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LETHAL CONCENTRATIONS OF HEAVY METALS IN TISSUE OF  
EARTHWORMS (D) MONTPELLIER-2 UNIV (FRANCE)  
M B BOUCHE ET AL. OCT 87 DAJ45-87-C-0813

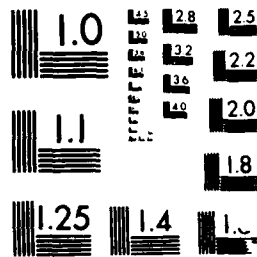
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Laboratoire de zooécologie du sol  
INRA/CNRS, CEPE, Route de Mende  
BP 5051  
F-34033 MONTPELLIER Cédex  
France

②  
Université des Sciences et Techniques  
du Languedoc  
Laboratoire de chimie analytique  
Place Eugène Bataillon  
F-34060 MONTPELLIER Cédex France

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Contract Number : DAJA 45-87-C-0013  
2n interim report  
OCTOBER 1987

→ This Toxicological research report addresses  
LETHAL CONCENTRATIONS OF HEAVY METALS IN TISSUE OF EARTHWORMS.

by

Marcel B. BOUCHE  
Patrick BRUN  
J.Y. GAL  
ABDUL RIDA A.M.

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# ERRATUM

In the first interim report, on the cover page, the last name of the authors A. REZA has to be replaced by ABDUL RIDA A.M.

Contu From P & A

# I. INTRODUCTION

Following the objectives of the contract we have presented the work in progress in the first interim report to improve

- 1) ecotoxicological test,
- 2) field procedures and
- 3) standardization of analysis.

This second interim report deals mostly with toxicity tests because the point (3) is now in order and the work in field was impossible during the last dry season. Nevertheless, analysis of soil sample are now currently in progress and most data on computer files.

~~So we chose~~ here to develop more in detail point (1) new results or state of the art in the improvement of toxicity tests.

Cadmium, Copper, Arsenic and Mercury

- 1) We have repeated the toxicity tests trying to get the LC50 for the different metals : Cd, Cu, As and Hg.
- 2) We worked out a method to analyse earthworms.

~~Thus~~ in the first part, we shall present the results of the toxicity test with their interpretations and, in the second part, we shall examine the analytical method and give results of the test-earthworms analyzed, trying to establish a relation between earthworms and artisol from which they come. Met. 2002 - FRANCE

# II. TOXICITY TEST

## 2.1. Results

The tests has already been described in the first report.

Since this report, we have carried out a second toxicity serie of tests during 14 days.

The different test concentrations and results (percentage of dead earthworms) are presented for each metal in the following table

| METAL | CONCENTRATION (ppm) OF HEAVY METAL AND CORRESPONDING DEATH RATE (%) |       |       |       |       |
|-------|---------------------------------------------------------------------|-------|-------|-------|-------|
| Cu    | 10                                                                  | 30    | 90    | 270   | 810   |
|       | 0 %                                                                 | 20 %  | 30 %  | 90 %  | 100 % |
| HG    | 1                                                                   | 6     | 36    | 216   | 1296  |
|       | 0 %                                                                 | 0 %   | 0 %   | 50 %  | 100 % |
| As    | 100                                                                 | 200   | 400   | 800   | 1600  |
|       | 0 %                                                                 | 100 % | 100 % | 100 % | 100 % |
| Cd    | 100                                                                 | 300   | 900   | 2700  | 8100  |
|       | 0 %                                                                 | 0 %   | 0 %   | 100 % | 100 % |



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## 2.2. Interprétation

Results have been expressed on a Gauss Logarithmic Diagram i.e. the different concentrations of tested substances (in mg per kg dried weight of Silica) and the corresponding percentages of dead earthworms.

The best curve is drawn among these different points. We need at least 3 points for which death rates are between 10 and 90 %.

Thus, we may find the LC50 for each tested substance (concentration of substance which kills 50 % of the test animals during the test period). We obtained LC50 only for copper : 110 ppm (see Figure 1 ) because this is the only metal for which we have 3 concentrations giving mortality rate between 0 and 90 %.

Thus, the other metals tested (Hg, Cd and As) do not allow the estimation of LC50. So we decided to make new tests with a shorter range of concentrations.

| METAL | CONCENTRATIONS IN ppm OF ARTISOL |     |      |      |      |      |
|-------|----------------------------------|-----|------|------|------|------|
| As    | 100                              | 120 | 140  | 160  | 180  | 200  |
| Cd    | 600                              | 900 | 1200 | 1500 | 1800 | 2100 |
| Hg    | 40                               | 80  | 160  | 320  | 640  | 1280 |

Nevertheless, we may deduce from the precedent results that :

- earthworms Eisenia fetida seem to be less sensitive to cadmium than to other metals ;
- arsenic (as sodium meta-arsenite) seems to be the contaminant whose range is the shortest (between 100 and 200 ppm) ;
- for mercury, the most interesting range to study, extends probably from 50 to 500 ppm.

