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THE INITIAL STEP IN PROVIDING INFORMATION ON THE BEHAVIOR OF HAZARDOUS CHEMICALS WAS MADE WITH THE IDENTIFICATION OF LABORATORY BIOASSAY PROCEDURES. RMA HAS CHOSEN AS THE SITE TO INITIATE FIELD EVALUATION OF BIOASSESSMENT PROCEDURES. VOLUME I REPORTS ON FY 1982 RESEARCH, AND VOLUME II REPORTS ON THE REVISED PLAN FOR FY 1983.

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April 1984

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EXECUTIVE SUMMARY

Based on the results of our 1982 studies (see Volume 1 of this report), we devised a new project plan (Appendix A) for 1983. The overall goal of the plan was to demonstrate that field tests using honeybees could be useful in detecting likely areas of chemical pollution, to further demonstrate the usefulness of PNL devised laboratory bioassays (microbial and plant phytoassay) and to attempt to demonstrate a relationship between laboratory derived phytotoxicity results and field observations of plant community structure and diversity. Field studies were again conducted through a cooperative arrangement, at the U.S. Army arsenal in Commerce City, Colorado (RMA). Principal findings were:

- A comparison of microbiological bioassay results indicates that migration of Basin F (BF) water into ground water has resulted in water that is less toxic to the soil respiration assays (alfalfa mineralization and dehydrogenase) and less stimulatory to the fungal assay (sclerotia). However, sclerotia isolated from BF water containing media generally produced fewer sclerotia in subsequent germination studies.

- Based on the results of the alfalfa mineralization and dehydrogenase assays, we speculate that the release of undiluted BF water to the soil would have a long-term toxic effect on mineralization of plant material (in the absence of rainfall).

- Predicting the effects of BF water based on microbiological assays of specific chemical components can be misleading.

- It appears that the alfalfa mineralization and dehydrogenase assays can be used interchangeably in the system studied.

  Based on the compounds tested, sclerotial formation is more sensitive than mycelial growth as an assay end point. Special advantages compared to other fungal assays include no maintenance of viable stock fungal cultures and the ability to measure several end points in one experiment.

- Honeybees at Derby Lake and Basin F exhibited statistically higher (α = 0.01) brood mortality compared to hives at a control site during July and early August of 1983 (72 and 85% compared to 21%). Based on no evidence of food shortages or brood diseases, we conclude that the increased levels of brood mortality resulted from foraging bees returning contaminated nectar or pollen.

- We have shown that Basin F (BF) water used as a toxicant in Neubauer, modified Neubauer and pot culture phytoassays produces similar results. Because the modified Neubauer procedure is cheaper and safer for phytoassays of toxic chemicals in soils, we advocate its use.

- The results of an experiment to assess the toxicity of the inorganic elements, alone and in combination, found in high concentrations in BF water were negative for wheat and indicated that only part of the toxicity for lettuce can be explained by inorganic elements. Thus, a prediction of the phytotoxic properties of BF water based on an assay of specific components would be misleading.

- Phytoanalysis of soil samples collected as a function of depth during 1982 at three RMA locations, revealed that the area around a waste ditch in Basin A was phytotoxic and exhibited a pattern that could tenuously be associated with Kochia size and abundance. In addition, we found lettuce seeds to be much more sensitive to the toxicant(s) in these soils.

- Phytoassay of logarithmically spaced soil samples collected from four equally spaced transects in 1983, indicated that toxic material had migrated from the 0 - 15 cm fraction to >15 to 30 cm. This observation was also supported by a kriging analysis of the data. Moreover, using our kriging results, and 30% lettuce seed mortality as a site cleanup criterion, we showed where soil should be removed. Unfortunately, the cleanup decision would be different for the 0 - 15 cm and <15 to 30 cm fractions. Our maps simply indicate that the field situation is complex and that cleanup based on the <15 to 30 cm map would remove all the contamination we know of. However, samples were not taken below this depth.

- As expected, isolated areas of contamination were observed in Basin C using the lettuce seed phytoassay. While assay of all samples could not be completed because of financial limitations, it appears that the systematic grid was effective in locating "hot spots." Using single Basin F and Basin A soil sample, we found LD-50s of 2.5 and 70% soil respectively.

- Results from the plant cover analyses in Basin A indicated a possible toxic gradient. The plots least impacted were furthest from the waste ditch while the most impacted were near or in the ditch. These results also indicated that a multivariate analysis of plant cover may be a useful rough guide in separating areas with relatively high levels of impacts (contaminants) from unimpacted areas.

- Based on a dendrogram of vegetative composition and cover and the kriging estimate of 30% lettuce seed mortality in Basin A soils, we demonstrated a qualitative correspondence of the two data sets (0-15 cm soil fraction only). While our result could be fortuitous and is not definitive, we believe that because the lettuce seed bioassay, the vegetative cover measurements and analyses, and the interpretation of the kriging analyses of lettuce seed mortality were carried out by four different investigators, there is little chance for any bias in the result.

ACKNOWLEDGEMENTS

The cooperation of Dr. Dave Thorn, Dr. Bill Trautman and Mr. Brian Anderson at the Rocky Mountain Arsenal is again acknowledged. Without their help, this research would not have been possible. Mr. Bart Utley from Madhava Honey Ltd., Longmont, Colorado helped with hive placement and field work associated with the honeybee study, while Steve Aulenbach and Maureen O'Shea-Stone of the University of Colorado aided with soil sampling and plant cover measurements. At the Pacific Northwest Laboratory the technical contributions of Marji Cochran, Mary Jo Harris, and Shu Mei Li contributed to the successful completion of this work. Rene Hinds edited the report and Gail Poole typed the manuscript.

CONTENTS

TABLES

FIGURES

1.0 INTRODUCTION

The original statement of work for the project as well as the research task plan for 1982 are in Volume 1, Appendix B and Section 1.0, respectively. Based on the research conducted in 1982, a revised project plan for 1983 was prepared (Appendix A) and a subsequent series of research tasks outlined. These research tasks were to be somewhat flexible in that additional field site selection and expenditures of limited funds could be redirected to reflect research needs as they evolved.

The following research goals were to be addressed during FY 1983 (comments follow to indicate where changes were made during the studies to take advantage of evolving research results):

1. Find and characterize (via phytoassay) a hazardous waste site at Rocky Mountain Arsenal (RMA). Since phytoassays were to be run first, the subsequent survey of plant community structure and diversity would be conducted on a known contaminated area. In 1982 we attempted to do both tasks concurrently. We were unable to establish a relationship between bioassay and field measurements because little phytotoxicity was found.

2. Demonstrate the usefulness of the Neubauer phytoassay, characterize sites using the technique and perform an experiment to determine if copper, arsenic, nickel or sodium (alone or in combination) might explain the phytotoxicity of F-Basin water to wheat seeds (observed in 1982).

3. Study the usefulness of honeybees and/or pollen as fate and effects biomonitors at hazardous waste sites.

4. Further develop and demonstrate the usefulness of the dehydrogenase and sclerotial bioassays as an adjunct to or replacement for the EPA CO_2 bioassay.

Both some unbudgeted needs and field circumstances caused us to redirect our efforts during the year. First, there were higher than anticipated costs to produce volume one of this report and an unbudgeted peer review of the entire project in Corvallis. In the field, an extra trip to Denver had to be scheduled in part because of the very cold and snowy spring and a lack of much phytotoxicity in our primary field site (Basins C, F, D). After we established and sampled a secondary field site in Basin A, we devoted most of our remaining funds to studying that site.

Because of these extra expenses, phytoassay of the primary site was terminated and a complete assay (using lettuce seeds) of the secondary site completed. Funds from the chemistry task and technical review were also used to cover some of the unanticipated costs. In spite of these problems, the results reported herein are in most cases definitive and complete. Finally, we were able to expand the "hot spot" task (04-01, Appendix A and described in Appendix B) using additional funds from a Nuclear Regulatory Commission contract, and prepared a journal paper on the Neubauer technique. Both documents are available on request. Additional journal papers on the microbiological (Section 3.0) and intercomparison (Section 7.0) aspects are planned.

2.0 ADDITIONAL SITE SELECTION AND SOIL SAMPLING

2.1 INTRODUCTION

In the statement of work for 1983 (Appendix A) several tasks and subtasks were outlined. Exactly which were to be done and their respective order depended on available funding (i.e., we would spend more of the available funds on the most promising tasks, as data accumulated) and our ability to discover field sites at Rocky Mountain Arsenal (RMA) where soil phytoassays revealed contamination.

Based on the statement of work, we attempted to select sites at RMA for the following three purposes:

- to relate results of plant field surveys to soil phytoassay results
- to provide soil samples with a high likelihood of contamination for phytoassay
- to aid in evaluating the feasibility of group-testing procedures to detect chemical hot spots.

The last objective was abandoned when we learned that complete chemical analyses would be available for two samples only. As a result, funding for phytoassays was devoted to meeting our first objective. However, the basis for our third objective is included as Appendix B.

2.2 STUDY SITE SELECTION AND ESTABLISHMENT

During 22-25 February, 1983, a 400 m x 400 m (16 ha, ~40 acres) study plot was established in section 26 at RMA. The northeast corner of the study plot was located in the southwest corner of F-Basin. The remainder of the plot included the northwest corner of C-Basin, the northern tip of D-Basin and a relatively undisturbed region (Figure 2.1). Thus, the plot included areas known to have high levels of chemical contamination (Basin F), as well as areas with possible intermediate contamination (Basins C and D) and little or no contamination (control area; see Figure 2.2).

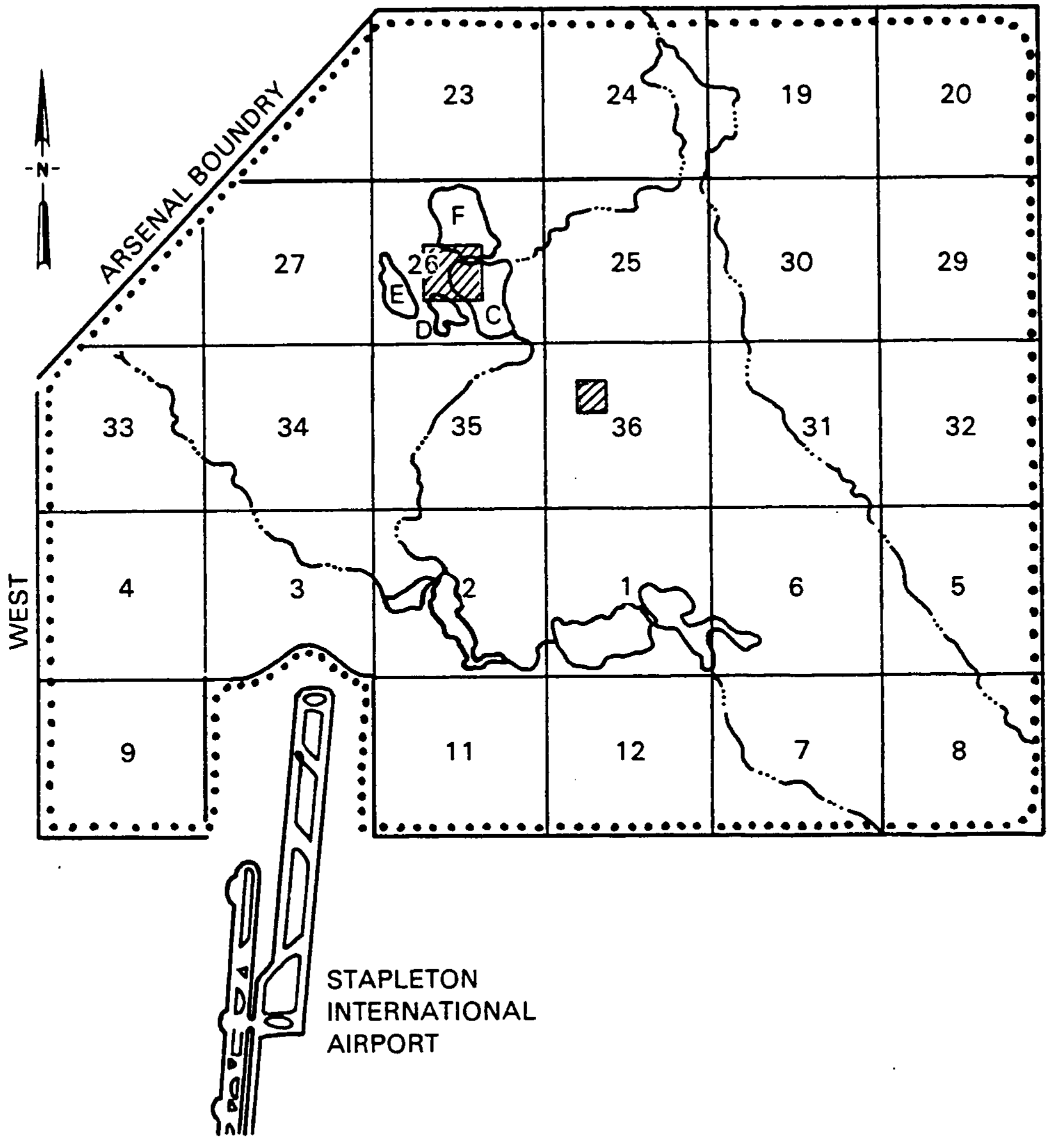

FIGURE 2.1. Location of Plots for Soil Samples and Vegetative Cover in Sections 26 and 36

We did not take advantage of our prior knowledge about likely contamination in the sampling design for two reasons. First, we wished to test the ability of our phytoassay to "discover" contamination; and, second, we wanted to use the results from a systematic design to evaluate the use of stratified designs. The grid consisted of 100 sample locations in a 10 x 10 design, each placed 40 m apart (Figure 2.2).

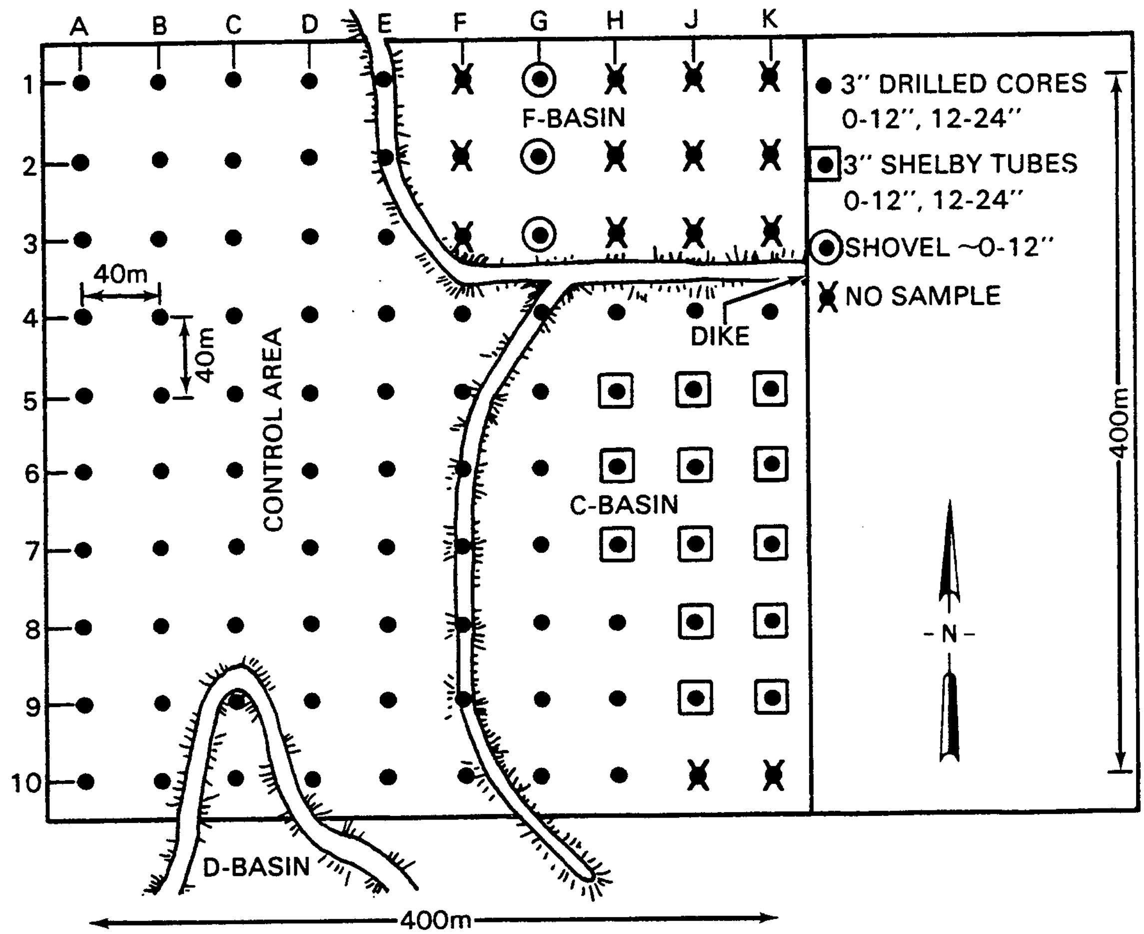

FIGURE 2.2. Location of Systematic Sampling Points in Section 26 at the Rocky Mountain Arsenal

Each sample point was located using a compass and meter tape and then marked with a stake. East-west coordinates were designated A through K; north-south coordinates were labeled 1 through 10. Fifteen sample points, numbers 1 through 3 in transects F, G, H, J and K, fell within Basin F. Basin C contained 27 sample locations: numbers 4 through 10 in transects H, J and K, and 3 through 10 in G. Sample numbers F6 through F9 and E1 through E3 were on dikes or roads. Two sample points, C9 and C10, fell within Basin D. The area with no known contamination (control) contained the remaining 48 points: F4, F5, and F10; transects A, B and D, numbers 1 through 10; transect C, numbers 1 through 8; and transect E, points 4 through 10.

Subsequent discussions at our laboratory resulted in the decision that extensive sampling in Basin F would be very difficult and expensive. In addition, since there was no vegetation in Basin F, it was obvious that plant gradients did not exist, and that the results of plant germination bioassay tests on soils would likely result in 100% mortality. Thus, we decided to sample only three points in Basin F and to augment the sampling scheme by establishing a second plot in section 36, Basin A (Figure 2.1).

Previous phytoassay results (October 1982; see Section 5.3.2) indicated a possible gradient of contamination on the west side of section 36, extending north-south from a trench that drains Basin A and runs to the west. Thus, on June 22, 1983, four parallel transects were established on the west side of Basin A, each beginning on the north bank of the trench and running south for ~90 m. A logarithmic scale was used beyond the south trench edge because we thought contamination might have moved by some physical means (e.g., wind or water). The transects were 15 m apart and labeled L, M, N and P. The first three sample points of each transect fell within the trench and the fourth was on the top of the south bank. Sample numbers 5 through 9 were 15, 20, 30, 50 and 90 m, respectively, south of the north trench edge (Figure 2.3). Each of the 36 sampling points was marked with a stake.

2.3 SOIL SAMPLING

Fox Drilling Company (Denver, Colorado), was hired to do most of the soil sampling. At each sampling point, a split spoon was used to take two soil cores, 3 in. in diameter, one from 0 to 15 cm depth, and a second from 15 to 30 cm. Together, these cores weighed approximately 4 kg. Between sampling points, the split spoon and drill bit were decontaminated by washing with methanol and rinsing with distilled water. All samples were put in plastic bags, sealed and labeled. The area being sampled and any problems encountered (e.g., mud, accessibility) dictated exactly how the cores were taken and variations on the basic sampling scheme.

In Basin A, the first two points in each transect were in the trench, which was very wet and soft, and the samples from these points were difficult

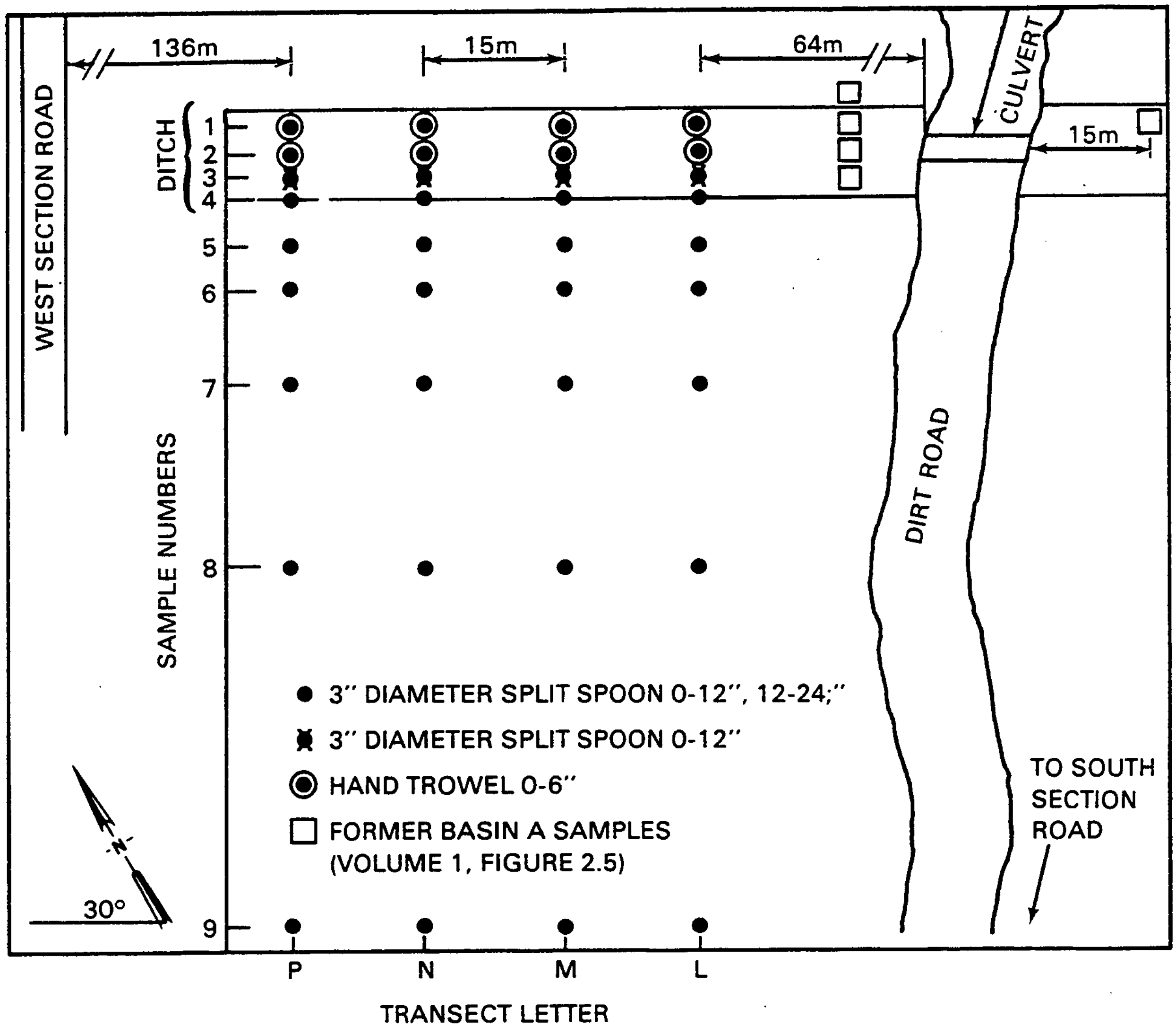

FIGURE 2.3. Location of Logarithmic Sampling Points in Section 36 at the Rocky Mountain Arsenal

to obtain. It was impossible to sample by depth; two surface samples (to ~6 in. deep) were taken from these points using a hand trowel. A split spoon with a 3-in.-diameter bit was mounted on a hydraulic drill rig and used to take most of the other samples in Basin A: points 4 through 9 in transects L, M, N and P. Sample points L, N and P-3 were just over the south bank of the trench and could not be reached from the drill rig. At these points, the split spoon was hammered into the ground and extracted by hand. Only a 0-15 cm sample was obtained from each of these points; the soil from 15 cm to 30 cm was too wet to stay in the split spoon.

A surface sample of undefined depth was taken from M-3 since the entire profile was very wet. The Basin A samples taken with the split spoon were collected on June 23, 1983. The hand-collected samples were taken on June 24.

Because of high levels of contamination, only three samples were taken from Basin F. Soil to a depth of about 15 cm (there was a liner at ~20 cm) was collected with a shovel at points G1 through G3 (Figure 2.3). Each 40 lb sample was packaged in a plastic bucket and labeled. Samples were collected on June 22, 1983.

All samples in the control area were taken using the split spoon and the hydraulic drill rig on June 23, 1983.

The hydraulic drill rig could not be used in Basin C because of the soft surface and uneven terrain. In this area, two sampling methods were used. A 230-lb hammer, suspended from an A-frame mounted on a sled and pulled by a pick-up, was used to pound the split spoon into dry hard-packed areas. The core was extracted by hand or with the aid of a high-lift jack. In soft or wet areas, samples were taken using Shelby tubes (hollow stainless steel tubes). These were pounded in by hand using a 12-lb sledge hammer, and retrieved with a high lift jack. Samples were not taken from points J10 and K10; the soil was too sandy to stay in the Shelby tubes or the split spoon.

