 72 cm depth. The iron phases are mixed with the minerals commonly occurring in the soils developed on Phlegrean volcanic materials (K-feldpars, clinopyroxenes, mica) which are the only mineral phases recognisable in the 3CB primary volcanic soil layers. Indication of goethite and hematite presence in the < 2 μm fractions of these horizons was denoted from the XRD diagrams obtained summing 10 times the signal. Considering that the association of metals and their complexes to fine grained particles is an important factor of metal mobility and fate,our results could indicate that in the studied soil metals are transported within the profile to the subsoil as colloids or adsorbed to fine-grained particles.

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CHROMIUM ACCUMULATION IN MARIGOLD

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1. Introduction

Among heavy metals, chromium has received little attention, in comparison to f.i. Cd, Cu, Zn, Pb, though Cr^{VI} is known as toxic, while Cr^{III} plays an essential role as a microelement to animals and man. In a previous work, designed to highlight the effects of wine waste spreading on soils and plants (Bini et al., 1997), the ability of marigold to accumulate heavy metals, and especially chromium, was ascertained. Therefore, it was suggested that this plant could be utilized for remediation of Cr-contaminated soils, though in the literature it is considered unlikely that soil Cr will be remediated, owing to the barrier effect of the roots (Chaney et al., 1997).Chromium is a "dispersed" trace element, with generally limited concentrations in the earth's crust and the related soils. However, Cr accumulation may occur at some locations, owing to different Cr sources. Possible "natural" accumulation may be related to Cr-bearing rocks, like serpentines, while anthropogenic accumulation is related to industrial activities (varnish, leather, fertilisers).In Italy, Cr concentrations in soils range from 5 to 1500 mg/kg (mean 70 mg/kg; Angelone & Bini, 1993). In some regions, like Tuscany, chromium-rich (0.9 – 3% Cr) by-products of leather factories (dead-roasted and/or hydrolysed leather) are introduced in the environment as organic N fertilizers in crop fertilization (Ciavatta & Gessa, 1997). This may determine Cr accumulation in the soil and potential toxicity to plants and the food chain.
The aim of this work was to assess the effective heavy metal, and especially chromium, accumulation ability by marigold, and the sorption mechanism. For this purpose, two species of marigold were selected, the wild-growing *Calendula arvensis* and the cultivated *C. officinalis*, which is largely used in medical botany and cosmetics as a scaring, anti-inflammatory, antimicrobic, and as a colourant plant in gastronomy.

2. Materials and Methods

Plants of marigold, after incubation in phytocells and germination, were transferred in pots prepared with common garden soil (silty-clayey, pH 7.43, O. M. 3.1%) and sprinkled with a solution containing different metal concentrations (5, 10, 50, 100, 250, 500 mg/kg Cr respectively). Sprinkling was carried out over 15 days at a rate of 10 ml each day. Globally, every plant received 750, 1500, 7500, 15000, 37500, 75000 mg/kg of chromium, respectively. Three replicates of each laboratory trials were carried out, in order to obtain significant mean values.After sprinkling, the plants were preserved and harvested at two different stages of their phenological cycle, i.e. during the maximun vegetative period (autumn, 1997) and during blossom (spring, 1998).Roots and leaves were gently washed and handly separated. They were air dried and then digested with suprapure HNO_3. The heavy metal content in both roots and leaves was determined by AAS.

3. Results and Discussion

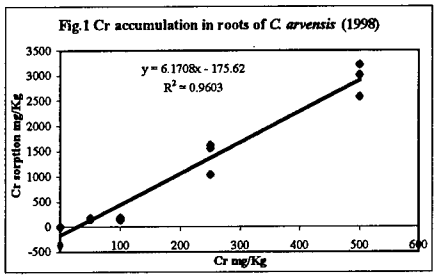
Relevant quantities of Cr were absorbed by marigold during the experimental period. However, differences were recorded between the two species, as well as among the different parts of the plant, over the vegetative cycle. Indeed, *C. arvensis* presents higher Cr concentrations than *C. officinalis*, both in leaves and in roots. Roots seem to represent a biological barrier at low Cr

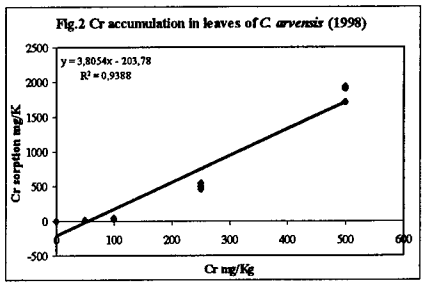
concentrations (5 – 10 mg/kg Cr), since the metal concentration in roots overcomes 40 mg/kg Cr in *C. officinalis* and 400 mg/kg Cr in *C. arvensis*, while in leaves it is lower than 10 mg/kg Cr in both species. At higher Cr rates (50 – 500 mg/kg), Cr concentrations in plants are more and more increasing, and overcome 2900 mg/kg in roots, and1000 mg/kg in leaves of both the species, thus suggesting that a relevant part of the metal is translocated from roots to leaves in proportion to the amount received. Though in the literature it seems unlikely (Barcelo & Poschenrieder, 1997), this behaviour is characteristic of indicator plants (Baker, 1981).

The soil – plant transfer factor for chromium is fairly low, with values (relative to the whole plant) ranging from 0.02 to 0.34, *C. officinalis* showing the lowest ratios, especially at low Cr rates (5 – 10 mg/kg). On the contrary, at the same Cr rates *C. arvensis* presents the highest soil – plant ratios, thus confirming this plant to be more effective than the former in Cr uptake. Moreover, in the aerial parts of the plants examined the values of the transfer factor were quite lower (by at least a factor of ten), varying between 0.002 and 0.024.

The Cr transfer factor within the plant (root – leave ratios) ranges from 28 to 2.5 in *C. officinalis*, and from 40 to 1.5 in *C. arvensis*, suggesting that, unlike to what is stated in recent literature (Silva et al., 1997), in this case the root system is a faint biological barrier for chromium.

An univariate statistical analysis of variable couples showed very good correlation between Cr concentration and plant uptake for all the laboratory trials, with R^2 = 0.88 – 0.99 in range, asillustrated for istance . in the figures1 and 2 for Cr sorption in roots and leaves of *C. arvensis.*





4. Conclusions

Marigold is able to uptake chromium in quite large quantities. Most of the metal is retained by roots, but at higher Cr concentration a relevant part of the metal is translocated from roots to leaves.*Calendula arvensis* seems to absorb more Cr than *C. officinalis*, thus suggesting it could be suitable for phytoremediation of Cr affected soils.

The current use of *C. officinalis* in medical botany and gastronomy makes it unlikely in phytoremediation, since it may constitute a potential toxic hazard for man.

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PETROMETALS AND CERTAIN ECOLOGICAL PROBLEMS

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This paper generalizes data on the content of a large number of microelements in crude oils from different fields; an examination is made of ways in which heavy metals interact in the environment. Extracting heavy metals at the oil refining stage are discussed, as well as the scope for preventing their emission into the atmosphere and industrial waste waters. The study of composition of crude oils is long-standing, and the level, scale and geography of these investigations have now become much broader. This has occurred because of problems: the need for an improvement in the technical and economic indices of oil production and refining and in the use of crude oil and petroleum products; the efficient use of many valuable petroleum components; environmental protection and many others.

Over 30 metallic elements and 20 nonmetallic elements have now been found in crude oils. The average concentrations of microelements in oils decrease in the following order: Cl, V, Fe, Ca, Ni, Na, K, Mg, Si, Al, I, Br, Hg, Zn, P, Mo, Cr, Sr, Cu, Rb, Co, Mn, Ba, Se, As, Ga, Cs, Ge, Ag, Sb, U, Hf, Eu, Re, La, Sc, Pb, Au, Be, Ti and Sn. Transition and alkaline-earth metals, which are capable of forming π-complexes, warrant most attention; these include V, Ni, Fe, Zn, Ca, Hg, Cr, Cu and Mn. Petroleum organics (mainly polynuclear arene and heteroatomic compounds) can act as complex-forming extractants, in which donor-acceptor bonds are localized on their collective π-systems, and on N; S and 0 heteroatoms.

As assumed, possible sources of metals in crude oils are oil-forming organisms and also microelements adsorbed or getting into the formation (by migration from rock or waters), and here a rectilinear relation is observed between the contents of particular elements. For example, the concentration of V is greater the higher the sulfur content, and the concentration of Ni is greater the higher the nitrogen content - in general, the higher the content of the ligand atom. Some metals in crude oils are in the form of organic acid salts of the R-COOM type or chelate complexes, in which the metal atom is located at the coordination center of the porphyrin ring or in the cavities of condensed aromatic fragments. The bulk of the metal is in the form of polydentate complexes, many of which can enter into ion exchange with the metals present in M^+A^- forms or on the surface of the rock $(MA)_x$ directly in contact with the crude oil.

Such as V, Ni, Mo, Co, Cr, Sb, Ga, Ge, La etc. are concentrated mainly in resinous- asphaltene fractions, where they are present in the form of metaloporphyrins (V, Ni), salts of metals (V, Ni, Mo, Ge etc.). The group of metals Pb, Zn, Cu, Hg, Se and As is found in high-boiling fractions of crude oils and predominates in bitumoids. These metals form organometallic compounds such as, for example, alkylmercury $Hg(Alk)_2$ or aryl- and alkyl-lead $Pb(Alk)_2$ compounds, or complexes with different organic ligands of oil.

Will be the heavy metals (HM) the main pollutants of the XXI century? We are answering positive - yes, they will, on the following mentioned chemical, medical, social and other reasons. Everybody can see today the close connection of chemical properties and exposure time with LD_{50} and LC_{50} of acute toxicity (therefore with population health) effects of mercury, lead, cadmium, nickel, vanadium, molybdenum, chromium, manganese, zinc, copper, cobalt, beryllium. They are penetrating into human body by inhalation of dust, fume, gases, vapors from air, even with drinking water and food. Orhus (Denmark) Conference-1998 of Ministers of

Environmental Protection from European Committee forwarded the actual problem of «the biosphere contamination by HM», as example, by usage leaded gasoline; the last problem is not only well timed, but is not postponing business. Concerning Russian Standards "GOST", the next metals are referring to (on alphabet): the first class of danger - Be, Cd, Hg, Pb, Zn and the second class of danger - Co, Cr, Cu, Mo, Ni, V.

At our time the high level of exploration of HM in environment takes place. That situation is the result of wide mining and usage of HM in industry; high volatility of HM-compounds as well influences on contamination. One can see the specific chemical properties of HM as **supertoxicants,** especially the property to change their states of oxidation. Above that HM are practically nonbiodegradable, the same time HM are essential. HM have possibility to catalyze many of organic reactions, but that spontaneous catalysis can't be ruled and in many cases is even unknown. HM are the components of living organisms (Fe, Cu, Zn, Co), their extravagance in water and atmosphere (V, Ni from termoenergetics) intercept the normal physiological processes.

We have to emphasize, that many HM have the different influence upon the representatives of two sexes - we say **gender influence**, having in mind not biological, but social differences in-between men and women. As example, gender influence by microsaturnism [by poisoning Pb^{2+} and Pb^{4+}-containing compounds, as example, by TEL $Pb(C_2H_5)_4$] will be discussed. As an illustration to the gender influence upon poisoning by HM we shall give the picture of different «trank», where influences mentioned were given by male - female as well by adults - children scheme.

The common tasks on evaluation of the contamination by HM - for understanding the spheres of their influences upon human being and for recommendations against HM pollution - are, in principle, quite clear and can be mentioned as the next ones: the assessment of HM - contamination and determination of industrial HM - sources; accurate analysis in different spheres for definite metal (metals); not only M - concentrations (in comparison with maximum permeable concentrations) but as well its (their) oxidation states $M^n \Leftrightarrow M^{n+1}$. Than to be followed medical-ecological and -demographical evaluation of the HM-impact upon workers at metal making or using factories and population of industrial zones in heavy contaminated regions. Important is evaluation of HM-actions together with another chemical compounds (N; S; C -oxides, pesticides and so on).

Yes, definitely HM can be, even will be, the main pollutants of the next century.

ARSENIC AFFECTED SOILS IN THE SURROUNDINGS OF A MINE IN ZACATECAS, MEXICO

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1. Introduction

Arsenic is commonly concentrated through industrial processes involved in the commercial extraction and concentration of certain metal, such as Pb, Zn and Ag. Mine tailings are therefore a hazard to environmental health, specially in the surroundings of mines. As tailings consist at very small particles, they can easily be transported by water or by wind; even if they are discharged into dams specially designed to avoid this problem.

In an extraction and concentration mining unit in Zacatecas, Mexico, a problem concerning the transportation by air into the surroundings of the tailings dam has been identified. A study to quantify arsenic in superficial soils, as a dispersion tracer was proposed. This approach is expected to show a picture of the effect of dispersion and accumulation of mine tailings through 15 years of operation.

Study Area

The mine is located in Zacatecas. Its altitude averages 2200 masl. The climate is dry (BS_1, according to García, 1988), mean annual temperature is 16°C, mean annual precipitation is 510mm. Superficial aquatic resources in Zacatecas are very scarce. Soil are shallow, with petrocalcic horizons or duripans, at 50cm depth, medium textured and of alluvial origin in most cases (Litosols and Xerosols). Their productive exploitation is therefore very limited (mainly rain-fed corn and beans). Plant cover corresponds to a specific type of desert vegetation (Desierto Chihuahuense). The main economic activity is mining.

Considering the dry climate and the absence of artificial irrigation, severe lixiviation problems are not expected. Due to this condition and to flat topography, air transportation is viewed as the main pathway of tailings dispersion. Vegetation condition (small coverage index) is though to oppose little interference to tailings dispersion, suggesting a large affected area.

2. Materials and Methods

Soil sampling: To define the sampling area, the direction of the dominant winds and the town position were considered.

The design was done according to geostatistical recommendations: a regular grid (250x250m) covering an area of 3.5x5km was traced (excluding the tailings dam). For soil profiles, 8 sites were selected at random over the grid (sampling was done by horizons). For tailings 7 sites were selected into the tailings dam. Sampling was done at two depths: superficial crust (clay size particles) and subjacent layer (sand size particles). For each site its georefereed position was registered.

Analytical techniques. Total arsenic: Sample digestion was done according to Moffet (1988) in a HCl and HNO_3 mixture and microwave digestor. Quantification by AAE-HG (Varian SpectrAA10 Plus-Varian, VGA 77). pH: With a potentiometer in a 1:2.5 relation (solid:1M KCl

pH=7) (Ortiz *et al.*, 1993). Electric Conductivity (E.C.): In a 1:5 relation (solid:water) (Ortiz *et al.*, 1993). % Carbon (%C): An ignition technique had to be adapted due to interferences observed in the chemical oxidation technique, according to Hesse (1971). Lost of weight after a 8h 450-500°C treatment.

3. Results

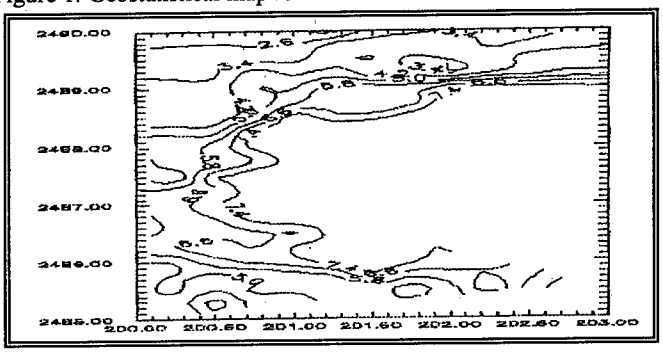
Table 1 summarizes the laboratory results obtained for soil and tailings samples.

Table 1: Intervals of values for superficial soils and tailings in the area surrounding the mine.

Sample	Total As ($mgkg^{-1}$)	Ph	E.C. ($mmhoscm^{-1}$)	%C
Tailings				
Soil ARL*(n=164)	50.2-7957.0 sd=1728.6	2.6-8.0 sd=0.8	0.04-18.53 sd=1.75	0.7-20.0 sd=3.45
Soil BRL**(n=45)	3.7-48.5 sd=12.7	4.3-7.6 sd=0.7	0.03-0.63 sd=0.16	1.4-15.0 sd=2.66

*Above remediation limit **Below remediation limit. Remediation limit=$50mgkg^{-1}$ (Floss and Hellerer, 1991)

Figure 1. Geostatistical map for arsenic



4. Conclusions

Soil depths were shallow, maximum of 40cm. Medium texture soils were obtained for the profiles. pH values were mostly neutral, tending to decrease in the proximity of the tailings pond. E.C. and %C values obtained were low, and apparently homogenous over the sampling area. Most of the area showed high total arsenic contents, above the remediation limit (80.3% of the total samples). Total arsenic was mainly found in superficial horizons of soil profiles. The higher total arsenic concentrations belonged to the nearest tailings pond samples. Spatial dependence of the data could be proved by geostatistical analysis. An alternative dispersion path was identified in addition to the N-S direction that was expected from the beginning, this one showing a E-W direction.

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TRACE ELEMENTS IN THE SOILS NEAR THE CENTERS OF MINING INDUSTRY

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1. Introduction.

The numerous researches show, that the soil cover passive accumulates from the air man-caused pollutions and reflects an outcome of long-term anthropogeneous effect on an environment. The aerosols of various genesis and dimensions are transferred by air streams on various distances from a source, fall out and strongly fixed in the upper levels of soil. Thus, the soil cover can be used as the good natural indicator for space variability of levels contamination of atmospheric air (DOBROVOLSKYI, 1980, YHANSSON, 1993). Especially it is brightly exhibited on urban areas. In these conditions the influence of many natural factors become weak to accumulation and distribution of microelements in a soil cover. Anthropogeneous factor is dominant. It is difficult to select autonomous, subordinate or transit forms of relief on urban areas accordingly with strict geochemical sense. The top-soil is divided into sectors, the exchange of substances between which is infringed. However, this fact improves indicator abilities of soils as index of air contamination on a comparison with natural territories.

In this work the results of a research of the upper genetic level of soils on territory of Apatity city - center of a mining industry of the Kola peninsula are represented. We represented the results of a research of atmospheric air quality in this region earlier (GOLOUBEVA, 1997). Priority contaminants in the present region are Sr, Cu, Ni, Pb, Zn, Mn. They arrive from local ore mining and processing enterprises, so from a regional source - non-ferrous metallurgical plant SEVERONICKEL.

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2. Materials and Methods

The sampling was carried out in July 1992 on territory of Apatity in knots of a grid 300x300m. During selection of samples the genesis of soil, mineralogical composition, landscape and hydromorphological conditions was taken into consideration. Totally 75 samples were selected. For determination of heavy metals atomic absorption spectrophotometry with flaming atomization of liquid was used. Transformation of metals into a solution was carried out with application of a hydrochloric acid as a standard technique.

3. Results and Discussion

The researches of a soil cover of the city have shown, that in upper humus-accumulative levels there is an intensive accumulation of Pb, Sr, Zn, Mn. Significances of eluvial-accumulative factors (ratio of the average contents of the chemical element to it's average contents in soil genesis layer) are the following: Pb: 0.5 - 40; Sr: 0.7 - 55; Zn: 0.5 - 10. The accumulation of Cu and Ni was much more poorly (Cu: 0.5 - 4; Ni: 0.7 - 3.7). For an estimation of influence of industrial emissions on the contents of metals in soils of the city, the results were compared to the concentration of heavy metals in soil of the background region. The analysis has shown, that concentration of Pb, Sr in 5-10 times and in isolated points more than 20 times, exceed background values and reached 120 mg/kg and 140 mg/kg, respectively. The greatest accumulation of Sr was found in the zone of city, which based on data of a tool research of air most exposed to influences of a mining industry. The greatest accumulation of Pb was found along the main highway. The concentrations of Cu and Ni did not exceed 55-80 mg/kg, 10-17 mg/kg respectively. These contaminants originate from a regional source and distribute on territory of city. In general on territory of city two main regions of an accumulation of metals can be distinguished (fig.1). The forming of Western zone of

contamination is promoted by a combination of unfavorable geomorphological and microclimatic features, here Sr, Pb, Cu accumulated in soils. In a South-East part of the city Pb, Sr, Cd predominate the contamination. Zones of heavy metals' accumulations in soil practically coincide with zones of the biggest contamination of the same heavy metals in snow cover. That allows to say that zones of the increased contents of microelements in soils have a lawful character and are stipulated first of all by contamination of air.

4. Conclusions

The obtained results show appropriateness of use the upper genetic level of soils for a research of space distribution of metals on urban territories at Polar regions. The zones of the increased contents of heavy metals are determined by proximity of the plants of a mining industry and combination unfavorable for dispersal microclimatic and geomorphological conditions. Level of contaminants in a soil cover of city is moderate. However, the separate areas exist, where the accumulation of metals in soils reaches a dangerous degree of contamination for health of the person and it should be taken into consideration in the strategy of development of production and town-planning in the present region.

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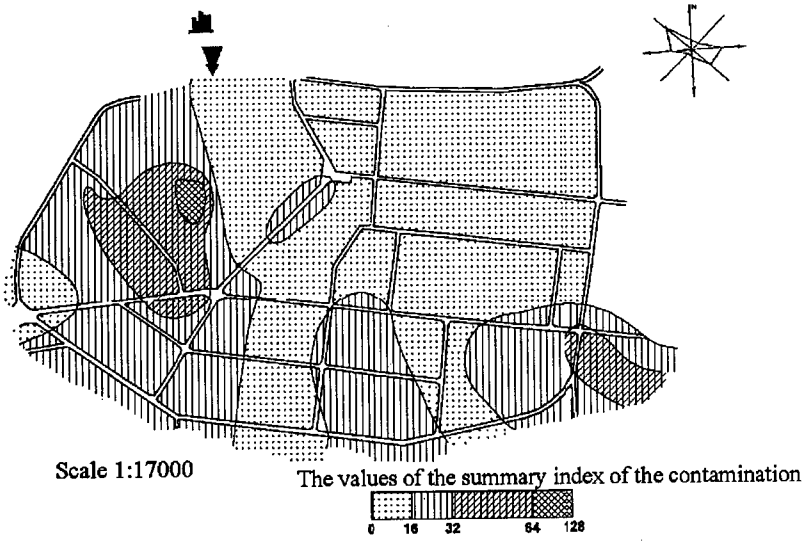

Fig.1 The scheme of the soil contamination with the heavy metals in the Apatity city.

CADMIUM, LEAD AND ZINC CONTENT IN SOIL AND IN SOME VEGETABLES NEAR THE LANDFILL OF LEAD SMELTER MEZICA AFTER ACCIDENTAL FIRE

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1. Introduction

The Mezica valley has been exposed to more than three hundred years of active lead mining and smelting. Soils in the valley are highly polluted with heavy metals, especially with Pb, Cd and Zn. In 1990 lead ore mining and smelting stopped and recycling old car batteries started. At present there are two main sources of pollution: The lead smelter in Zerjav and the landfill at Glancnik, where scoria and plastic parts of batteries are deposited. An accidental fire at the landfill in December 1995 caused additional pollution. People in this area produce plants for their own consumption. Therefore the most common vegetables were sampled and analyzed for Pb, Cd and Zn content. The aim of this study was to establish the effect of contaminated soil and atmospheric lead emissions from both lead smelter and landfill on commonly grown vegetables in the Mezica valley.

2. Materials and Methods

In 1996 soil and vegetables from the garden near the landfill were sampled. The following year garden soil and endive (Cichorium endiviae) were sampled from 16 sites at different distances from lead smelter. Samples of topsoil were taken (0 - 20 cm). An average soil sample from each sampling site was prepared as a composite of 18 sub-samples taken from an area of 250 m^2 in size. Leaves of test plants were collected from the same area and washed and unwashed leaves were analyzed. Plant tissue samples (250-500 mg) were treated with HNO_3 acid. Pb, Cd and Zn concentration in plants were determined using ICP atomic emission spectrometry after the acid dissolution technique with microwave heating (1). Heavy metal concentrations in soil were determined by aqua regia dissolution.

3. Results and Discussion

Lead, Cd and Zn content in soil near the landfill were 1920, 13,3 and 1788 mg/kg DW respectively. Endive and salad leaves, parseley roots and leaves and carrot roots accumulated high concentrations of Cd. Most of metals in plants originate from root uptake, but also foliar uptake of some metals, especially of Cd, can be relatively high (2). Ease of removal of Pb by washing suggests that the metal was largely a superficial deposit on the leaf surface. In the contrast the small fraction of Cd that can be washed off indicates a greater leaf penetration (figures 1-4).

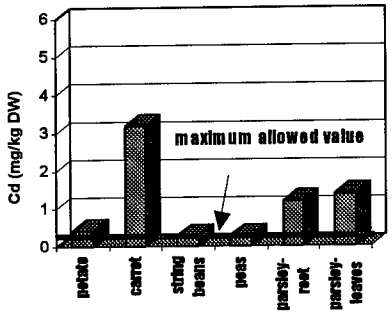

Figure 1: Cd content in vegetables from the garden near the landfill

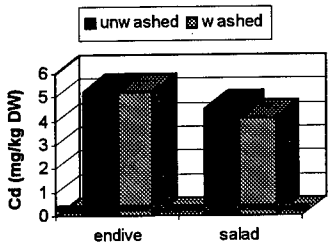

Figure 2: Cd content in unwashed and washed endive and salad leaves

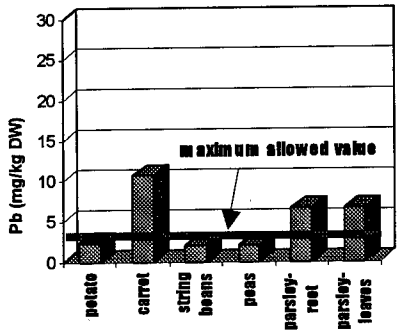

Figure 3: Pb content in vegetables from the garden near the landfill

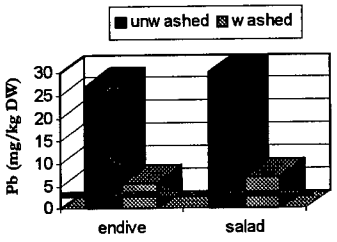

Figure 4: Pb content in unwashed and washed endive and salad leaves

4. Conclusions

Investigations in the wide area of upper Mezica valley confirmed soil and plants to be contaminated with Cd and Pb, especially in Zerjav where lead smelter is located, and in Crna, 1-3 km from the smelter (3). Cd and Pb concentrations in washed and unwashed leaves of sampled crops showed plants to be strongly affected by emissions from lead smelter.

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THE STUDY OF FLUORINE MOBILE FORM CONCENTRATIONS IN SOIL CONTAMINATED WITH ACIDOUS FLUORIDES

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1. Introduction

The use of fluorine-consisting phosphoric fertilizers and ameliorants, and sedimentation of flying pollution of chemical and metalurgical industry lead to the increased fluorine concentrations in soil (2, 3). Thus, in the zones of excessive humidity almost 20% of fertilizers being brought into soil appears in water sinks, and sewage cleaners have been disable to prevent drink water from toxic compounds, including fluorides. Fluorine accumulation in soil solutions is especially undesirable, because it has became available to plants (1). In this case the real hazard appears that forage for agricultural animals and human food stuff will be contaminated with fluorine. On our opinion, any fluoride compounds water-soluble and acid-soluble are the most mobile fluorine forms, that is why their contents in soil require to be monitored exactly within framework of regional and national projects.

2. Materials and Methods

To elicidate the soil activity ability to accumulate gaseous fluorides from down air the model experiments had been carried out using defined zonal soil: ordinary low in humus chernozem and medium loess-like loam with the agrochemical characteristics, as follows (per 100 g of soil): 208.4 mg, N_{total}; 13.8 mg, P_2O_5; 30.3 mg, K_2O; 4.49% humus; water extract pH 7.7. Soil samples were fumigated with HF concentrations (mg/m^3): strong- 0.4; middle - 0.2; weak - 0.02.The acid-soluble fluorine forms were extracted with 20 ml $HClO_4$ (pH=1.0), and 3 ml 30% sodium citrate; the water-soluble fluorine forms amounts were determined in standard water extract with volume aliquote of buffer, consisting of (in g/l): 61.5, CH_3COONa; 58.5 NaCl: 0.348; and 14 ml CH_3COOH. Fluorine contents were measured by potenciometric method with aid of ionomer "EV-74" (Russia), when fluorine-selected electrodes "Crytur" (Czechia) or EF-IV (Russia) were used.

3. Results and Discussion

Data in table 1 indicate that ordinary chernozem accumulated fluorine from atmospheric air in dependence on duration of soil exposure to HF and its concentration levels. Thus, when soil samples were fumigated with the weak HF concentration (0.02 mg/m^3), 10 days after F-ion of water-soluble form content was enhanced by 48.7%, 20 days after - by 46.9%, 30 days after - by 8.9 times to those in zonal soil, and 5 months after - by 41.5 times.

Table 1. Contents of mobile fluorine forms in soil samples under the model experimental conditions, $p<0.05$, $n=5$.

Variant of experiments	Fluorine content, mg/kg			
	Water-soluble forms		Acid-soluble forms	
	1	2	1	2
Control	1.56 ± 0.03	-	11.27 ± 0.33	-
0.02 mg HF/m^3 10 days	7.04 ± 0.29*	6.7	17.20 ± 4.0*	40.0
0.02 mg HF /m^3 20 days	7.27 ± 0.07*	74.9	218.67 ± 2.67*	77.1
0.02 mg HF /m^3 30 days	14.0 ± 0.56*	21.4	264.0 ± 11.99*	21.2
0.02 mg HF /m^3 5 months	64.67 ± 0.33*	190.5	430.67 ± 9.33*	44.9
0.2 mg HF/m^3 10 days	11.67 ± 0.44*	8.2	281.33 ± 16.67*	16.2
0.2 mg HF /m^3 20 days	12.93 ± 0.43*	26.4	406.67 ± 6.67*	59.2
0.2 mg HF /m^3 30 days	23.5 ± 1.0*	21.9	858.67 ± 21.33*	39.7
0.2 mg HF /m^3 5 months	225.3 ± 10.68*	20.9	2698.67 ± 61.33*	43.8
0.4 mg HF/m^3 10 days	22.33 ± 0.67*	30.9	526.67 ± 13.13*	38.7
0.4 mg HF /m^3 20 days	25.83 ± 1.17*	20.7	713.33 ± 6.67*	105.1
0.4 mg HF /m^3 30 days	28.0 ± 0.5*	52.8	1026.67 ± 26.68*	38.1
0.4 mg HF /m^3 5 months	348.6 ± 4.33*	80.1	5060.0 ± 265.58*	19.0

In column 1 - M ± m; in column 2 - ratio of deviation between paired data means to standard Student's "t"-coefficient.

The same regularity of F-accumulation was found in soil samples fumigated with both HF concentrations middle and strong. Content of fluoride-ion of water-soluble forms considerably exceeded that in non-contaminated soil under the all experimental conditions to be used. Therefore, a process of water-soluble fluorine form accumulation depends on both the duration of influence and the concentration of fluorine-consisting compounds. Furthermore, as it has been shown with data in table 1, exposure of soil samples to gaseous fluorine compounds causes an increased content of fluorine acid-soluble forms in treated soils. Thus, soil fumigation with strong HF concentration (0.4 mg/m^3) during 10 days resulted in increased content of acid-soluble fluorine forms by 47 times, 20 days - by 53 times, 30 days - by 90 times above, and 5 months - by 450 times comparing to zonal soil means.

It was interesting to study a dependence between content ratio of water-soluble/acid-soluble fluorine forms and HF concentration or duration of HF influence upon soil samples. The percent ratio of both fluorine form contents: water-soluble/acid-soluble ranged from 4 to 15% (in zonal soil, ordinary chernozem, it was 14%). Observed changes in those ratio means were nither significant no dependent on toxicant influence.

To determinate the concentration ratio of fluorine forms with various mobility in soils near an effective source of gaseous emissions we selected the samples in zones with different contamination levels. Data in table 2 showed that contents of the more mobile (water-soluble) fluorine forms weren't increased to the contents of less mobile (acid-soluble) forms. Concentration values of the water-soluble fluorine forms ranged from 9 to 16% in soil samples selected in all the contamination zones. In particular, percent ratio means of the water-soluble/acid-soluble fluorine form contents were, as follows: 9.9% for soil samples from the zone of strong contamination, 15.8% - for the middle contamination, and 13.1% - for the weak one.

Table 2. Contents of mobile fluorine forms in soils near a source of gaseous emissions, $p<0.05$, $n=5$.

Sites of sample selection	Fluorine content, mg/kg			
	Water-soluble forms		Acid-soluble forms	
	1	2	1	2
Strong contamination	121.25 ± 10.63*	89.1	1216.21 ± 88.99*	240.3
Middle contamination	84.53 ± 13.45*	41.5	533.68 ± 81.79*	53.8
Weak contamination	32.40 ± 10.12*	18.1	194.52 ± 44.06*	41.1
Sanitary-protection zone	4.73 ± 0.44*	10.1	36.08 ± 4.16*	38.5
Background	1.11 ± 0.02		15.91 ± 0.69	

In 1 column - M ± m; in 2 column - ratio of deviation between pairred data means to standard Student's "t"-coefficient.

Executed investigations resulted in conclusion that either in native soil a content of the most mobile fluorine forms (water-soluble) was 6-17% to a content of the less mobile acid-soluble forms, or in soil contaminated.

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METAL CONTAMINATION OF SOILS AND CROPS FROM TEXTILE AND DYEING FACTORIES IN NARAYANGANJ INDUSTRIAL AREAS.

ISLAM M., ULLAH S. M. and MOLLAH D. H..

Industrial pollution due to heavy metals and organic toxic substances released from industrial wastes and effluents is a serious problem and a matter of great concern. They affect soil, water and air quality. The pollutants both organic and inorganic enter into food chain through their accumulation in vegetables, fruits and cereal crops. The heavy metal enriched food may endanger human health causing serious diseases such as kidney and liver damages, genetic deformation, cancer and many other complications. Some of the heavy metals such as Zn, Ni, Cd, Cu, etc., are also phytotoxic. Moreover these pollutants especially of organic origin affect aquatic lives resulting in decline in fish population, species and quality in natural canal and river water systems.

In the developed countries the wastes and effluents are treated to safe level before their discharge into the natural ecosystems. In Bangladesh they are disposed of at random into soils, canals and rivers without any treatment. Therefore, they pollute and impair soil sediments and water quality affecting human health, terrestrial and aquatic lives due to the presence of Cu, Zn, Cd, Ni, Hg, As, Pb, Cr, phenols hydrocarbons, PCBs etc. in excess amounts in them.

Narayanganj dyeing and textile industrial area was selected to investigate the extent of pollution and their transfer into plants and food chain. The experimental site is located 8km west from Narayanganj Municipality. It is within the greater Dhaka City. It is 20km north from the capital. Sampling was carried out in 4 spots. Three spots were at Enayetnagar and one at Bholail. This area is becoming heavily polluted with untreated wastes and effluents. Soils effluents and agricultural crops were collected and analysed to assess the extent of pollution.

The soil pH of investigated area varies from 4.5 to 6.0. Low pH was obtained at Enayetnagar (4.5). This was due to the use of acid and phenolic compounds. Soil pH was not influenced by the wastes and effluents of the factories because of buffering capacity of soil, although pH of effluents were 7 to 9.6. This area was highly contaminated with Pb(188ppm), Ni(152ppm), Cr(642ppm), Fe(18614ppm). The uptake of heavy metals found higher in grass and waterhycinth than rice. Higher concentration of Cr was found in grass (450ppm). Accumulation of Cr in grains was higher than shoots of rice plants (30ppm). Uptake of Pb was high in waterhycinth and low in grass and rice. Accumulation of Ni was higher in grass (160ppm). Uptake of Fe was higher in waterhycinth than in grass and rice.

HEAVY METALS IN SOILS AND PLANTS IN THE AREA OF HISTORICAL COPPER MINING CENTRE IN THE LANDSCAPE PARK „CHELMY", POLAND

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1. Introduction

Non-ferrous metal mining and smelting is known as one of the major sources of soil and plant pollution with heavy metals (ALLOWAY, KABATA-PENDIAS and PENDIAS). Soils and plants were examined in the area of historical mining and smelting activities from 15th -19th centuries. Copper was excavated from the ores deposited in a shallow flat beds inside the hill Mlynik. Spoil material and slack from the smelting process, containing relatively high amounts of Cu and other metals, were dumped on the top and slopes of the hill and in the vicinity of a smelter in the village Chelmiec. About 100 years ago, both mines and smelters were closed and the area was successively aforested with decidous tree species. Neither trees nor herbaceous plants growing now in those sites show any evident signs of copper or other metal toxicity. However, on closer examination it can be easily seen that copper rich minerals are very common in stones remaining on the soil surface. This fact prompted the authors to take on the study on metal concentration, mobility and bioavailability in this area.

2. Materials and Methods

Soil material and plant samples were collected in 9 spots - representing dumps, the most likely localisation of former smelters and in their vicinity. Basic soil characteristics were determined by common methods of soil analysis. Pseudototal concentrations of heavy metals in soil samples were measured after wet digestion in the mixture of nitric and perchloric acids. Easily soluble forms of metals were determined after extraction with neutral salt solution 1 M NH_4NO_3 (KARCZEWSKA et al. 1996). Heavy metal uptake by plants was determined by analysis of plant tissues of the most abundant herbaceous species present in a forest undergrowth: *Impatiens vulgaris, Galium odoratum, Asarum europaeum, Aegopodium podagraria* and a mixture of different grass species with domination of *Festuca ovina*

3. Results and Discussion

The results obtained from the study are presented in Tables 1 and 2. Parent rocks from which examined soils developed, contained high amounts of heavy metals. In many cases, they occurred in easily mobilisable forms, as determined in the solution 1 M NH_4NO_3, which can be easily explained if considering the reaction pH of soil material - either acidic (soils 3,4) or slightly alkaline (soils 1,2), with enhanced solubility of organo-mineral complexes (BRÜMMER 1986; MC BRIDE 1989). Those high amounts of extractable metals in soils did not correspond with equally high metal concentrations in plant materials, even though some cases should be considered as cases of metal hyperaccumulation.
A question should be put here what is the mechanism of plant tolerance to such high concentration of soluble metals. Is this an effect of non-equilibrium processes with inhibited metal desorption (HOGG et al. 1993, MC BRIDE 1989), or rather the result of metal complexation with relatively big organic particles forming non-bioavailable compounds, or maybe these results reflect the specific physiological adaptation of selected plants?

4. Conclusions

- The weathering process of dump material led to development of shallow soils rich in heavy metals: Cu, Pb, Zn, Mn and Cd.
- Despite tha fact that metals occurred in soils in easily soluble forms, their bioavailability for plants was relatively low. In such situation, further research is needed focussing on metal forms in soils and soil solution, their transformation, and the mechanisms of plant tolerance in such conditions.

- More detailed examination should concentrate on the fate of heavy metals in this extremely interesting ecosystem, with special attention put on the processes of organic matter transformations (humification and mineralisation).

Table 1. Description of selected study objects and basic features of soils.

No	Object	Horizon	Depth	pH 1M	Corg	Soil texture (1), %			Total concentrations				
						>1	<0.02	<0.002	Cu	Pb	Zn	Cd	Mn
			cm	KCl	%	mm			mg/kg				
1	mine dump	Olf	10-5	6.1	49.5	-	-	-	847	206	510	5.0	2150
		Oh	5-0	6.7	36.3	-	-	-	8330	342	440	8.8	3890
		Can	0-30	6.3	1.27	40	50	17	9850	37.5	105	4.2	2980
2	mine dump	Olf	15-9	6.5	44.7	-	-	-	2260	512	760	8.6	4200
		Oh	9-0	6.9	33.1	-	-	-	4720	734	1410	16.6	7850
		Can	0-40	6.9	0.07	81	31	15	9940	188	422	5.9	3560
3	hill top	Of	10-5	3.9	45.8	-	-	-	252	226	270	5.7	8530
	distance 50 m	Oh	5-0	3.1	42.6	-	-	-	398	524	285	5.3	2890
	from dump	A	0-8	3.0	3.60	1	19	5	1160	158	280	3.5	2320
		IIC	30-60	3.2	0.26	6	68	44	51.5	61.6	308	1.8	6750
4	dump	(O)	0-3	3.8	38.9	-	-	-	376	9250	1390	11.1	3230
		ACan	20-30	3.9	1.78	75	23	5	840	4340	1505	8.0	1280
5	smelter	A	0-10	5.8	5.84	20	6	1	497	60.1	265	0.55	1930
	site	ACan	15-25	6.4	1.59	52	21	5	2700	38.6	223	0.25	2320
		IIC	40-60	6.2	0.23	7	36	13	68	15.5	170	2.10	1980

Soil texture (1) expressed as: fraction >1 mm - % of total, fractions <0.02, <0.002 mm -% of fine soil (< 1 mm)

Table 2. Mobile forms of metals and metal uptake by herbaceous plants and grass.

	NH_4NO_3 extractable metals, mg/kg					Plant	Metals in plant tissues, mg/kg				
	Cu	Pb	Zn	Cd	Mn	species	Cu	Pb	Zn	Cd	Mn
1	7.7	0	4.5	0.65	234	*Aegopodium podagr.*	17.5	8	82	1.1	121
	62	0	1.7	0.60	204	*Galium odoratum*	13.5	4	125	5.6	528
	1105	0	0.7	0.42	136						
2	25	0	4.0	0.70	148	*Asarum europaeum*	15	8	83	4.1	138
	39	0	3.9	0.65	159	*Galium odoratum*	16.5	7.5	65	5.1	216
	1988	0	5.0	0.62	108						
3	4.1	0	28.5	0.50	1700	*Impatiens vulgaris*	17.5	5	138	6.6	1010
	6.5	12	32.2	0.55	705						
	8.2	11.8	9.5	0	216						
	0.3	0	16.3	0.45	325						
4	13.2	141	185	1.68	440	grass (*Festuca ovina*	8.5	31	100	3.0	141
	26.8	650	160	2.62	35.5	et al.)					
5	7.6	0	3.2	0	41.0	*Aegopodium podagr.*	11.5	8	88	3.7	187
	46	0	1.8	0	51.8						
	0.9	0	0.5	0	5.5						

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PB DISTRIBUTION PATTERNS IN VEGETATIVE ORGANS OF PLANTS IN THE AGROSYSTEM UNDER DIFFERENT LEVELS OF POLLUTION

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1. Introduction

Regularities of the behaviour of heavy metals such as lead in the systems "soil-plant" are to be studied for developing a database that can make a basis for working out recommendations for ecologically safe agriculture.

2. Materials and Methods

The analysis of a lead accumulation in different organs of agricultural plants was carried out on the data of vegetation, microfield experiments and field studies. Pb accumulation was studied in the vegetative organs of grain and bean crops, grown on different soils (chernozem, soddy podzolie and peat). In Pb level simulation in vegetation and microfield experiments different Pb doses (from 150 to 40000 mg/kg of soil) and compound forms (water-soluble salt Pb(NO3)2) and hard soluble oxide PbO were applied.

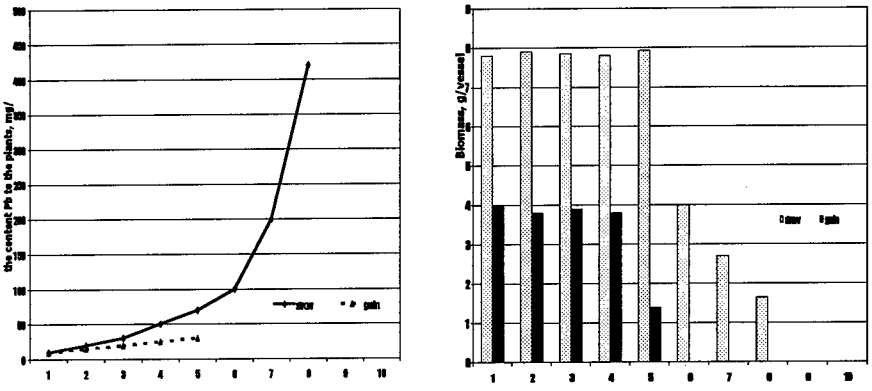
3. Results and Discussion

As a result of the studies it has been established that Pb contents in plants increase with increasing Pb doses, applied to the soil, achieving high values, until sometimes exceed the allowable limits for forage and food chains. However, Pb supply to plants is determined by the properties of the soil itself in addition in each case. Thus, considerable differences have been revealed between Pb accumulation levels in the vegetative organs of the plants, grow, on acid soddy podzilie soil and cultivated chernozem and peat soils. Pb absorption is not constant in the process of vegetation and changes depending of a phenophase and physiological features of plants. The highest levels of Pb accumulation were observed in plant leaves and stems. Lower levels were found in generative organs (grain).

For analysing the Pb behaviour in the system "soil-plant" multifactor regression models, non-linear for the general case, have been produced. Gross Pb content in the soil, soil pH, cation exchange capacity, humus content and amounts of other accompanying heavy metals (Zn,Cd) were used as factors. The content of Pb in plants, crop yields, and the concentration of the movable forms of Pb compounds in the soil was defined as a response.

The regression models have confirmed that soil composition and properties determine Pb translocation in plants and, as a consequence, a form of Pb compound in the soil. That is why the effect of the following factors is particularly distinct: soil pH, humus content, biological features of plants and other heavy metal content. Thus, high doses of Zn decreased Cd supply to plants in the experiment of PbO, ZnO and CdO into the soil.
The highest mobility of Pb compounds and their access to plants are characteristic of acid soddy podzolic soils. The soil has a neutral pH and high humus content and contains lower amounts of the lead, accessible for plants. Thus, lime and mineral fertiliser application and high humus content improve buffer properties of the soil which results in a transfer of the movable Pb

compounds, introduced into the soil, into forms non-accessible for plants, which result in a drastic decrease of its content in the vegetative organs of plants.



DOSE OF ENTRANCE PB (MG/KG) ACCORDING 1-10, 2-150, 3-300, 4-600, 5-1200, 6-2 500, 7-5 000, 8-10 000, 9-20 000, 10- 40 000

Figure 1. Influence Pb in the Crop of oats and entrance it to plants

4. Conclusion

The concentrations of the lead in soil, having a toxic effect on plants, obtained here, are to be improved in field studies for each soil and climatic zone and the level of pollution of an area. Thus, the ratio of Pb movable form concentrations for different natural zones and soil is dynamic in time and determined by soil properties and composition, agrotechnical methods and weather conditions. Because of this, "adaptation" coefficient, that adjusts the specific features of various natural and climatic zones has been introduced into the regression models.

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HEAVY METAL CONTAMINATION OF AGRICULTURAL LANDSCAPES IN THE MOSCOW REGION

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1. Introduction

The Moscow region is the largest industrial and urban agglomeration in Russia. The agricultural lands here are subject to permanent impact of various toxic chemicals including heavy metals (HM). Therefore it is important to evaluate the existing contamination of the area and to forecast the HM accumulation in arable soils. This data is required for adaptation of crop cultivation technologies to HM contamination in order to reduce the risk of HM uptake by the crops.

2. Materials and Methods

The study area is situated in the centre of the Russian plain composed of sedimentary rocks, mainly, of mantle, moraine loams and fluvioglacial sands. The region's climate is moderately continental with periodical excess of moisture in the northern part. The agricultural lands occupy 37,4% of total area, about 70% of which being arable lands. The arable soils for the most part are soddy-podzolic. Swamp-podzolic, grey forest, alluvial soils and chernozems have less extension. The present study summarises the preceding research results (ACHKASOV, 1987; AKIMOVA et al., 1994 and others). Data processing includes preliminary geochemical analysis, mapping and balance estimates.

3. Results and Discussion

Three main sources of contamination were disclosed. They are: fallout from atmosphere, non-standard fertilisers and meliorants, organic and mineral fertilisers. The HM input from the atmosphere causes considerable accumulation of Cu, Zn, Pb in soils. Moreover, atmosphere fallout contains sulphur and nitrogen oxides and therefore has low pH values enhancing HM mobilisation in soils (SIZOV et al., 1990). The spatial distribution of HM fallout rates was received by AKIMOVA et al. (1994). The compiled map takes into account the indexes of: industrial stress, energy consumption density, degree of urban development, ecosystem stability, air contamination, water resources utilisation.

The agriculture also contributes to the soil contamination. The agricultural chemicals are enriched in Hg, Ag, Cr, Zn,Cu, Ni, Co, V, Pb. The danger of HM pollution decreases in the following sequence: wastewater sediments > composts of solid domestic wastes > polluted river water > mineral fertilisers > organic fertilisers. Along with the HM amount increase in soil, the fertilisers change HM mobility and therefore influence the plant uptake. Organic fertilisers are capable to fix HM in immobile forms. Organic fertilisers with high exchange capacity form stable and low-soluble metal-organic complexes. The other mentioned chemicals enhance the HM mobility. The field experiments with wastewater sediments (SADOVNIKOVA and KASATIKOV, 1995) showed that they promote the HM exchange fraction increase accompanied by stable compounds reduction even with agricultural lime.

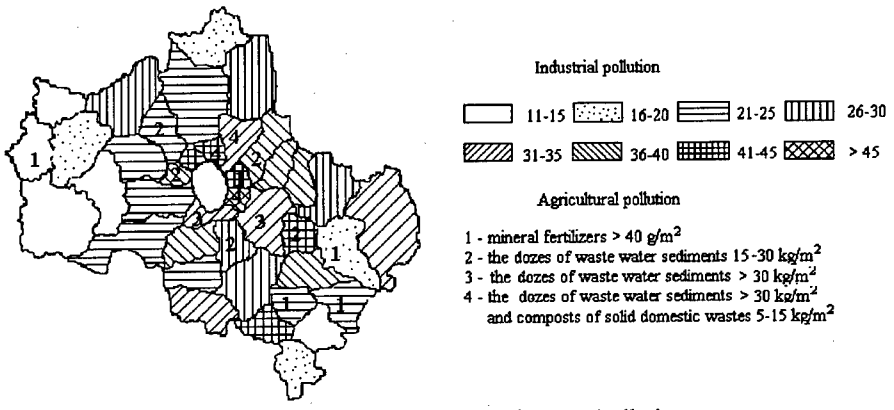

Figure 1. Pollution map

The information available on the subject of increased loads of fertilisers (ACHKASOV, 1987) was marked on the map of HM atmosphere fallout rates. The result map allows defining the districts with high levels of crops contamination risk (figure 1). The combined impact of industrial and agricultural contamination sources is most significant in districts, which are nearest to Moscow. So they require quality control of agricultural production.

To obtain ecological forecast for separate parts of the region HM balance in soils was estimated for two key areas located to the west and to the south of Moscow. The results revealed the positive balance of all HM. In the southern area the main source of HM is the input with fertilisers, in the western area the aerial incomings are. From the balance equation the rates of HM accumulation in soils and the period for which HM contents will reach threshold limit, were also determined.

4. Conclusions

The further research implies the field measurements of input and output balance components in different parts of the region and the numerical modelling of various scenarios of anthropogenic impact.

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ZN-FLOW IN SOIL-PLANT-ANIMAL SYSTEM WITHIN A POLLUTED AREA

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1. Introduction

The continuous emission into atmosphere of sulphur oxides and particles loaded with heavy metals from a non-ferrous metallurgical factory in Copsa Mica, for more than 50 years, caused a severe environmental pollution. The main polluting heavy metals are cadmium, copper, lead, and zinc.

An evaluation of Cd and Pb pollution in this area is presented by Lacatusu et al. (1997, 1998). The results have shown a strong pollution of soil and plants. The translocation rate of these elements from plants to animals was 18 and 8 times, respectively, higher than within a non-polluted area. This explains numerous cases of saturnism and chronic intoxication, especially with horses and cattle.

This paper presents some aspects regarding the zinc effects on soils, plants, and animals within the considered area as compared to a non-polluted area located in the same landscape conditions.

2. Materials and Methods

Six pastures, lying at different distances (between 0.5 and 14.0 km) from the emission source were investigated. Soil and plant samples were collected from these pastures. The soil samples were collected in the first 0-20 cm layer, and the plant samples were collected around the soil sampling point, up to a distance of 20 m.
The total and mobile zinc content in soil and zinc content in samples of vegetation and animal organs were determined using atomic absorption spectrometry. The fractionation of total Zn in soil was performed using the procedure proposed by Lacatusu and Kovacsovics (1994).

3. Results and Discussion

The soil in pastures is represented by Argilluvic Brown soils and Regosols. On an average, the total Zn content in topsoil of the polluted area represents 62% of the maximum allowable limit (300 mg/kg), but is 2.5 times higher than in a non-polluted area (figure 1). This means that the soil is being slowly polluted with Zn.
The general acidification of soils, to which acid rains contribute, determine the mobilisation of zinc in soil. This is reflected by the fractions dependent on the soil solution (17.2% of the total Zn), exchangeable complex (16.2%) and bound by the organic matter (22.9%) as compared to the fractions bound by iron and manganese oxides (12.5%) or the crystalline lattice of soil silicates (31.1%).
The floristic associations of pastures are predominantly characterised by species of *Festuca rubra, Poa pratensis, Agrostis tenuis* having a moderate grazing value and a high content of Zn, on an average, up to 2-3 times higher than the plants in a non-polluted area (figure 1). From the vegetal and animal nutrition point of view this content is placed in the normal-high range.

Animals ingesting low quality and rather toxic forage present progressive health deterioration. Numerous cases of saturnism and chronic intoxication, especially with Cd and Pb, have been recorded.

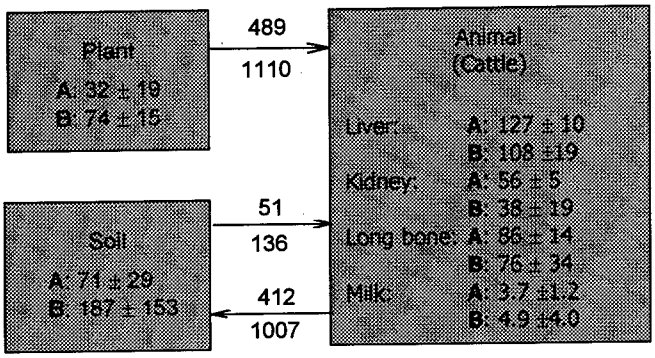

Figure 1: Zn flow in soil-plant-animal system within a non-polluted area (**A**) and a polluted one (**B**) with heavy metals during grazing period.
(Units of measure: *mg/kg* for soil, plant and animal organs and *mg/day* for the transfer between environmental components)

There was no record of a high difference between the values of Zn content in the target organs (liver, kidney), long bone, and milk of animals from the two areas: polluted and non-polluted. Much more, in the samples of animal organs from the polluted area smaller quantities of Zn than in samples from non-polluted area were determined, however the zinc translocation rate between plant and animal and between soil and animal was 2.2 and 2.7 times, respectively, higher in the polluted area than in the non-polluted one. This contradiction is due to the antagonistic effect between Cd-Zn and Cu-Zn, on one hand, and the synergetic effect of Pb-Zn on the other hand (Ghergariu, 1980).

Otherwise, one can say, on the basis of normal Zn content in cattle organs (Chesters and Quarterman, 1979), that Zn in animals from the Copsa Mica polluted area is at a deficiency level.

4. Conclusions

- The soils and plants in the pastures of the Copsa Mica area are being slowly polluted with Zn, but are strongly polluted with Cd and Pb.
- The Zn translocation rate between plant and animal and between soil and animal in the Copsa Mica area was 2.2 and 2.7 times, respectively, higher than in a non-polluted area.
- Antagonistic effect of Cd and Cu has determined the accumulation of Zn in organs and long bone of cattle at a deficiency level.

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CONTAMINATION OF SOILS, SEDIMENTS, PLANTS AND WATERS BY NATURAL OR MINED ORE DEPOSITS IN SWITZERLAND

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1. Introduction

The more than 200 mostly small ore deposits of the central and western Alps have only occasionally been subject of scientific interest in the last 30 years, and, apart from uranium and tungsten in the 70s, only of very limited economic interest either. However, most of these ore deposits had been mined during several historical periods (middle ages, 19th century, first and second world war) but were subsequently forgotten, overgrown by vegetation and filled with water which could leak to local ground and surface water. In Switzerland, little attention has yet been payed to possible natural or anthropogenic contamination of these sites through weathering and erosion of old addits and mine tailings.

2. Materials and Methods

For elements such as U, As, Ni, Cr, Pb, Zn and Cu recent environmental data are available.The environmental compartments influenced by ore deposits are different parts of a catchment and include: (1) the immediate neighbourhood, (2) the river area downstream from the ore deposit and (3) local or regional lakes. In each of these compartments, analysed potentially contaminated materials are soils, sediments, land plants and water with its mosses. For the first two, X-ray fluorescence spectrometry on solid samples has been used, whereas the analyses of the latter three are based on ICP-MS methods.

3. Results and Discussion

The possible environmental impact depends largely on (1) the geomorphological situation of the mine or natural ore deposit in question (e.g. steep slopes enhance soil creep and erosion of weathering products or mine tailings), (2) the climate (precipitation rate, vegetation cover) and (3) the solubility of the gangue or ore minerals. The natural dispersion of *Uranium* from pitchblende veins, situated on natural outcrops on steep slopes in the Martigny region (Rhone valley), has been investigated (PFEIFER et al. 1994). Soils containing between 100 and 2000 mg/kg U (uncontaminated refererence: 2 mg/kg), locally U-enriched blueberry plants and and regionally elevated U-contents in drinking water up to 40 µg/l have been observed. On the scale of the whole Rhone catchment, every year about 12 tons of dissolved uranium enters the lake of Geneva). Several former gold mines are related to arsenopyrite-veins (PFEIFER et al. 1997) and are at the origin of considerable *arsenic* contamination of soils and spring waters. In the Astano-Malcantone area (Ticino), known for its many small ore deposits, local till sediments contain between 80 and 600 mg/kg As (PFEIFER et al. 1999; world mean 7mg/kg!) and in around 12 communities of the Ticino area arsenic in the drinking water exceeds the recommended treshold value of the WHO of 10 µg/l, locally going up to 80 µ/l (even 200 µg/l in not captured surface waters). Locally grass, shrubs and trees contain 0.5 - 2 mg/kg As. A similar situation can be found at Salanfe/Wallis, where arsenopyrite rich mine tailings are situated within a pasture area

for cows. Locally low pH ponds exist (pH 5), but since snow melt waters are in the same range, they do not create environmental problems. *Nickel and chromium* are well known companions of peridotite-serpentinite lithologies, which typically display a restricted vegetation cover. Locally high pH waters can occur (PFEIFER et al. 1999), but are readily neutralized by CO_2 of the air. Although these waters exhibit high Mg-, Ni-and Cr- contents (40 mg/liter, 7 µg/l, 4 µg/l respectively), they remain below WHO guide line maximum values (20 µg/l Ni and 50 µg/l Cr). Plants such as birch trees or Erica herbacea can contain up to 40 mg/kg Ni and 3.5 mg/kg Cr. *Lead* related to abundant silver mining also is a relatively well studied element (WOODTLI et al. 1985). Natural enrichment in soils in the vicinity of galena- veins can reach values of several thousand mg/kg Pb (Astano/TI: 17'000, Goppenstein/VS 5000 mg/kg), however waters show extremely small Pb contents (max. 0.1 µg/l). *Zinc* is often found together with lead and shows similar concentration levels in soils. However it is more soluble in water: local creek water near Astano show up to 140 µg/l Zn, which remain nevertheless largely below dangerous values for drinking water (WHO guide line maximum 3000 µg/l). Only little is known yet about *copper* contamination. Close to chalcopyrite-bearing veins natural contamination in soils can reach 400 mg/kg (WOODTLI et al. 1985).

4. Conclusions

The environmental impact in terms of metal contamination of soils, plants, sediments and water in Switzerland is usually limited to the nearby neighbourhood of the ore deposits. Systematically soils and plants are heavily contaminated. Problems arise when former mine tailing are used for recreational purposes (e.g. secondary residences, tourism) or if percolating waters are used as drinking water (e.g. As in the Ticino area).

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EFFECT OF SOME MICROPOLLUANTS ON THE SOIL.

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1. Introduction

To get a reliable picture on the accumulation of elements, a statistically sufficient number of samples has to be analyzed. Such analyses of a hundred of soil, plant and fertilizer samples were carried out earlier, using ICP technique (FODOR, 1995; KÁDÁR, 1995; SZABÓ & FODOR, SZABÓ & KÁDÁR, 1994; SZABÓ-WILLIN, 1995, 1996 a, 1996 b).
The present paper aims to supply data on soil contamination caused by soluble salts of micro-pollutants added to the soil in a field experiment. Plant uptake of micro-elements in the experiment is presented elsewhere (FODOR, 1998).

2. Materials and Methods

The field trial on acidic brown forest soil with clay illuviation - discussed in the present paper - includes 8 toxic elements (Al, As, Cd, Cr, Cu, Hg, Pb, Zn) and 3 levels (30, 90, 270 kg/ha), i.e. 24 treatments all in triplicate with 72 plots altogether. Each plot has a size of 35 m^2. The experimental plants were: wheat in 1995, maize in 1996 and sunflower in 1997. The main characteristics of the soil of the experimental site (Tas-puszta Experimental Station ploughed layer) were: pH_{KCl} 6.2 upper limit of plasticity (K_A) 43, humus content 3%, clay content 30-35%. Compozite soil samples consisting of 20 subsamples are collected yearly from the ploughed layer of each plot. Choosing 20-40 plants or plant parts per plot randomly, plant samples are taken annually during the vegetation period and at harvest. The total amount of elements in homogenized soil and plant samples is measured after microwave digestion cc. HNO_3+H_2O_2. In the soil samples, the so-called "mobile" fraction is determined by NH_4 acetate+EDTA extraction according to LAKANEN & ERVIÖ (1971).

3. Results and Discussion

Considering that 3 kg/ha applied element equals 1 mg/kg in the ploughed layer, it can be stated that after the first year of the experiment nearly the total amount of the applied elements could be detected in the soil in "mobile" form in the case of Cu, Pb, Cd, Zn and As, while 70-80% of the applied Cr and Hg "disappeared" in the soil in other fractions. Two years later, in 1997, the NH_4 acetate+EDTA soluble mobile fraction of the elements Cu, Pb, Zn and Cd also decreased drastically. About two-thirds of the applied amount cannot be followed in soil by this method. At the same time, Cr and Hg almost completely "disappeared". There is also a transformation or fixation of these elements in less mobile form.
After harvesting the second crop in 1996, the "total" and "mobile" fractions were both measured in the 0-30, 30-60 and 60-90 cm soil layers of the plots with the highest (270 kg/ha) load. The conclusions concerning the vertical movement of the elements can be summarized as follows: As the elements are basically left in the upper 0-30 cm layer, deeper layers have not been contaminated significantly yet. Cd mobility cannot be proven either, deeper layers did not show any increase in total or in mobile concentration. Cr, applied as Cr(VI) was washed down to the 60-90 cm layer with precipitation, which might have been caused by the rainy and wet weather of 1996.
Cu, Hg, and Zn were obviously fixed in the upper layer, the soil profile showed no contamination with these elements. Pb, like As moved downwards somewhat, the mobile fraction in the 30-60 cm layer increased as compared to the control. Of course, the leaching of elements with time cannot be excluded in the long run (Table 1).

4. Conclusions

In a field trial on acidic brown forest soil the effect of 8 micro-pollutants was studied on the soil's total and mobile element content. The As, Cd, Cr, Cu, Hg, Pb, and Zn salts were applied in soluble form with the rate 30, 90 and 270 kg/ha in 1995. After the first year of the experiment, nearly the total amount of

applied Cu, Pb, Cd, Zn and As could be detected in the ploughed layer in mobile form, while the Cr and Hg basically "disappeared" from this fraction. Two years later only about one-third of the applied Cu, Pb, Cd, Zn and As was traced in available forms, while Cr an Hg nearly completely disappeared. With time there is a fixation of these elements in less mobile/soluble forms. Cd, Cu, Hg and Zn showed no downward movement, while As, Cr(VI) and Pb mobile fractions reflected some accumulation in the 30-60 and 60-90 cm layers following the harvest of the second crops.

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Table 1: The effect of 270 kg/ha As and heavy metal load on the total and mobile element content (ppm) of the acidic brown forest soil with clay illuviation (Tas-puszta Experimental Station, 1996)

Depth of sampling	"Total" content				"Mobile" content			
	I	II	III	Average	I	II	III	Average
				As				
0-30	54	61	110	75	12.6	13.6	33.6	19.9
30-60	12	11	13	12	0.3	0.0	0.5	0.3
60-90	11	11	11	11	0.1	0.0	0.1	0.1
				Cd				
0-30	99.6	108	61	90	83.6	88.1	58.2	76.6
30-60	0.8	0.6	0.5	0.6	0.2	0.3	0.2	0.2
60-90	0.4	0.4	0.5	0.4	0.1	0.1	0.2	0.1
				Cr				
0-30	96	128	80	101	2.0	2.3	2.2	2.2
30-60	37	35	35	36	0.4	0.3	0.4	0.4
60-90	38	30	34	34	0.2	0.2	0.3	0.2
				Cu				
0-30	59	88	100	82	39.0	62.2	49.5	49.9
30-60	22	23	21	22	5.4	4.5	5.5	5.1
60-90	16	18	20	18	3.5	3.6	3.6	3.6
				Hg				
0-30	19	17	80	39	0.8	0.5	8.7	3.3
30-60	0	0	0	0	0.0	0.0	0.0	0.0
60-90	0	0	0	0	0.0	0.0	0.0	0.0
				Pb				
0-30	61	29	49	46	4.9	15.3	32.1	29.4
30-60	33	16	15	21	17.2	5.4	5.5	9.4
60-90	14	14	12	14	3.8	4.2	3.4	3.8
				Zn				
0-30	136	114	200	150	35.4	19.5	92.6	49.2
30-60	89	88	68	82	7.9	9.1	3.7	6.9
60-90	74	74	65	71	4.7	2.9	2.0	3.2

In the control soil:
"Total" ppm: Hg 0, Cd 0.6, As 9, Pb 20, Cu 25, Cr 30, Zn 85
"Mobile" ppm: Hg 0, Cd 0.3, As 0, Pb 7, Cu 7, Cr 0, Zn 10

HEAVY METALS IN A MINE DUMP IN GALICIA, NW SPAIN

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1. Introduction

Many recent investigations studied the effect of surface mining on heavy metals mobilisation, revealing high toxicity indices in mine spoils and soils affected by sulphide oxidation (Pietz et al., 1989; Perez and Calvo, 1992; Monterroso, 1995). The fate of metals coming from sulphide oxidation and mineral weathering depends on physico-chemical properties of metals as well as on metal speciation in soil solution and interactions with solid phases. Metals can be leached, taken up by vegetation or adsorbed onto soil solid phases.