## III. ANALYSIS

### 3.1. Mineralization of samples

Samples are earthworms.

Before mineralization, samples are dried in a oven at 105° C during 24 hours. They are weighted before and after drying to know their percentage of water (see table 1 ). Then earthworms are mineralized in the same flask (glass containers of 30 or 65 ml), in nitric acid (Merck) at 65 % in a rate of 5 ml HNO<sub>3</sub> for 100 mg dry weight. Flasks are then closed by pressure with plastic tops unattacked by acid vapours and leaved at 70° C during 24 hours. A blank is also made in the same conditions than other samples (5 ml HNO<sub>3</sub> at 70° C during 24 hours). After mineralization, samples

are diluted with deionized water in volumetric flasks of 50 or 100 ml, according to the volume of acid used for mineralization, to get an acid rate of 10 %. If there is too many solid matters in the mineralization flasks, samples must be filtered.

Finally, they are stored in polyethylene flasks.

### 3.2. Analysis

For heavy metals analysis, we use an atomic absorption spectrophotometer : Pye Unicam Sp 1900. We only can analyse Cadmium and copper among the heavy metals studied because our spectrophotometer is not equipped for mercury and arsenic (we are solving this limitation now for arsenic).

We use a hollow cathode for each metal, to create the beam and we measure the absorption of radiation by the element to analyse, which is nebulised by a flame system composed with a mixture of air and acetylene. The absorption of the radiation emitted by the cathode is proportional to the metal concentration for small concentrations.

This characteristics of these hollow cathodes are given in the following table :

| METAL | WAVE LENGTH<br>in mm | RECOMMENDED<br>INTENSITY<br>in mA | MAXIMAL<br>INTENSITY<br>in mA |
|-------|----------------------|-----------------------------------|-------------------------------|
| Cd    | 228,8                | 3,5                               | 5                             |
| Cu    | 324,8                | 3,5                               | 5                             |

After a first reading to Absorbance with the Atomic Absorption Spectrophotometer, we can prepare the standards for the different metals, in the range of absorbance measured.

As usual, the standards are prepared between 0 and 1 or 2 ppm and contain 10 % nitric acid to be similar to samples. We also store them in polyethylene flasks. The standards are used to establish the linear relation between absorbance and concentration.

For copper, we prepared standards between 0 and 1 ppm because all samples to analyse are included in this range (see Figure 2 ).

For cadmium, we distinguished 2 kinds of samples : those lower than 1 ppm and those upper than 1 ppm. Thus, we drew 2 curves for these two ranges (see Figures 3 and 4 ).

Thanks to these curves (or their equations), the absorbance reading of each sample, could be expressed as metal concentration (in mg/l) (see Tables 2, 3 and 4 ).

### 3.3. Results and Interpretations

From the heavy metal concentrations (mg/l) in the solutions

analysed by Atomic Absorption Spectrophotometer, we can calculate heavy metal quantities in mg. Then, we express these quantities as a concentration versus earthworm dried weight (see Tables 5, 6 and 7). Means values of samples are given, for each artisol concentration in tables 8 and 9.

These results can be drawn on a graph for Cu and Cd (body burden versus Artisol concentrations) ( Figures 5 and 6 ).

Copper and cadmium the earthworm concentrations increase till a horizontal asymptote.

For copper, the upper limit seems to be reached at 270 ppm Artisol Cu. This is normal because in the test 80 % of earthworms are dead for this concentration. Whereas for Cadmium, we still have 0 % of dead earthworms at 900 ppm Cd in Artisol while earthworms are initially more contaminated in cadmium than in copper.

#### IV. CONCLUSION

This investigation shows the narrow relation between metal concentrations in the soil "Artisol" and the tissues of earthworms living in this soil. It lets think that earthworms are a good bio-sampling material of soil.

At least, we notice that uncontaminated earthworms give variable results depending on the test metal.

Roughly, uncontaminated earthworms contain about 5 to 7 ppm of copper and 150 to 160 ppm of cadmium. This last level gives us a very high background. This reflect both some contamination of the rearing initial manure and also the great accumulation power of earthworm for Cadmium, associated to a low lethal susceptibility.