3.0 MICROBIOLOGICAL BIOASSAYS

3.1 INTRODUCTION

Microbiological bioassays were designed to serve two basic purposes. During the first year (see Volume 1, Section 7.0), experiments were conducted to assess the feasibility of using Basin F water to contaminate RMA soil and to supply information for the design of a proposed field study. In a second and overlapping effort, we examined the utility of microbiological assays to assess the potential environmental impact of hazardous wastes sites. For this effort, we conducted a laboratory study to determine how Basin F (BF) water and Basin F well (BFW) water would affect the production of CO_2 from alfalfa amended soil collected from the proposed RMA study site. We also examined the effects of the waters on two microbiological assays, soil dehydrogenase and sclerotia formation. Interpretations were based partially on examinations of the sensitivities of the soil dehydrogenase and sclerotia assays to selected chemical components present at high concentration in BF water.

3.2 MATERIALS AND METHODS

3.2.1 Soil

The soil sample was a composite of individual 15.2 cm^3 samples taken from 39 randomly selected squares within a site located in Section 24 of the Rocky Mountain Arsenal (Volume 1, Section 2.1.2 and Figure 2.1). Upon receipt at the Pacific Northwest Laboratory, the soil was air-dried and sieved (2 mm).

3.2.2 Collection of Basin F Water and Basin F Well Water

Basin F water was collected from a sample of Basin F water that had been collected in June of 1982 and stored in a steel barrel for six days. Our sample was transferred to a 30 L teflon-lined beer keg. Basin F well water was collected from well 26008 at RMA by bailing and transferring the water to a 30 L teflon-lined beer keg. The water samples were shipped to the Pacific

Northwest Laboratory, where they have been stored at ambient room temperature.

3.2.3 Chemicals

All chemicals used are analytical reagent grade and were used without further purification. Ammonium sulfate, zinc sulfate, sodium sulfate, nickel (II) sulfate and sodium borate were obtained from J. T. Baker Company. Sodium fluoride was obtained from Aldrich. Copper (II) sulfate was obtained from Fisher Scientific and magnesium sulfate from MCB Manufacturing Chemists. Sodium arsenate, 2,3,5-triphenyl tetrazolium chloride (TTC) and 2,3,5-triphenyl tetrazolium formazan (TTC-formazan) were purchased from Sigma. Potassium nitrate, sodium phosphate dibasic and potassium phosphate monobasic were obtained from Mallinckrodt. Glucose, yeast extract, casamino acids and agar were purchased from Difco Laboratories.

3.2.4 Characterization of BF Water and BFW Water

Water samples were analyzed for pH, Eh, conductivity, dissolved organic carbon (DOC), inorganic carbon (IC), ammonium ion, and other trace anions and cations. In the laboratory, pH was measured with an Orion Model 611 meter with a 810200 (Ross) combination probe; Eh was measured with Markeson 1220 probe combined with an Orion 611 meter. Conductivity was measured with a Beckman RC-20 meter. DOC and IC were quantified using a Dohrmann DC-80 carbon analyzer; ammonium ion, using an Orion-95-10 Or HNU Model ISE-10-10-00 ammonium ion selective electrode coupled to an Orion-701 meter. The anions, sulfate and chloride, were quantified with a Dionex Model 16 ion chromatograph with conductivity detection, while cations were quantified by inductively coupled plasma emission spectrometry.

3.2.5 Alfalfa Mineralization Assay

Air-dried, sieved soil (2 mm) combined with dry alfalfa (1% w/w, for chemical analysis; see Volume 1, Table 7.1) was mixed until homogeneous in a V-blender. Subsamples (400 g) of the homogenate were added to reaction vessels and the soil moisture was adjusted in each case to 60% of field capacity by the addition of a test solution. Test solutions consisted of 100, 10, 1.0, 0.1, and 0% of either BFW water or BF water. After addition of the test solutions, reaction vessels were incorporated into an aeration apparatus that supplied moisture and CO_2-free air to each vessel. Carbon dioxide was subsequently trapped by passing offgases through a 0.75 N NaOH trap. The amount of CO_2 in the trap was measured periodically using an oceanographic carbon analyzer operating in the inorganic mode.

3.2.6 Soil Dehydrogenase Assay

Soil dehydrogenase activity was assayed by the method Klein et al., (1971) modified by our research team. Assays were initiated by adding 0.5 mL of a 0.5% (w/v) glucose solution which did or did not (control) contain a test compound, to 25 mL centrifuge tubes containing 1 g of air-dried alfalfa-enriched or unenriched soil, which had been amended with 0.2 mL of a 3% (w/v) solution of TTC. To account for compounds in the assay mixture which absorbed at 485 nm, a reagent control was prepared by replacing the TTC solution with distilled water. Assay and control tubes were incubated at 27°C in the dark. At the end of 24 hr, 10 mL of methanol was added to each of the tubes to extract TTC-formazan formed during the incubation. The tubes were mixed well, the liquid fraction decanted from the soil, and the absorbency of the resulting solutions was determined at 485 nm with a Spectronic 20 colorimeter. Soil dehydrogenase activity, expressed as µg TTC-formazan produced per g soil per 24 hr, was quantified by comparing adsorbence values to a standard curve, prepared with reagent grade TTC-formazan, ranging from 0 to 30 µg per mL methanol. All assays were performed in duplicate.

Enriched soil was prepared by combining 400 g of the composite soil sample from RMA with 4 g dry alfalfa, adding water to 20% of field capacity and incubating the moist soil mixture at room temperature in the dark. After 6 days incubation, the soil mixture was air-dried and stored in plastic bags at room temperature until needed.

3.2.7 Sclerotia Assay

A single mature sclerotium was placed in the center of each of the three replicate nutrient agar plates containing the test compound. Three control plates containing the same nutrient base, but no test compound, were also included. All inoculated plates were incubated (10 to 14 days) at 25°C until mycelial growth had reached the edge of control plates. At this time the diameter of the mycelial mat was measured and sclerotia were counted. Observations were made on mycelial morphology and on the size and shape of the sclerotia.

The sclerotia formed in these plates were subsequently collected, washed with sterile distilled water to remove attached mycelia, air-dried overnight, and then examined for their ability to germinate and grow on an uncontaminated agar medium. In this assay, nutrient agar plates were inoculated with 89 sclerotia placed 0.5 in. apart and incubated at 25°C for 14 days. During this period, mycelia from each of the inoculating sclerotia would grow radially until they intercepted mycelia from adjacent sclerotia, whereupon growth would terminate. Growth in this manner resulted in the formation of 89 0.25 in. square minicolonies. We recorded the number of sclerotia that germinated and the number of sclerotia formed within the individual colonies.

3.2.8 Growth Medium

Agar medium contained (per liter): 1 g KNO_3, 0.5 g $MgSO_4$, 0.71 g Na_2HPO_4, 10.21 g KH_2PO_4 (or 80 mM phosphate buffer), 2 g glucose, 5 g casamino acids, 1 g yeast extract and 15 g agar. Test compounds were dissolved in distilled water and the solutions sterilized by filtration (0.22 μ membrane) before addition to the liquid agar medium, which had been maintained at 47°C. Before addition, the sterile solutions were warmed to 47°C to insure proper mixing. Immediately following the addition of a test compound, the medium was dispensed to 105 x 10 mm plates. To avoid accumulation of excess moisture on the agar surface, fresh agar plates were incubated overnight at 25°C before plates were used in toxicity assays.

3.2.9 Isolation of Seed Sclerotia

Mature sclerotia were isolated from 14-day-old agar cultures of an *Aspergillus* species isolated from soil. Mature sclerotia were harvested by irrigating 2-week-old cultures with sterile distilled water and dislodging the sclerotia with a rubber spatula. After harvesting, the sclerotia were

TABLE 3.1. Chemical Analysis (ppm) of Basin F Water and Basin F Well Water

Constituent	Basin F	Basin F Well Water
pH	6.25	7.22
Eh	+273.4	+2.95
Conductivity (μmhos)	52610	26300
Color	black	light yellow
C (organic)	67770	0.0843
C (inorganic)	2667	480
NH_4^+	27200	130
SO_4^{2-}	51200	5060
Cl	106167	8980
B	16.8	1.61
Na	89600	8320
As	16.8	1.13
P	11500	244
Cu	3620	0.05
Ni	31.6	1.38
Zn	25.7	0.15
F	6500	88
Mg	95	730
Ca	105	744
Sr	3.55	20

washed with sterile distilled water until mycelia on sclerotia surfaces were undetectable; they were then air-dried. Sclerotia prepared in this manner can be stored at room temperature for at least one year without loss of viability.

3.3 RESULTS

3.3.1 Characterization of Basin F Waters

Both BF water and Basin F well water were analyzed for pH, Eh, conductivity, DOC, IC, ammonium ion, and major trace anions and cations (Table 3.1; the table and the following discussion as well as a small portion of 3.3.2 are also in Volume 1, Section 7.0, and are repeated here to provide continuity). Basin F water was slightly reduced in pH (6.25 vs. 7.22), but was considerably more aerobic (+273.4) than the reduced BFW water (+2.95). The conductivity of BF water was twice that of the well water; however, this was not reflected in the concentrations of major anions and cations, which were generally 10 times higher. This discrepancy is probably a result of the exceedingly high organic carbon and salt concentrations of BF water, which could lead to the formation of ion pairs metal organic complexes, thus reducing the conductivity.

The high ratio of organic carbon to phosphorous for both waters would indicate that the major organic contaminant may be diisopropylmethylphosphonate, a major waste product at RMA.

The concentrations of several anions and cations in BF water were exceptionally high (ppm); NH_4^+, 27200; SO_4^{2-}, 51200; Cl^-, 106000; Na^+, 89600; As, 16.8; Cu, 3620; F, 6500. Basin F well water showed high levels of SO_4^{2-}, Cl^-, Na^+, Mg^{2+}, Ca^{2+}, and Sr^{2+}. The high concentration of Mg^{2+}, Ca^{2+}, and Sr^{2+} in BFW water presumably resulted from high Na^+ in BF water, which has entered the ground water system and replaced these elements on the substrata, increasing their soluble concentration.

3.3.2 Inhibition of CO_2 Production from Alfalfa-Amended Soil

The breakdown of organic matter to replenish the limited supply of CO_2 available for photosynthesis is generally considered the most important function of soil microorganisms. Carbon comprises approximately 40 to 50% of the dry weight of plant tissue. When plant tissue is mineralized by microorganisms, O_2 is consumed and CO_2 is liberated, in accordance with the following equation:

$$(CH_2O)_x + O_2 \rightarrow CO_2 + H_2O + \text{intermediates} + \text{cellular material} + \text{energy}$$

Generally, only 60 to 80% of the carbon is liberated as CO_2 because of incomplete oxidation and synthesis of cellular and intermediary materials. The quantities of CO_2 evolved will depend on the type of substrate, the environmental conditions, and the microorganisms involved. In the following experiments, we have examined the effect of two potentially hazardous waters on the mineralization of a plant tissue, alfalfa, to CO_2.

Inhibition of the initial rate of the mineralization of alfalfa to CO_2 was only observed with the two highest concentrations of BF water and the highest concentration of BFW water (Table 3.2). Essentially complete inhibition was observed with 100% BF water. A 10-fold dilution of BF water

TABLE 3.2. Effect of Basin F Water and Well Water on Soil Respiration as Measured by Alfalfa Mineralization and Dehydrogenase

Sample	Concentration (%)	Rate CO_2 Production (% Control)	Total CO_2 Produced[a] (% Control)	TTC-Formazan[b] Produced (% of Control)
Control	0	100 (0.95)[c]	100 ± 9[d]	100 ± 18
Basin F Water	100	2 (0.77)	7 ± 0.4	36 ± 8
	10	61 (0.82)	93 ± 9	293 ± 76
	1.0	99 (0.85)	87 ± 15	82 ± 10
	0.1	117 (1.00)	107 ± 7	222 ± 63
Basin F Well Water	100	63 (1.00)	87 ± 6	233 ± 54
	10	99 (0.90)	100 ± 9	272 ± 64
	1.0	97 (0.87)	107 ± 10	226 ± 29
	0.1	84 (0.87)	93 ± 15	250 ± 74

[a] Total CO_2 produced in 47 days.

[b] This analysis was conducted after termination of the alfalfa mineralization study at 47 days.

[c] R^2 values, from linear least squares regression for days 1-4.

[d] Mean ± standard deviation, n=3.

resulted in 40% inhibition. A 40% inhibition was also observed with 100% BFW water. No inhibition was observed with either BF water diluted 100 or 1000-fold or BFW water diluted 10, 100 or 1000-fold. After 47 days of incubation, only 100% BF water showed reduced levels of total CO_2 produced (Table 3.2). Apparently, the decrease in the initial rate of degradation observed for the 10% BF water or 100% BFW water did not affect the extent to which the degradation of alfalfa would occur during the entire 47 day incubation period.

After 47 days incubation, the alfalfa mineralization experiment was terminated and the soils were subsampled to determine soil dehydrogenase activity. For the majority of test solutions, including all four BFW water concentrations, an approximate 2-fold enhancement of dehydrogenase activity was observed compared to control values (Table 3.2). A 60% reduction in dehydrogenase activity for 100% BF water was found.

TABLE 3.3. Effect of Basin F Water and Well Water on the Soil Dehydrogenase Activity

Sample	Concentration Added (%)	TTC-Formazan Produced (% of Control) Enriched Soil[a]	Unenriched Soil[b]
Control	0	100 ± 20[c]	100 ± 20
Basin F Water	100	5 ± 1	30 ± 4
	10	12 ± 2	6 ± 1
	1.0	101 ± 15	166 ± 25
	0.1	93 ± 17	109 ± 17
Basin F Well Water	100	111 ± 19	89 ± 13
	10	101 ± 22	129 ± 20
	1.0	91 ± 15	116 ± 18
	0.1	107 ± 17	123 ± 19

[a] Preincubated with alfalfa (see Materials and Methods).

[b] Soil not preincubated with alfalfa.

[c] Mean ± standard deviation, n=3.

3.3.3 Inhibition of Soil Dehydrogenase Assay by Basin F Water and Specific Chemical Constituents of Basin F Water

Dehydrogenases are the major representatives of the class of oxido-reductase enzymes. They catalyze the oxidation of substrates by transfer of electrons through the electron transport system (ETS). Specific dyes such as triphenyl tetrazolium chloride (TTC) can be used as indicators of ETS activity. TTC is reduced to formazan, an insoluble red precipitate, during the process. The dehydrogenase activity test, using TTC, has been used with soils (Casida et al., 1964 and Schaeffer, 1963) and a significant correlation between TTC activity and soil respiration has been reported (Stevenson, 1959 and Stevenson, 1962).

Comparisons of BF water on soil dehydrogenase activity in enriched and unenriched soil reveal a similar inhibition pattern for both soils (Table 3.3). Basin F water was inhibitory at concentrations of 100 and 10%. Basin F well water did not inhibit dehydrogenase activity.

The sensitivity of the dehydrogenase assay to several major inorganic components of BF water indicated that Cu^{2+} (Table 3.4) alone could account for the inhibition of the original waters (Table 3.3). Of the inorganic components tested, the cations were generally the most inhibitory (Table 3.4). Cupric ion was the strongest inhibitor, followed by $Ni^{2+} > Zn^{2+} > NH_4^+ > Mg^{2+}$. Arsenate was the most inhibitory of the anions (Table 3.5), falling between Cu^{2+} and Ni^{2+}. Of remaining anions, borate was inhibited at concentrations > 500 ppm, fluoride showed no effect at concentrations up to 5,000 ppm, and sulfate was inhibitory at concentrations > 25,000 ppm. No major differences were found between enriched and unenriched soil.

3.3.4 Inhibition of the Sclerotia Assay by Basin F Water and Specific Chemical Constituents of Basin F Water

In some fungi, hyphae become interwoven to form small hyphal aggregates. These hyphal aggregates have the ability to resist adverse conditions for longer periods than the ordinary mycelial hyphae. When sclerotia-forming fungi are maintained on solid medium, sclerotia are formed in concentric circles as the fungal mycelia radiate out from the initial point of growth. We have used this property of a sclerotia-forming species of *Aspergillus* to develop a bioassay to measure the effects of potential toxic compounds or mixtures on mycelial growth, sclerotia formation and sclerotia germination. In this study, the effects of BF and BFW water and specific major components of these waters on mycelial growth and sclerotial formation were examined.

No apparent effect of these waters on mycelial growth was observed; however, enhanced sclerotia formation was observed at some concentrations (Table 3.6). Enhancement was especially evident with BF water where a 5% (v/v) test solution caused a 2-fold increase in sclerotial formation. Basin F well water produced only a marginal increase at all concentrations examined. In all cases, when sclerotia (89) isolated from each of the assay plates were transferred to fresh agar media, 100% germination was observed. However, the number of mature sclerotia formed in the minicolonies of each plate were markedly different (Table 3.7). Application of Basin F water generally led to the formation of greater numbers of sclerotia compared to

TABLE 3.4. Effects of Cations on Soil Dehydrogenase Activity[a]

Cation Concentration (ppm)	Cu^{2+}		Mg^{2+}		Ni^{2+}		Zn^{2+}		NH_4^+	
	A[b]	B[c]	A	B	A	B	A	B	A	B
0	100 ± 9	100 ± 0	100 ± 5	100 ± 0	100 ± 18	100 ± 14	100 ± 3	100 ± 0	100 ± 6	100 ± 7
30	49 ± 4	72 ± 9	101 ± 3	107 ± 5	86 ± 11	61 ± 6	93 ± 2	99 ± 2	105 ± 7	103 ± 11
150	13 ± 6	12 ± 0	97 ± 7	107 ± 17	70 ± 9	54 ± 6	65 ± 3	85 ± 0	74 ± 7	100 ± 17
300	9 ± 4	6 ± 0	104 ± 6	95 ± 2	32 ± 4	31 ± 3	55 ± 2	69 ± 4	66 ± 11	102 ± 14
500	4 ± 0.2	6 ± 0	96 ± 4	99 ± 7	11 ± 1	12 ± 9	34 ± 1	47 ± 4	71 ± 13	108 ± 10
1,000	1 ± 0.2	0 ± 0	101 ± 7	94 ± 9	7 ± 2	6 ± 1	15 ± 1	18 ± 2	79 ± 11	109 ± 10
3,000	0 ± 0	0 ± 0	97 ± 3	95 ± 24	1 ± 0.1	0 ± 0	3 ± 0.4	0 ± 0	81 ± 7	107 ± 18
5,000	0 ± 0	0 ± 0	97 ± 4	102 ± 22	0 ± 0	0 ± 0	3 ± 0.05	0 ± 0	84 ± 3	134 ± 9
25,000									52 ± 3	39 ± 10

[a] Soil dehydrogenase activities are measured as μg formazan formed per gram dry soil per 24 hours. Data are expressed as % of control (mean ± standard deviation, n=3).

[b] Soil was enriched with 1% ground alfalfa (see Materials and Methods).

[c] Soil was not enriched.

TABLE 3.5. Effects of Anions on Soil Dehydrogenase Activities[a]

Anion Concentration (ppm)	F^-		AsO_4^{3-}		BO_3^{3-}		SO_4^{2-}	
	A[b]	B[c]	A	B	A	B	A	B
0 (control)	100 ± 0	100 ± 4	100 ± 18	100 ± 26	100 ± 9	100 ± 16	100 ± 0	100 ± 0
30	100 ± 11	103 ± 11	94 ± 12	78 ± 18	94 ± 10	110 ± 13		
50							103 ± 2	134 ± 6
150	108 ± 9	107 ± 11	66 ± 9	37 ± 7	99 ± 9	104 ± 13		
250							100 ± 0	119 ± 3
300	124 ± 0	116 ± 4	35 ± 5	28 ± 6	101 ± 6	92 ± 13		
500	134 ± 3	120 ± 4	28 ± 4	28 ± 6	101 ± 6	81 ± 12	99 ± 13	134 ± 0
1,000	137 ± 0	144 ± 7	23 ± 3	29 ± 7	82 ± 6	33 ± 12	103 ± 2	155 ± 13
3,000	167 ± 12	136 ± 5	21 ± 3	26 ± 7	30 ± 3	21 ± 16		
5,000	155 ± 6	70 ± 8	21 ± 4	32 ± 6	29 ± 2	12 ± 3	91 ± 14	133 ± 19
25,000							89 ± 5	144 ± 32
50,000							52 ± 1	74 ± 6
65,000							36 ± 3	63 ± 6

[a] Soil dehydrogenase activities are measured as µg formazan formed per gram dry soil per 24 hours. Data are expressed as % of control (mean ± standard deviation, n=3).

[b] Soil was enriched with 1% ground alfalfa (see Materials and Methods).

[c] Soil was not enriched.

TABLE 3.6. Effects of Basin F Water and Well Water on Fungal Sclerotial Formation

Sample	Concentration (%)	Number of Sclerotia (% of Control)
Control	0	100 ± 10[a]
Basin F Water	10	148 ± 20
	5	195 ± 14
	0.5	149 ± 14
	0.05	117 ± 18
Basin F Well Water	10	120 ± 14
	5	108 ± 10
	0.5	123 ± 21
	0.05	119 ± 10

[a]Mean ± standard deviation, n=3.

controls, whereas increased sclerotia formation was only observed at the two highest concentrations of BFW water and a considerable reduction was found at the lower concentrations (Table 3.7).

In contrast to the enhancements observed with BF and BFW waters, the major inorganic chemical components of the waters were either inhibitory to mycelial growth, sclerotial formation, or resulted in no effects. The most effective inhibitors of mycelial growth were Ni^{2+}, Cu^{2+} and Zn^{2+} (Table 3.8). All other elements tested (Mg^{+}, NH_4^{+}, F^{-}, AsO_4^{3-}, BO_3^{3-}, and SO_4^{2-}) were not inhibitory to mycelial growth at the concentrations tested. Although Ni^{2+} was the strongest inhibitor of growth, Zn^{2+} and Cu^{2+} were the most effective inhibitors of sclerotia formation, followed by $Ni^{2+} > Mg^{2+} > NH_4^{+}$ (Table 3.9). The only anions which showed appreciable sclerotia inhibition were BO_3^{3-} (Table 3.10), where sclerotial formation at a concentration of 300 ppm or greater was completely eliminated, and F^{-}, which was 50% inhibitory at 500 ppm. The remaining anions tested (AsO_4^{3-} and SO_4^{2-}) were not inhibitory.

TABLE 3.7. Number of Sclerotia Formed by Each of 89 Minicolonies in a Germination Test

Treatment	Number of Sclerotia Formed Per Colony															Total Sclerotia Formed
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	Number of Fungal Colonies[a]															
Control Plate	1	4	14	24	21	18	4	3	0	0	0	0	0	0	0	323
BF[b] - 10%	0	0	2	7	10	12	13	12	12	11	6	2	1	0	1	590
BF - 5%	1	6	19	15	15	14	9	6	3	1	0	0	0	0	0	348
BF - 0.5%	0	2	3	13	25	21	14	11	0	0	0	0	0	0	0	413
BF - 0.05%	0	0	2	5	21	26	24	7	2	0	1	0	0	0	0	452
BFW[c] - 10%	0	1	0	12	31	26	16	1	1	1	0	0	0	0	0	411
BFW - 5%	0	2	4	17	21	24	15	0	2	2	0	2	0	0	0	411
BFW - 0.5%	32	27	24	4	2	0	0	0	0	0	0	0	0	0	0	95
BFW - 0.05%	34	17	10	18	7	2	0	0	1	0	0	0	0	0	0	137

[a]Number of sclerotia that developed from each of 89 single test - sclerotia in the germination test. All 89 sclerotia inoculated into each plate germinated.

[b]BF = Basin F Water.

[c]BFW = Basin F Well Water.

TABLE 3.8. Effect of Cations on the Diameter of Mycelial Mats

Cation Concentration (ppm)	Zn^{2+}	Cu^{2+}	Ni^{2+}
	(% of Control)[a]		
0 (Control)	100 ± 0	100 ± 0	100 ± 0
15	90 ± 2	100 ± 0	96 ± 2
30	89 ± 0	100 ± 0	92 ± 4
100	80 ± 6	100 ± 0	79 ± 7
150	73 ± 6	100 ± 0	57 ± 3
200	63 ± 4	81 ± 4	11 ± 0
300	56 ± 4	11 ± 2	0
500	43 ± 2	0	0

[a]Mean ± standard deviation, n = 3.