The present study, carried out on the dump of the tungsten mine San Finx, in Galicia, NW Spain, aims to investigate heavy metals in the dump solid and liquid phases as well as in the dump vegetation. Zn, Cu, Cr, Ni, Pb and Cd, present in accessory minerals and object of environmental concern, as well as Fe and Mn, abundant in acid media, were studied.

2. Materials and Methods

The mine San Finx is located in Western Galicia, NW Spain. The mining activity ended in 1989, leaving a large (25000 m^2) unreclaimed dump.

30 surface soil (0-20 cm) samples were collected from the dump. *Blechnum spicant, Festuca sp., Erica arborea, Frangula alnus, Quercus robur* and *Salix atrocinerea* were sampled in three small locations where they existed as spontaneous vegetation (most dump was unvegetated). The whole plant was sampled in the case of herbaceous vegetation, leaves and twigs from the higher third of trees.

The following analytical determinations were carried out on the spoil materials: (1) pH in water and in 1 M KCl (1:2.5), (2) available heavy metals, extracted by Mehlich 3 reagent (Mehlich, 1985), (3) exchangeable heavy metals, extracted by 1M NH_4Cl, (4) soluble heavy metals in 1:10 aqueous extracts. Total heavy metals were determined on spoils and plant materials after wet digestion in H_2SO_4 and H_2O_2. Fe, Mn, Zn, Cu, Cr, Ni, Pb and Cd were determined in all cases by flame atomic absorption spectrophotometry.

3. Results and Discussion

pH in water ranged between 3.1 and 5.0 and in KCl between 3.0 and 4.4. The pH values correlated negatively to sulphate contents ($r = -0.46$, $P<0.01$), so the most acid conditions derive from H^+ release from sulphide oxidation.

Ranges of total and different heavy metal fractions are presented in Table 1. According to the toxicity limits proposed by Tietjen (1975) and Kabata-Pendias and Pendias (1984) for total heavy metal contents, a high risk of Cu and Zn phytotoxicity is expected in the dump, along with Mn phytotoxicity risk in some cases.

Available heavy metals, being likely more meaningful than total heavy metal contents, follow the same sequence as total heavy metals (Table 1).

Table 1. Ranges of different heavy metal fractions

	Total (mg kg^{-1})	Melhich-3 (mg kg^{-1})	1M NH_4Cl (mg kg^{-1})	1:10 aq. extract (mg kg^{-1})	Vegetation (mg kg^{-1})
Fe	4316-31579	40-1550	0.8-37	1.0-5.0	122-1278
Mn	295-2105	5-109	1-133	<<	83-2744
Cu	274-5421	28-954	20-1866	1.0-83	19-107
Zn	74-895	2-100	0-106	0.5-13	30-1038
Cr	0.01-30	<<	<<	<<	<<
Cd	<<	<<	<<	<<	<<
Ni	<<	<<	<<	<<	<<
Pb	<<	<<	<<	<<	<<

<<: undetectable

Organic C, effective cation exchange capacity, pH-H_2O and total S explained 70% to 39% of available Fe, Cu, Zn and Mn variance. Several authors attributed the difficulty to correlate available heavy metals in mine dumps to individual soil parameters to the usual heterogeneity of spoil materials (Monterroso, 1995). According to the toxicity limits proposed by Schafer (1979) for DTPA-heavy metals and correlations between Mehlich 3- and DTPA-extracts derived by Monterroso et al. (1999), only Fe and Cu exceeded the toxicity limits.

Available and exchangeable contents of Cu, Mn, Zn and Fe correlated significantly ($P<0.01$). The exchangeable fraction was a high portion of available Cu, Zn and Mn, whereas a minor fraction (0- 6%) of available Fe was exchangeable. In 1:10 aqueous extracts, Cu prevailed, followed by Zn and Fe (Table 1). The low Fe concentrations, contrasting with Mehlich 3 extracts, result from the low solubility of iron compounds: iron oxide or hydroxide precipitate even at pH<3 (Nordstrom, 1982). The heavy metal contents in plant materials (Table 1) indicated that the herbaceous vegetation accumulated more Fe and Cu, whilst the trees exhibited higher Mn and Zn concentrations. According to Kabata-Pendias and Pendias (1984), most plant samples present critical Mn and Cu levels and some *Salix atrocinerea* samples present critical Zn levels.

4. Conclusion

Total heavy metal contents in spoil materials point to copper toxicity and, in some cases, manganese and zinc toxicity. Available heavy metal contents point to copper and iron toxicity, whilst contents in plant materials reveal critical Mn and Cu levels in all species and critical Zn levels in *Salix atrocinerea.*

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Remediation and Restoration of Polluted Environments

(Technical Session 15)

REMEDIATION OF THE JALES MINE SPOIL, TOLERANCE OF *HOLCUS LANATUS* L. TO ARSENATE

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1. Introduction

To prevent erosion from bare industrial sites, revegetation has often been proposed as a solution against the dispersal of contaminants. The spoils of the former Jales gold mine, Portugal, are heavily contaminated with As. The spoils are sparsely colonized by a few plant species. Survival and growth on heavily As-contaminated mine spoils led to the assumption that several species are able to evolve mechanisms to tolerate arsenic. Reduced influx of arsenate by suppression of the arsenate/phosphate uptake system has been shown to be a mechanism of arsenate tolerance in *Holcus lanatus* (Meharg and Macnair, 1990). In this study it is investigated whether the grass *H. lanatus*, colonizing the Jales mine spoil, has potential to be used for revegetation purposes and whether a small concentration of phosphate can ameliorate the effect of As toxicity, for this genotype.

2. Materials and Methods

A As-sensitive genotype of *Holcus lanatus* came from an uncontaminated site at the university campus, Vila Real. An As-tolerant genotype was collected at the Jales mine spoils. In the lab, plants were grown in a 1/4 strength Hoagland solution (pH 5.0). Arsenate was added as $Na_2HAsO_4 \cdot 7H_2O$. During root growth experiments the nutrient solutions were renewed every 3 days. The solutions contained subsequently 0.5, 10, 20, 40, 80, 160, 320, 640 and 1280µM arsenate (As), in the presence as well as absence of 10 µM phosphate (P). Tillers of both genotypes were allowed to root during 7 days in a phosphate free nutrient solution prior to experiments.

Root growth is used to assess As-tolerance. The root growth experiments took place in a growth chamber at 20 °C, 85% RH and a 16 h. photo-period. Roots were stained black in an suspension of active coal followed by rinsing in deionized water (Schat and Ten Bookum, 1992). Root growth measurements were done after 3 days esposure to the different treatments. Maximum root growth (MRG; De Koe *et al.*, 1992) data were used to calculate the tolerance index: TI% = [(mean MRG in As treatment)/(mean MRG in control)]x100.

3. Results and Discussion

In a preliminary experiment it was determined, that from day 7 till day 10 after initial rooting, maximum root growth (MRG) of both genotypes of *Holcus lanatus* is constant. Further more it was observed that without the presence of As, *H. lanatus* grows longer roots in the absence of P, probably due to its adaptation to low P concentrations in the spoils.

MRG of *Holcus lanatus* shows a reduction due to As in the solution, for both As-sensitive and As-tolerant genotypes. *H. lanatus* from Jales have a higher MRG, even in the lowest As concentrations (Fig. 1). The EC_{50} values for the As-sensitive Vila Real genotype and the As-tolerant Jales genotype were at 15 and 40 µM As respectively. For *H. lanatus* from Vila Real, the EC_{100} was measured at an external As concentration of 640 µM As. The Jales genotype showed still root growth at 1280 µM external As.

In the presence of 10 µM P, it appears that the EC_{50} value of the As-sensitive genotype is higher (EC_{50}=24), while the EC_{50} value of the As-tolerant Jales genotype seems not to be positively affected (EC_{50} = 34).

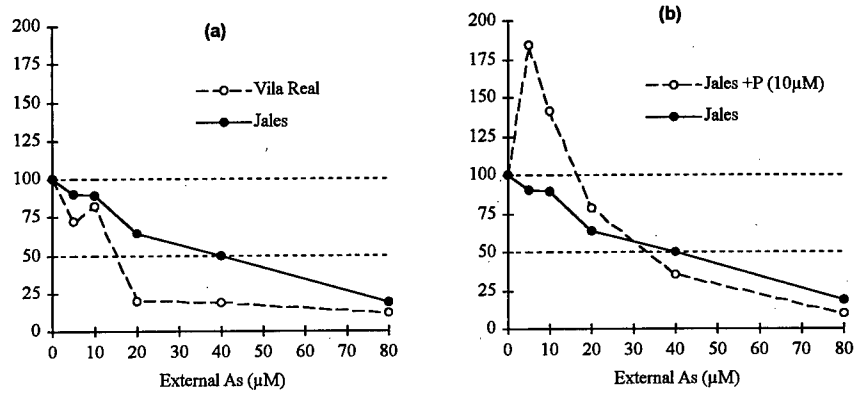

Figure 1. Effect of increasing arsenate concentrations on maximum root growth in As-sensitive (Vila Real) and As-tolerant (Jales) *Holcus lanatus* in the absence of P **(a)**. Effect of 10 µM P on maximum root growth in As-tolerant *H. lanatus* growing on increasing As concentrations **(b)**. (Growth is expressed as mean tolerance index, TI (%) n=10).

Only at low external As concentrations, addition of 10 µM P diminishes the toxic effect of As for *Holcus lanatus* from Jales (Fig. 1b). The same was observed in tolerance tests with P addition (500µM) for As-tolerant *Agrostis castellana* from Jales (De Koe and Jaques, 1993). At higher As concentrations the effect of P on root growth of *H. lanatus* is strongly diminished.
While root growth of *Holcus lanatus* from Jales decreases already on the lowest external As concentration (5µM As), in the As-tolerant genotype of *Agrostis delicatula* MRG only started to diminsh above 80µM As (De Koe and Jaques, 1993).

4. Conclusions

Compared to the non-tolerant *Holcus lanatus* genotype from Vila Real, *H. lanatus* from Jales exhibits tolerance to As. It is expected that *H. lanatus* from Jales can be used for revegetation purposes of As-contaminated spoils. Addition of P-fertilizer will help to diminish the toxic effect of As to As-tolerant *Holcus lanatus*.

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BUNKER HILL SUPERFUND SITE: ECOLOGICAL RESTORATION PROGRAM

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1. Introduction

The Bunker Hill Superfund site in the Coeur d'Alene River Basin is the second largest Superfund site in the United States. This area had been the nation's largest and richest mining district, with mining and smelting of Zn and Pb taking place through a period beginning in 1916 and continuing until the early 1980's. Tailings from the processing of these ores were disposed of directly into the Coeur d'Alene river or left near milling facilities. As part of their remediation effort, EPA Superfund has been collecting contaminated materials and placing them in a Central Impoundment Area (CIA). A field study was installed in June of 1997 to test the ability of residuals including biosolids, wood ash, and logyard to support a vegetative cover on this high metal waste material. The study consisted of 2 phases. The initial phase was primarily a demonstration, to illustrate the potential of residuals use to EPA officials. The second phase involved smaller plots with direct comparison of residuals amendments to conventional amendments.

2. Materials and Methods

For both phases of the study, amendments were mixed and then surface applied. Residual amendments were similar for both studies. Treatments included high N biosolids applied at 44 and 66 dry Mg ha^{-1} and low N biosolids applied at 66 and 99 dry Mg ha^{-1}. All biosolids were mixed with 224 Mg ha^{-1} wood ash (equivalent to 66 Mg ha^{-1} limestone) and wood waste at a 20% volume ratio. The second study used 2 x 4 m plots in a randomized complete block design with 3 replicates. In addition to these treatments, a range of conventional amendments including "Kiwi Power" and "Biosol" were included in the experiment. Properties of the tailings and residuals are presented in Table 1.

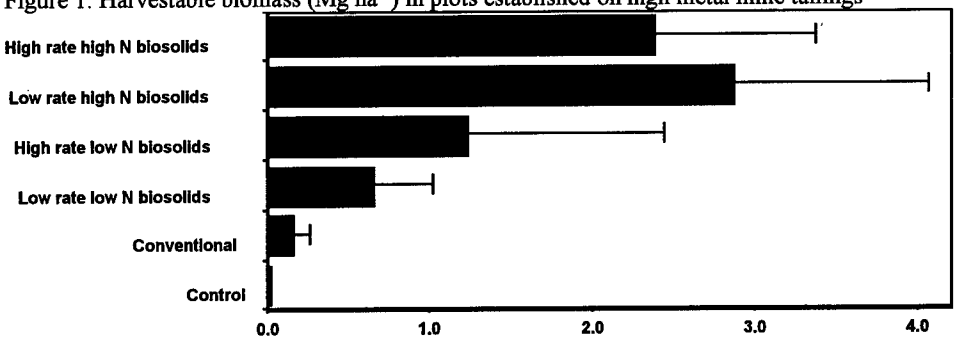
3. Results and Discussion

Plots were sampled in August, 1998. All residuals amended treatments showed a significantly greater yield response than control or conventional treatments. Harvestable biomass from the second phase of the study is presented in Figure 1. Metal concentrations in Western wheat grass from the biosolids amended treatments were within normal ranges (30-60 mg kg^{-1} Zn across all biosolids treatments). There were indications that surface application of amendments reduced the toxicity of the tailings. Plants showed extensive root growth into the tailings. In addition, application of the both the high and low rates of both biosolids treatments reduced 0.01 M $CaNO_3$ extractable Zn in soil below the amended surface from 56 mg kg^{-1} to 5.2 mg kg^{-1}. Extractable Zn in the soil below the conventional treatments averaged 43 mg kg^{-1}. Initial results indicate that application of biosolids in combination with other residuals is highly effective at establishing a vegetative cover on metal contaminated tailings.

Table 1. Characteristics of the materials used in the field trial of biosolids, wood ash, and logyard waste at the Bunker Hill, ID CERCLA site.

	Zn	Cd	Pb	pH	Carbon	Nitrogen
		mg/kg			%	
Biosolids						
High N	915	3.5	141	8.1	29	4.4
Low N	1652	21.5	380	6.5	26	2.8
Wood Ash	534	4.9	69	12.8	23	0.1
Logyard Waste	85	0.5	15	7.2	8.8	0.2
Tailings	10900	50	2550	7.2	0.5	0.05

Figure 1. Harvestable biomass (Mg ha^{-1}) in plots established on high metal mine tailings



EFFICACY OF LIMESTONE AND FERRIC HYDROXIDE AS SOIL AMELIORANTS FOR NI PHYTOTOXICITY - A POT STUDY

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1. Introduction

Emissions from a nickel refinery at Port Colborne, Ontario, Canada, resulted in soil contamination with Ni. Vegetable crops grown on an adjacent farm with a muck soil had visual symptoms of Ni toxicity, and their marketable yields were substantially reduced (Frank et al., 1982). Pot experiments were designed to test the effectiveness of lime ($CaCO_3$) and ferric hydroxide in remediation of Ni toxicity in the Ni-contaminated muck soil. Two batches of soil differing in total Ni contents were used. The "high Ni" soil contained 3090 mg kg^{-1} of total Ni, and the "Low Ni soil" containing 1360 mg kg^{-1} . Ameliorants were applied in factorial combinations.

2. Materials and Methods

Hard red spring wheat (*Triticum aestivum*) cv 'Grandin', oat (*Avena sativa*) cv 'Ogle' and redbeet (*Beta vulgaris*) cv 'Detroit Dark Red' were grown as test plants. These species represent various degrees of sensitivity to Ni phytotoxicity. Wheat is relatively resistant, while redbeet and oat are very sensitive to excessive soil Ni (Hunter and Vergnano, 1952). Experiments were conducted in a growth chamber with controlled environmental conditions. Reagent grade $CaCO_3$ was applied in both High-Ni and Low-Ni experiments at rates of 0 and 18% of soil dry weight. Freshly precipitated ferric hydroxide was amended at rates of 0 and 7% in the High-Ni experiment and 0 and 2.3 % in the Low-Ni study. The $Fe(OH)_3$ rate was reduced in the Low-Ni study because the rate used in the first experiment induced severe P and Mn deficiency and had an adverse effect on soil structure due to the pozzolanic properties of the ferric hydroxide. Basal fertilizers were added to all pots at the following rates P-510, K-504, N-190, Mg-200, Zn - 2.62, Cu-1.28, B-1.0 and Mo-0.18 mg kg^{-1}. Plants were harvested after 22 days growth in the High-Ni study, and 29 days in the Low-Ni study. After harvest, plant shoots were dried at 65 EC and dry weights were recorded. Plant samples were ashed at 480°. Ash was dissolved in concentrated HNO_3 on a hot plate and then refluxed with 3 M HCl. Nickel, Fe, Zn, Cu, Mn, Mo, K, Ca, P and Mg were determined by ICP with internal standard.

3. Results and Discussion

Wheat accumulated much less Ni than did oat or redbeet, which corresponded with the relative sensitivity of each species to Ni toxicity (Table 1). Redbeets and oats grown in the High-Ni and Low-Ni control (untreated) soils suffered from Ni toxicity. Redbeets were stunted and chlorotic. White chlorotic bands perpendicular to veins indicative of Ni phytotoxicity appeared on oat leaves. Lime and iron applications decreased Ni uptake by crops and lowered Ni:Fe ratios in plant shoots. The high Ni:Fe ratio is believed to be a better indication of the Ni toxicity than the Ni concentration in plant tissue (Crooke et al., 1954). Both amendments at least partially ameliorated Ni toxicity in oat and redbeet as Ni toxicity symptoms were not observed in plants grown on lime or iron amended soils. Decrease in shoot-Ni concentrations did not always result in yield increase because each ameliorant induced various degrees of Mn deficiency and decreased P concentrations in plants, in some cases, to deficiency levels. Plant yield was a net

effect of the amelioration of Ni toxicity and nutrient deficiencies already existing in soil or induced by the amendment application. Response to the amendments was species specific but generally $CaCO_3$ was more effective than $Fe(OH)_3$. There was no clear advantage from adding both ameliorants.

4. Conclusions

The evaluation of ameliorative effects of limestone and ferric hydroxide was complicated by a severe Mn deficiency induced by the amendments. Such lime-induced Mn deficiency may have confounded previous Ni-remediation tests with low Mn muck soils. Further study with adequate Mn and P for calcareous soil showed that these ameliorants could fully remediate Ni phytotoxicity to sensitive crops. Although application of enough Mn fertilizer to prevent Mn deficiency of calcareous muck soil would be expensive, it would be much more cost effective than soil removal and replacement. Sufficient limestone application could prevent soil acidification for long periods, achieving required remediation of such contaminated soils. Application of Fe may provide further amelioration over time as Ni becomes occluded in the Fe oxides. Plant nutrition evaluation should be an integral part of soil remediation technology development.

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Table 1. Yield and elemental composition of plant shoots grown in the High and Low-Ni soils

Treatment	High-Ni soil				Low-Ni soil			
	Yield	Ni	Mn	Ni:Fe	Yield	Ni	Mn	Ni:Fe
	g/pot	mg kg^{-1}			g/pot	mg kg^{-1}		
				Wheat				
Control	1.63a	5.27a	20.0a	0.16a	2.34a	4.54a	15.1a	0.11a
Lime	2.16a	2.77b	10.5b	0.07b	1.07bc	2.98b	6.88b	0.08a
Iron	0.38b	5.38a	7.2b	0.14a	1.45b	3.66b	9.97b	0.10a
Lime +Iron	0.45b	2.84b	11.9b	0.08b	0.70c	2.93b	7.35b	0.10a
				Oat				
Control	2.00a	78.0a	21.2a	2.3a	4.53a	45.2b	10.3a	1.8a
Lime	2.26a	50.3b	16.8ab	1.4b	3.18a	40.0b	5.36b	1.3a
Iron	0.65b	59.1ab	7.96b	1.9ab	3.22a	57.3a	4.87b	1.8a
Lime +Iron	1.32c	61.1ab	13.6ab	1.6b	2.49a	42.7b	5.52b	1.7a
				Redbeet				
Control	1.54b	92.1a	110. a	2.3a	1.98b	55.3a	58.5a	1.1a
Lime	2.96a	17.4b	13.1b	0.4bc	2.42ab	13.5c	7.49b	0.3b
Iron	0.43c	69.7c	25.5b	0.5b	3.95a	42.6b	41.9a	0.7c
Lime +Iron	1.87b	14.7b	10.1b	0.2c	3.32ab	15.9c	9.47b	0.3b

Means in column within the same plant species followed by different letters are significantly different at $P<0.05$ according to Duncan's multiple range test.

REMEDIATION OF HEAVY METAL CONTAMINATED GARDENS BY SOIL COVERING

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1. Introduction

Covering of heavy metal contaminated sites with natural unpolluted soil material can be a quite simple but nevertheless promising remediation technique. It can be applied mainly in cases where (i) direct contact between men, especially playing children and contaminated soil as well as contaminant transfer via foodchain (soil to plant pathway) for example in house or allotment gardens should be minimized, (ii) ambient atmospheric deposition of heavy metals is too low to be of relevance for a further contamination/re-contamination of plants/soils and (iii) heavy metal contamination of groundwater needs not to be considered. Results from the 'Stolberg Soil Covering Experiment' give information about the required cover thickness of the unpolluted soil layer in order to avoid a soil to plant transfer of heavy metals.

2. Materials and Methods

The experimental site is located in the western part of North Rhine-Westphalia, near Aachen. High concentrations of Cd, Pb and Zn are characteristic for the regional soils because of geogenic and anthropogenic factors (occurrence of Pb/Zn ore; rubble of historical ore mining, emissions of Pb/Zn smelters). In autumn 1989 the experiment was installed on a former meadow with a total of 7 treatments with 4 replicates (20 m² per plot; no randomization because of technical reasons). The treatments are described in Table 1, however, treatment 7 is not further discussed in this contribution. The soil material used for covering originated from a field located in the Cologne-Aachen-basin (loess derived Alfisol). Until 1997 the plots of treatment 1 to 6 were planted with 16 different vegetables[1] and in 1994 with winter wheat once. All cropping practices (e.g. fertilizing, irrigation) were done uniform and conformed to local practice. Only the edible parts of the crops were analysed for their Cd, Pb and Zn concentrations after wet digestion with HNO_3 by means of ICP or AAS. The experimental data were statistically analysed using SPSS PC+ (ANOVA, MANOVA, TUKEY-test).

Table 1: Treatments of the Stolberg Soil Covering Experiment

	treatment						
	1	2	3	4	5	6	7
topsoil material	0	40	40	40	40	40	40 cm
subsoil material				30	65	30	30 cm
drainage layer*)			15			15	15 cm
block layer*)						20	20 cm
total covering height	**0**	**40**	**55**	**70**	**105**	**105**	**105 cm**

*) additional technical barriers inaccessible to plant roots

3. Results and Discussion

Table 2 shows the high Cd, Pb and Zn concentrations in the original topsoil of the control plots (also representative for the former, now covered topsoils of the other treatments). The soil material used for covering had distinctly lower concentrations which were even below background levels of garden soils in North Rhine-Westphalia. Control and treatment plots show

[1] french beans, radish, mangold, kale and several times lettuce (several varieties), cellery, carrots, endive, spinach

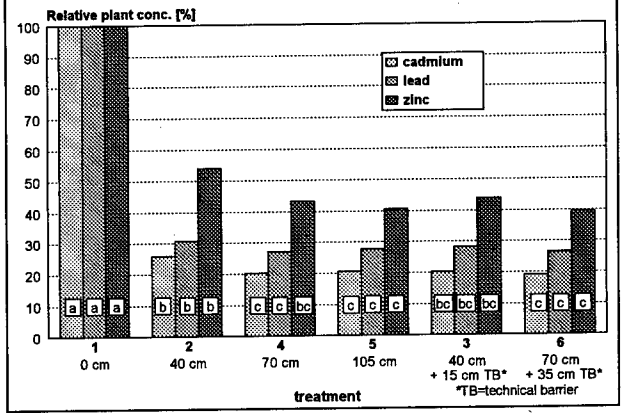
Table 2: Soil characteristics at the beginning of the Stolberg Soil Covering Experiment (heavy metals after aqua regia extraction)

	treatment		
	1	2 - 7	4 - 7
	A_p (0-30 cm)	topsoil mat.	subsoil mat.
pH-value	7,0	7,0	7,1
Corg [%]	2,7	0,9	0,5
Cd [mg/kg]	10,3	0,7	0,5
Pb [mg/kg]	610	28	18
Zn [mg/kg]	1830	76	59

equal pH-values of 7 in topsoil so that a low heavy metal mobility could be expected. Nevertheless, Cd and Pb concentrations in vegetables grown on control plots exceeded frequently by far the German threshold values for foodstuff. Zn concentrations in 10 of 16 vegetables were in the range possibly causing yield depressions. However, heavy metal concentrations in plants grown on covered treatments were below or in the range of foodstuff threshold values or yield related limit concentrations, respectively. Covered treatments differed relatively little although sometimes significantly. Only Zn concentrations were often markedly reduced by soil coverings exceeding 40 cm (treatment 2). The same applies for Cd and Zn in wheat grain.

On average, heavy metal concentrations of edible vegetable parts were reduced due to soil covering by about 80% for Cd, 70% for Pb and 60% for Zn (Figure 1). Soil coverings exceeding 70 cm and/or additional barrier techniques resulted in no further significant reduction of heavy metal uptake.

Figure 1: Average relative heavy metal concentrations in vegetables from the Stolberg Soil Covering Experiment (means of 16 vegetables; within one element different letters denote statistically significant differences at a 5% level)



4. Conclusions

In order to reduce the soil to plant transfer of heavy metals on polluted garden soils a covering of about 40 to 70 cm with unpolluted soil material is required but also sufficient to achieve tolerable plant concentrations. Obviously 70 cm are generally sufficient to cut contact between plant roots and contaminated soil. Additional technical barriers showed no significant effect in comparison to other treatments. However, a simple 'signal layer' below the unpolluted soil layer (e. g. geotextile) may be useful. No evidence was found for recontamination of unpolluted cover soils after 9 years of experiment .

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DESORTPTION OF HEAVY METALS BY SURFACTANTS AND COMPLEXING AGENTS IN THE CONTAMINATED SOILS

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1. Introduction

The contamination of heavy metals in soil environments has recently received much urgent attention. Many superfund sites are contaminated with toxic trace heavy metals in Taiwan. The use of surfactants to enhance remediation of contaminants has recently received increasing interest (Doong et al., 1997). Nivas et al. (1996) reported that the addition of surfactants could enhance the remediation of subsurface chromium contamination. Also, the complexing agent has a good capability in extracting heavy metals from contaminated soils. However, the role of surfactants and complexing agent in remediation of metal-contaminated soil is site specific, depending on the pH, matrix of soil, speciation of metal, nature of surfactant, and organic content. Also, the combination roles of surfactant and complexing agent on the remediation of contaminated soil still remains unclear.

The purpose of this study to determine the optimal surfactant system for enhancing the remediation efficiency of contaminated soil. Anionic (sodium dodecyl sulfate, SDS), nonionic (Triton X-100, TX100) and cationic (cetyltrimethylammonium bromide, CTAB) surfactants were used to elucidate the extraction efficiency of different types of surfactant. Also, complexing agents, such as EDTA and citric acid were added in combination with surfactants to enhance the extraction efficiencies of surfactants. Moreover, the pH effect was examined for determining the optimal surfactant systems.

2. Materials and Methods

Two soils were used in this study. The cadmium-contaminated (CAD) and chromium-contaminated soils (CHR) were obtained from northern Taiwan and central Taiwan, respectively. The average 0.1 N HCl extractable concentrations of nickle (Ni), zinc (Zn), lead (Pb), cadmium and chromium (Cr) of the soils were 64-112 mg/kg, 45.9-110 mg/kg, 29.2-41.9 mg/kg, 1.64-14.9 mg/kg and 27.3-68.1 mg/kg, respectively.

Batch experiments were performed by containing 5g of soil with 50mL surfactant solution in 125mL Erlenmeyer flasks. The series of reactors were equilibrated in an orbital shaker for 24 hr at 150 rpm and at 25 ± 0.2 °C. The concentrations of surfactants ranged from 0.25CMC to 10CMC. The initial concentrations of complexing agent ranged between 1 mM to 100 mM. Equilibrated samples were then centrifuged at 6000 rpm and digested with HNO_3 and $HClO_4$ to remove soil particles and interferences. The supernatants were then analyzed by ICP-AES for Ni, Zn, Cd and Cr or ICP-MS for Pb. The pH values of solutions were adjusted with phosphate buffer solution to yield pH 2.3, 7.1 or 12.3.

3. Results and Discussion

The addition of surfactants can enhance the desorption of different heavy metals from contaminated soil. SDS and TX 100 was shown to be the better surfactants in extracting zinc, lead and cadmium, whereas the addition of CTAB could enhance the desorption efficiency of chromium. Also, different desorption efficiencies among the heavy metals were observed. The desorption efficiency increased in the order of lead > zinc > cadmium> chromium. The

enhancement ratio, based on the concentration ratio of heavy metals extracted from surfactant-amended system to that of blank control, were 1.6 to 2.5 and 1.2 to 1.9 for SDS- and TX100-amended systems, respectively, showing that SDS is a better surfactant than TX100 in extracting heavy metals.

Surfactant concentration is an important factor influencing the desorption efficiencies of heavy metals. The desorbed concentration was found to linearly increase with the increasing surfactant concentration below CMC value, and remained relatively constant above the CMC.

The addition of EDTA and citric acid can significantly enhance the removal of heavy metals and the extraction efficiency increased with an increasing concentration. However, the extents of desorption differed among the heavy metals. Better desorption efficiency was found for cadmium and least for chromium. Different complexing agent concentrations at maximum heavy metal removal were observed. The optimal concentrations of EDTA in enhancing the desorption of heavy metals in CAD soil ranged from 1mM to 25mM, whereas 10 mM to 50 mM EDTA were needed to reach the maximum heavy metal removal in CHR soil. Also, EDTA is a better complexing agent than citric acid to enhance the desorption efficiencies of heavy metals.

pH value is an important factor influencing the desorption of metals with the amendment of different surfactants and complexing agents. The extraction capabilities of surfactants decreased with the increase of pH value in the EDTA- and citric acid-amended solutions. This implies that counterion binding and/or precipitation may be the major mechanisms for the extraction of heavy metals under alkaline environment. Moreover, pH value is a more important factor than the nature of surfactant in remediating lead-contaminated soils.

4. Conclusions

The results of this study demonstrate that surfactant in combination with complexing agents can be effectively used to treat the cadmium-contaminated soil by proper selection of type and concentration of surfactant and complexing agent at different pH values. The addition of complexing agents can significantly enhance the desorption of heavy metals. The anionic surfactant with EDTA was shown to be the most effective treatment in remediating cadmium-contaminated soils, whereas the cationic surfactant amended system was more sultable for chromium-contaminated soils.

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EFFECT OF ZEOLITE AND APATITE ON MOBILITY AND SPECIATION OF METALS

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1. Introduction

One of remediation techniques that could be used commonly and widely is in situ stabilization. In this technique are used easily available and inexpensive amendments such as natural minerals (zeolite or apatite) or waste by-products (Chlopecka and Adriano, 1996, 1997). These amendments do not produce drastic changes in the physical, biological, or chemical properties of the soils. Another important advantage of this technique is that soil is not excavated and amendments could be applied on a large-scale (Knox et at., 1998). This study evaluates the efficacy of zeolite and apatite in reducing metal mobility in contaminated soil. Additionally, this study focused on the effect of these amendments on metal retention in the solid phase of soil.

2. Materials and Methods

A greenhouse pot experiment was conducted using 7 kg of top soil from an Appling silt loam. The soil had the following properties: pH 5.4, particles<0.02 mm diameter, 21.5%; organic matter (OM), 25 g kg^{-1}. This soil contained 27, 15, and 0.2 mg kg^{-1} of total Zn, Pb, and Cd, respectively, which are considered as background levels. Metals such as Cd, Pb, and Zn were added separately to the soil at the two following rates (in mg kg^{-1}): Cd - 20, 40; Pb - 1500, 3000; and Zn - 1000, 2000. Metals were added to the soil as a defined mixture of various metals sources (40% as sulfate, 25% as carbonate, 20% as oxide and 15% as chloride). After soil equilibrium natural zeolite (phillipsite) and apatite were added to the soil at a rate of 25 g kg^{-1}. There were four replicates in each treatment and all pots were arranged in a completely randomized design. Three plants were grown on the potted soil: rye (*Secale cereale L.*), maize (*Zea mays L.*) and oats (*Avena sativa*). Rye and maize were harvested after 6 weeks but oats was harvested at mature stage. Aboveground parts of plants were taken for chemical analysis. The sequential extraction procedure was used to partition metals into five fractions (exchangeable, carbonate, Fe-Mn oxides, organic and residual). Metal concentrations in plant tissues were determined by wet method (HNO_3 and H_2O_2). Metal contents in all solutions were determined by AAS or ICP.

3. Results and Discussion

Stabilization of metals in contaminated soils could be expressed by stabilization factors such as: distribution coefficient K_d (the ratio of the total metal concentration in the soil to that in the solution phase at equilibrium), factor S/T(the ratio of metal content in the solution to the total content of the metal in the soil and expressed in percentage), and factor RT (ratio of residual fraction of the metals to total content of metals in soil). The lowest K_{dCd}, K_{dZn}, and K_{dPb} were observed for the highest level of metals in the blank treatment (Table 1). Addition of zeolite and apatite increased values of the distribution coefficient, i.e, decreased metal mobility. Factor S/T was very high for all treatments with metals only and decreased significantly in treatments with apatite and zeolite (Table 1). The greatest decrease in S/T was observed for Cd with apatite application. Factor RT indicates metal retention by the solid phase. Both zeolite and apatite significantly increased RT for all metals (data not shown); i.e., increased Cd, Pb, and Zn retention by the solid phase. In in situ stabilization methods, the main strategy is to reduce the concentration of metals in labile fractions and increase the least mobile fraction, i.e., the residual fraction. Data from this study clearly show a redistribution of metals to the residual fraction.

Apatite and zeolite significantly enhanced the yield of plants (Table 2) and reduced metal concentrations in the plant tissues (Table 3). Both levels of Zn were toxic to rye and maize in the blank treatment i.e., where only Zn was applied to the soil, plants died a few days after germination. Generally, the higher reduction of metal uptake was obtained in the treatment with apatite (Table 3). However, zeolite was also a very effective amendment and in most cases reduced Pb and Cd concentrations in all tested plants by more than 50 %.

Table 1. Stabilization indexes: distribution coefficient (K_d) of Zn, Pb, and Cd and factor S/T

Treatments	Distribution coefficient (K_d)			Factor S/T - % of metal in the solution/total metal		
	Blank	Zeolite	Apatite	Blank	Zeolite	Apatite
Ctrl	38.6	40.2	45.0	2.6	2.5	2.2
Zn 1000	3.5	6.0	11.4	28.2	16.6	8.8
Zn 2000	3.6	5.7	8.6	27.8	17.4	11.6
Ctrl	37.7	50	51.7	2.6	2.0	1.9
Pb 1500	25.8	87.6	126	3.9	1.1	0.8
Pb 3000	24.5	57.9	94.2	4.1	1.7	1.1
Ctrl	4.8	6.7	7.1	21.0	15	14.0
Cd 20	3.5	5.8	7.2	28.6	17.3	13.9
Cd 40	2.1	4.1	5.2	46.8	24.6	19.1

Table 2. Plant yield (g/pot); * - yield was not obtained due to Zn toxicity

Treatment	Rye			Maize		
	Blank	Zeolite	Apatite	Blank	Zeolite	Apatite
Ctrl	6.2	6.3	7.9	17.0	22.1	22.3
Zn 1000	-*	2.8	8.3	-	17.2	22.7
Zn 2000	-	0.51	4.3	-	4.2	22.2
Ctrl	6.0	6.5	7.8	16.7	22.7	24.1
Pb 1500	3.9	6.2	9.7	13.8	22.4	23.9
Pb 3000	1.6	5.9	7.8	15.4	21.9	21.8
Ctrl	6.2	6.4	7.4	16.8	22.4	22.9
Cd 20	2.5	6.0	7.1	2.5	18.3	22.3
Cd 40	2.4	5.4	6.6	2.4	17.4	21.8

Table 3. Influence of zeolite and apatite on Zn, Pb, and Cd concentrations (mg kg^{-1}) in rye and maize

Treatments	Rye leaves			Maize leaves		
	Blank	Zeolite	Apatite	Blank	Zeolite	Apatite
Ctrl	28.2	24	23.7	25.0	22.1	16.7
Zn 1000	-	1354	774	-	1093	530
Zn 2000	-	1960	1748	-	4177	1748
Ctrl	1.3	1.1	1.0	2.0	1.5	1.4
Pb 1500	101	48.0	34.0	156	60.0	45.0
Pb 3000	138	66.0	54.0	192	83.0	63.0
Ctrl	0.4	0.35	0.32	0.35	0.42	0.40
Cd 20	42.8	31.7	23.2	41.4	11.2	5.8
Cd 40	79.6	32.1	27.8	98.0	18.3	10.4

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REMOVAL OF HEAVY METAL FROM SOIL IN A CLOSED-CYCLE-BIOLEACHING PROCESS

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1. Introduction

For removing heavy metals from contaminated soil high amounts of water and acid are necessary. After processing, the water used is contaminated by heavy metals and must be cleaned. The amount of water used for decontamination has to be approximately fifty to a hundred times higher than the soil volume. Under the objective to save water and to avoid large water storage facilities, a dynamic waste water treatment is needed. In this investigation heavy metals were removed from contaminated soil using organic acids produced by heterotrophic fungus.

Fungus are not only able to produce organic acids and thus lower the pH-level, they can also use organic acids as a source of carbon needed for their growth, while the pH-value is increasing again. This mechanism of fungal metabolism can be used to develop a closed cycle process where organic acids are produced to remove heavy metals from soils and after this to clean the contaminated water in a biosorption process.

2. Materials and Methods

In shaking experiments, solutions of 0.1 mol/l citric acid concentrations were used to remove heavy metals from soil.

For optimal growing conditions for *Aspergillus niger* the contaminated waste water from the shaking experiments was diluted and adjusted to pH 4.9. Afterwards nine parallel solutions were inoculated with a culture of *Aspergillus niger* and aerated for two weeks. After the pH-values started to increase, one experiment was stopped per day and the heavy metal content was measured by using ICP-OES and ICP-MS.

3. Results and Discussion

Due to the decreasing pH-value of the soil solution not only heavy metals but also the metals from the soil buffering system are mobilised (Fig.1). For a biosorption process the mobilisation of Fe and Al is not disadvantageous however, because at pH values higher than 8 Fe forms amorphous hydroxide-complexes with heavy metals which can be removed from the solution together with the fungi biomass. In Figure 2 the elimination of zinc, lead and iron is illustrated.

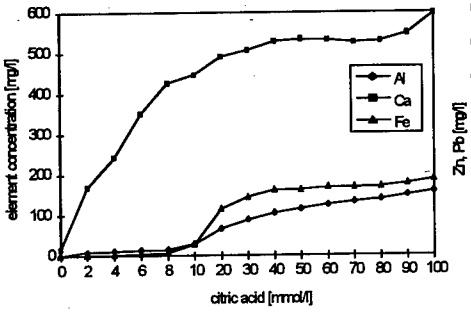

Fig.1: Mobilisation of heavy metals under the influence of increasing citric acid solutions.

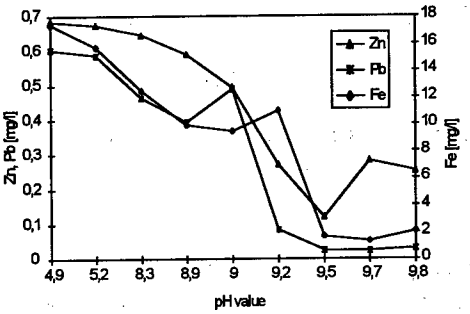

Fig.2: Immobilisation of heavy metals in a growing culture of *Aspergillus niger*. As *A. niger* grows the pH-value increases and a combination of biosorption and anorganical heavy metal elimination occurs.

The heavy metal content in solution is significant correlated with the Fe content and about sixty percent of the eliminated heavy metals can be found in the fungi biomass. So it can be assumed, that forty percent were eliminated inorganically. For different heavy metals, different results and correlations with Fe can be described. For Zn it seems that the element is not only adsorbed to the biomass surface but accumulated actively by the fungi because at higher pH levels, when the fungi is starving, Zn can be mobilised again rapidly which may result from an autolysis of the cells. In further investigations the biosorption process will be optimised to use the water again for the leaching process and to close the water cycle.

REMOVAL OF LEAD FROM SOIL BY ELECTROKINETIC PROCESS

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1. Introduction

An electrokinetic (EK) soil process involves the application of an electrical field across a porous medium to induce the movement of electrolyte solution and the transport of soluble contaminants toward the electrodes. In this process, the advection of elecroosmosis (EO) flow driven under electrical field, the movement of H^+ ions generated from H_2O electrolysis at the anode advancing through soil toward the cathode, and the migration of charged ions toward the opposite electrodes are the major mechanisms leading to the removal of contaminants from the soil. EK process has been demonstrated to be successful and cost-effective in removing both organic and inorganic contaminants from soil in many laboratory and field studies (Acar and ALSHAWABKEH 1993; LAGEMAN 1994). In this study, the feasibility and efficiency of EK process in the treatment of artificially Pb-contaminated agricultural soil were investigated. The removal efficiency of Pb pertaining to the applied voltage, the processing fluid used, and the soil Pb concentration was presented.

2. Materials and Methods

The tested soil was sandy loam collected in northern Taiwan, which has an average particle size of 190 μm, a pH_{zpc} of 2.5, a soil pH of 4.2, a specific surface area of 27.3 m^2/g, and an adsorption maximum of 18.5 mg-Pb/g at pH 5. Pb(II) in the solution was determined with an atomic adsorption spectrophotometer. The EK test specimens were assembled as shown in Fig. 1. Operating conditions are listed in Table 1. The electric current, reservoir pH, Pb concentration, and the amount of effluent were monitored during the tests. Soil pH and residual Pb profiles along the soil specimen were determined at the end of each test.

3. Results and Discussions

In all tests, the following phenomena were observed: 1. a decrease of current density with time reflecting an increase of electrical resistance that may be owing to the clogging of pore spaces by salt precipitates near the cathode, 2. lead precipitates formed in the cathodic reservoir, 3.pH in the reservoir maintained at 2-3 near the anode and 10-12 in the effluents (cathode), 4. an acidic front generated at anode reservoir flushed across the soil specimen, consequently lowering the soil pH to values around 2-4 except that in the vicinity of the cathode where the pH increased to approximately 8-10 due to the OH^- production at the catholyte, and the consequent migration of OH^- ion into soil. In a 13-day EK operation, Pb removal efficiency higher than 93% Pb was achieved under various operating conditions (Table 1). The rate of removal is directly related to the potential gradient developed in the test column. Increasing the applied voltage resulted in a better removal efficiency. A removal efficiency higher than 95% was achieved when the applied voltage was raised above 35 Volts. It was also found that use of EDTA solution as processing fluid nearly doubled the EO permeability (K_e). However, the Pb extraction rate was not affected. Nevertheless, the Pb accumulated in the vicinity of the cathode was effectively removed with the addition of EDTA to the processing fluid. Energy requirement per unit volume of soil treated ranged from 100-200 kWh/m^3 in all tests. Test results suggest that electrokinetic is an effective process for the removal of Pb from contaminated sandy loam soils.

4. Conclusions

This study shows that Pb can be effectively removed from artificially contaminated agricultural soil using the EK process. The efficiency of this process can be further improved by choosing appropriate processing fluid and by manipulating operating conditions.

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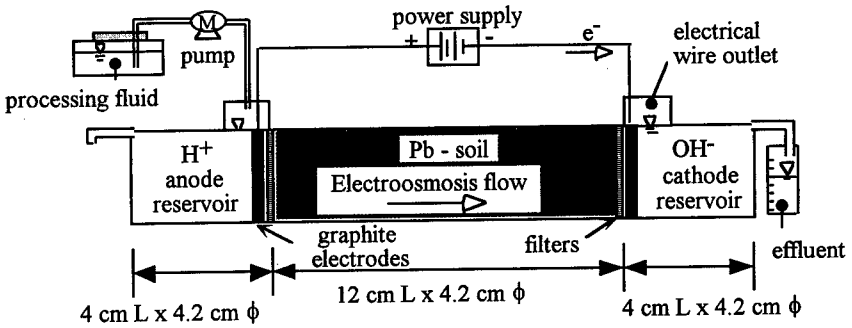

Figure 1: System setup of an electrokinetic test.

Table 1: Lead removal efficiency of contaminated sandy loam soil using EK process.

Test no.	Soil Pb (mg/kg)	Voltages (V)	Potential gradient (V/cm)	Processing fluids	Organic matter (%)	Q_e x10^{-5} (mL/day)	K_e x10^{-5} (cm^2/V-s)	% removal
1	500 Pb	25	1.67	simulated rain	0.67	27.6	0.97	93.7
2	500 Pb	35	2.33	simulated rain	0.41	38.1	0.96	95.4
3	500 Pb	45	3.00	simulated rain	0.51	44.2	0.87	97.2
4	250 Pb	35	2.33	simulated rain	0.84	37.3	0.94	97.3
5	100 Pb	35	2.33	simulated rain	0.48	36.2	0.92	98.0
6	250 Pb	35	2.33	10^{-2} M EDTA	0.31	78.2	1.98	97.6
7	250 Pb	35	2.33	10^{-2} M HAc	0.48	21.8	0.55	97.6

Notes: All tests were operated for 13 days at room temperature. Simulated rainwater was prepared in accordance with the standard methods of American National Bureau of Standards (NBS certified SRM 2694).

IMMOBILISATION OF HEAVY METALS IN CONTAMINATED SOIL: EVALUATING THE USE OF SYNTHETIC ZEOLITES

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1. Introduction

Extensively contaminated soils are difficult to remediate. Common techniques (e.g. excavation and extraction) are economically and physically impossible. In agricultural soils the remediation technology should not only eliminate the negative effects of contamination, but also retain the fertility of the soil. Much attention is nowadays paid to phytoremediaton and immobilisation. We will focus on *in situ* immobilisation of cadmium and zinc by adding highly adsorptive materials to the soil.

A binding agent must obviously possess a high adsorption capacity. However, that is not the only important property. The optimal amendment should not affect plants and soil organisms negatively, may not increase (facilitated) transport of heavy metals, needs to be durable under different environmental conditions and has to be cost-effective. Many products have been screened. Materials used in immobilisation studies can be divided in four groups: 1) Al, Fe and Mn-oxides, 2) incinerator waste products (fly-ashes, slags), 3) clays and (natural and synthetic) zeolites, and 4) organic matter products (e.g. sludge, compost, and peat).

We present a study on the (side-)effects of using synthetic zeolites to immobilise cadmium and zinc in contaminated soils. While natural zeolites appeared to have a relatively low capacity for heavy metal binding, several synthetic zeolites proved their effectiveness in different plant uptake studies (Gworek, 1992; Rebedea et al., 1997).

2. Materials and Methods

The study consists of the following experiments.

1. We started with 7 zeolites: zeolite A (A(I) en A(II), from two different companies), zeolite X, zeolite P, mordenite and faujasite, and one natural zeolite (often used in waste water treatment): clinoptilolite. In a batch experiment 0.2 g of zeolite was suspended in 180 ml of a 0.005 M mixed electrolyte solution and pH was adjusted to 5 or 6.5. Added cadmium concentrations were: $1.5*10^{-8}$, $5.0*10^{-8}$, and $1.0*10^{-7}$M. Zinc concentrations were 100 times higher.
2. Three zeolites were selected for further research: zeolites A(I) en A(II) and zeolite X. For these zeolites adsorption isotherms were determined at pH 5, 5.7 and 6.5. 0.2 g zeolite was suspended in 180 ml of 0.005 M $CaCl_2$ solution. Added cadmium concentrations were up to $5*10^{-6}$M. Zn concentrations were 100 times higher.
3. To evaluate long-term behaviour of zeolite treated soils, the leaching from columns was studied according to the Dutch protocol (NEN7343). Approximately 600 g of zeolite treated soil (moistened, incubated for 4 weeks, and dried) was put in a column (width: 5 cm, length: 20 cm) and leached with acid water (pH 4) from the bottom to the top. In 17 days 6 litres of leachate were collected and different fractions were analysed.

3. Results and Discussion

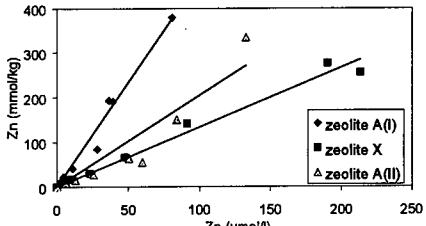

Figure 1: Zinc adsorption isotherms at pH 5.7

From experiment 1 we selected three zeolites. Zeolites A(I) and A(II) had the highest adsorption capacity for Zn and Cd and Zeolite X worked very well for Cd. Furthermore, the large amount of Na released from the zeolites and the high concentrations of Al measured at pH 5 were remarkable.

Figure 1 shows the adsorption isotherms for Zn. The adsorbed amount of Cd and Zn on zeolite A(I) was approximately 50% of the amount adsorbed on a very strong immobilising agent (*-MnO_2, Mench et al., 1994) at the same concentration in solution at pH 5.7 . Compared to other additives such as clays, natural zeolites and incinerator waste products, this is a good result. The column experiments show a high DOC concentration in the leachate. The large amount of Na on the synthetic zeolites is exchanged by divalent cations, predominantly calcium. The increased pH and the decreased calcium concentration in solution after adding zeolites causes organic matter to disperse. To prevent this phenomenon we

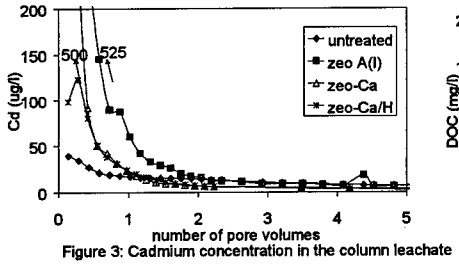

Figure 3: Cadmium concentration in the column leachate

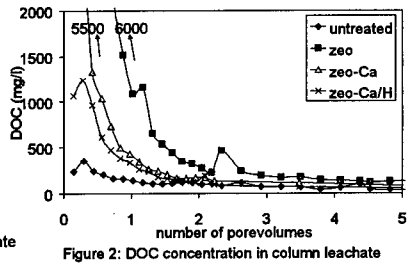

Figure 2: DOC concentration in column leachate

pretreated the zeolites with calcium and protons. Figure 2 shows the effect on DOC-leaching. The metal concentrations in the leachate show a similar trend (Fig. 3).

4. Conclusions

Zeolites A and X were the most effective binding agents among the zeolites tested. These zeolites became unstable below pH=5.5, though zeolite X proved to be slightly more durable than zeolite A. The most important result is the extreme increase of DOC leaching, which results in a mobilisation of metals instead of immobilisation. Pretreatment of zeolites with calcium and protons can reduce this effect.

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REMEDIATION OF HIGHLY SALINE PETROLEUM AND HEAVY METAL CONTAMINATED FINE TEXTURED SOILS

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1. Introduction

Existing technologies for the cleanup of contaminated soil are poorly suited for treating fine textured soils without adversely affecting the associated humic matter or soil mineralogy. The Institute for Chemical Process and Environmental Technology (ICPET), part of Canada's National Research Council (NRC), has developed a remediation process called Solvent Extraction Soil Remediation (SESR). SESR is the concurrent application of liquid phase agglomeration and solvent extraction of organic contaminants; heavy metals may be fixed concurrently by incorporating metal binding materials into the soil agglomerates as they form during solvent extraction of organic contaminants.

This presentation will discuss the results of recent tests on the application of SESR to the remediation of highly saline, fine textured, solid wastes containing both hydrocarbon and heavy metal contaminants. Plant growth studies on the remediated material will also be presented to show that the process is capable of producing material acceptable for use as topsoil.

2. Materials and Methods

The details regarding materials and experimental procedures has been reported elsewhere (Majid et al 1996, 1999).

3. Results and Discussion

Two highly saline, industrial soil samples, one contaminated with a heavy oil and several heavy metals and the other contaminated with diesel and lead were tested for remediation by NRC's SESR process. Concurrent removal of the hydrocarbon contaminant and fixation of heavy metals was achieved by incorporating metal binding agents such as peat, coal combustion ashes and phosphates into the soil agglomerates formed during processing. The efficiency of solid-solvent separation was greatly improved by selecting the agglomerate size in the 0.5-2 mm range. Over 90% of the oily contaminant was removed in the first stage of treatment. After five stages, close to 100% of the oily contaminant had been separated. Residual contaminant levels were close to the Ontario Ministry of the Environment and Energy (OMEE), guidelines for residential and recreational land use.

Dried agglomerates were leached with water, in a fixed bed system, to remove residual brine in order to meet OMEE, guidelines for soil conductivity. Agglomerated solids required less water and time to reach the conductivity guideline than unagglomerated soil extracted by conventional means.

After brine removal remediated material was evaluated for stability to plant enzymes by using it as soil in plant growth tests. Barley was used as the test species. A summary of the test results is shown in Table 1. These studies showed that the remediation process is capable of producing material suitable for use as topsoil. Analyses of lead in plant tissues suggests that only a small proportion of the lead absorbed by the plant roots was transported to the shoots. The additives used for the fixation of heavy metals were beneficial in reducing the bioavailability of lead to the plants. Biochemical activity, as a result of plant growth, did not render the fixed heavy metals leachable.

4. Conclusions

NRC's solvent extraction soil remediation process was successfully applied to the remediation of two highly saline industrial soil samples contaminated with both hydrocarbon and heavy metal contaminants. The remediated soil sample was demonstrated to be suitable for agriculture use by growing barley plants.

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Table 1. Lead up-take by Barley Plants Grown on Remediated Soil

Sample ID	Pb up-take by plants (mg/kg, dry matter)		TCLP Pb (mg/L)	
	Shoots	Roots	Before plant growth	After plant growth
Blank, remediated	2 ± 0.1	950 ± 40	53	3.0
With Na_3PO_4	Not detected	460 ± 30	<1	<1
With $Ca_3(PO_4)_3$	10.4 ± 2.2	370 ± 20	4.7	1.9
With CCA 'FBA'	3.2 ± 0.2	140 ± 20	21.5	1.6
With CCA 'C'	7.3 ± 0.9	290 ± 20	31.3	<1
With CCA 'F'	16.5 ± 3	260 ± 10	43.7	<1
With Lignin	9.5 ± 1.0	140 ± 20	22.1	1.7
With Peat	8.2 ± 1.1	380 ± 20	20.7	<1

TCLP: EPA's Toxicity Characteristics Leaching Procedure; CCA: Coal combustion ashes

EFFICIENCY EVALUATION OF SOME METHODS FOR REMEDIATION OF METAL CONTAMINATED SOILS: PRELIMINARY RESULTS

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1. Introduction

The well known problem of the contamination of soils by heavy metals or compounds containing heavy metals has assumed great importance in recent years. Heavy metals are among the most difficult contaminants to treat because they cannot be destroyed. Heavy metals are, moreover, usually found in soils in association with other types of contamination, especially organic compounds. These organic pollutants may complicate the removal of metals from the soil (Rulkens et al. 1995). In the European Union there are at least 120,000 to 150,000 contaminated sites, containing 1,000,000,000 m^3 of waste and contaminated soil. In Italy estimates of provisional data from some regions indicate that there are about 20,000 contaminated sites, including unauthorised dumps. In Sardinia the problem of soils polluted by heavy metals is particularly relevant in the mining areas (Melis et al. 1997), where such activity has created high artificial hills composed of waste-rock and tailings rich in heavy metals. This has contaminated large areas of the surface and the groundwater, through the effect of wind erosion and rainfall. Our research aims to evaluate remediation techniques using compost and *Trichoderma viride* as heavy metal immobilising agents. In this paper some preliminary results are presented.

2. Materials and Methods

Samples of soils, composts and *Trichoderma viride* were characterised physically and chemically (M.A.F.1992). These materials were then artificially polluted with three heavy metals (Pb, Zn, Cd) present in the contaminated areas. Tests were carried out using a single element each time. The results of the adsorption isotherms were elaborated using the Langmuir equation (Alberti et al. 1997). After contamination, the samples were treated with extracting agents at different strengths ($CaCl_2$ 0,2 N, EDTA 0,05 N) to study the contribution of the different interaction mechanisms.

3. Results and Discussion

The preliminary results are based on extraction tests made on a soil classified, by Soil Taxonomy, as Typic Xerochrept with a pH of 7.7, an organic matter content of 5.9%, a natural background of heavy metals of 70 mg/k^{-1}Pb, 66 mg/k^{-1} Zn, 66 mg/k^{-1} Cu, 1150 mg/k^{-1} Mn, 6.7mg/k^{-1} Cd, 20 mg/k^{-1} Cr and artificially polluted with $ZnCl_2$ and $Pb(NO_3)_2$. After pollution the Zn content of the soil was 1156.7 mg/k^{-1} and the Pb content 3700 mg/k^{-1}. In extraction tests made with $CaCl_2$ and EDTA, $CaCl_2$ extracted 21% of Zn and 9% of Pb adsorbed. When used sequentially, the EDTA extracted a further 47% of Zn and 11% of Pb. When used alone it extracted 36% of Zn and 44% of Pb. The soil, compost, and *Trichoderma viride* adsorption isotherms with Zn and Pb show different adsorption rates. The Pb – compost isotherm curves, for all the types of compost examined, are similar in shape, characteristic of materials showing an affinity proportional to the concentration of the contaminant as the type C in the Giles classification. The isotherms Zn- compost, Cd- *Trichoderma* and Cd- compost curves are S shaped, characteristic of substrata with an adsorption affinity variable at different concentrations.

The isotherms obtained putting Zn and Pb in contact with *Trichoderma* have a similar shape. The adsorption results elaborated by the Langmuir model show greater adsorption of Pb by the compost. *Trichoderma* has, by contrast, greater binding affinity. The metal saturated material was treated with extracting solution of $CaCl_2$ and EDTA to evaluate the contribution of the different interaction mechanisms. Cd is weakly retained by the *Trichoderma*: 80% of the adsorbed metal is released after treatment with both extracting agents. The Zn was even more weakly retained. It is completely extracted by $CaCl_2$ (40%) and EDTA (60%). Only 40% of Pb adsorbed by the *Trichoderma* is released after treatment with EDTA. $CaCl_2$ does not extract any Pb. 60% of Pb is strongly immobilised, probably inside the fungal cells. The Cd immobilising ability of *Trichoderma* and compost were also tested with growing tests made, using the water-cress (Lepidium sativum L.), on an artificial substratum of sand, compost, with or without *Trichoderma,* contaminated with 50 ppm of Cd. The plants grown on the substratum with *Trichoderma* showed, at chemical analysis, metal contents 30% less of these observed in plants grown in its absence. The extraction results with $CaCl_2$ and EDTA in the substrata, at the end of the growing tests, confirmed the capacity of the fungus to immobilise the metal and make it unavailable to the plants. Indeed, with $CaCl_2$ 58% less Cd and with EDTA 17% less Cd were extracted in the substratum containing the *Trichoderma*.

4. Conclusions

The results obtained, even if preliminary, demonstratethe ability of compost and *Trichoderma* to hold the metals and to prevent them, at different rates for the different metals, being leached and adsorbed by the roots.

Finally, the positive connection between the Cd contents found in the plants grown in contaminated substrata and the metal extraction results using $CaCl_2$ and EDTA sequentially can be inferred. This justifies the use of this method for rapid laboratory evaluation of the metals bioavailability.

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ELECTRODIALYTIC REMEDIATION OF DIFFERENT CU-POLLUTED SOILS

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1. Introduction

Electrodialytic soil remediation is a method that is develloped at the Technical University of Denmark in collaboration with the Danish company AS Bioteknisk Jordrens. The method is based on using an electric current as the cleaning agent for the polluted soil. In the method ion exchange membranes are used as separators between the soil and concentration compartments see Figure 1.

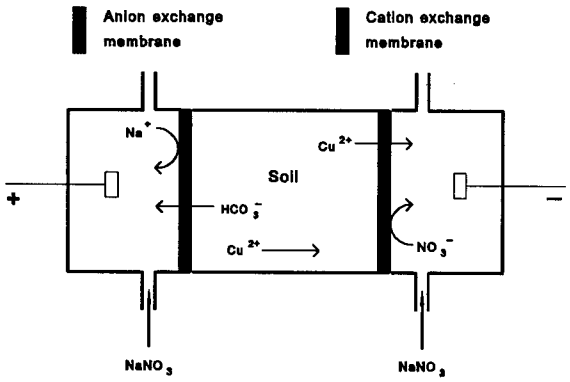

Figure 1: Principle of the electrodialytic soil remediation cell

The principle was first described in OTTOSEN and HANSEN (1992). More details in e.g. OTTOSEN et al. (1997) and HANSEN et al. (1997). At present two types of pilot plants are tested. In laboratory scale characterization and remediation of different soil and pollution types are done to make a matrix with remediation parameters for a wide range of heavy metal polluted soils.

2. Materials and Methods

A series of six remediation experiments with three different Cu polluted soils was made. Some characteristics of the three soils are outlined in Table 1. The cell used is based on the principle shown in Figure 1. The soil compartment has an inner diameter of 4 cm and the length of 1.5 cm. In each electrode compartment 0.5 L 0,01 M $NaNO_3$ with pH adjusted to 2was circulated. The first set of experiments were carried out with the soils in the conditions in which they were sampled. In the second set of experiments a desorbing reagent was applied to each soil. The reagent was chosen from knowledge of soil characteristics and added to air dried soil. To soil 1 and 3 was added 1 M HNO_3 . Soil 2 had a high carbonate content and the acid amount for lowering pH was very high. Instead 5% NH_3 was added to soil 2.The calcareous part of the soil was not dissolved due to high pH and Cu remain mobile as $Cu(NH_3)_4^{2+}$ OTTOSEN et al (1998). The current density (d.c.) was the same in all six experiments - 0.1 mA/cm^2. No optimization on the current strenght was done. The duration of all experiments was chosen to be 2 weeks.

Table 1: Some characteristics of the three experimental soils

Soil 1	Soil 2	Soil 3
Loamy sand polluted from wood preservation industry Weathered top soil. pH 5,9 Cu conc. 1200 mg/kg dry soil	Sand polluted from wire production Contains 12% of carbonates pH 7,5 Cu conc. 2240 mg/kg dry soil	Bottom sediment from a lake Very rich in organic matter. pH 5.5 Cu conc. 1680 mg/kg dry soil

3. Results and Discussion

In Table 2 pH and Cu concentration in the soil after the application of current for 14 days are given together with the calculated Cu removal percentage.

Table 2: pH, Cu concentration in the soil after 14 days of applied current in the six experiments. Percentage of Cu removed.

	Soil 1	Soil 2	Soil 3
Without pretreatment	pH 2.7 Cu conc. 105 mg/kg (91%)	pH 7.5 Cu conc. 2200 mg/kg (0%)	pH 4.3 Cu conc. 1200 (29%)
With pretreatment	Pretreated with 1 M HNO_3 pH 2.7 Cu conc. 90 mg/kg (93%)	Pretreated with 5% NH_3 pH 7.9 Cu conc. 1800 mg/kg (18%)	Pretreated with 1 M HNO_3 pH 4.3 Cu conc. 500 mg/kg (70%)

In the weathered soil the removal rate for Cu was high and the rate was only increased slightly by acid addition. In the bottom sediment from a lake the percentage of Cu removed was increased from 29% to 70% after addition of acid. In a calcareous soil 14 days of current was not enough to removed Cu without addition of ammonia but after this addition 18% Cu was removed.

4. Conclusion

Based on characterization of a Cu polluted soil a proper desorbing reagent to be added before electrodialytic remediation can be found. The desorbing agent can improve the remediation according to both energy consumption and remediation time.

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IN-SITU REMEDIATION OF CADMIUM CONTAMINATED SOILS: A FIELD INVESTIGATION

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1. Introduction

Rapid, cost effective strategies to remediate soils contaminated by heavy metals are considered desirable. Current protocols are either environmentally insensitive and expensive (eg. excavating the soil) or may require a lengthy period for significant reduction in toxic metal contamination (eg phytoremediation). Both synthetic and natural sorbent materials have been screened for their ability to decrease phytoavailability of metals in metal contaminated soils (for reviews see Mench *et al.* (1998) and Vangronsveld & Cunningham, (1998)). These screening processes in the main, have been in the form of pot experiments (Lothenbach *et al.* (1998); McLaughlin *et al.* (1998); Rebedea *et al.* (1997)), which do not necessarily give a good indication of the amendment performance in a field situation. In this investigation a field trial was conducted to observe the efficacy of a range of *in situ* sorbent amendments (Zeolite 4A, $FeSO_4$, Lime and Fe grit) in reducing the bioavailability of Cd to crop plants, decreasing bioaccumulation of the metal and hence reducing the risk of food chain amplification. Soil was treated at a domestic garden site which was contaminated by emissions of CdO from a nearby pigment manufacturer in the 1970s.