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A N N E X E S

TABLE I  
DATE : 22/07/87  
WEIGHTS OF EARTHWORMS  
AND PERCENTAGE OF WATER

| NUMBERS OF<br>SAMPLES | WEIGHTS OF FRESH<br>EARTHWORMS (mg) | WEIGHTS OF DRIED<br>EARTHWORMS (mg) | PERCENTAGE<br>OF WATER |
|-----------------------|-------------------------------------|-------------------------------------|------------------------|
| B1                    | 1041.80                             | 212.80                              | 79.57                  |
| B2                    | 719.10                              | 130.90                              | 81.80                  |
| Cu1                   | 527.50                              | 87.20                               | 83.47                  |
| Cu2                   | 881.70                              | 142.85                              | 83.80                  |
| Cu3                   | 962.95                              | 177.75                              | 81.54                  |
| Cu4                   | 828.50                              | 146.30                              | 82.34                  |
| Cu5                   | 478.45                              | 79.90                               | 83.30                  |
| Cd1                   | 983.35                              | 144.35                              | 85.32                  |
| Cd2                   | 835.80                              | 141.80                              | 83.03                  |
| Cd3                   | —                                   | 47.70                               | —                      |
| Cd4                   | 920.15                              | 144.85                              | 84.26                  |
| Cd5                   | 512.90                              | 93.60                               | 81.75                  |
| Cd6                   | 1013.10                             | 151.80                              | 85.02                  |
| Cd7                   | 1048.70                             | 159.80                              | 84.76                  |
| Cd8                   | 988.70                              | 166.50                              | 83.16                  |

TABLE 2  
CONCENTRATIONS OF SAMPLES

IN COPPER (in mg/l)

| NUMBER OF<br>SAMPLES | ABSORBANCE<br>SPECTRO | CONCENTRATION<br>(in mg/l) |
|----------------------|-----------------------|----------------------------|
| E1                   | 0.003                 | 0.011                      |
| E2                   | 0.005                 | 0.022                      |
| Cd1                  | 0.008                 | 0.037                      |
| Cd2                  | 0.019                 | 0.093                      |
| Cd3                  | 0.016                 | 0.078                      |
| Cd4                  | 0.072                 | 0.306                      |
| Cd5                  | 0.053                 | 0.208                      |
| Cd6                  | 0.005                 | 0.022                      |
| Cd7                  | 0.003                 | 0.011                      |
| Cd8                  | 0.001                 | 0.001                      |
| Cd9                  | 0.003                 | 0.011                      |
| Cd10                 | 0.002                 | 0.006                      |
| Cd11                 | 0.005                 | 0.022                      |
| Cd12                 | 0.004                 | 0.016                      |
| Cd13                 | 0.003                 | 0.011                      |

TABLES 3 AND 4  
CONCENTRATIONS OF SAMPLES  
IN CADMIUM (in mg/l)

RANGE LOWER THAN 1 P.P.M

TABLE 3

| NUMBER OF<br>SAMPLES | ABSORBANCE<br>SPECTRO | CONCENTRATION<br>(in mg/l) |
|----------------------|-----------------------|----------------------------|
| B1                   | 0.090                 | 0.214                      |
| B2                   | 0.232                 | 0.535                      |
| Cd1                  | 0.117                 | 0.275                      |
| Cd2                  | 0.203                 | 0.484                      |
| Cd3                  | 0.103                 | 0.300                      |
| Cd4                  | 0.173                 | 0.402                      |
| Cd2                  | 0.424                 | 0.973                      |
| Cd3                  | 0.154                 | 0.359                      |
| Cd5                  | 0.374                 | 0.859                      |

RANGE UPPER THAN 1 P.P.M

TABLE 4

| NUMBER OF<br>SAMPLES | ABSORBANCE<br>SPECTRO | CONCENTRATION<br>(in mg/l) |
|----------------------|-----------------------|----------------------------|
| Cd1                  | 0.446                 | 1.029                      |
| Cd2                  | 0.424                 | 0.968                      |
| Cd4                  | 0.544                 | 1.233                      |
| Cd6                  | 0.627                 | 1.529                      |
| Cd7                  | 0.705                 | 1.744                      |
| Cd8                  | 0.805                 | 2.020                      |

