Evaluating Potential Groundwater Contamination from Contaminated Soils

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Contamination of soils at toxic and hazardous waste sites can adversely affect groundwater and surface water. Water soluble materials can move in soil by leaching and percolation and by runoff. This project evaluated the toxicity of leachable toxicants from seven soils, five of which were obtained from designated toxic or hazardous waste sites. Acidified, dechlorinated tap water was used to extract toxic materials from surface soils. Extracts were used as complex mixtures in acute toxicity tests using Daphnia and in chronic effect tests using microcosms. Three classes of effects were observed. Some leachates (including control soils) showed no toxicity. Some soil leachates had moderate acute toxicity (50-80% diluted leachate) and no chronic toxicity. Very toxic soils showed both acute and chronic toxicity at < 3% leachate. Toxicological evaluations of contaminants in waste site soils can provide information not available from chemical analyses and may be useful in verifying the effectiveness of cleanup effort.

**Key Words:** Soil, Leachate, Toxic Waste, *Daphnia*, Microcosms, Toxicity
INTRODUCTION

National attention has focused on the problem of cleaning up the nation’s toxic and hazardous waste sites. Although a great deal of this concern has been for mismanaged dump sites, even well-managed industrial sites have a finite lifetime. Two approaches seem likely in the cleanup and rehabilitation of these sites: 1) removal of on-site toxic materials with permanent sealing of contaminated soils and long-term restriction of access to the site; and 2) removal of all contaminated materials from the site followed by returning the site to normal use. In the former case, it will be necessary to assess the adequacy of the cleanup effort and continue to monitor the sealed, contaminated material to be sure that no leakage takes place. In the latter case, it will be necessary to make extensive evaluations of the adequacy of cleanup efforts before the site can be rehabilitated for alternative uses. In either case, consideration of effects on both humans and local ecosystems will need to be made.

Removal of obvious toxic materials at hazardous waste sites is a major problem. Toxic materials remaining in the soil at these sites may be a complex mixture whose components may have several environmental fates. Materials strongly adsorbed to soil particles may have little probability of movement into groundwater or surface waters, and may only enter human food chains or natural food webs if forage plants are grown on the site and the material is mobilized by biological activity. Water soluble materials are more likely to be immediate problems at these sites. Percolation and runoff from contaminated soils may enter groundwater or aquifers that serve as drinking water sources or may enter local ecosystems contaminating surface water, drinking supplies, and local biota. It is likely that the composition of water soluble materials from each site will be quite different, and no simple chemical means are available for assessing toxicity of these complex mixtures to the variety of organisms exposed.

Protozoa occur in great number in soils, commonly reaching $10^6$ per gram of soil (dry weight). Protozoa play important roles in maintaining bacterial populations at rapid growth rates and in processing dead organic matter (Pratt and Cairns 1985; Campbell 1983). They respond rapidly to soil wetting (Bamforth 1980) and can be extracted from soils quite readily. The complexity of the soil community is not great and can be quickly evaluated using standard quantitative sampling methods. Protozoa, in general, are sensitive to low levels of toxic materials (Ruthven and Cairns 1973; Niederlehner et al. 1985) and are useful indicators of toxic stress. Since they represent higher trophic levels in soils than the dominant bacterial populations, they may integrate the effects of concentration of toxic materials by bacterial processors. Examination of the nature of the soil protozoan community is a first step in assessing possible contamination of soils.
The Resource Conservation and Recovery Act (RCRA) designates a number of chemicals as hazardous wastes and includes containers and contaminated soils which have come in contact with hazardous chemicals in its definition of hazardous materials (USEPA 1984). Methods are specified in regulations to test toxicity of waste materials. A number of chemical mixtures may be present at such waste sites. Many of these are heavy metal residues (Table 1) but a large number are complex organic pesticides and solvents. For example, the Commonwealth of Virginia has designated a number of compounds as class I compounds, or those for which the Commonwealth desires the greatest amount of information and control. Most of these substances (Table 2) are listed in EPA’s list of hazardous chemicals. The premier toxic compound on the Virginia list is pentachlorophenol. Several sites in Virginia (mostly wood preservers) have been designated hazardous waste sites because of the storage and dumping of this material (Water News 1984).