2. Materials and Methods

The soil was prepared and a series of 15 test plots ($2m^2$) were established on the site. Plots were treated either with Lime; $FeSO_4$ & Lime; Iron Grit and Zeolite 4A at a rate of 1% w:w with unamended control plots (n=3). The amendments were incorporated into the top 10cm of the soil and left to stabilise for two weeks prior to planting. A range of vegetable crops were cultivated, selected for their known ability to accumulate Cd in their foliage. Seedlings were transplanted into the trial plots, giving a replication of at least 15 plants per treatment. The plots received conventional husbandry (weeds removed manually, no fertiliser) and the crops were allowed to grow to a marketable size whereupon they were harvested and their fresh weights taken immediately. The crops were then dried overnight at 80°C or until their weight became constant. The metal content of the plant foliage was determined by wet digestion using 70 % HNO_3. Mean data are expressed for fresh weights and total foliage metal concentrations for two of the test crops, lettuce (*Lactuca sativa* L. cv Cindy) and spinach (*Spinachia oleracea* L. cv F1 Space).

3. Results and Discussion

The effects of the sorptive minerals on plant growth are shown in Table 1. It was observed from these data that none of the amendments had any significant effect on the growth of lettuce. However in the case of spinach the growth of test crops were significantly altered by some of the amendments. Growth was enhanced when spinach plants were grown in plots treated with Zeolite 4A, whereas growth was significantly reduced when the same cultivars were grown in plots treated with $FeSO_4$ & lime.

Table 1. The effect of sorbent amendments on the fresh weight (g) of lettuce (*Lactuca sativa* cv. Cindy) and spinach (*Spinachia oleracea* L. cv. F1 Space) grown in Cd contaminated soils. Mean ± SE.

	Treatments				
	Control	Zeolite 4A	$FeSO_4$ + Lime	Fe grit	Lime
Lettuce	135.07 ± 27.5	101.86 ± 21.5	75.54 ± 20.5	112.19 ± 21.3	113.38 ± 14.6
Spinach	30.57 ± 4.33	56.1 ± 11.0	17.82 ± 1.72	38.49 ± 5.0	24.72 ± 4.63

Data obtained from the metal analysis indicated a significant decrease in Cd uptake in the plants grown on treated plots compared with the control. Uptake of Cd into the foliage of both lettuce (Figure 1) and spinach (Figure 2) was reduced consistently by the Zeolite 4A treatment which reduced uptake by >50% in the lettuce and by >33% in the spinach. Other amendments were less effective, including the lime, but in particular the Fe based treatments which caused an actual increase in Cd uptake in lettuce when applied as Fe grit, along with an increase in foliar Cd in the spinach when applied as $FeSO_4$.

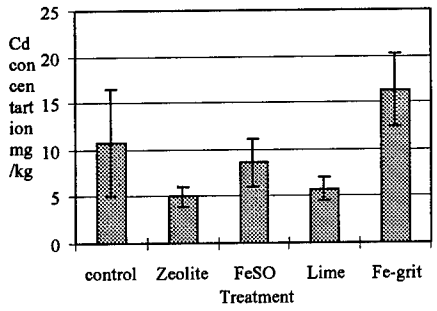

Figure 1. Cd concentration (mg/kg) in lettuce (*Lactuca sativa* cv. Cindy) foliage. Mean and SD

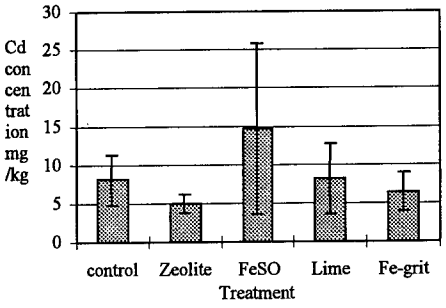

Figure 2. Cd concentration (mg/kg) in spinach (*Spinachia oleracea* cv. F1 Space) foliage. Mean and SD

4. Conclusion

It is evident from these first seasons data that there are significant differences between plant biomass and Cd concentration in the control and treated plants. Further data are required and these ongoing trials will be replanted to give clearer indications of the long-term effects of these sorbent amendments.

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REMOVAL OF TRACE ELEMENTS FROM WASTE WATER WITH BIOLOGICALLY ACTIVE MOVING-BED SANDFILTERS

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1. Introduction

Contamination of water by trace elements originates from a variety of industrial activities as well as from natural, geochemical processes. Conventional chemo-physical treatment plants may not always prove successful due to the high costs of processing streams of high volume and low contamination or due to the presence of complexing organic matter hindering metal removal.

Biotechnological approaches have met with some success in these areas. Certain micro-organisms are able to immobilise trace elements from aqueous solutions by a variety of biological (e.g. uptake), biologically mediated (e.g. bioprecipitation, biocrystallisation) and also chemo-physical (e.g. biosorption) mechanisms.

The sandfilter process presented here was designed to combine the optimal conditions for more than one of these well-known microbial processes. In addition to the removal of metals and organic matter it aims at the production of a biosludge with a high metal content acceptable for pyrometallurgical metal recycling.

2. Materials and Methods

In the moving-bed Astrasand filter (Astraco Water Engineering BV) the water flows upwards through a sand bed in a cylindrical vessel. Inoculated bacteria grow on the sand grains forming a biofilm, which traps trace elements from the feed water. From the bottom of the filter the laden sand is moved upwards by an internal airlift. Thereby the sand grains are cleaned by attrition. On the top of the filter particles of metal containing biofilm leave the system with some wash water. The sand grains keep a residual biofilm and fall back to the top of the bed, where bacteria re-start to grow and to immobilise the metals.

The sandfilter forms the core of a water treatment system which consists of a **buffer tank** to smooth concentration spikes, the **filter** itself, a **lamella separator** to separate the laden biomass from the wash water, **bag filters** to dehydrate the biomass, **dosing equipment** to supply nutrients, and **on-line measuring devices**.

After four years of fundamental collaborative research five bacterial strains were finally selected to proof laboratory experiments in pilot plants. Besides their basic properties to immobilise metals the natural strains were demonstrated to be genetically stable, to have no special requirements concerning nutrition, to be resistant against the metals of interest, and to pose no risk for men, animals and environment (risk-class 1).

It was decided to inoculate the filters with a mixed population, to follow the surveillance of each of these strains by means of differentiating microbial growth tests, and to observe the appearance of autochthonous micro-organisms.

In 1998 four pilot plants have been operated with the following waters:

- Olen (B): non ferrous industry, broad spectrum of elements, main interest: Fe, Co, Ni
- Hoboken (B): non ferrous industry, broad spectrum of elements, main interest: Co, Ni
- Hartenstein (D): mine drainage, main interest: As, U
- Vienna (A): rinsing water of chemical nickel plating, main interest: Ni, organic acids

The concentrations of the elements of interest range between several hundreds of µg/L and about 10 mg/L.

3. Results and Discussion

With all four pilot plants the biological operation could be initiated by inoculating adapted bacteria. Depending on the water used, various nutrients and electron acceptors have been dosed to achieve sufficient growth of the micro-organisms.

The operation of the biggest pilot plant (30 m^3/h), which was used for treating the Olen waste water, could be optimised to a removal of 90-100% of the metals Co, Ni, Zn, Cu. The system was run anaerobically with the dosing of a cheap carbon source and an electron acceptor.

A smaller plant (1-8 m^3/h) has been used to treat rinsing water from a chemical nickel plating line. Although the present nickel is complexed by organic acids, 50% of the metal could be removed with the influent water being saturated with oxygen. Organic acids from the bath were completely degraded. With a feed concentration of 2 mg/L of Nickel, the dried bio-sludge contained 2% of Ni, which gives a concentration factor of 10,000.

Detailed data analyses over several months of operation showed up the way for further improvements of the systems and are being used for a mathematical modelling of the processes. Final results will be presented for all pilot plants.

4. Conclusions

Moving-bed sand filters inoculated with biosorbing and bioprecipitating bacteria were demonstrated to remove trace elements such as Ni, Co, Cu, Zn, U, As from various industrial effluents. The micro-organisms are also able to cleave organo-metal complexes like Ni-lactate and to degrade the organic acids completely. The produced bio-sludge contains several percent of heavy metal and could be treated pyrometallurgically for the recycling of the metals. The process may be used as an economic alternative to conventional systems in the final polishing of industrial waste water or in the processing of contaminated ground- or surface water.

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POLYACRYLATE POLYMER REMEDIATION OF SANDY SOILS CONTAMINATED WITH TOXIC METALS

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1. Introduction.

The polymers we used are insoluble in water, have monovalent cations as counter ions, and can retain large quantities of water. They are commonly used to increase the water holding capacity of coarse textured soils. Swelling of these hydrophilic polymers is affected by the presence of soluble salts. The ions reduce the osmotic imbalance between the polymer and the external solution and screen the charges on the chains thereby reducing the potential for water absorption. Divalent cations can also provide ionic cross-links between fixed charges, resulting in a further depression of water absorption. These polymers can also retain toxic metals, probably through chelation with the carboxylic groups present in their chains (Vasheghani-Farahani *et al.*,1990). We studied the effects of polyacrylate polymers on the availability of toxic metals in free solution and in sandy soils.

2. Materials and Methods

For work in free solution, samples of polymer were left to expand for 48 h in 250 ml of de-ionized water or salt solutions containing 0.01 M K, Na, Mg, Cu, Cu-EDTA, Ni, Zn, Cd, Mn or Cr (III) at pH 5.0. Excess water was removed by filtration. The polymer was weighed and then equilibrated with 250 ml of de-ionized water for 72 h before being drained and reweighed.
For work in soil, three replicate pots were filled with 10 kg of a sandy soil with a N-P-K-Mg basal fertiliser dressing. In separate experiments, salts of Cu or Cd, and polymer were added to the soil. Eighty plants of perennial ryegrass (*Lolium perenne* L. cv Victorian) were grown in each pot, and sampled by four cuts. Additional N fertiliser was added after the first three cuts. The shoot material from each cut was dried at 65 °C and weighed.

3. Results and Discussion

We found that K, Na, Mg and Cu-EDTA caused a reduction in water-holding capacity that was wholly or partly reversible. In contrast, metal ions such as Cu, Ni, Cd, Zn, Mn and Cr, when present in free solution, were rapidly adsorbed on the polymer and irreversibly reduced its water holding capacity (Table 1). For Cu, we measured a proportion of four carboxylic groups for each Cu ion, suggesting that this is the number of ligands that participate in the formation of each complex (Torres and de Varennes, 1998).
Compared with the controls, increased plant growth was obtained with perennial ryegrass, when polyacrylate polymers were applied to the sandy soil artificially contaminated with Cu or Cd (Table 2). For copper, we showed that the Cu content of shoots from plants in polymer-amended contaminated soil was less than that of plants in unamended soil (Torres and de Varennes, 1998). Water extractable Cu was also considerably reduced in the polymer amended contaminated soil, and polymer particles extracted from this soil were found to contain high levels of Cu (Torres and de Varennes, 1998). -

Table 1. Effects of soluble salts on water absorption (g swollen polymer g^{-1} dry polymer)

	Cation in the salt										
	H_2O	K	Na	Mg	Cu-EDTA	Cu	Ni	Cd	Zn	Mn	Cr
1	443 B	213 B	254 B	33 B	103 B	18 A	5 A	9 A	4 A	2 A	6 A
2	572 A	487 A	614 A	189 A	360 A	14 A	3 A	6 A	3 A	3 A	8 A

1 – After equilibration with the salt; 2 – After re-equilibration with water
Means in a column followed by the same letter are not significantly different as judged by the Scheffé's test at a level of p=0.05.

In uncontaminated soil, yield was also improved by the polymers, presumably due to the supply of water, and to the cations present as counter ions (Table 2).

Table 2. Dry weight of perennial ryegrass grown in a sandy soil with Cu or Cd, with or without polymer

Element (mg kg^{-1} of soil)	Polymer	Dry weight (g per pot)
Cu – 0	-	47 B
Cu – 0	+	53 A
Cu – 50	-	11 D
Cu – 50	+	29 C
Cd – 0	-	49 B
Cd – 0	+	62 A
Cd – 50	-	14 C
Cd – 50	+	44 B

For the same element, means followed by the same letter are not significantly different as judged by the Scheffé's test at a level of p=0.05.

4. Conclusions

We conclude that polyacrylate polymers can be used to remove toxic metals from solution, and that plant growth can be enhanced by amending metal-contaminated soils with these polymers.

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ARSENATE EXCHANGE BETWEEN SOIL AND SOLUTION IN SOILS TREATED WITH STEEL SHOTS AND BERINGITE

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1. Introduction.

In percolation vats, the leaching and soil-plant transfer of arsenate (As) were decreased by the addition of steel shots (SS, an iron bearing material), beringite (B, a modified aluminosilicate) and a combination of the two (SS+B) to an As polluted soil (BOISSON et al., 1998). One of the determining processes concerning the leaching and soil-plant transfer of As is the As exchange between the solid soil phase and the solution. In this paper, we investigate for untreated (UNT) and SS, B and SS+B treated soils changes in the kinetic of As exchange between the solid soil phase and the soil solution using an isotopic method.

2. Materials and Methods.

The As polluted soil originates from a maize-field in the vicinity of a former industrial As site. It is a sandy soil with a pH of 6.6 and a total As content of 170 mg kg^{-1}. Subsamples were mixed with SS (1% by soil dry weight), B (5%) and SS+B (1% and 5%) and placed into percolation vats. After 6 months soil samples were taken from the vats. The amount (E_t) of isotopically exchangeable As was determined using the isotopic dilution principle, i.e. the isotopic composition of E_t equals the isotopic composition of As in solution; $E_t = Q_{As}/(r_t/R)$ (equation 1) in which Q_{As} is the amount of As in solution ($Q_{as} = 10C_{As}$). The isotopic dilution method (FARDEAU, 1993) was used to determine E_t. A known amount (R) of ^{73}As is added to a soil-solution (ratio 1:10) system in quasi equilibrium and the time dependent radioactivity in the solution (r_t) is followed in time (t = 1, 4, 10, 40 and 100 min). R and r_t were measured by liquid scintillation, the As concentration in the soil solution (C_{As}) at time 1 min was measured by Graphit Furnace Atomic Absorption Spectrometry. Speciation analyses of the solution samples by coupled high performance liquid chromatography - inductively coupled plasma - mass spectrometry indicated that 97 - 99% of the total arsenic in solution was present in the form of the arsenate ion.

3. Results and Discussion.

E_t as a function of time is described by the equation proposed by FARDEAU (1993) for phosphate: $E_t = Q_{As} / (m[t + m^{1/n}]^{-n} + r_{eq}/R)$ (equation 2) in which r_{eq} is the radioactivity in the soil solution after equilibrium is reached (r_{eq}/R can be estimated by the Q_{As}/As_{tot} ratio in which As_{tot} is the total As concentration in the soil) and m and n are regression parameters. The values of Q_{As}, m and n are given in Table 1 and experimental and fitted values of E_t in figure 1. Q_{As} decreased by 71 and 78 % respectively after SS and SS+B introduction. SS is known to oxidise in soil to form iron oxides (SAPPIN-DIDIER et al., 1998). Arsenate can form inner-sphere complexes with iron oxides (MANCEAU, 1995). The mechanism of the effect of B is unknown, As might form surface complexes with B as well. E_t in the SS and SS+B treated samples was lower but increased more quickly with time than in the UNT samples for the experimental time (100 min) (Table 1, fig. 1). With the parameters from Table 1, E_t has been calculated for $0 < t < 10000$ min. A Mobility Index, $MI = (E_{t,treated}-E_{t,UNT})/E_{t,UNT}*100$ (equation 3), has been calculated to analyse changes in As exchangeability when the additives were introduced into the soil (figure 2). The time corresponding to $MI = 0$ ($E_{t,treated} = E_{t,UNT}$) depends on the additives and decreases

with decreasing Q_{As}. The additives have two distinguished effects: i) transformation of quickly exchangeable As forms into more slowly exchangeable forms (MI < 0, Table 1) and ii) transformation of some of the slowly exchangeable As forms into more quickly exchangeable forms (MI > 0, Table 1). The additives immobilize quickly exchangeable As and mobilize slowly exchangeable As, thereby increasing the As pool with a medium exchangeability. It can therefore be concluded that the immobilized As did not become fully inert when SS and SS+B are introduced into the soil. As is still present in an exchangeable form.

Table 1: Values of Q_{As} (mg kg soil^{-1}), m and n (with their 95% confidence interval) and of time (t in min) at which MI = 0 for the untreated (UNT) soils and soils treated with Steel Shots (SS), Beringite (B) or Steel Shots + Beringite (SS+B) (n = 2).

Soil	Q_{As}	m	n	R^2	t for MI = 0 (min)
UNT	8.2 (1.5)	0.890 (0.165)	0.098 (0.057)	0.887	
B	6.5 (0.7)	0.851 (0.096)	0.118 (0.037)	0.932	17581
SS	2.4 (1.0)	0.987 (0.229)	0.258 (0.058)	0.994	4485
SS+B	1.8 (0.7)	0.922 (0.162)	0.322 (0.043)	0.998	1120

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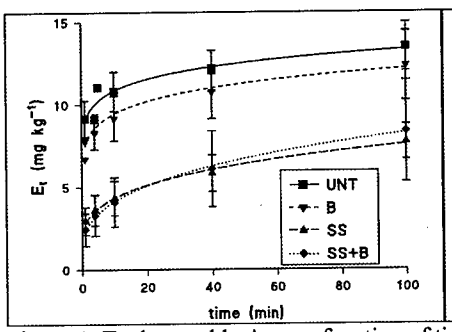

Figure 1: Exchangeable As as a function of time.

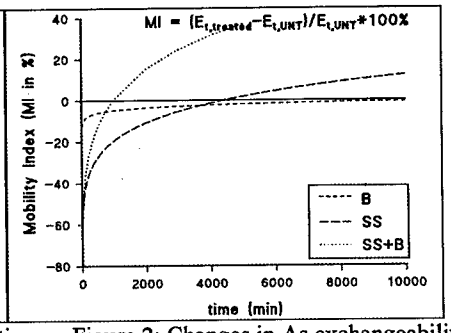

Figure 2: Changes in As exchangeability in the treated soils as % of in the UNT soil.

CHEMICAL REMEDIATION METHODS INFLUENCE ON THE UPTAKE OF CADMIUM AND LEAD BY VEGETABLES IN CONTAMINATED SOILS

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1. Introduction

Some chemical remediation techniques were used to immobilize the heavy metals in the contaminated soils and to reduce the soluble concentration of trace elements in soils by the reaction of precipitation, adsorption, or complexed with organic matter in some studies (Mench et al., 1994). Many research reports also indicated that the application of hydrous manganese oxides mixed into the polluted soils could reduce the concentration of cadmium (Cd) and lead (Pb) in the soil solution (Tiller et al., 1984). More than 100 ha of rural soils have been contaminated by Cd and Pb in northern Taiwan (Chen, 1991). The objectives of this study are (1) to identify the differences on the extraction concentration of Cd and Pb in contaminated soils treated with different chemical materials, and (2) to examine the effect of different chemical treatments on the total uptake of Cd and Pb in two vegetables growing in treated soils.

2. Materials and Methods

Two rural soils contaminated by Cd and Pb in northern Taiwan, clayey soil A and sandy soil B, were selected for this study. The treatments including (1) check, (2) applying organic composts 30 tons/ha, (3) applying organic composts 30 tons/ha and calcium carbonate to increase the soil pH to 6.8, and (4) applying organic composts 30 tons/ha, calcium carbonate to increase the soil pH to 6.8, and 1% of manganese oxide. These treatments are incubation for two months in 50% of water holding capacity. Pickled cabbage (*Brassica chinensis* Linn.) and water convolvulus (*Ipomoea aquatica* Forsk) were grown in these treated soils by pot experiments in three replicas. Different extraction solutions were used to evaluate the extractability of concentration of Cd and Pb in the soils after these treatments (Mench et al., 1994). The concentration of Cd and Pb in the soil solution and in the vegetables was determined by AA spectrophotometer (Hitachi 180-30 type). The statistical significance in this analysis was defined at level of p =0.05.

3. Results and Discussion

The results of pot experiments indicated that the extraction concentration of Cd and Pb in contaminated soil were significantly reduced after treating with organic composts (30 tons/ha) mixed with calcium carbonate, or treating with organic composts mixed with calcium carbonate and 1% of manganese oxide (Table 1). There were no significant effect on the treatment following the application of the organic compost. The concentration and uptake of Cd and Pb by two vegetables are also significantly reduced on the treatments of organic compost (30 tons/ha) mixed with calcium carbonate and 1% of manganese oxide (Table 2), but there are no significant effect on the treatment only applying the organic compost.

4. Conclusions

The extraction concentration of Cd and Pb in contaminated soil were significantly reduced after treating with organic composts (30 tons/ha) mixed with calcium carbonate, or treating with organic composts (30 tons/ha) mixed with calcium carbonate and 1% of manganese oxide. These treatments are also significantly reduced on the uptake of Cd and Pb by cabbage or water convolvulus grown in these treated soils.

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Table 1. Cadmium concentrations (mg/kg) extracted in soil A for different chemical treatments.

Treatments	Cd concentration (mg/kg) extracted from soil A				
	Water	DTPA	EDTA	HOAc	HCl
			cabbage		
Check	ND	4.0 a	4.6 a	3.9 a	4.8 a
Compost	ND	3.7 b	4.4 ab	3.5 b	4.8 a
Compost+$CaCO_3$	ND	3.5 c	4.3 b	3.4 b	4.5 b
Compost+$CaCO_3$+MnO_2	ND	3.5 c	4.4 ab	3.4 b	4.5 b
			water convolvulus		
Check	ND	4.0 a	4.7 a	4.3 a	4.9 a
Compost	ND	3.8 b	4.4 b	4.1 ab	4.8 a
Compost +$CaCO_3$	ND	3.7 b	4.6 a	3.9 b	4.8 a
Compost +$CaCO_3$+MnO_2	ND	3.9 ab	4.6 a	3.9 b	4.8 a

Data are expressed as mean values of three duplicates in the pot experiments.
The same letter within a column are not significantly different at $p = 0.05$; ND: not detectable.

Table 2. Mean concentration, dry weight and total uptake of Pb by cabbage in two contaminated soils with different treatments.

Soil	Treatments	Mean concentration	Dry weight	Total uptake
		— mg/kg —	– g/plant –	–g/plant –
Soil A	Check	32.1 a	0.28	6.30 c
	Compost	28.1 b	0.76	17.1 b
	Compost+$CaCO_3$	19.9 c	1.12	25.2 a
	Compost+$CaCO_3$+MnO_2	19.9 c	0.82	18.5 b
Soil B	Check	17.3 a	0.38	4.29 b
	Compost	14.6 b	0.61	8.90 a
	Compost+$CaCO_3$	15.5 b	0.60	9.30 a
	Compost+$CaCO_3$+MnO_2	14.6 b	0.62	8.05 a

Data are expressed as mean as mean values of three duplicates in the pot experiments.
The same letter within column are not significantly different at $p = 0.05$.

AMENDEMENTS TO REDUCE TRACE ELEMENT MOBILITY

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1. Introduction

Metal contamination of soils by human activities always involves a certain danger of either leaching the metals into the groundwater or transfering them into the food chain (Adriano, 1986). A wide range of in-situ soil remediation technologies has been developed. One possibility to lower the risk of bioavailable trace metals in the soil is the use of soil amendments (Phillips, 1998). In this study pot experiments were carried out to investigate the effects of three different substances

2. Materials and Methods

Four Austrian soils from locations named Untertiefenbach (UNT), Weyersdorf (WEY), Reisenberg (REI) and Arnoldstein (ARN) were collected. Last-mentioned soil came from the vicinity of a former lead-zinc smelter and is highly contaminated with Pb, Zn, Cd and As by long-term deposition. The other soils have been contaminated artificially in 1987 by adding Zn, Cu, Ni, V and Cd compounds. They show a wide range of soil properties; WEY displays a low capacity of trace metal binding. Table 1 shows some characteristics of the experimental soils.

Table 1: Characteristics of the soils used in the experiments.

	UNT	WEY	REI	ARN
Cd-total [mg kg^{-1}]	6.8	6.0	7.1	19.7
Zn-total [mg kg^{-1}]	713.6	533.1	701.2	2713.2
Pb-total [mg kg^{-1}]	-	-	-	12322.5
Clay content [g kg^{-1}]	325	58	190	203
pH [$CaCl_2$]	7.0	5.9	7.3	6.9

The amendments tested were red mud (10 g kg^{-1} soil), zeolite (20 g kg^{-1}) and lime (3 g kg^{-1}). Red mud is a byproduct of the aluminum industry that is alkaline and rich in Fe/Al-oxides (Wong and Ho, 1995). Zeolite is a natural occuring hydrous aluminosilicate with a three-dimensional crystal structure and a high CEC (Ming and Mumpton, 1989). Lime is widely used as a soil amendment to increase the pH and consequently to decrease the solubility of metals (Chlopecka and Adriano, 1996). The amendements were compared to a non-amended control.

The soils were mixed with the amendements in a concrete mixer and filled into plastic pots 20 cm in diameter. After two weeks incubation time *Amaranthus hybridus* was sown. The plants were harvested after two months, dried and digested in HNO_3 and $HClO_4$. The metal

concentrations were measured by ICP-AES techniques. The mobile fraction of the soils was extracted by 1 M NH_4NO_3.

3. Results and Discussion

The effects of red mud, zeolite and lime on Cd uptake by *Amaranthus hybridus* are indicated in Table 2.

Table 2: Effects of different amendements on Cd plant uptake, as % of non-amended controls.

	UNT	WEY	REI	ARN
red mud	**38.4**	**87.1***	**29.1**	**30.4**
Zeolite	**12.8**	0	-4.4	-2.9
Lime	-14.9	**26.0**	**4.1**	**2.1**

* Relative to the zeolite treated soil, because no plant was growing on the Weyersdorf control.

Red mud treatment reduced the Cd uptake to a greater extent (Table 2). Plant uptake of Zn was reduced on ARN by 29 %; on REI by 41 % and on UNT by 13 %. On WEY Zn uptake was reduced by 80 % relative to the zeolite treatment. On ARN the Pb uptake was reduced by 17 %. The addition of red mud had only little effect on pH (between 0 and 0.3 units), only the pH WEY increased by 1.0 unit.

Zeolite treatment indicates different effects. Cd uptake was reduced only on UNT. Zn uptake decreased only on ARN by 5 %. The pH was not influenced by adding zeolite (20 gkg^{-1}).

Lime application decreased Cd uptake on ARN, REI only slightly, but WEY by 26 % . Plant uptake of Zn was reduced on ARN by 16 %, on REI by 6 %, and on WEY* by 25 %. On ARN lime treatment decreased Pb uptake by 9 %. Lime treatment had no influence on pH.

4. Conclusions

Only red mud decreased plant uptake of Cd, Zn and Pb continously. The strongest effect was obtained for WEY, associated with an increase soil pH by 1 unit. Zeolite and lime were less consistent. Red mud performed well in the short-term pot study, but the results have to be evaluated by long-term field experiments.

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LEAD IMMOBILIZATION IN LEAD CONTAMINATED SOILS

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1. Introduction

Lead immobilization was examined on artificially Pb contaminated soils using 8 different treatments. Three different criteria were used to evaluate the efficiency of the soil additives applied.

A selective extraction using $Ca(NO_3)_2$ was used to evaluate the effect on metal mobility. Secondly, phytotoxicity of the soils was studied. Morphological as well as biochemical parameters were determined on beans plants (*Phaseolus vulgaris* L. cv. 'Limburgse vroege') grown on the soils. Phytotoxicity degrees were calculated for each treatment combining these data and classifying the soils into 4 toxicity classes: (1) not toxic, (2) slightly toxic, (3) moderately toxic, (4) strongly toxic (Vangronsveld & Clijsters, 1991). Further, lettuce plants were cultivated on the same soils and the Pb content of above ground plant parts was determined.

2. Materials and Methods

Different concentrations of Pb (as Pb-acetate) were added to an acid sandy soil (pH-H_2O=4.27): 0, 250, 500, 1000, 1500, and 2000 mg Pb kg^{-1}. Except the untreated (no additive), following soil amendments were applied (w/w): 1 % bentonite, 0.5 % zeolites (13-X), 5 % cyclonic ashes, 5 % compost, 1 % lime, 1 % steelshots and 1 % hydroxylapatite.

After a minimum period of stabilization (c 100 days), 5 g soil was shaken for 24 h with 50 ml 0.1M $Ca(NO_3)_2$ (Sappin, 1995). The extracts were analysed for Pb by AAS.

Beans were sown on the soils after 2 days vernalisation and 1 day imbibition. The plants were cultivated for 14 days under controlled environmental conditions. Shoot length, primary leaf area and root weight were measured at harvest (14 days after sowing). Plant material was stored at –70°C, enzyme capacities in primary leaves (Guiacolperoxidase (GPOD), Malic Enzyme (ME), Isocitratedehydrogenase (ICDH)) and roots (GPOD, ME, Glutamatedehydrogenase (GLDH)) were determined spectrophotometrically as described by Van Assche et al. (1988).

Lettuce seedlings (3 weeks old) were transplanted to the soils and grown for 4 weeks. Plants were harvested and above ground plant material was used for Pb analysis with AAS after extraction with $HNO_3/HClO_4$.

3. Results and Discussion

Results from $Ca(NO_3)_2$ extractions demonstrate that in the control soil the available Pb fraction increased when the total Pb content was enhanced (up to 520 mg kg^{-1} at 2000 mg kg^{-1}). The bioavailable fraction declined when bentonite was applied (333 mg kg^{-1}); a further reduction was found with zeolites (140 mg kg^{-1}) and steelshots (130 mg kg^{-1}). Strong immobilization however occured when compost (40 mg kg^{-1}), cyclonic ashes (36.7 mg kg^{-1}), hydroxylapatite (36.8 mg kg^{-1}) or lime (9.4 mg kg^{-1}) were applied. At 2000 mg kg^{-1}, application of lime reduced the bioavailable Pb fraction to the same level as was found in the control soil (9.4 mg kg^{-1} vs 8.3 mg kg^{-1}). From table ,1 it is clear that in the untreated soil (no additive application) phytotoxicity increased (from not toxic (1) to moderately toxic (3)) with increasing Pb concentration. Root growth inhibition was an early criterion of phytotoxicity; it was already visible at 500 mg Pb kg^{-1} (up to 65 % growth reduction at 2000 mg kg^{-1}) together with an increased capacity of ME, GLDH and to a lesser degree of GPOD.

Every soil treatment studied reduced phytotoxicity. With bentonite and steelshots Pb was not toxic at all at 1000 mg kg^{-1} and only slightly toxic at 1500 and 2000 mg kg^{-1}. Treatment with

cyclonic ashes, compost, lime and hydroxylapatite resulted in a complete elimination of phytotoxicity at all Pb concentrations examined. Application of zeolites induced slight toxic responses independent of the Pb concentration. This is due to its destructive effect on soil texture, making normal development of the test plants impossible.

Table 1: Phytotoxicity indices for 8 different treatments on soils polluted with 6 different concentrations of Pb (mg kg^{-1}).

	Un-treated	1% bento-nite	0.5% zeolites	5% cyclonic ashes	5% compost	1% lime	1% steel-shots	1% hydroxyl-apatite
0	1	1	2	1	1	1	1	1
250	1	1	2	1	1	1	1	1
500	1	1	2	1	1	1	1	1
1000	2	1	2	1	1	1	1	1
1500	2	2	2	1	1	1	2	1
2000	3	2	2	1	1	1	2	1

At 2000 mg kg^{-1} Pb, up to 24.5 mg Pb kg^{-1} fresh weight was found in the leaves of lettuce. When compost was applied, still 13.5 mg kg^{-1} was present and soil treatment with cyclonic ashes and bentonite further reduced the leaf content to 8.9 and 8.5 mg kg^{-1} respectively. When hydroxylapatite or lime were applied Pb content decreased to 6.1 and 4.3 mg kg^{-1}. Application of zeolites was problematic at 2000 mg kg^{-1}, but analysis at 1500 mg kg^{-1} (9.6 mg kg^{-1}) suggests the effect being more or less comparable with that found with the compost treatment. No results could be obtained with steelshots since this additive released toxic amounts of Fe and Mn and therefore killed the test plants.

4. Conclusions

An important conclusion to be made is that different approaches need to be used to evaluate immobilization and phytotoxicity of lead polluted soils: $Ca(NO_3)_2$ analysis for example indicated that zeolites have an excellent immobilizing effect on Pb. Problems raise however when plants are cultivated on a soil treated with this additive, since it demonstrates phytotoxicity due to destruction of the soil texture. Phytotoxicity however is not always caused by elevated Pb concentrations: in some cases test plants are under stress due to mineral deficiency, caused by too strong immobilization of essential elements in the soil.

It can be concluded from these data that lime, hydroxylapatite, cyclonic ashes and compost give satisfying results: the bioavailable fraction of Pb in the soils is reduced, soil phytotoxicity disappears and the Pb content in lettuce declines.

The experiments described however cover only short term effects of the additives on Pb immobilization. For field application of immobilization long term effects over years must be considered; practical aspects should also be taken into account, e.g. price, degree of contamination, destination of the site after remediation, etc.

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REMEDIATION OF AN ACID SOIL USING HYDROXYAPITITE AND ZEOLITES

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1. Introduction

Typical drainage of an acid soil results in low pH-groundwater with elevated concentrations of Fe, Al and sulfate. The contaminants of concern are Ni, Cd, Cr and Pb may be released to groundwater from acid soils, particularly those affected by coal pile leachate. A number of studies have been reported on the use of different ameliorants to stabilize and immobilize the release of contaminant metals from acid soils (Chlopecka and Adriano, 1996; Laperche et al., 1996; Bowman et al., 1997). Present study was conducted to determine the remedial effects of hydroxy apatite and two zeolites on two acid soils collected from Savannah River Site (SRS) near Aiken, South Carolina.

2. Materials and Methods

Acid soils Blanton sand (Grossarenic Paleudult) and Orange loamy sand (Typic Paleudult) collected from Savannah River Site were air dried and passed through a 2 mm-sieve. Selected physical and chemical properties of these soils are presented in Table 1. One and a half (1.5) kg samples of sieved soils were packed separately in pre-washed PVC columns (40 cm long and 6 cm internal dia.). Before packing, duplicate sets of columns were given four soil treatments: (1) Control, (2) 2.5% hydroxyapatite, (3) 2.5% clinoptilolite, and (4) 2.5% phillipsite. The bottom part of each column was fitted with a sintered plastic Buchner funnel lined with Whatman No. 42 filter paper and a layer of glass wool. Soil columns were saturated with deionized water and were kept at field capacity. Each soil column was leached twice with 100 mL deionized water at intervals of two and four weeks. The pH of leachates was checked and then acidified for elemental analysis using ICP instrument.

3. Results and Discussion

Initial properties of the two study soils are comparable in concentrations of Mg, K and Zn but differ for Ca, Fe, Al and Mn (Table 1).

Table 1. Selected physical and chemical properties of the Blanton and Orangeburg soils.

Properties	Unit	Blanton	Orangeburg
pH		4.71	4.70
Ca	mg kg^{-1}	113.50	174.00
Mg	„	14.50	18.50
K	„	24.50	24.00
Fe	„	4.86	7.84
Al	„	5.94	14.10
Mn	„	18.50	53.50
Zn	„	1.50	1.00

Application of hydroxyapatite increased the pH and amount of Ca and Mg leached from Blanton sand, but decreased Al and Fe (Table 2). Leached Na increased markedly due to the composition of both zeolites, but there was some increase in the leached Mg with phillipsite treatment.

Table 2: Major elements leached from Blanton sand columns under different treatments.

Treatment	Leaching Interval	pH	Ca	Mg	Na	K	Al	Fe
			mg kg^{-1}					
Control	2 wks.	3.94	0.69	0.26	0.46	0.43	0.11	0.03
	4 wks.	5.20	0.23	0.08	0.23	0.31	0.17	0.84
Hydroxyapatite	2 wks.	6.18	8.71	0.94	1.82	0.42	0.07	0.02
	4 wks.	6.05	7.97	0.76	1.47	0.32	0.07	0.60
Clinoptilolite	2 wks.	5.11	0.88	0.41	23.77	0.31	0.31	0.14
	4 wks.	6.12	0.53	0.12	12.78	0.12	1.08	1.09
Phillipsite	2 wks.	4.25	4.48	2.83	30.43	0.84	0.27	0.07
	4 wks.	5.46	0.28	0.14	13.40	0.16	0.41	0.20

Table 3: Major elements leached from Orangeburg loamy sand columns under different treatments.

Treatment	Leaching Interval	PH	Ca	Mg	Na K	Al	Fe	
			mg kg^{-1}					
Control	2 wks.	6.00	1.39	0.72	0.18	0.41	0.04	0.43
	4 wks.	6.10	1.29	0.62	0.16	0.40	0.02	0.48
Hydroxyapatite	2 wks.	6.61	9.05	2.49	1.02	0.61	0.04	0.58
	4 wks.	6.50	6.52	1.57	0.92	0.49	0.04	0.79
Clinoptilolite	2 wks.	6.97	1.00	0.37	14.46	0.13	0.46	0.97
	4 wks.	6.56	0.90	0.33	15.92	0.20	0.26	1.11
Phillipsite	2 wks.	6.38	1.21	0.66	20.02	0.33	0.14	0.40
	4 wks.	6.71	0.75	0.34	12.16	0.19	0.22	0.95

Hydroxyapatite treatment also increased the pH and leached Ca and Mg in Orangeburg loamy sand (Table 3). Application of both zeolites resulted in marked increase of leached Na, but other elements were practically unaffected by all three materials. Additional data showed some reduction of leached Mn with both zeolite treatments in Orangeburg loamy sand, while Ni, Cd, Cr and Pb were unaffected in both soils.

4. Conclusion

Our preliminary results indicate certain changes in the nature of acid soil with the application three ameliorants hydroxyapatite, clinoptilolite and phillipsite. Increase in pH of the leachates occurs with small doses of 2.5%. Results show good promise for further studies.

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WEST PAGE SWAMP WETLAND RESTORATION PROJECT AT BUNKER HILL, ID.

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1. Introduction

The Bunker Hill Superfund site in the Coeur d'Alene River Basin in Idaho is the second largest Superfund site in the US. This area had been the nation's largest and richest mining district, with mining and smelting of Zn and Pb beginning in 1916 and continuing until the 1980's. Direct deposition of mine tailings, alone, has contributed an estimated 70 million Mg of Pb, Zn, Cd, and As contaminated material into the river basin, and associated wetlands. The primary ecosystem risk associated with elevated Pb levels in wetlands is to the migratory fowl that use these wetlands as a seasonal feeding and nesting area. Birds using these wetlands as a habitat have died as a result of acute lead toxicity.

West Page Swamp (WPS) is an approximately 11-ha wetland that was used for direct tailings deposition for the Hayes Company Mill from 1918-1929. As part of a closure agreement with Region 10 U.S. Environmental Protection Agency (EPA), the mining companies involved with the site excavated a 2-ha portion of the swamp. Tailings were removed in 1997 to a depth of 0.7 m to reduce the potential for exposure of wildlife to metal contamination. The remaining sediment contains up to 30,000 and 16,000 mg kg^{-1} Pb and Zn.

The goal of this project was to test the feasibility of using biosolids compost in combination with other residuals to accelerate revegetation and to limit the ecosystem impact of metals contaminated wetlands. If successful, this remediation strategy could be used in the approximately 25,000 ha of tailings-affected wetlands downstream of the mining area.

2. Basis for Amendment

Previous work in lead-contaminated soils has shown the potential for soil amendments to reduce the bioaccessibility of lead. This reduction can be achieved in two ways: (1) by altering Pb mineralogy, and (2) through a physical separation of the contaminated sediments from edible plant tissue. By adding approximately 15 cm of fertile surface to WPS, plant species should be able to rapidly reestablish, with rooting concentrated primarily in the compost surface horizon. For waterfowl, this suggests that the bulk of ingested sediment will be from the newly created soil horizon, so that the risk associated with the elevated Pb concentrations in the underlying horizon will be reduced.

3. Project Installation

Application of composts to a pre-existing wetland provides a challenge from an operational perspective. Standing water and a low bearing capacity limit the amount of traffic the floor of the swamp can bear. To gain access to the center of the swamp, logyard debris and wood ash were used to build a road. Road building fabric was laid down and dump truck loads of material were spread over the fabric using a bulldozer. Compost and wood ash were mixed and thrown with an AeroSpread from both the constructed road and the county highway. Coverage was up

to 25 m with most extensive occurring between 0-15 m. Installation was completed in late October.

4. Future Monitoring Scheme

Physical Changes. Depth of treatment will be measured along transects at 0, 12 and 24 months to establish the fate of the treatment (i.e., movement, and settling).

Carbon-Nitrogen Dynamics. Decomposition and N mineralization of organic C and N will be monitored at 12 and 24 months by porous ceramic tubes filled with treatment mixtures, and inoculated with wetland water (for introduction of wetland microbial populations).

Plant Establishment and Elemental Uptake. Changes in plant cover, species diversity, and metal concentration of select species will be monitored in permanent plots within the excavated and vegetated areas.

Effluent Quality. Grab samples on water leaving the wetland will be analyzed monthly for suspended solids, pH, total elements, snd N species

Bioaccessibility of lead. Measures of changes in bioaccessibility of sediment Pb will be made through laboratory incubations using a wet DTPA extraction as well as physiologically based extraction procedure (PBET).

CAN FBC ASH BE USEFUL FOR METAL INACTIVATION IN CONTAMINATED MATERIALS AND FOR REVEGETATION ?

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1. Introduction

Addition of alkaline fly ash to acidic mine spoils, contaminated soils and wastes can neutralise acidity, decrease bulk density, and increase water-holding capacity (Carlson and Adriano, 1993). This may alleviate some of the problems associated with these environments such as metal exposure, allowing the establishment of vegetative cover on previously barren sites (Mench et al, 1998). High amount of coal fly ashes from fluidized bed combustion boiler with limestone injection (FBC ashes) are available. These products contain Ca, Mg, S and some interesting trace elements for living organisms such as Cu, Mn, Se, and B. However, detrimental effect can occur due to soluble salts, e.g. sulphates, and trace elements, e.g. As. The aim was to assess the effect of FBC addition into either acid Zn/Cd-contaminated soils or an acid mine spoil on growth and mineral composition of plants. Special attention was paid on metal (Cd, Cu, Pb, Zn) and As exposure.

2. Materials and Methods

Four metal-contaminated soils, control uncontaminated soils, and a mine spoil have been tested in pot experiments (Table 1). FBC ash provided by EDF was added alone or in combination with other additives (Table 2). For each treatment, amendment and material were mixed by rotating plastic flask. Materials were rehydrated with distilled water and a nutrient solution aliquot. Plants were grown after a 3-wk reaction period. Dwarf bean (vroege limburgs) or maize (volga) were cultivated for two weeks (five plants per pot, 2 or 3 replicates). Plant biomass, chlorophyll density, and mineral composition of plant parts were determined. Metals were measured by either ICP-AES or GFAAS after wet-digestion of plant materials in nitric acid and hydrogen peroxide. Reference material (Bureau Community Reference ryegrass 281) and blanks were included in each series.

Table 1 : Main characteristics of the materials (all collected in the topsoil layer)

Site	pH	Soil type	Org. C	Cd	Cu	Ni	Pb	Zn
			g/kg	mg/kg soil dry weight				
Louis Fargues (France)	6.4	sandy soil	9.8	25	17	70	22	43
Mortagne (France)	5.7	sandy soil	18.9	3.4	19		71	409
Nivelle (France)	6.05	sandy soil		3.1			392	1926
Overpelt (Belgium)6.2	sandy soil	45.7	151	2302	70	4760	18250	
Pyhäsalmi (Finland)	2.5	sandy spoil	0.5	23	1100	8	390	970
Pierroton (France)	5.3	sandy soil	25.7	0.08	3	<2	10	12
Control LUC (Belgium)	6.9	sandy soil	50.5	0.8	10		30	106
Control C24 (France)	5.9	loamy sand		0.7	11		32	91

3. Results and Discussion

(Table 2) Pyhäsalmi spoil is a very acidic material with high soluble Fe and metals. Consequently, maize and bean did not germinate on it. FBC addition either alone or combined with compost resulted in the restoration of maize growth. Despite high metal contents in this spoil, metal concentrations in maize 3rd-leaf grown on FBC treated spoils were similar compared with those found for uncontaminated LUC soil. Dwarf bean growth was improved by FBC addition in 3 contaminated soils (Louis Fargues, Mortagne, Overpelt). Zinc and Cd (except for Overpelt) concentrations in bean primary leaves generally decreased. Maize grown on Nivelle soil showed also a decreased Zn concentration in aerial parts while shoot yield remained constant. Changes in soil pH may likely explain decrease in Zn plant exposure. Lead taken up by

bean was limited in Overpelt soil treated by FBC. Copper plant availability was generally not affected by FBC addition. However, Cu concentration and speciation in solution may be mainly regulated by dissolved organic matter. No detrimental effect was observed when FBC was added to acid uncontaminated soils such as Pierroton. Moreover, metals and As concentrations in dwarf bean primary leaves were similar in treated and untreated Pierroton soils. Arsenic was not increased by FBC addition in contaminated soils.

Table 2 : Shoot yield (g fresh weight/plant) and trace element concentrations in plant parts (3rd-leaf of maize, primary leaves of dwarf bean, mg/kg dry weight)

Site	Treatment	pH	Plant spec	Shoot yield	As	Cd	Cu	Pb	Zn
Louis Fargues	Unt	5.6	bean	1.10a	0.36a	17.1a	17a	2.0a	251a
	FBC (0.17%)	7.2	bean	1.75b	0.15a	1.7b	14b	0.7a	55b
Mortagne	Unt	5.7	bean	1.15a		6.8a	13a	0.7a	1010a
	FBC (0.27%)	nd	bean	2.70b		1.1b	11ab	0.4a	146b
	Control C24	5.9	bean	2.50b		0.1c	10b	0.4a	49c
Nivelle	Unt	6.05	maize	2.84a		0.9a	11a	0.7a	1129a
	FBC (0.2%)	nd	maize	3.12a		1.0a	11a	0.9a	680b
	FBC (0.2%)+SS (1%)	nd	maize	2.94a		0.9a	10a	1.1a	460c
	Control C24	5.9	maize	2.64a		0.5b	5b	<0.3b	67d
Overpelt	Unt	6.3	bean	0.80a	0.83a	3.1a	27a	12.9a	160a
	FBC (0.3%)	8.2	bean	1.75b	0.44a	3.2a	24a	2.6b	87b
Pierroton	Unt	5.3	bean	2.10a	<0.1a	0.05a	14a	<0.3a	54a
	FBC (0.2%)	7.0	bean	2.00a	<0.1a	0.06a	13a	<0.3a	49a
Pyhäsalmi	Unt	2.5	maize	0.00c	-	-	-	-	-
	FBC (9.7%)	7.5	maize	3.06b	<0.2a	0.31a	13.3a	0.3a	58a
	FBC (9.7%)+C (5%)	7.8	maize	3.30b	0.3a	0.21a	11.0a	0.2a	45a
	Control LUC	6.9	maize	5.00a	0.7a	0.18a	4.4b	0.2a	45a

(Amendments : % by air-dried soil weight ; Unt : untreated, C : compost, SS : steel shots . For each soil series, mean values within a column followed by the same letters are not different according to standard deviation)

4. Conclusions

Short-term plant bioassays on a material series demonstrated that FBC addition can be useful to restore plant growth and/or to decrease metals such as Zn and Cd taken up in aerial plant parts depending on soil samples. Decrease in Zn exposure may be often involved in phytotoxicity alleviation. Mechanisms are probably related to changes in pH. Additional adverse effect was not observed in contaminated materials and uncontaminated soils treated by FBC. The sustainability of soil and spoil remediation by alkaline amendments is however questionable. Therefore, long-term experiments will be carried out to assess the duration of beneficial effects related to the FBC addition. These further studies need also to provide data on wild plant species, especially trees, fauna, and material-solution transfer.

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ELECTROKINETIC REMEDIATION OF METAL CONTAMINATED SOILS: PRELIMINARY STUDIES USING ARSENIC CONTAMINATED SOIL

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1.Introduction

Heavy metal contamination of soils through anthropogenic activities is a widespread and serious problem confronting scientists and regulators throughout the world. In most countries reporting contaminated sites, metals account for much of the contamination. For example, metals have accounted for contamination at 65% of the sites at superfund sites in the USA (USEPA, 1997). Remediation of metal contaminated soils is challenging and often an expensive process. Until recently, most remediation technologies involved either development of strategies to manage metal contaminated sites (e.g. via chemical immobilisation) or *ex-situ* remediation. While chemical immobilisation technique minimises risks through reduced bioavailability of metal contaminants, the *ex-situ* technique generally involves removal of contaminants via a soil washing process. Recently, electrokinetic remediation technology has been receiving increasing attraction in soil decontamination. This technology uses a low level direct current to mobilise contaminants via both electroosmotic and electrophoretic processes. The objectives of this study were to assess the potential for electrokinetic remediation of arsenic (As) contaminated soils.

2. Materials and Methods

Arsenic contaminated soils adjacent to cattle tick dips in Northern New South Wales were sampled, air-dried and crushed to pass through a 2-mm sieve. The sieved soils were thoroughly mixed, homogenised and packed into a PVC column to a bulk density of 1.3 g cm^{-3}. Graphite electrodes (10 mm) were attached to the top and bottom covers of the column . After saturation with deionised water the column was connected to a DC converter that subjected the column to either 0, 0.5, 1 or 2 V cm^{-1} of soil. This generated a current of between 2.5 to 3 mA during the electrokinetic process. To facilitate the movement of ions generated in the charged column, a mobile phase was passed through the column at a flow rate of between 5 to 15 ml hr^{-1}. Leachates were collected at intervals of one hour using an automatic fraction collector for a period of 150h. The leachates were passed through a 0.2 μm millipore filter and then used for measurement of pH using an Orion pH meter and total composition using an ICPES. Where the As concentrations were low, measurements were conducted using hydride generation atomic absorption technique.

3. Results and Discussion

There was a significant effect of applied current on the pH and the composition of leachate. The pH of the leachate decreased markedly from 7 in the absence of applied voltage to approximately 3 when the column was subjected to 2 V cm^{-1} of contaminated soil (Figure 1a). This decrease in leachate pH is not surprising and has previously been attributed to the electrolysis of water at both anode and cathode end. In marked contrast to pH, the concentration of As in the leachates increased appreciably with both increasing applied voltage and the duration of leaching (Figure 1b). This led to significantly higher amounts of As being released with increasing applied voltage. In marked contrast to As, there was no effect of applied voltage on the amount of S in

the leachates although the concentration of S decreased with increasing duration of applied voltage. The amounts of metal ions released during the electrolysis process decreased with increasing duration of applied voltage although there was no apparent effect of voltage on the concentration of metal ions in the leachate.

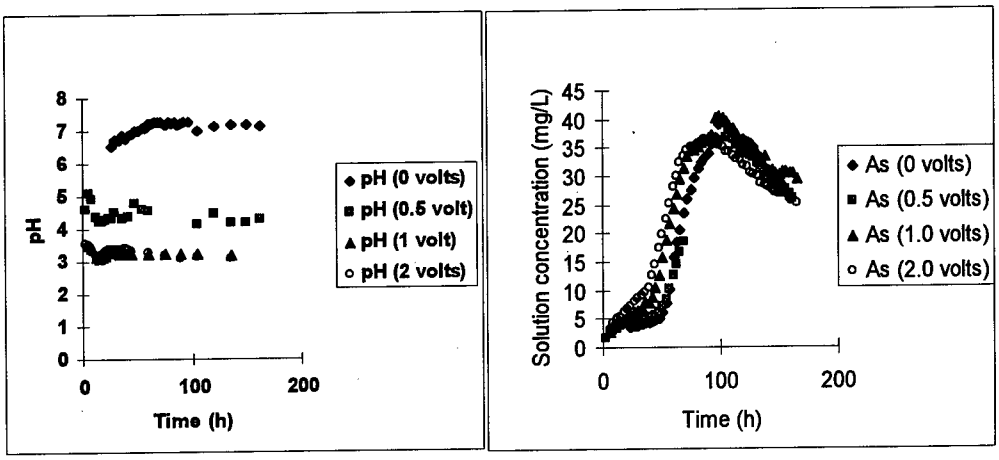

Figure 1. Effect of applied voltage and duration of leaching on the (a) pH and (b) concentration of As in the leachate.

4. Conclusion

The results from our preliminary study reveal that electrokinetic remediation may be a useful technology for the removal of As from contaminated soils. However, considerable effort needs to be directed towards maintaining the soil pH as close as possible to its natural value. While a reduction in pH may enhance desorption of contaminants and dissolution of heavy metal contamination, alkalization of the soils at the cathode end may favour sorption of contaminants and formation of heavy metal precipitates. Current studies in our laboratory focus on techniques for neutralising the electrolytic effect of water molecules on soil and solution pH and the development of optimal conditions for electrokinetic remediation of As.

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SOIL WASHING TECHNIQUES FOR REMEDIATION OF ARSENIC CONTAMINATED SOILS

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1. Introduction

'Plunge dips' filled with pesticides have been used in the past in Australia to rid cattle and sheep of ticks. Unfortunately, this has led to soil contamination with arsenic (As) and 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) at many sites. Although both of these chemicals have been banned for agricultural use since mid-1960's, the high concentrations used in the dips plus the run-off and the sludge that was disposed around the dip have led to extensive contamination of soils at many localised points, particularly in New South Wales (Smith et al., 1998) where there are over 1600 cattle dips. Arsenic and DDT are highly persistent and can be passed into the food chain. The mobility of both As and DDT in soil are generally recognised to be low, but this depends on the physical and chemical properties of the soil at the contaminated site. Major concerns have been raised by the public and regulatory bodies across Australia about the environmental and health risks that these chemicals pose to animals and humans. Although much effort has been directed by various agencies towards assessing the As status of contaminated soils, less effort has been given to examining the potential for remediating contaminated soils. At present there are no well-established techniques for the remediation of As and DDT contaminated soils. In this study we assessed the potential for removing As from the contaminated soils using a soil washing technique at various soil:solution ratios and ambient temperature (the remediation of DDT is being studied as part of a separate investigation). Two approaches were used during the development of the soil washing technique. These included (a) the use of an extractant that stripped As binding sites on the soil colloids and (b) the use of an extractant that enhances the release of As through increased desorption of bound As. These results were compared with As extracted following equilibration with water.

2. Materials and Methods:

Surface (0 to 10 cm) and subsurface (10 to 20 cm) soils were sampled from 12 contaminated sites (composited – 4 samples per depth), air-dried and crushed to pass through a 2-mm sieve. These soils were used for all laboratory studies described in this paper. Total As in the contaminated soils was estimated following microwave digestion and analyses using atomic absorption spectrophotometry equipped with an acetylene–nitrous oxide flame. For the 12 sites that were examined, the concentration of As ranged from <50 to 3000 mg kg^{-1} soil. Generally, As content was low in the surface of sandy soils but high in clayey soils.

To test soil washing approach (a) soil samples were extracted (1:4 soil:solution ratio) with trisodium citrate solution at various temperatures (20 to 100°C) using a hot water bath, centrifuged and passed through a 0.45μm millipore filter. Soil washing approach (b) was tested by extracting As (1:4 soil:solution ratio) using a desorbent that had the capacity to increase the accessibility of desorbing ions to As in the contaminated soils. These extractions were compared with As desorption in water using conditions similar to that adopted for approaches (a) and (b). Following equilibration, As in the filtered extracts were determined as described above. Because of commercial considerations it is not possible to give full details of the processes used.

3. Results and Discussion

Approach (a) – washing with water and sodium citrate

The amount of As extracted by water ranged from < 0.01 mg L^{-1} to approximately 0.1 mg L^{-1} in the clayey and sandy soils, respectively. Sequential extraction of the soils with water led to a gradual increase in the cumulative amount of As especially in the highly contaminated (total As > 1000 mg kg^{-1}) soils. Increasing the temperature of the extraction process did not have any significant effect on the amount of water-extractable As. In contrast to water, the amount of As extracted by sodium citrate increased with both temperature and the duration of extraction process. The increase in concentration of As in the extract may be attributed to the mode of action of citrate that removes As both by displacement of bound As and the by dissolution of the active iron oxide binding sites. The dissolution of iron oxide was confirmed by the Fe content of the extracted solution. For all soils with As concentrations exceeding 2000 mg kg^{-1} between 20 to 30 % of the As was released in the first 15 minutes of extraction with another 20% being released in the subsequent two extractions. However, when the soils were subjected to longer extraction periods with citrate and at temperatures exceeding 80°C, between 85 to 90% of the As was removed in the first extract. In contrast to these highly contaminated soils, there was 100% removal of As at elevated temperatures in the first extract for all soils with lower As concentration ranging from 100 to 1000 mg kg^{-1} soil. These results indicate that by stripping the soil constituents binding As it may be possible to remove As from the contaminated soils.

Approach (b) – washing with enhanced As extractant

The same soils were washed with the extractant that had the capacity to enhance the displacement of and accessibility of extractant to As. The amount of As released in this extract was similar to that observed using the citrate solution at elevated temperature for soils containing less than 1000 mg As kg^{-1} soil. However, the desorbing solution released 20 to 30% more As in the first extract from the highly contaminated soils at room temperature indicating considerable potential for the development of an *ex-situ* technique using this electrolyte solution. There is, however, a need to develop a technique to separate As from the extracted solution to facilitate storage and disposal processes. Preliminary studies in our laboratory indicate that As in the extract could be separated by a simple precipitation technique.

4.Conclusions

Preliminary studies using two different electrolyte solutions under a range of soil:solution ratios and ambient temperatures revealed that all the As in the contaminated soils could be removed by a soil washing process. However, further studies are required to optimise the extraction process for both further enhancing the effectiveness of the extractant solution and for making it economically attractive.

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PERFORMANCE OF AN EXTRACTION PROCEDURE FOR HEAVY METAL REMOVAL FROM A CONTAMINATED SILTY CLAY SOIL

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1. Introduction

Effective remediation technologies for soils contaminated with heavy metal are limited (SHEPPARD and THIBAULT, 1992). We investigated the performance of a counter-current metal extraction procedure (MASSCHELEYN *et al.*, 1996) for the removal of selected heavy metals (Cd, Cu, Pb, and Zn) from a contaminated silty clay soil. In the procedure put forward (Figure 1), soil metals are extracted in a three-step process including decarbonation, solubilization, and washing. Metal mobilization occurs in an acid solubilization step. The acid extract is then used to pre-treat (decarbonate) the contaminated soil in the first step of the counter-current extraction procedure. The soil leaving the solubilization step undergoes a final washing step.

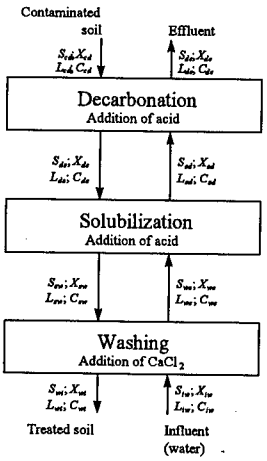

Figure 1. Counter-current metal extraction scheme

2. Materials and Methods

A simple mass-balance model was developed to describe metal mass flows in the counter current procedure (Figure 1). Distribution coefficients (K) of the equilibrium expressions were experimentally determined in a series of batch equilibration experiments. To validate the model, an array of batch-equilibrations was used to simulate the counter-current extraction technique. The soil used was characterized by a silty clay texture, a pH 7.4, 2.0 % organic carbon, 4.9 % carbonate, and a cation exchange capacity of 19.5 $cmol_+$ per kg soil. The soil had total Pb, Cu, Zn, and Cd contents of 240, 105, 1500, and 8.7 mg kg^{-1} dry soil, respectively.

3. Results and Discussion

The counter-current metal extraction scheme set forward makes optimal use of the acid added. A model was developed to describe metal flow during the counter-current metal extraction procedure (Figure 1). Operating conditions necessary for soil metal cleaning were determined and the feasibility of the

extracting procedure evaluated. We considered the treated soil to be decontaminated when metal levels became lower than the Dutch "A" reference values for soil metal content (VROM, 1990).
The influence of varying the pH during the decarbonation step (pHdeca) while solubilizing at pH 1 and washing with deionized water, on the soil Pb content is shown in Figure 2. To decrease the soil Pb content to below 78 mg kg^{-1} dry soil, the decarbonation step must be conducted at pH values below 1.2. According to the model predictions, Pb extracted in the solubilization step would accumulate in soil entering the decarbonation step if pH_{deca} is allowed to raise above 1.4. Moreover, when decarbonation would occur above pH 3.6, no net removal of Pb would occur.

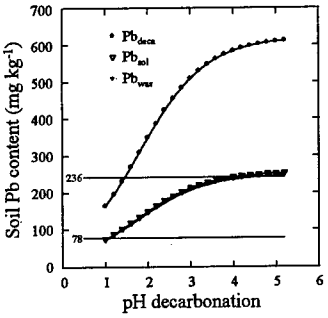

Figure 2. Predicted Pb content in decarbonated (Pb_{deca}), solubilised (Pb_{sol}), and washed (Pb_{was}) soil during the counter-current soil cleaning process as a function of the pH in the decarbonation step (pH_{sol} = 1, deionized water wash

The predicted behavior of soil Cu (data not shown) was similar to Pb. With a solubilization pH of 1, pH_{deca}-values below 1.4 were required in order to obtain a 'clean' soil, containing less than 32 mg Cu kg^{-1} dry soil. At pH_{deca} above 4.2, no net removal of Cu occurred.
Cadmium, in contrast, was easily removed from the soil. Model calculations (data not shown) predicted Cd concentrations in the washed soil to be below 0.65 mg kg^{-1} dry soil when the following operating parameters were used in the extraction procedure: pH_{sol} = 1, deionised water as washing agent, and pH_{deca} below 4.5. Cadmium never accumulated during the decarbonation step.
The effectiveness of the counter-current metal extraction procedure for the removal of Zn was predicted to be limited. Zinc concentrations in the cleaned soil were reduced to only 1195 mg kg^{-1} dry soil after extraction at pH_{deca} = 1, pH_{sol} = 1 and deionized water wash.
Model predictions showed that of the metals studied Cd, Pb and Cu will be extracted from the soil by the counter-current extraction procedure to a level for 'clean' soils under the the following operating conditions: pH_{deca} = 1.2, pH_{sol} = 1.0, 2.5 mol HCl per kg dry soil are required. The overall removal efficiency for Zn was too low in order to reach acceptable soil levels for this contaminant.
In order to execute the soil metal cleaning strategy at pH_{deca} 1.2 and pH_{sol} = 1.0, 2.5 mol HCl per kg dry soil are required. This leads to the need of approximately 200 L of conc. (12 *M*) HCl for each tonne of soil to be extracted.

4. Conclusions

A counter-current metal extraction procedure was examined to remove heavy metals from a silty clay soil. Model calculations allowed for the optimization of extracting conditions. Although our results are limited to the specific experimental conditions used in our study, an approach similar to ours can be of help in predicting feasibility of other (metal) soil cleaning strategies, in a rapid and cost effective manner.

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ENERGY CROPS FOR REMEDIATION OF CONTAMINATED LAND

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1. Introduction

When agricultural perspectives must be abandoned in highly contaminated territories (e.g. following the Chernobyl disaster) due to irremediably high levels in food products, economically and technically unrealistic or socially unacceptable corrective options, interest arises in sustainable rehabilitation approaches. Industrial crops with non-food end use, are potential candidates for the valorisation of contaminated land with restricted use. Under the EC-RECOVER project, short rotation forestry (SRF) for energy production is proposed as alternative land-use. Fast growing willow species are harvested in a 3-5 year cutting cycle and a 22-25 year rotation. Harvested biomass (10-12 t ha^{-1}) is converted into heat and power.

2. Materials and Methods

When evaluating remediation options for radioactively contaminated sites, not only radiological but also technical, economic and socio-political issues should be considered. All these aspects are dealt with under RECOVER (for details we refer to VANDENHOVE et al., 1998):

- *Flux of Cs (and its analogue K) during willow cultivation and wood combustion*

Study of radiocaesium behaviour in a coppice ecosystem on lysimeter scale (detailed cycling study, 2 soil types, 1 clone), on farmers sites in Sweden (different stand ages, soil types and clones) and on test sites in Belarus (2 soil types, 4 clones). The aim is to understand the radiocaesium cycling and the effect of soil (K-status, mineralogy, ...) and plant characteristics (yield, clone, age) on uptake in order to make predictions on radiocaesium levels in wood and assess the radiological sustainability of a SRF system. Lysimeter (1996) and Belarus trials (1997) are established and sampling is continuously on-going. 5 sites in Sweden are sampled and 10 more sites selected.

- *Dose acquired during coppice (and other bio-energy crops) culturing, harvest and conversion*
- *Comparison of SRF at a technico-agricultural level with other energy crops*

Based on crop demand and energy efficiency of production, a series of crops (sugar beet, winter wheat, rape seed, Jerusalem artichoke, sweet sorghum, miscanthus or willow) are evaluated. This evaluation is backed-up with a growth model, developed to assess yield and crop suitability for given climate (Belgium/Sweden/Belarus), soil conditions and input system (GOOR and JOSSART, 1998).

- *Energy balance and economic cost benefit of SRF and other energy crops*

Comparison for high (Western Europe) and low input (CIS, Belarus) systems from production up until conversion (different conversion units considered) based on detailed data collection. In a final comprehensive evaluation, parameters will be identified which affect the viability of the

SRF-concept, providing us with the necessary tool to validate this site rehabilitation option critically.