TABLE 5  
QUANTITIES OF COPPER (in P.P.M.)  
IN ANALYSED EARTHWORMS

| NUMBER OF<br>SAMPLES | VOLUME<br>(in ml) | CONCENTRATION<br>(in mg/l) | CON.VOL<br>(µg) | WEIGHT OF<br>SAMP.(mg) | Mg METAL/kg<br>DRIED WEIGHT |
|----------------------|-------------------|----------------------------|-----------------|------------------------|-----------------------------|
| B1                   | 100               | 0.011                      | 1.10            | 212.80                 | 5                           |
| B2                   | 50                | 0.022                      | 1.10            | 130.90                 | 8                           |
| Cu1                  | 50                | 0.037                      | 1.85            | 87.20                  | 21                          |
| Cu2                  | 50                | 0.093                      | 4.65            | 142.85                 | 33                          |
| Cu3                  | 100               | 0.078                      | 7.80            | 177.75                 | 44                          |
| Cu4                  | 50                | 0.366                      | 18.30           | 146.30                 | 125                         |
| Cu5                  | 50                | 0.268                      | 13.40           | 79.90                  | 168                         |
| Cd1                  | 50                | 0.022                      | 1.10            | 144.35                 | 8                           |
| Cd2                  | 50                | 0.011                      | 0.55            | 141.80                 | 4                           |
| Cd3                  | 50                | 0.001                      | 0.05            | 47.70                  | 1                           |
| Cd4                  | 50                | 0.011                      | 0.55            | 144.85                 | 4                           |
| Cd5                  | 50                | 0.006                      | 0.30            | 93.60                  | 3                           |
| Cd6                  | 50                | 0.022                      | 1.10            | 151.80                 | 7                           |
| Cd7                  | 50                | 0.016                      | 0.80            | 159.80                 | 5                           |
| Cd8                  | 50                | 0.011                      | 0.55            | 166.50                 | 3                           |

B1 and B2 : earthworms from uncontaminated "Artisol"  
Cu1 and Cu2 : earthworms from 10 p.p.m. Cu "Artisol"  
Cu3 : earthworms from 30 p.p.m. Cu "Artisol"  
Cu4 : earthworms from 90 p.p.m. Cu "Artisol"  
Cu5 : earthworms from 270 p.p.m. Cu "Artisol"  
Cd1, Cd2 and Cd3 : earthworms from 100 p.p.m. Cd "Artisol"  
Cd4 and Cd5 : earthworms from 300 p.p.m. Cd "Artisol"  
Cd6, Cd7 and Cd8 : earthworms from 900 p.p.m. Cd "Artisol"

TABLES 6 AND 7  
QUANTITIES OF CADMIUM (in P.P.M.)  
IN ANALYSED EARTHWORMS

A)  
TABLE 6

| NUMBER OF<br>SAMPLES | VOLUME<br>(in ml) | CONCENTRATION<br>(in mg/l) | CON.VOL<br>(µg) | WEIGHT OF<br>SAMP.(mg) | Mg METAL/kg<br>DRIED WEIGHT |
|----------------------|-------------------|----------------------------|-----------------|------------------------|-----------------------------|
| B1                   | 100               | 0.214                      | 21.40           | 212.80                 | 101                         |
| B2                   | 50                | 0.536                      | 26.80           | 130.90                 | 205                         |
| Cu1                  | 50                | 0.275                      | 13.75           | 87.20                  | 158                         |
| Cu2                  | 50                | 0.484                      | 24.20           | 142.85                 | 169                         |
| Cu3                  | 100               | 0.380                      | 38.00           | 177.75                 | 214                         |
| Cu4                  | 50                | 0.402                      | 20.10           | 146.30                 | 137                         |
| Cd2                  | 50                | 0.973                      | 48.65           | 141.80                 | 343                         |
| Cd3                  | 50                | 0.359                      | 17.95           | 47.70                  | 376                         |
| Cd5                  | 50                | 0.859                      | 42.95           | 93.60                  | 459                         |

B)  
TABLE 7

| NUMBER OF<br>SAMPLES | VOLUME<br>(in ml) | CONCENTRATION<br>(in mg/l) | CON.VOL<br>(µg) | WEIGHT OF<br>SAMP.(mg) | Mg METAL/kg<br>DRIED WEIGHT |
|----------------------|-------------------|----------------------------|-----------------|------------------------|-----------------------------|
| Cd1                  | 50                | 1.029                      | 51.45           | 144.35                 | 356                         |
| Cd2                  | 50                | 0.968                      | 48.40           | 141.80                 | 341                         |
| Cd4                  | 50                | 1.299                      | 64.95           | 144.85                 | 448                         |
| Cd5                  | 50                | 1.529                      | 76.45           | 151.80                 | 504                         |
| Cd7                  | 50                | 1.744                      | 87.20           | 159.80                 | 546                         |
| Cd8                  | 50                | 2.020                      | 101.00          | 160.50                 | 607                         |

B1 and B2 : earthworms from uncontaminated "Artisol"  
Cu1 and Cu2 : earthworms from 10 p.p.m. Cu "Artisol"  
Cu3 : earthworms from 30 p.p.m. Cu "Artisol"  
Cu4 : earthworms from 90 p.p.m. Cu "Artisol"  
Cd1, Cd2 and Cd3 : earthworms from 100 p.p.m. Cd "Artisol"  
Cd4 and Cd5 : earthworms from 300 p.p.m. Cd "Artisol"  
Cd6, Cd7 and Cd8 : earthworms from 900 p.p.m. Cd "Artisol"

A) RANGE LOWER THAN 1 P.P.M.