TABLE 3.9. Effects of Cations on Fungal Sclerotial Formation

Cations Concentration (ppm)	Cu^{2+}	Mg^{2+}	Ni^{2+}	Zn^{2+}	NH_4^+
		(% of Control)[a]			
0 (Control)	100 ± 4	100 ± 10	100 ± 31	100 ± 2	100 ± 17
15	11 ± 3	83 ± 19	82 ± 19	14 ± 3	97 ± 17
30	32 ± 9	73 ± 7	53 ± 19	14 ± 1	92 ± 13
100	20 ± 7	83 ± 9	23 ± 12	9 ± 1	95 ± 13
150	6 ± 7	99 ± 13	11 ± 4	0 ± 0	94 ± 12
200	3 ± 4	82 ± 15	0 ± 0	0 ± 0	114 ± 14
300	0 ± 0	73 ± 16	0 ± 0	0 ± 0	109 ± 14
500	0 ± 0	74 ± 6	0 ± 0	0 ± 0	121 ± 25

[a]Mean ± standard deviation, n=3.

TABLE 3.10. Effects of Anions on Fungal Sclerotial Formation

Anion Concentration (ppm)	F^-	AsO_4^{3-}	BO_3^{3-}	SO_4^{2-}
		(% of Control)[a]		
0 (Control)	100 ± 11	100 ± 3	100 ± 17	100 ± 8
15	82 ± 10	87 ± 9	104 ± 14	101 ± 12
30	85 ± 8	104 ± 4	103 ± 13	82 ± 7
100	97 ± 14	93 ± 3	97 ± 17	100 ± 10
150	95 ± 15	94 ± 9	91 ± 12	93 ± 6
200	104 ± 13	103 ± 4	82 ± 12	92 ± 5
300	82 ± 15	113 ± 4	0 ± 0	81 ± 5
500	56 ± 8	122 ± 9	0 ± 0	82 ± 5
10,000				81 ± 6

[a]Mean ± standard deviation, n=3.

3.4 DISCUSSION

Basin F well water was collected downgradient from the Basin F holding pond in Section 26 of RMA (see Figure 2.1). Judging from the high conductivity values and organic carbon to phosphorous ratios found in both BF and BFW water (Table 3.1), it appears that water from the holding pond has leaked to the adjacent groundwater system. Apparently the movement of BF water through the substrata has resulted in a general decrease in chemical concentrations (by diffusion and dispersion as a function of travel time to the monitoring well). However, certain constituents (e.g. As, P, Cu, Zn, Ni, and F) appear to have been removed from solution by sorption or precipitation, whereas other elements (e.g. Mg^{2+}, Ca^{2+}, and Sr^{2+}) increased in concentration, perhaps as a result of the high Na^+ in BF water. After Na^+ entered the groundwater it replaced these elements on the substrata and thereby increased their soluble concentration. Comparison of bioassay results from BF and BFW indicates that migration of BF water into ground water has resulted in a water that is less toxic to the soil respiration assays (alfalfa mineralization and dehydrogenase) and less stimulatory to the sclerotia assay. However, sclerotia isolated from BFW water containing media generally produced fewer sclerotia in germination studies.

Using the combined results from alfalfa mineralization (Table 3.2) and dehydrogenase assays (Tables 3.2 and 3.3), some of the potential effects of BF water released to soil can be estimated. Undiluted BF water would have a long-term toxic effect on mineralization of plant material (assuming the water is not diluted by natural rainfall). If rainfall events are included in the analysis, long-term effects would be reduced if the initial BF water was immediately diluted. Short-term effects would not be reduced until natural water input exceeded the BF water by greater than 10-fold; long-term effects would be minimal at this point. Predictive scenarios of this type could be invaluable in the development of manageable and meaningful field studies to assess the toxicity of hazardous waste sites.

Basin F water is a complex solution of potentially hazardous organic and inorganic components (Table 3.1). Thus its effects on environmental bio-assays may also be expected to be complex. Predicting the toxicity of BF water to environmental assays by comparing the sensitivity of the assays to specific components identified by chemical analysis may be misleading. For example, if the concentration of Cu^{2+} in BF water (Table 3.1) was used to estimate the toxicity of BF water to sclerotia formation based on the sensitivity of the assay to Cu^{2+} shown in Table 3.9, BF water would have been considered toxic to sclerotia formation when in fact it was stimulatory (Table 3.6). This is an extreme illustration. However, a similar estimation (compare Tables 3.1 and 3.4) with the soil dehydrogenase assay would have suggested that BF water would be slightly more toxic than was actually observed (Table 3.3). Results such as these emphasize our need to improve our understanding of the effect of complex mixtures on environmental bioassays. Complex mixtures will probably be the norm in assessing the potential adverse environmental conditions associated with hazardous wastes sites.

Although the data are limited, similar effects of BF and BFW water on the alfalfa mineralization and the soil dehydrogenase assays indicate they may be used interchangeably. The dehydrogenase assay is cheaper and requires less time (48 hr), whereas the mineralization assay required up to 30 days. The dehydrogenase assay may therefore be the preferred assay for most studies.

The sclerotia assay, unlike other microbiological assays, is primarily based on a differentiation process, i.e., the formation of sclerotia. In this sense, it is similar to the seed germination/root elongation and fish egg bioassays. It is not limited to examination of sclerotia formation, however, since radial mycelial growth can also be measured. With respect to the series of compounds examined herein, the formation of sclerotia was more sensitive than was mycelial growth (Tables 3.8, 9, and 10). The assay requires less than 40 man hours to complete, and results are available within 14 days.

The sclerotia assay offers several advantages over other fungal assays that have been developed. The assay does not require the maintenance of a viable fungal culture. Sufficient sclerotia need only be harvested on a yearly basis. Stored in the dry state, sclerotia will remain viable for 1-2 years. Previously developed assays require that test plate be inoculated from freshly grown precultures (Babich et al., 1982). The sclerotia assay is initiated with sclerotia that may have been stored for up to a year. Many other fungal assays require individual experiments to investigate growth effects, spore formation, and spore germination. We have combined these experimental end points into two simple assays so that a greater number of fungal growth parameters can be measured.

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4.0 INVERTEBRATE STUDIES

4.1 INTRODUCTION

During the preliminary study on the Rocky Mountain Arsenal (RMA) in 1982, several invertebrate groups were evaluated for potential use as indicators of the presence of toxic materials in the environment. The most promising was the use of honeybees and the determination of their brood survival (Thomas et al. 1983).

This test is based on the sensitivity of the honeybee's larval stage, a period when all cells are rapidly dividing and differentiating; the most sensitive time in a honeybee's life cycle. The young larva are very sensitive to the quality of food they receive. Their food is a protein rich fluid that is secreted by young adult bees that have fed heavily on pollen. Toxicants contained in the pollen or nectar can be passed onto the brood and result in mortality. Honeybee colonies contain large numbers of larva during the growing season which can provide good sample sizes for quantitative studies of brood mortality.

Foraging bees collect pollen and nectar mostly from sources near the hive but may range up to 6.5 km, depending on the source (Gary 1975). There also appears to be a preference for a diversity of plant species instead of a single source (Gary et al. 1972). These wide ranging foraging habits are attractive attributes for collecting environmental samples from a relatively large area. A series of honeybee colonies at a single location can provide information on the presence of environmental pollutants through samples of pollen collected for chemical analysis and through the effects on brood mortality. Our study compares the results of brood mortality tests conducted on colonies located within areas containing environmental pollutants to an area free of pollutants.

4.2 METHODS

In May 1983, twenty-four colonies of honeybees were inspected and equalized according to number of bees and frames of brood in each prior to shipment to the RMA. During the week of 1 July 1983 the colonies were taken to Denver, Colorado. Treatment sites were established on the RMA with eight colonies each near F-Basin and Derby Lakes (see Thomas et al. 1983 for complete site description). A control site with eight colonies was located approximately 35 miles northwest of RMA near Lyons, Colorado.

Brood mortality was measured in each hive on two different occasions; between 18 July and 2 August, and again between 15 and 30 August 1983. Bromenshenk et al. (1984) defines the sampling procedures in detail. The procedure consists of locating a frame of brood that contains as many eggs as possible. Six rows of 20 cells are selected and marked at the end of each row with colored pins. The content of each cell is recorded on a data sheet, e.g. egg, larval stage, pollen etc. Fourteen to sixteen days later, the rows of cells are re-examined and the contents and developmental stages are recorded again.

Developmental stages of honeybees are very distinct and allow relatively accurate predictions of the time eggs were laid, i.e. within 1-3 days (Butler 1975). The following are the developmental stages utilized for our study:

- egg, 1-3 days old, resembling a small white bean
- young larva, 4-5 days old, small grub floating in a milky fluid
- old larva, 6-7 days old, large grub with no fluid
- just capped, 8-9 days old, white ball that fills the cell
- tail up, 10 day old larva with what appears to be a tail pointed up
- reversed pupae, 14-17 days old, rare condition where pupae develop in a face down orientation
- non-pigmented eyes, 11 days old, eyes of pupae are white
- pink eyes, 13-14 days old, eyes are pink and head still white
- brown eyes, 15-17 days old, eyes are dark brown and head is beginning to show color
- black eyes, 18-21 days old, eyes are black and head shows definite color.

TABLE 4.1. Honeybee Brood Mortality (%) for July 18 - August 1, 1983

Colony	Lyons (Control)	F-Basin (RMA)[1]	Derby Lakes (RMA)
1	7.1	97.9	84.1
2	14.0	10.1	89.6
3	33.3	21.4	97.9
4	34.5	100.0	98.8
5	16.0	63.5	97.6
6	20.0	86.4	64.2
7	20.6	100.0	65.7
8	25.6	100.0	--
$\overline{X} \pm SE$	21.4 ± 3.3	72.4 ± 13.2	85.4 ± 5.7

[1] RMA = Rocky Mountain Arsenal.

The data were analyzed by computer for percent mortality and comparisons made between treatment and control sites using the Bonferonni T test (Miller 1966). Pollen samples were collected from each colony on the days following the brood mortality tests for possible future analysis.

4.3 RESULTS AND DISCUSSION

Brood mortality estimates were made for two periods during the summer of 1983. A summary of brood mortality estimates for the first period (18 July - 2 August) is shown in Table 4.1 for the three study sites. Brood mortality for individual hives ranged from a low of 7.1% for a control colony located near Lyons, Colorado to a high of 100% for some colonies located on the RMA (Table 4.1).

Comparisons of average brood mortality rates for the three study sites showed that the control colonies near Lyons experienced significantly less mortality than colonies located at either of the two RMA sites (α = .01; Bonferonni T Tests, Miller 1966). The average brood mortality at the Lyons site was 21.4 ± 3.3% ($\bar{x}$ ± SE) as compared to 72.4 ± 13.2% and 85.4 ± 5.7% respectively for the F-Basin and Derby Lake Sites located on the Rocky Mountain Arsenal.

Normally brood mortality for strong honeybee colonies should be less than 26% (Garofalo 1977). The size of the colony and any brood disease present however, can affect brood mortality rates. Bad weather may also prevent bees from foraging and this can result in a colony running low on food reserves. If this happens, the queen will stop laying and the workers may pull and eject larvae from the cells. A lack of pollen may also result in brood mortality. Finally, exposure to even moderate amounts of some toxic materials will cause increased levels of brood maturity. We saw no evidence of any shortages of food reserves (either honey or pollen), or brood diseases and can only conclude that the increased levels of brood mortality observed were due to the foraging bees returning nectar or pollen to the hive that was contaminated with some toxic material.

Interestingly, the variability associated with brood mortality was much greater for colonies located at the F-Basin site in comparison with those located near Derby Lakes (standard deviation = 37.2 for colonies near F-Basin, s = 15.0 for colonies near Derby Lakes). This may indicate the availability of a more widespread source of materials toxic to bees in the Derby Lakes vicinity since all colonies were experiencing approximately the same high levels of brood mortality or that all colonies were foraging near the same location. At the F-Basin Site, some colonies experienced very low levels of brood mortality (10.1%) while others experienced 100% mortality of their brood (Table 4.1). This indicates that the colonies at F-Basin were probably foraging within different areas and that some of those areas were highly contaminated while other areas were contaminant free.

Honeybees are known to communicate the direction and distance of food sources to other workers within the hive (von Frisch 1971). This allows individual colonies to take advantage of nectar and pollen sources and particular locations very quickly. We also know that honeybees flying 4 km in all directions from a single hive have access to over 5,000 hectares (Martin 1975). Consequently, it seems reasonable that for localized sources of contamination some colonies within an apiary might be expected to collect contaminated materials while other colonies within the same apiary would not.

TABLE 4.2. Honeybee Brood Mortality (%), August 15-19

Colony	Lyons (Control)	F-Basin (RMA)[1]	Derby Lakes (RMA)
1	76.7	69.6	98.7
2	91.6	N.A.[2]	72.8
3	80.8	93.7	98.8
4	75.0	89.1	N.A.[2]
5	100.0	N.A.[2]	66.7
6	N.A.[2]	96.6	N.A[2]
7	100.0	44.7	15.8
8	64.3	66.7	N.A.[2]
$\bar{X} \pm SE$	84.1 ± 5.1	76.7 ± 8.2	70.6 ± 15.2

[1]RMA = Rocky Mountain Arsenal.

[2]Colony queenless and/or no young worker brood to sample.

A summary of brood mortality for the second sampling period (15 August - 30 August) is shown in Table 4.2 for the three study sites. Unfortunately, the period of active brood production was drawing to a close for that part of Colorado. According to B. Utley of Madhava Honey Ltd., Longmont, Colorado, although the summer months represent a period of intensive foraging activity by honeybees, the season is relatively short. This is mostly dictated by the elevation of the study areas (e.g. above 5,000 ft.). As a consequence, we were unable to obtain a valid estimate of honeybee mortality for this second sampling period. The high mortality values shown in Table 4.2 for all three study sites during the second sample period probably occurred as a consequence of the honeybees preparation for winter.

Our conclusions from these results is that honeybee colonies do appear to serve as useful biomonitors for the presence of toxic materials in the environment. We are confident that placement of honeybee colonies near contaminant sources similar to those at the Rocky Mountain Arsenal would result in detection of increased brood mortality in comparison with colonies located remote from such areas. However, only personnel well versed in apiculture should conduct tests of this nature since the occurrence of disease or natural changes in brood production patterns could be interpreted as a response to toxic materials. Additional studies could be conducted to evaluate this variable, using analysis of covariance techniques. This technique, however, is only useful during those seasons of the year when honeybees are actively foraging and raising brood. This period of time is relatively short for high elevation sites such as those near the Rocky Mountain Arsenal and may consist of only a few months.

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5.0 NEUBAUER PHYTOASSAYS

5.1 INTRODUCTION

Neubauer phytoassays are useful because of their sensitivity, use of actual soil samples instead of water extracts of soils (as used in the current EPA seed germination and root elongation test; see Porcella 1983) and cost effectiveness. Our major modifications of the standard Neubauer test (Vandecaveye 1948) included the use of a plastic bag and the substitution of disposable petri dishes for crystalizing dishes so the entire apparatus could be disposed of at the termination of each test (Figure 5.1). These modifications enabled us to avoid periodic irrigation, and to contain toxic materials. Volume 1, Section 8.0 of this report contains the initial results of our research on the development of a modified Neubauer phytoassay as a test for hazardous environmental chemicals in soil and water samples.

In Volume 1, Section 8.0 we presented the standard deviation of wheat shoot lengths calculated on the basis of the standard deviation of plants in all replicate plates of a treatment. A more representative expression of error (to compare with germination) is the weighted standard deviation of the mean of the three plates or an estimate from a nested analysis of variance (ANOVA). Thus we have repeated the results from three of last year's studies using Basin F water to supply the corrected standard deviation and to provide some continuity for the experiments. Additional results of phytoassay studies may be found in Volume 1, Section 8.2 (Methods). The rationale for selection and the locations for additional 1983 study sites in Basin A, F, C, and D are in Section 2.0.

5.2 BASIN F WATER STUDIES

5.2.1 Methods

We conducted an initial range finding experiment using Basin F water, locally available, commercially treated wheat seeds and the standard Neubauer technique. In this and all subsequent experiments, control soil was wetted with distilled water (control) or with different dilutions of contaminated

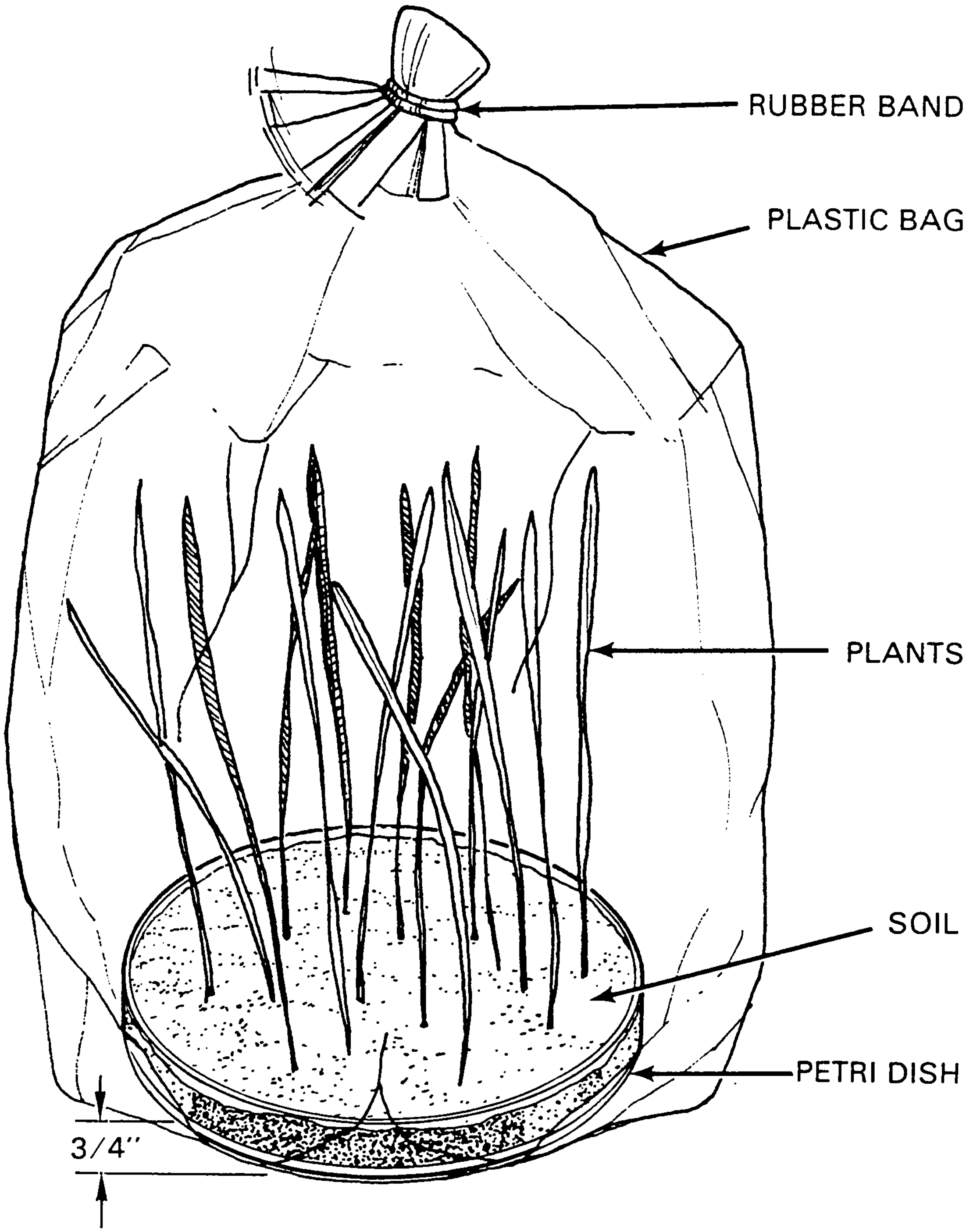

FIGURE 5.1. Modified Neubauer Apparatus Used in Seed Germination and Leaf Elongation Phytoassays

solutions (to 85% of water holding capacity). In a subsequent experiment, we compared the toxicity of holding basin water using the modified Neubauer technique on untreated wheat seeds to results obtained in a pot experiment. In the pot experiment, 1 kg of soil was mixed with holding basin water in plastic bags, the soil was then seeded with 15 seeds in three replicate quart-sized ice cream cartons. In a third experiment, we prepared solutions containing four elements (alone and in combination) that duplicated the concentrations found in Basin F water. Sodium, nickel and copper were added as sulfates, while arsenic was added as the sodium salt ($NaAsO_3$). Both germination and shoot length were measured.

5.2.2 Results

Holding basin water caused a reduction in wheat seed germination and shoot length at a dilution of 1% (Table 5.1). Measurements of both parameters indicate that a dilution somewhere between 0.1 and 1.0% would also be toxic. We attribute the poor germination of control seeds to their treatment with fungicide. In all subsequent experiments with untreated seeds, control seed germination improved (usually greater than 90%).

Experiments conducted with the modified Neubauer and pot cultures showed that 1% holding basin water was toxic to wheat seeds (Table 5.2). Seed germination was about one-half that of control values in this experiment (using either technique) compared to a little less than one-half using the standard Neubauer test (Table 5.1). Both the pot culture and Neubauer test showed that 1% holding basin water caused a severe reduction in shoot length, while the modified Neubauer test results showed about a 50% reduction. It appears that all three procedures lead to similar conclusions. Since the modified Neubauer procedure is cheaper and safer for phytoassays using toxic chemicals, we advocate its use.

Chemical analysis of the holding basin water (pH 6.25, Eh 273.4) showed that large quantities of inorganic ions and salts were present (Table 3.1). Electrical conductivity was 55 $dS.m^{-1}$. The high ratio of organic carbon to phosphorous suggests abundant phosphorolated organics. One major waste produced at RMA was diisopropylmethylphosphonate. Richards (1950)

TABLE 5.1. Effect of Basin F Water on Wheat Seed Germination and Shoot Length

Basin Water (%)	Germination[a] (%)	Shoot Length[a] (%)
Control	46.7 ± 3.1	8.4 ± 0.82
0.01	47.0 ± 6.0	7.7 ± 0.86
0.10	45.0 ± 1.7	8.9 ± 0.54
1.00	17.3 ± 11.6	3.0 ± 0.89
10.00	0	0
50.00	0	0

(a) Mean ± standard deviation, n = 3.

TABLE 5.2. A Comparison of the Effect of Basin F Water on Wheat Seed Germination and Shoot Length Using the Modified Neubauer Technique and Pot Culture

Basin Water (%)	Germination[a] (%)	Shoot Length[a] (%)
	Modified Neubauer	
Control	95.0 ± 4.0	10.4 ± 0.51
1.0	48.0 ± 11.0	5.0 ± 0.60
5.0	0	--
	Pot Cultures	
Control	96 ± 7.5	8.7 ± 0.34
0.1	89 ± 3.5	9.6 ± 0.38
0.25	93 ± 0.0	6.8 ± 0.75
1.0	53 ± 18.0	1.3 ± 0.57
2.5	0	--

(a) Mean ± Standard deviation, n = 3.

showed that salinity values of 15 $dS.m^{-1}$ in irrigation water was deleterious to the growth of crop plants. The concentration of copper in the basin water was high (3620 mg L^{-1}); copper has been shown to be toxic to plant growth in trace amounts (Bowen 1966). Mathur and Levesque (1983) reported that copper becomes phytotoxic when it exceeds 5 percent of the soil cation exchange capacity (or 16 ppm for every milliequivalent of cation exchange capacity per 100 g soil). The highest copper concentration in our experiment was 91 ppm (2.5% basin water). Thus, the soil would only need a cation exchange capacity of 6 to make the copper insoluble and unavailable for plant uptake. Clearly, phytotoxic materials were present and their effects detectable in the basin water using the modified Neubauer procedure.

We conducted a third experiment to evaluate the toxicity of some of the important inorganic elements (alone to lettuce, Table 5.3; alone and in combination to wheat, Table 5.4; summarized results for germination of wheat and lettuce and wheat shoot growth are in Table 5.5). Again, 1% holding basin water reduced wheat seed germination about one-half and caused a decrease in shoot length, while no germination occurred in 2.5% Basin F water. Results of a one-way analysis of variance (ANOVA) for germination of wheat (Table 5.6) or a nested one-way ANOVA for shoot length (Table 5.7) followed by Duncan multiple range test (Duncan 1955) revealed some statistically significant differences. However, only the basin water treatments were significantly different from the control for either parameter. No other important differences were found that might explain holding basin water toxicity. Thus, it does not appear that inorganic salts are the causative agent of Basin F water toxicity to wheat seeds. We believe the high organic carbon to phosphorous content implicates an organic toxicant (Section 3.3.1).