3. Results and Discussion

The willow soil-to-wood TFs are up to a factor 1000 lower than for forests (10^{-4}-10^{-3} m^2 kg^{-1}). Willow variety hardly affects the TF and effect of stand maturity is not straightforward. Soil type plays an important role. For Sweden and Belgium, the soil-to-wood TF (m^2 kg^{-1}) is 2-9*10^{-6} for loamy, 2-6*10^{-5} for sandy and 8*10^{-4} for peaty soil. The respective Belarus first-year TFs are a factor 100 higher, both for the sandy and peaty soil. This is partly due to a low yield (resulting in a Cs concentration effect and relatively more bark: TF bark > TF wood) and low soil fertility. Given the preliminary TFs for Belarus and the exemption limit for fuelwood (370 Bq kg^{-1}) coppice fuelwood production should be restricted to sandy or peaty soil with contamination levels of < 1200 and 290 kBq m^{-2}, respectively. Some significant correlations between soil parameters and TF were found. However, future data (end of 1998) from continued sampling in existing trials and additionally selected sites in Sweden will be indispensable to make reliable long-term predictions for the effect of soil type and crop maturity on the TF.

A comparison at a technico-agricultural level of coppice with other bio-energy crops reveals that willow is the most promising crop: suitable for various soil and climate conditions, limited fertiliser demand and efficient production system (energy efficiency of 20-30 compared to 2-22 for other crops). In Belarus, actual first-year yield was low (0.4-1.4 t ha^{-1}, compared to 2.5 t ha^{-1} in the West) due to low soil fertility and water availability. Potential yield estimates for Belarus are, however, comparable. Fertilisation, especially with K, will increase yield and decrease the TF.

A preliminary comparison of energy balance and economic cost-benefit of SRF and other bio-energy crops shows SRF is more profitable and that yield, mechanisation level and scale of conversion unit are critical parameters determining system viability.

4. Conclusions

On general radioecological (low TF), technico-agricultural and energetic grounds and relying on presently available data, SRF can be recommended for rehabilitation of contaminated land for Western conditions. For Belarus, the results are less optimistic, but 1998 results have to be awaited to make firm conclusions. Final conclusions on the economic and comprehensive evaluation are expected in June 1999 when the RECOVER project will be finalised (VANDENHOVE et al., 1999).

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Retention and Adsorption of Trace Elements

(Technical Session 16)

ZINC ADSORPTION BY ACRIC SOILS OF THE STATE OF SÃO PAULO, BRAZIL

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1- Introduction

Due to their mineralogy, highly weathered soils have variable electric charges as a function of pH and ionic concentration. Under natural conditions, Oxisols with acric properties show a positive balance of charge in the subsoil, due to abundant amounts of Fe and Al oxides and low contents of organic carbon. Knowledge about zinc adsorption in this type of soils is incipient, although Oxisols are dominant in Brazil and are used in the cultivation of many crops.

2- Materials and Methods

The objective of this study was to evaluate the effect of pH and zinc concentration on zinc adsorption in an Anionic "Rhodic" Acrudox (RA), an Anionic "Xanthic" Acrudox (XA) and a Rhodic Kandiudalf (RK). Soil samples were collected from the A and B horizons.

In order to vary soil pH, samples were incubated with calcium carbonate and hydrochloric acid. Zinc adsorption was evaluated by equilibrating the samples with solutions containing either 2, 16 or 64 mg dm^{-3} of Zn, employing $CaCl_2$ 0.0025 mol L^{-1} as the support solution. Two grams of soil were stirred for 24h with 20 ml of each solution, in duplicate. The content of adsorbed Zn was estimated by subtracting the concentration of Zn in equilibrium from the initial level of added zinc.

3- Results and Discussion

In the surface horizons, the values for the zero point of salt effect, ZPSE (Table 1) were almost the same for all soils. Zinc adsorption, at pH 4.0, was higher for RK, probably due to its higher CEC. On the other hand, in the subsurface horizons, where the organic matter level was similar in all soils, the ZPSE was 3.6 in RK, 5.7 for RA and 6.1 for XA, suggesting that there was a high charge reversion in the acric soils, as indicated by delta pH values. When pH varied from 4.0 to 8.0, similar amounts of adsorbed zinc were observed in the three soils, even considering the higher CEC values for RK (Table 2). Thus, it is reasonable to suppose a higher zinc affinity for the iron and aluminum oxides in the XA and RA as compared to the Alfisol (RK).

At pH 4.0, when 2 mg dm^{-3} of Zn were added, at both depths, the adsorption was similar for all soils, being slightly lower for XA. When zinc in solution was higher, RK adsorbed significantly more zinc than acric soils. This did not happen in pH 8.0 (Table 2).

Table 1. Chemical, physical and mineralogical characteristics of the soils.

Soil	Depth cm	C gkg^{-1}	ΔpH	ZPSE	ki	CEC pH 4.0	CEC pH 8.0	Clay gkg^{-1}	Kaolinite %	Gibbsite %
XA	0-20 (A)	16	-0.6	3.4	0.98	14	71	350	-	-
	40-60 (B)	6	+0.6	6.1	0.97	9	30	450	37	32
RA	0-20 (A)	20	-0.9	3.6	0.93	15	98	600	-	-
	80-100 (B)	5	+0.3	5.7	0.87	19	56	640	41	41
RK	0-20 (A)	26	-0.9	3.6	1.89	103	247	820	-	-
	75-95 (B)	7	-0.7	3.7	1.74	71	148	700	57	10

Letter in parenthesis indicates soil horizon; C = organic carbon; ΔpH= pH KCl N - pH H_2O; ZPSE = zero point of salt effect, determined by potentiometric titration; ki = SiO_2 x 1.7/Al_2O_3; CEC = cation exchange capacity.

When each soil was individually studied, zinc adsorption was similar for A and B horizons at a given pH and added zinc. Organic carbon apparently have had little influence in the extent of Zn adsorption. The mineralogical constitution of the acric soils, with pH - dependent electrical charge, seems to control zinc adsorption at pH 4.0 to 8.0.

Table 2. Zinc adsorption as a function of pH, zinc concentration and soil horizon.

		Added zinc ($mg\ L^{-1}$)								
		2	16	64	2	16	64	2	16	64
Horizon	pH	Adsorbed zinc ($mg\ dm^{-3}$)								
		RK			XA			RA		
A	4,0	10	110	464	6 36	191	10	62	276	
B	4,0	14	96	325	6 52	193	12	51	258	
A	8,0	20	157	556	20	147	638	20	160	636
B	8,0	20	158	640	19	157	638	20	160	638

4- Conclusions

Considering the increasing of zinc adsorption as function of pH, the level of added zinc and the CEC of the soils, acric Oxisols showed a high bonding affinity for zinc, when as compared to the Alfisol, indicating the presence of specific adsorption sites in the acric soils.

COPPER ADSORPTION AND PH EFFECTS IN HIGHLY WEATHERED BRAZILIAN OXISOLS

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1. Introduction

Surface sorption is the most important mechanism which regulates Cu concentration in the soil solution. As the pH increases, the number of negative charges on soil surface sites increases, and Cu adsorption tends to be higher. We examined highly weathered Brazilian soils where the colloidal fraction was mostly consisted of Fe and Al oxides and hydroxides and 1:1 layer silicates. The objective was to evaluate the effects of pH on Cu adsorption in the B horizons of three soils, two of them with effective CEC lower than 15 $mmol_c\,kg^{-1}$ clay.

2. Materials and Methods

Subsurface (B-horizon) samples were taken from three soils from the state of São Paulo, Brazil: Rhodic Kandiudalf (RK), Anionic "Rhodic" Acrudox (RA), and Anionic "Xanthic" Acrudox (XA). Some chemical attributes and clay contents are shown in Table 1.

Table 1. Soil chemical attributes and clay contents.

Soil	Depth	C	Clay	Fe_t	Fe_d	Fe_o	Al_t	Al_d	Al_o	Kt	ki	ZPSE	pH H_2O	ΔpH
	m	----------					g kg^{-1}			----------				
RK	0.7-0.9	7	700	284	150	14	195	21	7	570	1.74	3.6	6.0	- 0.7
RA	0.8-1.0	5	640	341	54	9	195	21	7	410	0.87	5.8	5.3	+ 0.3
XA	0.4-0.6	6	450	96	45	7	125	31	8	370	0.97	6.0	5.1	+ 0.6

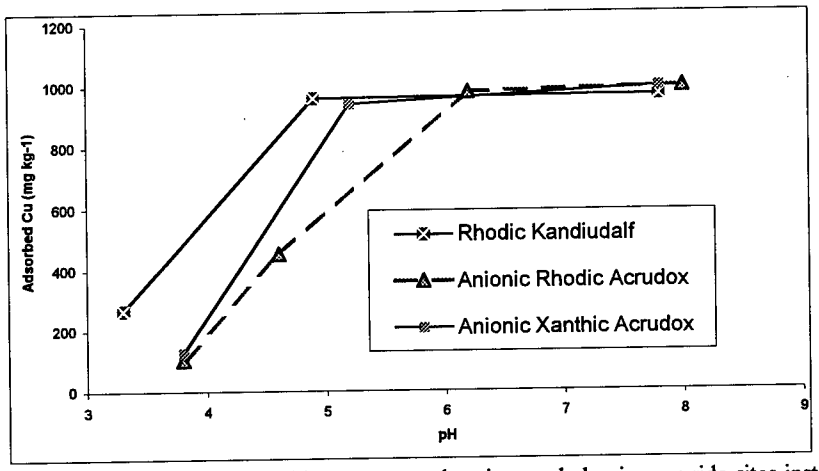
C = organic carbon; Fe_t = total iron oxide; Fe_d = "free" iron oxide; Fe_o = amorphous iron oxide; Al_t = total aluminum oxide; Al_d = "free" aluminum oxide; Al_o = amorphous aluminum oxide; Kt = kaolinite; ki = SiO_2 x $1.7/Al_2O_3$; ZPSE = zero point of salt effect, determined by potentiometric titration; ΔpH = pH KCl 1N - pH H_2O.

The experiment was conducted in duplicate. Addition of HCl or $CaCO_3$ followed by incubation for 3 months altered pH between 4 and 8. Afterwards, 20 mL of a 100 mg kg^{-1} of copper ($CuCl_2$) solution were added to 2 g of soil and shaken for 24 hours. Copper was determined by atomic absorption. Soil adsorbed Cu was obtained by subtracting the copper in the solution, after centrifugation and filtration, from the copper added (in the different concentrations). The quantity of Cu originally present in the samples, although small, was also subtracted to calculate this content. The pH at which 50 % of the added Cu had been sorbed (pH_{50}) was used as a measure for Cu adsorption by these soil samples.

3. Results and Discussion

Copper adsorption increased in the three soils, with increasing pH values up to 8.0, when the maximum adsorption was achieved (Figure 1). The sequence of pH_{50} was RK (≅ 3.9) > XA (≅ 4.5) > RA (≅ 4.8) and indicated decreasing affinity for soil Cu adsorption, since adsorption at increasing pH occurs for sorbents on the adsorption sites with decreasing binding energy.

Figure 1. Copper adsorption-pH curves.



Soil Cu adsorption was probably concentrated on iron and aluminum oxide sites instead of silicate clays or organic matter. The low values of organic carbon in the three soils and the prevalence of kaolinite in the clay fraction of the Oxisols explain this predominance of Cu bonding to sesquioxides. On the other hand, some peaks of hydroxy-interlayered vermiculite were found in the X-ray diffractograms of the Kandiudalf (not shown) and these minerals adsorb higher amounts of copper than 1:1 minerals. The positive charge balance in both Oxisols ($\Delta pH > 0$) was important in defining the lower Cu adsorption in the Oxisols, where the ZPSE were 5.8 and 6.0 for RA and XA, respectively. This finding reinforced the action of the oxides in charge balance, because their zero points of charge vary from 8 to 9. The higher Cu adsorption in the XA, compared to the RA, was probably due to the higher Fe_o/Fe_d ratio and the effect of free aluminum (in XA, Al_d represents 25 % of total Al, while in RA, Al_d is 11 % of total).

4. Conclusions

Copper adsorption increased with increasing pH. In the B horizon of Brazilian Oxisols, with ZPSE at 5.8 and 6.0 and net positive charge balance, adsorption was lower than in the Alfisol, with ZPSE = 3.6 and net negative charge balance.

INFLUENCE OF PLANT RESIDUES ON ZINC ADSORPTION AND DESORPTION IN AN INCUBATION EXPERIMENT

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1. Introduction

The increasing contamination of soils by trace elements and the associated environmental hazards are proof for several studies of their reactions with soil naturally occurring adsorbents i.e. mineral oxides and organic matter (TILLER et al. 1984). The purpose of the current work was to assess the influence of two plant residues on zinc sorption in an incubation trial.

2. Materials and Methods

A light loamy sand soil: 6.0 g C/kg; $pH_{(CaCl2)}$ 6.3; $CEC_{(Mehlich8.2)}$ 5.3; $Zn_{(1M\ HCl)}$ 39.7, was incubated with dried and ground wheat straw, WS (0.64% N) and sugar beet leaves, SB (4.2% N). Plant materials were added at 1% (dry weight basis) and three treatments were set: without organic additions -Or, 1% WS and 1% SB. Zinc (as $ZnSO_4$) was applied at the rates of 50, 150 and 250 mg/kg. Treatments were duplicated and kept moist at 70% FWC, 20°C. The incubation lasted 65 days and soil samples for adsorption studies were taken at 14 and 65 days since the start of the incubation. Different concentrations of zinc: 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 $mmol_c/dm^3$ were prepared by dissolving $Zn(NO_3)_2$ in 0.1M $Ca(NO_3)_2$ as a background electrolyte. These solutions were added to soils at 1:10 soil/solution ratios, shaken for two hours, equilibrated overnight and filtered. Zinc after equilibration (C_e) was determined by AAS flame method. Zinc adsorption curves were established by plotting C_o (initial Zn concentration) versus adsorption coefficient expressed by $1\text{-}C_e/C_o$.

3. Results and Discussion

Figures **1a, 1b** and **1c**, and **2a, 2b** and **2c**, show Zn sorption curves for all treatments (see Materials and Methods). Zinc added at 50 ppm was entirely adsorbed irrespective of the treatment and the sampling time. The similar curves trends for –Or and WS may be attributed to the slow decomposition rate of wheat straw resulting in a shortage of new organic substances being one of the most important soil adsorbents. The highest Zn sorption in the case of SB (Fig. **1b, 2b**) is a proof of intensive mineralization of sugar beet leaves hence an input of organic substances enchancing Zn adsorption.

At Zn application rates higher than 150 ppm a possible Zn desorption may take place especially for treatments –Or and WS (Fig. **1a, 1c** and **2a, 2c**). Zn adsorption in all treatments, may be ranged as follows: Sugar beet leaves (SB) > Wheat straw (WS) ≈ without Organic addition (-Or)

4. Conclusions

Zinc adsorption was notably influenced by the type of plant residues. The strongest Zn adsorption occurred in the treatment SB, irrespective of the application rate. At Zn inputs higher than 150 ppm its desorption in the case of WS as well as -Or is possible.

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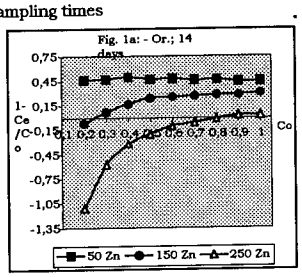
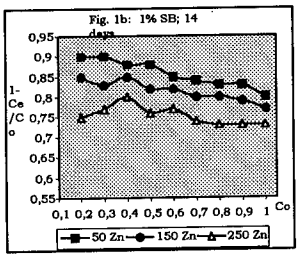
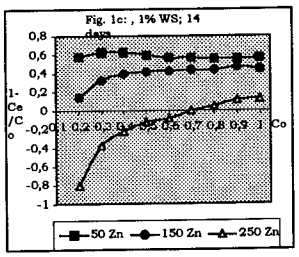
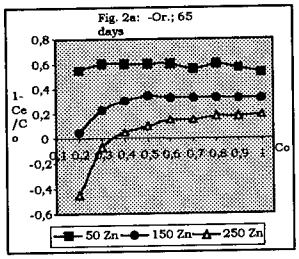
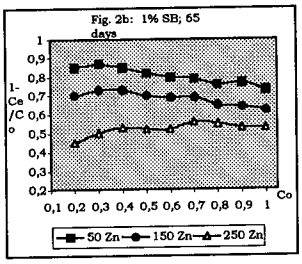
Zinc adsorption and desorption for treatments without organic additions (-Or), with sugar beet leaves (SB) and wheat straw (WS) at two sampling times

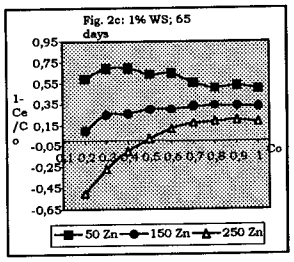












THE USE OF LANGMUIR EQUATION FOR THE ESTIMATION OF ZINC ADSORPTION IN AN INCUBATION EXPERIMENT

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1. Introduction

The adsorption of zinc by soil has importance in determining its bioavailability and its movement through the soil layers (TAN et al., 1971; DIATTA and KOCIALKOWSKI, 1998). The objective of this work was to evaluate using Langmuir one-surface adsorption equation the effect of two plant residues on Zn sorption in an incubation experiment.

2. Materials and Methods

The preparation of soil samples for adsorption studies is widely described in one of the authors extended abstract (this issue). The amounts of Zn adsorbed (S) were calculated as the difference between initial Zn concentration (C_o) and that remaining in solution after equilibration (C_e). Zinc adsorption parameters were calculated according to the Langmuir one-surface adsorption equation:

$$C_e/S = 1/a_{max} \cdot b + C_e/a_{max}$$
$$S = (C_o - C_e) \cdot V/W + S_o$$

where,
S: amount of Zn adsorbed ($mmol_c/kg$); C_e: equilibrium solution concentration ($mmol_c/dm^3$); a_{max}: adsorption maximum, ($mmol_c/kg$); b: bonding energy term ($dm^3/mmol_c$); C_o: initial solution concentration ($mmol_c/dm^3$); V: volume of the solution (cm^3); W: soil sample weight (g), S_o: initial metal content in soil ($mmol_c/kg$). Amounts of Zn extracted by 0.05M $Cu(CH_3COO)_2$ (pH 6.5) in the respective treatments were used for S_o calculation.

3. Results and Discussion

Adsorption parameters are listed in Tables 1, 2 and 3 for all treatments (see materials and methods). In the treatment without organic additions (-Or) (Table 1) maximal absorption (a_{max}) of Zn occurred at 250 ppm where simultaneously the values of the bonding energy (b) reached their lowest level. No striking differences in the maximal buffering capacity (MBC) parameters were noted at 50 and 150 ppm of Zn added. This state suggests that up to 150 ppm of Zn the soil alone may sufficiently bind Zn without excessive saturation of the sorbing complex.
The treatment receiving sugar beet leaves (SB) was characterized by higher values of all adsorption parameters in comparison with other treatments (Table 2). Unexpectedly at the first period (14 days) the MBC values increased simultaneously with the amount of Zn added.

Table 1: Adsorption parameters for the treatment without organic additions

Without organic additions (- Or.)						
14 day				65 day		
Zn (ppm)	a_{max}	b	MBC*	a_{max}	b	MBC
50	9.37	3.10	29.1	11.2	4.95	55.3
150	11.0	2.58	28.4	10.9	5.41	59.2
250	12.3	1.35	16.6	13.9	3.59	49.9

*: Maximal Buffering Capacity = $a_{max} \cdot b$, (dm^3/kg)

Sugar beet leaves richer in nitrogen underwent a quick decaying process. This probably increased the concentration of organic acids enchancing soil buffering properties.

Table 2: Adsorption parameters for the treatment with sugar beet leaves

Sugar beet leaves (SB)						
14 days				65 days		
Zn (ppm)	a_{max}	b	MBC	a_{max}	b	MBC
50	12.4	16.8	207.0	11.0	16.0	175.0
150	14.1	16.0	225.0	12.3	10.7	131.0
250	15.6	15.1	236.0	14.6	7.39	108.0

In the case of the treatment with wheat straw (WS), b and MBC parameters notably and simultaneously decreased with Zn application rates at 14 and 65 days of incubation, (Table 3). Furthermore one may point out a certain stability of all adsorption values at 150 ppm Zn for both treatments at the second period.

Table 3: Adsorption parameters for the treatment with wheat straw

Wheat straw (WS)						
14 days				65 days		
Zn (ppm)	a_{max}	b	MBC	a_{max}	b	MBC
50	10.2	4.89	49.9	7.71	12.6	97.2
150	14.5	2.53	36.7	10.1	5.67	57.1
250	13.1	1.36	17.8	13.7	1.88	25.9

4. Conclusions

The highest values of adsorption parameters were found in the treatment with sugar beet leaves. On the basis of MBC values all treatments, irrespective of Zn application rates may be ranked as follows: Sugar beet leaves (SB) > Wheat straw (WS) > without Organic additions (-Or).

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SORPTION OF LEAD AND CADMIUM ON DIATOMOTES FROM LESZCZAWKA DEPOSIT

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1 Introduction

Purification of soil from the excesses of heavy metals may be performed by binding those elements with minerals and humus or with other organic materials or minerals used for soil treatment. Such materials should bind harmful elements in a selective and permanent way. Diatomites may well fulfil that role. (Borowiak at al., 1986).
They occur in large volume as sedimentary rock in the south-eastern parts of Poland, are characterised by considerable sorption capacities (over 20 mgR/100g of soil) and can induce approx. 60 % of saturation of sorption complex with base cations.

2. Materials and Methods

The diatomite rock obtained from deposit in Leszczawka was ground in ball grinder, with 0.00-0.25 mm fraction being separated for further testing. Lead and cadmium cations of concentrations 2.5, 5.0, 7.5 mg/dcm^3 were added in the form of water solutions of chloride salts in a proportion of 250 ml of the solution per 100 g of diatomites.
The concentration of cations was measured directly in the slurry by ASA method every day for 20 days. The durability of bonding was determined after 20 days by acidulating the slurry with 0.1 HCl, adding 1 ml of acid for five consecutive days, while measuring the variation of acid reaction (Rybicka and-, Kyziol 1991).

3. Results and Discussion

The lead sorption rate was high. During the first measurement its concentration in the solution was reduced from 2.5 and 5.0 to below 0.5 mg/dcm^3 and reached a state of equilibrium. From initial concentration of 7.5 mg Pb/dcm^3 , a reduction to approx. 1.0 mg/dcm^3 occured after 24 hours and then, after three consecutive days, there was a further reduction to 0.5 mg and an equilibrium state was reached.
Sorption rate for cadmium was even higher. After just 6 hours its concentration became reduced from 2,5 mg to - levels below 0.25 mg Cd /dcm^3 and from 5.0 mg to below 1.0 mg Cd /dcm^3 at eguilibrium. Initial concentration of 7.5 mg became reduced to 1.5 after 6 hours and,- reached the state of equilibrium at approx. 1.0 mg Cd /dcm^3 during the subsequent three daily measurements.
Acidulation of water slurries of lead and cadmium salts in the presence of diatomites with hydrochloric acid caused systematic variations of pH reaction (Table 1) and the desorption of cations.
Desorption of lead from diatomites occured only at the highest initial concentration and the concentration of lead in the slurry increased from 0.5 to approx. 1.0 mg Pb/dcm^3 .

Release of cadmium under the effect of acidulation was stronger and commensurate to initial concentration. After adding 5 ml of 0.1N HCL its concentration increased to approx a bit confusing. 1, 3 and 4 mg for initial concentrations of 2.5, 5.0 and 7.5 mg Cd /dcm^3, respectively.

4. Conclusions

The state of equilibrium between lead and cadmium in the solution and in diatomites is achieved 24 hours after Incubations, where , approx. 95 % of lead and 85 % of cadmium was subjected to sorption processes.

Lead sorption at lower concentrations is more pronounced, however, the lowering of solution pH 3.2 increased the concentration of lead from 0.5 to 1.0 mg/dcm^3.

Table 1 Variation of pH of water slurry of lead and cadmium in diatomites versus the dose of added acidulant (0.1N HCl)

Element	Initial concentration, mg/cm^3	pH Value					
		Initial	After adding 0.1n HCL (ml)				
			1	2	3	4	5
Lead	2,5	4,78	4,26	4,07	3,55	3,55	5,51
	5,0	4,23	3,88	3,69	3,37	3,32	3,22
	7,5	3,83	3,56	3,38	3,17	3,14	3,02
	Average	4,28	3,90	3,71	3,36	3,34	3,25
Cadmium	2,5	5,63	4,93	4,50	4,10	3,97	3,90
	5,0	5,33	4,51	4,23	3,78	3,71	3,70
	7,5	5,12	4,59	4,31	3,86	3,73	3,71
	Average	5,36	4,68	4,35	3,91	3,80	3,77

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KINETIC AND EQUILIBRIUM STUDY OF ZN SORPTION BY GREY FOREST SOILS OF RUSSIA

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1. Introduction

Data concerning Zn sorption obtained for different soils and experimental conditions (Chairidchai and. Ritchie, 1990, Stahland James, 1991) suggest that a single mechanism does not control Zn distribution between solid phases and soil solution. While Zn retention by soils is likely to be complicated process, involved several different reactions, it was shown (Shuklaand Mittal, 1979) that the quantitative equilibrium between dissolved and adsorbed zinc in soils could be described by the Langmuir's or Freundlich's equations. Equilibrium studies, however, have a limited value: equations allow to predict the levels of metal sorption, but do not give any information of the reaction mechanisms. Kinetic approaches can provide valuable insights into the reaction pathways and mechanisms of chemical interactions. The purpose of this investigation was to apply chemical kinetics and equilibrium studies to gain data about the nature and energies of Zn retention by the grey forest soils of Russia.

2. Materials and Methods

Samples used in the study were collected from genetic horizons of two soil profiles of the Tulskaya province of Russia. Soil samples were dried and passed through a 1 mm sieve. Sorption-desorption equilibrium experiment was carried out in duplicate. Series of 2 g samples of each soil were shaken on a mechanical shaker for 1 hour with 40 mL of 0.005 M $CaCl_2$ (as a background electrolyte) containing from 1 to 500 μg Zn/mL. Then suspensions were allowed to stand 24 hours to attain equilibration, after which they were filtered and Zn in the filtrates was determined by atomic absorption spectrophotometry. The amount of sorbed Zn were calculated as a difference between initial and final concentrations. Immediately after sorption the desorption procedure was conducted. After finishing of the sorption experiment soil samples were treated by 0.005 M $CaCl_2$ (immitation of natural surface waters), ratio soil-solution and the procedure of equilibrating were the same as in the sorption experiment. The number of treatments, required for the complete removal of all exchangeable Zn varied from 2 to 5 dependantly on the soil horizon and initial Zn loading. The amount of Zn desorbed were measured in the filtrates after each treatment and thereafter the overall Zn removed from the soils was calculated. Kinetic experiment was carried in triplicate at the ratio soil-solution 1:20. Soil samples were mixed with solutions containing 5, 20, 50 μg Zn/mL (at the 0.005M $CaCl_2$ or bidistilled H_20 as a background). Suspensions were continuously stirring, and the sampling was performed at 10 sec-intervals during the first minute, at 30-60 sec - over the interval 1-10 min, then interval was increased. Phases were separated by a syringe equipped with a replaceable filter. Changes of Zn concentration through the sorption process were examined within 24 hours.

3. Results and Discussion

The values of C_e/x vs C_e were plotted to fit the data to the Langmuir's isotherm: $\frac{Ce}{x} = \frac{1}{kb} + \frac{Ce}{b}$, where Ce is equilibrium Zn concentration, μM/mL, x - Zn adsorbed by soil, μM/g, k and b - are constants representing the binding energy and adsorption maximum coefficients. The obtained curves can be split into two linear parts (I and II) with the different slope, that may indicate 2 types of adsorption sites or reactions. Complete saturation was observed only for arable horizons: b=77-155 μM Zn/g. The binding energy coefficients were higher and adsorption maximum were lower in the Part 1 than in Part 2 of the curves for all investigated soils. The high k-values in the Part 1 showed that at low equilibrium concentrations (up to .0.15 μM/mL) Zn is retained at the sites with high affinity (so called specific adsorption). This share of most firmly retained Zn measures 4-14% from the total adsorbed amount. The desorption experiment confirmed that strength of Zn bonding by soils is different. The number of desorptions required for the complete extraction of weakly tied Zn measures 2-3 times for those soil samples where loading range corresponded to the Part I of the curve at the Langmuir's plot, and 5 times for higher loading. Desorption demonstrated that share of firmly tied Zn ranges from 60 to 82% that is much greater in comparison with amount can be assigned to the so named specific sorption. At the same time soil horizons which had the highest k_1 -values after exhaustive desorption were found to contain more firmly bonded Zn. Kinetics demonstrate that sorption process is very fast: the quantity of sorbed Zn in the first 5 sec of the experiment ranges from 43-60% (in $CaCl_2$) to 56-97%.(in H_2O) The equilibrium is reached within 5-20 min dependantly of solution composition. Sorption rate may be governed both by the rate of diffusion and chemical reactions. It was shown that external diffusion may influence Zn sorption kinetics if rate of stirring is less than 200 rot/min, whereas for the given experiment it was fixed at 300 rot/min. Within the first minute of the experiment the rate of Zn sorption is described by the 1st order reaction. Reactions constants measure 0.0086±0.0011 sec^{-1} for the Zn-$CaCl_2$ systems and 0.0147±0.0012 sec^{-1} for Zn-$H_2$0-system. Nature of soil and horizon don't influence constants values. This result suggests that the reactions between the soil and the Zn-containing solution are the same for all investigated soils within 1st min of the interaction.

3. **Conclusions**

The maximum applied loading 15.4 mM Zn/100g allows complete saturation only for arable horizons of the investigated soils. More than 60% of the sorbed amount of Zn is not extracted after the treatment by the weak electrolyte (with ionic strength 0.03 M/L). These values are larger than the amounts of "specifically" sorbed Zn calculated from the Langmuir's equation. Within the first minute the rate of the sorption process is described by the 1st order reaction.

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ENHANCEMENT OF CU ADSORPTION ON SOILS IN PRESENCE OF AMITROLE

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1. Introduction

Soils receive many metals as a result of the use of agrochemicals and the disposal of industrial and domestic wastes containing heavy metals. At the same time, large additions of pesticides are necessary to achieve the demand of high agricultural productivity.

The environmental fate of heavy metals and pesticides is mainly determined by their capacity to be sorbed onto soils. The presence of pesticides could influence the sorbing properties of soils with respect to heavy metals, and viceversa.

In spite of the fact that there are many studies on adsorption of heavy metals and pesticides on soils independently, little attention has been paid to the phenomena which take place when both are present together (Undabeytia et al., 1994; Morillo et al., 1997; Maqueda et al., 1998). So, the aim of this paper is to study the effect of the presence of the pesticide amitrole (AMT) on the adsorption of Cu by soils amended with different waste fertilizers.

Amitrole is a non-selective herbicide. It is a basic compound that is able to protonate in aqueous solution depending on the pH, and is also able to form complexes with various transition metals, Cu among them (Lukasiewicz et al., 1992).

2. Materials and Methods

The soil used was a sandy soil (SR) from Huelva (SW, Spain). This soil was treated with several wastes used as fertilizers. The wastes used were: urban solid residues (RSU), waste from paper industry (RP) and wastes from olive mill industry mixed with other plant refuse. All samples were taken from the upper horizon (0-25 cm).

The pesticide used was amitrole (3-amino-1,2,4-triazole). The heavy metal selected was Cu due to its extensive use for agricultural purposes.

The adsorption experiments were done in triplicate in 50 mL polypropilene centrifuge tubes by mixing 0.5 g of soil samples with 20 mL of solutions containing various concentrations of Cu and AMT. The concentrations used were 5-60 ppm for Cu and 25-200 ppm for AMT. All experiments were carried out in 0.01 N $Ca(NO_3)_2$ medium to keep the ionic strength constant.

3. Results and Discussion

The amount of Cu adsorbed on the four soils used after reaching the equilibrium gave the following sequence: RP > RSU > RF > SR.

The same sequence presents the kinetic study, 24 h being sufficient for reaching equilibrium in the original soil (SR), 3 days for RF and 15 days for RSU. Cu adsorption in soil RP did not reach the equilibrium after 22 days of treatment.

The wastes added to the soil have changed some of their properties, such as organic matter content and pH. The pH value for soil SR is 6.54, but when this soil receives the different wastes the pH values obtained are 6.93 for RF, 7.27 for RSU and 7.67 for RP. This circumstance, together with the organic matter content and its quality, favours a higher adsorption of Cu.

Moreover, in the case of soil RP, these residues contain lignosulfonates, which are substances normaly used as ion exchangers, so that cationexchange will contribute to Cu retention.
In presence of AMT the amount of Cu adsorbed on the four soils increases as the pesticide concentration in solution increases. The equilibrium pHs in the presence of AMT are slightly lower than in its absence, so the increase in Cu adsorption is not due to pH effect. AMT forms complexes with some heavy metals in solution (Lukasiewicz, 1992) and it could be contributing to the increased adsorption of Cu.
In order to check whether this phenomenon was related with the presence of Cu-AMT complexes in solution, an experiment was carried out in which the soils were previously treated with a solution of AMT, and later with Cu solution. In this case, the presence of AMT adsorbed on the soil did not affect Cu adsorption.

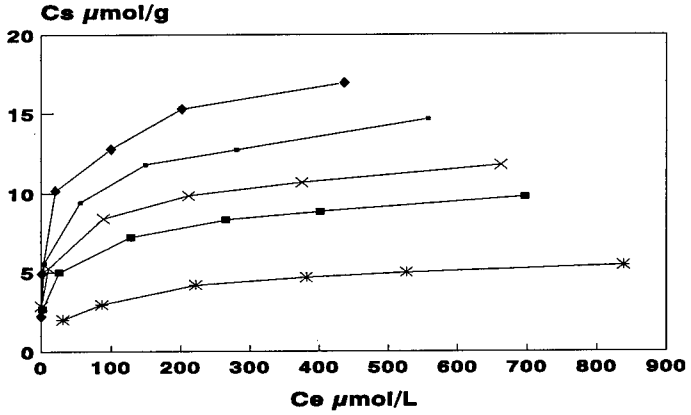

Fig.1. Adsorptions isotherms of Cu on soil SR without AMT (✳) and in the presence of 25 (❑), 50 (×), 100 (·) and 200 (□) mgL^{-1} of AMT in solution.

4. Conclusions

The addition of wastes to the soil increases the amount of Cu adsorbed. Cu retention increases in the presence of AMT, both in the original and amended soils. This is due to the formation of a Cu-AMT complex which has more tendency to be adsorbed than free Cu.

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COPPER AND ZINC COMPETITIVE ADSORPTION IN ACID SOILS

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1. Introduction

The retention - release reaction of heavy metals by soils plays a significant role in their availability to plants, leaching losses and potential contamination of groundwater supplies. Adsorption of trace metals is pH dependent, Cu adsorption beginning at a lower pH than Zn adsorption and more Cu than Zn is adsorbed at the same pH. Copper and Zn adsorption is depressed by mutual competition to the adsorption sites. Competitive sorption has been studied by several researchers. Christensen (1987) established a model for Zn competition with Cd using a simplified competitive Langmuir equation. Murali & Aylmore (1983) applied to multicomponent adsorption models the competitive Langmuir equation as well as Freundlich-type isotherms.

Competitive Langmuir equation.- The competitive Langmuir equation may be written in the linear form as a function of the distribution coefficient K_d^i :

$$1/K_d^i=1/(Q_i\, k_i)+1/(Q_i)\, C_i, \qquad (1)$$

and the competitive Langmuir equation for binary adsorption

$$1/K_d^i=1/(\, Q_i\, k_i)+(1/\, Q_i)\, C_i+1/\, Q_i\, (k_j/k_i)\, C_j, \qquad (2)$$

k_i and k_j the affinity Langmuir equation parameters. The first two terms on the right hand side of the equation correspond to single species adsorption, and therefore the last term could be considered as the interaction term. The parameters of this equation can be calculated by multilinear regression analysis.

Distribution coefficients - Distribution coefficients $K_d = q/C$ (q the amount of adsorbed cation and C the cation concentration in the equilibrium solution) relating adsorbed cation with cation concentration in the equilibrium solution were used to study Cu and Zn affinity to the soil, to assess their interaction and give a measure of metal interaction and of their relative mobility.

2. Materials and Methods

Surface samples from a schistic soil (Eutric Leptsol) - A, a granitic soil (Eutric Cambisol), - B, and from an alluvium soil (Dystric Fluvisol), - C, were used in this work..

The soils were air dried, passed through a 2 mm sieve and ground to 200 mesh before use. Some soil characteristics are presented on Table 1.

Table 1- Soils characteristics.

Soils	Clay %	pH	O.M. g kg^{-1}	CEC $cmol_c\, kg^{-1}$	*Fe_2O_3 g kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}
A	24	5.1	9.0	10.1	48.4	23.0	39.5
B	14	5.0	20.0	12.0	9.0	6.0	56.0
C	14	5.3	6.9	3.5	9.0	6.5	19.5

O.M.- organic matter; CEC- cation-exchange capacity; *Fe_2O_3 - Free iron.

To study Cu and Zn adsorption, duplicate soil samples from the surface layers (0-20 cm) were treated by aliquots of solutions containing Zn, Cu, (single solution) and Cu+Zn (binary solution) as nitrate salts with concentrations up to 40 mg L^{-1}. Adsorbed Cu and Zn were calculated by difference between initial and final concentrations in the equilibrium solutions. Exchangeable Cu and Zn were extracted with 0.5 M $Mg(NO_3)_2$ and analysed for Cu and Zn. Specifically adsorbed cations were evaluated by difference between "total" and exchangeable Cu and Zn.

3. Results and Discussion

Adsorption and desorption data fitted Langmuir type equations and the parameters of the respective equations are presented in Table 2 and 3.

The Langmuir parameters of maximum adsorption (mg kg^{-1}) for Cu and Zn added by a single solution were lower than the soil cation exchange capacity and similar to the experimental values implying that adsorption sites may be saturated in the experimental conditions. Copper adsorption was more depressed

by Zn than Zn by Cu but both Cu and Zn were significantly affected by the competitive ion. Soil C presented a slightly different behaviour, certainly due to the lower O.M. content. Desorbed Zn did not fit the Langmuir equation.

Table 2- Parameters of Langmuir adsorption and desorption isotherms.

Soil		$^{a}Q_{Cu}$	k_{Cu}	r^2	$^{a}Q_{Zn}$	k_{Zn}	r^2	$^{b}Q_{Cu}$	k_{Cu}	r^2	$^{b}Q_{Zn}$	k_{Zn}	r^2
	q_{tot}	63.694	2.907	0.999	17.544	0.404	0.943	48.780	0.735	0.996	14.749	0.509	0.991
A	q_{exc}	22.222	0.452	0.993	13.106	0.285	0.995	21.739	0.048	0.994	9.823	0.510	0.992
	q_{spc}	47.619	10.500	0.998	4.785	0.666	0.982	35.088	1.707	0.998	4.931	0.505	0.988
A*	q_{spc}*	51.813	8.773	0.999	5.302	4.840	0.999	35.336	20.214	0.997	33.898	0.167	0.997
	q_{tot}	41.494	0.290	0.998	15.200	0.471	0.998	20.202	0.469	0.997	12.330	0.339	0.999
B	q_{exc}	12.346	0.148	0.987	9.497	0.555	0.999	10.060	0.291	0.996	5.621	0.284	0.997
	q_{spc}	29.412	0.382	0.999	5.831	0.740	0.999	10.627	0.835	0.998	6.627	0.463	0.998
B*	q_{spc}*	50.251	1.171	0.971	30.864	0.329	0.962	15.723	1.951	0.971	15.408	1.338	0.942
	q_{tot}	28.571	0.417	0.997	111.111	0.043	0.982	31.250	0.182	0.950	31.646	0.138	0.976
C	q_{exc}	12.821	0.186	0.988	10.989	0.097	0.917	10.204	0.233	0.943	11.765	0.066	0.906
	q_{spc}	16.129	0.738	0.997	97.087	0.045	0.984	21.277	0.149	0.935	67.114	0.132	0.962
C*	q_{spc}*	18.868	3.786	0.979	*0.617*	*-1.050*	*0.982*	21.277	0.153	0.934	33.333	1.667	0.972

A - schistic soil, B - granitic soil; C Alluvial soil q_{tot} - total adsorbed; q_{spc} - specifically adsorbed; q_{exc} - exchangeable; * desorption isotherms; Q_m,, k - Langmuir parameter; [a]-single solution; [b]- binary solution.

Competitive Langmuir equation

Table 3 - Parameters of the competitive Langmuir equation for adsorption reactions

		Cu				Zn			
soil		Q_{Cu}	k_{Cu}	k_{Zn}	r^2	Q_{Zn}	k_{Cu}	k_{Zn}	r^2
A	q_{tot}	62.50000	0.75829	0.20853	0.996	17.23336	0.06558	0.39275	0.991
	q_{exc}	22.95684	0.04583	0.00260	0.993	11.22460	0.05472	0.39441	0.992
B	q_{tot}	20.98724	0.64705	1.59961	0.998	14.72169	0.05275	0.27605	0.996
	q_{exc}	6.28208	0.70802	8.63261	0.999	10.02577	0.11678	0.15071	0.997
C	q_{tot}	32.75467	0.14931	0.00240	0.996	55.15720	0.05891	0.07511	0.998
	q_{exc}	11.82562	0.16501	0.01879	0.999	14.48016	0.01445	0.05410	0.994

Q_i-maximum adsorption: k_{Cu} and k_{Zn} Langmuir coefficients;.

Table 4 - Parameters of the competitive Langmuir equation for desorption reaction

		Cu				Zn			
soil		Q_{Cu}	k_{Cu}	k_{Zn}	r^2	Q_{Zn}	k_{Cu}	k_{Zn}	r^2
A*	q_{spc}*	36.23188	69.00000	4.50000	0.998	36.60322	0.00303	0.15315	0.998
B*	q_{spc}*	21.32651	1.05918	0.06664	0.998	16.88305	0.07976	1.23413	0.942
C*	q_{spc}*	*8.05802*	*9.55350*	*-8.10008*	*0.905*	75.52870	0.19201	0.51358	0.985

q_{spc}* - specifically adsorbed as a function of desorbed cation, Q_i-maximum adsorption: k_{Cu} and k_{Zn} Langmuir coefficients;
Copper desorption for soil C does not fit the competitive equation, presenting negative values for k_{Zn}.

Distribution coefficients Distribution coefficients decreased when concentration of the added cation increased, decreasing Cu or Zn affinity with the soil and increasing their mobility.

4. Conclusions

Adsorption - desorption reactions were represented by Langmuir isotherms. The soils adsorbed more Cu than Zn, Cu mainly specifically adsorbed and Zn in the exchangeable form. Cu and Zn adsorption was depressed by their simultaneous presence in solution. Competitive Langmuir equations described quantitatively Cu-Zn interaction.

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METAL ATTENUATION BY PRECIPITATES FORMED FROM ACID SULFATE WATERS

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1. Introduction

Effective management and remediation of acid mine drainage is only possible if the processes influencing metal release and transportation are fully understood. Acid sulphate water resulting from pyrite oxidation may precipitate large quantities of Fe-rich minerals (Nordstrom, 1982, Bigham et al., 1992), and some investigations have implicated their importance in the regulation of heavy metal solubility through such adsorption/precipitation reactions (Chapman et al, 1983; Winland et al, 1991; ...). In this study the scavenging potential of the precipitates collected from a coal mine in Galicia (NW Spain) was evaluated by comparing the heavy metal content in the precipitates with those in solutions associated drainage waters.

2. Materials and Methods

Twenty six samples of precipitates and associated solutions emanating from the As Pontes lignite mine dump (Galicia, Spain) were sampled. Water samples were analyzed for pH, Eh, electrical conductivity, sulfate and total dissolved metals (Fe, Mn, Zn, Ni, Co, Cd and Pb). Precipitate samples were analyzed for chemical and mineralogical composition. The mobility of trace metals was characterized by a distribution coefficient, Kd, defined as the ratio of the metal concentration in the solid phase (precipitates) to that in solution at equilibrium (waters) (Andersom and Christensen, 1988).

3. Results and Discussion

Water pH ranged between 2.1 and 8.0 and redox potential values between 150 and 750 mV, however most of the water samples were strongly acidic (pH 2.5-3.5) and oxidizing (Eh 600-750 mV); these conditions are usually found in mine drainage systems where pyrite is present. In general, the water samples were characterized by the presence of elevated concentrations of Fe, SO_4^{2-} and H^+, liberated from the oxidation of pyrite, and of Si, Al, Ca and Mg derived from the accelerated mineral hydrolysis occurring under these conditions. At the same time, very high concentrations of heavy metals, in particular Mn, Zn, Ni and Co, which were liberated as a result of both processes, were recorded.

Results showed that the major secondary phase which precipitated was dependant upon pH, solution composition and moisture content. Precipitates formed at pH<4 and their high sulfate content (>2000 mg l^{-1}) was composed predominantly of schwertmannite (ideally $Fe_8O_8(OH)_6SO_4$) or jarosite ($KFe_3(OH)_6(SO_4)_2$), while goethite was the dominant phase at pH>4. In addition, several soluble sulfate minerals were commonly formed during dry periods.

The variation in mineralogy and water pH influenced metal retention by the precipitates. Distribution coefficients for Mn, Co, Cu, Zn, Ni, and Cd showed a preferential accumulation of these elements in the soluble sulfate phase. Schwertmannite adsorbed small amounts of these cationic metals due to the very low pH of the solution and goethite adsorbed significant amounts of Zn only. Lead appeared to be strongly associated with the jarosite phase due to the ability of

this mineral to incorporate metals into the crystal structure; a very low concentration of Pb was found in the solution.

4. Conclusions

The water pH was the main influential factor on both mineralogical composition of the formed precipitates and their metal retention capacity. Pyrite oxidation generates geochemical conditions which result in the high mobility of Mn, Co, Ni and Zn, but the low mobility of Pb. The mobility sequence was: Mn > Co > Ni > Zn > Cd > Cu > Pb (Fig. 1)

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ADSORPTION OF FERRIOXAMINE-B (FEDFOB) ONTO MONOSATURATED ME^{N+} BENTONITE

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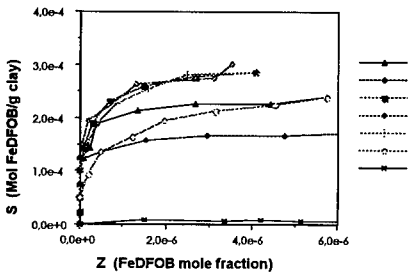
1. Introduction

Deficiency of micronutritives, specially Fe and Mn, can arise in soils characterized by high pH and redox potential. Siderophores produced by some plants and many soil microorganisms in response to Fe deficiency may reduce these negative effects on the plant nutrition. The adsorption of siderophores onto the soil colloidal surfaces hinder their effectiveness for the Fe mobilization and their utility in the plant nutrition. With the aim to have useful informations about the micronutritive mobility in the root-soil system, we studied the Ferrioxamine-B (FeDFOB) interactions with K-, Na-, Ca-, Mn-, Cu- and Al-monosaturated bentonites by adsorption isotherms and IR-spectroscopy. FeDFOB is a complex of Fe(III) with the Deferrioxamine-B siderophore (DFOB), a trihydroxamic acid derivative, soluble but stable at pH >10, with a $K_S=10^{30.6}$.

2. Materials and Methods

The FeDFOB/Me-clay systems were obtained by suspending 20 mg of dry Me^{n+}-clay powder in 10 ml of high-purity water containing variable amounts (0-10 μmol) of FeDFOB. Sorbed FeDFOB was calculated from the difference between the initial and final concentrations of the siderophore in the supernatant solutions. The adsorption data were treated according to BOWMAN (1982). IR spectra of FeDFOB and the siderophore/clay complexes were obtained with the KBr pellets method.

3. Results and Discussion



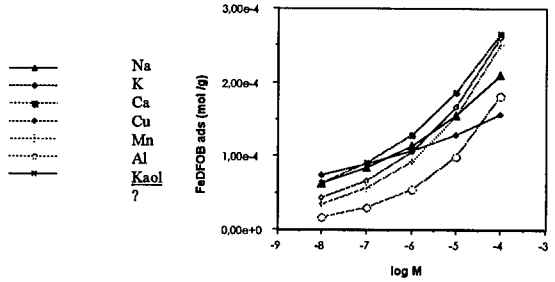


Fig. 1: Adsorption isotherms of FeDFOB on Na-, K-, Ca-, Cu-, Mn- and Al-monosaturated bentonite and on kaolinite (kaol) elaborated according to BOWMAN.

Fig.2 : Sorbed FeDFOB at selected FeDFOB molar concentrations in external solutions of FeDFOB/Me-bentonite suspensions, calculated by the BOWMAN elaboration.

The adsorption of FeDFOB seems related to the nature of the saturating cation and also to the clay mineral expandability: in fact, the adsorption of the Fe-complex onto kaolinite is negligible compared with that onto Me-bentonites (Fig. 1). All the Me-bentonite systems

adsorb FeDFOB by H-type isotherms, with very different maxima, depending on the nature of the saturating cation on the clay exchange sites and then on the adsorption reaction type. The sorption maxima in Ca-, Cu-, Mn- and Al-bentonite, higher than those in Na- and K-bentonite, suggesting the involvement of complexation reactions in the former and of ionic exchange in the later systems. Calculation of FeDFOB sorptiona at selected FeDFOB molar concentrations in external solutions by the Bowman elaboration (Fig.2), shows that the adsorption is well related to different expandability of Me-clays at low concentrations, in which the Na- and K-systems show adsorption values higher than those of other Me-systems. At higher FeDFOB concentrations ($>10^{-5}$), in spite of the size of the FeDFOB complex sorption is lower for Na- and K systems. Therefore, when the saturating ions forms , the adsorption seems due principally to ionic exchange at low concentrations, but complexation at high concentrations. The FeDFOB adsorption onto clays saturated by bivalent complexing ions is higher than in Al-bentonite suggesting that in Al-bentonite the hindrance to the interlayer expandability, due to the trivalent ion presence, inhibits the affinity of the clay surface for FeDFOB. The IR results suggest that the high affinity of FeDFOB for clay surfaces can be attributed to the formation of bonds with the exchange cations through the carbonilic O of amidic groups, or directly by complexation bonds, or by ion-dipole interaction, or by means of "water bridges" (MORTLAND and BARAKE, 1964).. The higher are the charge and polarizing power of exchange cation, the stronger are the bonds. Therefore, the adsorption by bentonites saturated with bi- and trivalent cations can be justified. The results of desorption carried out by AcOH and $CaCl_2$ solutions confirm the high affinity of FeDFOB for bentonite surfaces and the different adsorption depending on the nature of the saturating ion AcOH shows an extracting power much lower than that of $CaCl_2$ and negligible when the siderophore is sorbed onto bentonite saturated by complexing ions. On the contrary, $CaCl_2$ is able to exchange significant amounts of sorbed siderophore.

4. Conclusions

The high affinity of FeDFOB for the soil clay surfaces can be considered a serious problem for its mobility and then for its accessibility to the plant nutrition. However, this is true when the clay surface is bentonitic and saturated by complexing ions. If the clay is low expandable (i.e., kaolinite) or saturated by alkaline ions (a very real case, in common soils), FeDFOB can be removed by acidic excrets of plants and saline soil solutions and so becomes accessible to plants.

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CHROMATE REMOVAL BY DITHIONITE-REDUCED CLAYS

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1. Introduction

Chromium contamination in soils and water is consequence of many industrial activities, including mining, electric-power production, electroplating, leather tanning, and chemical manufacturing. Aqueous form of Cr(VI) is of particular concern because it is toxic to both plants and animals at low concentrations. Speciation, mobility, and toxicity of metals in soils are largely controlled by chemical reactions at the solid-water interface. In neutral and alkaline soils, Cr(VI) is mobile due to the weak adsorption on mineral surface. In acidic soils Cr(VI) is removed from solution by adsorption on positively charged sorption sites. Reduction of Cr(VI) to Cr(III) is of particular interest as the process immobilizes Cr in soils. Chromium(VI) can be reduced by soil organic and inorganic reductants. While organic matter may be primary source for Cr(VI) reduction in surface soil, Fe(II) containing minerals are more important for Cr(VI) reduction in subsurface soil and aquifer. The main objective of this study was to determine chromate removal by dithionite-reduced clays and to characterize Cr oxidation state on clay surfaces with X-Ray Absorption Near Edge Structure (XANES) spectroscopy.

2. Materials and Methods

The pure clay minerals used in this study include smectite, vermiculite, illite, and kaolinite to represent natural clay minerals. These clays were either in original or reduced (reduced with sodium dithionite) state. They were chosen to represent a wide range of clay mineral types and structural Fe contents. The smectite and illite samples were Na-saturated by washing with 1*M* NaCl three times, then four times with de-ionized water to remove excess salts. Impurities were removed from the clay by centrifugation. The clay fraction(<2μm) was freeze-dried before being used in the experiment. For Fe-reduction and Cr reduction study, a 200-mg portion of each clay sample was weighed and placed in a 50-ml centrifuge tube. The clay was first washed with 1*M* KCl by centrifugation then suspended into 10 ml de-ionized water and mixed with 20 ml potassium citrate-bicarbonate buffer. The tubes were then transferred to an inert-atmosphere glove box. Sodium dithionite was added to each tube to form a concentration of 0.1*M*. All samples were reacted in the glove box for 4 hours at 70^{0}C. For comparison, another 200-mg portion of the clay was treated identically except that 0.1*M* sodium sulfate was used in place of sodium dithionite to maintain similar electrolytes, but without Fe reduction. After the reaction, the clay suspension was centrifuged to discard the supernatant and the clay washed with deoxigenated, de-ionized water to remove excess salt under N_2-gas flow. All the treatments were done in triplicates. One replicate of each treatment was used for determination of total structural Fe content and Fe(II) to total Fe ratio in the clay. Under N_2 gas flow, the tube with the reduced or unreduced clay was mixed with 20 ml 1 mmol Cr(VI) solution, and gently shaken with 3 days at 22^{0}C. The reaction mixture was separated by centrifugation at 22^{0}C. The supernatant was measured for total Cr and Cr(VI) concentrations. The clay sediment was used for spectroscopic characterization. Total Cr and Fe was determined by atomic absorption. Total Cr was assumed as the sum of Cr(III) and Cr(VI). Chromium(VI) was determined by colorimetric assay (Bartlett

and James (1979). Chromium(III) in the solution was calculated as the difference between the total Cr and Cr(VI). The total structural Fe content and Fe(II) to total Fe ratio in clays was determined by the method of Komadel and Stucki (1988). For XANES study the clays after treatments were freeze-dried and packed in plastic sample holders. The X-ray absorption spectra at the Cr K-edge(5989eV) was collected at Beamline X23B of the National Synchrotron Light Source (Brookhaven National Laboratory, New York). The raw data collected from the beam line was converted. The pre edge background was then subtracted. The absorption coefficient was normalized relative to the intensity of the „white line" peak so that all the spectra were plotted on the same scale.

3. Results and Discussion

The study indicated that the reduced clays acted as an efficient remover of Cr(VI) from an aqueous system. The XANES spectra of Cr-treated clays provided the evidence that the clays reduced Cr(VI) to Cr(III) and immobilized Cr in the clays. Sodium dithionite applied directly into aqueous systems reduced Cr(VI) to Cr(III) but could not immobilized Cr even in the presence of clays. The Cr(VI) removal capacity varied with the clay mineral type and the structural Fe content. For clays used in this study, the removal capacity follows the orders of smectite>vermiculite and illite> kaolinite. Within the same type of clay minerals, reduction of Cr(VI) is highly related to the ferrous iron content of the clays, which indicated that the reduction of Cr(VI) to Cr(III) results from the oxidation of structural Fe(III). The results of this study can be used to develop a method for remediation of Cr in waste water.

Table 1. Adsorption of Cr in dithionite-reduced or unreduced clays from 1 mmol sodium dichromate solution.

Clay Type	Clay Sample	Fe reduction Levels (FeII)/FeTotal (mmol kg^{-1})	Cr absorbed by Untreated clay (mmol kg^{-1})	Cr absorbed by reduced clay (mmol kg^{-1})	Difference of adsorbed Cr (mmol kg^{-1})
Smectite	Swa-1	34.9	3.7	78.5	74.8
		89.7	5.6	50.0	44.4
		99.8	4.1	52.3	48.2
Illite	Imt-1	49.2	5.3	26.6	21.3
	Fithian	58.7	9.4	38.6	29,2
Vermiculite	Libby	27.6	22.2	43.2	21.0
Kaolinite	KGa-1	99.8	2.1	19.9	17.8
	KGa-2	78.3	2.0	20.9	18.9

4. Conclusions

Clays rich in structural Fe(III) under anoxic conditions can reduce toxic Cr (VI) to less toxic Cr(III) resulting in surface immobilization and remediation of waste water and contaminated ground water.

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THE EFFECT OF SOLUTION COMPOSITION ON THE SORPTION KINETICS OF AS^V IN SOIL

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1. Introduction

Sorption reactions are one of the important processes that control the fate and mobility of As^V in the soil. Much of our present understanding of sorption reactions are derived from equilibrium studies. These studies have provided valuable information on time independent behaviour and the driving force of chemical reactions (Skopp, 1986). In soils, sorption reactions between ions and sorbing surfaces are often not at equilibrium (Sparks, 1989). For better understanding of the dynamics of the sorption process, further study of the kinetics of these reactions in soils is required. To better understand and predict how these soil variables may affect the sorption of As^V with time, the kinetics of these reactions need to be studied. A modified stirred-flow technique was used to study the effect of solution composition on the sorption kinetics of As^V by a soil.

2. Materials and Methods

The Ah horizon (0 to 150 mm) of a soil (Soil A) was collected from an uncultivated and non-polluted site in northern New South Wales, Australia. Soil samples were air dried and passed through a 2-mm stainless steel sieve. Properties of the selected soil used in the study are given in Table 1.

Table 1: Selected characteristics of soils studied.

Soil Group	pH 1:5H_2O	CEC (mmol kg^{-1})	TC (g kg^{-1})	Particle Size (g kg^{-1})			Extractable Fe and Al (mmol kg^{-1})					Native As (mg kg^{-1})
				Clay	Silt	Sand	Oxalate		Citrate-dithionite			
							Fe	Al	Fe	Al	Mn	
Alfisol	6.01	32	1.6	70	40	870	12	0.06	86	52	<0.01	0.67

TC - Total Carbon

The sorption kinetic studies were conducted using the modified stirred-flow chamber developed by Carski and Sparks (1985). The effect of ionic strength (I) on the kinetics of As^V adsorption was studied in either 0.03 or 0.3 mol L^{-1} of $NaNO_3$ with the addition of 0.027 mmol As^V L^{-1} to the solutions. The effect of different index cations was studied using either 0.03 mol L^{-1} of $NaNO_3$ or 0.01 mol L^{-1} $Ca(NO_3)_2$. Anion competition was studied using P. Phosphate (0.032 mmol P L^{-1}) was added in solution with 0.027 mmol As^V L^{-1} in a background electrolyte of either 0.03 mol L^{-1} $NaNO^3$ or 0.01 mol L^{-1} $Ca(NO_3)_2$. A 0.5 g of soil was placed in the chamber with the magnetic stirrer and a known volume of solution was carefully added to the chamber with a syringe. The speed of the magnetic stirrer was standardised for all the experiments conducted. The top quickly tightened and any air trapped in the chamber was removed with the adjustable plunger base. Effluent from the stirred-flow reaction chamber was collected with a fraction collector at various time intervals ranging from 0.3 to 10 min. Arsenic remaining in the effluent solution was analysed by atomic absorption spectroscopy with hydride generation.

3. Results and Discussion

The kinetics of As^V sorption by Soil A with different treatments are shown in Fig. 1. The sorption of As^V was initially very fast and then was followed by a slower sorption rate which continued in all treatments for the duration of the study period.

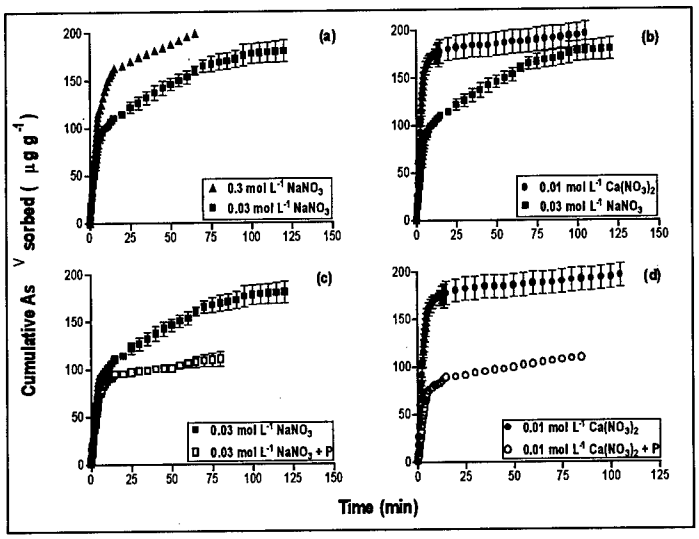

Figure 1: The effect of solution composition on the sorption kinetics of As^V by Soil A. Bars represent the standard errors of means. Where no bar is seen, error is smaller than the symbol.

The effect of the different treatments on the sorption capacity of As^V can be divided into two categories: (i) those treatments without the presence of P in the influent solution, and (ii) those treatments with P in the influent solution. Treatments of different *I* and index cation made little difference to the As^V sorption capacity of Soil A. However, the presence of P clearly decreases the sorption capacity of Soil C. The presence of P decreased the estimated sorption capacity from 183.7 to 97.4 and 177.2 to 115.2 in 0.03 mol L^{-1} $NaNO_3$ and 0.01 mol L^{-1} $Ca(NO_3)_2$, respectively. Kinetic equations were fitted to describe the apparent rate of As^V sorption on soil, but all had limitations in describing the apparent reaction rate of As^V sorption by soil. However, the one-site second order equation (Yin et al., 1997) described the data well.

4. Conclusions

This study showed that As^V sorption on Soil A was influenced by the solution composition of the influent. Increasing the *I* of solution or changing the index cation made no difference to the sorption capacity of the soil. The presence of P, in contrast, decreased the sorption capacity of Soil A.

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LONG-TERM REDUCTION OF TRACE ELEMENT SORPTION CAPACITY IN A SOIL-AQUIFER TREATMENT (SAT) SYSTEM

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1. Introduction

Soil-aquifer treatment (SAT) is a wastewater treatment scheme which uses the filtration characteristics of soil, vadose zone, and aquifer for purification and final "polishing" of mechanically-biologically treated effluents before their reuse e.g. for irrigation purposes. The removal of suspended solids, biodegradable materials, bacteria, viruses, nitrogen, phosphorus, heavy metals, trace organics, and detergents from effluents in SAT systems was studied before and found to be efficient (Bouwer, 1993). Significant accumulation of trace elements in the top layer of the soil profile was reported by, e.g., Chang and Page (1985) and Bouwer (1993), and lead to the feeling that sufficient retention of heavy metals is achieved during SAT operations over long periods of time. Although the concentrations of heavy metals in domestic sewage are generally low, the presence of some elements may become critical when large amounts of treated effluent are recharged to the ground water. In the present study we addressed the changes of the soil characteristics under the influence of continued long-term recharge of secondary effluent in a wastewater treatment plant, and the impact of these changes on the heavy metal sorption behavior of the recharge-basin soil.

2. Materials and Methods

Soil samples were taken from a recharge basin operated for 11 years at the Dan Region Sewage Reclamation Project near Tel Aviv, Israel. Pristine, unrecharged soil was obtained from an adjacent sand dune. Disturbed samples for batch isotherm tests and quasi-undisturbed samples for leaching-column experiments were taken at a depth of 40-50 cm. Cu solutions of various concentrations were prepared in a matrix solution similar in its major cationic components and ionic strength to the actual recharge effluent. Sorption isotherms were established by shaking one gram samples of air dry soil in 25 ml of Cu solutions with various concentrations and measuring the Cu sorption by the soil. Column experiments were performed by percolating Cu solutions with various concentrations through the soil columns at a flow rate of approximately 1 pore volume per hour. All experiments were conducted at a temperature of 25 ±1°C. Cu analyses were performed by AAS and ICP-AES.

3. Results and Discussion

The unrecharged, pristine soil is a sandy soil with a carbonate content of 4 ±1% and an organic matter content of around 0.25 ±0.05%. As a consequence of more than 10 years of effluent recharge, the 30-60 cm horizon of the basin soil, from which the samples for this study were taken, generally has a reduced carbonate content of 1.5 ±1.2%, a significantly diminished total Mn concentration, and an increased average organic matter content of 0.4 ±0.1% (Banin and Shachar, 1996). The basin soil samples actually used for this experimental study have a somewhat lower carbonate and higher organic matter content than the average values (0.1-0.3 % and 0.4-0.5 %, respectively). Adsorption isotherms (Figure 1) reveal a striking difference in Cu sorption behavior between the recharged and the pristine soil. The distribution coefficient K_d, calculated from the initial slope of the isotherms by fitting linear isotherm segments to low

concentration data points, is one order of magnitude lower for the recharged soil (3,133 l/kg) than for the pristine soil (24,572 l/kg), indicating a drastically reduced sorption affinity of Cu in the recharge basin soil. The non-linear sections of the isotherms, at higher concentrations, show a significantly reduced sorption capacity of the recharged soil towards Cu. The reduced sorption capacity of the recharged soil is confirmed by the results of the column experiments (Figure 2). Maximal Cu loads in the soil columns are significantly lower in samples from the recharge basin (300-350 mg Cu/kg soil), compared to those in the pristine soil (> 2000 mg/kg). We interpret the reductions in the sorption affinity and capacity of the soil towards Cu as being the result of the reduced content of carbonates and manganese oxides due to acidolytic and reductive dissolution, respectively, both driven by the decomposition of added organic matter in the top layer of the soil. The accumulation of organic matter during the effluent recharge, which itself may increase Cu sorption, was not sufficient to compensate the loss of such capacity caused by carbonates and oxides dissolution.