B) RANGE UPPER THAN 1 P.P.M.

TABLES 8 and 9  
Concentrations of Copper and Cadmium in Artisol and corresponding  
concentrations in earthworms

| CONCENTRATION OF<br>"ARTISOL" IN COPPER<br>in ppm | CONCENTRATION OF<br>EARTHWORMS IN COPPER<br>in ppm |
|---------------------------------------------------|----------------------------------------------------|
| 0 (Blank)                                         | 6,5                                                |
| 10                                                | 27,0                                               |
| 30                                                | 44,0                                               |
| 90                                                | 125,0                                              |
| 270                                               | 168,0                                              |

| CONCENTRATION OF<br>"ARTISOL" IN CADMIUM<br>in ppm | CONCENTRATION OF<br>EARTHWORMS IN CADMIUM<br>in ppm |
|----------------------------------------------------|-----------------------------------------------------|
| 0 (Blank)                                          | 153                                                 |
| 100                                                | 358                                                 |
| 300                                                | 453,5                                               |
| 900                                                | 552,0                                               |

Figure 1 : COPPER (Toxicity tests)

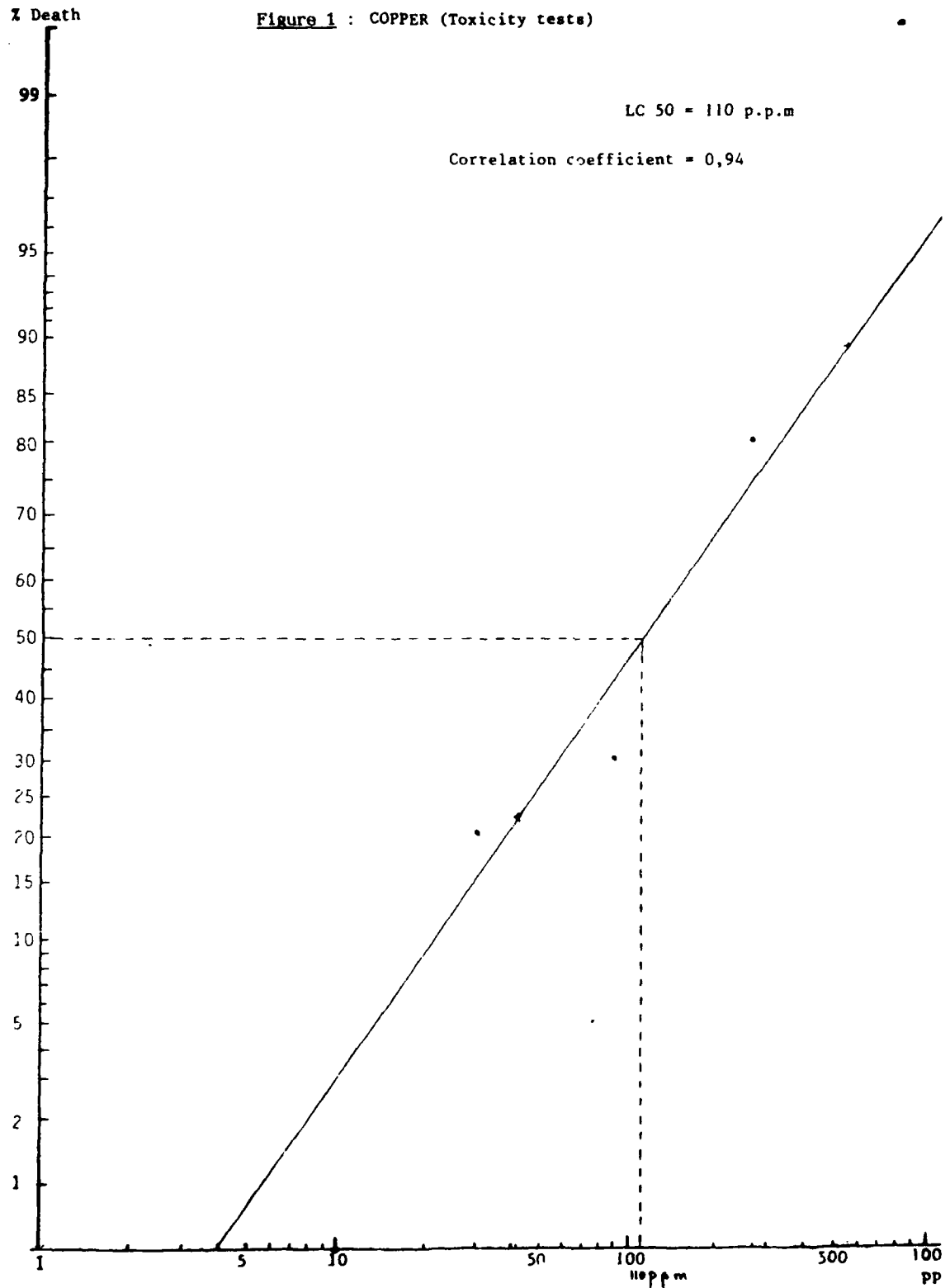
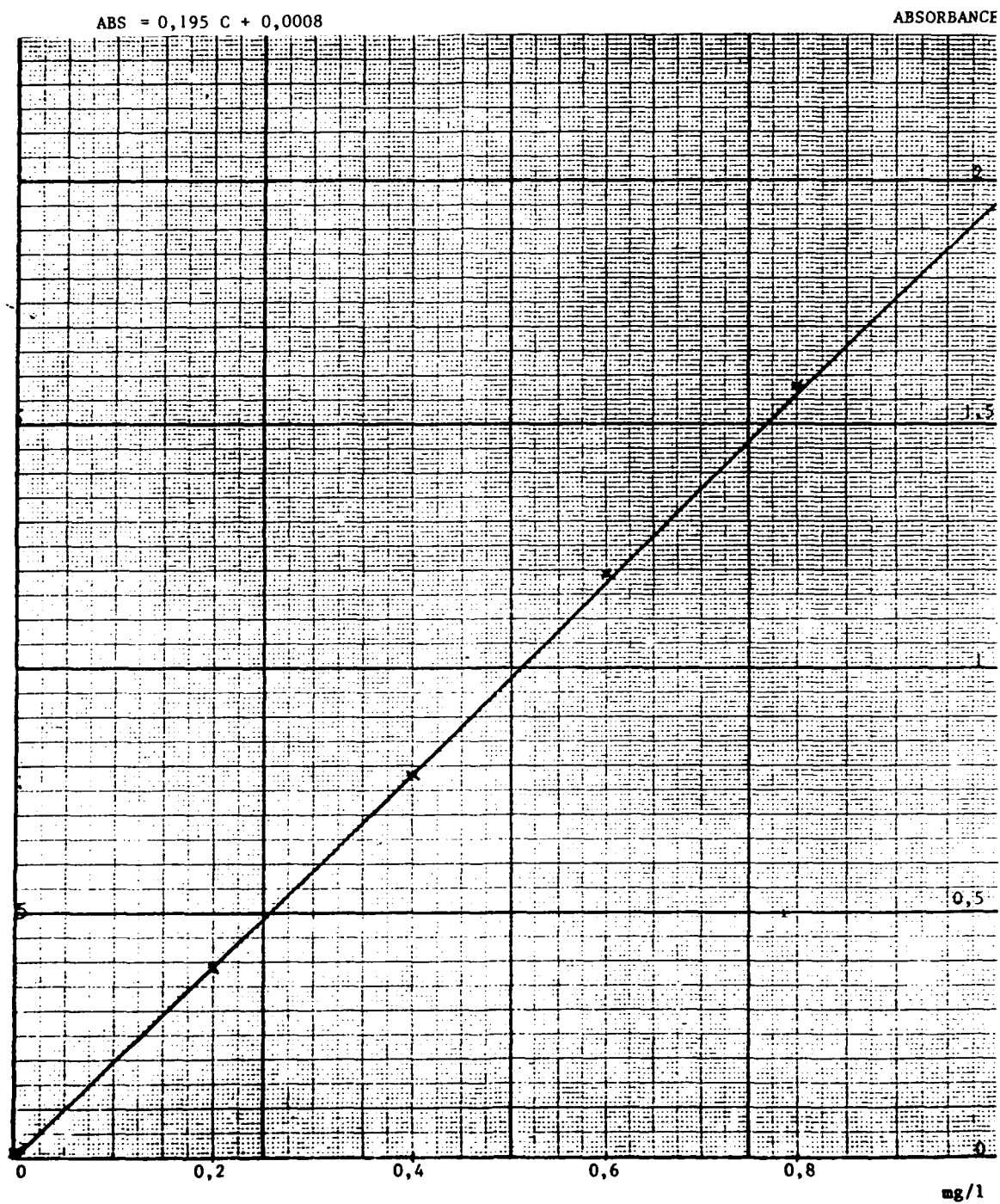


Figure 2 : COPPER (Range with  $\text{HNO}_3$  10%)



CADMIUM (Ranges with  $\text{HNO}_3$  10%)