The results of the lettuce phytoassay (Tables 5.3, 5.5, and 5.8) indicate that either 2.5% sodium (2.24 mg/g soil) or copper (0.09 mg/g soil) significantly depressed lettuce germination compared to controls ($P < 0.05$, Table 5.8). Moreover, 1% sodium (1.0 mg/g) was also inhibitory. In general, we have found lettuce seeds to be more sensitive than wheat in soil studies

TABLE 5.3. Effect of Four Elements Found in High Levels in F-Basin Water on Germination of Lettuce Seeds

Element	Fraction of F-Basin Water (%)	Dish Number	Number of Seeds Germinated/40 Planted	Mean	Standard Deviation
Control		13	12		
		113	19		
		46	20	17.00	4.36
F-Basin Water	0.1[a]	22	3		
		80	7		
		55	4	4.67	2.08
	1.0	33	0		
		123	0		
		73	0	0	0
	2.5	104	0		
		101	0		
		71	0	0	0
Sodium	0.1	95	20		
		106	16		
		2	14	16.67	3.06
	1.0	78	3		
		110	5		
		32	2	3.33	1.53
	2.5	49	1		
		89	0		
		11	0	0.333	0.577
Nickel	0.1	20	19		
		53	10		
		98	15	14.67	4.51
	1.0	8	13		
		7	16		
		34	17	15.33	2.08
	2.5	18	21		
		86	9		
		5	12	14.00	6.24
Copper	0.1	24	12		
		25	11		
		64	6	9.67	3.21
	1.0	75	10		
		67	14		
		72	14	12.67	2.31
	2.5	87	9		
		108	11		
		19	3	7.67	4.16
Arsenic	0.1	51	12		
		115	9		
		116	17	12.67	4.04
	1.0	38	19		
		45	21		
		103	20	20.00	1.00
	2.5	111	9		
		15	18		
		37	9	12.00	5.20

[a] Amounts of elements actually added resulted in the same soil concentrations produced by Basin F water. For instance, concentrations at the 2.5 % level were 0.09 mg/g copper, 2.24 mg/g sodium, 0.43 mcg/g arsenic and 0.80 mcg/g of nickel.

TABLE 5.4. Effect of Sodium, Nickel, Copper, and Arsenic on Germination of Wheat Seeds

Element	Fraction of F-Basin Water (%)	Dish Number	Number of Seeds Germinated/40 Planted	Mean	Standard Deviation
Control		70	36		
		36	36		
		120	38	36.67	1.15
F-Basin Water	0.1[a]	41	37		
		85	38		
		82	37	37.33	0.58
	1.0	88	19		
		90	18		
		81	18	18.33	0.58
	2.5	57	0		
		68	0		
		65	0	0	0
Sodium	0.1	47	38		
		119	38		
		52	39	38.33	0.58
	1.0	54	38		
		60	39		
		59	39	39.67	0.58
	2.5	14	36		
		29	36		
		94	38	36.67	1.15
Nickel	0.1	16	37		
		4	38		
		56	36	37.00	1.00
	1.0	3	37		
		6	36		
		43	40	37.67	2.08
	2.5	62	37		
		84	39		
		96	38	38.00	1.00
Copper	0.1	83	37		
		92	40		
		30	40	39.00	1.73
	1.0	39	35		
		63	39		
		9	38	37.33	2.08
	2.5	21	36		
		100	35		
		28	35	35.33	0.58
Arsenic	0.1	76	38		
		93	38		
		77	38	38.00	0
	1.0	23	37		
		40	36		
		35	37	36.67	0.58
	2.5	91	39		
		105	38		
		12	38	38.33	0.58

TABLE 5.4. (Continued)

Element	Fraction of F-Basin Water (%)	Dish Number	Number of Seeds Germinated/40 Planted	Mean	Standard Deviation
Copper + Nickel	0.1	26	38		
		102	36		
		61	37	37.00	1.00
	1.0	58	40		
		74	38		
		31	36	38.00	2.00
	2.5	121	37		
		114	33		
		79	39	36.33	3.06
Arsenic + Copper	0.1	17	39		
		69	39		
		117	38	38.67	0.58
	1.0	107	38		
		48	38		
		112	38	38.0	0
	2.5	99	36		
		66	36		
		118	37	36.33	0.58
Arsenic + Copper + Nickel + Sodium	0.1	122	38		
		97	39		
		109	39	38.67	0.58
	1.0	44	38		
		27	36		
		42	34	36.00	2.00
	2.5	50	37		
		10	37		
		1	37	37.00	0

[a] Amounts of elements actually added resulted in the same soil concentrations produced by Basin F water. For instance, concentrations at the 2.5 % level were 0.09 mg/g copper, 2.24 mg/g sodium, 0.43 mcg/g arsenic and 0.80 mcg/g of nickel.

TABLE 5.5. Summary of the Effects of Sodium, Nickel, Copper and Arsenic on Germination of Lettuce and Wheat and Wheat Shoot Length

Element[a]	Fraction F-Basin Water (%)	Mean Number of Seeds Germinated: Lettuce[b]	Mean Number of Seeds Germinated: Wheat[b]	Mean Shoot Length[c] (cm)
Control		17.0 ± 4.4	36.7 ± 1.2	10.97
F-Basin Water	0.1	4.7 ± 2.1	36.3 ± 0.6	9.61
	1.0	0.0 ± 0.0	18.3 ± 0.6	6.87
	2.5	0.0 ± .00	0.0 ± 0.0	--
Sodium	0.1	16.7 ± 3.1	38.3 ± 0.6	11.36
	1.0	3.3 ± 3.1	38.7 ± 0.6	10.51
	2.5	0.3 ± 0.6	36.7 ± 1.2	10.82
Nickel	0.1	14.7 ± 4.5	37.0 ± 1.0	11.58
	1.0	15.3 ± 2.1	37.7 ± 2.1	11.55
	2.5	14.0 ± 6.2	38.0 ± 1.0	11.75
Copper	0.1	9.7 ± 3.2	39.0 ± 1.7	11.82
	1.0	12.7 ± 2.3	37.3 ± 2.1	10.71
	2.5	7.7 ± 4.2	35.3 ± 0.6	10.76
Arsenic	0.1	12.7 ± 4.0	38.0 ± 0.0	11.67
	1.0	20.0 ± 1.0	36.7 ± 0.6	10.89
	2.5	12.0 ± 5.2	38.3 ± 0.6	10.97
Copper + Sodium	0.1		37.0 ± 1.0	11.72
	1.0		38.0 ± 2.0	10.77
	2.5		36.3 ± 3.1	10.98
Copper + Arsenic + Nickel	0.1		38.7 ± 0.6	11.39
	1.0		38.0 ± 0.0	12.05
	2.5		36.3 ± 0.6	10.71
Copper + Arsenic + Nickel + Sodium	0.1		38.7 ± 0.6	11.51
	1.0		36.0 ± 2.0	10.98
	2.5		37.0 ± 0.0	9.80

(a) Elements tested were all sulfates except arsenic ($NaAsO_2$). Levels were the same as those in F-Basin water at the concentration of F-Basin water indicated.

(b) Mean ± standard deviation of three replicate plates of 40 seeds each.

(c) Seedlings were measured between 7/13/83 (p.m.) and 7/15/83 (a.m.). Plates were randomly selected. The pooled estimate of the standard deviation of plants in plates was 2.75 ($\sqrt{7.535}$, Table 5.7) The estimate of the standard error of the mean for three plates, which averaged 36.67 seeds/plate that grew was 0.448 cm ($\sqrt{226.83/110}$, Table 5.7).

TABLE 5.6. Results of a Statistical Analysis of the Effect of Sodium, Nickel, Copper and Arsenic on Germination of Wheat

Treatment	Mean % Germination[a]					
0.1 Cu	97.5	A				
1.0 Na	96.6	A	B			
0.1 AsCuNi	96.6	A	B			
0.1 AsCuNiNa	96.6	A	B			
0.1 Na	95.8	A	B			
2.5 As	95.8	A	B			
1.0 Cu + Na	95.0	A	B			
2.5 Ni	95.0	A	B			
1.0 AsCuNi	95.0	A	B			
0.1 As	95.0	A	B			
1.0 Ni	94.1	A	B	C		
1.0 Cu	93.3	A	B	C		
0.1 F-Basin Water	93.3	A	B	C		
0.1 Ni	92.5	A	B	C		
0.1 Cu + Na	92.5	A	B	C		
2.5 AsCuNaNi	92.5	A	B	C		
2.5 Na	91.7	A	B	C		
1.0 As	91.7	A	B	C		
Control	91.7	A	B	C		
2.5 Cu + Na	90.8		B	C		
2.5 AsCuNi	90.8		B	C		
1.0 AsCuNaNi	90.0		B	C		
2.5 Cu	88.3			C		
1.0 F-Basin Water	45.8				D	
2.5 F-Basin Water	0.0					E

[a] Means without a common letter are significantly ($P < 0.05$) different using Duncan's (1955) multiple range test.

Analysis of Variance

Source	df	SS	MS	F
Treatment	23	6953.81	302.34	30.55**
Experimental Error	48	475.00	9.89583	
Total	71			

**$P < 0.01$

TABLE 5.7. Results of a Statistical Analysis of the Effect of Sodium, Nickel, Copper and Arsenic on Wheat Shoot Lengths

Treatment	Mean Shoot Length[a] (cm)
1.0 Cu + As + Ni	12.05 A
0.1 Cu	11.82 A
2.5 Ni	11.75 A
0.1 Cu + Na	11.72 A
0.1 As	11.67 A
0.1 Ni	11.57 A
1.0 Ni	11.55 A
0.1 Cu + As + Ni + Na	11.51 A
0.1 Cu + As + Ni	11.39 A
1.0 Cu + As + Ni + Na	10.98 A B
2.5 Cu + Na	10.98 A B
Control	10.97 A B
2.5 As	10.97 A B
1.0 As	10.89 A B
2.5 Na	10.82 A B
1.0 Cu + Na	10.76 A B
1.0 Cu	10.71 A B
2.5 Cu + As + Ni	10.71 A B
1.0 Na	10.52 A B
2.5 Cu + As + Ni + Na	9.80 B
0.1 F-Basin Water	9.61 B
1.0 F-Basin Water	6.87 C

(a) Means without a common letter are significantly ($P < 0.05$) different from one another using Duncan's (1955) multiple range test.

Analysis of Variance

Source	df	SS	MS	F
Treatment	23	1853.31	80.579	3.552**
Experimental Error	48	1088.78	22.683	3.010**
Sampling Error	2562	19305.50	7.535	
Total	2633	22247.594		

**$P < 0.01$

TABLE 5.8. Results of a Statistical Analysis of the Effect of Sodium, Nickel, Copper and Arsenic on Germination of Lettuce

Treatment	Mean % Germination[a]					
1.0 As	50.0	A				
Control	42.5	A	B			
0.1 Na	41.7	A	B			
1.0 Ni	38.3	A	B			
2.5 Ni	35.0	A	B			
1.0 Cu	31.6		B	C		
0.1 As	31.6		B	C		
2.5 As	30.0		B	C		
0.1 Cu	24.2		B	C	D	
2.5 Cu	19.2			C	D	
0.1 F-Basin Water	11.7				D	E
1.0 Na	8.3				D	E
2.5 Na	0.83					E
1.0 F-Basin Water	0.0					E
2.5 F-Basin Water	0.0					E

(a) Means without a common letter are significantly ($P < 0.05$) different using Duncan's (1955) multiple range test.

Analysis of Variance

Source	df	SS	MS	F
Treatment	13	7861.31	604.716	7.68**
Experimental Error	28	2204.16	78.7201	
Total	41	10065.5		

**$P < 0.01$

(see Section 5.3). In this experiment, control germination was also poor (42.5%). We attribute this to an overwatering error at planting. We also note that the toxic amounts of sodium (≥1%) and copper (2.5%) exceeded the amounts in 0.1% Basin F water which, in this experiment, also caused statistically depressed germination. Thus, it appears that for lettuce, only part of the toxic properties of Basin F water can be associated with as little as 1% sodium, 2.5 copper, or both.

5.3 CONTAMINATED SOIL STUDIES

5.3.1 Phytoassay of Soils Collected in 1982

Soil samples collected in 1982 along a transect in Basin C and in a waste ditch in section 1 did not result in detectable biotoxicity using the *Selanastrum*, clover and lettuce, and Neubauer bioassays (Volume 1, Tables 2.4

and 8.2). Thus, we were unable to relate biotoxicity to field observations, even though differences in plant cover were observed (Volume 1, Section 5.0). In October of 1982 we returned to RMA and collected the following soil samples as a function of depth; 1) Basin C near plot 27, because that sample (20 cm) appeared to suggest phytotoxicity (Volume 1, Table 8.2); 2) a waste ditch in section one, because a chemical analysis indicated that toxic components had migrated below 15 cm (Volume 1, Table 2.2); and 3) a location in Basin A previously shown by EPA, Corvallis to be toxic in bioassays. We hoped that phytotoxicity testing of these samples would aid us in the selection of a study site where phytotoxicity testing could be compared to plant cover analysis in the 1983 field season. Sample locations, methods of collection and maps are in Volume 1, Section 2.0. Each sample was dried, sieved, mixed and subsequently divided. One half of each sample was labeled COR and the other BCL, in anticipation of the needs of the two laboratories. We used portions of each in our studies in such a way that enough soil could be retained for these other needs.

5.3.2 Results for 1982 Samples

Germination results for five subsamples (BCL1A-5A) of a large mixed sample from the northeast location in the Basin A trench indicate that the 0-60 cm fraction was toxic to wheat (mean of 15 replicates was 8.8% germination) and 100% toxic to lettuce seeds (COR1A-5A, Table 5.9). Four of the five samples showed excellent reproducibility for wheat germination, while BCL3A was clearly different (21.8%). We speculate that our mixing of the original 170 kg sample was incomplete, since adjacent samples (within 1/2 m) taken at three separate depths (COR11A-13A, Table 5.9), indicate decreasing wheat phytotoxicity as a function of depth. In contrast, little wheat phytotoxicity is evident for Basin C (COR1C-3C) or the Section 1 ditch (COR1D-3D) samples obtained from similar depth increments. It appears that the 30-60 cm sample from Basin C (COR3C) caused slightly depressed wheat germination. However, the sample standard deviation was relatively large and control germination was only 90.8%. Thus, the mid-part of Basin C and the

TABLE 5.9. Germination of Wheat and Lettuce Seeds as a Function of Location and Depth in Three Waste Areas

Sample Identification	Location[a]	Depth (cm)	Germination[b] (Percent)	Plant Species
Basin A - East				
BCL1A	Northeast	0-60	5.0 ± 2.5	Wheat
BCL2A	Northeast	0-60	4.2 ± 5.2	Wheat
BCL3A	Northeast	0-60	21.8 ± 7.8	Wheat
BCL4A	Northeast	0-60	5.0 ± 6.8	Wheat
BCL5A	Northeast	0-60	8.3 ± 5.8	Wheat
COR1A-COR5A	Northeast	0-60	all 0%	Lettuce
COR11A	Northeast	0-15	0.0	Wheat
COR12A	Northeast	15-30	15.8 ± 3.0	Wheat
COR13A	Northeast	30-60	34.3 ± 5.8	Wheat
Basin A - West				
BCL6A	Southwest	0-60	0.0	Wheat
COR6A	Southwest	0-60	0.0	Lettuce
BCL7A	Mid-ditch - west	0-15	0.0	Wheat
COR7A	Mid-ditch - west	0-15	0.0	Lettuce
BCL8A	Northwest	0-30	7.5 ± 5.3	Wheat
COR8A	Northwest	0-30	0.0	Lettuce
BCL9A	North bank	0-50	86.8 ± 7.3	Wheat
COR9A	North bank	0-50	29.3 ± 5.3	Lettuce
Basin C				
COR1C	Volume 1, Figure 2.7	0-15	95.0 ± 5.0	Wheat
COR2C	Volume 1, Figure 2.7	15-30	98.3 ± 3.0	Wheat
COR3C	Volume 1, Figure 2.7	30-60	81.8 ± 12.8	Wheat
Ditch - Section 1				
COR1D	Volume 1, Figure 2.6	0-15	92.5 ± 2.5	Wheat
COR2D	Volume 1, Figure 2.6	15-30	95.8 ± 3.8	Wheat
COR3D	Volume 1, Figure 2.6	30-60	91.8 ± 3.8	Wheat
Control				
	Volume 1, Figure 2.8	0-15	90.8 ± 8.0	Wheat
	Volume 1, Figure 2.8	0-15	92.5 ± 6.5	Lettuce
Negative Control 1% Basin F Water	Volume 1, Figure 2.8	0-15	20.0 ± 2.5	Wheat

(a) North-south in the ditch, east-west relative to the road and culvert (Volume 1, Figure 2.5).
(b) Mean ± standard deviation, n=3.

waste ditch in section 1 did not appear to be promising field sites for an intercomparison of phytotoxicity results and a field vegetation survey.

Soil samples collected from a transect across the western part of the Basin A trench were also used to identify areas of potential phytotoxic contaminants. Three locations in the ditch bottom were very toxic, resulting in zero or very little germination for either wheat or lettuce seeds (Table 5.9). The sample taken from 0 to 15 cm depth (COR11A) on the northeast edge was more toxic than counterpart 15 to 30 or 30 to 60 cm (respective percent germinations were 0, 15.8 and 34.3%), indicating that some of the toxic material had moved from the surface to greater depths after abandonment, or that the last material in the ditch was most toxic. The sample from the northwest edge (BCL8A, 0-30 cm) appears to integrate (or average) the results obtained from the 0-15 cm and 15-30 cm fractions of the northeast sample (COR11A and 12A). A soil sample taken above the north bank (BCL9A) was not toxic to wheat seeds (86.8 ± 7.3%), while lettuce germination was significantly reduced (COR9A, 29.3% compared to 92.5% for controls). Thus, ditch bank soil would have been judged "not toxic" based only on a wheat seed phytoassay. It appears that the modified Neubauer technique can be expanded to assess germination of additional species; such studies will aid us in quantifying potential phytotoxic effects of contaminated soil. Based on these results, it appeared that Basin A and particularly areas near the trench offered the possibility of a contamination gradient for 1983 field studies. In fact, as Figure 2.5 in Volume 1 shows, no vegetation grew in the ditch bottom; some apparently stunted kochia on the north edge and tall and fairly vigorous kochia above the ditch edge. These field observations do track the wheat phytoassay results, even though only three locations were studied. This observation is mentioned again in Section 7.0.

5.3.3 Phytoassay of Soils Collected in 1983

Details of sample collection, the rationale for site selections, and maps are in Section 2.0. We elected to use lettuce seeds exclusively in the phytoassays conducted on samples from the 1983 study sites because our prior results (Section 5.3.1) showed that they are more sensitive than wheat (at least for the samples studied thus far). In addition, limited funding precluded conducting the phytoassay on all samples collected. We were able to assay all samples from Basin A (72 = 36 x 2 depths) and a limited number from Basin C (Figure 2.2). Only ten samples from the control area were assayed, since an initial experiment and field observations indicated little chance of contamination. Two samples from Basin C (K-9, 0-15 and 15-30 cm; Figure 2.2) and one from Basin F (G2, Figure 2.3) were sent to Battelle Columbus Laboratories for organic and inorganic chemical analyses. The available elemental results to date are in Table 5.10. The results of the organic analyses should be available soon.

5.3.4 Results for 1983 Samples

5.3.4.1 Basin A

Replicate lettuce germination values as well as summary statistics for both soil depths in each transect (Figure 2.3) are in Tables 5.11 and 5.12. A comparison of the mean percentage mortality at each depth is in Table 5.13 and the respective percent mortalities are presented at their respective sample point locations in Figures 5.2 and 5.3. The mortalities observed at points 1-3 (all transects) were based on the same soil sample (Section 2.3) and are a measure of experimental reproducibility, since the 0-15 cm soil fractions were run as one experiment and the 15-30 cm samples were assayed two weeks later (Table 5.13). Since the maximum mortality difference between the two experiments was about 15% (6 seeds of 40) for samples which were reassayed (Points 1-3 and 0.1% Basin F water), we arbitrarily selected this value as a cutoff point to assess mortality differences at the two depths. Using this rule, only one sample (L-7) in transects L or N showed any lettuce seed mortality differences attributable to depth. However, four samples in Transect M and three in Transect P showed rather large differences, suggesting that the contaminants had either migrated below 15 cm or were purposely placed there. We have no records to support that latter argument and conclude the toxic material has migrated. We have attempted to depict these relationships using the original data in Figures 5.2 and 5.3.

TABLE 5.10. Elemental Analyses of Two Samples from Basin C and One from Basin F

Constituent	Sample Concentration (ppm)		
	K-9		
	0 - 15 cm	15 - 30 cm	G-2
Al	11000	18000	8600
As	63	81	40
Ba	150	180	79
Ca	1500	1600	5900
Cd	<2.5	<2.5	<2.5
Cu	18	23	860
Fe	12000	16000	10000
Mg	2900	4000	2300
Mn	170	150	190
Na	<1000	<1000	9500
Ni	<10	14	<10
P	440	1200	1400
Pb	14	<13	<13
Se	11	20	5.2
Sr	43	44	56
Ti	560	590	230
Zn	740	820	4.7

TABLE 5.11. Original Observations and Mean ± Standard Deviation for Three Replicate Plots Containing 40 Lettuce Seeds Each, Grown in the 15-30 cm Fraction of Basin A Soils

Control	0.1 Basin F Water
34	37
35	25
36	34
35.00 ± 1.0	32.00 ± 6.3

Transect			
L	M	N	P
L1	M1	N1	P1
0	0	9	0
0	0	10	0
0	0	6	0
0	0	8.33 ± 2.08	0
L2	M2	N2	P2
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
L3	M3	N3	P3
9	9	0	1
12	15	2	8
5	2	1	2
8.67 ± 3.51	8.67 ± 6.51	1 ± 1	3.67 ± 3.79
L4	M4	N4	P4
33	4	0	2
32	6	0	1
29	11	0	2
31.33 ± 2.08	7.00 ± 3.61	0	1.67 ± 0.58
L5	M5	N5	P5
36	33	35	15
35	35	39	12
35	33	35	14
35.33 ± 0.58	33.67 ± 1.15	36.33 ± 2.31	13.67 ± 1.53

TABLE 5.11. (Continued)

Transect			
L	M	N	P
L6	M6	N6	P6
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
L7	M7	N7	P7
27	17	1	0
28	15	4	0
22	28	1	0
25.67 ± 3.21	20.00 ± 7.00	2.00 ± 1.73	0
L8	M8	N8	P8
34	28	35	0
34	26	36	0
28	31	37	0
0	0	0	0
L9	M9	N9	P9
34	14	23	32
35	12	17	37
33	16	18	35
34.00 ± 1.00	14.00 ± 2.00	19.33 ± 3.21	34.67 ± 2.52

TABLE 5.12. Original Observations and Mean ± Standard Deviations for Three Replicate Plates Containing 40 Lettuce Seeds Each Which Were Grown in the 0-15 cm Fraction of Basin A Soils

Control	0.1% Basin F Water
28	37
33	25
34	27
31.67 ± 3.21	29.67 ± 6.43

Transcect L	Transect M	Transect N	Transect P
L1	M1	N1	P1
0	0	6	0
0	0	9	0
0	0	5	0
0	0	6.67 ± 2.08	0
L2	M2	N2	P2
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
L3	M3	N3	P3
9	5	0	9
4	3	2	11
4	3	2	9
5.67 ± 2.89	3.67 ± 1.15	1.33 ± 1.15	9.67 ± 1.15
L4	M4	N4	P4
38	35	3	35
36	37	4	36
37	35	2	34
36.00 ± 1.00	35.60 ± 1.15	3.00 ± 1.00	34.00 ± 1.00
L5	M5	N5	P5
37	38	33	30
34	30	35	34
32	29	36	36
34.30 ± 2.52	32.30 ± 4.93	34.67 ± 1.53	33.30 ± 3.06

TABLE 5.12. (Continued)

Transect			
L	M	N	P
L6	M6	N6	P6
0	28	0	0
0	31	0	2
0	23	0	2
0	27.30 ± 4.04	0	1.33 ± 1.15
L7	M7	N7	P7
32	36	0	0
37	37	0	0
38	37	2	0
35.70 ± 3.21	36.70 ± 0.58	0.67 ± 1.15	0
L8	M8	N8	P8
32	36	38	38
38	32	37	37
33	31	36	38
34.30 ± 3.21	33.00 ± 2.65	37.00 ± 1.00	37.70 ± 0.58
L9	M9	N9	P9
38	35	28	34
32	35	21	27
36	33	20	33
35.30 ± 3.06	34.30 ± 1.15	23.00 ± 4.36	31.33 ± 3.79

TABLE 5.13. Comparison of Percent Lettuce Mortality in 0-15 cm and 15-30 cm Fractions of Basin A Soil[a]

	Transect											
	L			M			N			P		
	0-15 cm	15-30 cm	Difference	0-15 cm	15-30 cm	Difference	0-15 cm	15-30 cm	Difference	0-15 cm	15-30 cm	Difference
1	100	100	0	100	100	0	83	79	+4	100	100	0
2	100	100	0	100	100	0	100	100	0	100	100	0
3	86	78	+8	91	78	+13	97	98	-1	76	91	-15
4	10	22	-11	11	83	-72	92	100	-8	15	96	-81
5	14	12	+2	19	16	+3	13	9	+4	15	66	-51
6	100	100	0	32	100	-68	100	100	0	97	100	-3
7	11	36	-25	8	50	-42	98	95	+3	100	100	0
8	14	20	-6	17	29	-12	7	10	-3	6	100	-94
9	12	15	-3	14	65	-51	42	52	-10	22	13	+9

Control			0.1% Basin F Water		
0-15 cm	15-30 cm	Difference	0-15 cm	15-30 cm	Difference
20.82	12.50	+8.03	29.67	20.00	+9.67

[a] Samples PNML 1-3 (0-15 and 15-30 cm), control and Basin F water were from the same source in each experiment.