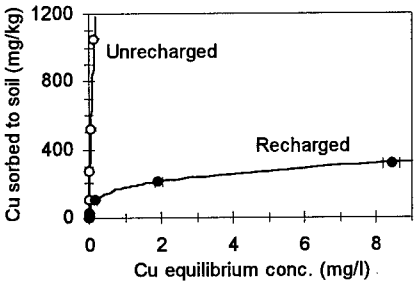

Figure 1: Cu adsorption isotherms for recharged and pristine sandy soil.

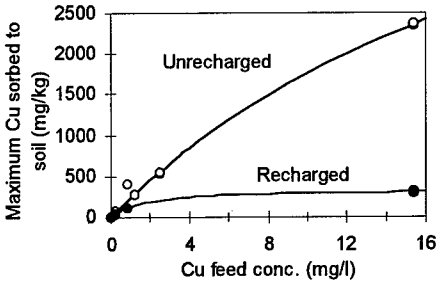

Figure 2: Maximum Cu load in the soil columns at various Cu feed concentrations

4. Conclusions

These findings reveal that slow but significant long-term changes of soil properties taking place during effluent recharge in a SAT system may profoundly affect the ability of the soil to effectively remove trace metals from the recharged effluents.

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Long-Term Trends of Trace Elements Deposition and Accumulation

(Technical Session 17)

HOLOCENE CONCENTRATION CHANGES OF MERCURY IN TWO OMBROTROPHIC BOGS IN SOUTHERN SWEDEN

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1. Introduction

An important gap in the understanding of large-scale Hg pollution is the limited data on historical trends of atmospheric Hg deposition, particularly of natural conditions. This gap can only be filled by environmental archives such as lake sediments and ombrotrophic peat bogs. Because of natural transport processes such as sediment focusing, lake sediments mainly allow reconstruction of *relative* trends in atmospheric deposition. Ombrotrophic peat bogs, which by nature only receive their nutrients, and pollutants as well, from the atmosphere, offer the potential of reconstructing *absolute* atmospheric deposition rates.

Although complete peat profiles have been used successfully to reconstruct long-term atmospheric Pb pollution (BRÄNNVALL et al. 1997, WEISS et al. 1997), previous reconstructions of atmospheric Hg deposition rates have relied only on short ($\leq$50 cm) ^{210}Pb-dated peat cores (e.g., JENSEN and JENSEN 1991, BENOIT et al. 1994, NORTON et al. 1997). These studies have not assessed Hg accumulation or behavior in deeper peat layers. A long-term perspective is especially crucial in reconstructing deposition in Europe, where atmospheric heavy metal pollution has existed for >3,000 years (RENBERG et al. 1994, BRÄNNVALL et al. 1997).

Our objective is to assess the potential of peat cores for reconstructing long-term, Holocene changes in atmospheric Hg deposition using two peat cores. These cores were shown to have trends in Pb accumulation similar to those established for lake sediments in Sweden (BRÄNNVALL et al. 1997). A long lake sediment core from southwestern Sweden is analyzed for a comparison between peat and sediment of relative trends in Hg deposition.

2. Materials and Methods

Peat cores were collected from the ombrotrophic bogs, Store Mosse and Trolls Mosse, in southern Sweden, using a Wardenaar corer for the top one meter and a Russian peat corer for deeper layers. Lake sediment cores were collected in Måkevatten using a freeze-corer for unconsolidated surface sediments and a Russian peat corer for consolidated sediments below. Samples were freeze-dried before digestion in aqua regia (HNO_3:H_2SO_4:HCl) and analyzed for Hg by CVAAS. Concentrations of Hg, ng/g, are given on a dry weight basis.

3. Results and Discussion

Analysis of two complete bog profiles, ca 650 cm deep, reveal similar trends with an increasing Hg concentration gradient downwards in the cores (Fig. 1). This pattern is not observed in the lake sediment profile from Måkevatten, where Hg concentrations are relatively stable, ca 50-80 ng/g, from 400 cm upwards to 18 cm depth; thereafter, in correlation with flyash particles, concentrations increase markedly upwards and decrease at the surface. Although the Pb trends are very similar in the peat and lake sediment cores, there is an apparent dissimilarity in Hg trends in the peat compared to the lake sediment. Although Pb and Hg are assumed to have similar biogeochemical behavior, e.g. binding capacity to organic matter, there may be post-diagenetic processes affecting the distribution of Hg, but not Pb, in the bogs, e.g. those processes

affecting the vertical distribution of S, which may also have a downcore concentration gradient in bogs (WEISS et al. 1997).

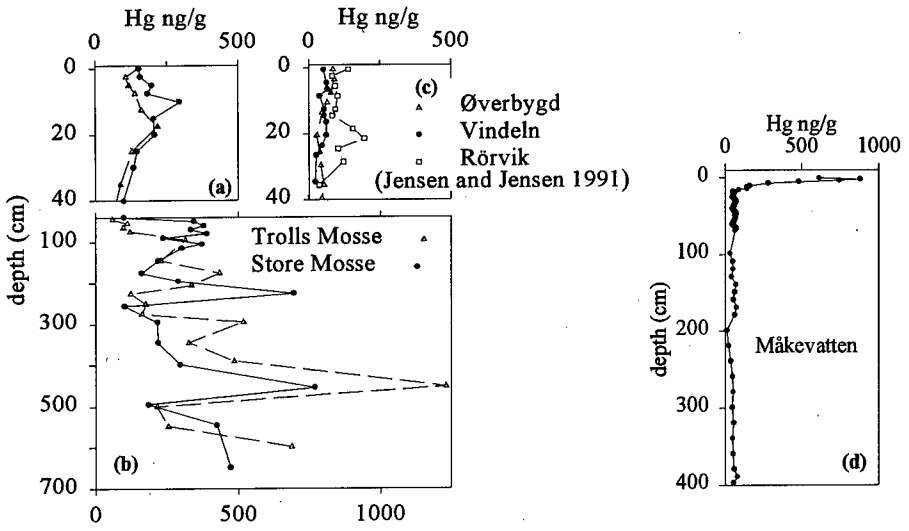

Figure 1. Mercury concentrations in: (a) Store Mosse and Trolls Mosse, 0-40 cm, and (b) 40-660cm; (c) bog data redrawn from JENSEN and JENSEN (1991); and (d) sediments of Måkevatten. The peat cores from Store and Trolls Mosse cover ca 8,000 years and the sediment core from Måkevatten ca 12,000 years.

4. Conclusions

Analysis of two peat cores and one lake core, spanning the post-glacial period, reveal different Hg concentration trends. Future studies must resolve the discrepancy between peat and lake Hg records, e.g. whether post-diagenetic processes alter Hg distribution in peat layers or alternatively in lake sediments, in order to reconstruct reliable deposition records.

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ATMOSPHERIC LEAD POLLUTION TRENDS IN SWEDEN DURING 4000 YEARS

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1. Introduction

The perception of a "clean" environment before industrialization is gradually changing since discovery of at least 3000 years old atmospheric pollution in Greenland ice, and European lake sediments and peat deposits (HONG et al., 1994; RENBERG et al., 1994; BRÄNNVALL et al., 1997; SHOTYK et al., 1998). While previous studies mainly rely on concentration analyses, we combine here concentration and stable isotope analyses ($^{206}Pb/^{207}Pb$ ratios) from many lake sediment and peat records in Sweden to provide a comprehensive history of long-range transported atmospheric lead pollution in northern Europe. Isotope analysis is a powerful tool to distinguish lead from different sources.

2. Materials and Methods

Our investigation includes three ombrotrophic peat bogs from southern Sweden and 25 lakes located all over Sweden (Fig. 1). Lake sediments and peat cores covering at least the last 4000 years were collected using a large Russian peat corer for older sediments, a gravity or freeze corer for recent sediments and a Wardenaar corer for the top meter of peat. Chronology is based on calibrated radiocarbon dates and varve counting in some lake sediments. Lead concentration and isotope ratios were determined using ICP-MS, after digestion in HNO_3 + $HClO_4$ (10:1). Analytical errors are $<\pm$ 10 % for Pb concentrations and $<\pm 0.010$ for the $^{206}Pb/^{207}Pb$ isotope ratios.

3. Results and Discussion

Figure 1 summarizes the results of the Pb concentration and $^{206}Pb/^{207}Pb$ isotope ratio analyses. The $^{206}Pb/^{207}Pb$ isotope ratio in unpolluted sediments, peats and mineral soil horizons in Sweden is significantly higher (mean = 1.53±0.28; range=1.28-3.11; n=50 sites), than in sulphide ores exploited during the Greek-Roman period and in Medieval Europe (1.174 ± 0.017; n = 80, GRÖGLER et al., 1966; WEDEPOHL, 1978), or in alkyl Pb from gasoline. The large difference in isotope ratios between natural Pb in Sweden and pollution Pb makes isotope analysis a more sensitive method than concentration analysis to trace pollution Pb.

The first signs of pollution Pb occur about 2000 BC. A major trough in isotope ratios and a small concentration peak occur about 2000 years ago, which is caused by large-scale transport of airborne pollutants produced during the Greek and Roman cultures. According to estimates from historical sources, the annual world production of Pb increased from a few tons, 3000 BC, to about 80,000 tons during the Roman empire (SETTLE and PATTERSON, 1980). The concentration declined and the isotope ratio increased after the fall of the Roman Empire. Starting in the late 10th century AD, with the rise of mining and metal production in Europe (NEF, 1987), the influx of pollution Pb increased rapidly; there was simultaneously a rapid decline in the isotope ratio and an increase in Pb concentrations in the sediment and peat records. With the Industrial Revolution and particularly following the extensive use of alkyl-Pb in the middle of this century, the atmospheric deposition of Pb increased significantly, peaking in the 1970's, and declining thereafter.

4. Conclusions

This investigation demonstrates that large-scale atmospheric Pb pollution has been in progress for several thousand years and reflects the economic history of Europe. Particularly from 1000 AD and forward the atmospheric Pb deposition has been extensive. Most importantly, the accumulated pre-industrial antropogenic load is of the same order as the load of the Industrial period (RENBERG et al., 1994). The pre-industrial load and its fate must be considered when assessing present-day environmental metal problems.

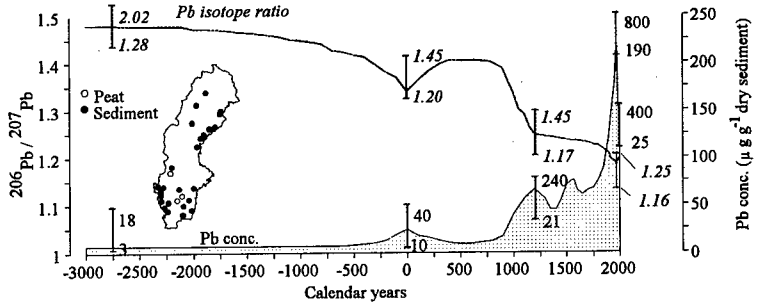

Fig.1. Schematic diagram of Pb concentration and $^{206}Pb/^{207}Pb$ ratio changes in sediments and peat records in Sweden reflecting the atmospheric lead pollution history. There is a considerable south to north gradient within Sweden and the diagram presents average values with ranges. The inserted map shows the 28 sampling sites.

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GEOCHRONOLOGY AND METAL DEPOSITION IN THE SOUTH FLORIDA ECOSYSTEM, U.S.A.

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1. Introduction

Managing an ecosystem ideally requires a full knowledge of the environmental dynamics. If no historical environmental records exist, the employment of other methods is necessary to obtain this information. A well-known geochemical procedure that supplies time information is the use of short-lived isotope chronological methods. Of the many naturally occurring nuclides, ^{210}Pb was found to be the best suited for gauging the timing of environmental changes in Florida Bay and in the Everglades of South Florida. The age-depth relationships in cores were calculated using the ^{210}Pb method at 35 sites within Florida Bay and at 58 sites in the Everglades. In the Florida Bay system, ages were independently confirmed by comparing the distribution of the known concentrations of atmospherically anthropogenic total stable lead recorded in dated cores to similar data in an annually banded coral. In the Everglades system the ages were confirmed by photographic evidence that bracketed changes measured in the cores.

2. Results and Discussion

Cores from three sites in the central part of Florida Bay were selected for further analysis. X-radiographs revealed laminae over most of their length that indicated negligible sediment disturbance. The ^{210}Pb and ^{226}Ra were measured at 2-cm increments throughout these cores. Maximum activities of total ^{210}Pb were not much higher than ^{226}Ra activity. Thus, high-frequency measurements of radium were needed to construct accurate chronologies from excess ^{210}Pb (total ^{210}Pb minus ^{226}Ra). The ^{210}Pb profiles were nearly exponential, indicated evidence of little sediment mixing, and were consistent with a constant rate of delivery of excess ^{210}Pb and sediment mass. Mean accumulation rates ranging from 0.17 $\forall$ 0.02 to 0.92 $\forall$ 0.04 g/sq cm/yr (ca. 0.42 to 1.22 cm/yr) provided age-depth assignments to about 100 years BP.

Because ^{210}Pb dating is usually based on several plausible but untested assumptions, age-depth assignments generally must be validated by independent means. Fallout ^{137}Cs and stable lead profiles in the cores were compared with time records of atmospheric deposition at Miami (1964 maximum) and continental atmospheric lead concentrations (1972 maximum), respectively. For lead, profiles were also compared with lead/calcium ratios (1978 maximum) in annual coral bands from a specimen (*Montastrea annularis*) located on the Atlantic side of the Florida Keys (Shen and Boyle, 1987). Age-depth assignments in the cores were confirmed by the correlation of the ^{137}Cs peak as well as the nearly perfect match with the lead distribution in the coral (Robbins and others, 1998). The analysis of the cesium distribution also indicated that there is a time averaging of the distribution of metals in the bay of approximately 16 years. $^{239+240}$Pu measurements confirmed this time averaging model (Robbins and others, 1998). This means that the system requires approximately 16 years to remove any metal that is introduced into it. Two cores were analyzed for mercury, barium, and uranium in addition to the lead. The barium and uranium concentrations are constant, whereas a mercury maximum occurs in the cores around 1960 and lead around 1970, confirming the ^{210}Pb dates.

Three cores were selected for metal analysis in the Everglades system. These cores are on a north-south traverse transecting an impacted area. High phosphorous concentration and the high growth rate of cattails distinguish the impacted area. Because of the high growth rate of the cattails and consequent increased production of peat at the surface, the concentration ^{210}Pb became diluted, resulting in declining profiles. However, at all sites the convergence of the ^{210}Pb and ^{226}Ra activities at depth demonstrate that an equilibrium level is clearly defined. This level is estimated to be the 100∀25 year horizon. The determination of the metal inventory above this horizon indicated that most metals were deposited at a similar rate. One core taken outside of the impacted zone was dated. The distribution of metals in these cores has a unique separation between normally coherent metals. Mercury, aluminum, and titanium are very coherently peaking around 1950 and around 1900, whereas copper, arsenic, cadmium and manganese show only a continuous increase through time. Lead peaks around the 1950. The conclusion of this study is that short-lived isotopic chronologic methods are valuable in determining the history of metal deposition in this subtropical environment.

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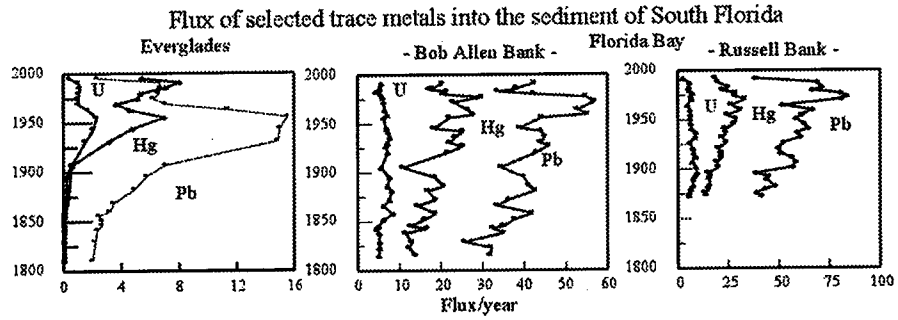

The Everglades site is in the impacted zone with the sediment composed of cat-tail and sawgrass peat. The Florida Bay sites are from carbonate banks and the sediment is wholly carbonate. The apparent increases began in 1900's

The flux for lead (Pb) is given as □/g x10/year; for mercury (Hg) as □/g/year; and for uranium (U) as □/g /year.

SEPARATING NATURAL AND ANTHROPOGENIC FRACTIONS OF HG USING THERMAL LABILITY ANALYSIS OF A PEAT CORE

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1. Introduction

Numerous studies using lakes and peat bogs as historical archives of the Hg fluxes showed elevated concentrations of Hg in superficial layers as compared to deep layers belonging to pre-industrial times. Nevertheless for RASMUSSEN et al. (1997) the geological component of the Hg cycle is widely variable and the postdepositional diagenetic processes can greatly affect the validity of ice, lake and bog records. In their recent review FITZGERALD et al. (1997) have pointed out that not all the profiles can be explained by diageneis but they show a coherent spatial and temporal pattern for areas so distant as Europe and North America.

In this paper we present the results obtained for Hg in the peat bog of Penido Vello (NW Spain), PVO, where Cd, Zn and Pb were previously analyzed (MARTINEZ CORTIZAS et al., 1997). This bog is interesting because mining in Almadén, the biggest Hg mine in the world which is located at SE Spain, extends back at least to the Roman times. Another important but local source of Hg appeared at the end of the 70s of the present century, when the lignite-fired power station of As Pontes began to operate some 25 km to the west of the bog.

2. Material and Methods

A core was sampled to a depth of 250 cm. It was inmediately sliced into 2 cm slices for the upper meter and into 5 cm slices for the rest. Wet samples were kept a 4° C in a refregirator until analysis. Peat samples were also dried at 30° and 105° during three weeks. Samples of fresh granitic rock substratum, weathered rock (saprolite), interface peat-sediment and interface peat-vegetation were also taken.

Mercury was measured in wet and dried samples using a LECO-ALTEC AMA-254 mercury analyzer conected to an automatic sampler. For each sample triplicates were measured. Differences between replicates never exceeded 0.5 ng g^{-1} being always less than 1% of the mean value of the sample. Mercury was measured in the wet (Hg_T), dried at 30° ($Hg_{30°}$) and dried at 105° ($Hg_{105°}$) samples. Standard reference materials (SMRs) were run within each set of analysis. All the determined values were in the precision range for each SMRs and mean reproducibility for triplicates was 5.2%.

3. Results and Discussion

Total Hg_T concentrations ranged from 22 to 436 ng g^{-1}. Concentrations in dried samples ranged from 18 to 315 ng.g^{-1} and 11 to 285 ng.g^{-1} for $Hg_{30°}$ and $Hg_{105°}$ respectively. The Hg_Tprofile showed maximum concentrations at the surface of the peat representing enrichments of up to 290 times the rock content. But also some other individual peaks were found: at 17 cm with 191 ng.g^{-1}, at 41 cm with 65 ng g^{-1}, at 97 cm with 45 ng g^{-1} and from 190 to 250 cm with concentrations up to 47 ng g^{-1}. Most of the secondary peaks dissapeared in the profiles of dried samples.

In part the Hg_T profile can be related to the history of Hg mining and metallurgy in the Iberian

Península, but some striking findings indicate that processing-production releases were not the only factor in the accumulation of Hg by the peat. Results suggest that accumulation was deeply affected by the environmental conditions at the time of deposition. As a general rule peat layers formed uner cold climate conditions showed enhanced accumulation, while peat layers from warm to very warm climate phases showed the lowest Hg concentrations. Even more, the thermal lability of Hg also depended on a climatic control. It was found that a multiple regression function using as predicting variables the thermal lability of Hg explained 95% of the variance of the Hg_T for samples older than 2200 years (previous to Hg mining and metallurgy in the Iberian Península). For these samples Hg accumulation is assumed to depend only on natural processes, so the function is a good model to estimate both the natural (Hg_{NAT}) and the anthropogenic fractions of the Hg accumulation (Hg_{ANT}: as the difference between Hg_T and Hg_{NAT}). The variations of the estimated anthropogenic Hg through time are in very good agreement with the history of mining and metallurgy in Spain and is also consistent with that of other trace elements, as Pb, previously analyzed. A reemision factor (RF: the ratio between looses of Hg from the peat and deposition) for the natural fraction as well as total deposition were also estimated. The results indicate that total deposition for preindustrial times ranged from 3 $\mu g\ m^{-2}\ y^{-1}$ in the deeper sections of the core to 18 $\mu g\ m^{-2}\ y^{-1}$ with an average of 7.0 ±3.0 $\mu g\ m^{-2}\ y^{-1}$; for industrial times the average is 112.3 ±36.3 $\mu g\ m^{-2}\ y^{-1}$, with a maximum in the upper most sample of the peat of 165 $\mu g\ m^{-2}\ y^{-1}$.

4. Conclusions

Our findings suggest that environmental conditions at the time of deposition controled Hg accumulation in the PVO peat bog. This effect is expressed as an enhanceed accumulation and a greater proportion of low estability Hg under cold conditions, while warm climatic episodes promoted Hg losses, lower total Hg concentrations but an increased thermal estability.

The results are consistent with the behaviour of the processes affecting the Hg cycle as a whole: volatilisation, cold condensation effect, deposition processes, partioning, etc..., and point out, in agreement with MACKAY et al. (1995), that Hg may be subjected to the type of long range transport of persistent organic pollutants.

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CHANGES AND DISTRIBUTION OF EXTRACTABLE CU AND ZN IN A SPODOSOL PROFILE UNDER A LONG-TERM DIFFERENTIALLY LIMED AND FERTILIZED SWARD

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1. Introduction

Pensacola bahiagrass (*Paspalum notatum* Flugge) alone or associated with white clover (*Trifolium repens* L.) has been the choice over the past several decades for pastures on the Spodosols in North Central Florida (Ref). For the establishment and maintenance of such pastures, liming materials and fertizers are usually applied. Nutrient distribution in a soil profile is affected by soil characteristics, lime and fertilizer application rates, sources, and frequency of application, specific nutrient mobility, rainfall amount, and vegetation.

The principal objective of this experiment was to study the changes and distribution of extractable Cu and Zn in a Myakka fine sand profile that had supported a permanent legume-grass sward for 32 years, under differential lime and fertilizer treatments.

2. Materials and Methods

The experimental area was established with Pensacola bahiagrass and white clover 32 years before this experiment (Ref.). Phosphate and K fertilizers were applied at establishment of the experiment, and were reapplied annually to all experimental plots. Soil samples were taken at nine soil depths: 0 to 2.5, 2.5 to 5.0, 5.0 to 7.5, 7.5 to 15.0, 15.0 to 30.0, 30.0 to 45.0, 45.0 to 60.0, 60.0 to 75.0, and 75.0 to 90.0 cm. Four main plots, each having two subplots, and replicated four times, were sampled at each soil depth.

The four main plots were: 1. Control: no lime and micronutrients were applied; 2. Lime: this treatment received lime at the establishment and was limed periodically to maintain soil pH near 6.0. Micronutrient-containing fertilizers were not applied; 3. Lime+MN: this treatment received lime, and a mixture of Cu, Fe, Mn, and Zn sulfates was applied in the first year to supply 4.2, 3.2, 4.6, and 3.9 kg ha^{-1} of each micronutrient, respectively. Copper and Zn were reapplied 3, 6 and 7 years after the establishment, through the same sources and at the same rates. The total amount of Cu and Zn were 16.8 and 15.6 kg ha^{-1}, respectively; 4. Lime+MNS : this treatment received lime, Cu, Fe, Mn, and Zn, as described in the Lime+MN treatment. Borax was applied at the same time as the other micronutrients to supply 1.2 kg B ha^{-1} each time or a total of 4.8 kg B ha^{-1}. Sulfur was added at 30 kg ha^{-1} as gypsum 30 years after the sward establishment.

Subplots were with and without S fertilizer during the last 4 years. Where S was applied as gypsum and potassium sulfate at a total rate of 200 kg ha^{-1} in the 4 years.

Soil samples were air-dried,and Cu and Zn determinations were done after extraction with Mehlich-I double-acid (0.05 M HCl + 0.025 M H_2SO_4) extractant.

3. Results and Discussion

Mehlich-I extractable micronutrients (Ref.) in the soil were significantly affected by lime+MN+S treatments and soil depth interactions. Extractable soil Cu and Zn as a function of Lime+MN+S treatments x soil depths are presented in Fig.1. Extractable Cu was significantly higher in the Lime+MN and Lime+MN+S than in the limed or control treatments at all except the 75 to 90 cm soil depth. Lime+MN treatment had higher extractable Cu than the Lime+MN+S. The lower

extractable Cu in the Lime+MN+S treatment can not be attributed to higher plant uptake than in the Lime+MN. Sulfur fertilization did not significantly affect extractable Cu. Detailed study of the Lime+MN+S combinations x S fertilization interactions indicated once more that extractable Cu was higher in soils with Lime+MN than with Lime+MN+S, and that both of these treatments had higher extractable Cu than lime or control treatments. In treatments where micronutrients were not applied (control or lime), extractable Cu decreased with soil depth to 7.5 to 15 cm only. Below the 15 cm depth no significant changes occurred. However, under Cu fertilization (Lime+MN and Lime+MN+S treatments), extractable Cu was higher in the top 30 cm of soil, with no further changes at deeper soil layers. The highest extractable Cu occurred at the 5.0 to 7.5 cm soil depth.
Extractable soil Zn was higher in the Zn-fertilized plots (Lime+MN and Lime+MN+S treatments) than in the control or limed treatments, at the four soil layers in the top 15 cm of the profile. Below that soil depth and down to 90 cm, no distinction could be made in extractable Zn between soil with or without applied Zn. Extractable Zn was higher under Lime+MN than Llime+MN+S. This can not be explained through plant uptake of Zn and removal from the area. Sulfur fertilization also resulted in a significant decrease in extractable Zn. In limed soils, extractable Zn decreased as soil depth was increased to 15 cm. In the control treatment, only the 0 to 2.5 cm soil depth had more extractable Zn than the other soil layers sampled. In both Lime+MN and Lime+MN+S, extractable Zn was highest in the 0 to 2.5 cm soil depth.

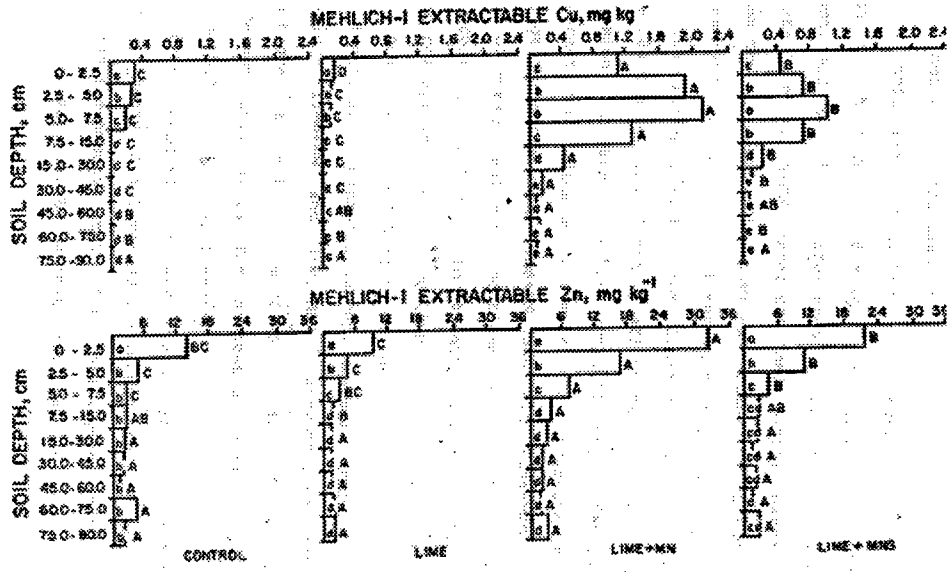

Fig.1 Effects of lime-MN-S combinations and soil depth on Mehlich-I extractable Cu and Zn in the Ryakka fine sand profile. Bars with the same lower-case letters within subfigures, and bars followed by the same upper-case letters within soil depth are indicative of no significant difference among means at the 5% level of probability (DMRT).

CUMULATIVE LOADS OF ANTHROPOGENIC LEAD AND ITS FATE IN BOREAL FOREST SOILS, SWEDEN

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1. Introduction

Studies of lake sediments and ombrotrophic peat bogs have revealed the presence of atmospheric Pb pollution in Europe for 3,000 years (Renberg et al., 1994, Brännvall et al., 1997). In lake sediments the cumulative load of pollutant Pb deposited prior to the Industrial period (pre-1800) is at least equal to that deposited since (RENBERG et al. 1994). Stable Pb isotope analyses offer a powerful tool to assess the cumulative load of Pb pollution to the boreal forest soil and to study the fate of this Pb in soils. Isotope analyses have shown that the $^{206}Pb/^{207}Pb$ ratios naturally found in Swedish soils and lake sediments are considerably higher than the ratios of either pollutant Pb or continental European soils. Consequently, it is possible, using simple mixing models, to asses the burden of Pb in soils and sediments, which has been atmospherically transported and deposited from non-Swedish sources. Basal lake sediments and soil C-horizons in Sweden have a mean value of Pb 1.53 (n=50, range=1.28-3.11), while pollutant Pb and European soils have values typically $\leq$1.2. Here, we present data on the cumulative load of airborne anthropogenic Pb and the distribution of this Pb in the soil profiles of old-growth Scots pine forests.

2. Materials and Methods

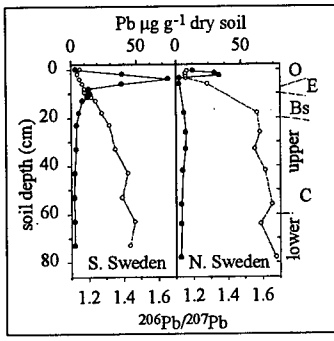
Samples from four soil profiles, each located in either a national park or nature reserve with 200-350 year-old pine-dominated forests (*Pinus sylvestris* L.), were collected using a stainless steel soil corer in hand dug soil pits after the sampling face was first scraped clean with a stainless steel trowel. Lead concentrations and isotope ratios were determined on freeze-dried soil samples using ICP-MS following digestion in HNO_3 and $HClO_4$ (10:1). Concentrations are reported on a dry weight basis. Pollution Pb in soils is calculated applying a simple mixing model using the background ratio at each site and assuming that the $^{206}Pb/^{207}Pb$ ratio of the pollutant Pb was 1.17 up until the post-war period, after which a ratio of 1.14 (mixing of 1.17 and a lower ratio for alkyl Pb) was used.

3. Results and Discussion

Results of analyses of Pb concentrations and $^{206}Pb/^{207}Pb$ ratios in the four soil profiles, of which two are presented as examples (Figure 1), reveal a characteristic pattern: the highest concentrations are found in the organic humus horizon and decrease with depth, with stable values in the C horizon; and the $^{206}Pb/^{207}Pb$ ratios are lowest in the surface organic horizon, ca 1.13-1.16, and increase with depth to a relatively stable ratio in the lower C horizon. The ratio found in the organic horizons are comparable to that of pollutant atmospheric aerosols, 1.13-1.17 (Hopper et al., 1991), while the underlying mineral soils have higher values of 1.36, 1.45, 1.57, and 1.63.

Application of a two-component mixing model, with pollutant Pb and local Pb (determined from C horizon ratios), reveals that anthropogenic Pb has been transported downwards to a depth of ca 60 cm in S. Sweden soil profiles and ca 40 cm in the N. Sweden profiles.. Although the concentration of pollution Pb is highest in the surface organic horizon, the major fraction of the anthropogenic pollution load is accumulated in the underlying mineral soil horizons. Due to the steep south to north gradient in long-range atmospheric lead deposition in Sweden, the total

inventory of anthropogenic Pb in southern Sweden (2.3, 2.4, and 2.8 g Pb m^{-2}) is three times higher than that in northern Sweden (0.7 g Pb m^{-2})



Soil horizon	Anthropogenic Pb inventory g m^{-2} $horizon^{-1}$		Natural Pb inventory g m^{-2} $horizon^{-1}$	
	S Sweden	N. Sweden	S. Sweden	N. Sweden
O	0.402	0.136	0.005	0.001
E	0.330	0.205	0.036	0.230
Bs (to 20 cm)	0.906	0.090	0.541	1.154
Upper C (20 to 60 cm)	0.399	0.148	0.894	2.248
Lower C (60 to 100 cm)	0.000	0.000	1.245	1.767
Pb inventory	2.038	0.737	2.722	5.400

Figure 1. Lead concentrations and $^{206}Pb/^{207}Pb$ ratios in a soil profile from S. Sweden and N. Sweden and the inventories of anthropogenic Pb and natural Pb in each soil horizon.

4. Conclusions

Models of Pb fate and transport in forest soils are commonly based on Pb concentrations, but because of the natural pool of Pb in soil mineral horizons it is difficult to distinguish the influence of pollutant Pb. By applying simple Pb mixing models, based on stable Pb isotope ratios, it is possible to assess the cumulative load of pollutant Pb and its fate in boreal forest soils.

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HISTORICAL ENVIRONMENTAL MONITORING USING BARK POCKETS AS POLLUTION TIME CAPSULES

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1. Introduction

Historical changes in environmental pollution, especially air pollution are of considerable interest for evaluating the present situation in regions of the world with different environmental and historical backgrounds. It has been demonstrated that bark pockets enclosed between annual rings in tree trunks or branches contain some of the most available historical specimens for monitoring of air pollution (Satake et al., 1995). The bark pockets investigated so far have included those of the conifer *Cryptomeria japonica* formed around 1760-1780 at Nikko, about 100 km north of Tokyo, and around 1786-1809 on Yakushima, a remote island in the southern region of Japan. These specimens have confirmed that bark pockets can be applied for historical monitoring of air pollution, and have revealed a drastic increase of lead pollution in recent times. Bark pockets are common in tree trunks. Here we report the analytical results obtained using laser-ablation-ICP-MS on pollution recorded in bark pockets of *C. japonica* collected from the grounds of a temple with a view to developing the use of bark pockets for environmental monitoring.

2. Materials and Methods

Trees containing bark pockets were collected in the precincts of Muro Temple in Nara prefecture, about 60 km south of Kyoto and about 37 km south of Nara. Tree trunks of *C. japonica* containing bark pockets were cut with an electric saw. The sections were observed and recorded photographically or photocopied to clarify the processes of encapsulation of the outer bark. Then the sections including the annual rings, cambium layer, inner bark and outer bark (bark pocket) were analyzed by laser-ablation-ICP-MS (CETAC LSX-100 Laser Ablation System, Yokogawa Analytical Systems HP-4500 ICP-MS) in chronological series within the bark pocket (David et al., in preparation).

3. Results and Discussion

The analysis of bark pockets of *C. japonica* from Muro Temple using laser-ablation-ICP-MS revealed clearly the historical changes in mercury and lead pollution over a period from 140 years ago to 70 years ago and up to the present time (Table 1). There have been many reports on the concentrations of heavy metals that accumulate in tree rings in relation to historical changes in pollution (e.g., Baes and Ragsdale 1981). However, most air pollutants in the form of wet and

Table 1 Historical changes in relative levels of mercury and lead pollution in the precincts of Muro Temple

Year	1858	1928 (-140 y)	1998 (-70 y)	(0)
Hg		1	1	2.8
Pb		1	0.8	39

dry deposits on trees accumulate on the leaves or outer bark. The concentrations of pollutants in annual rings are very low compared with those on the outer bark. This is due to the difference in the roles of the outer bark and xylem. The outer bark, which is in contact with the atmosphere, protects the inner bark, cambium and xylem layers from physical, biological and chemical damage, whereas the roles of the xylem layer are to pass water and nutrients from soil to the leaves, and to provide physical support for the tree. The radial transportation of pollutants through the bark to the xylem is prevented or limited. In contrast, most atmospheric pollutants that accumulate in tree rings are transported as water-soluble compounds via the soil and roots. Thus lateral movement of pollutants may occur between adjacent rings (Donnelly et al., 1990). Therefore, it is difficult to obtain direct information on air pollution from annual rings, although some historical trends do have an indirect and limited impact on tree rings.

Many natural and artificial materials have been used for historical monitoring of pollutants transported to terrestrial and aquatic ecosystems. Typical of these materials are polar ice, tree rings, lake sediments, peat, freshwater shells, corals and herbarium specimens. However, these materials are not always suitable for the historical monitoring of air pollution, which increased drastically at the start of the Industrial Revolution. A number of difficulties still remain with the use of such specimens for monitoring. The major ones are: (1) dispersal and translocation of pollutants; (2) contamination during natural and/or artificial preservation; (3) difficulty in obtaining materials representing a suitable time scale at defined monitoring sites such as urban areas, and also remote and background areas; (4) correct dating.

4. Conclusions

Laser ablation ICP-MS study of bark pockets collected from Muro Temple in Japan revealed a drastic increase of lead and mercury pollution at the present time in comparison with that 70 and 140 y ago.

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INDEPENDENT RECORDS OF LONG TERM ATMOSPHERIC PB DEPOSITION TO SWISS FOREST SOILS USING PEAT CORES FROM OMBROTROPHIC BOGS

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1. Introduction

To quantify the extent of anthropogenic metal contamination of forest soils, the true, natural, „background" metal concentrations must be clearly established. One first step to meet this challenge is to clearly separate the measured metal concentrations into natural (lithogenic, pedogenic) and anthropogenic compartments (Blaser and Zimmerman, 1993). For soils receiving anthropogenic inputs exlusively from the air, an alternative approach would be to compare the metal inventory in a given soil profile with an independent record of atmospheric metal deposition. For example, the history of atmospheric metal deposition can be quantified using peat cores from ombrotrophic bogs (Shotyk, 1996; Shotyk et al., 1997).

The surface layers of ombrotrophic bogs are isolated from the influcence of local groundwater, and receive their inorganic solids exclusively from the atmosphere. Isotopic studies have shown that Pb is effectively immobile in peat profiles (Shotyk et al., ,1996, 1997), allowing peat cores from bogs to be used as archives of the changing rates and sources of atmospheric Pb deposition. Recently, a core from a Swiss bog has been used to reconstruct a complete history of atmospheric Pb deposition from 12,370 ^{14}C yr BP to the Present (Shotyk et al., 1998). Here we use peat cores from selected bogs across Switzerland to provide an independent record of anthropogenic, atmospheric Pb deposition to Swiss forest soils since the Roman Period.

2. Materials and Methods

Peat cores ca. 10 x 10 x 100 cm long were collected from two neighbouring bogs in the Jura Mountains (Etang de la Gruère, EGR and La Tourbière des Genevez, TGE), and from one bog in the southern Alps, in Canton Graubunden (Suossa, SUO). In addition, one peat core was collected from a minerotrophic fen (Gola di Lago, GDL) south of the Alps, in Canton Ticino; even at this site, however, the Pb inventory by far is dominated by atmospheric inputs. Lead was measured using the EMMA miniprobe XRF analyzer (Cheburkin and Shotyk, 1996) and Sc using instrumental neutron activation (INAA) at ACTLABS (Ancaster, Ontario, Canada). Age dates were determined using ^{14}C (decay counting) at the Radiocarbon Lab, Physics Institute, University of Berne.

3. Results and Discussion

The *anthropogenic* Pb inventory of each sample (3 cm slices) was calculated as the difference between total Pb and lithogenic Pb, with lithogenic Pb calculated as

$$Pb_{lithogenic} = Sc_{sample} \times Pb/Sc_{crust}$$

where $Pb/Sc_{crust} = 1.8$ (Taylor and McLennan, 1995). The anthropogenic atmospheric Pb flux at each site was expressed as cumulative anthropogenic Pb since the Roman Period; this was taken as 2000 ^{14}C yr BP, and the depth corresponding to this time was estimated using the

chronologies established for each core. The results for the two Jura bogs are remarkably consistent at 2.1 and 2.2 g/m^2 for EGR and TGE, respectively; these values correspond to the total mass of anthropogenic Pb deposited since the Roman Period per square metre of bog surface. Because anthropogenic Pb inputs from 3000 ^{14}C yr BP until Roman times are comparatively small, these values are approximately equal to total anthropogenic Pb. In contrast, at Suossa the cumulative anthropogenic Pb flux since the Roman Period is 4.0 g/m^2, and at GDL it is 13.1 g/m^2. The much higher net rate of anthropogenic Pb deposition at GDL reflects the influence of the heavily industrialized region of northern Italy, centred on Milan. Comparing the results from SUO with those from EGR and TGE indicates that there is also a measurable influence of Italian industry on the southern Alps.

4. Conclusions

Dated peat cores from ombrotrophic bogs can be used to provide an independent record of anthropogenic, atmospheric Pb deposition to forest soils and other terrestrial ecosystems. For comparison with these data, we are now using Pb and Sc concentrations in Swiss forest soil profiles to calculate the anthropogenic Pb inventory of selected soils and to calculate net losses of metals from leaching.

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Speciation
(Technical Session 18)

MERCURY SPECIATION IN TAILINGS AND RIVER SEDIMENTS OF THE IDRIJA MERCURY MINING AREA

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1. Introduction

Five hundred years of intensive mercury mining activity in the Idrija/Slovenia, the second largest Hg mine in the world, left a legacy of highly polluted soils and sediments (Kosta *et al.*, 1974, Palinkaš *et al*, 1995, Gosar *et al.*, 1997). Besides soil pollution by atmospheric derived Hg^0 considerable amounts of cinnabar (HgS) bearing ore residues were spilled into river Idrija Hg pollution in the Gulf of Trieste has been reported to be related to the influx of sediments of the rivers Idrijca and Soca draining the mining area.. Moreover, Hg methylation in the Gulf of Trieste was estimated to be potential high (Planic *et al.*, 1993). However, only little is reported about inorganic Hg binding forms in tailings and sediments which determine the potential of Hg species transformation. In this study Hg binding forms in tailings and river sediments have been investigated to estimate the amount of Hg which may undergo transformation.

2. Materials and Methods

Samples were taken from tailings of different ages located alongside river Idrijca in the vicinity of the former mining area about 20 km distant from the roaster (WII). Sediment samples were taken from different locations down the rivers Idrijca and Soca from the city of Idrija to the mouth of the Soca river (Gulf of Trieste). Overbank sediments of river Idrijca were taken from terraces of different ages located 20 km distant from the mining area. At the same location a forest soil sample was taken as a reference of atmospheric derived Hg soil pollution. Solid phase Hg binding forms in all samples were analyzed by means of a Hg-Thermo-Desorption technique described elsewhere (Biester and Scholz, 1997). Identification of Hg binding forms was achieved by comparing Hg-Thermo-Desorption-Curves (TDC) with those of standard materials (Fig. 4). Solubility and reactivity of Hg in tailings was determined by leaching tests and operationally defined aqueous Hg speciation analysis.

3. Results and Discussion

Results show that total Hg concentrations vary from 40 to 2000 mg/kg in the tailings and can reach up to 1000 mg/kg in bottom sediments. Hg concentrations in overbank sediments range from 1 to more than 500 mg/kg. Solid phase Hg-TD measurements indicate that in older tailings Hg predominately exist as cinnabar (HgS) whereas in younger tailings adsorbed metallic Hg is the predominant Hg form besides unbound Hg^0 and traces of HgO (Fig. 1). In younger tailings soluble Hg can amount up to 2 % of total Hg, whereas more than 50 % can exist as reactive soluble Hg. In older tailings Hg solubility was much lower (0.005 %) due to the predominance of insoluble cinnabar. Hg in all sediments was found to occur mostly as cinnabar (Fig. 2). Accordingly, Hg in overbank sediments has been found to be mostly cinnabar, as well (Fig. 2). In the oldest terraces considerable amounts of matrix bound Hg from atmospheric Hg desposition were found besides cinnabar (Fig. 3). Forest soil samples from the same location, only show atmospheric derived matrix bound Hg which is attributed to Hg^0 emissions from the smelter

(Fig. 3). Cores taken from bottom sediments show that cinnabar is the predominant Hg species even in deep layers (Fig. 2).

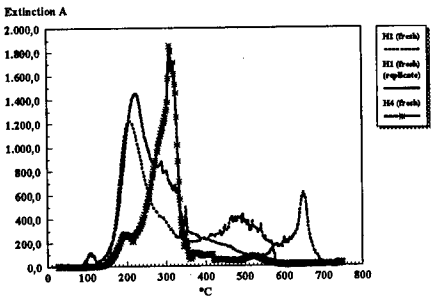

Figure1: TDC of Hg in young and old tailings of the Idrija Hg mining area

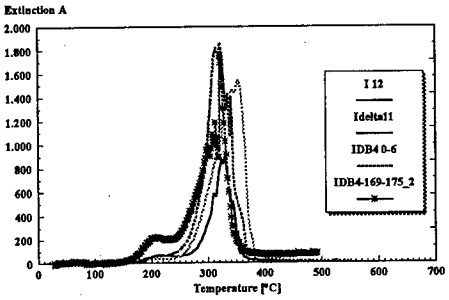

Figure 2: TDC of Hg in sediments of the rivers Idrijca and Soca

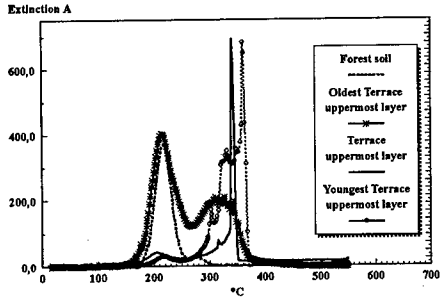

Figure3: TDC of Hg in Idrijca overbank sediments of different age

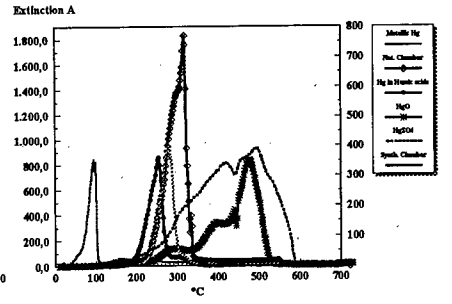

Figure 4: Hg-TDC of standard Hg compounds

4. Conclusions

Despite the lower total Hg concentrations found in younger tailings the long term risk potential of the mobile matrix bound Hg^0 found in this material is higher than that of older tailings bearing mostly immobile cinnabar. Hg binding form analysis in bottom and overbank sediments do reflect the predominance of cinnabar in the mining residues spilled into the rivers throughout the 500 years of Hg mining activity in Idrija. Therefore, we concluded that most of the Hg introduced into the Gulf of Trieste by Soca river is cinnabar.

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SPECIATION AND MOBILITY OF TRACE METALS IN POLLUTED SOILS AND SEDIMENTS FROM ROAD ENVIRONMENT

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1. Abstract

Road traffic is the source of trace metal contamination of runoff waters and roadside soils. In order to evaluate the trace metal mobility and the risk of groundwater contamination due to stormwater infiltration, trace metal speciation has been studied in road dust, roadside soil and a sediment from a retention pond by means of single (deionised water, NaCl, EDTA, HNO_3) and sequential chemical extractions. Generally the risk of metal release is poor, especially as regards road dust. Cd that is mainly in exchangeable form appears as the most mobile metal.

2. Introduction

Exhaust gases, tyre and brake lining wear, pavement wear, as well as corrosion phenomena are the origin of the chronic trace metal pollution of highway runoff waters and roadside soils, especially as regards Cd, Cu, Pb and Zn (Brinkmann, 1985). In this work, trace metal speciation has been studied in order to evaluate their potential mobility and the risk of groundwater and soil contamination due to stormwater infiltration.

3. Materials and Methods

Trace metal mobility has been studied by means of single chemical extractions : deionised water, sodium chloride 0.1 mol/l at pH=7 in order to simulate the effects of de-icing salts, EDTA 0.05 mol/l at pH=7, complexing reagent generally used to evaluate trace metal bioavailability of agricultural soils (Ure et al., 1993) and nitric acid solution at pH ranging from 2 to 8 to study the effect of acidity on metal release. Besides, trace metal speciation has been determined by using the sequential extraction procedure from Tessier et al. (1979). The studied samples are road dust taken on the pavement surface and soil taken at 0.50 m from the asphalt edge on the RN12 highway (50 km from Paris, 25,000 vehicles/day). A sample of sediment has been taken in a retention pond at the Nantes ring road (60,000 vehicles/day).

4. Results and Discussion

The samples studied are strongly contaminated by Pb, Zn, Cu and Cd (Table 1).

Tab. 1 : Results of EDTA trace metal extraction (% extracted and total content mg/kg).

Sample	Pb	Zn	Cu	Cd	Cr
Soil	66 (2580)	24 (880)	73 (200)	83 (3.1)	0.4 (63)
Sediment	59 (490)	41 (2680)	60 (250)	79 (2.5)	0.5 (100)
Road dust	20 (1450)	11 (840)	6 (1070)	14 (1.7)	0.3 (52)

Metals are weakly extracted by deionised water and NaCl, less than 1% of total content. Metals are better extracted by EDTA, and metals bound to soil appear more mobile than metals associated to road dust.

Results from the sequential extractions (figure 1) show that lead is mainly associated with the acid-soluble and reducible fractions, copper with the oxidizable fraction, cadmium with the exchangeable and acid-soluble fractions, zinc with the reducible and acido-soluble fractions for the soil and the sediment and with the residual and oxidizable for road dust. Finally, chromium, that seems to be essentially from geochemical origin, appears mainly in the residual fraction.

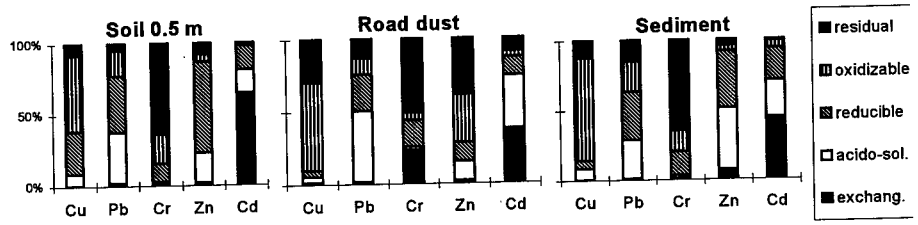

Fig. 1 : Results of the sequential extraction of trace metals.

From these results, the metal mobility can be placed in the order Cd>Pb>Zn>Cu>>Cr for the soil and road dust, and Cd>Zn>Pb>Cu>>Cr for the sediment that is very polluted by zinc.
As regards the effect of pH on metal mobility, it appears that Cd, Cu and Zn are released at pH less than 6 and Pb at pH less than 4. Metals are less extracted from road dust than from other materials. Metal mobility varies in the order Cd>Zn>Cu>Pb>Cr and is more important in the sediment than in other materials.

5. Conclusions

The various behaviours of metals could be explain by the different chemical compositions of the studied materials and by the origin and speciation of metals. The mobility of metals contained in road dust, close to their emission point, is weak. They are strongly associated to exhaust particles or abrasion products. The risk of dissolution in runoff water is poor: generally metals are bound to particles in runoff water, especially lead (Legret et al, 1997). Cadmium, that is mostly in exchangeable form, appears to be the most mobile pollutant.

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ARSENIC OXIDATION STATES IN CHEMICALLY EXTRACTED SOILS

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1. Introduction

The mobility and bioavailability of As in soils depends on its interaction with the solid phase, redox and pH conditions. Chemical fractionation methods have been tested to estimate the distribution of As in soils and, by implication, assign As to different solid phase pools. It is generally agreed that assigning As to specific soil constituents is difficult but fractionation methods have value for estimating relative solubility (Gruebel et al 1988; Onken and Adriano, 1997). Using a combination of chemical fractionation and spectroscopic techniques, e.g., x-ray absorption near edge structure (XANES), important information may be obtained about the forms and interactions of As in soil. This report focuses on oxidation states of As(III) and As(V) added to soil following an incubation period and chemical sequential extraction.

2. Materials and Methods

Soil samples from a bioremediation study were collected before flooding (pre- flooded) and 5 months after flooding (post flooded). Soil samples were air-dried, ground, and sieved through a 1 mm opening. The soils were digested with HNO_3-H_2SO_4 and the extracts analyzed by ICP(Huang and Fujii 1996). The pre-flooded soil contained 5.0 mg/kg As and had a pH of 7.9 while the post flooded soil contained 5.6 mg/kg As and had a pH of 8.4. Pre and post- flooded soil samples were treated with either $NaAsO_2$ (As(III)) or Na_2HAsO_4 (As(V)) in a 1:1 (v/w) mixture to give a final As concentration of 5 μmol As/g. The pH of the samples was maintained near 7.5 for a 10-day incubation period. At the end of the incubation period, the pH and Eh of the mixtures were measured. Samples were sequentially extracted with NH_4Cl, NH_4F, NaOH, and H_2SO_4 solutions according to the method of Onken and Adriano (1997) without drying and As determined. Parallel samples were extracted and soils saved at each step for XANES analysis.

3. Results and Discussion

The pH of both the pre- and post flooded soils after As treatment and incubation was nearly the same ranging from 7.5 to 7.8. However, the Eh was much higher for the pre-flooded soils (433-455 mV) compared to post-flooded soils (130-240 mV). Fig. 1 shows XANES spectra for pre- and post flooded soils 10 days after treatment with either As(III) or As(V) and compares this with As dissolved in water. In the As(V) treated pre- and post-flooded soils only As(V) was observed as shown by the edge at 11876 eV. However, in the As(III) treated pre- and post-flooded soils both As(III) and As(V) were seen as indicated by the edges at 11873 and 11876 eV. This suggests that some As(III) was oxidized during incubation. Manning et al.(1997) found As(V) in phosphate extracts of several clay minerals treated with As(III) indicating oxidation. Chemical fractionation of the soils showed that more NH_4Cl extractable As was obtained from pre- and post-flooded soils treated with As(III) than with the As(V) treatment. Amounts of As extracted using NH_4F, NaOH, and H_2SO_4 were nearly the same for both the As(III) and As(V) treated pre- and post-flooded soils.

Residual As was greater in As(V) treated soils. XANES spectra showed a decrease in absorption edge intensity for As(III) on the solid phase after each extraction step starting with NH_4Cl while the As(V) absorption edge showed a more gradual decrease for the As(III) treated soils. Chemical extraction may have selectively removed As(III) first, leaving As(V) enriched on the solid phase. An alternative explanation is that the extraction caused As(III) to oxidize. In either case, chemical extraction significantly changed the distribution of oxidized forms of As on the solid phase. XANES spectra for the As(V) treated soils showed only As(V) on the solid phase (Fig.1) that decreased in relative intensity with successive extractions.

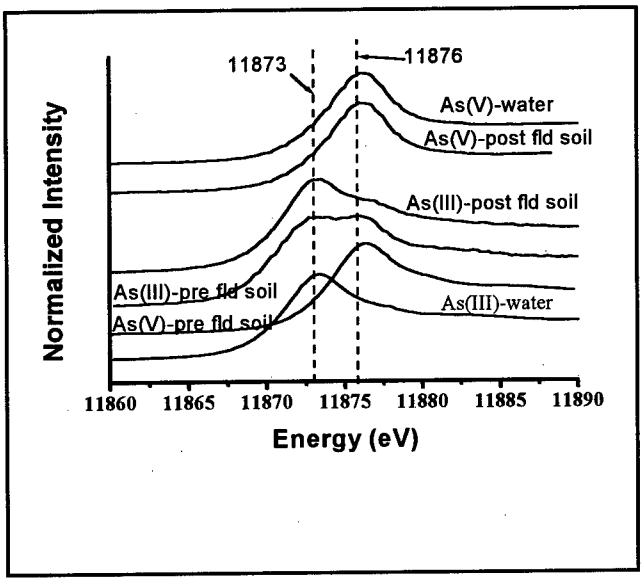

Fig. 1 XANES spectra of As(III) or As(V) treated pre- flooded (pre fld) and post-flooded (post fld) soils compared to water soluble As(III) and As(V).

4. Conclusions

Incubation of As(III) in both pre- and post-flooded soils resulted in partial conversion of As(III) to As(V) on the solid phase. Sequential chemical extraction of As(III) treated soils resulted in depletion of As(III) and partial removal of As(V) on the solid phase. This may be the result of either selective removal of As(III) or its oxidation to As(V) during extraction.

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CORRELATING MANGANESE X-RAY ABSORPTION NEAR-EDGE STRUCTURE (XANES) SPECTRA WITH EXTRACTABLE SOIL MN.

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1. Introduction

Manganese redox chemistry in soil plays an important role in Mn uptake by plants, plant availability and ecotoxicology of trace elements associated with Mn-oxides, and the etiology of some soil-borne plant fungal diseases. In previous work, we showed that XANES spectroscopy can be used to quantify Mn^{II} and Mn^{IV} in mixed systems (Schulze et al., 1995). Our long-range goal is to use micro-XANES spectroscopy to study Mn chemistry in the rhizosphere of live plant roots. The objective of this study was to determine if we could correlate sequential chemical extraction of Mn with changes in the micro-XANES spectra of the soils.

2. Materials and Methods

Micro-XANES spectroscopy was done on beamline X26A at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York, using a 30 x 30 μm incident x-ray beam. Analysis of $MnSO_4$ (Mn^{II}), Mn-substituted goethites (Mn^{III}), and a synthetic Na-birnessite (Mn^{IV}) provided the position of the x-ray absorption K edge for different Mn redox states. The following sequential extraction procedure was used to sequentially extract increasingly less soluble fractions of Mn from 4-gram samples of the surface horizons of four soils cropped to wheat: NH_4-acetate (1 M, pH 7; exchangeable), $CuSO_4$ (0.5 M, pH 2; organically-bound), hydroquinone (0.02% in 1 M NH_4-acetate, pH 7; readily reducible), and dithionite-citrate-bicarbonate (DCB; reducible). A XANES spectrum of the soil paste was obtained after each extraction. The soils were studied in both a field-moist (aerated) condition, and after being saturated with water for 7 days following the addition of 0.5g $(NH_4)_2SO_4$ and 1g sucrose per kg of soil as a food source for the soil microorganisms (to enhance reduction).

3. Results and Discussion

The results were reproducible and similar for all four soils. Under aerated conditions, the XANES spectra changed little after NH_4-acetate and $CuSO_4$ extraction (on average, only 3% of the total reductant-extractable Mn was removed), but after subsequent hydroquinone and DCB extractions, the Mn^{IV} peak decreased in proportion to Mn removed (Figure 1). After Mn^{IV} reduction and removal, the XANES spectra indicated residual Mn^{II} and Mn^{III}, presumably within the crystal structures of aluminosilicate minerals.

Under reduced conditions, NH_4-acetate extracted 37% and $CuSO_4$ an additional 25% of the total reductant-extractable Mn. A progressive reduction in the intensity of the Mn^{II} peak indicated that both extractants were removing Mn^{II} that had been solubilized by microbial activity (Figure 2). A prominent Mn^{III} peak remained after $CuSO_4$ extraction. This peak sharpened slightly after extraction with hydroquinone, which removed only 1% of the total reductant-soluble Mn. The

Mn^{II} remaining after the NH_4-acetate extraction, and removed by $CuSO_4$ may be present as $MnCO_3$, or as Mn^{II} sequestered by microorganisms.

4. Conclusions

Using XANES spectroscopy, we can distinguish between Mn^{II}, Mn^{III}, and Mn^{IV} in soils. Bulk extraction data from gram-sized samples correlates well with microspectroscopy data from 30 x 30 μm spots. Hydroquinone appears to extract the Mn available for microbial reduction. Under reducing conditions, there may be a sizable fraction of Mn^{II} sequestered by microorganisms or precipitated as $MnCO_3$.

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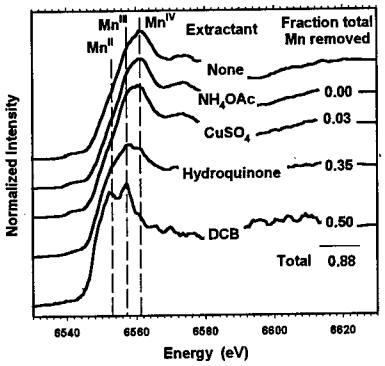

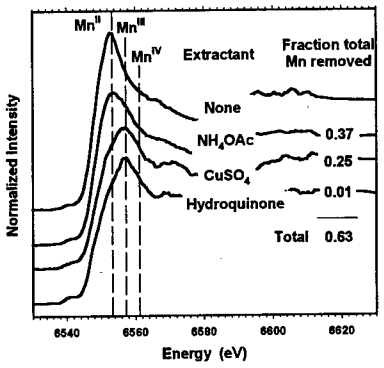

Figure1: XANES spectra of the aerated soil after each extraction. (The fraction of total Mn removed is based on the total Mn removed after 3 successive overnight DCB extractions at room temperature.)

Figure 2: XANES spectra of the reduced soil after each extraction. (DCB extractions were not done.)

6. Acknowledgments

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SPECIATION OF HEAVY METALS IN LANDFILL-LEACHATE POLLUTED GROUNDWATER

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1. Introduction

Landfill leachate and landfill leachate polluted groundwater may contain high concentrations of heavy metals. The mobility of heavy metals may be enhanced by the complexing capacity of organic matter leaching from landfills and by the content of colloidal matter in leachate and leachate-polluted groundwater (*e.g.*, Christensen, et al., 1996; Gounaris, et al., 1993. The objective of this study was to determine the distribution of heavy metals between different species (different size-fractions, organic species, and dissolved inorganic species) in leachate-polluted groundwater sampled downgradient of a landfill.

2. Materials and Methods

The landfill has no engineered leachate collection system. Therefore, the leachate is leaking into the groundwater aquifer. Two samples (L1 and L2) were taken downgradient of the landfill. The pollution plume in this area is strongly anaerobic with a high content of organic matter. The sampling procedure was designed to ensure that the samples reflected the *in situ* conditions with respect to, *e.g.*, colloidal content and redox conditions. The content of heavy metals in the leachate-polluted groundwater samples was very low. Therefore, the groundwater samples were spiked with heavy metals at concentrations within the range of concentrations of heavy metals in landfill leachates and leachate polluted groundwater (100 µg Cd/L, 500 µg Ni/L, 1000 µg Zn/L, 1000 µg Cu/L, 1000 µg Pb/L) and divided under anaerobic conditions into colloidal (screen-filtration and cross-flow ultrafiltration) and dissolved species. Subsequent, each size fraction was divided into organic (anion-exchange) and inorganic species of the heavy metals.

3. Results and Discussion

The major cations in the leachate-polluted groundwater samples were Na, Ca, K, and NH_4 and the major anions were HCO_3^- and Cl. Total organic carbon was found primarily (more than 87 %) in the smallest size-fractions (< 0.010 µm), with the main part in the smallest colloidal fraction (0.001 - 0.010 µm). The results of the size-fractionation and the anion-exchange speciation were combined in a "total speciation" for the heavy metals (Figure 1). The heavy metals are divided into four different species: inorganic colloidal metal species, organic colloidal metal species, organic dissolved metal species, and inorganic dissolved metal species. The distribution of the heavy metals between the different size-fractions showed that a substantial, but highly varying part of the heavy metals was associated with the colloidal fractions (Cd: 38-45 %, Ni: 27-56 %, Zn: 24-45 %, Cu: 86-95 %, Pb: 96-99 %). These colloidal bound metals were primarily associated with the organic fraction, except for Zn, which was associated mainly with the inorganic fractions. Dissolved Cd, Cu, and Pb were mainly associated with dissolved organic carbon, while Ni also was present as carbonate complexes and Zn as carbonate complexes and free divalent Zn. The results indicate that heavy metals in leachate-polluted groundwater are strongly associated with small-size colloidal

matter and organic molecules. Thus, the behavior of heavy metals in aquatic systems is expected to differ from that of dissolved heavy metals.

4. Conclusions

The distribution of heavy metals between different size-fractions showed that a large part of the heavy metals was associated with the colloidal fractions (24-99 %). The heavy metals especially Cd, Cu, and Pb complexed strongly with the organic matter in leachate-polluted groundwater. The results confirm the importance of colloidal and organic matter in controlling the transport of heavy metals in leachate-polluted groundwaters. Therefore, the chemical processes affecting the distribution of dissolved metals cannot be modeled simply in terms of reactions involving inorganic aqueous complexes and free ions.

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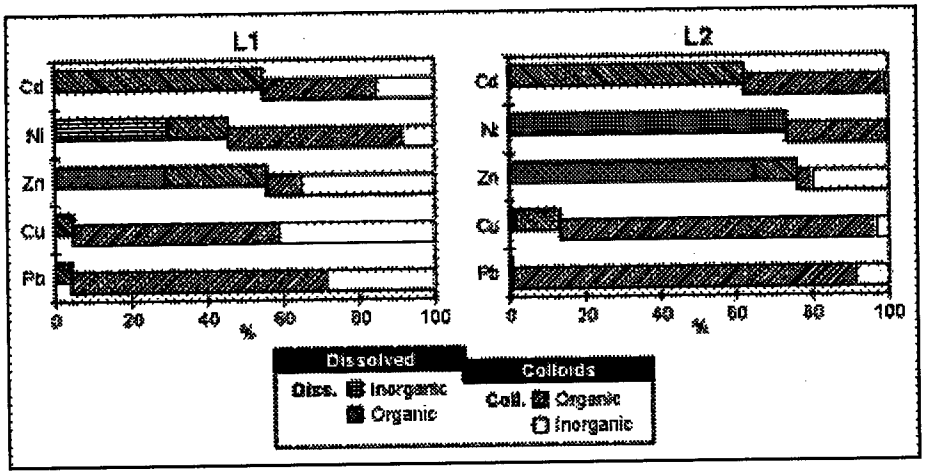

Figure 1. "Total" speciation of Cd, Ni, Zn, Cu, and Pb in the leachate-polluted groundwater samples L1 and L2.