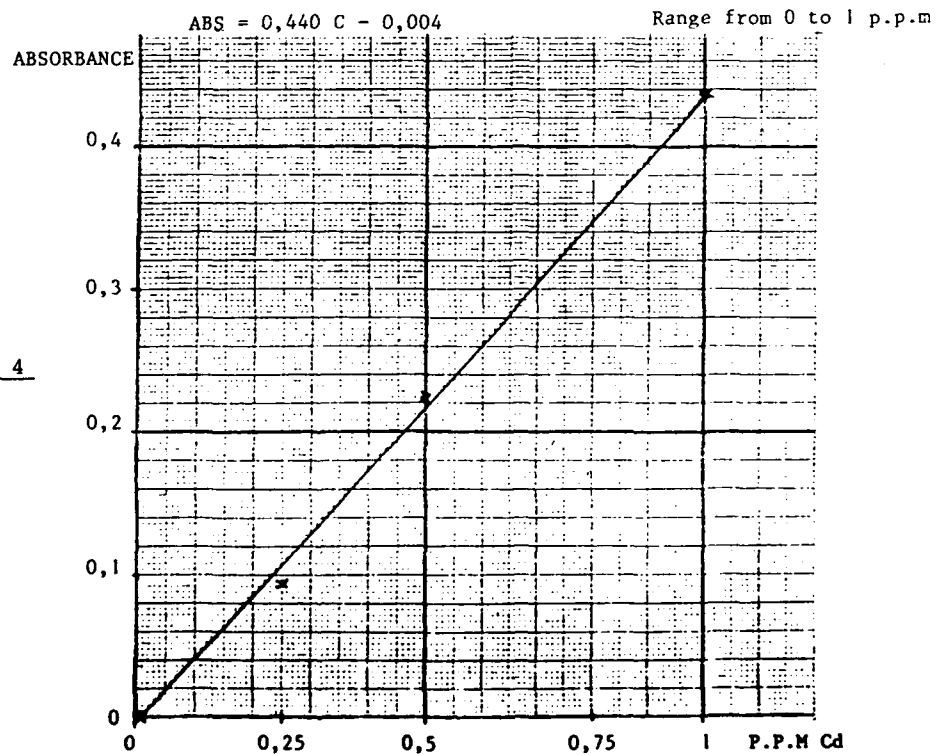
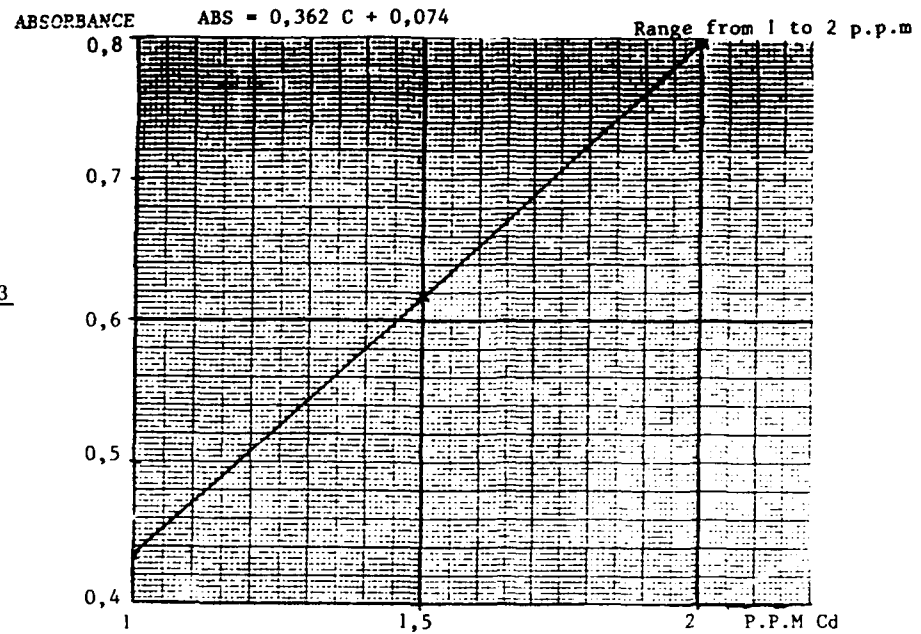