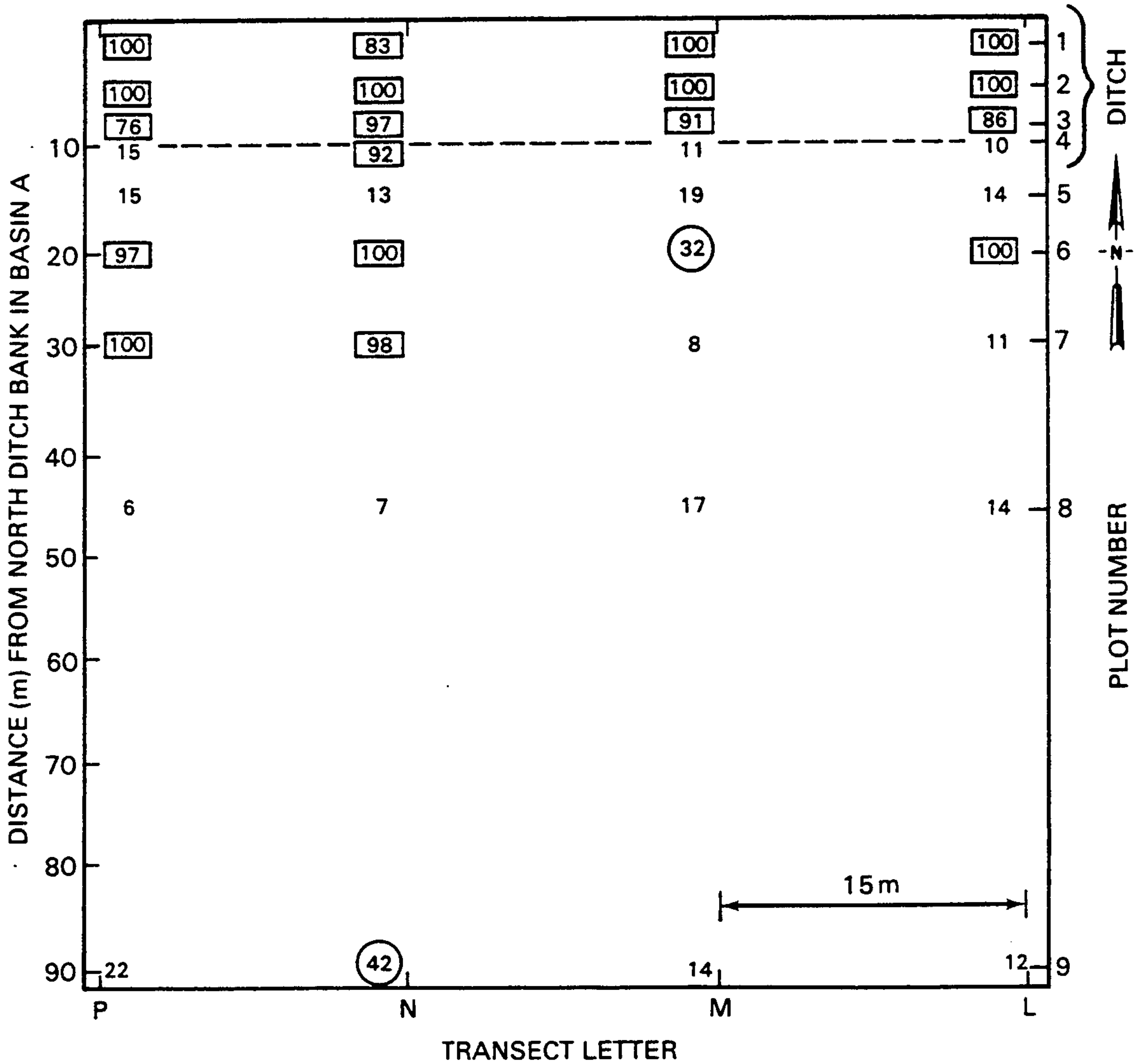

FIGURE 5.2. Observed Mean Lettuce Seed Mortality at Each Basin A Plot (0-15 cm Soil Fraction). Means enclosed with a □ are greater than 75%, a O 30 to 75% and numbers with no symbol are less than 30%.

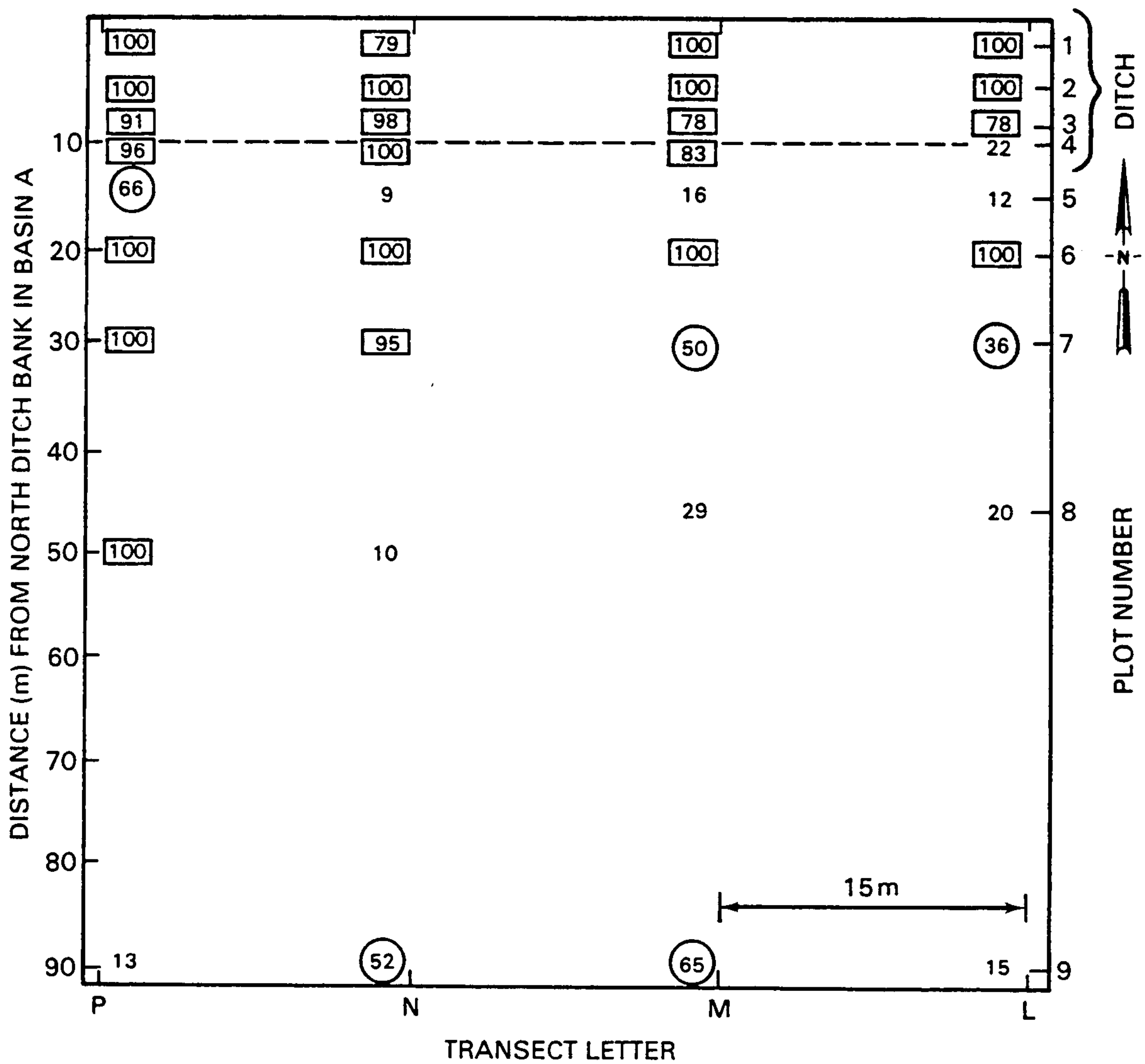

FIGURE 5.3. Observed Mean Lettuce Seed Mortality at Each Basin A Plot (15-30 cm Soil Fraction). Means enclosed with a □ are greater than 75%, a O 30 to 75% and numbers with no symbol are less than 30%.

Another way to depict the mortality patterns at each depth is to calculate some sort of contour map, based on the observations. We elected to use a relatively new statistical technique called kriging developed for use in the mining industry and used principally in Europe and South Africa (Journal and Huijbregts 1978, Clark 1982). Kriging is a weighted moving average technique that calculates point estimates or block averages over a specified grid. The derivation of the kriging weights takes into account the proximity of the observation to the point or area of interest, the structure of the observations (i.e., the relationship of the squared difference between pairs of observations and the intervening distance between them) and any systematic trend or drift in the observations. Additionally, kriging provides a variance estimate that can be used to construct a confidence interval for the kriging estimate. Contour maps are prepared from the kriging estimates. In order to be fairly confident about kriging results, a well behaved and appropriate semivariogram model is needed. Since we were unaware of any previous attempts to krige lettuce seed mortality (or any similar variable), we had no basis to select a semivariogram model except to use a best fit. Because of this, we elected not to present the predicted kriging error structure. Our results, presented in Figures 5.4 and 5.5, clearly show the lettuce seed mortality differences at the two depths. Predicted contamination is far greater at 15-30 cm than at 0-15 cm; also indicated by our analyses of results in Table 5.13.

We believe such maps could be useful in site cleanup decisions (especially when we become more confident about error estimates). As a possible scenario, we have selected 30% mortality as a criterion for cleanup of this Basin A site. The kriging estimates for the 30% mortality level of toxicity in the two soil fractions are in Figures 5.6 and 5.7. The areas enclosed by the solid lines would be targeted for cleanup. Unfortunately, the cleanup decision would be different for the 0-15 cm (Figure 5.6) and the 15-30 cm (Figure 5.7) fractions. While this complicates decision making, the data available and the subsequent maps aid in showing that the field situation is complex, and that decisions based on the 0-15 cm samples alone could

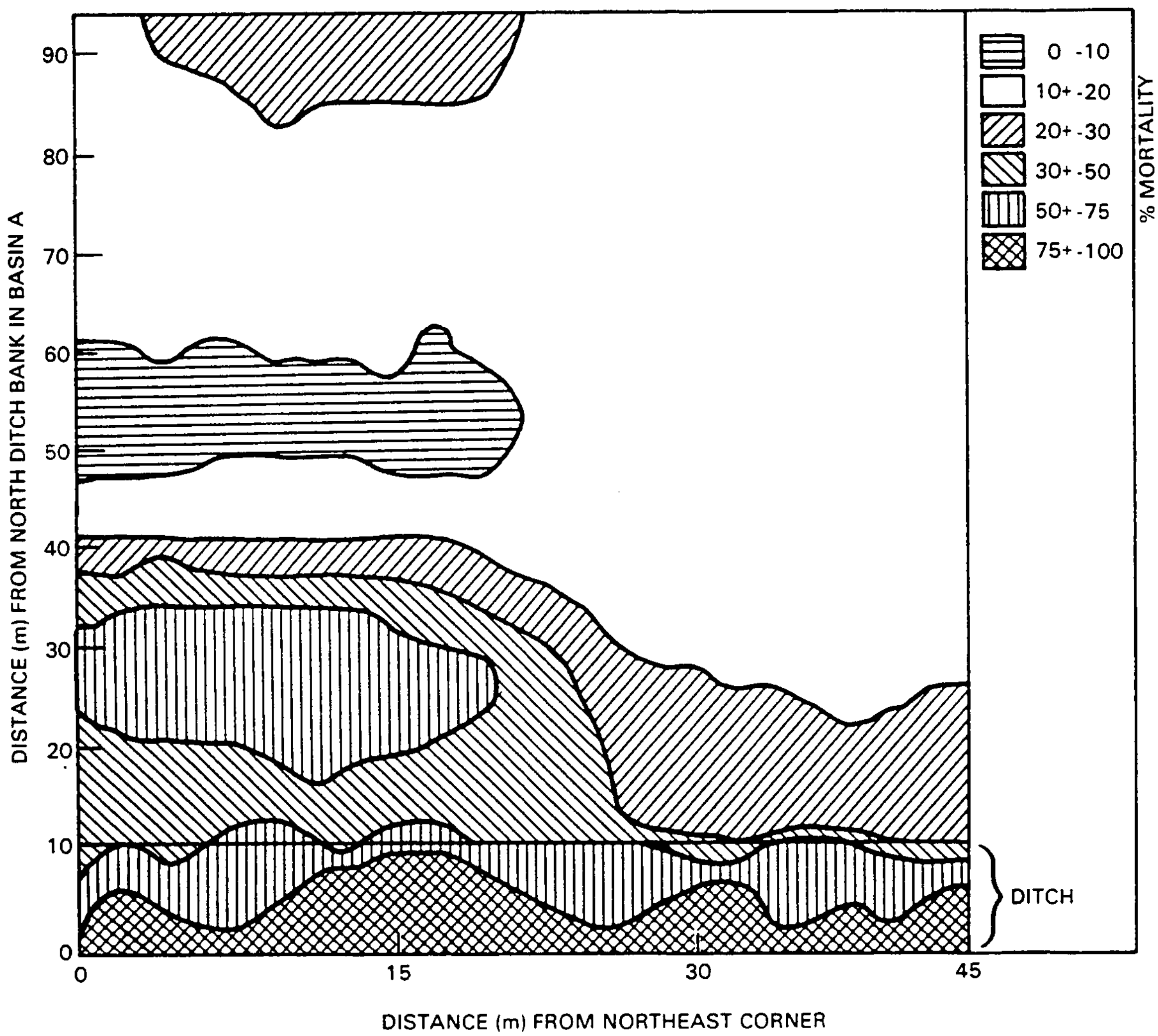

FIGURE 5.4. Predicted Mean Lettuce Seed Mortality Using Kriging (0-15 cm Soil Fraction)

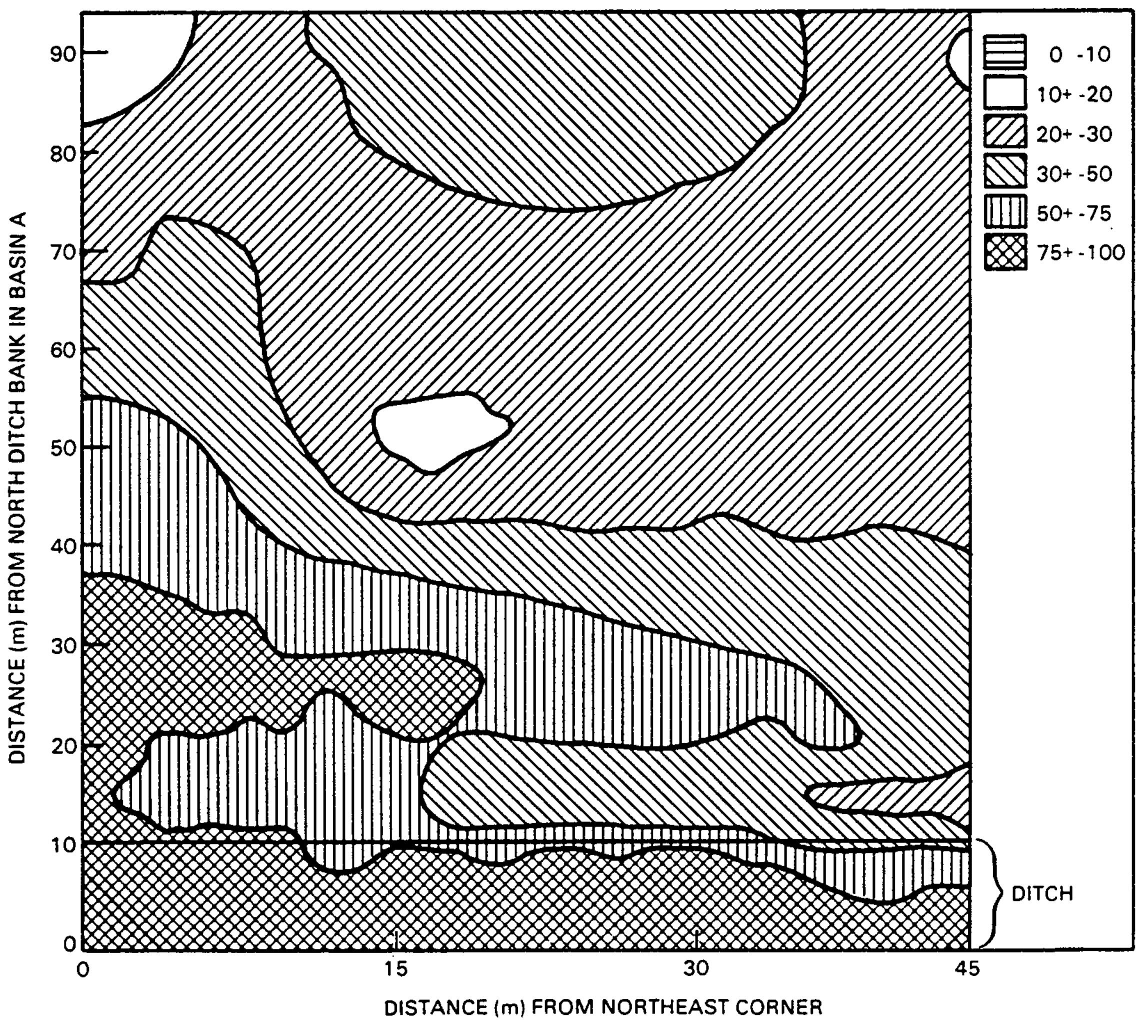

FIGURE 5.5. Predicted Mean Lettuce Seed Mortality Using Kriging (15-30 cm Soil Fraction)

have unwanted consequences. Cleanup based on the 15-30 cm contour would remove all existing contamination that we know of, but samples were not taken below this depth.

Finally, we have added crosshatching to each figure to show where the observed data indicate that mortality was actually greater than 30%. We constructed these "boxes" by simply drawing a line halfway between grid points with observed mortalities greater than 30% and those less than this value. Thus, the crosshatched box at the top of Figure 5.6 is a consequence of the observed mortality at plot N-9 (Figure 5.2, 42%) and all other surrounding plots (P-9, N-8, and M-9) exhibiting mortalities less than 30%. The kriging >30% contour does not include this area or a real hot spot at L-6 (100% observed mortality) because it is a weighted moving average technique. If mimicking the observed data is the criteria for how well kriging performed, then Figure 5.7 shows almost perfect agreement between observed and predicted mortality. We believe this exercise in bioassay of field samples and subsequent kriging analyses offers prospects for aid in cleanup decisions. However, more demonstrations in other environments would be needed to provide evidence for general applicability.

5.3.4.2 Basin F, C, D, and Control Area

No lettuce phytotoxicity was evident in the 15-30 cm fraction for any of the ten control area samples or the two Basin D samples (Table 5.14). As expected, the Basin F sample (G-2) was highly toxic and three of the samples from Basin C (H-5, H-7 and J-7) also resulted in depressed germination (<50%, see Figure 2.2 for locations). The results presented in Table 5.14 for 32 treatments replicated three times represent the limit of our facilities for a single experiment. When this experiment was completed, we decided to devote the remaining resources available for this task to completing the Basin A study (Section 5.3.4.1) since the preliminary results from the first experiment on those soils appeared to indicate a contamination gradient compared to the hot spot situation we found (as planned, Section 2.1) in Basin C (based on the limited data in Table 5.14). Nine samples from the 15-30 cm fraction remain to be assayed as well as all 0-15 cm samples. Under the

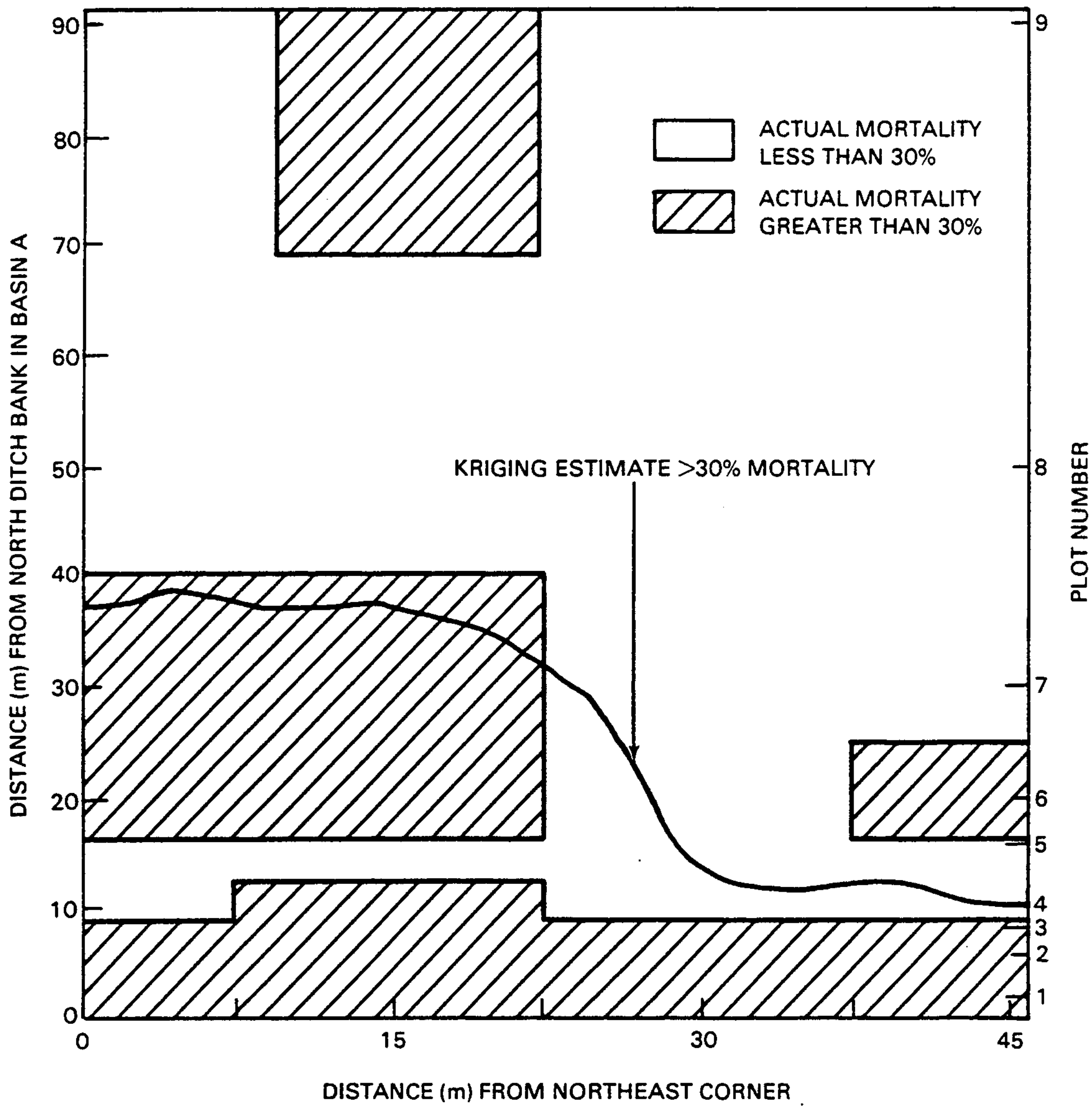

FIGURE 5.6. A Comparison of Lettuce Seed Mortality Predicted from Kriging to Actual Lettuce Seed Mortality (0-15 cm Soil Fraction)