ZINC SPECIATION IN A SMELTER-IMPACTED SOIL BY A COMBINATION OF EXAFS DATA AND CHEMICAL EXTRACTIONS

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1. Introduction

Current understanding of metal speciation in contaminated soils is largely based on indirect methods such as thermodynamic modeling or "selective" chemical extractions (Tessier et al., 1979). In this study, we combined XAFS spectroscopy with chemical extractions in order to directly determine the speciation of zinc in smelter-impacted soils from Northern France (Ildefonse et al., 1999).

2. Materials and Methods

The study soil is located in Northern France near a Pb-Zn smelter. Two soil profiles were sampled: one in a cultivated field, about 1000 m NW of the smelter, and a second one, around 500 m NW of the smelter in a forestsed area. Profile 1 (500 $mgkg^{-1}$ Zn, pH=7.5 and TOC=15 gkg^{-1}wt% in topsoil layer) is characterized by a 30 cm thick tilled horizon, related to cropping and the second one (1400 $mgkg^{-1}$ Zn, pH=5.5 and TOC=64gkg^{-1} in topsoil layer) had not been disturbed for about 50 years.

A combination of separation procedures was used to isolate different metal-bearing fractions from both topsoils. Chemical and mineralogical characterization of these different fractions were conducted by XRD, EMPA and SEM-EDS. Synchrotron-based techniques (μ-SXRF and EXAFS) were used for *in situ* localization of the different pools containing zinc and were combined with chemical extractions to directly determine the speciation of this element.

3. Results and Discussion

Based on μ-SXRF observations, two distinct pools from both topsoils were identified and analyzed separately: i) the dense fraction (d > 2,89) extracted from the 50-200 μm fraction and ii) the < 2 μm fraction.

In the dense fraction, glassy-like particles containing up to 100 gkg^{-1} Zn and 80 gkg^{-1} Pb were identified by EMPA and SEM-EDS analyses and can be considered as part of the source of heavy metals in the soil. EXAFS evidenced a partial substitution of Fe^{2+} by Zn^{2+} in the tetrahedral sites of magnetite (Fe_3O_4) identified by XRD as a major component of this dense fraction. In this fraction, small amounts of zinc sulfides (30 gkg^{-1}) were also detected using Rietveld refinement of XRD powder patterns.

In the < 2 µm fraction of both topsoils, EXAFS data suggested that the major part of zinc was incorporated in mixed-Zn/Al hydroxides (hydrotalcite-like structures). These structures consist of positively charged mixed-cations octahedral layers (brucite-like sheets) alternating with disordered negatively charged interlayers (containing either carbonate, nitrate, sulfate, chloride anions and water) required for the electrical balance of the structure (Allmann, 1968; Allmann and Jepsen, 1969). The positive charge observed for the octahedral layer results from the partial substitution of divalent cations (Mg, Fe, Ni, Co, Zn) by trivalent cations (Al, Fe, Cr).
In addition, combination of selective chemical extractions and EXAFS data revealed the occurence of a significant fraction (57 %) of organically-bound Zn in the < 2 µm fraction of the soil with the higher organic matter content and the lowest pH. This "organically-bound" Zn was mainly found to be exchangeable (38%) indicating that zinc mainly sorbs onto organic soil components as outer-sphere Zn complexes or as weakly bound inner sphere complexes.

4. Conclusions

This study gives the first evidence of the incorporation of zinc in hydrotalcite-like structure in a natural system and emphasizes the importance of mixed-cation hydroxides for controlling pollutant mobility (especially divalent transition ions, i.e. Co, Ni, Cu, Zn) in contaminated soils. These species represent an important way to bind metals released in contaminated soils because their formation occurs in solutions undersaturated with respect to the homogeneous hydroxide phases. Further studies will be undertaken to assess the kinetics of formation and the stability of mixed-cation hydroxides (hydrotalcite-like structure) in soils.

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RAMAN INVESTIGATION OF ASSOCIATIONS OF LEAD AND ZINC WITH IRON-CONTAINING PHASES WITHIN A CONTAMINATED SOIL.

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1. Introduction

Chemical speciation understanding of metal pollutants in contaminated soils is essential for predicting their fate in the environment, especially in regards to their bioavailability. In soils polluted by Zn, Pb and Cd atmospheric deposits, first investigations including chemical extractions and physical fractionation of soil showed that lead and zinc were mainly associated with calcite and iron-containing phases. Direct investigations of the bindings between iron-containing phases and lead and zinc were then achieved using Raman spectroscopy at a micrometer scale in combination with multi-element microanalysis (SEM-EDX). The investigations were done on iron-enriched fractions separated by particles colour, density and magnetic susceptibility. Besides, iron-phases loaded with lead were synthesised, in order to give references on Raman spectra modifications.

2. Materials and Methods

Samples were collected in the subsurface layer (20 cm) of soil at 1 km from a lead smelting factory. The samples were dried at 35°C and gently ground to pass a 2 mm sieve. Particles of size from 2000 to 1000 µm and 1000 to 500µm were separated by sieving in water. Red particles were then isolated from these fractions under a binocular lens and identified as oxy-hydroxide compounds and rock fragments. Besides, the ferromagnetic fraction of soil was separated using a hand magnet in water suspension of soil, and the heavy fraction (>3.3g/cm^3) was separated by diiodomethane.
Major crystallised phases were identified by XRD (X-Ray diffraction). Total elemental content in soil and in each sub-fraction was determined by ICP after hot acid digestion (table 1).

Table 1. Zn, Pb, Fe and Mn total analyses and XRD characterisations of sub-fractions of soil

Fractions	total	2000-1000 µm red	1000-500 µm red	Magnetic	Heavy
Zn (mg/kg)	965	1756	1704	1519	2750
Pb (mg/kg)	1060	1133	2288	1482	2309
Ca (mg/kg)	24400	56350	99242	19658	21770
Fe (mg/kg)	51300	272311	186743	432858	170797
Mn (mg/kg)	350	3164	2940	2631	1230
Main crystallised phases	Quartz Calcite Goethite	Goethite Ferrihydrite	Hematite Magnetite Wuestite		Goethite Hematite Magnetite Magnesioferrite

The Raman scattering of samples was excited with the 514.5 nm line of an Argon laser or with the 632.8 nm line of a He-Ne. Measurements were performed at room temperature through the micro-spectrometry technique with a spatial resolution of the micron size : the Laser beam was focused on particles through a high-aperture microscope objective and the scattering light was collected by the same objective.
As few references exist in Raman spectroscopy of minerals, chemical models were synthesised to identify molecular species in which the metals are involved. Hematites loaded with lead where synthesised by steering a solution of lead nitrate (10^{-4}mol/L) with 100 mg of the iron oxide, in presence of KNO_2 (10^{-2}mol/L). pH was adjusted to 8 with soda. XRD analysis of the resulting solid shows no difference in the spectra compared to genuine hematite.

3. ESEM-EDX Results

X-cartographies of the heavy fraction of soil revealed that zinc is associated with large particles (10µm) containing Fe and S. Pb is found in smaller particles (1 µm) in association with Fe and Si. The SEM-EDX analysis of magnetic fraction showed geometric particles of FeO and Fe_3O_4 (result from semi-quantitative analysis). Some of them are associated with Zn ; their quantitative analysis gave a stochiometry of $ZnFe_2O_4$ (JCPDS n° 22-1012). Moreover, non-crystallised particles containing Zn with a vitreous aspect were observed. The X-ray analysis permitted us to calculate the formula $Fe_{0.85-x}Zn_xO$, and then this compound was identified by XRD as a scoria particle from the factory. Concerning lead associations, we observed small particles (2-3µm) with an amorphous aspect containing Fe and S elements or small particles of Pb fixed on the surface of Fe-Mn-Ti particles. ESEM-EDX analyses of loaded hematite showed that the repartition of lead is heterogeneous as particles contain from 0 to 10% of lead.

Raman Results

The major mineral compounds of the soil were found to be similar to those identified by XRD. More interesting were the Raman spectroscopic investigations of particles loaded with lead identified by ESEM-EDX experiments. Investigations of the red particles, of heavy and magnetic fractions permitted the identification of lead sulfate associated with particles containing iron(III) oxy-hydroxides. Lead species appear to be associated with iron(III) oxides because of some significant vibrational changes in the Raman spectra of particles loaded with lead, whereas particles without lead do not exhibit such changes. In particular, spectra 1 and 2 from figure 1 show external mode band shifts (from 288 to 275 cm^{-1} and from 404 to 388 cm^{-1}) when hematite is loaded with lead. Spectrum 3 of a particle from the magnetic fraction shows the same vibration modes than that of hematite loaded with lead.

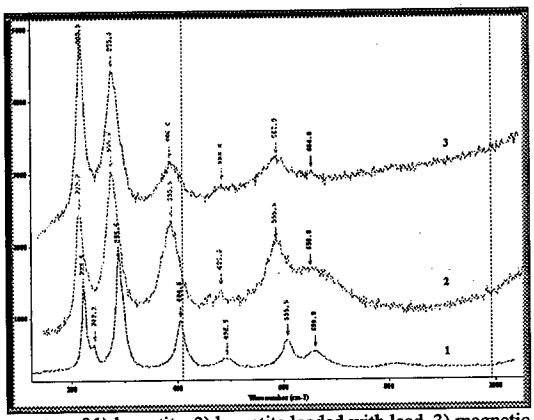

Figure 1. Raman spectra of 1) hematite, 2) hematite loaded with lead, 3) magnetic fraction particle.

4. Conclusions

The identification of the molecular species was made through the frequencies (cm^{-1}) and relative intensities of Raman signals. This method gave us a molecular information about chemical species in which the metals are involved through the vibrational properties. It should be noted that minor compounds and non crystallised phases could be detected. The use of models was efficient to identify compounds loaded with lead. Other experiments are in progress to locate metals associated with calcite. However, the quality of spectra (peak intensities) depends mainly on the Raman Scattering cross section of the compounds which is very low in the case of zinc compounds.

CHEMICAL FORMS OF LEAD IN A SOIL DEVELOPED ON A PB-MINERALIZED SANDSTONE (ARDÈCHE, FRANCE)

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1. Introduction

Lead is a widespread pollutant which generally accumulates in topsoil horizons as a result of surface reactions (adsorption or co-precipitation) with soil components (Fe/Mn-(hydr)oxides, organic matter or clay minerals) (e.g. Morin et al., 1999) or precipitation of Pb-bearing secondary minerals (e.g. Cotter-Howells et al., 1994). In order to identify immobilization processes that may be efficient at the geological time-scale, the present study focused on the chemical forms of lead in a naturally enriched soil which exhibits lead concentration in the same order of magnitude than those in polluted soils.

2. Materials and Methods

Three horizons were sampled in the soil profile studied: A-organo-mineral horizon (pH=5.5, 5.5% total organic carbon, T.O.C.), B-horizon (pH=6.0 and 1.6 % T.O.C.), saprolitic C-horizon (pH=6.5 and 0.1% T.O.C.). EXAFS analysis was combined with XRD, SEM, EPMA and μSXRF in order to determine and quantify the various chemical forms of lead along the profile.

3. Results and Discussion

EXAFS and XRD analyses evidenced that plumbogummite ($PbAl_3(PO_4)_2(OH)_5.H_2O$), a low solubility mineral ($K_S=10^{-29.3}$), is the main lead species in the soil studied. Chemical micro-analyses (EMPA, SEM-EDS and μ-SXRF) also indicated the occurrence of Pb/Mn-(Fe) associations, especially in the C and B horizons. Analysis of EXAFS data from bulk soil samples indicated that these associations correspond to Pb(II)-sorbed onto manganese (hydr)oxides. In addition, EXAFS revealed that a significant fraction of lead is bound to soil organic matter, especially in the topsoil horizon.

Changes in lead speciation along the soil profile were assessed by least-square fitting of EXAFS raw data from bulk soil samples, using appropriate model compounds (plumbogummite, Pb(II) sorbed onto birnessite, and Pb(II)-humate) Figure 1). The proportion of plumbogummite was found to be almost constant (50 - 55 % of total lead) along the whole profile. In contrast, Pb(II) - manganese (hydr)oxides complexes are gradually replaced by Pb(II) - organic matter complexes upward the soil profile. Normalization of the lead concentration to the zirconium concentration evidenced a loss of lead in the A-horizon with respect to the C-horizon. The disapearing of the

Pb(II) - manganese (hydr)oxides complexes and the possible dissolution of about half of the plumbogummite could explain such loss of lead upward the soil profile.

4. Conclusions

The present study evidences the high affinity of lead for organic matter, especially in topsoil horizons where T.O.C. is high. In addition, our results seem to indicate that sorption onto manganese (hydr)oxides does not constitute an effective way for long-term binding of lead in organic rich Pb-contaminated soils. Precipitation of insoluble minerals like Pb-phosphates seems to be more appropriate for the sequestering of lead in soils at geological time-scales. However, further work is needed to study the weathering kinetics of these low solubility minerals, especially in soils where organic matter and micro-organisms can enhance their solubility.

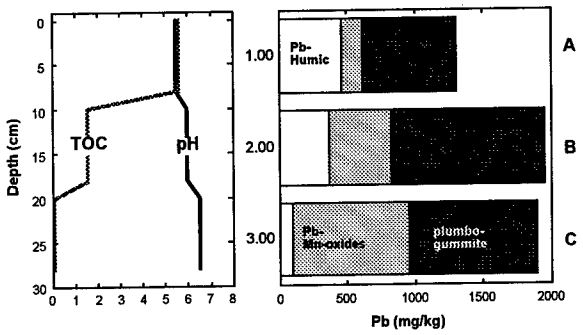

Figure 1. Chemical forms of lead along the soil profile, as determined from EXAFS data. Soil pH and Total Organic Carbon content (wt.% TOC) are also reported.

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EXAMINING THE FATE OF CHROMIUM(VI) IN SOIL BY SPECIATION

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1. Introduction

Speciation in case of chromium measurements is particularly important, because the toxicity of Cr(III) and Cr(VI) is very different. While Cr(III) is an essential micronutrient for mammals and Cr(VI) is potentially toxic (Bartlett and James 1988, Katz and Salem 1994). In the recent 5 years we tested and developed different chromium speciation methods, standardised the measurement and introduced it to the measurement networks of environmental and soil testing laboratories (Prokisch et al. 1997). We attempted to prepare a soil reference material for Cr(VI), and examined the fate of Cr(VI) in soil. Reduction rate, adsorption isotherms and toxicity of Cr(VI) in soil were investigated as well.

2. Materials and Methods

For the separation of chromium ions an acidic activated aluminium oxide filled microcolumn was used which was directly connected to an ICP-AES or flame AAS instrument. Four synthetic or natural Cr(VI) contaminated soil samples were tested in a circle test for standardisation and validation of measurement. Cr(VI) contaminated areas were used for studying the leaching and reduction of Cr(VI) in natural circumstances. Laboratory experiments were set up for measuring Cr(III) and Cr(VI) adsorption by soil. Cr(VI) toxicity was studied in a toxicity test using bioluminescent bacteria (*vibrio fishery*).

3. Results and Discussion

Acidic activated aluminium oxide do not adsorb the Cr(III) but adsorbs the Cr(VI) after conditioning with aluminium nitrate solution. The adsorbed Cr(VI) can be eluted with 3M hydrochloric acid. On this basis there is a chance for the separation of chromium ionic species from soil extracts both off-line and on-line method. The alumina was filled to a microcolumn and the steps of speciation were controlled by the autosampler. The detection limits obtained were 4 µg/l and 0.5-1 µg/l for the Cr(III) and Cr(VI) respectively. When the off-line separation and the GF-AAS measurement were applied the detection limits obtained were in the range of 0.01-0.05 µg/l for both chromium ion.

The adsorption of Cr(III) and Cr(VI) by soil are very different. In the case of Cr(III) the total precipitation was observed in the meadow solonetz soil (B level) while the Cr(VI) was not adsorbed. Langmuir-isotherms were applied for the description of chromium adsorption by the soils. The adsorption capacity values were calculated on the basis of this model. The Cr(III) adsorption capacity of sandy soil, chernozjom A and B level were 200, 538 and 727 mg/kg respectively. The Langmuir-isotherms are convenient only for a specific concentration range for modelling of adsorption because of the presence of different adsorption on the soil surface. The Cr(VI) adsorption capacity of the meadow solonetz soil (B level) was negligible. The Cr(VI) adsorption capacity of sandy soil, chernozjom A and B level were 2.36, 4.75 and 2.14 mg/kg respectively. The adsorption capacities of the soils were higher at least by two order of magnitude for Cr(III) than Cr(VI) at least.

Tab. 1. Parameters of Langmuir isoterm ($1/q_e = 1/Q + k_L/(Q*C_e)$) for Cr(III) and Cr(VI) for soils

soil	Cr(III) k_L [mg/l]	Cr(III) Q [mg/g]	Cr(III) r^2	Cr(VI) k_L [mg/l]	Cr(VI) Q [mg/g]	Cr(VI) r^2
sandy soil	0.614	0.2	0.9674	12.043	0.0236	0.9465
chernozem A level	3.88	0.538	0.938	18.771	0.0475	0.8913
chernozem B level	1.097	0.727	0.987	15.25	0.0214	0.9984

The rate of Cr(VI) reduction was measured for the sandy soil, chernozjom A and B level soils at different levels of water content. There was no detectable Cr(VI) reduction in the meadow solonetz soil (B level). The Cr(VI) reduction can be separated into two parts. At the first week it is faster and later it is slower.. The first order reaction rate model gives a good description for the reduction rate. Therefore the half-life of reaction is a good parameter for the comparison of soils. There was no reduction in the alkaline meadow solonetz soil (B level). Among the other soil samples the slowest reaction was obtained in the chernozjom B level and the fastest in the A level. The water content of soil has significant effect on rate of reduction. .the rate of reduction increased with decreasing water content. The half-life of Cr(VI) reduction was between 4-8 weeks on the top soils, and it was higher than 10 weeks in the deeper soil layers.
We obtained proof of chromium(III) oxidation in the waste incineration processes, which can increase the ecological risk of high-temperature waste burning (Prokisch et al. 1997).
On the basis of toxicity measurement the acceptable level of Cr(VI) in soil should be below 500 µg/kg. Soil solution was tested for chromium as well. The concentration of soil solution was 8-10 µg/l, and only 2-3 µg/l , respectively

4. Conclusions

The developed method is applicable for studying the fate of chromium contamination in soil. The environmental and agricultural laboratory networks are trained for this measurement, and we have suggestion for the permitted concentration limit of Cr(VI) in soil.

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SPECIATION AND COMPLEXATION OF CADMIUM IN SOIL SOLUTIONS

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1. Introduction

The relationships observed between soil properties and solution speciation of Cd are reported for 64 field-collected contaminated soils and 46 metal-spiked soil samples containing between 0.1 and 56 mg Cd kg^{-1}. The soils were analyzed for pH, soil organic matter, total dissolved Cd, dissolved organic carbon, free Cd^{2+} and total metal content.

2. Materials and Methods

Free Cd^{2+} activity in solution was determined using differential pulse anodic stripping voltammetry (DPASV), assuming DPASV is sensitive to easily dissociated inorganic ion-pairs and free Cd^{2+} while excluding organic complexes (Florence 1986). The total cadmium dissolved in solution was determined using graphite furnace atomic absorption spectrometry.

3. Results and Discussion

The solid/liquid partition coefficient (K_d) varied over a range from 10 to 100 000, and the fraction of the dissolved Cd present in solution as the estimated free Cd^{2+} species varied between 0 and 80%. The soil solution free Cd^{2+} activity can be predicted from a semi-mechanistic equation using a simple competitive adsorption model (McBride et al. 1997):

$$pCd^{2+}(\pm 0.66) = 4.39(\pm 0.32) + 0.69(\pm 0.05)\cdot pH - 0.97(\pm 0.08)\cdot \log_{10}(Total\ Soil\ Cd)$$
$$R^2 = 0.736,\ p < 0.001,\ n = 102,\ (\pm S.E.) \quad (1)$$

where the standard errors are given in parentheses, pCd^{2+} represents the free Cd^{2+} activity (by analogy to pH, it is the negative $\log_{10}$ free ion activity), the *pH* was measured in the equilibrating solution (similar to pH in 0.01 *M* $CaCl_2$) and *total soil Cd* was measured using hot nitric acid digestions (in mg Cd•kg^{-1}).

Total dissolved Cd can be predicted using a similar model which includes an organic matter component (Eq. 2) allowing the description of the solubility relationships for the whole dataset (including, acidic, neutral, and alkaline soils):

$$\log_{10}(Dissolved\ Cd)(\pm 0.60) = -0.09(\pm 0.52) - 0.36(\pm 0.04)\cdot pH$$
$$+0.86(\pm 0.07)\cdot \log_{10}(Total\ Soil\ Cd) + 1.59(\pm 0.27)\cdot \log_{10}(Soil\ Organic\ Matter) \quad (2)$$
$$R^2 = 0.708,\ p < 0.001,\ n = 110,\ (\pm S.E.)$$

where the standard errors are given in parentheses, dissolved Cd is in $\mu g \cdot L^{-1}$, soil organic matter is in g $C \cdot kg^{-1}$, and other input variables are the same as for Eq. 1.

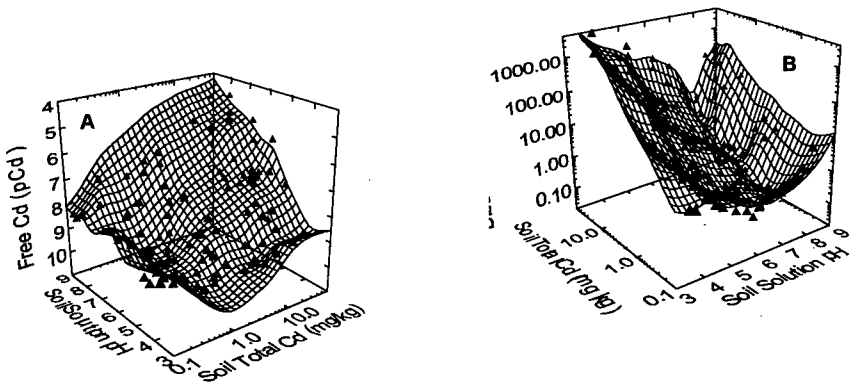

Fig. 1: The free Cd^{2+} activity (pCd^{2+}-A) and the dissolved Cd ($\mu g \cdot L^{-1}$-B) in the soil solutions as a function of pH and total metal content. The graphic surfaces are obtained using a distance-weighted least-square smoothing algorithm.

4. Conclusions

The solubility and free metal speciation of Cd^{2+} in a set of field-collected and metal salt-spiked soils could be predicted using a simple semi-mechanistic linear regression model which accounts for soil solution pH, total soil metal concentrations and possibly soil organic matter.

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CHARACTERIZATION OF ARSENIC IN SOIL FROM THE LÖCKNITZ SITE USING SYNCHROTRON X-RAY ABSORPTION SPECTROSCOPY

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1. Introduction

Chemical speciation is one of the principal factors affecting the mobility, bioavailability, and treatability of potentially toxic soil contaminants at hazardous waste sites. Preliminary results are presented which used x-ray absorption spectroscopy (XAS) to characterize different forms of organic and inorganic arsenic species in soil from the Löcknitz site in eastern Germany, which is contaminated with an arsenic-based chemical warfare agent - Clark I. Analyses were conducted on contaminated soil samples from the site, and on samples of uncontaminated soil that were reacted with various forms of arsenic (e.g., phenyl arsines or m-arsenite). Shifts in the x-ray edge energy were used to determine changes in oxidation state of the arsenic.

2. Materials and Methods

Two soil samples (BR1D3 and BR1E4) containing high levels of As (196 and 440 mmol/kg, respectively) were collected in one region of the site. A soil sample uncontaminated by As but typical of the soil in the rest of the site was also collected for the spiked soils studies. Standard compounds used included sodium m-arsenite, triphenylarsine, and triphenylarsineoxide. These samples were diluted using BN to give arsenic concentrations of 50 mmol/kg for the XAS measurements. Spiked soils samples were prepared by adding known amounts of the latter three standard compounds to the uncontaminated soils from Löcknitz. The concentration ranges for the spiked samples were 2-4 mmol/kg of As.) XAS measurements were carried out on beam line X-11A at the National Synchrotron Light Source at Brookhaven National Laboratory. Measurements were made at the As K edge in the fluorescence mode using a 13 element Ge detector.

3. Results and Discussion

All of the spiked samples showed some changes as evidenced by changes in the near edge structure (XANES) as shown in figures 1 and 2. In the case of the sodium arsenite and triphenylarsine, there appeared to be some oxidation as evidenced by a shift of the main absorption edge to higher binding energy. It can not be ruled out, particularly for the triphenylarsine, that this oxidation is induced by the x-ray beam. In all of the spiked samples an enhanced absorption appears at the edge. For arsenic compounds, this has been found to be due to an adsorbed complex being formed. The two contaminated soil samples also show large white lines and an edge position consistent with As(V) (fig. 2) . They are generally similar in appearance to the sodium arsenite spiked soil. Some differences in the height and width of the white line are apparent between the two samples.

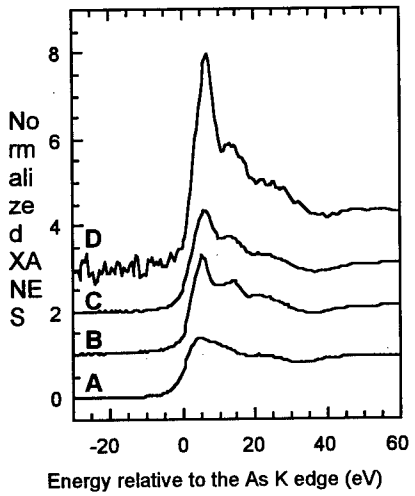

Fig. 1 The As K edge XANES for (A) triphenylarsine, (B) triphenylarsineoxide, (C) triphenylarsine spiked soil, and (D) triphenylarsineoxide spiked soil.

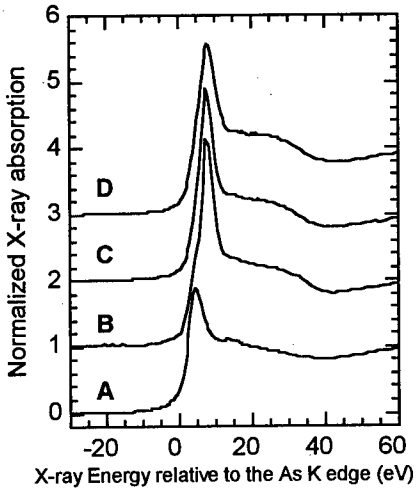

Fig. 2 The As K edge XANES for (A) sodium m-arsenite, (B) arsenite spiked soil, (C) Soil BR1D3, and (D) Soil BR1E4.

4. Conclusions

Preliminary studies of arsenic contaminated soils from the Löcknitz site along with soils spiked with standard arsenic compounds have shown that arsenic compounds react when in contact with the soil, generally forming an adsorbed complex. In the two soils samples studied, the arsenic was present as As (V) and appeared to be part of an adsorbed complex. These results suggest that oxidation-reduction reactions in the contaminated soil may transform arsenic into less mobile or less hazardous forms.

RADIO-LABILE CADMIUM AND ZINC IN CONTAMINATED SOILS

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1. Introduction

The radio-labile soil Cd fraction (or E-value) is an operationally defined *capacity* term. It identifies the fraction of metal in the solid phase which is in dynamic equilibrium with the solution activity of (Cd^{2+}) ions in the soil pore space. Labile soil metal concentrations are determined from the distribution of an appropriate isotope (eg, ^{109}Cd) between the solid and solution phases in a soil-electrolyte suspension (eg, Nakhone and Young, 1993).It is commonly observed that metal ions freshly added to soil undergo prolonged sorption reactions. Thus Nakhone and Young (1993) added relatively high concentrations of Cd (100 and 1000 mg kg^{-1}) to four different soil types and found a steady decrease in the radio-labile Cd fraction with time. Several commonly used soil extractants gave poor estimates of labile Cd: organic complexants *overestimated* and neutral salts *underestimated* the labile Cd pool. This paper reports initial results from a long-term study of time-dependent changes in metal lability and the investigation of an alternative method (1M $CaCl_2$ extraction) for measuring labile soil Cd on historically contaminated soils.

2. Materials and Methods

Cadmium and Zn, equivalent to the UK-ICRCL concentration limits (3 and 300 mg kg^{-1}), were added in solution (nitrate salt) to 23 UK soils (field-moist, 4 mm seived) varying widely in land-use, pH, %C and texture. The soils were incubated (16°C) under aerobic conditions and maintained at 80% field capacity. Radio-labile measurements of Cd and Zn were made at designated times; the method used was that of Nakhone and Young (1993) except that the equilibrating electrolyte was 0.1 M $Ca(NO_3)_2$.

The effectiveness of 1M $CaCl_2$ (1:5 soil:extractant) to estimate labile Cd and Zn was determined using samples collected from Pb/Zn mine spoil from Derbyshire, UK and a large dedicated sewage disposal site in the East Midlands, UK. Specific sites were chosen to provide a range of pH and soil metal contents. Soil (air-dried, <2mm, ≈5 g) was shaken with 25 cm^3 1M $CaCl_2$ for 24 hours, and centrifuged for 20 minutes at 2500 rpm. Total soil metal contents were estimated by HNO_3 digestion. Analysis of Cd and Zn was by F/GF-AAS.

3. Results and Discussion

Figure 1 shows that the relative lability (% of total) of both metals had decreased considerably after 296 days contact; a pronounced influence of pH was evident. Variation due to factors other than soil pH (texture, mineralogy) were minor by comparison.

Apparent metal fixation may occur through several mechanisms, such as co-precipitation, occlusion or solid-phase diffusion which are likely to show some degree of time dependence. Thus, for example, solid phase diffusion and fixation of Cd on Goethite has been shown to increase with time (Bruemmer et al 1988). Figure 1 indicates that liming to immobilize potentially toxic metals not only increases metal sorption but has the added advantage of progressively reducing the chemical reactivity of adsorbed metal.

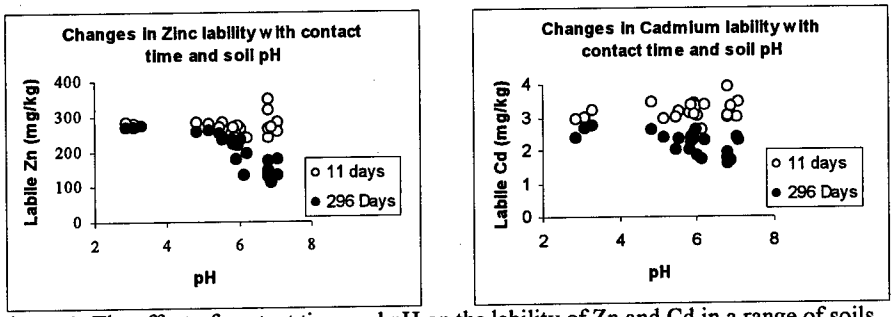

Figure 1. The effect of contact time and pH on the lability of Zn and Cd in a range of soils.

Figure 2 shows the relationship between radio-labile Cd and 1M $CaCl_2$ measurements for soils which have been contaminated by the disposal of Pb/Zn minespoil (1-2 centuries) or sewage sludge (up to 1 century). Soils used for this analysis had total Cd values ranging from <1 to >600 mg kg^{-1}. Results show that the use of 1M $CaCl_2$ gives an accurate estimate of labile Cd in a wide range of soil types, metal contents and contact times.

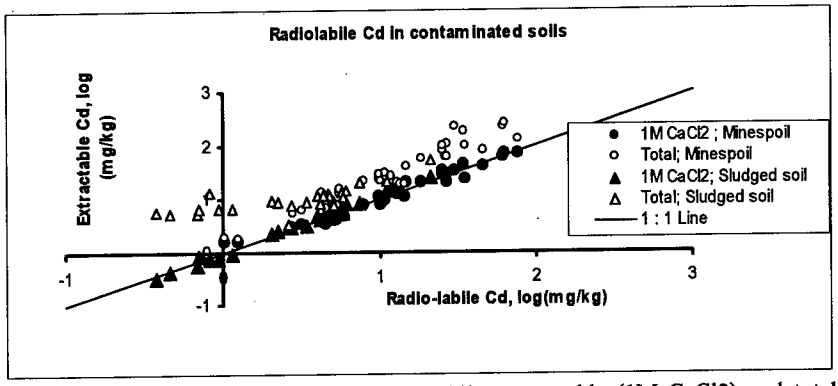

Figure 2. Comparison of soil Cd pools: radiolabile, extractable (1M CaCl2) and total (HNO_3 digest). Soils were contaminated with minespoil (100-200 y) or sewage sludge (up to 100 y).

4. Conclusions

Initial results examining changes in Cd lability have shown that the transfer of metal from labile to non-labile forms is affected by time and soil pH. The use of 1M $CaCl_2$ extraction is a viable alternative to the isotopic dilution technique for estimating labile Cd but cannot be applied to Zn because of relatively weak chloro-complexation.

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DETERMINATION OF DISSOLVED INORGANIC SELENIUM SPECIATION IN WATERS BY ION CHROMATOGRAPHY-HYDRIDE GENERATION-ATOMIC FLUORESCENCE SPECTROMETRY (IC-HG-AFS)

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1. Introduction

Speciation is an important factor affecting selenium toxicity and biogeochemistry in aquatic ecosystems. It is traditionally determined by an indirect operationally-defined approach (1), in which all Se species are successively converted chemically to selenite [Se(IV)], and then determined by atomic spectrometry after hydride generation (HG). This way, Se(IV) can be measured directly, whereas selenate [Se(VI)] and organic Se compounds [Se-org] are calculated differentially from the operationally-defined Se fraction "total inorganic Se" (TISe), which is supposedly the sum of Se(IV) and Se(VI), and the total Se content (TSe). This technique (referred to as the "Cutter-method" in the following) is very sensitive and works well in comparably clean waters (notably in seawater), but encounters problems in complex aqueous matrices (such as soil leachates or waste waters). In those kinds of samples, matrix components can interfere with the reduction of Se(VI) to Se(IV), yielding low (or even negative) results for Se(VI) and, consequently, artificially high concentrations of Se-org. Apart from this, the method cannot identify any other Se species than Se(IV), Se(VI) and Se-org, so that completely wrong speciation patterns are obtained (and remain undetected) when other Se species are present. One example of a Se species that cannot be analyzed by the "Cutter-method" is selenocyanate [SeCN], which represents a major fraction of the total Se in some refinery and mining waste waters (2). To overcome these shortcomings, Se speciation has to be determined by direct hyphenated techniques, coupling chromatographic separation to highly sensitive and specific detection (3).

2. Method

The developed technique consists of an ion chromatographic separation with detection by on-line hydride generation atomic fluorescence spectrometry. The three selenium anions selenite, selenate and selenocyanate are separated on a low-capacity anion-exchange column within 15 min, using perchlorate as the eluent anion. Se(VI) is then reduced on-line to Se(IV) by reaction with HBr at 100 °C. The temperature of the reaction mixture is lowered in a cooling loop to reduce water vapor evasion in the following HG step. Se(IV) is then converted to SeH_2 by reaction with KBH_4 in HBr medium. Water vapor is removed from the gas stream by a semi-permeable dryer membrane, before Se is determined by atomic fluorescence at 196.0 nm in a hydrogen diffusion flame.

3. Results and Discussion

Under the chosen chromatographic conditions, temporal resolution between Se(IV)/Se(VI) and Se(VI)/SeCN is on the order of 3 min (fig. 2). This allows for accurate quantification of small amounts of one species in the presence of much larger amounts of another one. Detection limits, as determined by the USEPA approach (based on the reproducibility of low-level spikes), are on the order of 0.1 µg/L for Se(IV) and SeCN, and around 0.2 µg/L for Se(VI). Instrumental detection limits (based on the signal/noise ratios) are approximately 0.01 µg/L for all three species. The higher detection limit for Se(VI) is due to worse reproducibility, likely caused by temperature variations during the prereduction step. These detection limits are among the lowest ever reported for direct selenium speciation. The method's dynamic range extends from the detection limits up to concentrations of several hundred µg/L Se per species, or four to five orders of magnitude, which demonstrates the unique sensitivity and linearity of AFS detection.

The developed method has been applied to a wide variety of aqueous samples, including the tailings pond of a zinc/lead mine, which receives the waste water from the two metal extraction processes (fig. 3). This example clearly demonstrates the superior performance of IC-HG-AFS vs. the "Cutter-method" for Se speciation in complex aqueous matrices. The IC-HG-AFS analysis yielded 11.5 ± 1.2 µg/L Se(VI) and 20.9 ± 1.8 µg/L SeCN, with no detectable Se(IV). The sum of these two species agrees well with the TSe

of 31.2 µg/L determined by the "Cutter-method". That method also found 3.5 µg/L Se(IV), which is below the detection limit of IC-HG-AFS analysis with the chosen sample dilution. However, the "Cutter-method" determined a "negative" Se(VI) concentration, because TISe was lower than Se(IV) due to matrix interferences. This is not only nonsense, but it also implies that 90 % of the Se in the sample is Se-org, while there's no indication for the presence of SeCN. IC-HG-AFS demonstrates the absence of organic Se compounds, which would elute with the dead volume after ca. 2 min, and also confirms the presence of significant amounts of Se(VI). The presence of SeCN in this sample probably results from the use of cyanide in the metal extraction process, and has major implications for ecotoxicology and remediation.

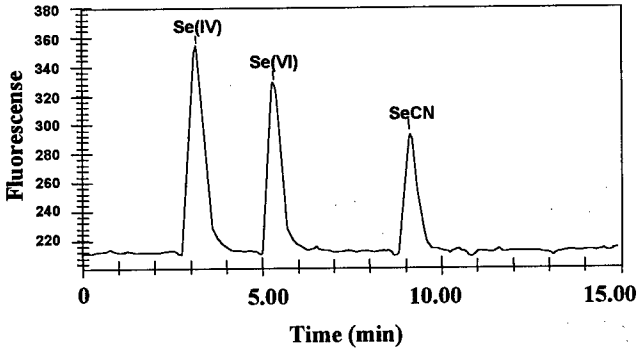

Figure 1: Separation of selenite, selenate and selenocyanate by IC-HG-AFS; c(Se) = 1 µg/L per species

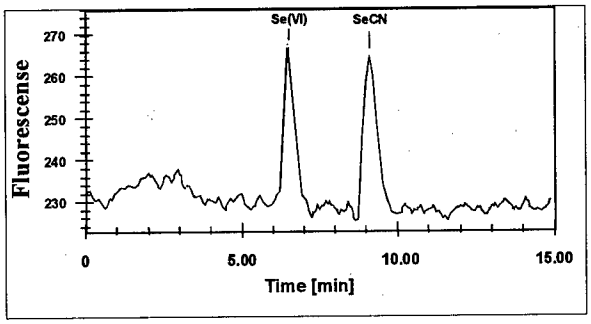

Figure 2: Dissolved inorganic selenium speciation in a discharge pond for waste water from a Zn/Pb-mine

4. Conclusions

The developed method permits direct, robust and sensitive determination of Se(VI) in complex aqueous matrices. It also enabled us to detect significant amounts of selenocyanate in industrial waste waters.

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SEQUENTIAL FRACTIONATION OF TRACE ELEMENTS IN POLLUTED AND NON-POLLUTED SOILS.

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1. Introduction

The total soil concentration of metals gives little information on the behaviour of the trace elements in the soil and consequently about their environmental impact. It is more useful to determine the chemical form of the metal, its mobility, bioavailability and consequently the degree of risk. Numerous sequential extraction schemes are described in existing literature (i. e. Ritchie and Sposito, 1995, Tessier et al.,1979). Recently the Community Bureau of Reference (BCR, Ure et al., 1993) proposed a three steps sequential chemical extraction, in which results four species: 1) HOAc soluble metal, 2) Fe/Mn oxides bound metal, 3) Organically bound metal and 4) Residual. Information on the speciation of trace elements in mediterranean soils is relatively scarce. The aim of this work is apply the BCR-speciation method to polluted (PP) and apparently non-polluted reference pedons (RP) of the Viladecans community (Catalonia, Spain).

2. Materials and Methods

The pedons of Viladecans were described, sampled and classified during the soil mapping by Bech et al. (1989-91) . Clay, organic matter content, carbonate, pH etc. were determined using standard methods. Total contents of the trace elements (Sr, Ba, Ce, V, Ni, Cu, Zn and Pb) were determined with X-Ray Fluorescence. The sequential fractionation was determined with the BCR procedure. The extractants were: (1) acetic acid (0.11 M); (2), hydroxylammonium chloride (0.1M, pH 2); (3), hydrogen peroxide (8.8 M) oxidation followed by extraction with ammonium acetate (1M pH 2). The Residual fraction (4) is derived by subtracting these 3 fractions from the total (XRF). Analysis of the extracts was carried out by an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES, a Thermo Jarrell Ash Polyscan 61E).

3. Results and Discussion

Both soils are *Xeralfs* and the conventional properties of both pedons, the polluted and reference, are similar, with the exception of the organic matter and carbonate contents of the surface horizon. The results show that the total concentrations of trace elements of the reference soil are within the normal range of concentrations reported for uncontaminated soils, while the concentrations of Cu, Zn and Pb of the surface horizon of the polluted soil are clearly larger.

4. Conclusions

In the surface horizon the available species are larger than in deeper horizons of this pedon and the whole reference pedon. This fact confirms the anthropogenic pollution, which in this case is a short or medium-term environmental hazard.

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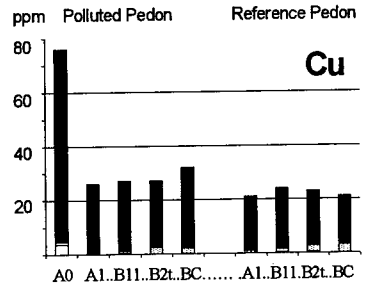
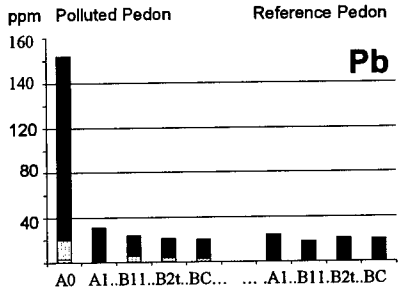

ppm
Polluted Pedon
Reference Pedon
Cu
80
60
40
20
A0 A1..B11..B2t..BC..... .A1..B11.B2t..BC


ppm
Polluted Pedon
Reference Pedon
Pb
160
140
120
80
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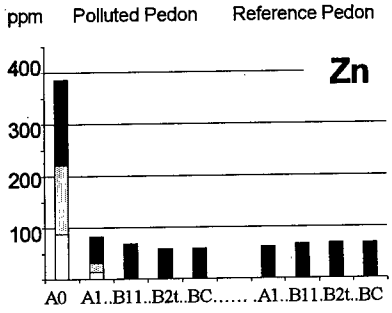
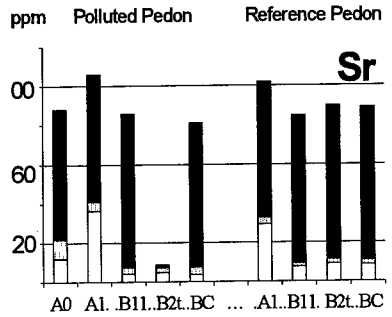
A0 A1..B11..B2t..BC... ... .A1..B11.B2t..BC


ppm
Polluted Pedon
Reference Pedon
Zn
400
300
200
100
A0 A1..B11..B2t..BC..... .A1..B11.B2t..BC


ppm
Polluted Pedon
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Sr
00
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20
A0 A1. .B11..B2t..BC ... .A1..B11. B2t..BC

METAL SULFIDE SPECIATION IN A SEDIMENT FROM THE INFLUENCE OF PH AND OXIDATION KINETICS ON SOLUBILITY

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1. Introduction

A method is presented to determine the speciation of metal sulfides in sediments. The method is based on measuring the solubility upon the titration of the sediment with acid followed by an extraction with EDTA of the liberated and readsorbed or repreciptated metals .
The results from this procedure are compared with speciation calculations (CHESS, Vanderlee, 1993) and are verified by the analysis of results from the oxidation kinetics of the sediment.

2. Experimental

Sludge from the canal Gent-Terneuzen (Belgium) was studied as a transition metal polluted sediment. All experiments were performed in a glove box to avoid the interference of oxidation.
- First the solubility of the heavy metals was examined as a function of pH. The sediment was first suspended in a background solution, which mimicked the concentration of the water from the Canal Gent-Terneuzen. Different samples were slowly titrated to different pH values and were allowed to equilibrate overnight. The supernatant was withdrawn after centrifugation and was analysed for metal ions and pH. The centrifugate was further extracted with a solution of 0.7 M NaAc – 0.14 M EDTA at pH 4.65 to reach a final concentration of 0.5 NaAc and 0.1 M EDTA. The EDTA concentration was sufficient to complex all Fe, Zn and Mn which were present in fairly large amounts in the sediment. After overnight equilibration and centrifugation the supernatant was again analysed for metal ions and pH.
In a second series of experiments the same procedure was repeated, but the initial solution was spiked with ^{109} Cd and ^{65} Zn . cadmium(Cd) and zinc(Zn) were analysed radiochemically.
All these experiments were conducted in a glove box to avoid oxidation.

- The oxidation kinetics of the sediment was performed on 20 g batches of wet sludge in 1 liter of a solution of 0.02 mol/l $NaNO_3$ and 0.02 mol/l EDTA. The presence of EDTA is necessary to avoid the readsorption or reprecipitation of the metals released upon oxidation of the sulfides. The pH was automatically held at pH 8.5. These experiments were studied outside the glove box using compressed air bubbling for oxidation. A blank test was run inside the glove box to check for the influence of time on the dissolution of metals by EDTA. Samples were taken at regular time interval and were analysed by AAS.

The following metals were monitored: K, Ca, Mg, Fe, Mn, Cu, Zn, Cd, PB, Co.

3. Results and Discussion

Solubility as a function of pH

The dissolution edge for Fe starts around pH 6. The experimental dissolution edge lies in between the edges expected for FeS and $FeCO_3$. Apart for Fe in clay minerals (released at lower pH values) the dissolution around pH 5 to 6 is taken to be due to the presence of a mixture of both FeS and $FeCO_3$.

The dissolution edge for cadmium , copper, lead and zinc only starts below pH 2 in agreement with the low solubility product of the metal sulfides. However the EDTA extracts revealed that, depending on the metal ion, part of the metal sulfide was released into solution and readsorbed or reprecipitated at higher pH values than the expected dissolution edge based on the pure metal sulfide form. The enhanced dissolution also appeared to coincide with the dissolution edge for FeS / $FeCO_3$. It was therefore concluded that part of some metal sulfides must have been present as a mixed precipitate with FeS.

The speciation as determined from the combination of the solubility versus pH curve and the EDTA extraction curve was as follows: Cd (100% CdS); Zn (65% ZnS, 35 % FeZnS); Pb (75% PbS, 25% FePbS); Cu (99% CuS, 1% FeCuS and a persistant but measurable 0.1 % Cu humic substance species).

The distributions measured with radiolabelled Cd and Zn were identical with previous distributions. Freshly added radiolabelled metals were therefore imediately (overnight) in isotopic equilibrium with the other metal phases.

Oxidation kinetics

Theoretical calculations (CHESS) revealed that FeS and $FeCO_3$, MnS, CoS are expected to dissolve in the EDTA solution at pH 8.5. The dissolution of CdS, CuS and ZnS is however expected to be below 1% of the total metal content.

For all metal ions the amount extracted in presence of oxygen increased with time. The results indicated a different oxydation kinetics for the various metal ions. The oxidation of Zn, Pb and Cu continued after all FeS was oxidized.

These curves were corrected for the amount dissolved in absence of air. The resulting curves were analysed as a sum of first order oxydation reactions on the pure metal sulfide form and the mixed iron/metal form. The obtained percentages assigned to the mixed Fe/metal ion form only qualitatively confirmed the previously obtained speciation results.

4. References

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FLUORIDE AND ALUMINIUM SPECIATION IN THE SOIL LIQUID PHASE IN THE VICINITY OF AN ALUMINIUM SMELTER IN NW SPAIN

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1. Introduction

The present paper aims to study fluoride contents in the soil liquid phase in an area surrounding an aluminium smelter in Galicia, NW Spain. Moreover the influence of fluoride on aluminium speciation in soil solution is studied. Fluoride contamination is typical from aluminium smelters, coming from the use of fluorine compounds, namely cryolite (Na_3AlF_6), in the electrolytic process.

2. Materials and Methods

The studied area surrounds the "Alúmina- Aluminio" factory in San Ciprián, in the Northern coast of Galicia, NW Spain. The soil, developed on granite, is mainly used for forest plantation, especially eucalyptus.

Two soil depths (0-10 and 10-20 cm) were sampled within a radius of 3.5 km around the factory, intensifying the sampling in the direction of prevailing winds, to a total of 35 samples. Control samples, far away from the factory, were taken for comparison.

To approximate the soil solution, 1:10 aqueous extracts were prepared (Alvarez and Monterroso, 1992). The extracts were analysed for: pH, electrical conductivity, sulphate, by turbidimetry (Bardseley and Lancaster, 1960), Ca and Mg, by flame atomic absorption spectrophotometry, Na and K, by flame atomic emission spectrophotometry, dissolved organic carbon (DOC), by wet oxidation by $KMnO_4$. Free and total dissolved fluorine were determined by using a selective electrode, with addition of reagent TISAB IV (Orion, 1976) for total fluorine and reagent ISA for free fluorine. According to Driscoll (1984), dissolved aluminium was speciated into total, reactive, acid-soluble, labile and non-labile aluminium. Labile Al was speciated by using the computer program SOLMINEQ (Kharaka et al., 1989).

The soil solid phase was characterised and total and available fluorine determined in a previous paper (Gago et al., 1997). An inverse relationship was found between available fluorine and distance to the factory.

3. Results and Discussion

Table 1 summarises the results of the aqueous extracts analyses. The pH, electrical conductivity, DOC and sulphate concentration values were normal for Galician natural soils (Calvo et al., 1987).

Total fluorine concentrations tended to decrease as the distance to the factory increased. The free fluorine concentration and the ratio free/ total fluorine increased with pH, this ratio approaching 1 in samples having relatively high pH.

The more acid surface samples had higher aluminium concentrations. A high portion of total Al (often more than 50%) was acid-soluble aluminium, i.e. colloidal polymeric and/or organically complexed aluminium (Driscoll, 1984). Non-labile aluminium (organic monomeric aluminium) amounted on average 40 percent of total aluminium and 70 percent of reactive aluminium. Labile aluminium (inorganic monomeric aluminium) was a tiny fraction in samples having pH>5 but the prevailing aluminium fraction in some more acid samples.

Table 1. Analytical data of aqueous extracts

	0-10 cm depth			10-20 cm depth		
	min	max	average	min	max	average
pH	4.50	7.85	5.46	4.89	7.12	5.60
CE (dS m^{-1})	0.041	0.318	0.079	0.046	0.208	0.077
DOC (meq L^{-1})	0.57	6.54	2.91	0.54	5.83	2.39
SULPHATE (mg L^{-1})	3.78	13.40	7.67	4.10	10.49	6.65
TOTAL FLUORINE (mg L^{-1})	0.18	1.19	0.54	0.23	0.80	0.43
FREE FLUORINE (mg L^{-1})	0.09	0.83	0.30	0.10	0.38	0.21
TOTAL ALUMINIUM (mg L^{-1})	0.04	3.24	1.13	0.22	1.82	0.87
NON-LABILE ALUMINIUM (mg L^{-1})	0.03	1.05	0.41	0.02	0.79	0.32

Most labile aluminium, nearly 100% in most samples, consisted of Al-F monomers. The lowest Al-F monomer concentrations appeared in extracts having low labile aluminium concentrations, irrespective of fluorine concentration. Aluminium was in most cases the limiting factor in the formation of Al-F complexes, so that free fluorine concentration was usually high. As pH increased, labile aluminium and Al-F concentrations decreased, and free fluorine increased. The prevailing Al-F complex depended on the ratio labile aluminium/total fluorine: AlF_2^+ and AlF_3 prevailed at low ratios, and AlF^{2+} at low ratios. The speciation of labile aluminium in soils near the aluminium smelter differed from that in control samples; in this case fluorine is the limiting factor in the formation of Al-F complexes, which are present at lower concentrations, while Al^{3+} or hydroxyaluminium complexes prevail.

Free aluminium, Al^{3+}, appeared in a few samples. Usually the free aluminium concentration was very low, as a result of aluminium binding to fluoride and, to a lesser extent, to OH^-. The high fluorine contents decrease the aluminium toxicity (Al^{3+} is the most toxic species) in comparison to control samples. On the other hand, aluminium in solution is not enough to bind all fluorine, so that much of it remains free.

4. Conclusions

Opposite to control samples, most labile Al is bound to fluoride. Fluoride decreases Al toxicity, but most fluoride remains free since there is not enough Al to bind it.

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THE OXIDATIVE BEHAVIOR OF MN OXIDES IN HIGH-MN SOILS

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1. Introduction

Soil manganese oxides are important reactive sites that control the behavior of many metals and organics in soil. These sites appear to be sensitive to both environmental change and sample handling. We used x-ray absorption near edge structure (XANES) spectroscopy and wet chemical methods to study the effect of sample drying and to examine the oxidation of added Mn(II).

2. Materials and Methods

The soils were collected from Vermont's Champlain Valley in areas of high-lime bedrock or glacial till (Eutrochrepts in the U.S. system). Total reducible Mn content ranged from 0.05 to 1.75%. XANES spectra were obtained at the National Synchrotron Light Source (NSLS), using the microprobe at beamline X26A. The effects of the x-ray beam and the effects of sample drying were examined. Oxidation of added Mn(II) was observed on separate subsamples treated at various times before examination and on the same sample over a 40 hour period. The oxidation capacity was estimated using the Chromium Net Oxidation Test (amount of Cr(III) oxidized in 15 min). The effects of sample drying and storage were examined over a 1 year period.

3. Results and Discussion

Air drying caused a considerable decrease in the oxidative capacity of all soils. This decrease continued over time and was matched by an increase in extractable Mn(II) (Figure 1). After one year, the drying effect was detected with XANES spectroscopy. Recovery of oxidative capacity occurred if samples were remoistened in the first 100 hours of drying. We did not find any pretreatment of long-term dried samples that caused recovery of oxidative capacity. By contrast, XANES spectroscopy of moist soils showed x-ray induced reduction. Recently air-dried samples were usually slightly more oxidized than their moist counterpart. Overall, XANES spectra suggested that soil oxides are similar in structure to layer oxides such as birnessite but may have a higher ratio of Mn(IV)/Mn(III). The oxidation of added Mn(II) occurred even after exposure to the x-ray beam, suggesting a nonmicrobial mechanism (Figure 2). The increase in oxidative capacity as a result of the Mn(II) additions was not reflected in the XANES spectra.

4. Conclusions

Soil manganese oxides are more sensitive to environmental conditions than known Mn oxide minerals. Soil drying causes reduction or disproportionation at the oxide surface. X-ray absorption spectroscopy causes reduction. Both these effects may be reversible. Changes in the oxidation behavior at the oxide surface may not be detected by XANES spectroscopy. Oxidation of added Mn(II) can be observed both by the Cr oxidation test and by XANES spectroscopy.

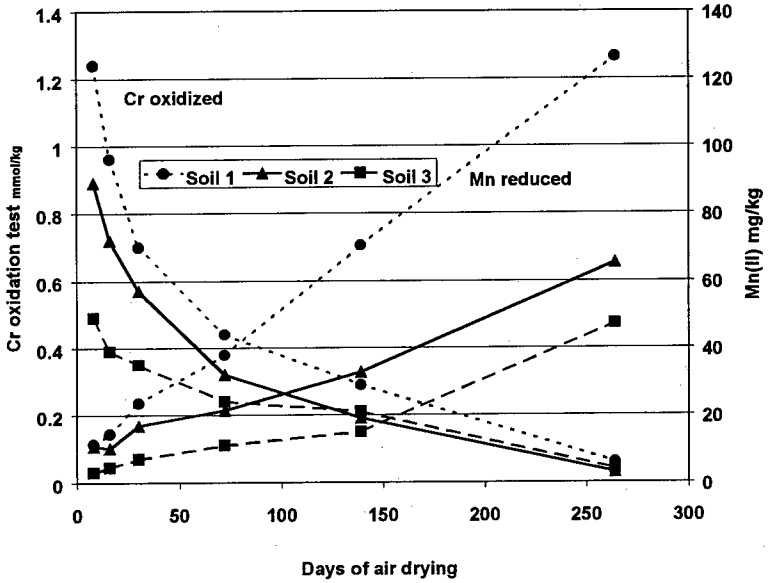

Figure 1. Effect of air drying on the oxidative capacity of three high-Mn soils.

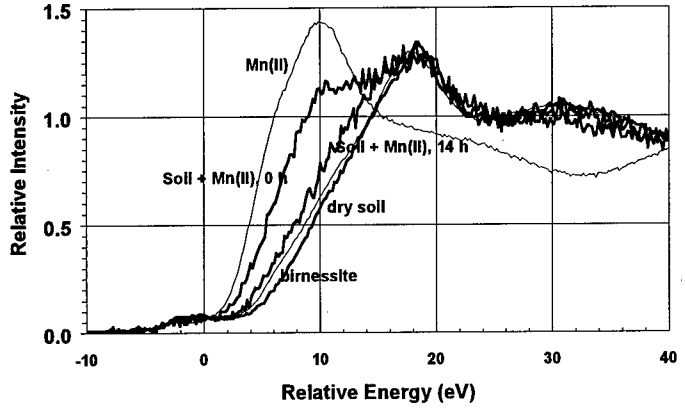

Figure 2. Oxidation of added Mn(II) shown in repeated XANES spectra of a high-Mn soil.

ASSESSMENT FOR CLEAN-UP PROCESSES OF POLLUTED SOIL BY SEQUENTIAL EXTRACTION AND XAFS

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1. Introduction

Aim of heavy metal remediation techniques from soil is the complete depletion of the metals from this mobile fractions. In this paper the effect of acetic acid leaching on the heavy metal distribution during remediation processes has been studied. Sequential extraction techniques are frequently used to access the toxicological potential of contaminated soils by description of the distribution of metal contaminants in operational fractions. To investigate what happens during the remediation process sequential extraction can be used as well.

2. Materials and Methods

A highly lead contaminated soil (25.000 mg/kg) from a former battery factory was used for this investigation. The decontamination was conducted by leaching with acetic acid solution and stopped before the decontamination was completed, getting a remaining concentration of 10.000 mg/kg. Then the treated soil was assessed by sequential extraction as well as by XAFS-spectroscopy (X-ray Absorption Fine Structure). The Sequential extraction consists of a seven step extraction procedure according to Zeien and Brümmer 1989. The fractions 1 to 4 are defined as the mobil fractions. The real existing binding form can be investigated with XAFS.

3. Results and Discussion

The results of the XAFS are shown in Table 1, compared with sequential extraction and with a geochemical modelled program (PHREEQC).

Table 1: Results from XAFS measurement

Untreated soil	Treated soil
32,3 % $PbSO_4$ anglesite	41,5 % $PbSO_4$ anglesite
21,4 %$PbCO_3$ cerusite	5,9 % $PbCO_3$ cerusite
33,9 % Pb humic substance 1	52,6 % Pb montmorrillonite
12,4 % Pb humic sustance 2	

The results obtained by XAFS show that the dominating binding forms in the soil before the decontamination are anglesite, cerusite and Pb associated to humic acids. During the remediation cerusite has been dissolved immediately and the humic acid associated fraction is shift to a fraction associated with clay. The anglesite fraction is reduced in according to the solubility product. The sequential extraction shows a different behaviour. The mobile fraction increases while the other fractions decrease (see Figure 1).

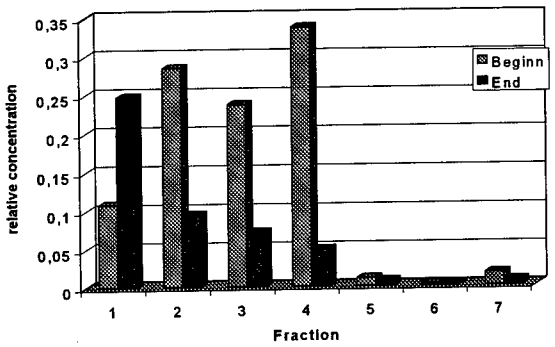

Figure 1: Results from sequential extraction

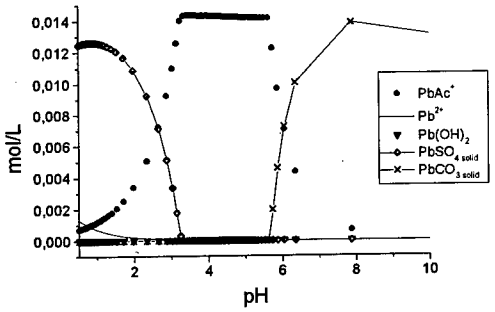

Figure 2: Pb-species with 1M acetate

Those differences can be understood using geochemical modelling. Figure 2 shows the dissolution of anglesite at high acetate concentrations used in the second step of the sequential extraction. This results has been verified by batch experiments with pure $PbSO_4$.

4. Conclusions

Dependent on the real binding form of heavy metal in the soil the operational fractions received from sequential extraction can give us a wrong answer. Specially in the presence of lead which build relatively strong complexes with acetate a shift from immobile to mobile operational fractions appear. This shift is just lead back to the high acetate concentration in the extraction solution. Using acetic acid could never reduce the lead concentration in the soil in the same range because of the small solubility of anglesite. Even for remediation procedure assessment sequential extractions cannot be used without verifying the results with other methodes or calculations.

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SENSITIVE DETERMINATION OF THREE ARSENIC SPECIES IN WATER BY ION EXCLUSION CHROMATOGRAPHY-HYDRIDE GENERAION-INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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1. Introduction

Arsenic in water occurs mainly in inorganic forms, such as arsenate (As^{V}) and arsenite (As^{III}), but also occurs in methylated forms, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), at very low concentration levels. [1)] Various methods for speciation of arsenic were developed so far [2)], and a hyphenated method of liquid chromatography – inductively coupled plasma mass spectrometry (LC-ICP-MS) has become popular recently. However, these methods have not been successful for the direct determination of MMA and DMA in water because of their low concentrations. In the present study, ion exclusion LC was examined for the first time for the separation of arsenic species. As a result, three species of As^{III}, As^{V} and MMA were separated by using diluted acid solution, although DMA could not be eluted. The detection limits obtained with the present method were approximately better than those reported before.

2. Materials and Methods

Stock solution of AsV at 1000 mg As l-1 was prepared by dissolving sodium arsenate dibasic heptahydrate (Na2HAsO4•7H2O) in water. Stock solution of AsIII at 1000 mg As l-1 was purchased from Wako, Japan. Stock solution of MMA was prepared by dissolving monomethylarsonic acid, disodium salt (CH3AsO(ONa)2•6H2O) in water. Stock solution of DMA was prepared by dissolving dimethylarsinic acid ((CH3)2AsO(OH)) in water. A 1% sodium borohydride solution was prepared by dissolving high-purity grade NaBH4 in a 0.1 mol l-1 NaOH solution just before use. Sodium hydroxide was purchased from Merck (analytical reagent grade) . Trifluoroacetic acid was purchased from Wako. LC-ICP-MS with hydride generation was used. An LC-6A liquid chromatograph (Shimadzu, Kyoto, Japan), equipped with a Shimadzu LC-6A pump and a sample injection valve (9725i, Rheodyne, Cotati, CA, USA) with an injection volume of 50 µl or 200 µl, was used. The ion exclusion column was a sulfonated polystylene type, Shim-pack SCR-102H (Shimadzu). The mobile phase was a diluted trifluoroacetic acid adjusted at pH 2.1 and the flow rate was 1.5 ml min^{-1}. The eluate from the liquid chromatograph was first mixed with 1.5 mol l^{-1} HNO_3 and then mixed with 1% $NaBH_4$ solution to generate the hydrides. The mixture was transferred to a laboratory-made gas-liquid separator through a polytetrafluoroethylene tube (50 cm long, 3 mm i.d.). An ICPM-8500 inductively coupled plasma mass spectrometer (Shimadzu), equipped with a miniaturized torch, was used.

3. Results and Discussion

The pK_1 values are 2.24, 2.6, 6.3 and 9.08 for As^V, MMA, DMA, and As^{III}, respectively and it is said that the protonation occurs for DMA at pH 3.85. Therefore, at pH 2.1, As^V exists as an anionic form, MMA and As^{III} exist as neutral forms, and DMA exists as a cationic form, respectively. A chromatogram of three species is shown in Fig.1. This retention behavior of As^V, As^{III}, and MMA is unique compared with those reported so far, in which the elution of As^{III} was always earlier than As^V. Attempts to separate MMA and DMA by using higher pH eluent were not successful. Six-point calibration graphs for three arsenic species were linear on plotting peak areas against the concentration of arsenic for each species in the range 0 - 10 ng ml^{-1}. The detection limits, defined as three times the standard deviation of the peak areas for 7 replicates of the blank were 0.5 pg/ml(As^{III}), 1.1 pg/ml(As^V) and 0.5 pg/ml(MMA), respectively .These values are better than those reported so far. To validate the present method, three riverine reference water samples (JAC0031, SLRS-1 and SLRS-3), an estuarine reference water sample (SLEW-2), a reference seawater sample (CASS-3) and a tap water sample were analyzed. Analytical results are shown in Table.1 along with their certified values. As^{III} was not detected in all the samples because it was oxidized to As^V with HNO_3 added for the storage of the samples. The sum of the concentrations of As^V and MMA for CASS-3 and SLEW-2 showed good agreement with their certified values. On the contrary, those for the riverine waters samples were out of the range of the certified values. Particularly, the deviations from the certified values were large for SLRS-1 and SLRS-3. The reason is not clear at this moment, but one possible reason might be the existence of other species such as DMA which could not be detected with the present method. The main disadvantage of the present method is the failure to elute DMA. This is probably because DMA is retained in the column due to the hydrophobic adsorption on polystylene resin. The use of a more hydrophilic resin will help to decrease this effect.