Figure 5  
Copper Concentration in Artisol and correspondent concentration  
in earthworms

$$Y = \frac{X}{4,36 \cdot 10^{-3} X + 0,456} + 6,5$$

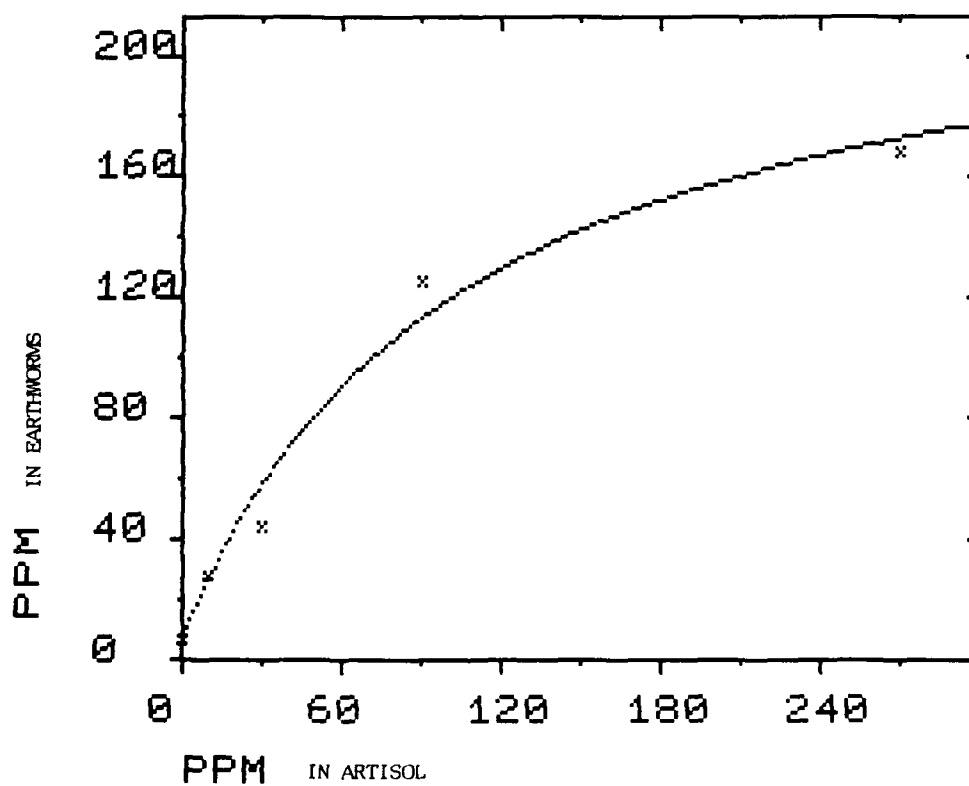
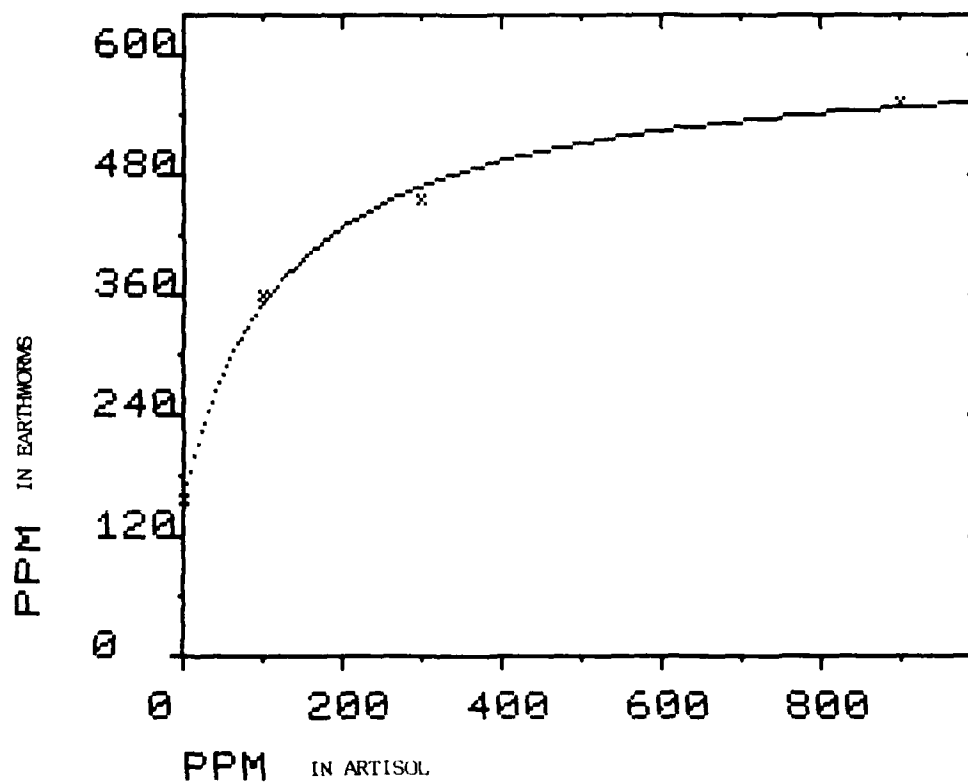


Figure 6

Cadmium concentration in Artisol and corresponding concentration in earthworms

$$Y = \frac{X}{2,23 \cdot 10^{-3}X + 0,386} + 153$$



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