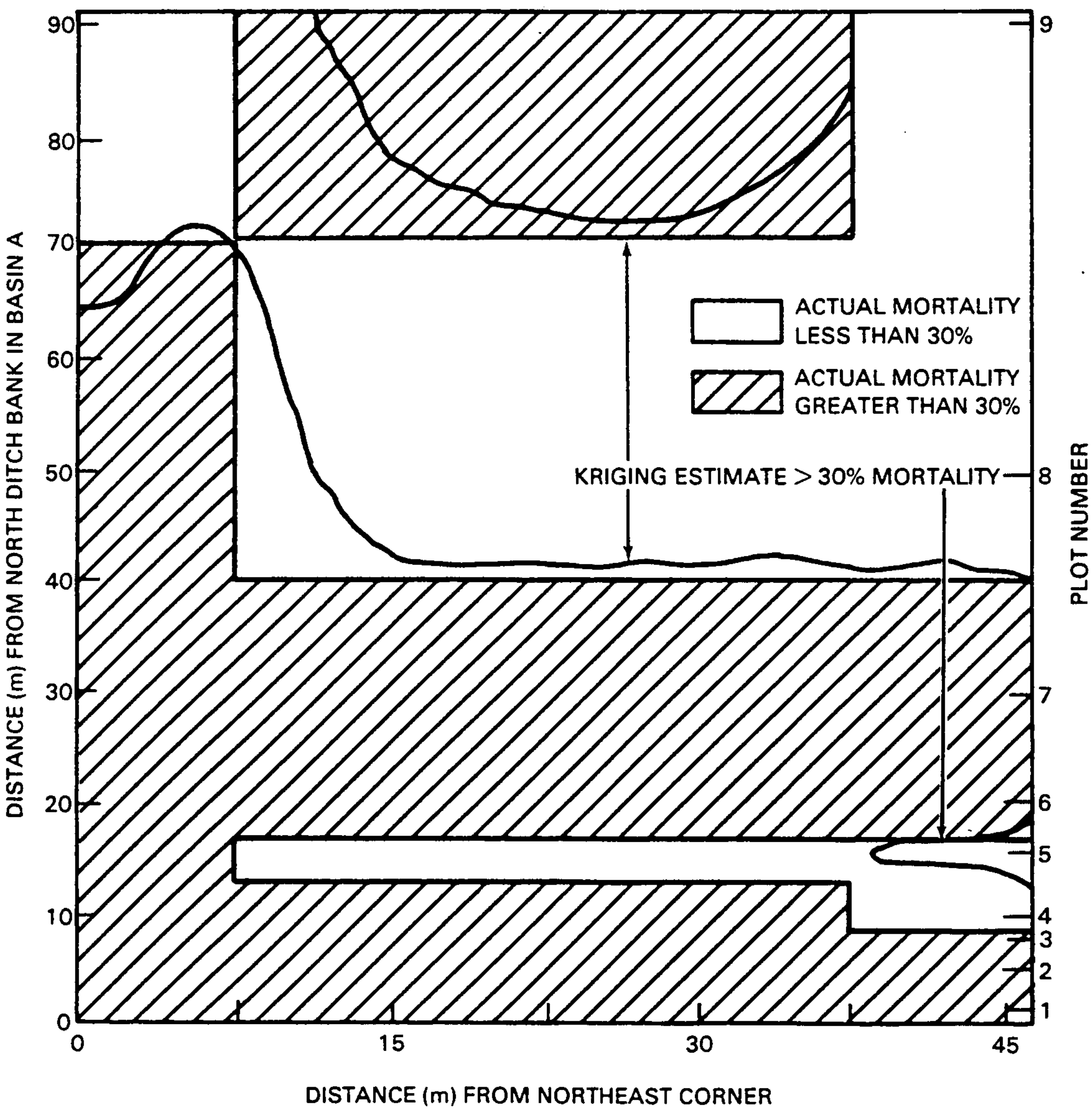

FIGURE 5.7. A Comparison of Lettuce Seed Mortality Predicted from Kriging to Actual Lettuce Seed Mortality (15-30 cm Soil Fraction)

TABLE 5.14. Original Observations and Mean ± Standard Deviation of Lettuce Seed Germination for Three Replicate Plates of 40 Seeds Each Grown in the 15-30 cm Fraction of Basins F, C, D and a Control Area Soils

		Basin F Water	
	Control	0.1%	1%
	33	33	0
	32	39	0
	31	38	0
	32.0 ± 1.0	36.7 ± 3.2	0.0

Basin				
F	G2			
	0			
	0			
	0			
	0.0			
D	B9	B10		
	35	38		
	34	35		
	36	36		
	34.0 ± 1.0	36.3 ± 1.5		
C	H4	H5	H6	H7
	36	24	36	22
	36	15(b)	36	14
	34	--	38	23
	35.3 ± 1.2	19.5	36.7 ± 1.2	19.7 ± 4.9
	J4	J5	J6	J7
	40	35	39	7
	34	36	36	3
	36	36	36	4
	36.7 ± 3.1	35.7 ± 0.6	37.0 ± 1.7	4.7 ± 2.1
	K4	K5		
	24	34		
	34	30		
	29	37		
	29.0 ± 5.0	33.7 ± 3.5		
	G5	G6	G7	G8
	36	37	35	36
	31	36	38	37
	37	39	37	37
	34.7 ± 3.2	37.3 ± 1.5	36.7 ± 1.5	36.7 ± 0.6

TABLE 5.14. (Continued)

Basin				
C (cont.)	G9	G10		
	38	37		
	33	36		
	29	35		
	33.3 ± 4.5	36.0 ± 1.0		
Control	E4	E5	E6	E7
	38	35	40	36
	34	36	28	32
	32	35	30	37
	34.7 ± 3.1	35.7 ± 0.6	32.7 ± 6.4	35.7 ± 1.15
	E8	E9	E10	
	35	35	39	
	35	36	36	
	37	34	37	
	35.7 ± 1.2	35.0 ± 1.0	37.3 ± 1.5	
	F4	F5	F10	
	35	32	34	
	32	35	38	
	34	38	32	
	33.7 ± 1.5	35.0 ± 3.0	34.7 ± 3.1	

(a) See Figure 2.2 for sample locations.
(b) Too little soil.

assumption that all other control area soil samples are not toxic, phytoassay of the nine additional Basin C samples (plus a few samples already assayed to provide an experimental intercomparison) would allow us to attempt a limited investigation of objective three in Section 2.1 (i.e., optimum designs for hot spot detection, further explained in Section 2.2 and Appendix B). Clearly, the most appropriate procedure would be to repeat the entire experiment since the intercalibration of data from two experiments can be difficult and confusing.

Since we had routinely used Basin F water as our negative control, we conducted an experiment to determine the LD50 of Basin F soil (G-2) and soil from the Basin A trench (N-1, see Figures 5.2 and 5.3). Two experiments gave mortalies of 83% and 79% in the latter soil (Figure 5.2 and 5.3) when 100% soil was used, while 100% lettuce seed mortality was observed in undiluted Basin F soil (Table 5.14). The LD50 results are in Table 5.15 and are presented graphically in Figure 5.8. It appears that the LD50 for Basin F soil is about 2.7% (95% confidence interval was 2.3 to 3.2%). Our previous results using Basin F water indicate that the LD50 between 0.1 and 1% (see tables in this section). Thus, Basin F water is over twice as toxic as the soil. The 100% Basin A soil resulted in about 14% germination, compared to 17% and 21% in other studies using the same soil (Figures 5.2 and 5.3). The LD50 was approximately 70% soil, a factor of 30 less toxic than Basin F soil.

5.4 REFERENCES

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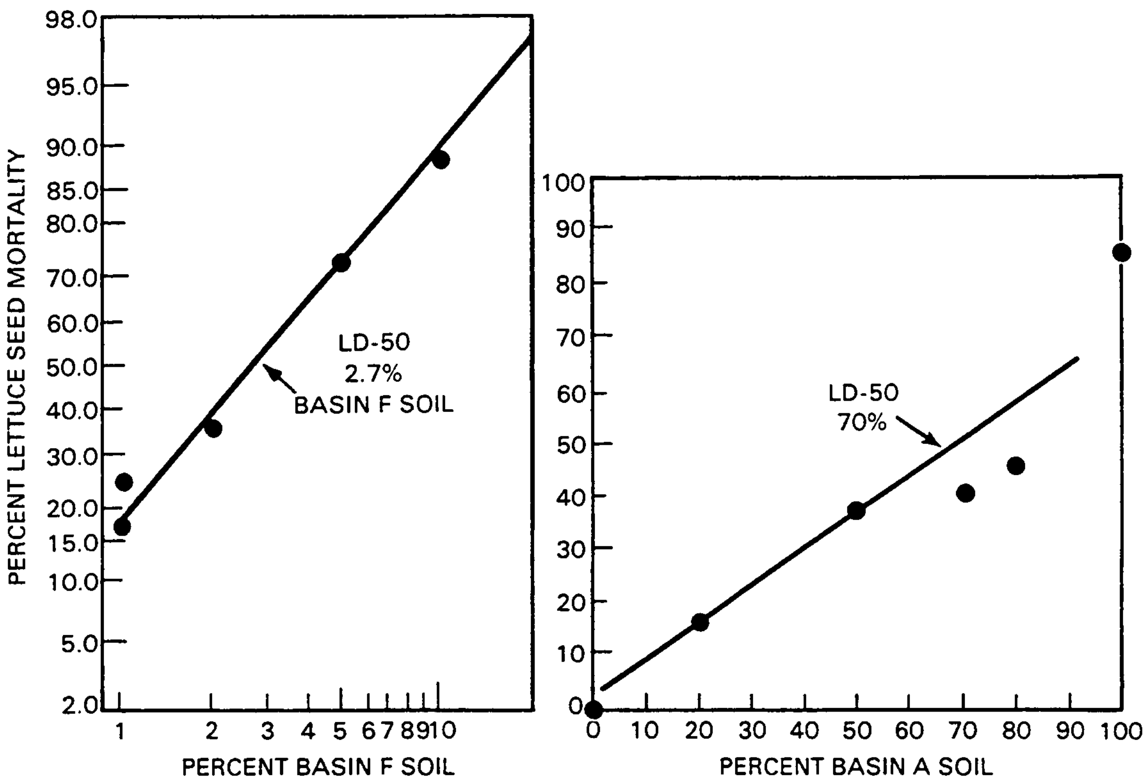

FIGURE 5.8. LD50 for One Basin A and One Basin F Soil

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TABLE 5.15. Percent Germination of Lettuce Seeds as a Function of Dose for a Basin F and Basin A Soil

Sample Numbers	Location	Proportion of Soil (%)		Seeds Germinated[a]	
		Basin	Control	Number ± SD	Percent
G-2	Basin F	50	50	0	0
		10	90	4.7 ± 4.0	11.8
		5	95	11.0 ± 5.6	27.5
		2	98	25.7 ± 4.2	64.3
		1	99	30.3 ± 3.1	75.8
N-1	Basin A	100	0	5.5 ± 0.7[b]	13.8
		80	20	21.3 ± 1.5	53.3
		70	30	23.7 ± 4.7	59.3
		50	50	24.3 ± 6.0	60.8
		20	80	33.7 ± 1.5	84.3
Positive Control		0	100	35.0 ± 1.0	87.5
Treatment Control	Basin F Water	0.1	100	32.3 ± 2.1	80.8

(a) Mean of three replicate plates of 40 seeds each.
(b) Mean of two replicate plates of 40 seeds each.

6.0 VEGETATION STUDIES

6.1 INTRODUCTION

The results of our earlier work (Volume 1, Section 5.0) indicated that gross changes in field vegetation along suspected chemical gradients can be detected by measurements of relative plant cover and species composition. However, analyses of soil samples were required to determine specific areas of potential contamination among plots along three separate transects. Unfortunately, phytoassay of 25 soil samples (Volume 1, Section 8.0) failed to reveal any contamination (top 20 cm only). Because of this, five additional soil profile samples were obtained for phytoassay (Volume 1, Section 2.3.2 and Figures 2.5, 2.6, 2.7). The results (reported in Section 5.0) obtained using Basin A waste ditch soil samples led us to believe that a gradient of contamination would likely exist near this location. Phytoassay of the profile samples from Basin C and an abandoned ditch were not encouraging. However, since Basin F water is extremely phytotoxic (Volume 1, Table 8.1), we thought that a Basin C site immediately adjacent to Basin F might exhibit a contamination gradient as opposed to the Basin C transect located in mid-basin (Volume 1, Figure 2.7). Two additional field sites were, therefore, established (Section 2.2); one in Basin A and one in the north end of Basin C. In this section, we report the results of the plant cover and species characterization for these two new study sites. The intercomparison with the phytoassay results based on soil samples from the same sites is in Section 7.0.

6.2 METHODS

Plant height and cover measurements were made on August 19-20, 1983. The same general scheme was used to estimate percent vegetative cover at each sample location. Each soil sampling location (Section 2.3) served as the reference corner of a 5 x 5 m plot; within the plot, the Daubenmire (1968) cover rank system (1 = 0.5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-95%, and 6 = 96-100%) was used to estimate percent cover by species and bare ground within six randomly chosen 0.5 x 0.2 m subplots (Daubenmire frames).

Since litter is an important indicator of past plant growth, this component was also estimated within each Daubenmire frame. The frames were always aligned north-south; the southwest corner of the frame was always placed on the randomly chosen coordinate pair. A list of all species (and the respective abbreviations) found and used in our analyses are in Table 6.1.

In Basin A, at sample location 4 for all transects, the plot size was restricted to 1.5 x 1.5 m because the distance between points 3 and 4 on each transect was only 1.5 m (Figure 2.3). In addition, only three random Daubenmire frames were used to estimate cover at these locations. No plots were established for locations 2 and 3 on all transects because these points were in the trench and water and soft mud made access impossible. Furthermore, no plants or litter were found in the vicinity of these points.

The heights of the three most common species in Basin A [*Kochia iranica* (kochia), *Lactuca scariola* (prickly lettuce) and *Conyza canadensis* (horseweed)] were measured. Five individuals from each of the chosen species closest to a soil sampling point were selected and measured from ground level to the highest vertical branch (or the tip, in the absence of branching).

Vegetative measurements were made at all sample locations in Basin C except G-10 (vegetation was too high so a visual estimate was made). Only locations E-4 through E-10 were evaluated in the control area adjacent to Basin C (Section 2.0, Figure 2.2). No measurements were made in Basin F.

Plant cover results were evaluated using a computer algorithm called TWINSPAN, which is based on principal component analysis, but incorporates a reciprocal average weighing scheme (Hill 1979). This procedure uses Daubenmire (1968) species cover ranks to create an indicator or "pseudospecies" (Table 6.2). For example, if the highest Daubenmire rank cover estimate for Kochia is 4, four pseudospecies are created by the TWINSPAN algorithm: Kochia 1, representing those instances where the average cover rank for Kochia over the six Daubenmire quadrants per plot is less than 0.5; Kochia 2, representing those cases where the average cover rank is between 0.5 and 1.5, and so on. The maximum number of pseudospecies is seven and only occurs if a particular species has an average cover rank greater

TABLE 6.1. Plant Species Recorded from Basin A, C, and a Control Area at Rocky Mountain Arsenal Soil Sample Locations (August 19-20, 1983)

Code	Scientific Name	Common Name
SPCR	*Sporobolus crytpandrus*	Sand dropseed
ARLO	*Aristida longiseta*	Red threeawn
AGSM	*Agropyron smithii*	Western wheatgrass
SIHY	*Sitaneon hystrix*	Squirrel-tail
BUDA	*Buchloe dactyloides*	Buffalo grass
BRTE	*Bromus tectorum*	Cheatgrass
SOTR	*Solanum triflorum*	Cutleaved nightshade
SORO	*Solanum rostratum*	Buffalo-bur
PHVI	*Physalis virginiana*	Ground cherry
LASC	*Lactuca scariola*	Prickly lettuce
KOIR	*Kochia iranica*	Kochia
AMAL	*Amaranthus albus*	Tumble pigweed
AMRE	*Amaranthus retroflexus*	Rough pigweed
LYJU	*Lygodesmia juncea*	Rush skeletonweed
HEAN	*Helianthus annus*	Common sunflower
HEPE	*Helianthus petiolaris*	Prairie sunflower
VEEN	*Verbesian encelioides*	Crownbeard
SAKA	*Salsola kali*	Russian thistle
COCA	*Conyza canadensis*	Horseweed
ARPO	*Argemone polyanthemos*	Prickly poppy
CHGI	*Chenopodium gigantospermum*	Giant goosefoot
CLSE	*Cleome serrulata*	Rocky Mountain bee plant
CHGL	*Chamaesyce glyptosperma*	Corrugate-seeded spurge
SCSP	*Scrophularia sp.*	Scroph species
BARE		Bare ground
LITT		Litter

TABLE 6.2. An Example of the Relationship between Percentage Cover, Average Daubenmire Cover Rank and the TWINSPAN-Generated Pseudospecies (*Kochia iranica* = KOIR).

Percentage Ground Cover	Average Daubenmire Rank Value (n=6)	Pseudospecies (Kochia iranica)
0-5	<.5	KOIR1
0-5	.5-1.5	KOIR2
6-25	1.5-2.5	KOIR3
26-50	2.5-3.5	KOIR4
51-75	3.5-4.5	KOIR5
76-95	4.5-5.5	KOIR6
96-100	>5.5	KOIR7

than 5.5 for at least one plot in the TWINSPAN analysis. It should also be noted that since bare ground and dead plant litter were estimated using the Daubenmire cover rank system, they were also included as pseudospecies in the TWINSPAN analysis. These indicator species were initially used to divide the sampling plots into two major groups and subsequently, to iterate the same scheme until the desired resolution was attained.

In addition to the TWINSPAN analysis, mean percentage cover by species was calculated from the Daubenmire rank values for each 5x5 m plot. Mean plant height was also estimated for the three most common species.

6.3 RESULTS

6.3.1 Basin A

The most obvious difference between plots located along the four transects in Basin A is illustrated by the first division of the TWINSPAN dendrogram (Figures 6.1 and 6.2). Plots with pseudospecies "Bare 6" and "Bare 7" were separated from those plots with pseudospecies of litter and forbs. Plots 1 through 4 of all transects had no or low (below 30%) vegetation cover and, when plants were present, species diversity was low (Table 6.3). The other plots along the Basin A transects were characterized either by relatively more vegetative and litter cover, higher species diversity, or both (Figures 6.1 and 6.2, Table 6.2).

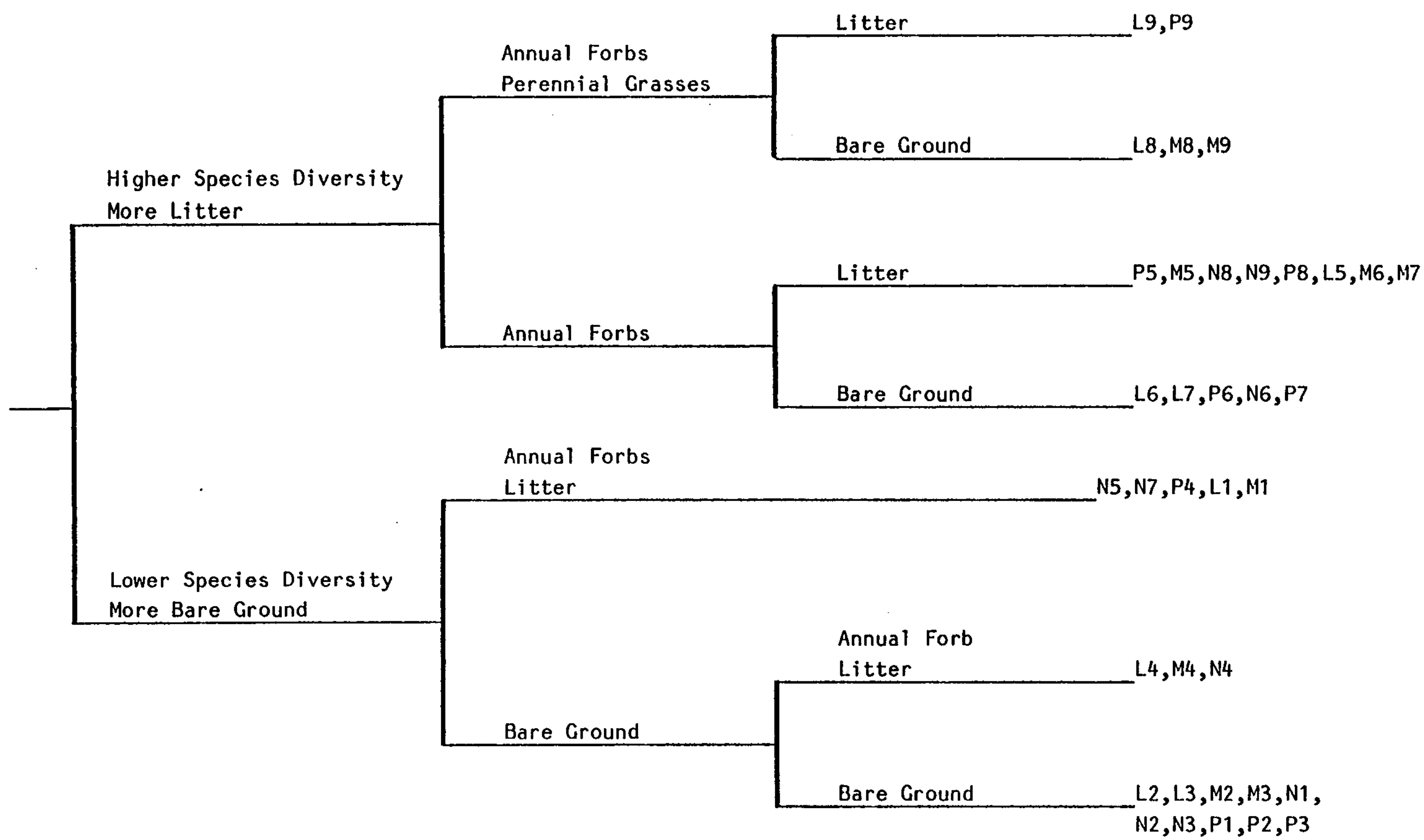

FIGURE 6.1. Summarized Results of TWINSPAN Analysis of Plant Species Composition and Cover for Plots along Four Transects in Basin A, Rocky Mountain Arsenal. The plots are identified by a transect letter and number which indicates relative position (see Section 2.0 and Figure 2.3).

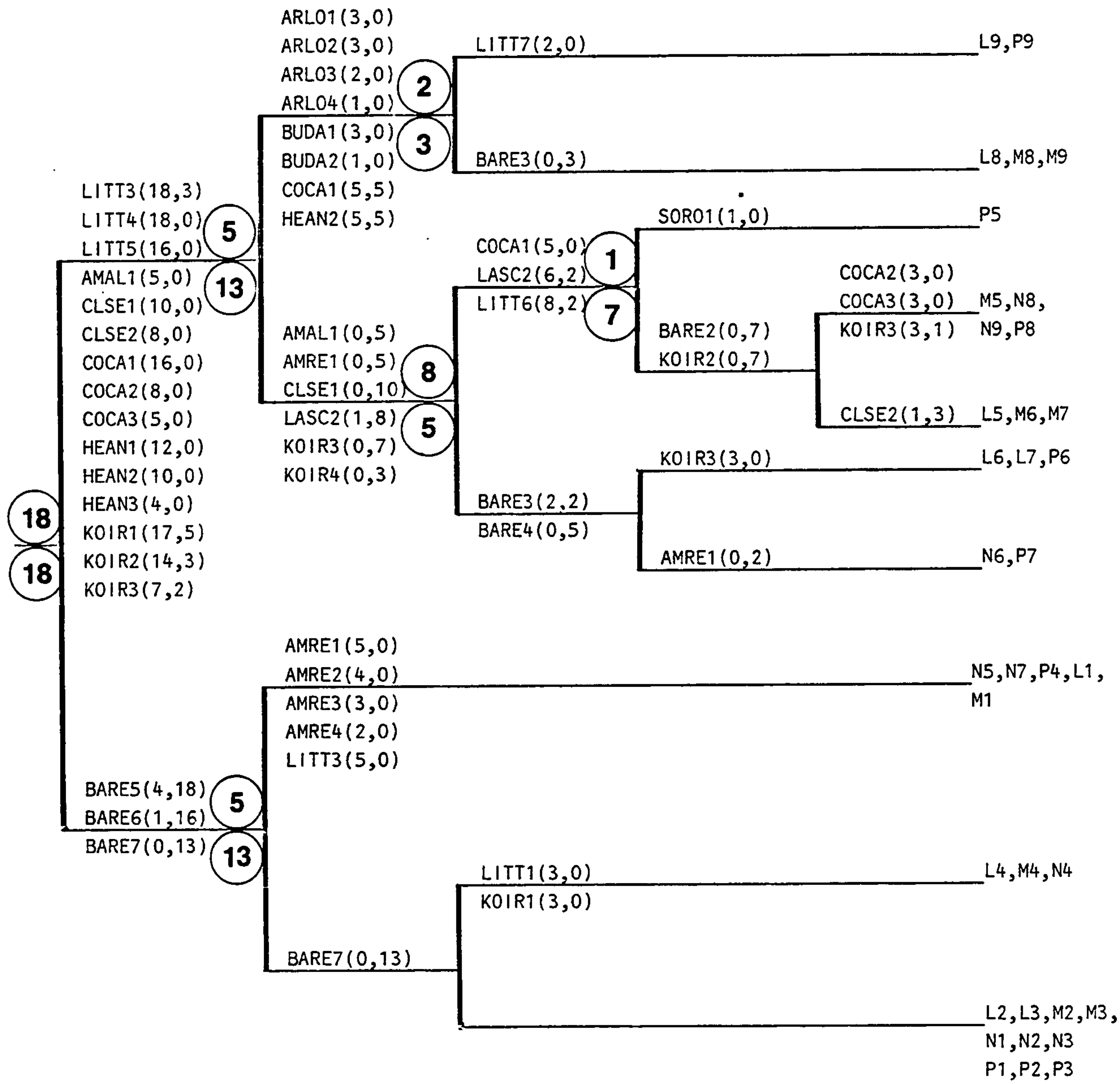

FIGURE 6.2. Detailed Results of TWINSPAN Analysis of Plant Species Composition and Cover for Plots along Four Transects in Basin A, Rocky Mountain Arsenal. Circled numbers indicate the number of plots included in each portion of a dendrogram split. Numbers in parentheses with each pseudospecies give an indication of the "strength" of a pseudospecies in defining a particular group of plots. The larger of the two numbers is the number of plots in each group created by a dendrogram branch which contains that particular pseudospecies; the lesser of the two numbers is the number of plots in the opposite group created by the dendrogram branch which contains that pseudospecies.