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Toxicity
(Technical Session 19)

EVALUATION OF CATION TOXICITIES TO SUNFLOWER; A STUDY RELATED TO THE ASSESSMENT OF ENVIRONMENTAL HAZARDS

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1. Introduction

Pharmacology and ecotoxicology studies have successfully applied principles of quantitative structure-activity relationships (QSARs) to the screening and prediction of toxicity and biological activity of organic chemicals for some time (DEARDEN, 1990). QSAR predictions for inorganic toxicity (especially for the toxicity of metal ions), however, are less developed. Therefore, as predictive toxicology has gained general recognition as an important tool in environmental toxicology, the study of metal ions in plants seemed very promising for a research project on the possibility to develop a QSAR system. We present here a number of significant models relating toxicological activity measured in our laboratory to some parameters of the metal ions.

2. Materials and Methods

Biological data: toxicity data for 10 divalent metal ions (applied as nitrates, Analar grade with the exception of zinc nitrate - reagent grade) were obtained by germinating decoated sunflower seeds (*Helianthus annuus* "Sunspot") in different concentrations of metal salt dissolved in distilled water (seeds germinated at 25°C in darkness). The concentration giving 50% inhibition of radicle growth one day after emergence was determined for each metal ion from the graphical interpolation of the data points of the corresponding dose-response curves (molar). The log of the inverse of this concentration was calculated as the toxic endpoint.
Physicochemical characteristics: a large array of physicochemical characteristics of metal ions was gathered from a variety of sources. The categories of parameter included in the analysis were: fundamental ionic properties, surrogate metal ion characteristics described to reflect metal-ligand binding tendencies, thermodynamic considerations, and equilibrium constants of metal ions with a variety of inorganic and organic ligands of biological significance.
Statistics: quantitative structure-toxicity relationships were examined using simple regression analysis using the Minitab software.

3. Results and Discussion

The range of observed toxicities covers approximately 1.5 orders of magnitude with magnesium and cadmium at the two extremes (Table 1). Several equations were statistically significant but the highest predictive models were obtained with the heat of formation of inorganic oxides (ΔH_o, WEAST, 1978), the chemical softness parameter (σ_P, PEARSON and MAWBY, 1967) and the heat of formation of aqueous ion ($\Delta E_{aq\ ion}$ at 298.1 K, Brewer et al., 1950) (Table 2). ΔH_o reflects metal-oxygen interactions which are important because metals associate with oxygen, nitrogen or sulphur atoms located in functional groups present in biologically active molecules. The softness parameter σ_P is a quantitative expression of the HSAB (Hard and Soft Acids and Bases) concept which explains trends in the chemical behaviour of metal ions in biological systems. More specifically σ_P reflects binding

preferences of metal ions to ligands. Better correlations and the production of statistically robust QSARs are expected with two-parameters models.

The present study is only part of a complex analysis of the toxicity of metal ions in different plant systems in relation to a large number of parameters known to play a role in metal ion toxicity modelling.

4. Conclusions

Correlations reported earlier (ENACHE et al., 1997) based on published toxicity data have been improved and extended here in an analysis based on toxicity data for 10 metal ions obtained under uniform laboratory conditions and using salts of high purity. These results confirm the possibility of obtaining high correlations of metal ion toxicity with physicochemical parameters of the ions.

Table 1: Critical concentrations (EC_{50}) of divalent metal ions to sunflower "Sunspot"

Metal ion	EC_{50} (Molar)	$\log \frac{1}{EC_{50}}$
Cd	0.0045	2.34
Cu	0.0072	2.14
Ni	0.0085	2.07
Pb	0.0122	1.91
Co	0.0172	1.76
Zn	0.0183	1.74
Mn	0.0242	1.62
Ba	0.0722	1.14
Ca	0.1199	0.92
Mg	0.1423	0.85

Table 2: Relevant results with a coefficient of determination greater than or equal to 0.83 of linear regression analysis of metal ion toxicity in sunflower and physicochemical ion characteristics

$-(\log EC_{50}) = f(x)$	r^2 adj.	s	p	F
$2.67 + 0.000012\ \Delta H_o$	0.87	0.19	0.000	61.54
$3.59 - 14.4\ \sigma_p$	0.83	0.21	0.000	45.70
$2.07 + 0.00876\ \Delta E_{aq\ ion}$	0.83	0.21	0.000	45.54

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CORRELATION OF FREE METAL ION ACTIVITIES WITH TOXICITY RESPONSE

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1. Introduction

Bioavailability is affected by the physico-chemical form of a heavy metal in the environment from which it is uptaken. For soils, the soil solution is an important physical phase from which uptake into organisms occurs. Aqueous complexation plays a vital role in determining the bioavailability of the heavy metals present in solution. Several studies have shown that the thermodynamic activity of the free (hydrated) metal ion is a good indicator of the bioavailability of that metal in solution (Campbell, 1995). This study systematically examines the relationship between free ion activity of the heavy metals, Pb, Cu and Zn in an artificial soil solution and the toxicological response induced in *lux* marked *Escherichia coli*, an organism sensitive to heavy metal toxicity (Paton *et al.*, 1997).

2. Materials and Methods

Solution composition: The composition of the artificial soil solution used in the experiments was simplified from the recipe given by Quist (1995). Test solutions containing the artificial soil solution and known heavy metal concentration (seven concentrations between 1 and 10 mg l^{-1} of Pb, Zn or Cu) were prepared and ionic strength adjusted by the addition of K_2SO_4 giving 0, 0.003, 0.006 and 0.01 M K_2SO_4 representing the range of ionic concentrations found in the soil solution. pH was recorded and bioassays performed over the range of concentrations for each metal at each ionic strength.

Bioassay: The test organism used was *Escherichia coli* HB101 (pUCD607) which had been genetically modified to contain the plasmid pUCD607 encoding the *lux* CDABE genes from *Vibrio fischeri*. The bioluminescent response of this bioassay is independent of pH between pH 4 and 8.4. The bioassay was prepared and stored as freeze-dried cells which were resucitated as required. The assay procedure involves the comparison of light output of cells in a control solution (artificial soil solution at each ionic strength) to those in increasing concentration of the three heavy metals. Triplicate 900 µl of solution sample were aliquoted into luminometer cuvettes and then 100 µl of cell suspension in 0.1 M KNO_3 was added (Paton *et al.*, 1997). Bioluminescence was then recorded after 15 minutes contact time in a portable luminometer. Four replicate vials were used to determine bioluminescence for each test solution.

Geochemical modelling: For each heavy metal concentration at each ionic strength the activity of the free heavy metal ion was calculated using GEOCHEM-PC (Parker *et al.*, 1995b) at the pH of the test solutions after correction for the assay matrix (0.01 M KNO_3).

EC_{50} values were calculated for each heavy metal in terms of both concentration and free ion activity from plots of bioluminescence against metal concentration or free ion activity. Regression analysis of bioluminescence on free ion activity was performed for each heavy metal by pooling the data from all ionic strengths.

3. Results and Discussion

All three heavy metals complex strongly with SO_4^{2-} which gave rise to a large variation in free metal ion activity from 90% to 50% of the total metal concentration. pH was between 5.4 and 5.7 for all test solutions. For each metal, as ionic strength increased there was an increase in the EC_{50} value expressed as total concentration. However, when EC_{50} values were expressed as free ion activity this difference was not observed.

These results give mean EC_{50} values of 0.365 mg l^{-1} (s.d.=0.0465) for Pb, 0.365 mg l^{-1} (s.d.=0.10149) for Zn and 0.297 mg l^{-1} (s.d.=0.13525) for Cu. The variation in bioluminescence response with ionic strength for total concentrations is clearly shown in Fig. 1a for Zn. Pb and Zn showed the expected trend of toxicity decreasing with increasing ionic strength although for Cu at high concentrations, this trend was not observed. It is clear from the plot of bioluminescence against free Zn^{2+} activity (Fig. 1b) that the data is tighter. The regression coefficients (r^2) for Pb, Zn and Cu were 0.84, 0.93 and 0.78 respectively.

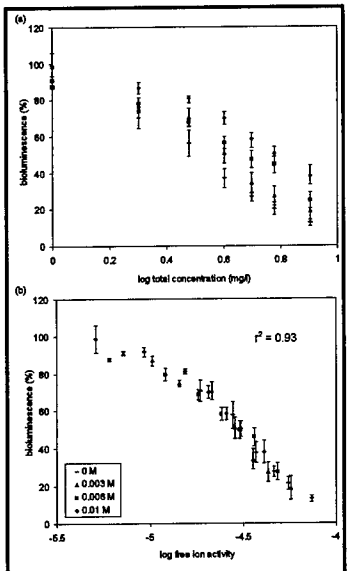

Figure 1 Plot of bioluminescence against a) total concentration and b) free ion activity of Zn.

This study has shown that, for Pb and Zn at least, expression of EC_{50} values in terms of free ion activity is independent of ionic strength. Although only one anion (SO_4^{2-}) has been investigated here, it supports the conclusions of other workers in this field (Campbell, 1995) that the free metal ion is the dominant bioavailable form. However, complexes formed by other metals with other anions may also be bioavailable. This is thought to be particularly true of Cu (Magnuson *et al.*, 1979) and is borne out by this study. Nevertheless, this approach has important implications for environmental quality standards of water which in the UK are currently expressed in total concentrations and listed separately for waters of different ionic strength, *i.e.* soft, hard and saline waters (Gardiner & Mance, 1984). If toxicity can be expressed in terms of the free ion activity, then it will be possible to state regulatory limits that are independent of water hardness or salinity.

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PHYTOTOXICITY OF SOME MICROPOLLUTANTS ON CALCAREOUS CHERNOZEM SOIL

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1. Introcduction

The purpose of this work was to evaluate the toxicity effect of some micropollutans on the soil fertility and crop growth (KÁDÁR, 1994) A long-term field trial was set up with extreme high loading rates at the Nagyhörcsök Experimental Station of our Institute on loamy calcareous chernozem soil, developed on loess (KÁDÁR, 1995).

2. Materials and Methods

The long-term field trial was set up in 1991. The site has in its plow layer 20 % clay, 3 % humus, 3-5 % $CaCO_3$. To ensure a sufficient macronutrient supply in the whole experiment, 100-100 kg/ha N, P_2O_5 and K_2O, respectively are given yearly. 13 elements were added each on 4 levels as soluble salts to plots once at iniciation and mixed into the plow layer. The 52 treatments were arranged in a split-plot design with 2 replications. The elements applied were Al as $AlCl_3$, As as $NaAsO_2$, Ba as $BaCl_2$, Cd as $CdSO_4$, Cr as K_2CrO_4, Cu as $CuSO_4$, Hg as $HgCl_2$, Mo as $(NH_4)_6Mo_7O_{24}$, Ni as $NiSO_4$, Pb as $Pb(NO_3)_2$, Se as Na_2SeO_3, Sr as $SrSO_4$, and Zn as $ZnSO_4$. Loading levels were 0, 90, 270 and 810 kg/ha.

Different crops are cultivated each year with commonly used agrotechnics, and composite soil and plant samples are collected randomly from each plot. The total amount of elements in homogenized soil and plant samples are measured after microwave digestion using cc HNO_3+H_2O_2, In the soil samples, the mobile (NH_4-acetate + EDTA soluble) fraction (LAKANEN AND ERVIÖ, 1971) is also determined by ICP-AES. Harvested plant material is partly used in animal feeding experiment at the Department of Animal Nutrition, University of Veterinary Sciences.

3. Results and Discussion

From the applied 13 elements, the following ones proved to be phytotoxic at the time of harvest, in decreasing line: for corn grain in 1991 Cr, Se, Mo; for carrot root in 1992 Cr, Se, Hg; for potato tuber in 1993 Se, Cr; for pea grain in 1994 Se, As, Cr; for beetroot in 1995 Se, Cd, Cr; for spinach leaves in 1996 Se, Cd, As; for winter wheat grain in 1997 Se, As, Cd, Cr.

At highest load (810 kg/ha), Se diminished yields and "cleared" the soil surface. Toxicity of Se-treatment has been very impressive and also increased with time. The given selenite from might have turned into more mobile and toxic selenate form in this well-aerated calcareous soil. Plants and plant parts accumulated Se intensively up to a few thousand ppm. Animal organs absorbed it also strongly, maximum concentration up to 40-60 ppm were measured in liver and kidney. Among the elements studied, Se presented the greatest capability of moving relatively unhindered through the soil-plant-animal chain/system.
Cr-toxicity lessened with time, given Cr(VI) mobile form basically has turned into less toxic Cr(III) form in the plow layer, and partly moved down into deeper layers as soil data proved

(Kádár 1995). Cr accumulated weakly in plant parts and generally showed low movement in the soil-plant system. So, only 2 % of added Cr could be extractted with NH_4-acetate + EDTA after 4 years of the experiment, while 12 % of Hg, 13 % of Mo, 22 % of As, 32 % of Se, 52 % of Cd and 94 % of Pb were found in mobile form.

Mo was not mobile in soil, but accumulated extremely in plant tissue up to few thousand ppm (like Se). Rabbit's organs absorbed it however weakly, the maximum concentration observed was in kidney 3-4 ppm D.W. and it was excreted in faeces and partly through urine. Mo-toxicity appeared only in the first experimental year. Hg was not mobile in this calcareous soil and accumulated moderately in plant tissues. It was absorbed extremely only in rabbit's kidney with more than 50 ppm D.W. concentration. The residue was excreted in the faeces. As was moderately mobile in soil but showed no definitive accumulation in plant tissues. It's toxicity, however increases with time, the given $NaAsO_2$ form could have turned into more toxic arsenate form in soil.

Cd was very mobile in soil, but it accumulated moderately in plant tissues. Rabbit's organs absorbed it also weakly with maximum concentration of 4-5 ppm in kidney. Cd was excreted through faeces. Cd-toxicity was found only redbeet and spinach. It is not clear yet whether these crops were too sensitive to Cd-load or the Cd-toxicity might increase with time.

4. Conclusions

From the 13 applied elements/chemicals proved to be phytotoxic on this calcareous loamy chernozem, in decreasing order Se, Cr, As, Cd, Hg and Mo. Uptake of microelements depended on plant species and parts. The lowest concentrations were measured in maize grain while the highest in spinach leaves. As a function of element load, As and Hg showed lowest accumulations in plant tissues compared to the control, while it was the highest in case of Mo and Se. Among the elements studied, Se presented the greatest capability of moving relatively unhindered through the soil-plant-aminal system.

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THE ECOTOXICOLOGY OF PARTICULATE COPPER EMISSIONS ON THE BRYOPHYTES OF A MANAGED GRASSLAND

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1. Introduction

The ecotoxicology of soil copper, originating from particulate emissions, on grassland bryophytes in the vicinity of the BICC Rod and Wire plant at Prescot, Merseyside, UK was investigated. Bryophytes, whilst not directly deriving minerals from the soil by conventional root systems (RICHARDSON, 1981), are well known for sensitivity to heavy metals and for ability to non-selectively accumulate metals from the atmosphere and surface water films. Their lack of a cuticle reduces barriers to direct cellular access of soluble metals, making these plants potential indicators of toxic effects of metals. The impact of differing rates of copper deposition from the rod - rolling plant, indicated by differing levels of soil copper contamination, were investigated with regard to their effect on composition and diversity of the bryophyte flora of the managed grassland surrounding the factory.

2. Materials and Methods

Previous work established that zones of soil copper contamination, ranging from <100 $\mu g\ g^{-1}$ to >2000 $\mu g\ g^{-1}$ HNO_3-extractable copper, occurred (LEPP et al., 1997). Six sites, covering the full range of copper concentrations, were selected for detailed ecological assessment (Table 1). At each site, an area (30 x 30 m) was sub-sampled based upon 20 0.5 x 0.5 m subplots; in each subplot, % vegetation cover was determined, together with identification of species present, % vegetation cover and growth form of all bryophytes present. This was repeated for each zone. Data was analysed using Pearson correlations to establish the significance of relationships between bryophyte distribution, soil chemical properties and vascular plant cover.

Table 1. Soil Copper concentrations at each site. Values in $\mu g\ g^{-1}$ dry wt

Site	H_2O-extractable Cu	HNO_3-extractable Cu
3	0.75	82.3
4	8.1	521
5	23.1	2017
7	4.0	305
8	4.0	434
10	0.85	312

3. Results and Discussion

Six species of bryophytes were identified: three acrocarpous species - *Barbula recurvirostra* L., *Bryum rubens* (Mitt.), *Pohlia nutans* (Hedw.) and three pleurocarpous species - *Brachythecium rutabulum* (Hedw.), *Eurhynchium praelongum* (Hedw.) and *Calliergon cuspidatum* (Hedw.). The sites at which each species were present, ranked in decreasing order of abundance, are given in Table 2. Statistical analysis of the field data indicated a significant negative correlation between total % moss cover and both water- and HNO_3 - extractable soil Cu content (Table 3).

Table 2. Bryophyte species present at each site. Numbers in brackets refer to sites in Table 1 and indicate descending order of abundance of each species

Acrocarpous	Pleurocarpous
Barbula recurvirostra (4, 10)	Brachythecium rutabulum (10, 8, 7, 4)
Bryum rubens (5)	Calliergon cuspidatum (3, 10, 4)
Pohlia nutans (4,5,7,10)	Eurhynchium praelongum (3,10,8,7)

Table 3. Correlation matrix for factors affecting bryophyte distribution
*p>0.01. **p> 0.05, ***p>0.001

Factor	% total cover Moss	% cover Pleurocarpous	% cover Acrocarpous
% Bare ground	**- 0.86***	**- 0.81***	**0.80***
H_2O - ext Cu	**- 0.85***	**- 0.83***	0.75
HNO_3 - ext Cu	**- 0.91*****	**- 0.85****	0.66

Differences existed between acrocarpous and pleurocarpous species in sensitivity to elevated Cu; in terms of % cover; the former were very sensitive, whilst the latter showed no adverse response.

The responses of the two types of bryophytes to increasing soil Cu content may be explained partly in terms of their growth forms and water relations. Pleurocarpous species, or "feather mosses" are ectohydric, taking up water over the whole stem and leaf surface. In the absence of a cuticle, metals in solution can access photosynthetic tissue and are thus, in theory, more phytotoxic. Acrocarpous mosses are endohydric, conducting water from the stem base to the leaves via stem surfaces and water-conducting tissues within the stem. The sensitivity of pleurocarpous species to copper may be due to ectohydric water uptake, coupled with the ability to grow through the grass stems in the managed grassland. The lack of sensitivity in acrocarpous species may result from endohydric water uptake, with copper binding to non-living tissue en route to the leaves, increased incidence of bare ground or to innate copper tolerance. Analysis of results does not allow for full separation of these factors for acrocarpous species, but this site supports a restricted bryophyte flora in comparison to managed grassland at an adjacent golf course (LINDSAY, 1993). *Pohlia nutans*, the most abundant acrocarpous species, has been reported as frequently associated with soils containing elevated heavy metals (including copper) (SMITH, 1982). Its capacity for asexual reproduction may be a contributory factor to apparent copper tolerance.

4. Conclusions

Pleurocarpous mosses are sensitive to copper and are largely absent from managed grassland where total soil copper >550 $\mu g\ g^{-1}$ dry wt. Acrocarpous species are less sensitive and their presence where soil concentrations are elevated may be a result of tolerance, coupled with lack of competition from vascular plants.

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MORPHOLOGICAL AND BIOCHEMICAL CHANGES OF *ZINNIA ELEGANS* UNDER CADMIUM STRESS.

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1. Introduction

Most of phytoremediation studies are mostly concerned about screening and selection of tolerant plants from the open field, mines and smelter (Baker et al., 1994). The tolerant species could accumulate heavy metals absorbed from polluted soil. However, cleaning of heavy metals in polluted soil with ornamental plants could be another better choice.

2. Materials and Methods

Zinnia elegans is a common wild plant with beautiful flowers and it is also a model plant for studying tracheid formation at cellular and whole plant levels (Robert , 1976). Plants treated with cadmium at the sub-lethal concentrations were morphologically and biochemically studied as previously described (Ma, 1995).

3. Results and Discussion

The major morphological abnormalities of cadmium-treated plants were the retardation of plant growth, chlorosis of leaves (especially young leaves), and earlier senescence of old leaves. Transfer cells were not only found in phloem tissues and minor veins of vascular bundles but also found in xylem tissues near by the vessels in 1st internode of cadmium-treated plants. However, transfer cells were not found in xylem tissues of root and leaf of test plants. Similar results were detected in zinc-treated plants, but not found in plants treated with other heavy metals.

Cadmium lowered the protein content, chlorophyll content and total amount of carotenoids. The isozyme patterns of superoxide dismutase, peroxidase and acid phosphatase were altered by the various applications and duration of cadmium treatment. Plants pretreated with low concentration (10μM) of cadmium and then treated with μM Cd induced more sulfur hydryl group and glutathione than that of plain 45 μM Cd-treated plants. The individual peaks of sulfur hydryl group did show a prominent increase of peak 3 and 4 in PHLC profile at 24 h, whereas they gradually decreased to intitial levels of sulfur hydryl after 96 h. The purified active fraction of LMW complex consisted of 2 peak lands in HPLC profile. The histidine content of the two peaks was more than 90% of the total amino acids. Five peaks in HPLC profile were obtained from HMW fraction. Peak 1 had high histidine content (>80%). However, peaks 4-7 were phytochelatins whose n numbers of repeated E-C unit were 3 and 4.

Phytochelatins with n numbers from 2 to 7 were the major cadmium-binding peptides (Rauser, 1990). In the present study, only peaks 4, 5, 6 and 7 of HMW Cd-binding complexes in HPLC profile contained high percentage (> 90 %) of glutamic acid, cysteine and glycine. The numbers in (E-C)n-G ranging from 3 to 4 were found in Cd-treated plants of *Zinnia elegans*. Transfer cells found in the vicinity of xylem tissue of first internode may relate to enhance lateral transport of cadmium in stem. It is a new finding about the distribution of transfer cells in plants (Mauseth, 1988).

4. **Conclusions**

Cadmium at sublethal concentrations morphologically resulted plant growth retardation and leaf chlorosis, and formation of transfer cell in stem as well as biochemical changes in pigments, isozyme patterns and phytochelatins.

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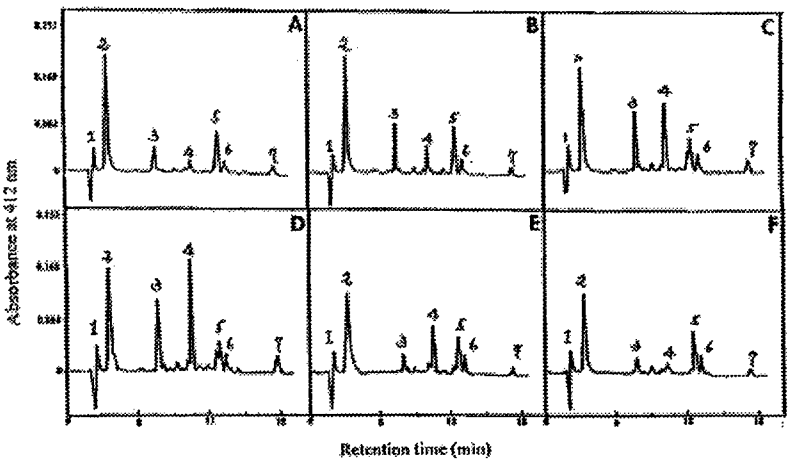

Fig.1.HPLC profile of hydryl sulfur in *Zinnia elegans* pretreated 1 ppm Cd^{++} and then treated 5 ppm Cd^{++} for a weak. Time A, B, C, D, E and F are 0, 3, 12, 24, 48 and 96 h, respectively. Ellman's regent in post column for detection and measured at 412 nm.

REEVALUATING THE FREE-ION ACTIVITY MODEL OF TRACE METAL TOXICITY: EXPERIMENTAL EVIDENCE WITH COPPER

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1. Introduction

Organic ligands alter the speciation and thus bioavailability of trace metals, and a free-ion activity model (FIAM) of availability is widely assumed (CAMPBELL, 1995). Much of the supporting data has been generated using unnaturally strong ligands such as EDTA, and exceptions to the FIAM have recently been demonstrated with weaker ligands such as citrate (ERRECALDE et al., 1998) and chloride (SMOLDERS and McLAUGHLIN, 1996). Based on equilibrium modeling simulations, PARKER and PEDLER (1997) suggested that departures from the FIAM were most likely with weaker ligands such as organic and amino acids. Our objective was to examine the validity of the FIAM in the case of Cu toxicity toward roots of higher plants. Nutrient solutions are convenient for manipulating metal speciation, but are complicated by the presence of Fe-chelates added to meet plant nutritional needs. We used split-root methods and short-term root elongation studies to circumvent this problem.

2. Materials and Methods

For the split-root experiments, we used custom-made acrylic solution-culture vessels with two 3-L root chambers. Four wheat (*Triticum aestivum* L., cv 'Yecora Rojo') seedlings were secured in the lid with half of their seminal roots immersed in a complete nutrient solution, and the other half immersed in a simple $Ca(NO_3)_2$-H_3BO_3 solution ("incomplete side") to which Cu and various ligands were added. Seedlings were grown for 16 to 21 d with frequent solution changes; a frozen subsample of each Aexhausted@ solution was retained for later analysis of the complexing ligand by ion chromatography (IC) or HPLC. Similar, short-term root elongation experiments were carried out in a simple $CaCl_2$ medium. Two-day-old seedlings were transferred to test solutions and grown for 48 h before measuring root length. To minimize microbial colonization, all test solutions were filtered to 0.2 μm, and replaced with fresh solution after 24 h of growth. Ligand analyses were as above. Copper speciation was computed using the GEOCHEM-PC program.

3. Results and Discussion

Split-root experiments: In the absence of complexing ligands, increasing Cu_T from 0.2 to 25 uM caused the dry-weights of the treated, incomplete-side roots to decrease by 80% (Table 1). The half of the root system not exposed to Cu exhibited compensatory growth such that the total weight of roots was unaffected; shoot growth was also not inhibited (data not shown). EDTA or citrate was then included in combination with 1 to 25 μM Cu_T such that the computed Cu^{2+} activity was constant at 0.13 μM (the value in the ligand-free 0.2-μM Cu_T solutions). With EDTA, root growth was constant with increasing Cu_T (but constant Cu^{2+} activity), the expected result based on the FIAM (Table 1). With citrate, however, root growth decreased markedly with increasing Cu_T (Table 1). But, subsequent analysis of these solutions by IC revealed significant biodegradation of the citrate had occurred, despite complete replacement of the culture solutions every 3-5 d. Thus, no direct refutation of the FIAM was provided by these experiments.

Short-term experiments: No ligand degradation could be detected in the comparable short-term elongation experiments, indicating that these were sufficiently axenic to allow proper evaluation of metal complexation by metabolizable organic acids. Again using designs with constant calculated Cu^{2+} activity, we obtained results that are consonant with the FIAM; growth was flat or decreased very slightly with increasing Cu_T (Fig. 1). Slight declines in growth, especially with citrate, may be due to small uncertainties in the published complexation constants. Thus, we are unable to duplicate the Zn- and Cd-citrate results of ERRECALDE et al. (1998), who ascribed marked departures from the FIAM to membrane transport of the intact metal-ligand complex.

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Table 1. Root weights (mg per pot; ∀1 SE) for Cu-treated roots in the Aincomplete@ sides of three split-root experiments. The 0.2-uM Cu_T solutions were all ligand-free; at ∃ 1 uM, EDTA or citrate concentrations yielded constant computed Cu^{2+} activities of 0.13 uM.

Total Cu (μ*M*)	No Ligand	EDTA	Citrate
0.2	231 ∀ 18	225 ∀ 25	244 ∀ 12
1	95 ∀ 13	233 ∀ 15	112 ∀ 16
5	53 ∀ 3	266 ∀ 10	61 ∀ 4
25	47 ∀ 6	244 ∀ 28	62 ∀ 2

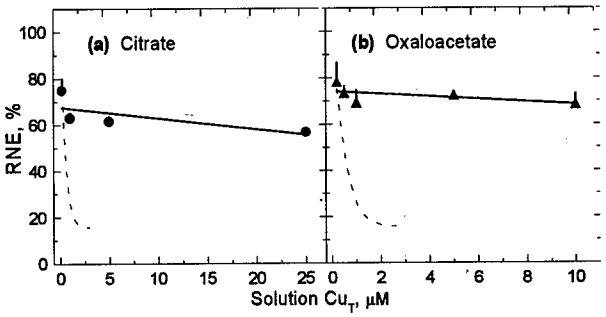

Figure 1. Relative net root elongation (RNE) of wheat seedlings as a function of total solution Cu. Citrate (a) or oxaloacetate (b) was added to maintain calculated Cu^{2+} activity at 0.14 uM throughout. Dashed lines are the root growth response in the absence of added ligand.

MERCURY POLLUTION IN EASTERN INDIA – GENOTOXIC EFFECTS IN PLANT SYSTEMS

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1. Introduction

Studies on the toxic effects of mercury compounds are receiving importance following extensive exposure of living organisms to mercurials. Recent reports indicate that mercury concentration in various soil samples from the eastern part of India is much higher than the level of 20-150ppb (0.2-1.5ppm) prescribed by WHO (1989). 3-6kg/year, depending on salt purity, is discharged from a chlor-alkali factory in Khardah (The Telegraph, 1996) ; alarming levels of sediment mercury were detected in the river Saptamukhi (0.121mg/kg⊗121ppm, CPCB). High levels of mercury have been observed in the effluents discharged from a chemical fertilizer in Meendweep Islands in West Bengal and also in Digha, Ganga and Prentice (CPCB, 1996). The objective of the present study is to find out the lowest effective concentration and the highest dose below lethality of the common inorganic mercurials on plant systems of economic importance.

2. Materials and Methods

The plant systems used were (i) Allium cepa L. var. aggregatum (multiplier onion) bulbs, and (ii) Hordeum vulgare L. (common barley) seeds. Inorganic form of mercury tested was mercuric chloride ($HgCl_2$). It is the common component of the effluents and is readily dissolved in water. The concentrations used were 1, 10, 100, and 1000ppm. The exposure was for a short period (6,12, 18 and 24 hours) for both plant systems and long term (48, 72, 96 and 120 hours) for Allium cepa only. The parameters for screening were cytogenetic ones, according to guidelines of EPA Gentox Program (Grant, 1982) and Kihlman (1966). The cytogenetic endpoints screened following usual acetic-orcein squash technique (Sharma and Sharma, 1994) were (i) the frequency of dividing cells (mitotic index), (ii) alterations in chromosome structure (chromosomal aberrations) and (iii) effects on the spindle fibres (turbagenicity).

3. Experiment

Allium cepa bulbs with fresh crop of roots were placed at the mouth of jars containing solutions to be tested (Levan, 1949). Root tips were removed at regular intervals and processed. On the completion of experiment the bulbs were transferred to Knop's nutrient medium for recovery and root tips were studied after every 24 hours. Seeds of barley were soaked in the solutions for 24 hours, washed thoroughly in distilled water and allowed to germinate in clean sawdust. Root tips were removed from germinating seedlings after every 24 hours.

Preparation of chromosome spreads – The excised root tips from each experiment at required intervals were fixed in (1:3) acetic-ethanol for 24 hours, washed and treated with 45% acetic acid for 10 minutes. The roots were then heated in 2% acetic-orcein-HCl mixture (9:1) for a few seconds and kept for 2 hours in the stain. Finally, the root tips were squashed in 45% acetic acid on a slide under a coverslip. 20.000 cells per set were screened for frequency of cell division and micronuclei and 500 dividing cells for chromosomal aberrations. The experiment was repeated thrice in three different seasons. Data obtained was analysed with the help of mean, standard deviation and two-way ANOVA.

4. Results and Discussion

The two plant systems were selected so as to test the different types of exposure from soil and water. *Allium cepa* bulbs included meristematic zones (roots) only, exposed for both short and long term periods and then allowed to recover. The barley seeds were immersed totally for 24 hours and then allowed to germinate.

1. In both plant systems, the frequencies of cell division i.e. mitotic index were reduced in direct proportion to the concentration of the chemical and duration of exposure. The frequencies of chromosomal aberration were directly proportional to the concentrations and duration of exposure.
2. Following exposure, the onion bulbs were allowed to recover. In general,
   - (i) The bulbs exposed to highest concentration of the toxicant for both short term and long term duration failed to recover. 1000ppm was the lethal concentration.
   - (ii) The bulbs exposed to 100ppm, i.e. next to lethal concentration for short term duration recovered,

(iii) whereas those exposed for longer periods could not recover.

The concentration at which exposed plants recovered after all duration was the **LECT** (Lowest Effective Concentration Tested) – **10ppm.**

Analysis of variane for the mean values of mitotic index revealed highly significant ($p < 0.01$) differences between the two test systems studied. Similar results were recorded following the analysis of variance of the mean values for chromosomal aberrations.

Table 1: Transformed Data on Mitotic Index for the Two Test Systems

Concentration (in ppm)	Mitotic Index		Percentage Reduction	
	Allium cepa	*Hordeum vulgare*	*Allium cepa*	*Hordeum vulgare*
0 (control in distilled water)	3.958	3.017	-	-
1	3.34	2.810	15.61	6.86
10	2.918	2.583	26.27	14.38
100	2.247	2.073	43.22	31.28
1000	1.157	1.773	70.76	41.23

Table 2: Transformed Data on Chromosomal Aberration for the Two Test Systems

Concentration (in ppm)	Mitotic Index		Percentage Reduction	
	Allium cepa	*Hordeum vulgare*	*Allium cepa*	*Hordeum vulgare*
0 (control in distilled water)	0.707	0.707	-	-
1	0.963	0.707	36.20	-
10	0.892	0.782	26.16	10.60
100	2.202	1.569	211.45	121.92
1000	6.902	1.851	876.22	161.81

A comparison of the divisional frequency following exposure to mercury shows that the reduction in mitotic index as compared to control is directly proportional to the concentration in both species but is greater in *Allium cepa* as compared to *Hordeum vulgare* in all concentrations (table 1). The increase in frequency of chromosomal aberrations shows a similar trend (table 2) indicating that the mitostatic and the clastogenic effects of mercuric chloride after 24 hours exposure are higher in *Allium cepa* than in *Hordeum vulgare*. Such differential response of the two plant systems may be attributed to species differences and also possibly to the procedure, since onion root tips are processed immediately after exposure while barley seeds are washed and the root tips collected after germination, at an interval of 8 to 12 hours after exposure.

5. Conclusions

Our observations on genotoxic effects of mercuric chloride on two diverse plant species indicate that mercury contamination of the soil and water upto 10ppm can be tolerated but 100ppm is lethal on longer exposure. The higher concentrations like 121ppm recorded at the rivers where effluents are discharged and adjacent areas are lethal for plants.

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ROOT STUNTING CAUSED BY ZINC TOXICITY IS AMELIORATED BY MICROMOLAR LEVELS OF MAGNESIUM AND POTASSIUM

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1. Introduction

The use of simple $Ca(NO_3)_2$ or $CaCl_2$ solutions for screening plant seedlings for metal tolerance has long advocated (JOWETT, 1958). However, severe root stunting under phytotoxic Zn (an effect clearly seen when plants are grown in simple solutions of $CaCl_2$ or $Ca(NO_3)_2$) is not apparent in studies where complete nutrient solutions are used. With short-term root elongation studies and split root experiments, we have shown that magnesium and potassium ions ameliorate the root stunting effects of Zn toxicity.

2. Materials and Methods

Short-term root elongation studies used a simple 2.0 mM $CaCl_2$ medium, buffered at pH 6.0 with MES, to which $ZnCl_2$, $MgCl_2$ and KCl were added in micromolar ranges. Wheat (*Triticum aestivum* L. cv Yecora Rojo) seedlings (2 days old) were transferred to test solutions, and grown, in the dark, for 48 h before measuring root lengths.

Split root experiments used custom-made acrylic solution culture tanks, with two 2-L chambers. Four seedlings were secured in the lid, with half of their seminal roots immersed in complete nutrient solution, and the other half immersed in a simple $Ca(NO_3)_2$-H_3BO_3 solution ('incomplete solution')to which the phytotoxic Zn (at 40 µM) and ameliorating Mg and K were added. Seedlings were grown for 16 days and solutions were replaced regularly.

3. Results and Discussion

Short Term Experiments. Wheat root growth was inhibited by 85% at 60µM Zn (Figure 1a). As little as 0.5 µM Mg will increase root growth by nearly 10% in seedlings grown at 60 µM Zn, and 200 µM Mg will almost completely ameliorate the effect (data not shown). In contrast, although 50 µM K will increase root growth by 20% at 60 µM Zn, 200 µM K is not sufficient to improve root growth above 40% of the control (data not shown). Thus, the ameliorating effects of Mg and K differ. Furthermore, a low K addition (50 µM) to the test solutions enhances the alleviation by Mg (Figure 1b).

Split-Root Experiments. The presence of Mg and K did not affect root growth in the absence of Zn (Table 1). Zn-induced stunting only occurred in the absence of Mg. The presence of Mg, and Mg with K, clearly ameliorated root stunting by Zn. In contrast to short-term results, K did not appear to enhance root growth. Surprisingly, Zn, in treatments with Mg and K, appeared to stimulate growth slightly. Shoot growth was similar for all treatments (data not shown). FRANCIS et al. (1995) have shown that phytotoxic Zn affects the population of mitotic cells by arresting the plant cell cycle, rather causing cell death. This would allow roots to live, but stunt growth. Cells in those experiments, exposed to 100 µM Zn, accumulated 40-fold more zinc than control cells. This Zn may be immobilized in cell vacuoles as Zn-phytate (VAN STEVENINCK et al, 1993).

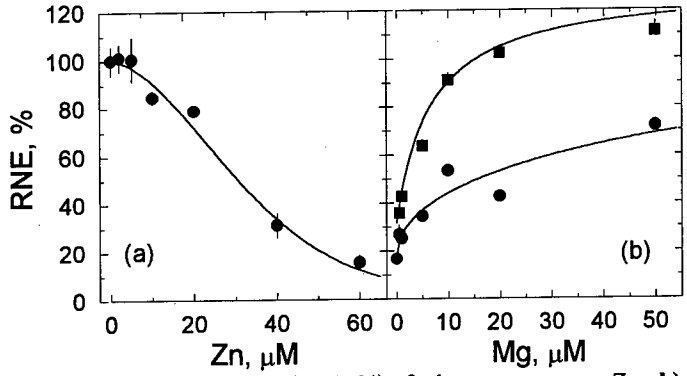

Figure 1 a) Relative net elongation (RNE, %) of wheat roots, versus Zn. **b)** RNE of wheat roots grown with Zn (60 μM) versus Mg, with (□)or without (□)50 μM K. Standard error bars may be hidden by the symbols.

Table 1. Mean dry weights (g) of wheat roots grown in split root experiments. Zn, Mg and K levels are given in μM. There were three replicates, and standard errors are given.

Zn	Mg	K	ROOT-RIGHT	s.e.	Root-left	s.e.
-	-	-	0.281	0.02	0.249	0.01
-	100	100	0.278	0.03	0.249	0.02
40	-	-	0.229	0.08	0.044	0.02
40	-	100	0.492	0.01	0.074	0.01
40	100	-	0.331	0.02	0.336	0.04
40	100	100	0.307	0.01	0.326	0.03

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PHYTOCHELATIN CONSTITUTIVE AMINO ACIDS AND HISTIDINE ABROGATE COPPER TOXICITY IN *CERATOPHYLLUM DEMERSUM* L. - AQUATIC MACROPHYTE

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1. Introduction

Pollution and contamination of the bio-geosphere with toxic metals is posing a major threat to the health of biota. Tolerant plants capable of scavenging metals have tremendous scope in phytoremediation. Copper is an essential element for plant growth and in excess cause oxidative stress (1). Plants normally are equipped with antioxidative enzymes and antioxidants to counteract the oxidative stress. Organics and inorganics viz., citrate, malate, calcium and a few amino acids are reported to protect plants from heavy metal toxicity (2-5). Hence, this study was initiated to examine how amino acids abrogate copper toxicity and to suggest the possible physiological basis for tolerance.

2. Materials and Methods

Ceratophyllum demersum L. were collected from local fish ponds and maintained under laboratory conditions in aquaria using 1/10 Hoagland solution. Plant material (1 gm) was transferred to 100 ml beaker and initially treated with 2 mM glycine, glutamate, cysteine and histidine individually for 12 h in light and the pH was adjusted to 5.5. Plants were thoroughly washed with water and were treated for 12 h with 4 μM Cu. Copper concentration in plants was analyzed by atomic absorption spectrophotometer). Lipid peroxidation and GSH content were measured according to Devi and Prasad (1).

3. Results and Discussion

Plants pre-treated with various amino acids didnot show any difference with regard to the uptake of copper compared to plants not subjected to amino acids pre-treatment. Copper accumulation was 6.5 times in both the cases when compared to control. This suggests that pre-treatment with amino acids has no influence on the copper uptake.. Plants treated with copper alone showed a significant increase in lipid peroxidation. However, plants pre-treated with amino acids showed a significant reduction in the lipid peroxidation compared to copper alone (no pretreatment with amino acids). Greater decrease in lipid peroxidation was observed with histidine and cysteine when compared to other amino acids tested. The order of protection being histidine > cysteine > glutamate > glycine. Copper considerably decreased the GSH levels due to oxidation or its diversion to phytochelatin synthesis. However, no such decrease was observed with copper upon pre-treatment with amino acids. Glycine, glutamate, and cysteine being the constituents of GSH and phytochelatins, their supplementation might have increased the synthesis of GSH and phytochelatins thereby reduces the free metals ions in the cells. Though the histidine is not a GSH constituting amino acid, the greater protection offered by it towards copper toxicity suggests a definite role in copper tolerance. Copper is known to block some of the metabolic steps in histidine biosynthesis. Thus, histidine and cysteine abrogate copper toxicity by maintaining the metabolic integrity of the enzymes and proteins (6,7). Further, cysteine and histidine are known to inhibit the production of free radical generation when compared to other amino acids. Thus, the greater

protection by cysteine and histidine can be related to their capacity to inhibit free radical generation and to protect enzymes and proteins from oxidation in addition to the cysteine being a constituent of phytochelatin, the prime metal chelating peptides in plants. Genetically engineered hyper-accumulators overexpressing the genes coding for these amino acids would certainly play an important role in phytoremediation of copper polluted and contaminated ecosystems.

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POSSIBLE RELATIONSHIP BETWEEN MANGANESE DEFICIENCY AND OESOPHAGEAL CANCER IN TRANSKEI

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1. Introduction

In Transkei the average oesophageal cancer incidence is not spectacularly higher than in some other southern African populations, but there is a marked gradient from low in the northeast to very high in the south, where the incidence rate is comparable to the three other highest incidence rates in the world (Laker *et al.*, 1981). In some Transkei areas oesophageal cancer is the major single cause of adult deaths. Rainfall decreases as the cancer incidence increases from the northeast to the south.Within the highest incidence districts there are huge differences between localities with extreme oesophageal cancer incidences ("black spots", "cancer gardens") and localities that are almost cancer free. In Transkei oesophageal cancer became an "epidemic" outbreak shortly after World War II, ie not long after severe depletion and impoverishment of especially the poor quality soils on Beaufort mudstones and shales in the 1930's when severe droughts were followed by gale force winds and then torrential rains, leading to devastating erosion. In addition long-term exhaustive cropping with little or no nutrient replenishment reduced the mineral nutrients in the latter soils to extremely low levels. Fertile soils on Jurassic dolerite, which are also inherently stable against erosion, were affected much less.

Previous research in Transkei and the Caspian littoal of Iran, the most intensively studied areas in regard to oesophageal cancer, did not include any **systematic quantitative comparative** studies on possible soil-oesophageal cancer relationships. Consequently a major interdisciplinary project was conducted, with close collaboration between epidemiologists and soil scientists (Laker *et al.*, 1981). Soil-oesophageal relationships were studied in a project comprising comparisons of (a) low and high incidence districts in Transkei (involving two studies), (b) low and high incidence areas in the Caspian littoral of Iran and (c) low and high incidence areas in a high incidence district of Transkei. Only the latter is reported here.

2. Materials and Methods

A detailed study was conducted in half of Butterworth district, one of the highest oesophageal cancer incidence districts in Transkei. Cancer registration records for 23 years and assistance of local authoritative individuals enabled plotting of the homestead of each victim on an aerial photograph. This revealed southwest-northeast running strips with very high cancer incidence separated by a very low incidence strip (Figure 1). Maize leaves, sampled according to a grid pattern in home gardens in six high and five low incidence areas identified on the aerial photograph, were analysed for mineral elements.

3. Results and Discussion

The low incidence strips coincided with large dolerite dykes, with the high incidence strips on Beaufort sediments. Of the maize leaves from the high incidence sites 83% had Mn levels below the discriminating value of 40mg.kg^{-1} identified during the initial study comparing high and low incidence districts in Transkei, whereas only 41% of the samples from the low incidence sites were below this value. This division was statististically significant at P = 0.001 according to Fisher's exact test. The dolerite in the study area contained 881mg.kg^{-1} Mn, compared with only

332mg.kg^{-1} in the Beaufort sediments (Kibblewhite *et al.*, 1984). In addition the soils on dolerite were far less depleted by erosion. The soils studied in the high incidence area in the Caspian littoral also had much lower plant-available Mn levels than those from the low incidence area. This is not unexpected considering that the high incidence area is in an extremely arid area in which soil pH is often above nine, thus sharply reducing trace element availability. (The low incidence area has very high rainfall and soils with relatively low pH.) Marjanen and Soini (1972) found a remarkably tight negative linear relationship between soil Mn content and cancer incidence in a study comparing 179 parishes in Finland. They also found that the Mn contents of old arable lands were **much** lower than those of new ones, indicating the danger of exhaustive cropping.

4. Conclusions

The group of four studies mentioned here, seem to suggest that manganese deficiences may increase oesophageal cancer incidence. They also illustrate the importance of understanding the inter-relationships between soil forming factors (especially climate and parent material), soils, crops and human health, especially in less developed areas.

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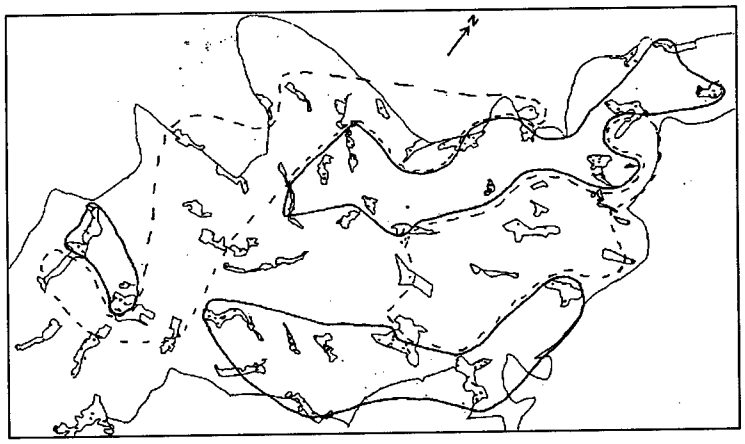

Fig. 1. High (solid lines) and low (broken lines) zones identified for maize leaf sampling. Dots indicate sites of cancer cases. Small, straight-line areas demarcate residential areas.

ASSOCIATION BETWEEN CHRONIC ARSENIC EXPOSURE AND CHILDREN'S INTELLIGENCE IN THAILAND

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1. Introduction

Previous reports presented high arsenic level in hair of children aged less than 10 years at Ronpiboon subdistrict. Many children had arsenic accumulated in their bodies though they had no skin changes. What are the health effects of chronic arsenic poisoning to these children? The present study aimed to test the association between chronic arsenic exposure, indicated by the arsenic levels in hair and children's intelligence among children living in Ronpiboon district since birth.

2. Materials and Methods

We selected 529 subjects from 838 children aged 6-9 years who were born in Ronpiboon district (63.1%) using simple random sampling. The arsenic level in hair was measured with atomic absorption spectrophotometry (AAS) method using a GBC 906 automatic multi-element atomic absorption spectrophotometer with the GBC HG 3000 hydride generator. Children's IQs were assessed with Wechsler Intelligence Scale Test for Children. Potential confounders were collected by child interview form, maternal interview form and father's questionnaire. The association result was explained by simultaneously adjusting for confounders in multiple classification analysis.

3. Results and Discussion

The range of arsenic level in hair was 0.48 to 26.94 μg/g. Around half of children (55.4%) had arsenic levels between 1.01 and 3 μg/g. Only 44 of 529 (8.3%) children had normal arsenic levels in hair (≤1 μg/g). The mean IQ of the study subjects was 90.44 points. The lowest IQ of total subjects was 54, the highest was 123. Percentage of the children in the average IQ group was remarkably decreased in the higher arsenic levels. Multiple classification was applied to selecting the parsimonious model for explanation of children's IQ variation. This model consisted of children's age and four significant variables associated with children's IQ as follows: arsenic level, father's occupation, mother's intelligence score and family income (Table 1). Arsenic could explain about 14% of the variance in IQ after controlling for other risk factors, ($p=0.002$).

Table 1: Multiple classification analysis of children's IQ (Grand mean = 90.44)

Variables	n	Unadjusted		Adjusted for Confounders		p-value
		Deviation	Eta	Deviation	Beta	
Arsenic (μg/g)			.13		.14	0.002***
Low (≤ 2)	190	1.98		1.97		
High (2.01 - 5)	244	-0.77		-.75		
Very high (> 5)	95	-1.97		-2.02		
Father's occupation			.24		.17	0.000***
Maternal Intelligence score			.28		.24	0.000***
Family income			.21		.10	0.059
Multiple R^2					.16	
Multiple R					.40	

Arsenic can damage the central nervous system, chronic encephalopathy symptoms include diminished recent memory and organic cognitive impairment. However, CNS impairment has been less frequently observed. A follow-up study in the Morinaga powdered-milk poisoning case in 1969-1971, reported the victims had a lower IQ than their siblings. The other study presented proportion of victims with an IQ of less than 85 which exceeded average numbers. The IQ of children in Ronpiboon district among the different arsenic levels showed an increasing proportion of the below average IQ level (< 90) with increasing concentrations of arsenic found in hair. The findings after controlling for other risk factors showed a significant inverse relationship between arsenic levels and IQ (p=0.002). These results suggest a possible association between hair arsenic concentrations and children's IQ. The weak point of this study was the difficulty in evaluating a causal relationship. It could not establish the time precedence of arsenic exposure, as the level of arsenic was measured at the same time as IQ. This association can be explained by the biological plausibility and dose-response trend. The association is more plausible as study subjects were born in 1986-1989, in a period of chronic arsenic poisoning problem at Ronpiboon subdistrict. At that time, a high proportion of drinking waters was contaminated with arsenic above 0.05 ppm. This birth cohort has been continuously exposed to arsenic since birth because of their non-mobility.

4. Conclusions

This study concludes that arsenic levels could explain why the mean of IQ were different in the varying arsenic groups of children. The management of risk to children should be the following: (1) The public health officers in Ronpiboon district should monitor health status of children for early detection of abnormal IQ findings. Especially, children who have high arsenic hair concentrations need to obtain frequent physical examinations. (2) The curriculum of primary education level need to be revised for the children with poor IQ. (3) The important implementation is the limitation of chronic arsenic exposure from ingestion of arsenic contaminated water. To clarify the association between arsenic and intelligence, further studies should be designed as a follow-up study beginning of birth. These children group should be evaluated for prolong or permanent intellectual impairment.

EFFECT OF LANTHANUM ON PHYSIOLOGICAL ACTIVITIES IN SEEDLING STAGE OF WHEAT

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1. Introduction

Lanthanum is one of the rare earth elements (REE). The REE consist of a group of metals including 17, lanthanides, Yttrium and Scandium. China has a share of about 80% of the world's resource. Fertilizers containing nitrate of REE or a mixture of complexes of REE (mainly La and Ce) have been used to increase crop yield in China for over 20 years. Increases reported in crop yield from all parts of China range between 5-50% with the common response being of the order of 8-14% (Guo, 1985). The area to which REE have been applied increased to more than 3 million ha in recent years. There have been few reports from other countries on the benefits of REE in increasing plant production. This work was designed to increase understanding of the physiological basis of crops response to REE application.

2. Materials and Methods

Winter wheat (Tritcum aestvicum, L., ssp. Ningmai No. 5 in Chinese) seeds were soaked in various concentrations of $LaCl_3$ for 8 hrs at 20 °C, and then put in culture dish lined with filter paper and kept at 20 °C. Germination was observed, the roots and shoots were weighed, and amylase activity of the seedling determined after 48 and 96 hr. The seedlings were cultured in Hoagland solution containing various amounts of La^{3+}. Catalase activity and chlorophyll content were determined 45 days later.

3. Results and Discussion

Seed germination was enhanced when the concentrations of La^{3+} were lower than 100 mg/l and inhabited when the concentrations were higher than 200 mg/l, the weight of roots and buds were increased at La^{3+} concentrations of between 0.05 to 100 mg/l and 0.05 to 50 mg/l respectively, amylase activity was enhanced on a wide range, from 0.05 to 300 mg/l (see Table 1). Chlorophyll content and catalase activity was increased (see Table 2).

Table 1. Effect of La^{3+} on germination, weight of root and bud, and amylase activity of wheat seedling

La^{3+} in Solution (mg/l)	Ger. Rate(%), 4d (SE)	Weight of root[1], 4d (mg)	Weight of bud[1], 4d (mg)	Amylase activity[2]
Control (0)	46.5(9.3)	26	37	1
0.05	49.5(10)	34	49	1.03
0.5	48.5(8.0)	36	45	1.10
5.0	53.5(4.6)	31	44	1.06
20	63.6(9.1)	39	44	
50	50.5(11)	39	52	1.08
100	51.5(11)	40	29	1.17
300	43.5(7.7)	13	23	1.03
500	44.4(1.7)	14	23	0.94
700	21.2(5.2)	6	12	0.91
1000	18.2(8.0)	8	13	0.84

[1] Average of 10 seedlings.
[2] Relatively value, take the value of control group as 1.

Table 2. Effect of La^{3+} on chlorophyll content and catalase activity in wheat shoot.

$C_{La^{3+}}$ (mg/l)	*0*	*0.05*	*0.1*	*0.2*	*0.5*	*1.0*	*2.0*	*5.0*	*10.0*	*20.0*
Cat. act[1].	1	1.36	1.20	1.34	1.14	1.31	1.19	1.48	1.41	1.25
Chl. Cont[1].	1	1.20	1.14	0.96	0.99	1.42	2.06	2.06	1.23	1.10

[1] Relative value, take the value of control group as 1.

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EFFECT OF INORGANIC LEAD ON GROWTH AND DEVELOPMENT OF *HORDEUM VULGARE* L.

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1. Introduction

Of late, there has been an alarming increase in the environmental concentrations of lead, especially in the developing countries, due to rapid urbanisation and industrialisation. In India, lead concentrations, that form a component of inorganic fraction in particulate matter, exceed the WHO guidelines of $0.5\mu g/m^3$ in seven cities, viz. Ahmedabad, Bombay, Calcutta, Delhi, Hyderabad, Kanpur and Kochi (NEERI, 1995). Recently, levels of lead have been estimated to be much higher than that permitted by WHO, in vegetables like *Beta vulgaris* var. *benghalensis, Chenopodium* sp., *Raphanus sativa, Brassica oleracea* var. *botrytis, Cucurbita moschata* and *Solanum melongena* grown in the waste-recycled areas in east Calcutta (Sengupta, 1997), further stressing the importance of the problems of lead toxicity and its amelioration.Lead pollution can affect all environments but its effects are most long lasting in soils. It is mostly concentrated near the soil surface with very little leaching due to its strong adsorption onto the surfaces of iron and manganese oxides, humus and clay colloids (Alloway and Ayres, 1993). Moreover, it is resistant to microbial degradation. Soils contaminated by airborne lead accumulate the mineral, and plants absorb it from the soil. Increasing acidity of soils from fertilizers and acid rain further increase the solubility of this metal.Reduction of concentrations of lead or its toxic effects introduced into the environment by the human activities is specially important because toxic lead ions, on entering plants, tend to accumulate in plant tissues and display the phenomenon of bio-magnification, resulting in loss of crop yield and contaminating the food supply (Beri and Setia, 1995). The present investigation was undertaken to study the effect of inorganic lead on growth and yield parameters in *Hordeum vulgare* L. Experiments were extended to explore the possibility of inducing tolerance in *Hordeum* plants to higher levels of lead salt by pre-conditioning in lower concentration. With this objective, plant populations raised in the first generation following exposure to low concentration were subjected to the same and to higher concentrations of the toxicant in the second generation.

2. Materials and Method

DETAILS OF TREATMENT:	← 100PPM (T_1) ← (1 + 100) PPM (T_2) ← (1 + 1) PPM (T_3) ← 1PPM + WATER (T_4) ← CONTROL (T_5)

Seeds of *Hordeum vulgare* L., belonging to family Poaceae, was divided into separate equal sample lots. They were presoaked in sterile distilled water for 6 hours and then in freshly prepared aqueous solution of lead nitrate [$Pb(NO_3)_2$] of known concentrations for 24 hours. Following the completion of the chemical treatment, seed lots were thoroughly washed in running tap water for 12 hours and on the following day sown in the field in a Randomized Block Design with three replications. Randomly selected twenty plants from each treatment per replication were observed for the following pre-and post harvest characters -- Plant height, number of total and fertile tillers, number of leaves, length of flag leaf and panicle, filled grain percentage, and 1000 seed weight. On maturity, seeds were harvested separately and kept for raising the next generation. Mean of each character for all the treatments were computed and variations were statistically analyzed with the help of Least Significant Difference Test (LSD).

3. Results and Discussion

The mean and the results of the LSD test of five treatments for each character are tabulated below. Analysis of the data showed that inorganic lead induced toxic effects as shown by alterations in all characters studied in *Hordeum*. The highest concentration viz. 100ppm caused statistically significant change in number of leaves, total tillers, fertile tillers and filled grain percentage as compared to control. Attempts were made to explore the possibility of inducing tolerance in treated plants. In the second year, seeds, harvested in the first generation from seeds exposed to 1 ppm, were further exposed to both low (1ppm) and higher concentration (100ppm) of lead nitrate and grown in the field. Statistical comparison of mean values of treatments for the characters studied in *Hordeum* revealed a significant deviation of the mean values for the two treatments viz. 100ppm (T_1) and (1+100)ppm (T_2) as compared to control (T_5) for number of leaves per plant, total and fertile tillers, length of panicle and percentage of filled grains. Though both treatments induced toxic effects in the characters studied, the degree of toxicity was more

pronounced following exposure to 100ppm alone without pretreatment. This indicates that pretreatment with 1ppm lead salt induces a degree of metal tolerance. Brown and Martin (1981) reported that Cd tolerance could be stimulated in *Holcus lanatus* L. following low level exposure of roots to the metal. The phenomenon of heavy metal tolerance in plants has for many years attracted the attention of plant scientists. Various hypotheses have been developed to explain heavy metal tolerance by plants: selective uptake of ions, reduced permeability of membranes, exclusion of metals from the plant, compartmentalization of metals in vacuoles, and cell walls, evolution of metal-tolerant enzymes and production of metal-chelating compounds. However, more recent identification and isolation of heavy metal-binding polypeptides, also known as phytochelatins, in higher plants (Rauser, 1990; Steffens and Williams, 1987 ; Xianga and Olivera, 1998) and discovery of its significant role in metal tolerance, have led to better understanding of the mechanism of metal tolerance. Furthermore, its discovery opened up a whole new range of possibility of inducing tolerance in plants, especially in relevance to crop plants, to higher concentrations of metals by inducing production of phytochelatins by prior pre-treatment in low levels. The tolerance to the effects of lead, after exposure to a low dose of the chemical in the previous generation may be attributed to the formation of the phytochelatin as well.

Table: Computation of mean of the treatments for each character of *Hordeum vulgare* studied in the 2nd generation.

	Plant Height	**Leaves per Plant**	**Tillers/ plant**	**Fertile Tillers/ Plant**
Treatments	Mean	Mean	Mean	Mean
T_1	71.744 A	22.350 D	3.150 D	2.133 D
T_2	69.912 A	28.633 BC	4.000 BC	3.333 B
T_3	70.555 A	27.217 C	4.117 AB	3.033 C
T_4	68.690 A	29.967 AB	4.143 AB	3.600 AB
T_5	68.433 A	31.550 A	4.433 A	3.883 A

	Length of Flag Leaf	**Length of Panicle**	**Filled Grain (%)**	**1000-Seed Weight**
Treatments	Mean(cm)	**Mean(cm)**	Mean	**Mean (g)**
T_1	13.047 A	10.980B	64.38C	19.95A
T_2	12.918 **AB**	12.165A	70.44A	20.56A
T_3	12.915 AB	10.467B	74.15AB	20.05A
T_4	12.983 A	10.937B	72.45A	21.10A
T_5	12.913 AB	10.445B	75.35AB	21.50A

Values are means of n = 60 observations. For each parameter, treatment means followed by a common letter (s) do not differ at the p = 0.05 level of significance based on LSD test.

4. Conclusions

The results obtained indicate that pretreatment of *Hordeum* seeds with 1ppm of toxicant prior to its exposure to 100ppm induced substantial alleviation of the toxic effects in *Hordeum* in contrast to the plants grown with single exposure to 100ppm. This technique may possibly be employed for growing plants in lead-contaminated soils.

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THE EFECT OF CR^{VI} ON THE SEEDLING DEVELOPMENT OF TWO SPECIES OF CALENDULA (*C.ARVENSIS* L. AND *C. OFFICINALIS* L.).

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1. Introduction.

Plants may present different responses to heavy metals. Some of them (*bioaccumulators*) translocate from the soil relevant quantities of metals, which are actively concentrated in the aerial parts, overcoming the soil metal concentration (Baker, 1981). Others (*bioindicators*) accumulate metal quantities in proportion to those present in the soil, in the leaves. Still others (*excluders*) uptake and translocate from roots to shoots very low metal quantities, until a critical concentration is reached, and toxicity symptoms occur. Of these three basic strategies the second is of particular consideration since plants following this strategy may be utilised as *biomonitors* of the environmental conditions (Witting, 1993).

In recent works Bini et al. (1998 and 1999) found that some species of *Calendula* are able to uptake chromium at quite high rate, thus suggesting that it may behave as a bioindicator. *Calendula* is a genus of the Compositae family growing in temperate areas. In Italy the species *C. arvensis* is largely diffused and grown wild especially in olive orchards and in vineyards, while *C. officinalis* is widely cultivated in gardens and in the field and utilised as colorant and medicinal plant. The aim of this work was to evaluate the effect of Cr^{VI} on the seedling development.

2. Materials and Methods.

Seeds of the two species were cultivated in vitro in Arnon solution (Arnon, 1938) at elevated Cr concentration (1,2,3,5 µg /ml). The germination percentage and the developing of seedling were recorded. Fifteen days after sowing, seedlings were gathered and the length of the roots and of the aerial part were measured.

Sections of root apices and leaflets, fixed in Bouin solution and embedded in Historesin, were examined by Light Microscopy (L.M.) in order to evidence possible differences between non treated and treated plants.

3. Results and Discussion.

The germination in *C. arvensis* was scarcely influenced by Cr in the nutrient solution, while in *C. officinalis* it was quite lower at 5µg/ml Cr treatment.

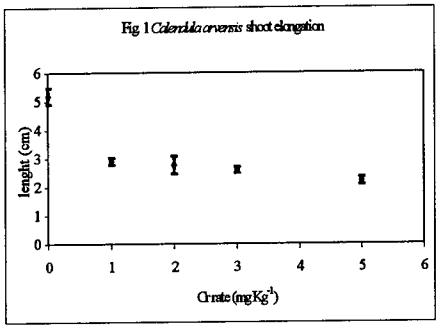
In both species, the treated seedlings, in comparison to the test plants, showed a darker green colour of the leaflets and pale brown roots. This was particularly evident at seedlings growing in the solution with the highest Cr concentration.

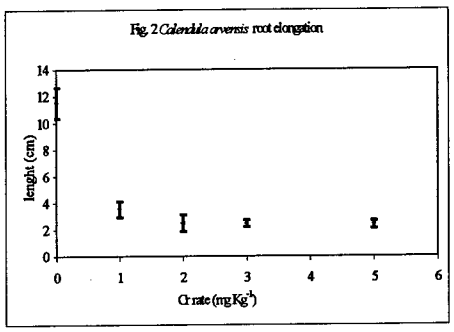
C. arvensis presented a marked reduction in root and leaf lengths even with the lowest Cr concentration, as shown in fig. 1 and 2.

Similar results were recorded also for *C. officinalis*.

Root apex observation by L.M. evidenced in both species a decrease of the meristematic zone and the presence of differentiated tissues, particularly tracheids with annular thickening. These characteristics were developed much earlier in *C. officinalis* than in *C. arvensis*, since in the former, supplying 1 µg/ml Cr, the length of meristematic zone was drastically reduced with

respect to the control plant (1mm the control plant, 30μm the treated one). Increasing of Cr concentration did not change the length significantly. On the contrary, in *C. arvensis* a
Fig. 1and 2 : Significant reduction of the length of shoots and roots in *C. arvensis*.





gradual decrease of the length of the meristematic zone with increasing Cr concentration, was observed. Only plants receiving 5 μg /ml Cr, presented a marked decrease in meristem length (80μm; 45μm) and the occurrence of tracheids.

4. Conclusions.

These preliminary observations allow us to suggest that the presence of Cr in the nutrient solution, even at the significant rate of 5 μg/ml, does not impede the seedling development of *C. arvensis* and *C. officinalis.* However, reduced dimensions of the seedlings were observed for both species, owing to the precocious ageing of the meristematic tissue.
C. arvensis seems to tolerate chromium better than *C. officinalis*, as indicated by higher germination percentage, better seedling development and the maintenance of a larger meristematic zone in the root apex. Therefore, chromium has a minor toxic effect on *C. arvensis* and this may explain its higher sorption capacity (Bini et al., 1999) , associated with the higher tolerance for this metal.