There is considerable controversy over the methods used to assess toxicity of even pure compounds, and it is likely that the sole use of single species testing protocols will soon be replaced by more environmentally realistic and scientifically defensible testing methods (Cairns 1981). There are several problems with reliance on single species tests, and no attempt will be made here to construct a detailed argument. The argument can be simply stated: Single species tests are incapable of predicting the responses of communities and other higher levels of biological organization.

Recent research trends have moved toward the development of microcosms and microecosystems in which important, complex biological phenomena not apparent in single species tests can be studied and the effects of toxic compounds on these phenomena assessed (Pritchard and Bourquin 1984; Odum 1984). These tests are quite sensitive (approaching the sensitivity of analytical equipment for heavy metal toxicants), produce reliable results, and can be carried out in a relatively short period of time (e.g., Niederlehner et al. 1985). The time required and the cost incurred in conducting such tests with qualified personnel compare quite favorably with long-term, single species tests of sensitive life history stages (Cairns et al. 1986). Because these tests use microbial communities that are often comparable among similar sites, indigenous species can be tested and the potential for harm to the local ecosystem assessed relatively quickly. In addition, it is possible to test predictions of adverse effects by measuring colonization processes in receiving ecosystems to determine if predictions of harm (or no harm) are accurate. Test systems are sufficiently well developed now so that either static (lentic) or flowing (lotic) systems can be simulated in laboratory tests.

I. Purpose

The purpose of this project was to examine the usefulness of microbial species
in detecting soil contaminants at toxic and hazardous waste sites which might adversely affect groundwater through percolation and surface water through runoff. Specific objectives include: 1) determine the response of soil microfauna to added toxicants; and 2) determine the efficacy of soil column extraction tests for evaluating the effectiveness of waste site rehabilitation.

II. Related Research

Current regulations specify the proper handling and disposal of 359 chemical substances identified as hazardous wastes. At many sites identified for cleanup, these wastes have been improperly disposed of, resulting in extensively contaminated soils. Analytic methods for identifying hazardous wastes at disposal sites and in contaminated groundwater (USEPA 1980, 1984) specify monitoring of chemical concentrations and in some cases assessing the relative toxicity of soil and groundwater contaminants. Chemical analyses can verify the presence of expected contaminants but cannot predict the toxicity of complex mixtures of toxic materials which may enter groundwater or surface water by infiltration and runoff.

If leached material from hazardous waste sites is considered a complex mixture, its relative toxicity can be measured using both acute and chronic toxicity testing procedures. The question of appropriateness of testing procedures remains a controversial issue. However, research in our laboratory has aimed at developing direct measurements of chronic toxicity which are cost-effective and applicable to a wide variety of situations. Testing procedures previously developed have relied on measurements of effects on complex natural communities, testing several dozen species simultaneously rather than sequentially testing a number of test species in single species tests. Recent evidence indicates that for pure compounds the microcosm, multi-species test yields predictions of toxic effects comparable to those estimated from long-term tests with single species (Niederlehner et al. 1986; McCormick et al. 1986) Such tests can yield predictions of adverse effects at low levels of toxic exposure and may, in some circumstances, replace data bases derived from single species tests which may take a minimum of 18 months to generate (Stephan et al. 1983).

Our long-term goal in developing such tests is to provide a practical and inexpensive alternative to long-term single species tests performed using laboratory-tolerant organisms which may or may not occur in the ecosystem receiving the toxic material. Further, single species tests cannot provide the degree of environmental realism necessary for predicting effects on species interactions, nor can these tests allow alternative endpoints other than mortality. It is very difficult to validate single species tests in the field because effects on functions of natural communities must be measured in total rather than as effects on specific, isolated species.
Present knowledge concerning the fate and transport of toxic materials in soils is comparatively sparse. Most of this research deals with the movement of materials through soil columns and suggests that groundwater contact may occur with possible adverse effects. However, concentrations of compounds may be extremely low, even below detection limits for analytical instruments. Nevertheless, exposed organisms may integrate these effects over several generations. For microbial species, several generations may mean only a few days, and measurements of effects on these naked cells provide a more rapid feedback of information which may be used to protect both aquatic ecosystems and human populations exposed to contaminated surface and groundwater.
MATERIALS AND METHODS