TABLE 6.3. Mean Vegetative Cover (n=6 Daubenmire frames) for Plots along Transects in Basins A and C and a Control Area in Section 27, Rocky Mountain Arsenal

Transect Location	Number of Species	Bare Ground (%)	Litter Cover (%)	Perennial Grass Cover (%)	Annual Grass Cover (%)	Forb Cover (%)	Total Cover (%)
Basin A							
L-1	2	25	74			6	6
L-2	0	98					
L-3	0	98					
L-4*	1	98	1			15	15
L-5	4	11	84			50	50
L-6	5	41	54			52	52
L-7	4	55	32			50	50
L-8	9	70	15	24		39	63
L-9	6	3	89	72		9	81
M-1	2	64	36			22	22
M-2	0	98					
M-3	0	98					
M-4*	1	98	1			26	26
M-5	3	8	84			25	25
M-6	5	18	81			43	43
M-7	3	19	80			39	39
M-8	4	20	75			59	59
M-9	6	23	62	12		14	26
N-1	0	98					
N-2	0	98					
N-3	0	98					
N-4*	1	98	2			5	5
N-5	3	81	18			34	34
N-6	5	42	57			29	29
N-7	2	75	24			30	30
N-8	4	7	89			55	55
N-9	5	23	70			27	27
P-1	0	98					
P-2	0	98					
P-3	0	98					
P-4*	1	82	18			34	34
P-5	8	1	98			73	73
P-6	3	73	27			53	53
P-7	4	25	74			39	39
P-8	4	3	82			66	66
P-9	5	1	98			33	33
Basin C							
G-4	4	54	17			51	51
G-5	2	33	65			1	1
G-6	1	82	20			<1	<1
G-7	0	93	5			0	0
G-8	1	81	19			<1	<1
G-9	1	25	75			5	5
G-10	4	74	26			99	99

TABLE 6.3. (Continued)

Transect Location	Number of Species	Bare Ground (%)	Litter Cover (%)	Perennial Grass Cover (%)	Annual Grass Cover (%)	Forb Cover (%)	Total Cover (%)
Basin C (continued)							
H-4	3	69	21			41	41
H-5	0	98	1				
H-6	0	98	2				
H-7	0	98	2				
H-8	2	83	17			13	13
H-9	2	62	38			39	39
H-10	3	78	22			32	32
J-4	3	46	34			29	29
J-5	0	98					
J-6	0	98	1				
J-7	2	62	38			7	7
J-8	0	90	6				
J-9	0	98	2				
J-10	1	85	15			<1	<1
K-4	3	93	6			61	61
K-5	0	98	2				
K-6	0	98	1				
K-7	0	98	1				
K-8	0	98					
K-9	0	98					
K-10	2	77	23			24	24
Control							
E-4	2	38	62			37	37
E-5	6	9	90			78	78
E-6	2	3	98	46		3	49
E-7	10	4	95	3	35	12	50
E-8	7	3	95		74	32	106
E-9	4		98		97	9	106
E-10	4		98	9	82	1	92

*n=3

Height measurements of the three most common species showed no discernible pattern between plots (Table 6.4). However, the coefficient of variation for height was relatively constant in all transects for prickly lettuce, (LASC) while kochia (KOIR) and horseweed (COCA) generally exhibited higher mean coefficients of variation and wider ranges (Table 6.5).

6.3.2 Basin C and Control Area

The TWINSPAN dendrogram first separated nearly all of the plots located along the transect in the control area (the plots along transect E; Figure 2.2) from those in Basin C (Figures 6.3 and 6.4). With the exception of plot E4, all plots located along the transect through the control area had relatively high plant and litter cover (Table 6.3). In addition, grasses were present in five of the seven plots along transect E (Table 6.3). The plots along the transects in Basin C were divided into two major groups by the TWINSPAN analysis. Plots G6 through G8, H5 through H7, J5 and J6 and J8 through J10 and K5 through K10 had essentially no plant cover (Table 6.3) and were grouped together in the dendrogram (Figures 6.3 and 6.4). The other plots in Basin C had relatively high plant cover, all provided by forbs (Table 6.1 and Figure 6.4).

6.4 SUMMARY

6.4.1 Basin A

Basin A has received toxic wastes for years and is, by its very nature, a disturbed area. Based on the results of the plant cover analysis, we conclude that some of the plots established along the transects in Basin A were more severely impacted or impacted in a different way than others. One interpretation of the TWINSPAN dendrogram of Basin A (Figures 6.1 and 6.2) would be to view it as a "gradient": the plots at the top of the dendrogram have the highest relative cover and species diversity and hence are the least impacted; the plots at the bottom of the dendrogram have the lowest relative plant cover and diversity and are therefore the most severely impacted. When the results of the lettuce phytoassay are compared to those of the plant

TABLE 6.4. Mean Height (cm), Standard Deviation and Coefficient of Variation of the Three Most Common Plant Species in 5 x 5 m Plots along Four Transects in Basin A, Rocky Mountain Arsenal (n=5)

	Plant Species								
	Prickly Lettuce (Lactuca scariola)			Kochia (Kochia iranica)			Horseweed (Conyza canadensis)		
Location	X	SD	CV	X	SD	CV	X	SD	CV
L-1	--	--	--	23.0	2.5	10.9%	56.8	14.9	26.2%
L-2	--	--	--	--	--	--	--	--	--
L-3	--	--	--	--	--	--	--	--	--
L-4	--	--	--	25.5	6.4	25.1%	--	--	--
L-5	116.8	14.8	12.7%	27.2	8.9	32.3%	73.0	11.7	16.0%
L-6	82.2	21.7	26.4%	37.1	13.1	35.3%	15.5[a]	--	--
L-7	103.8	15.5	14.9%	15.9	2.4	15.1%	43.7	6.8	15.6%
L-8	111.1	34.5	31.1%	15.8	2.6	16.4%	84.8	3.9	4.6%
L-9	102.6	17.8	17.3%	--	--	--	81.1	15.9	19.6%
M-1	--	--	--	13.2	5.4	40.9%	--	--	--
M-2	--	--	--	--	--	--	--	--	--
M-3	--	--	--	--	--	--	--	--	--
M-4	--	--	--	27.0	6.3	23.3%	50.0[a]	--	--
M-5	112.0	20.6	18.4%	27.6	7.9	28.6%	66.3[c]	19.7	29.7%
M-6	110.8	19.0	17.1%	42.8	4.6	10.7%	67.5[b]	17.7	26.2%
M-7	106.0	42.1	39.7%	9.7	4.6	47.4%	79.2	24.5	30.9%
M-8	87.2	13.6	15.6%	12.0	4.1	34.2%	57.0	26.9	47.2%
M-9	80.6	17.4	21.6%	6.6	2.2	33.3%	68.5	5.9	8.6%
N-1	--	--	--	--	--	--	--	--	--
N-2	--	--	--	--	--	--	--	--	--
N-3	--	--	--	--	--	--	--	--	--
N-4	--	--	--	31.2	11.8	37.8%	--	--	--
N-5	106.3	18.7	17.6%	26.0	6.5	25.0%	--	--	--
N-6	65.2	22.0	33.7%	24.2	8.9	36.8%	70.8	26.9	38.0%
N-7	52.8	15.2	28.8%	17.8	11.2	62.9%	83.0	21.4	25.8%
N-8	118.2	16.1	13.6%	6.9	1.4	20.3%	91.8	8.1	8.8%
N-9	122.2	21.5	27.6%	8.2	4.0	48.8%	40.0	14.1	35.3%
P-1	--	--	--	--	--	--	--	--	--
P-2	--	--	--	--	--	--	--	--	--
P-3	--	--	--	--	--	--	--	--	--
P-4	--	--	--	--	--	--	--	--	--
P-5	125.2	13.1	10.5%	31.0	7.1	22.9%	79.0	19.9	25.2%
P-6	83.7	14.8	17.7%	32.6	4.4	13.5%	--	--	--
P-7	108.8	39.7	36.5%	14.6	9.9	67.8%	74.0	33.9	45.8%
P-8	115.6	23.6	20.4%	34.6	4.2	12.1%	80.0	11.8	14.8%
P-9	111.6	21.9	19.6%	6.7	2.2	32.8%	71.0	12.8	18.0%

[a] n=1
[b] n=2
[c] n=3

TABLE 6.5. Mean and Range of Coefficients of Variation for the Three Most Common Plant Species in 5 x 5 m Plots along Four Transects in Basin A, Rocky Mountain Arsenal

Transect Letter	Prickly Lettuce (Lactuca scariola) N^a	CV	Range	Kochia (Kochia iranica) N^a	CV	Range	Horseweed (Conyza canadensis) N^a	CV	Range
L	5	20.5	12 - 31	6	22.5	11 - 35	5	16.4	5 - 26
M	5	22.5	17 - 39	7	31.2	11 - 47	5	28.5	9 - 47
N	5	24.3	18 - 34	6	38.6	20 - 63	4	27.0	9 - 35
P	5	20.9	11 - 37	5	29.8	12 - 68	4	26.0	15 - 46

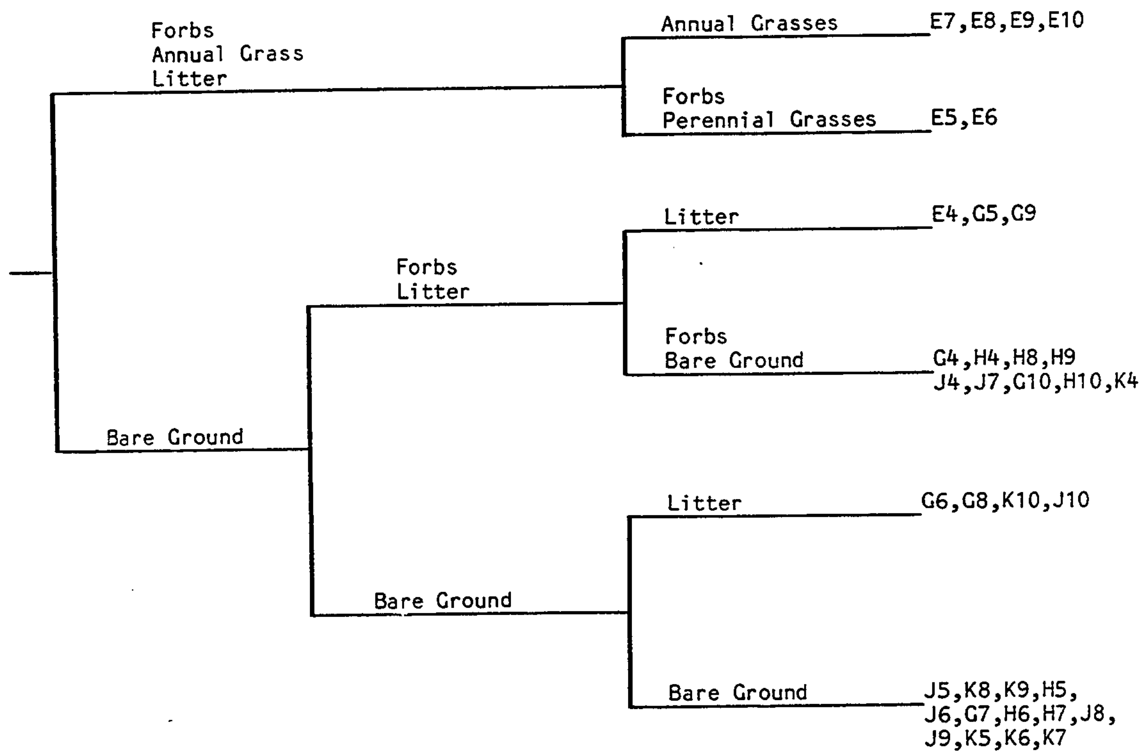

FIGURE 6.3. Summarized Results of TWINSPAN Analysis on Plant Species Composition and Cover for Plots along Five Transects in Section 27 (G, H, J and K in Basin C and E in a Control Area), Rocky Mountain Arsenal

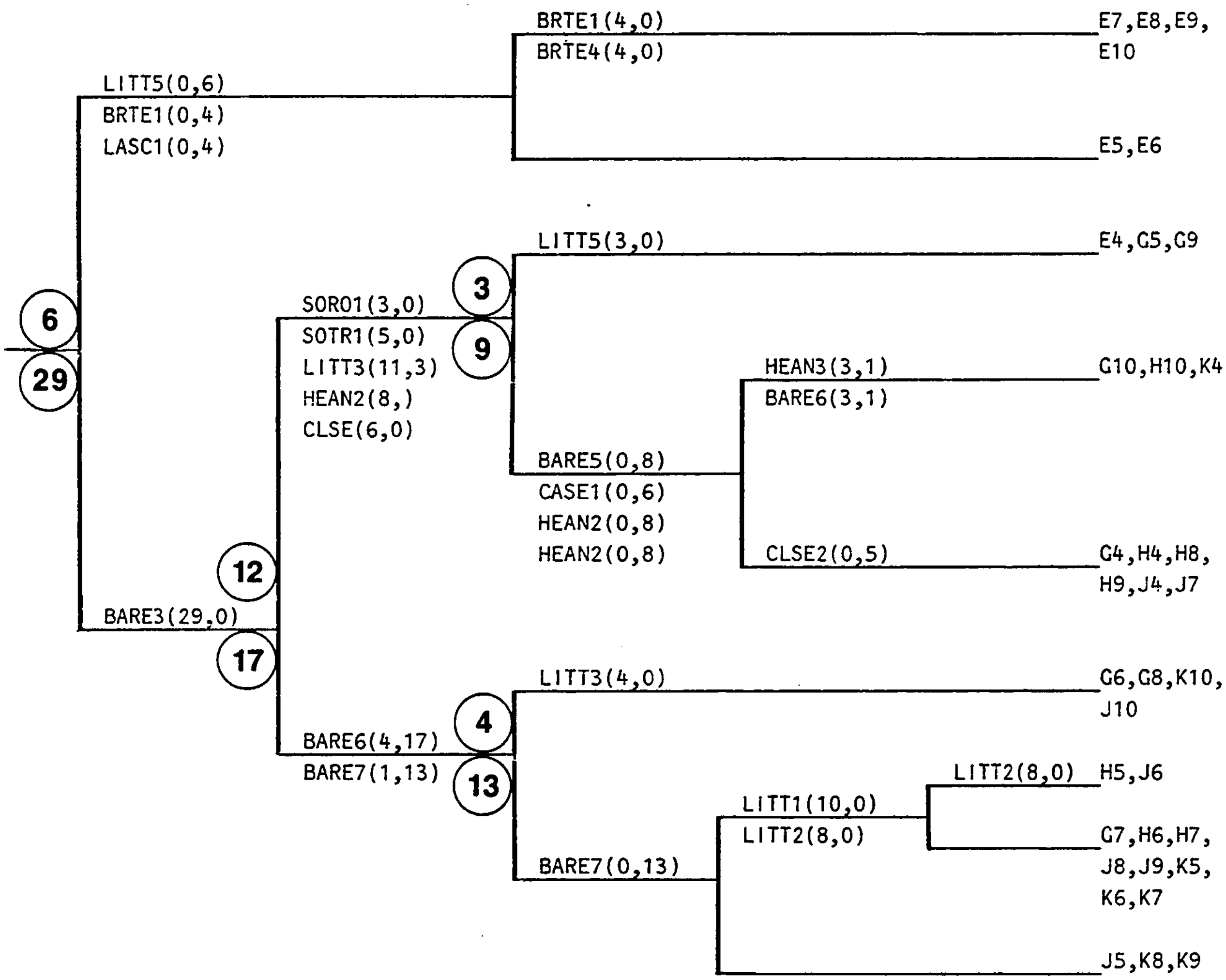

FIGURE 6.4. Detailed Results of TWINSPAN Analysis of Plant Species Composition and Cover for Plots along Five Transects in Section 27 (G, H, J and K in Basin C and E in a Control Area), Rocky Mountain Arsenal (Section 2.0, Figure 2.2). Circled numbers indicate the number plots included in each portion of a dendrogram split. Numbers in parenthesis give an indication of the "strength" of a pseudospecies in defining a particular group of plots. The larger of the two numbers is the number of plots in each group created by a dendrogram branch which contains that particular pseudospecies; the lesser of the two numbers is the number of plots in the opposite group created by the dendrogram branch which contains that pseudospecies.

cover analysis, the view of the TWINSPAN dendrogram as a "gradient" becomes even more convincing (Section 7.0). Thus, it seems likely that vegetation cover analysis in conjunction with a multivariate technique such as TWINSPAN may be a useful rough guide in separating areas with relatively high levels of impact (contaminants) from unimpacted areas. We caution that in semi-arid areas such as Rocky Mountain Arsenal, the results of such an analysis could be very different in years of low rainfall (1983 was a high moisture spring-summer). The techniques would be best used in conjunction with other methods (selected bioassays), to confirm field observations or, more importantly, as an aid in choosing sites for intensive soil sampling for laboratory bioassays.

6.4.2 Basin C

The results of the plant cover analysis of Basin C are difficult to relate to impact because these plots were in an area that was under water or saturated for much of the year. The impermeable clay layer underlying much of the basin limited drainage and severely restricted plant growth. However, the TWINSPAN analysis did confirm our field observations. This "validation" supports the usefulness and accuracy of the technique and lends credibility to the Basin A analysis.

6.5 REFERENCES

Daubenmire, R. 1968. Plant Communities, a Text Book of Plant Synecology. Harper & Row, New York.

Hill, M. O. 1979. TWINSPAN, a Fortran Program for Arranging Multivariate Data in an Ordered Two-Way Table by Classification of the Individuals and Attributes. Cornell University, Ithaca, New York.

7.0 INTERCOMPARISON OF PHYTOASSAY AND VEGETATIVE COVER RESULTS

7.1 INTRODUCTION

In Section 5.3.2 we noted a tenuous relationship between the size and vigor of Kochia and lettuce seed mortality in Basin A trench soils (n=3, 1982 samples). Because of this, a logarithmic sampling grid was established in the Basin in 1983 (Figure 2.3), in an attempt to relate results of a soil phytoassay to results from a field vegetation survey (Section 2.1).

In part, the principal component methods used to classify plant cover (e.g. TWINSPAN; Section 6.2) are qualitative and as such do not have a rigorous statistical basis. This statement is not meant to detract from the usefulness of such methods, but to point out that caution should be employed in interpreting the final dendrogram (in a quantitative sense). Similarly, the kriging methodology used to depict lettuce seed mortality observed in Basin A soil samples (Section 5.3.4.1) has never been applied (to our knowledge) to this kind of data. Thus, the following intercomparison of field vegetative cover results and laboratory-derived lettuce mortality should be considered qualitative.

To avoid bias, the lettuce seed bioassay, the vegetative cover measurements, the TWINSPAN analysis of these measurements, and the interpretation of the kriging analyses of lettuce seed mortality were carried out by four different individuals without any knowledge of other results. However, all investigators were aware (*a priori*) that the trench area itself was thought to be contaminated.

7.2 METHODS

Figure 7.1 is similar to the summary of plant species composition and cover in Basin A shown in Figure 6.1. However, Figure 7.1 also depicts lettuce seed mortality in both soil fractions (i.e., 0-15 and 15-30 cm). Reference to Figure 6.2 shows that the upper three "arms" of the dendrogram in Figure 7.1 coincide with less than 25% bare ground (i.e., pseudospecies BARE1 to BARE3). We took this to be a cutoff point between better and poorer

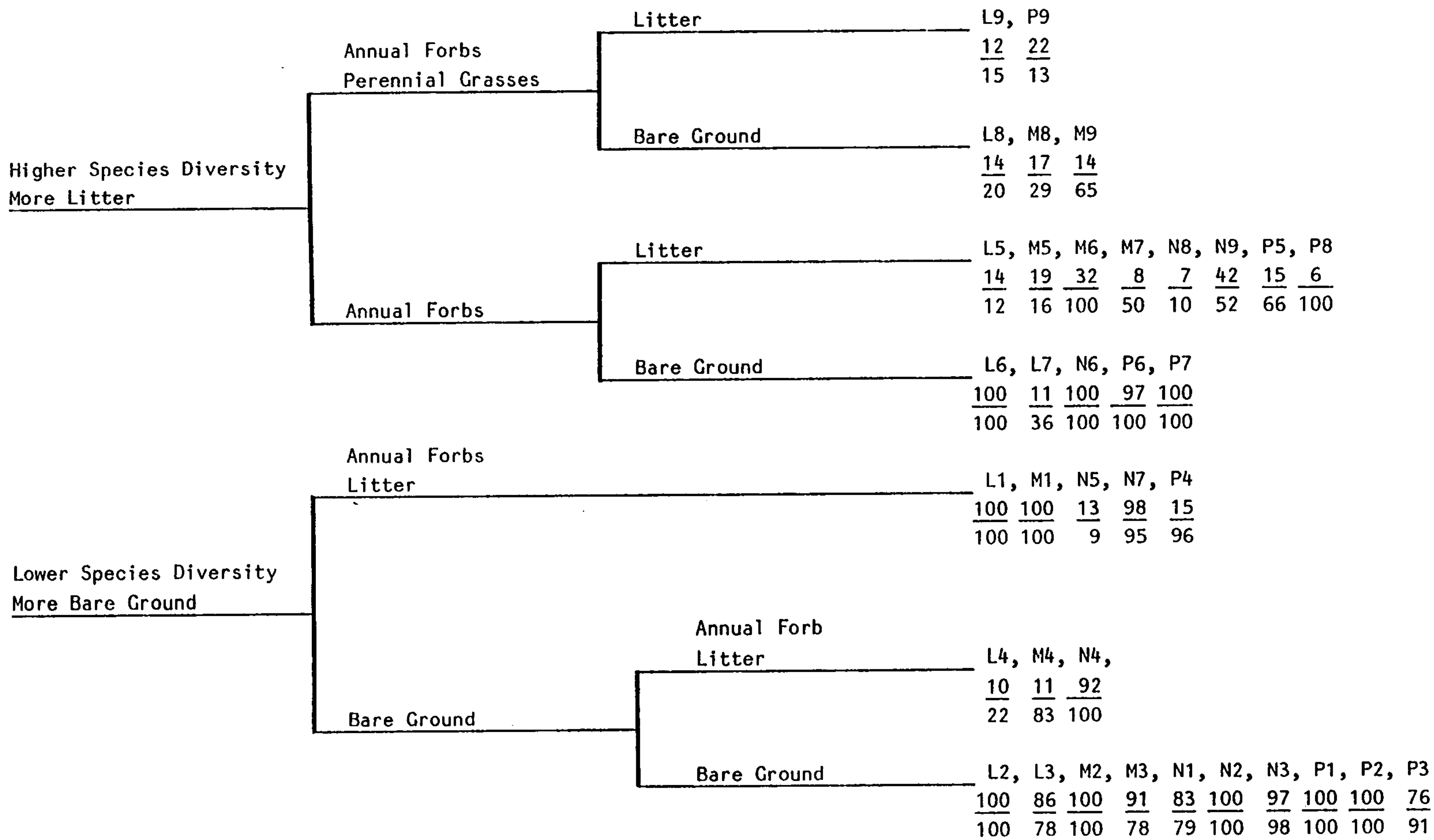

FIGURE 7.1. Summarized Results of TWINSPAN Analysis of Plant Species Composition and Cover for Plots along Four Transects in Basin A, Rocky Mountain Arsenal Compared to Percent Lettuce Seed Mortality. The top number represents mortality based on the 0-15 cm soil fraction; the bottom number is mortality based on 15-30 cm soil fraction.