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ZINC TOLERANCE IN METALLIFEROUS AND NON-METALLIFEROUS POPULATIONS OF *CARDAMINOPSIS HALLERI*

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1. Introduction

Metal tolerance is the ability of a species to grow and survive on contaminated sites, MACNAIR (1993). It has generally been studied as an example of adaptation to a particular environment under selective pressure, with contaminated sites being colonised by tolerant ecotypes. ANTONOVICS *et al.* (1971) declared that ' there is no evidence that a species has constitutional tolerance to heavy metals: evolution has always occurred when mine habitats are colonised'. In contrast to this paradigm is the phenomenon of constitutive tolerance. It is defined as a trait present in all members of populations growing either on contaminated or uncontaminated soils, MEHARG (1994). Constitutive tolerance exists and it has been found in several species. *Cardaminopsis halleri* is often associated with Zn, Cd and Pb polluted areas. The fact that *C. halleri* is able to grow and survive on these extreme biotopes indicates that is tolerant to these metals. Interestingly, *C. halleri* is also found on uncontaminated sites. Nevertheless, it is not known whether the distribution of *C. halleri* is related to its ability to evolve metal tolerance when it grows on contaminated sites, or whether it shows constitutive tolerance throughout its range. *C. halleri* populations from contaminated and uncontaminated sites have never been compared and it is not known whether populations from uncontaminated sites show metal tolerance.

2. Materials and Methods

Materials: During July 1997, seeds from 3 populations growing on uncontaminated soil in the Sumava mountains (Czech Republic) were collected: 30 seeds from Kubova Hut 1; 18 seeds from Kobova Hut 2 and 23 seeds from Stary Herstejn. In the same period, seeds were collected from 2 French populations found on industrially polluted areas highly contaminated with Zn, Pb and Cd: 30 seeds from Auby and 7 seeds from Courcelles-Les-Lens. *Arabidopsis thaliana* (30 seeds) and *Cardaminopsis petraea* (30 seeds) known as non-Zn-tolerant species were added to the experiment.

Method: The tolerance of the seedlings was measured by determining the concentration at which no new root growth is produced (the EC_{100}), SCHAT and TEN BOOKUM (1992). Roots of all seedlings were blackened with activated charcoal. The seedlings were transferred to 10µM Zn hydroponic solution for 1 week. Roots of the seedlings with new root growth visible beyond the charcoal coated roots, were reblackened and seedlings transferred in successive weeks to 100; 250; 500; 1000 and 2000µM Zn. At each transfer, those plants which had reached their EC_{100} were removed from the experiment and deemed to be non tolerant at this concentration.

3. Results and Discussion

Root growth of all the *A. thaliana* and *C. petraea* seedlings were completely inhibited at 100µM Zn and appeared to be unhealthy, with dry and chlorotic leaves. The seedlings of *A. thaliana* and *C. petraea* were assumed to be non-tolerant at 100µM Zn, as expected, and were removed from the experiment. In contrast, all the *C. halleri* seedlings, whatever their origin, showed continued root growth at 100µM Zn. All the seedlings appeared to be healthy, and did not show any dry or chlorotic leaves, and thus appeared to be tolerant when compared to *A. thaliana* and *C. petraea* seedlings. At 250 and 500µM Zn, there was no difference in the root growth of all seedlings from contaminated and uncontaminated sites and they looked very healthy. At 1000µM Zn (Figure 1), 3 seedlings out of 18 tested from Kubova Hut 2 (uncontaminated site) and 1 out of 30 tested from Auby (contaminated site) were unable to root. At 2000µM Zn (Figure 1), all populations from both sites segregated seedlings which were unable to root (non tolerant) and seedlings which were able to root (tolerant). The heterogeneity in the tolerant and the non-tolerant proportions was analysed using the G statistic. There was a significantly lower proportion of tolerant individuals in the populations from uncontaminated sites than in the populations from contaminated sites (G_H= 4.98, p=0.026).

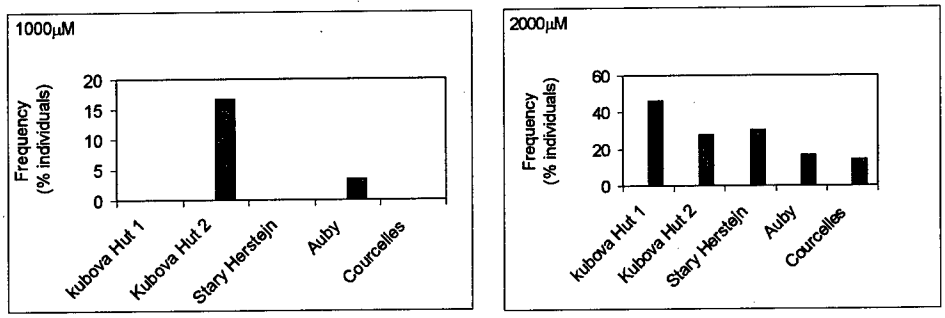

Figure 1: Frequency distribution (%) of the seedlings which have reached their EC_{100}.

4. Conclusions

In *C. halleri*, Zn tolerance is largely a constitutive property. From an evolutionary point of view, how to explain the presence of this trait in all members of the species? From its distribution, it is impossible to know whether the species has colonised normal habitats from contaminated sites or in contrast whether it has colonised contaminated sites from normal habitats. However, we can assume either that *C. halleri* has evolved its Zn tolerance by the formation of tolerant ecotype, having evolved the tolerant ecotype, the progenitor population became extinct, leaving the population isolated and totally tolerant. Then the species started to colonise uncontaminated sites from contaminated habitats. However, we can not rule out the alternative hypothesis that the species was tolerant and it has colonised uncontaminated from contaminated sites.

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THE ISOLATION OF A TONOPLAST PROTEIN POSSIBLY RELATED TO NATURALLY SELECTED ZINC TOLERANCE IN *SILENE VULGARIS* USING 2-D GELELECTOPHORESIS

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1. Introduction

Phytoextraction of heavy metals, the use of hyperaccumulating plants to remove metals from the environment, has been an important object of study for several years now. For a plant to be successfully applied in this technique the ability to accumulate metals and a large shoot biomass production are not the only prerequisites; they also have to be able to detoxify the metal once it accumulates in their tissues. According to Salt et al (1998) it is vital 'to understand the existing molecular and biochemical strategies plants adopt resist metal toxicity' in order to develop plants which can be used for phytoremediation. *Silene vulgaris* is a non-hyperaccumulator species with both heavy metal sensitive and highly tolerant populations, which offers an exellent model to study naturally selected heavy metal tolerance. Our research focusses on zinc tolerance in a sensitive (Amsterdam) and tolerant (Plombières) ecotype of *S. vulgaris*.

It has been shown that zinc tolerance in this species is not based on either reduced uptake (Harmens et al 1993a) or the intracellular binding of the metal to phytochelatins (Harmens et al 1993b). Therefore, it was hypothesized that tolerance is due to enhanced compartmentation in the vacuole. Recently, we have shown that tonoplast vesicles derived from tolerant plants take up more zinc than those derived from sensitive plants when zinc is supplied as Zn-citrate, in the presence of MgATP (Verkleij et al 1998). Subsequent research has been performed to characterize this uptake system (Chardonnens et al, unpublished). It was concluded that increased zinc uptake by vesicles from tolerant plants is not dependent on the formation of a proton gradient, whereas zinc uptake in the sensitive ecotype was inhibited by the presence of both gramicidine (22%) and NH_4Cl (52%). A similar difference between ecotypes was found for changes in the electrochemical gradient upon the addition of zinc. Ortho-vanadate did not affect zinc uptake in the tolerant ecotype, but inhibited uptake by 58% in the sensitive one. The substitution of ATP by GTP led to a 59% increase of zinc uptake in the tolerant ecotype, and a reduction of 39% in the sensitive one. In both ecotypes, zinc is actively transported across the tonoplast ($Q10>1.6$), most likely as a free ion, since citrate does not accumulate in vesicles . In short, zinc uptake characteristics differ notably between ecotypes. To obtain the protein responsible for the increased zinc uptake in the tolerant ecotype, the protein composition of the tonoplast of different ecotypes was investigated using 2-D gel electrophoresis.

2. Materials and Methods

Plant material

All experiments were performed with a zinc sensitive and a tolerant ecotype of *Silene vulgaris* (Moench.) Garcke. The sensitive ecotype was collected in Amsterdam (The Netherlands), the tolerant one in Plombières (Belgium).

2-D gel electrophoresis
Tonoplast vesicles of both ecotypes were isolated from roots as described by Verkleij et al (1998) and subsequently used to perform 2-D gel electrophoresis of tonoplast proteins. All samples were silver stained and compared to a standard mixture of proteins to estimate pI and MW.

3. Results and Discussion
At least one constitutive difference between 2-D gels of sensitive and tolerant ecotypes was found. This particular protein had a MW of ±39 kD and a pI of ±5.9.
This protein might be related to both zinc tolerance and the uptake difference found between tonoplast vesicles of the different ecotypes. However, until experiments have been performed using selected homozygous lines of crosses between sensitive and tolerant plants, no such conclusion can be drawn. Currently, such homozygous lines are being selected, and will subsequently be tested for both their tonoplast protein composition and zinc uptake by isolated tonoplast vesicles.
If, after testing homozygous lines, enhanced zinc uptake by tonoplast vesicles and a tonoplast protein spot turn out to segregate with zinc tolerance, this particulair protein would most likely be involved in naturally selected zinc tolerance in *S. vulgaris*. Further research would then have to focus on the sequencing of the protein and the cloning of this metal tolerance gene, and its potential use in developing plants suitable for phytoextraction.

4. Conclusions
A protein (MW ±39 kD, pI ±5.9) has been shown to be located on the tonoplast of a zinc tolerant ecotype of *S. vulgaris*, while it is absent on the tonoplast of a zinc sensitive ecotype. These ecotypes differ in their ability to transport zinc into isolated tonoplast vesicles. Therefore, the protein found might be related to zinc tolerance. This relationship, however, is speculative until experiments have been performed using selected homozygous lines of crosses between sensitive and tolerant plants.

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RESPONSES TO COPPER TOXICITY DURING *IN VITRO* CALLUS INITIATION IN SUNFLOWER

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1. Introduction

Plant tissue culture technology has made significant contributions to fundamental and commercially-orientated research in agriculture and industry. One potential application is as a test system for investigating phytotoxicity and/or metabolic fate of chemicals. Based on existing literature on sunflower tissue culture (PATERSON and EVERETT, 1985, PRADO and BERVILLE, 1990), a callus initiation test has been developed and used successfully in the quantitative determination of metal ion toxicity. This paper presents an outline of the testing procedure and the responses of sunflower cell cultures to copper.

2. Materials and Methods

Surface sterilization of decoated F.1. "Sunspot" sunflower (*Helianthus annuus*) seeds was carried out by 15-20 min exposure to 0.5% sodium hypochlorite solution, followed by several rinses in sterile distilled water. Seeds were then aseptically transferred to culture tubes containing 10 ml of agar medium (B5 salts, 5 g sucrose and 8 g agar (PATERSON and EVERETT, 1985)) and were germinated on illuminated shelves in a growth room (23-25°C, 3000 lx light level, on a 16h light - 8h dark cycle). After 7 days, transverse hypocotyl segments (2-3 mm) of the seedlings were excised and transferred to 100 × 15 mm plastic Petri dishes containing a range of copper concentrations incorporated into 10 ml of solidified callus induction/growth medium (10 explants/Petri dish, 12 Petri dishes/treatment). Copper was applied as nitrate by filter-sterilization, after the autoclaving of the basic medium. The NB callus induction medium of PRADO and BERVILLE (1990) was used, but MS basal salts were used instead of MS basal medium of the original recipe. The dishes were sealed with parafilm and kept in an incubator (25°C) in the dark. Growth was determined after 21 days by noting the fresh and dry weight (according to the method described by DODDS and ROBERTS, 1995). The rate of callus growth based on fresh weight measurements (RGR) was calculated according to WAJDA et al. (1989). The dried callus tissue was suspended in HNO_3 and was digested by microwave digestion, then copper concentration in the callus dry matter was determined by AAS.

3. Results and Discussion

The toxic range of copper was established by varying its concentration in a number of preliminary experiments. The effects of increasing copper concentrations in the culture medium on dry weight and fresh weight accumulation during callus growth, and the accumulation of copper within the explant/callus tissue, are shown in Figure 1. EC_{50} values (effective concentration producing 50% decrease in callus growth) in tissue and in solution were determined from the plotted dose-response curves. Based on fresh weight measurements the EC_{50} values are 21.6 mmol/kg in tissue and 0.0745 mmol/L in solution. Based on the dry weight measurements the EC_{50} values are 16.2 mmol/kg in tissue and 0.0629 mmol/L in solution.

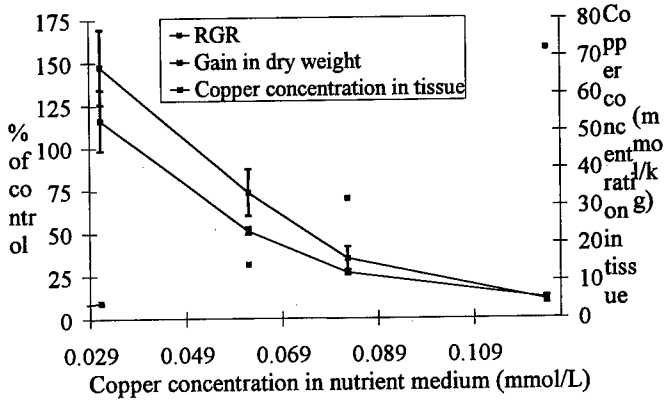

Figure 1: Dose-response curves for the toxic effect of Cu (II) on callus growth of sunflower "Sunspot" explants and accumulation of copper within the tissue (growth data are mean of two experiments ± standard deviation)

4. Conclusions

The results reported here indicate that this novel toxicity test, based on measurement of callus growth parameters during the initiation stage of the cultures, can be used in the quantitative assessment of metal ion toxicity. The use of the callus initiation stage was found to overcome the high variability inherent in most callus cultures and to provide an accurate and easy-to-measure toxicity parameter. The toxicity test with callus initiation does not need culture media for long-term maintenance of the cultures, because these are set up from the "0" stage (establishment of aseptic culture of seedlings) each time. Using this technique, it is no longer necessary to do all tests together (in order to avoid the variability induced by the time factor) and it is also possible to carry out the experiments at any time and as many times as needed in order to accomplish a specific task. Other advantages are the high number of replicates that can be achieved by using this method and the ease in handling the cultures, which also provides a very low contamination rate.

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SOME PHYSIOLOGICAL ASPECTS OF LEAD PHYTOTOXICITY

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1. Introduction

Lead phytotoxicity was examined using bean plants (*Phaseolus vulgaris* L. cv. 'Limburgse vroege') grown on hydroponics over a range of 14 concentrations of lead. Plant development was observed and the capacity of some enzymes known to be induced under oxidative stress was measured in primary leaves and roots to determine whether Pb induces oxidative stress. Soluble protein content was measured as another criterion for evaluating harmful effects of Pb. Additionally Pb content in primary leaves was determined.

2. Materials and Methods

Seeds of bean plants were germinated in wet rockwool at 22 °C during 96 h, after vernalisation (72 h). Seedlings with a root length of app. 1,5 cm were grown in 3-mm thick polystyrene squares (13 seedlings per 289 cm^2 polystyrene) by fixing the roots through 5-mm holes. The polystyrene was floated on 3 l of Hoagland solution in 3.5 l polyethylene beakers. The plants were grown for another 14 days under controlled environmental conditions (temperature: 22 °C, relative humidity: 65 %, photoperiod: 12 h light/12 h darkness, photosynthetically active radiation: 150 µmol m^{-2} s^{-1}). Lead was added as $Pb(NO_3)_2$, complexed with EDTA (1/1) in 15 different treatments: 0 (control), 2, 5, 10, 20, 30, 40, 50, 65, 80, 100, 125, 150, 200, 400 µM. In the control no EDTA was used since no effects on enzyme capacties have been detected due to EDTA (results not shown).

Morphological parameters were measured at harvest (14 days after sowing): weight of aerial parts and of primary leaves, primary leaf area (LiCor type LI-3000 areameter), stem length and root weight.

Plant material was stored at –70°C; enzyme capacities were determined spectrophotometrically (Shimadzu UV-1602) in primary leaves and roots: Ascorbate peroxidase (APOD), Dehydroxy ascorbate reductase (DHAR), Glutathion reductase (GLUR), Guajacol peroxidase (GPOD), Superoxide dismutase (SOD) and Syringaldazin peroxidase (SPOD). Preparation of the samples is described by Van Assche et al. (1988); results are expressed in mU per g fresh weight.

Soluble protein content was measured in primary leaves and roots using the Biorad method (Bradford, 1976). Enzyme capacities and protein content could not be measured on plants treated with 400 µM Pb due to the limited amount of biomass obtained. Primary leaves were used for Pb analysis with AAS (Perkin Elmer 1100B), after extraction with $HNO_3/HClO_4$ (Milestone MLS-1200 MEGA).

3. Results and Discussion

Morphological analysis showed that Pb strongly reduced the root growth, already visible at 80 µM Pb. Root weight was reduced by 90 % at 400 µM (0.22 g vs 2.15 g in control). Above ground parameters stem length and leaf surface started to decline from 150 µM. At 400 µM, both organs were reduced respectively by 42 and 57 % in comparison to control plants. Decline of aerial parts and primary leaves was only noticeable at 400 µM, and for both parameters decrease was around 50 % as compared to control plants.

In primary leaves, induction of enzyme capacity was measured for 2 enzymes. At 200 µM Pb, the capacity of DHAR and GPOD increased respectively with a factor 154 and 333 % in

comparison to the control. Induction was also noticeable at 125 µM for GPOD (fig. 1), and at 150 µM for DHAR. In roots, 5 enzymes were induced: GLUR (110 %), APOD (522 %), SOD (299 %), GPOD (300 %)(fig. 1) and SPOD (260 %). Increase was detectable at 100 µM for SPOD, and at 125 µM for GLUR, APOD, SOD and GPOD.
In roots, soluble protein content (fig. 2) was increased with 70 %, first detectable at 125 µM. In primary leaves, this content remained unchanged.
A linear relation was observed between lead concentration in the nutrient solution and lead content in primary leaves: at 400 µM Pb up to 2 370 mg Pb kg^{-1} dry weight was accumulated.

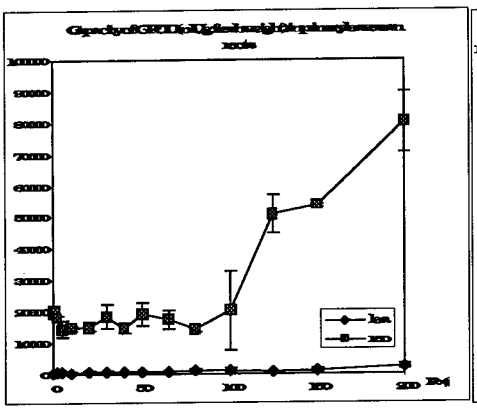

Fig. 1: Capacity of GPOD (mU/g fresh weight) as a function of Pb added in primary leaves and roots of 18 days old *Phaseolus vulgaris* seedlings grown on hydroponics.

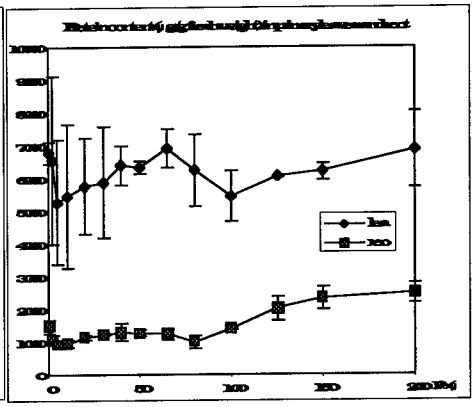

Fig. 2: Soluble protein content (µg/g fresh weight) as a function of Pb added in primary leaves and roots of 18 days old *Phaseolus vulgaris* seedlings grown on hydroponics.

4. Conclusions

Morphological analysis shows that roots respond faster and more intensively to elevated Pb concentrations than above ground plant parts: root weight is declined by 90 % while reduction of aerial plant parts is around 50 % when 400 µM Pb is added. The increased enzyme capacity in roots and partly in primary leaves indicates that oxidative stress is produced. The question however is open whether the capacity of the enzymes not induced here would increase at concentrations above 200 µM Pb. The high Pb content found in primary leaves suggests that Pb is present in the plant as Pb-EDTA since in this form lead can easely be transported to the upper plant parts (Blaylock at al., 1997; Huang et al., 1997). The linear relation found between Pb content in nutrient solution and in primary leaves suggests that this uptake is passive.

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COPPER TOLERANCE TESTING ON PLANT SPECIES GROWING NEAR A COPPER SMELTER IN CENTRAL CHILE

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1. Introduction

Studies about the effects of pollution generated by metal smelters on surrounding vegetation are very scarce in Chile. However, Ginocchio (1997) quantified the effects of a copper smelter located in the central zone of Chile on surrounding vegetation at both plant community and individual levels. The study, carried out on a grassland formation that has been polluted during the last 30 years by the smelter, indicates that has already been a 46% decrease in plant species richness over a distance of 5.5 km from the polluting source. However, species responses to increasing pollution levels have shown to be different; most plant species decreased in their abundance towards the smelter, eight species showed unchanged abundance while only two species were more abundant near the smelter (Ginocchio, 1997).
Although these observations are in agreement with vegetation changes produced by metal smelters described in the literature (e.g. Freedman & Hutchinson 1980, Moldan & Schnoor 1992, Galbraith et al. 1995, Gunn 1995, Helmisaari et al. 1995, Kapustka et al. 1995, McCall et al. 1995), it is necessary to prove if this selection process is mainly due to excess copper in soils or it is due to other environmental changes produced by the smelter. Therefore, the aim of this study was to test experimentally if the multiple patterns of change observed in plant abundance can be explained by differences in copper resistance to available copper in soils.

2. Methodology

Plant species resistance to elevated copper concentrations was tested on seedlings generated from seeds collected in the Ventanas copper smelter hinterlands, central zone of Chile, belonging to the species: *Oenothera picensis* and *Eschscholzia californica* (species more abundant on high-copper polluted areas), *Rhodophiala advena*, *Noticastrum sericeum*, *Convolvulus chilensis* (species abundant all along the polluted area), *Baccharis linearis*, and *Solidago chilensis* (species more abundant in the less polluted areas). Ten to fifteen day-old seedlings were grown in aerated one/fifth-strength Hoagland solution amended with 0.0, 0.5, 1.0, 2.5, 5.0, and 10.0 ppm of copper, on polystyrene beads floating on 1 l black plastic pots. After a growth period of 10 days the root lengths of the seedlings were measured and the root and shoot yields (dry weight) were registered. Seedlings of two copper tolerant species, *Eschscholzia caespitosa* and *Mimulus guttatus* were germinated from seeds collected from a mine site in California (Copperopolis) and also tested following the methodology described above. These copper tolerant controls were included in order to compare the response of the Chilean plants with the responses of known copper tolerant plants using the same methodology.

3. Results

Root length was significantly inhibited in all Ventanas copper smelter species when copper concentration was increased either to 0.5, 1.0, 2.5, 5.0, or 10.0 ppm (Table 1). Seedlings of these species exposed to 0.5 ppm copper showed chlorosis and reddish coloration in their leaves, indicating toxicity symptoms. Root yield was negatively correlated with increasing copper concentration and shoot yield was decreased in a smaller degree than shoot productivity. However, root length was only significantly inhibited in *E. caespitosa* and *M. guttatus* at copper concentrations greater than 1.0 ppm. Only those seedlings exposed to copper concentrations

higher than 1.0 ppm showed toxicity symptoms such as chlorosis and reddish coloration in leaves and a decrease in root yield.

4. Conclusions

We conclude that all plant species growing in the Ventanas area analysed are sensitive to increased copper concentration in nutrient solutions when compared to copper tolerant species. As some of the tested plant species are able to grow in highly polluted soils in the Ventanas copper smelter hinterlands, the results of this study may indicate that copper pollution can not be the most important factor explaining plant abundance changes produced near the smelter.

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6. Acknowledgements

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Table 1. Root length of seedlings exposed to several copper-enriched nutrient solutions after 10 days. Numbers indicate mean and standard deviation.

SPECIES	0.0 mg l^{-1} Cu	0.5 mg l^{-1} Cu	1.0 mg l^{-1} Cu	2.5 mg l^{-1} Cu	5.0 mg l^{-1} Cu	10 mg l^{-1} Cu
Ventanas:						
B. linearis	2.6 ± 0.97	1.9 ± 0.67	1.7 ± 0.67	1.2 ± 0.91	1.7 ± 0.41	1.9 ± 0.69
C. chilensis	20.5 ± 4.48	6.9 ± 1.34	6.0 ± 1.53	6.1 ± 1.23	5.8 ± 1.65	6.0 ± 1.46
E. californica	12.6 ± 3.90	5.9 ± 2.26	4.0 ± 1.33	n.d.	6.2 ± 2.09	5.0 ± 1.76
	15.2 ± 3.90	4.9 ± 1.18	5.0 ± 0.71	5.0 ± 0.71	4.7 ±0.76	5.4 ± 1.06
N. sericeum	8.0 ± 1.39	4.9 ± 1.24	5.7 ± 1.47	4.8 ± 1.36	5.4 ± 1.38	5.4 ± 1.70
O. picensis	4.8 ± 0.93	2.1 ± 0.44	2.8 ± 0.53	2.9 ± 0.39	3.4 ± 0.59	3.5 ± 0.69
R. advena	4.0 ± 1.58	4.3 ± 2.29	3.9 ± 1.96	4.6 ± 1.54	4.2 ± 1.67	3.4 ± 1.94
Copperopolis						
E. caespitosa	8.8 ± 4.41	7.4 ± 3.73	5.3 ± 3.07	n.d.	3.3 ±1.44	4.2 ± 1.95
M. guttatus	16.7 ±4.02	17.5 ± 3.90	17.9 ± 3.14	16.0 ± 5.27	15.1 ± 3.78	11.6 ± 4.98

INDUCTION OF PLANT PATHOGENESIS-RELATED PROTEINS BY HEAVY METALS

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1. Introduction

In Plants, growth arrest and chlorosis are among the common symptoms resulting from exposure to elevated concentrations of heavy metals, and therefore biomass production or root elongation are often used to determine metal tolerance. However, the mechanisms involved in the generation of metal toxicity symptoms are not well understood. Numerous studies support the idea that metals induce biochemical changes in metal sensitive as well as tolerant plants, for example the production of phytochelatins or free histidine, respectively (1,2). Metal exposure also induces changes in gene expression. Previous data from our laboratory showed that in barley seedlings metal exposure resulted in increased levels of mRNAs encoding for proteins that are known to be induced by pathogen attack, namely peroxidase, chitinase, thionin and 1,3-β-glucanase, and an increase in peroxidase activity(3). Here we report the results from a detailed investigation of metal-induced changes in the expression of barley thionin.

2. Materials and Methods

Barley seedlings were germinated in moist vermiculite over a container containing 0.25-strength Hoagland's solution. Seedlings were exposed to 100 µM Cd added to the nutrient solution for periods between 6 h and 10 d. Ten-day-old seedlings were harvested, root and shoot lengths determined, and plants were divided into root tips, upper root, leaf sheath, lower leaf and upper leaf. Cd content was determined in these tissues by ICP. Selected tissues were prepared for *in situ* hybridization. Steady-sate mRNA levels encoding for thionin were analyzed in root and shoot tissues by Northern blotting (4).

3. Results and Discussion

In 10-day-old barley seedlings thionin mRNA levels were very low in the shoots of control plants, but increased substantially upon exposure to Cd for 6 h (Fig. 1). A prolonged exposure to Cd resulted in further accumulation of thionin mRNA, with highest levels detected after the longest exposure period of 10 d. A significant growth reduction was observed after 2 d of metal exposure. After 10 d of Cd treatment highest Cd concentrations were found in root tip (3010 ± 310 µg g^{-1} dry biomass), and concentrations in the shoot were similar in upper leaf, lower leaf and leaf sheath (115 ± 53 µg g^{-1} dry biomass). Localization of thionin gene expression determined by *in situ* hybridization will be reported.

4. Conclusions

In response to exposure of barley seedlings to elevated concentrations of Cd in the rooting medium for six hours or longer shoot thionin mRNA levels increased considerably. Our data suggest that metal toxicity symptoms may be the result of the activation by Cd of a plant response which is similar to responses elicited by pathogen attack.

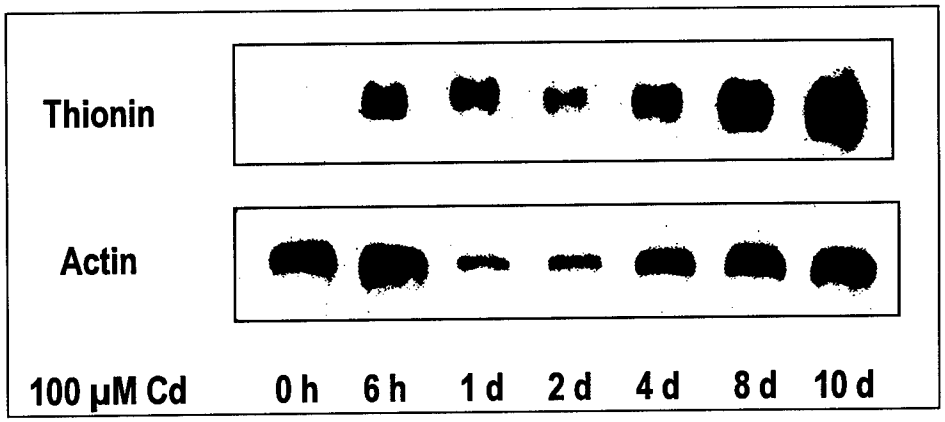

Fig. 1: Northern Blot showing thionin mRNA levels (barley actin as a control) in the shoots of 10-day-old barley seedlings after various periods of exposure to 100 µM Cd in the hydroponic rooting medium. RNA was extracted from shoot tissues. Twenty µg total RNA were loaded, blotted and hybridized to a digoxigenin-labeled probe.

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COPPER--INDUCED AMINO ACID SYNTHESIS IN THE XYLEM SAPS OF CHICORY AND TOMATO PLANTS

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1. Introduction

The transport of heavy metals in the xylem is only partly understood. White et al. (1981) indicated that Fe was bound to citric acid and Cu to several amino acids, while Zn, Mn, Ca and Mg to citric acid and malic acid. The presence of nicotianamine (NA) in tomato xylem sap suggests it is an important Cu transporter (Pich and Scholz, 1996). The objective of this study is to investigate the mechanisms of copper transport in the xylem sap of chicory and tomato plants as influenced by solution Cu concentrations at the root surface.

2. Materials and Methods

Chicory and tomato plants were grown in a nutrient film system using a modified Hoagland solution supplemented with 0, 5, 10, 20 ppm Cu supplied as $CuSO_4$. Plants were grown for 6 weeks before harvest. At harvest, shoots were removed and xylem sap collected. The ionic strength, pH and total Cu of xylem sap were measured (White et al., 1981). The separation and quantitative estimation of amino acids were conducted according to Fierabracci et al. (1991) with some modifications. The Cu^{2+} concentration/pH titration patterns of individual amino acids in aqueous solution and simulated xylem sap were compared with tomato xylem sap.

3. Results and Discussion

More than 99.68% of total Cu in tomato xylem sap was complexed, irrespective of the Cu concentration in the nutrient solutions. Graham (1979) found similar results for sunflower xylem sap. The concentrations of seven amino acids in xylem sap significantly increased with increasing Cu concentration in nutrient solutions. When exposed to high Cu concentrations in the root medium, the relative changes of some amino acids concentrations were much higher than other amino acids (Figure 1). Production of a free amino acid or organic acid in plants as a specific and proportional response to Cu treatment has not been previously published, although increased synthesis of histidine has been reported for Ni (Kramer et al., 1996). For a single complexing agent, the Cu^{2+}/pH titration curve of NA was most similar to the curves of the amino acid mixture and tomato sap (Figure 2). NA molar concentration in the sap of 0.05 mM could account for 99.57% complexation of xylem Cu at 0.033 mM. This suggested that NA plays the most important role in complexation of Cu in tomato xylem saps. However, NA is perhaps not the sole Cu translocator in tomato (Pich and Scholz, 1996). Histidine is an important complexing agent, especially in the absence of NA (Figure 2). We are not aware of any other direct simulation of the roles of NA and histidine in Cu transport in xylem sap. Although no titration work has conducted with chicory xylem sap because of limited sap volumes, it is reasonable to expect that most of the xylem Cu was transported as complexes with NA and histidine because NA and histidine concentrations are higher in chicory sap than tomato sap.

4. Conclusions

Cu treatment induces selective synthesis of certain amino acids which include NA, His, Gln and Asn which form highly stable (logK ≥ 13.4) complexes with Cu. Free NA and histidine are the major Cu chelators in tomato and chicory xylem sap.

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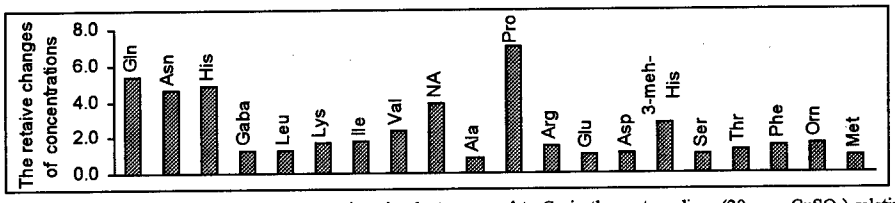

Figure 1 Ratios of xylem amino-acid concentrations in plants exposed to Cu in the root medium (20 ppm $CuSO_4$) relative to amino acid concentrations in the xylem sap of control plants (0.05 ppm $CuSO_4$). Amino acids are listed from left to right in the order decreasing abundance in the xylem sap of tomato plant. Only those amino acids present at ≥ 0.001 mM in any sample are shown.

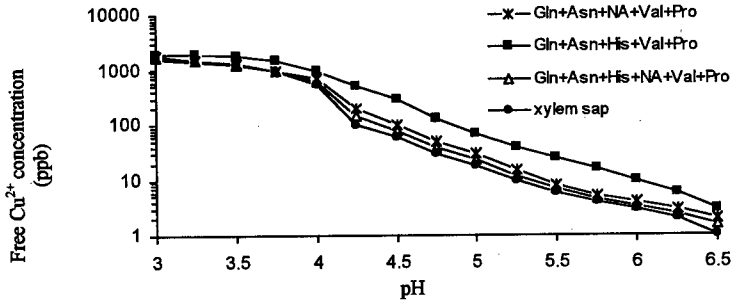

Figure 2 The Cu^{2+} concentration /pH titration curves. The amino acids and total Cu concentrations, the ionic strength were controlled exactly as detected in the xylem sap from high Cu treatment tomato plants. A cupric electrode, which only responds to free Cu^{2+}, was used to measure Cu^{2+} in the pH range 3 to 6.5.

TRACE ELEMENTS INFLUENCE ON CHRONIC BRONCHITIS DEVELOPMENT

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1. Introduction

The air pollution is one of the causes beginning bronchopulmonary pathology. In Prydneprovsk metal work region such metals as Mn, Cr, Pb, Cu, Ni along with gas pollution and dust unhealthy influence on respiratory system. The large importance in pollution of an environment by heavy metals belongs to vehicles. In this region in wintertime night hours about ground inversion of temperature forms, which is accompanied by weak winds. The harmful substances don't dissipate almost within a night. Penetrating into respiratory passages these pollution are realized by violation of mucous membranes with the depression of the local protection and cell regeneration, the changes of the most important metabolic processes. The reliable change of parameters of spreading and heaviness of course of lung chronic diseases has been found out. Thus the complicated forms of disease were registered. The physical inability under a chronic bronchitis does not come directly after disease beginning, and in some years, therefore we has carried out the correlation analysis between basic pollutant concentration, including salts of heavy metals, in atmospheric air in 1985-1987 years and level of physical inability. For this industrial area the positive correlation revealed between invalid rate level and dust concentration in atmosphere ($r = + 0.45$, $P < 0.05$). In display trace elements toxicity the main role was played by its interaction with biological membranes of lung cells (and also with its basic component - lipoproteins) to free radical mechanism with intensity of lipid peroxides oxidation process. The accumulation of heavy metals negatively influenced on enzyme activity of antioxidizing system.

2. Materials and Methods

104 patients suffering from chronic bronchitis and living in unfavorable environmental condition of big industrial centers, where level of air pollution exceeded by limit possible concentration from 2 till 8 times in beginning 80th years were examined. On the moment inspection (1992-1994 years) these patients had steady violation of the cardiorespiratory system, leading to limit their disability: 70,2 % these patients could not carry out the regular work. All examining patients were separated on 4 groups with different degree of the respiratory failure and violation of common blood circulation. They were ill more 6 years - 74,2 %. The indices of 30 healthy people without bronchopulmonary pathology were used as control ones. We used serum and washed erythrocytes for analyses.

Cholesterol, triglycerides, total lipids, low density lipoproteins (LDL), high-density lipoproteins (HDL), malone dialdehyde, haptoglobin, ceruloplasmin were assays by Cobas Mira.

Extra LDL and LDL cholesterol were calculated (1): extra LDL cholesterol $= \frac{\text{triglycerides}}{5}$; LDL cholesterol = Total cholesterol - HDL cholesterol - extra LDL cholesterol.

3. Results and Discution

Lungs participate actively in the lipid exchange. It doesn't only use but also regulate lipid level in the blood plasma. Patients with chronic bronchitis had higher content HDL, triglycerides and lower content LDL, total cholesterol, total lipids, LDL cholesterol. Levels of cholesterol LDL, cholesterol HDL and cholesterol extra LDL were increased. Change of energetic exchange under this pathology caused intensive use of LDL as substance of oxygen processes by tissues. Free-radical processes were activated. It has been found out that level of lipid peroxides accumulating in the blood plasma had correlated with bronchial obstruction degree and rising respiratory failure degree. The increase of ceruloplasmin activity, haptoglobin level as antioxidants had been found out in all groups. But it did not correlate with lipoproteins level. It caused imbalance between antioxidant system and free-radical processes, decreasing

resistance of erythrocyte membranes to negative influences. In the membrane structural functional changes accompanied with lowering toxic metabolic elimination by erythrocytes and increasing endogenic intoxication.

4. Conclusions

The heavy metals had expressed cumulative action and were capable to render negative action on active groups of proteins, enzymes and others physiologically active substances. Xenobiotics oppressed work of antioxidizing system in live organism. It resulted to intensification of free radical processes that had underlain development various, including bronchopulmonary pathologies. The carried out researches has allowed assuming participation of heavy metals in such disease formation, as a chronic bronchitis. It has been resulted in growth of physical inability. Thus, this investigation gave the possibility to reveal one of bronchopulmonary pathology causes and can be considered as the rehabilitation basis for the patients suffering from the disease.

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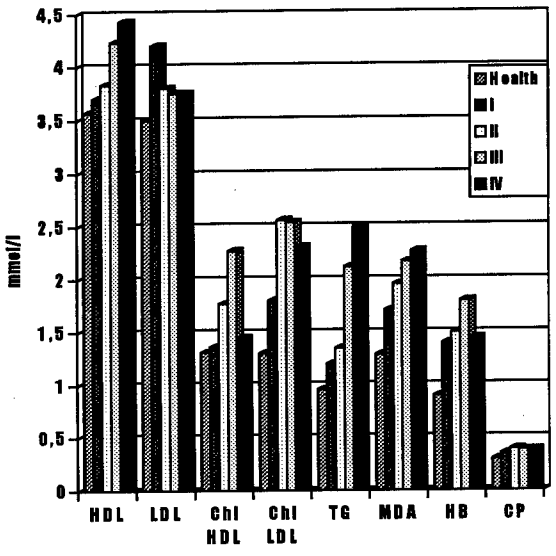
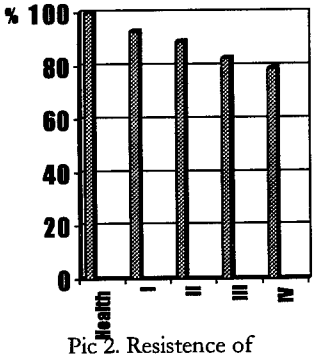


Pic1. Distribution of lipid components and antioxidizind elements in serum of patients with chronic bronchitis with different respiratory insufficiency



Pic 2. Resistence of erythrocyte membranes in the presence of 3% H_2O_2

GROWTH, MINERAL COMPOSITION, AND BIOMARKERS IN MAIZE EXPOSED TO METAL CONTAMINATED SOILS

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1. Introduction

Anthropic metal-contaminated sites in the North district of France allow studies on metal behavior in soils and on plant response to increasing exposure. In particular, a part of an agricultural field was contaminated by Zn/Pb smelter waste deposits on an old lane roughly 60 years ago. Coarse contaminated materials were removed. But plant species such as maize are still highly affected on a narrow lane (~5 m wide) and then displayed a progressive restoration of growth when they were sown more and more distant from the point source. Soil analysis indicated an increasing range of metal contamination on a short distance (~20 m) with the same soil texture. This particular *in situ* situation provides opportunities to assess i) plant growth and mineral composition of plant products at harvest depending on the contamination extent, ii) the phytotoxicity of soil samples in bioassay, and iii) techniques dedicated to restore plant growth and safer products. One aim was to compare plant growth and mineral composition at harvest and in short-term bioassays. Particular attention was paid to global biomarkers in plantlet organs in relation to metal exposure. On the other hand, several inorganic additives were tested to improve our understanding on soil remediation by the inactivation technique.

2. Materials and Methods

Soil samples were collected in the topsoil (0-0.25 m) along a transect away from the highest contaminated zone (table 1). They were labelled in relation to maize row at harvest (~0.80 m between each sample). Control soil was collected in a neighbour field with similar crop history. Maize and winter wheat are usually cultivated in both fields. The soil texture is loamy sand. Soils are classified as brown soil (Brunisols RPF, 1995) and developed in glaunite-rich Ostricourt sand deposits with possibly some eolian silt addition at the surface. Plant samples were collected at harvest in the field, weighed and washed, then oven dried and milled before wet digestion and ICP-AES analysis. To estimate metal mobility in soils, Cd, Cu, and Zn were extracted by a 0,1 M $Ca(NO_3)_2$ solution (1/5 ratio, filter porosity 0.2 µm). Soil samples phytotoxicity was assessed by the Plantox test using maize (MENCH et al, 1997). Phytochelatins in roots were determined by HPLC and monobromobimane derivatization.

Table 1 : Characteristics of the soil samples (metal concentration in mg/kg soil dry weight)

Labels	R1	R2	R3	R4	R5	R6	R8	R10	R14	R25	Control
Soil pH	6.18	6.05	6.18	6.45	6.59	7.09	7.67	7.9	7.77	7.59	5.9
Total Zn	1926	1926	1621	1603	1307	847	617	297	134	117	91
Total Pb	392	392	317	272	183	136	100	60	37	34	32
Total Cd	3.1	3.1	3.1	2.7	2.2	1.7	1.6	1.1	0.8	0.8	0.7

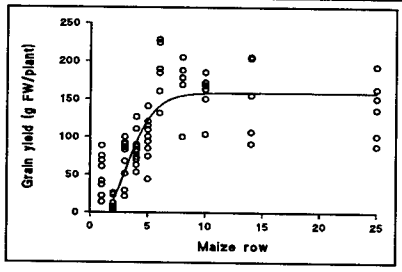


Figure 1 : Maize grain yield at harvest depending on maize row number.

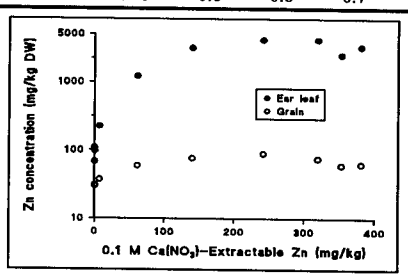


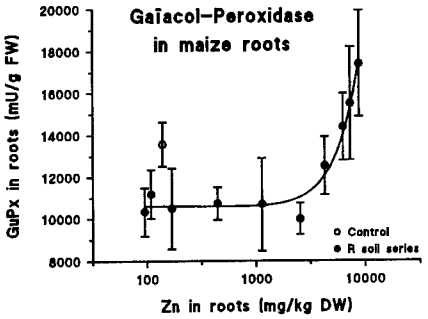
Figure 2 : Zn concentration in maize ear leaf and grain vs. soil Zn extracted by calcium nitrate

3. Results and Discussion

Maize at harvest: Grain yield (fresh weight, FW) (Fig. 1) and shoot yield (dry weight, DW) decrease as metal exposure raises across the transect. A 50% reduction in grain yield is found at row R4 with roughly 4500 mg Zn in the ear leaf and 100 mg Zn in grain per kg DW (Fig. 2). Maximum Zn concentration is about 50 times higher in the ear leaf than in grain. Highest values for Cd and Cu concentrations in grain are 0.12 mg and 2.8 mg per kg DW.

Metal exposure: Total metal contents highly increase across this soil series (Table 1), but metal concentrations in soil solution and soil buffer capacity are widely claimed as better parameters. For example, Zn extracted by the unbuffered solution varies from 0.03 to 381 mg/kg soil DW. Zn chemical species in extracts are not currently determined. Nevertheless, Zn concentration in plant parts such as ear leaf at harvest is well related to soil Zn extracted by calcium nitrate, even though metal exposure *via* the foliar pathway especially at juvenile growth stage cannot be ruled out (Fig. 2).

Figure 3 : GuPx activity in 15-day-old maize roots vs. Zn concentrations in roots



Plantox bioassay (15-day-old maize plantlets): As plants are exposed from R25 to R2 soil samples, mean values for growth parameters (i.e. shoot and roots yields fresh weight, shoot length, leaf area) numerically decrease. Minimum values are found for R2 and R3 treatments. However the differences are poorly or not significant across the treatments except for the 4th-leaf FW (50% decrease between R2 and R4). As metal exposure increases, chlorophylleous pigment densities decrease. Among global biomarkers measured in the 3rd- and 4th-leaves and in apical roots, gaïacol-peroxidase activity (GuPx) shows a marked raise in roots in relation to metal exposure. Significant (1% level) negative correlations are found between GuPx activity in roots and both shoot yield and Fe concentration in roots. Highest positive correlation is found between GuPx activity in roots and Zn content in roots, and in a lesser extent with Mn and Cu contents. GuPx activity in roots plotted vs. Zn in roots displays the best dose-response relationship (r^2=0.97) (Fig. 3). But Mn and Cu exposure can act in synergy with Zn. When maize is grown on R4 soil, GuPx activity starts to increase in apical roots of plantlets, and on the other hand, at harvest, Zn concentration in ear leaf levels off whereas grain yield decreases by 50%. Cd content in roots is not related to GuPx activity in roots. However, specific biomarkers such as phytochelatins (PC) have been analysed in roots. Maximum Cd content measured in maize roots (150 mg/kg DM for R2 soil) leads to the highest PC_2 and PC_3 in root extracts.

4. Conclusions

Wide scale risk assessments on a field basis are scarce. Case study on phytotoxicity can be useful especially when the investigation of plant exposure focuses also at cellular and subcellular levels. In parallel, metal speciation in soil and soil solution can be taken in account. Pluridisciplinarity information gained is relevant for people looking for the thresholds of toxicity for metals, and for those working on plant bioassays and on guidelines to assess soil ecotoxicology.

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EXOGENOUS AMINOACIDS AND ANTIOXIDANTS ALLEVIATE COPPER-INDUCED OXIDATIVE STRESS IN *SCENEDESMUS BIJUGATUS* - SCOPE FOR REMOVAL OF COPPER FROM CONTAMINATED AND POLLUTED WATERS

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1. Introduction

Copper is a bioelement, but is toxic at high levels. Accumulation of copper in aquatic ecosystems poses a threat to biota (1, 2). Copper generates free radicals and causes oxidative stress, damages pigments, lipids, proteins and nucleic acids. Copper retards/ inhibits growth (3, 9). The objective of this study was to examine as how the exogenous amino acids and antioxidants alleviate copper-induced toxicity in *Scenedesmus bijugatus*. Eventually, such an approch could lead to the development of strains/mutants capable of scavenging copper from contaminated and polluted waters (1).

2. Materials and Methods

Scenedesmus bijugatus was grown in sterile Kessler's medium under continuous illumination. The medium was supplemented with varying concentrations of copper as $CuSO_4$ (0-400 µM). The copper-treated and untreated cultures were supplied with aminoacids (glutamic acid 50 µM; glycine 100_µM; cysteine 50_µM) and antioxidants (mannitol and sodium benzoate 10 mM each; BHT 1 mM; GSH 2 mM). Growth was measured as the increase in chl. a content. The extent of peroxidation of membrane lipids was measured. Antioxidative enzymes APX, GPX, SOD and CAT (3) and enzymes of glutathione metabolism GSH-PX, γ-GCS, GSSG-R and GST (4) were assayed.

3. Results and Discussion

Growth was stimulated at 25 µM concentration of copper but was progressively inhibited from 50 µM onwards (3). The minimum inhibitory concentration (IC_{50}) was found to be 200 µM in 24 hrs. There were increases in the activities of antioxidative enzymes (3) and of GSH-PX, γ-GCS, GST whereas the activity of GSSG-R decreased. The modest increase in the activity of γ-GCS coupled to the decrease in the activity of GSSG-R reflects the decrease in glutathione content observed previously (3) under copper stress. The increase in the activities of these enzymes is a part of the cellular defence against oxidative stress and increases the tolerance level, thereby possibly increasing the accumulation level.

Reversal of toxicity was observed as a reduction of growth inhibition in the presence of aminoacids and antioxidants (3,6,7). Cysteine, glutamic acid and glycine are constituents of phytochelatins which are the metal-binding peptides of algae (5). In addition, cysteine also functions as an antioxidant in the cells as it is the most abundant thiol aminoacid and has the ability to bind and scavenge metal ions.

Hydroxyl radicals are the most damaging active oxygen species and are responsible for the initiation of peroxidation of lipids, oxidation of proteins and nucleic acids (3,9). Intermediate radicals like peroxyl radicals further react with lipids and amplify the oxidative chain reactions. Mannitol and GSH, being scavengers of hydroxyl radicals (8), were able to reverse copper-induced toxic effects to a greater extent than the other scavengers used. The exogenous supply of aminoacids and

antioxidants confers tolerance to copper in *Scenedesmus*. The isolation of mutants which overexpress the genes for the synthesis of aminoacids, intracellular antioxidants and antioxidative enzymes and which show increased tolerance and accumulation capacity is feasible and can be explored in the stategies being developed for pollution control.

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PHYTOCHELATIN INDUCTION IN PERIPHYTIC GREEN ALGAE OF THE GENUS STIGEOCLONIUM BY HEAVY METALS CONTAINED IN MINING WATER

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1. Introduction

Phytochelatins (PCs) are peptides of a general structure (γGlu-Cys)nGly, (n=2-7) synthesized during exposure of plants, some fungi and algae to some heavy metals. They sequester toxic metals, are involved in the intracellular detoxification, and could be also considered as an indicator of heavy metal bioavailability. In the present communication, the influence of water pH on the production of PCs in two strains of the green algae of the genus *Stigeoclonium* exposed to the mixture of heavy metals is shown.

2. Materials and Methods

Stigeoclonium sp. was collected from mining water containing heavy metals (17 μM), *Stigeoclonium tenue* was isolated from unpolluted lake water and cultivated under laboratory conditions. Phytochelatins were identified and determined in algal extracts, using HPLC with post-column derivatization with Ellman's reagent. Total metal content was determined by AAS, labile forms of Zn and Pb were determined by anodic stripping voltametry.

3. Results and Discussion

Both strains of *Stigeoclonium*, upon exposure to heavy metals (mainly zinc = 15 μM) contained in mining water of pH 8.2, responded by synthesizing thiol oligopeptides called phytochelatins which have been identified as a dimer (PC2) and trimer (PC3). Independently on the site of alga isolation , the studied strains produced similar amounts of phytochelatins (500 nmol -SH/g dry wt). Incubation of both algae in the slightly acidified mining water (pH 6.8) caused significant increase of phytochelatin level and appearance of longer-chain oligopeptide PC4 (Figs 1 and 2). Simultanous voltamperometric investigations showed a significant effect of pH on the content of labile forms of heavy metals (especially zinc). At pH 8.2, the concentration of labile zinc in water was 0.22 ppm, while at pH 6.8 - 0.91 ppm.

4. Conclusions

Heavy metals contained in mining waters induced the production of phytochelatins in the green algae of the genus *Stigeoclonium* . The obtained results suggest, that phytochelatin level in algal cells is connected with the concentration of the bioavailabile, labile metal fraction and that PCs can be considered as a potential biomarker and indicator of heavy metal bioavailability in aquatic environment.

Fig 1. The effect of water pH on the phytochelatin production in the alga *Stigeoclonium sp*. exposed to heavy metals contained in the water

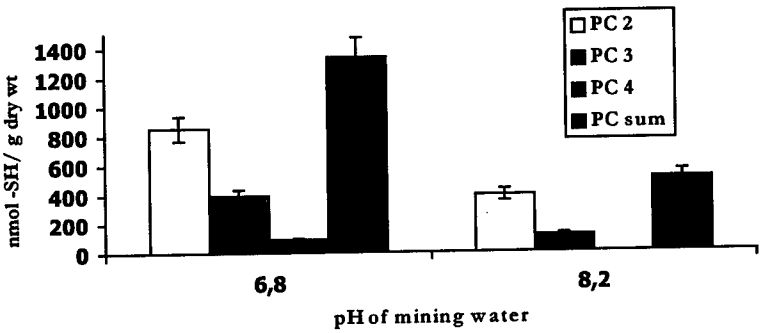
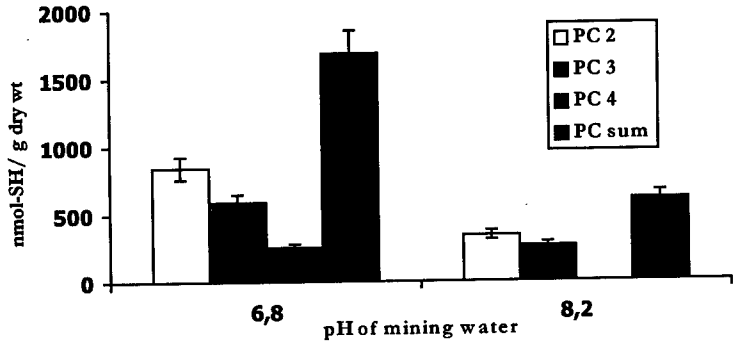


Fig 2. Phytochelatin level in *Stigeoclonium tenue* after incubation in mining water of different pHs, containing heavy metals



NON-FERROUS METAL BINDING PROPERTIES OF FERRITIN IN *VIGNA MUNGO* (L.) HEPPER (BLACK GRAM): ROLE IN HEAVY METAL DETOXIFICATION

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1. Introduction

Ferritin is ubiquitous (1) and stores iron as a polymeric hydrous ferric oxide core encased in a hollow spherical protein shell (2). This capacity is not normally utilized to the full and has enough space for other metal ions to be accommodated. Animal ferritins are known for their ability to bind non-ferrous metal ions viz. Cd, Al, Co, etc. The objective was to investigate the function of ferritin from *Vigna mungo* in heavy metal detoxification.

2. Materials and Methods

Ferritin was purified from *Vigna mungo* seeds by ammonium sulphate fractionation, ion exchange chromatography and gel filtration (3). Ferritin, thus purified, was incubated with Cd (as $CdCl_2$) and Cu (as $CuSO_4$). The UV difference spectrum was recorded against a ferritin blank. Fluorescence analysis as a function of metal binding was carried out by recording the fluorescence emission spectrum at 15 min intervals within an hour at an excitation wavelength of 286 nm. Ferritin was incubated with $^{109}CdCl_2$ (0.1 μCi) in the presence of cold Cd and analyzed by native PAGE and autoradiography.

3. Results and Discussion

Purified ferritin in *Vigna mungo* seeds showed significant binding with Cd and Cu as revealed by UV difference spectra. The spectra showed peaks at 242 nm and 290 nm and peaks at 242 nm and 275 nm, respectively. These difference spectra are generally considered to be characteristic of deprotonated tyrosine, suggesting the involvement of tyrosine residues in metal binding of the protein (4,5). Be^{2+} was bound to carboxyls of aspartic and glutamic acid residues or the hydroxyls of tyrosine residues on the protein shell of ferritin in rats which supports our results (5). *In vitro* binding assay with ^{109}Cd also showed a band corresponding to ferritin on an autoradiogram, confirming the binding ability. Ferritin from *Glycine max* was capable of binding heavy metals *in vitro* (6). Quenching of fluorescence at 340 nm due to Cd-binding to ferritin clearly points to the fact that, apart from iron storage, ferritin also chelates heavy metals *in vitro*. Unlike phytochelatins, which are inducible and chelate heavy metals, ferritins are expressed constitutively. However, in iron deficient conditions they are inducible by iron treatment.

Manipulation of genes for overexpression of ferritin would be relevant for chelation of toxic trace metals . Anaemia induced by iron deficiency is a serious health problem in various populations (7). Enhancement of the ferritin content in edible plant parts for human consumption would go a long way in treating anaemia and is of human health concern.

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METALLOTHIONEIN GENE OF *SILENE VULGARIS* INCREASES TOLERANCE OF HEAVY METAL SENSITIVE YEASTS

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1. Introduction

Metallothioneins are low-molecular-weight, cysteine-rich proteins found in plants, fungi and animals. They take part in the detoxification of non-essential metals and in maintaining homeosthasis of essential trace metals. In this study, metallothionein genes were searched for from a metal-tolerant *Silene vulgaris* (SCHAT and TEN BOOKUM 1992). The aim was to study their role in metal tolerance.

2. Materials and Methods

Screening of Silene vulgaris *cDNA library*: A lambda gt11 cDNA library was prepared from leaves of a metal-tolerant *S. vulgaris* (Imsbach population). Using *MT*2b-specific primers (sequence from GenBank) and *MT*2b plasmid DNA (ZHOU and GOLDSBROUGH 1995) as a template in PCR, a digoxigenin-labeled DNA probe was produced for screening the library. Similarly, a *MT*3 probe was prepared.

Complementation of metal-sensitive yeasts with Silene *metallothionein gene*: Metallothionein gene (*MT*) found from *S. vulgaris* was amplified with PCR from a lambda gt11 clone lysate using *MT*-specific primers containing restriction sites for cloning into pAJ401 yeast-*E. coli* shuttle vector. Recombinants were first introduced into *E. coli*, selected for ampicillin resistance and the presence of *MT* gene was confirmed with PCR. Plasmid DNA miniprep was made from a single *E. coli* colony and introduced into Cd-sensitive (JWY53, WEMMIE et al. 1994) and Cu-sensitive yeast mutants (DBY746, D. Botstein; DM771-6C, Yeast Genetic Stock Center). The first transformant selection in yeast was made by URA auxotrophy, and second by increased metal tolerance.

3. Results and Discussion

Using *Arabidopsis thaliana MT*2b probe for screening *S. vulgaris* cDNA library we found an *MT*-gene, which shares closest homology with the metallothionein gene of *Mesembryanthemum crystallinum* (Figure 1). When the gene was transferred to various Cu and Cd sensitive yeasts, a complementation of the metal sensitivity was found (Table 1). No *MT*3 clones were found.

4. Conclusions

We found one *Silene vulgaris* metallothionein gene, which complements mutations in metal-sensitive yeasts, increasing their metal tolerance.

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ZHOU, J. and P. B. GOLDSBROUGH (1995): Structure, organization and expression of the metallothionein gene family in *Arabidopsis*, Mol. Gen. Genet. 248:318-328.

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          1               15    16              30      31              45
1 ARABID.    MSCCGGSCGCGSACK     CGNGCGGCKRYPDLE      N***TATETLVLGVA
2 SILENE     MSCCNGNCGCGSACK    CGSGCGGCKMFPDFA      E*GSSGSASLVLGVA
3 M.CRYS.    MSCCGGNCGCGSACK    CGNGCGGCKMYPDMA      ENGASSTATLVTGVA
                46              60   61          75      76              90
1 ARABID.    PAMNSQYEASGETFV     AENDACKCGSDCKCN      PCTCK     77
2 SILENE     PMASYFDAEMEMGVA    TEN*GCKCGDNCQCN      PCTCK     78
3 M.CRYS.    PKISYFDNGSEMGVG    AENDGCKCGSDCKCD      PCTCK     80
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Figure 1: Alignment of amino acid sequences of *Arabidopsis thaliana Mt*2b, *Silene vulgaris MT* and *Mesembryanthemum crystallinum MT*.

Table 1: Growth of yeast mutants and *MT*-transformed yeasts in metal-containing medium. The highest metal concentrations where the yeasts grew are shown.

Yeast mutant	Untransformed yeast	MT-transformed yeast
DBY746 (Cu^S)	1 mM Cu	5 mM Cu
DM771-6C (Cu^S, Δ*cup*1)	0,5 mM Cu	1 mM Cu
JWY53 (Cd^S, Δ*ycf*1)	0,01 mM Cd	0,1 mM Cd

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RADIONUCLIDE AND HEAVY METAL DISTRIBUTION IN SOIL AND PLANTS FROM A 35-YEAR OLD RECLAIMED URANIUM MINING DUMP SITE

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1. Introduction

A compartment study on natural radionuclide and heavy metal distribution in soil and plants from a 35-year old reclaimed site on a uranium mining dump near Schlema (Saxony, Germany) is presented. Soil development on the former barren mining debris is described in relation to the radionuclide and heavy metal distribution in the soil profile and compared between two different vegetation types (stand dominated by *Alnus glutinosa* vs. *Pinus sylvestris*). This is related to plant uptake and accumulation in different parts of the trees. Conclusions are drawn for the perspective of reclamation measures taken for radiation protection with special regard to cost-effectiveness.

2. Materials and Methods

Soil was characterized and sampled under the two different stands area-related in order to calculate element storage for each horizon. Sampling points were chosen according to the plants sampled. Plants were selected randomly and divided into roots, stemwood, twigs and leaves/needles. Samples were then analysed for their radionuclide and heavy metal content by gammaspectrometry and ICP-MS.

3. Results and Discussion

After 35 years on both plots an approximately 5 cm thick organic soil layer has developed. Under the dense pine stand with no understorey the organic soil layer could be characterized as raw humus whereas under the stand dominated by alder trees with a well developed understorey a moder-like soil-type could be identified.

Within the underlying dump material (yC-horizon) activity concentrations are fairly high (530-800 Bq/kg U-238, 1025-1480 Bq/kg Ra-226 and 860-1120 Bq/kg Pb-210) but not significantly different for both stands. Compared to the dump material the radionuclide content in the organic soil layer strongly decreases towards the surface. Higher radionuclide concentrations in the lower organic soil layers may be explained by a concentrating effect due to mineralization since at least Ra-226 is thought to be very immobile. This underlines the importance of plants as „pumps" of radionuclides and heavy metals from mining material to developing humic layers. Bioturbation and dust disposal seem to play no important role at this stage. Differences of Pb-210 activity concentrations in the Of- and Oh-horizon between the alder and pine stand are ascribed to differences in humification whereas differences of Ra-226 can be attributed to different plant concentrations.

In trees highest concentrations of radionuclides and heavy metals are found in roots, lowest in stemwood. Overall low concentrations are found in the above-ground plant parts. Species specific differences can be stated with alder exhibiting slightly higher U-238 concentrations in above-ground plant parts. With respect to land-use of reclaimed uranium mining sites in this area it is concluded that forest management with timber production could be possible.

Tab.1: Percentage of activity concentration of selected radionuclides in the organic horizons compared to the dump material (yC-horizon) under stands dominated by *Pinus sylvestris* and *Alnus glutinosa*

	Stand with *Pinus sylvestris* % [from yC]	**Stand with *Alnus glutinosa*** % [from yC]
L-horizon		
U-238	3 - 4,5	4,5 -7
Ra-226	2 - 2,5	**4 - 8**
Pb-210	17 - 23	14 - 21
Of-horizon		
U-238	11 -16	11 - 21
Ra-226	5 - 10	**15 - 19**
Pb-210	**58 - 65**	33 - 46
Oh-horizon		
U-238	33 - 53	31 - 41
Ra-226	32 - 45	25 - 29
Pb-210	**66 - 70**	51 - 62

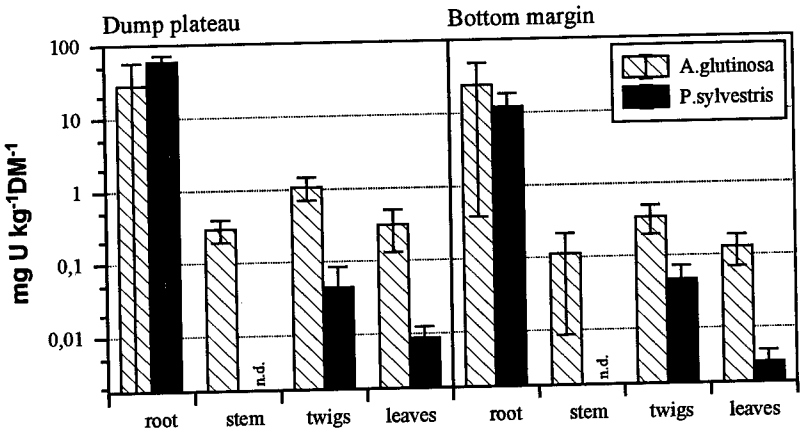

Fig.1: U-concentration in different plant parts of alder (*Alnus glutinosa;* n=10) and pine trees (*Pinus sylvestris;* n=5) on the dump plateau and at the bottom margin (logarithmic scale)

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