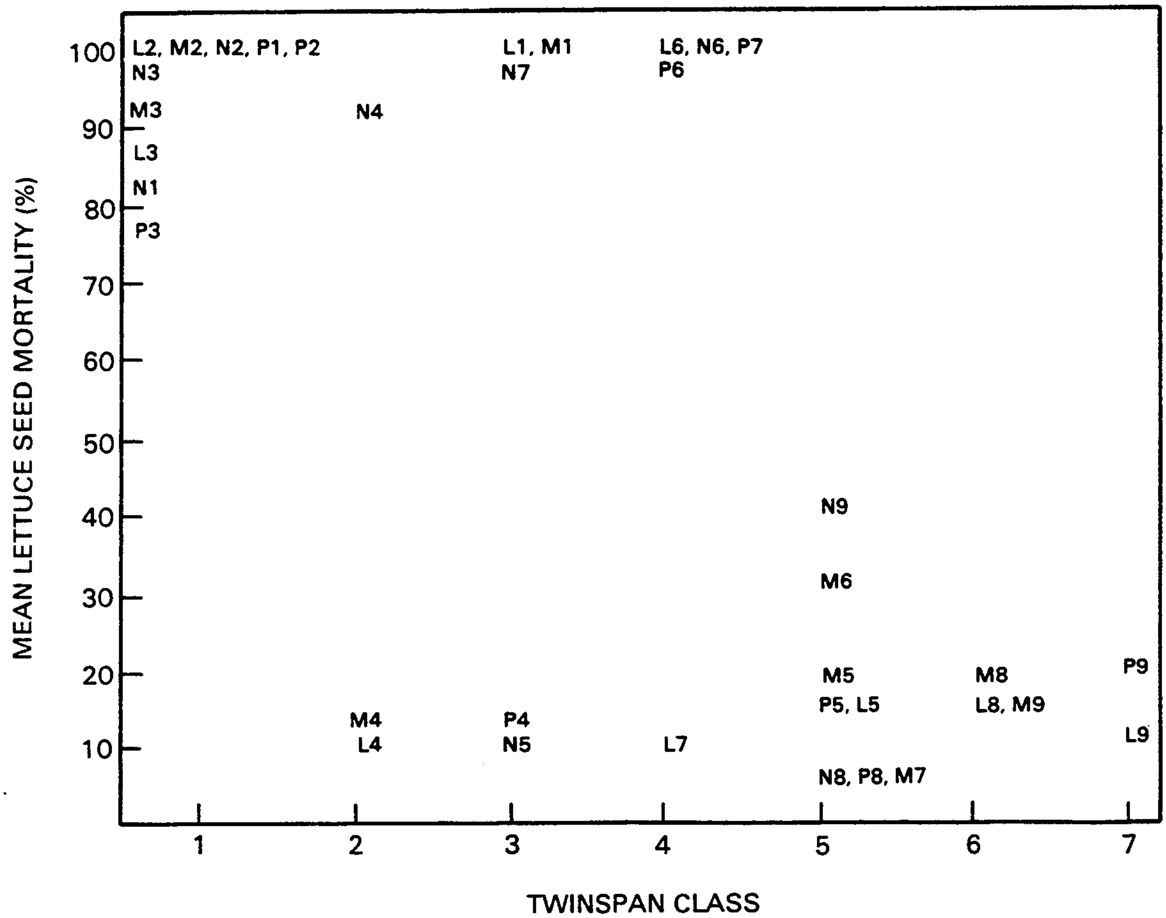

FIGURE 7.2. Relationship Between TWINSPAN Species Composition and Cover Classes and Mean Lettuce Seed Mortality (0-15 cm Soil Fraction). Control mortality was 20.8.

plant productivity. The data in Figure 7.1 have been replotted in Figures 7.2 and 7.3 to illustrate further the relationship between predicted TWINSPAN classes and lettuce seed mortality at the two soil depths. Since control mortality was 20.8% in the experiment using 0-15 cm soils, it appears that soil from the plots in TWINSPAN branches 5-7 caused less lettuce seed mortality than those in classes 1 through 4 (five exceptions are evident, M4, L4, P4, N5, and L7, Figure 7.2). When mortality from 15-30 cm soils are compared to TWINSPAN classes (12.5% control mortality, Figure 7.3), most of the relationship evident in Figure 7.2 disappears. We could speculate that since the 1983 spring-summer was abnormally wet in the Denver area, the

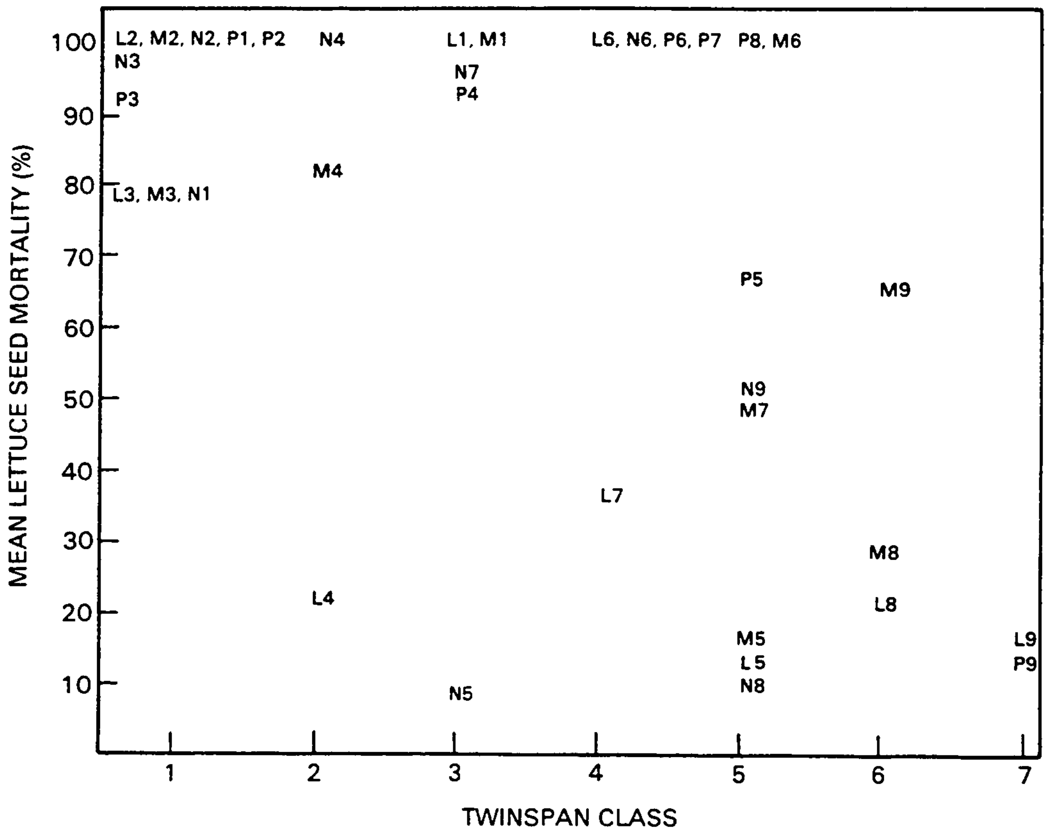

FIGURE 7.3. Relationship Between TWINSPAN Species Composition and Cover Classes and Mean Lettuce Seed Mortality (15-30 cm Soil Fraction). Control mortality was 12.5%.

predominate plant species in the upper three dendrogram arms were either shallow rooted or more resistant. However, no such speculation can be quantitatively supported.

7.3 RESULTS

The 30% kriging isopleth for lettuce seed mortality is compared to vegetative composition and cover (based on the rule that greater than 25% bare ground is relatively poor) for each soil fraction in Figures 7.4 and 7.5. The boxes, which enclose relatively bad and relatively good composition and cover, were constructed as outlined in Section 5.3.4.1 for Figures 5.10

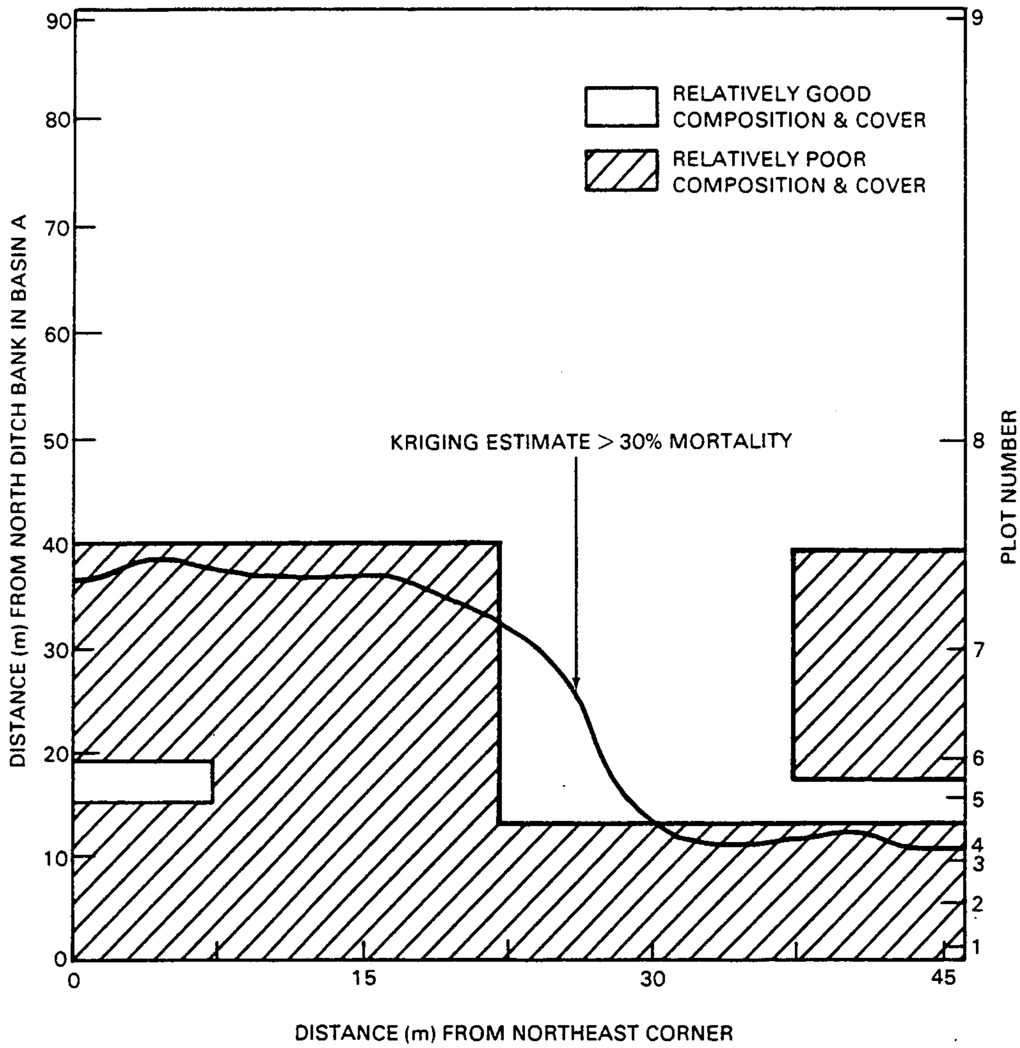

FIGURE 7.4. A Comparison of Lettuce Seed Mortality Predicted from Kriging to Species Composition and Cover Using TWINSPAN (0-15 cm Soil Fraction)

and 5.11, where, observed lettuce seed mortality (<30%) was compared to kriged mortality. Observed and kriged 30% mortality tracked one another fairly well in both Figures 5.10 and 5.11. Because lower mortalities were associated with the upper three dendrogram arms in the 0-15 cm soil fraction

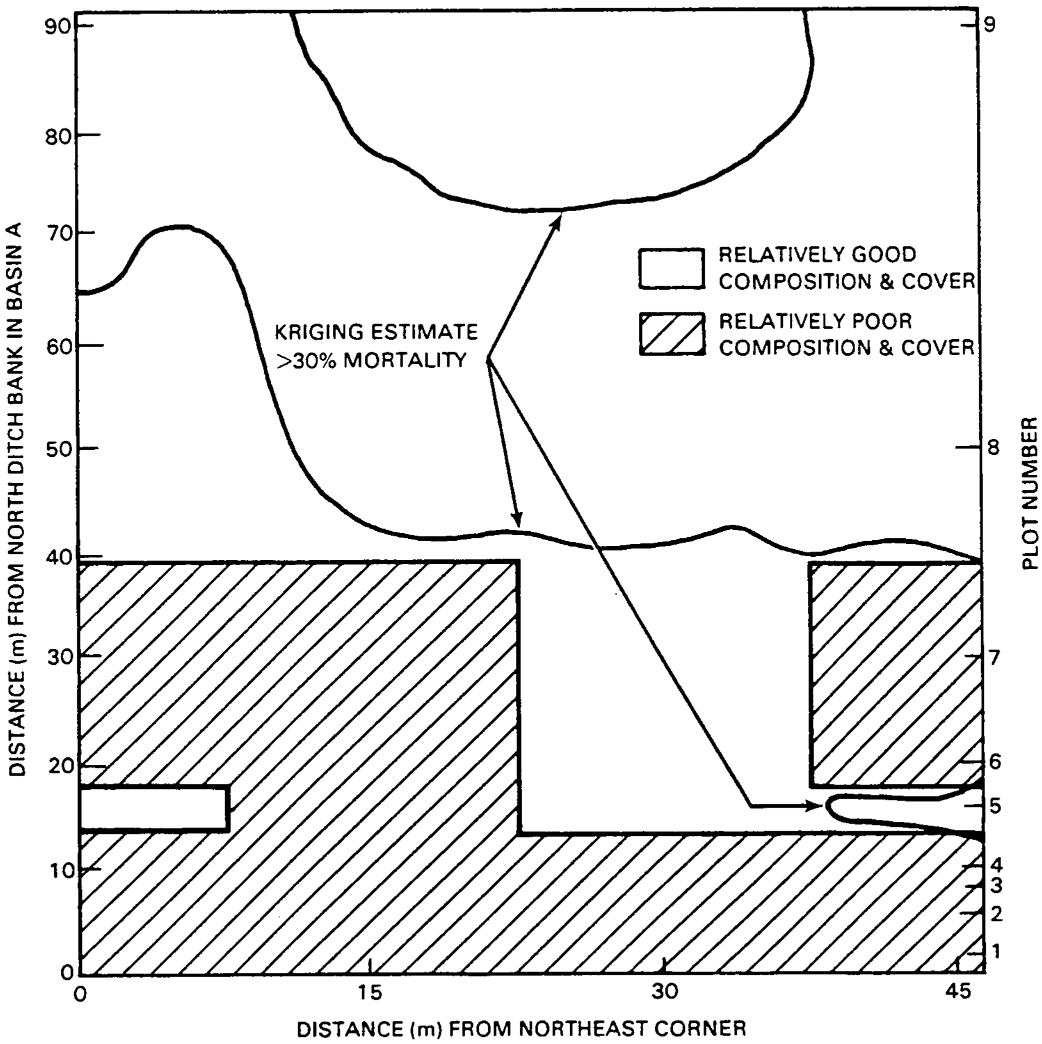

FIGURE 7.5. A Comparison of Lettuce Seed Mortality Predicted from Kriging to Species Composition and Cover Using TWINSPAN (15-30 cm Soil Fraction)

(Figure 7.2; but not the 15-30 cm fraction, Figure 7.3), the good agreement between cover and mortality in Figure 7.4 (0-15 cm fraction) is not surprising.

7.4 DISCUSSION

The excellent agreement presented in Figure 7.4 could simply be fortuitous. Since wheat seeds have been more resistant in our earlier experiments, it is likely that the kriged 30% wheat seed mortality prediction in Figure 7.5 would be displaced downward and the upper predicted 30% area in the figure would disappear. Thus, we speculate that better agreement between observed mortality and plant cover would result. In contrast, a similar downward displacement in Figure 7.4 (0-15 cm fraction) would result in poor agreement between predicted mortality and vegetative cover. It appears from our results that more than two seed species, depth decrements in 7.5 cm intervals to at least 60 cm (root zone limit for most grassland species), and increased sampling intensity between plots 6-9 should be considered if the best relationship between seed mortality and vegetative cover is to be established. The question as to whether similar results would be obtained in other years or other places on RMA remains for investigation.

We believe we have approached the intercomparison of results from a phytoassay of soils to vegetative cover in as unbiased and scientific manner as possible. Our results, while very encouraging, are not definitive.

APPENDIX A

PROJECT PLAN (FY 1983)

FIELD EVALUATION OF HAZARDOUS WASTE SITE
BIOASSESSMENT PROTOCOLS

APPENDIX A

Project Plan (FY 1983)

Field Evaluation of Hazardous Waste Site Bioassessment Protocols

The objective of this project is to conduct field studies to assess the utility of laboratory bioassay tests for evaluating the potential environmental impacts of hazardous waste sites. Field studies will be coordinated closely with laboratory bioassay tests being conducted concurrently by CERL-Corvallis, PNL, or at other laboratories (i.e., BCL). Field bioassessment tests, as well as additional bioassays, will also be evaluated and/or developed by PNL to supplement available laboratory bioassays. The eventual product of this study will be the development of information necessary to produce user-oriented documents that outline protocols for using bioassay techniques to assess the potential risks of hazardous waste sites.

Based on the results of FY-82 studies and a workshop review of PNL and BCL progress (held in Richland, November 30, 1982) the following project plan has been developed. Codes mentioned in the task discussion below refer to the work breakdown structure (attached).

Task I. Terrestrial Field and Laboratory Studies

Subtask 1. Characterize F-Basin Soils for Hazardous Substances Using Bioassays.

As soon as possible a field trip will be organized to collect as many RMA soil samples (hopefully 0-24", and about 4 kg each) as feasible to characterize "the southern half of F-Basin." The Neubauer (PNL) and either Daphnia and/or Selenastrum (CERL) bioassays will be used to assess approximately 30-40 samples. For purposes of this subtask this region of F-Basin (boundary as yet undesignated) will be treated as an unknown hazardous waste site. Additional soil samples, in excess of the 30-40, will be collected for additional bioassays if it is found that a better delineation of "site" contamination is needed. We hope to discover whether gradients, abrupt changes or "hot spots" exist. A small effort has been included (Code 04-01) to develop statistical procedures to decide whether a site has "hot spots" (in the absence of prior knowledge and where hot can be defined *a priori* in terms of chemical concentration or bioassay results). Such methods will increase the usefulness of bioassessment as a technique useful in ranking the relative hazard of sites. Depending on the contamination pattern found, an appropriate field plant transect will be located (in June or July) and evaluated using ordination techniques (Codes 05-03 and 04-02). Additional concurrent 15 kg soil samples will be collected for complete bioassessment by BCL and/or preliminary Neubauer evaluation by PNL and Daphnia/Selenastrum by CERL. Soils collected in this task may be also evaluated using the dehydrogenase or sclerotial assays being developed in Task II (Codes 06-01, 06-02) depending on their state of development. The objective is to correlate bioassay results with field measurements of plant community structure and diversity. Since *a priori* bioassay results will be available, the demonstration has a high probability of success. During the course of the field trip, seeds will be collected, if available (since most seeds are available in the fall), from the same three or four representative species in F-Basin, or Basin A or C, and a control area. PNL evaluation of any seeds collected (germination in the presence of F-Basin water) will depend on the availability of additional funding.

Subtask 2. Demonstrate the Usefulness of the Neubauer Techniques Using RMA Control Soil and Elements Found in High Concentrations in F-Basin Water

We expect to select three or four components of F-Basin water from the following list: copper, arsenic, an organic fraction, fluoride, or ammonia. These components will be assessed using the Neubauer assay. Initially, copper will be studied at levels similar to that found in F-Basin water to determine whether up to 100% (and perhaps higher) of that found in F-Basin water can produce 50% inhibition of germination. Since copper sulfate will be used, appropriate controls for sulfur may be needed. Relevant literature will be collected on the toxic properties of all elements contemplated for study.

An additional study will be conducted in a 3^3 factorial arrangement to determine the effects of individual elements as well as to assess possible interactions. Based on the results of this study, an additional factorial experiment will be conducted to validate and finalize the experimental results. We expect this experimental work to provide the following useful results:

1. A demonstration of the usefulness and economics of the Neubauer procedure as an additional bioassay technique.
2. To mimic, in part, the effect(s) of F-Basin water. We realize similar results will not be proof that the components studied are the toxicants of F-Basin water.
3. To aid us in interpreting the results of Subtask 1.

Subtask 3. Establish that Honey Bees Can Be Effective Fate and Effects Biomonitors for Hazardous Waste Sites.

As soon as feasible in the spring of 1983, 28 hives will be established at RMA. We currently plan to use one site at F-Basin, another possibly near Basin A or C and two control areas (seven hives/site). Brood mortality (Bromenshenk, personal communication) will be monitored three or four times during the spring-summer (effect) and pollen will be chemically analyzed for organics (principally pesticides) and trace elements (fate). Data from studies conducted by the University of Montana (personal communication) show that five hives/site are adequate to detect about a 26% change in brood mortality. However, since there is a possibility of either disease or rapid weather change at RMA, we believe the additional hives are warranted since the incremental cost is low.

Task II. Develop and Demonstrate the Usefulness of the Dehydrogenase and Sclerotial Bioassays.

Subtask 1. Dehydrogenase Bioassay as a Replacement for the Current CO_2 Bioassay.

Our work during 1982 indicated that the dehydrogenase bioassay (a reflection of biomass) correlated with results obtained from the CO_2 bioassay currently under consideration by EPA. Graded levels of F-Basin water were used as the toxicant and added to control soil amended with 1% alfalfa to obtain the comparable results. Since the dehydrogenase assay is more cost effective than the CO_2 test (48 hours compared to up to 30 days), we plan to conduct studies to further substantiate their intercomparability and to assess the inhibition sensitivity of the dehydrogenase procedure.

Initial Work Breakdown Structure

1983

Evaluation of Hazardous Waste Sites

Code	Task	Subtask	Investigator
01	Reports		All
01		82 Report	
02		83 Report	
03		Reserve	
02	Project Management		Thomas
03	Technical Review		Schreckhise
04	Statistics		
01		Hot Spot	Skalski
02		Vegetative Analysis	Simpson
03		Kriging	Simpson
05	Terrestrial		
01		Neubauer Phytoassays	Cline
02		Soil Collections RMA	Skalski/McShane
03		Vegetative Cover	McShane
04		Honeybees	Rogers
06	Microbiology		Rogers
01		Dehydrogenase/CO_2	
02		Sclerotia	
03		Field Work	
07	Chemistry		Thomas
01		81 Pollen	
02		82 Pollen	
03		Reserve	

APPENDIX B

<u>COMPOSITING AS A STRATEGY FOR MAXIMIZING DETECTION OF HOT SPOTS AT HAZARDOUS CHEMICAL WASTE SITES</u>

APPENDIX B

COMPOSITING AS A STRATEGY FOR MAXIMIZING DETECTION OF HOT SPOTS AT HAZARDOUS CHEMICAL WASTE SITES

Rapid, cost-effective sampling protocols are necessary if the hazards of uncontrolled chemical waste sites are to be successfully mitigated under the EPA mandates of CERCLA (Comprehensive Environmental Response, Compensation and Liability Act, 1980) or Superfund and RCRA (Resource Conservation and Recovery Act, 1976). As many as 1500 sites may require inspection under superfund provisions, and of these sites, 400 (100 interim priority sites currently selected) may be eventually chosen for priority cleanup (Collins and Tusa 1981). Pease and James (1981) further believe on-site sampling is needed in all studies of hazardous waste sites.

Using even a conservative estimate for numbers of sites requiring direct sampling, the cost of the chemical analysis alone for soil samples will be enormous. Schaeffer et al. (1982) report average analytic costs as $2000 - $5000 per soil sample. Currently, these analytic costs not only limit the number of sites that may be inspected, but also limit the sampling programs at the sites chosen for inspection. As a consequence, potential health and environmental risks may go undetected.

In this study, we hoped to investigate compositing procedures that would allow the numerous samples collected at RMA to be combined and thereby reduce bioassay or analytic costs. The anticipated result is a statistical technique that could maximize the detection of environmental contaminants while minimizing the cost of site inspections. These procedures could have direct applications to the detection of contaminant zones at waste sites and the validation of site cleanup activities, whether bioassay or chemical analyses or both are used.

The compositing procedure we were going to investigate is known as group testing. Group testing procedures were developed during World War II to reduce the laboratory costs of blood tests used in screening for infectious diseases among military personnel (Dorfman 1943). Since then group testing methods have been used successfully for numerous applications (Hwang 1976). This statistical technique for compositing samples is applicable whenever analysis costs are high, numerous samples may be collected, and the expected incidence of contamination is low. Only very recently (Schaeffer et al. 1982), however, has the potential application of group testing for environmental contaminants been recognized. Our purpose was to extend the statistical applications of group testing to unique requirements of hazardous waste site inspection. We had hoped to demonstrate the cost-efficiency of the techniques developed at RMA.

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