I. Study sites

Seven study sites were selected. Two of these sites were on the Virginia Tech campus. The remaining five sites were selected with the help of personnel of the Virginia Water Control Board and Department of Health. These agencies recommended a variety of potential study sites. Site managers and other corporate officers were contacted to obtain permission to sample at these sites. Some sites are or were involved in potential legal and regulatory action. This limited access to some sites. Because this project was experimental in nature, sites have been identified by codes rather than company names to prevent the unreviewed use of results of these experiments. A more complete description of each site follows. Sampling was frequently aided by directions from on-site personnel. We endeavored to sample in areas of known contamination and made no effort to objectively characterize the areal extent or depth of contaminated soil.

VAM01. This site was an agricultural field on the Virginia Tech campus. No toxic materials were expected to be in these soils, although some pesticide residues may have been in sampled soil.

VAM02. This site was the coal storage area near the Duck Pond on the Virginia Tech campus. The site has been used for a number of years to stockpile coal prior to its short-term storage at the Virginia Tech power plant.

VAAL1. Soil was obtained from a site receiving heavy, long-term (> 20 yr) contamination from solvents (toluene, MEK, TCE) used in the manufacture of rubber gaskets.

VARI1. Sandy soil was sampled from under a storage tank at this site. Contamination was from solvents and other chemicals used in wood preservation.

VARI2. Soil at this site received heavy metal contamination, primarily chromium, at a metal extrusion industry.

VAWE1. Soil from this site was obtained from a former waste pond that received waste from a wood preserving operation.

VAFL1. Soil at this site was contaminated by heavy metals (e.g., Cu, Cd, Ni, Pb, Zn) and acid. The site is presently closed.
II. Soil Collection and Characterization

Soil samples were randomly selected from each of the sites tested. Surface organic matter (when present) was removed prior to sampling. Samples were taken from surface soil (a cube ca. 25 cm on each side, Smith and Atkinson 1975). The samples were composited and thoroughly mixed in plastic buckets which were then sealed and transported to the laboratory. For purposes of ensuring that contaminated soils would be obtained, some soil samples were taken from limited areas of known contamination.

Subsamples of collected soil were air dried, passed through a 1 mm mesh screen and then used for soil characterization. Routine soil analyses were done by the Virginia Tech Soil Testing Lab using standard methods of soil analysis for the determination of pH, cation exchange capacity, and percent organic matter.

Soil bacteria were enumerated as follows. Five grams of each soil were placed in a sterile 125 ml Erlenmeyer flask to which 50 ml of sterile tap water was added. The soil water mixture was shaken on a rotary shaker at 50 cycles/min for 24 h. After 24 h, material in each flask was fixed with 6 ml filter-sterilized 37% formalin to achieve a final fixative concentration of 4%. Each sample was well-mixed and an aliquot removed for staining with DAPI for enumeration of total bacteria (Porter and Feig 1980). A second aliquot was removed for staining with FITC for enumeration of microflagellates (Sherr and Sherr 1983).

III. Soil Column Elution

Six columns (50 cm length, 9 cm i.d.) of high density polyethylene pipe (Plexco Plastics) were used for elution of soil samples (Figure 1). This material is resistant to most chemicals (c.f. Van Voris et al. 1984). Columns were placed on a Buchner funnel lined with cheese cloth. Each column was then placed in a 30 L plastic tub and filled to a depth of 23 cm with the soil being tested.

The elution water was dechlorinated tap water acidified to pH 4.5 with 1N HCl. Initially 500 ml of elution water was slowly added to each column in order to saturate the soil. After 1-2 h an additional 1500 ml of elution water was slowly added to each column. The columns were left until elution water was no longer visible at the top of the soil column (2 - 12 h). Similar methods have been used by other investigators (Khan 1980; Sebastian et al. 1981). The eluates were then filtered through Whatman number 4 qualitative filter paper to remove coarse particulates and combined in three 4 L plastic cubitainers. The eluate was adjusted to a pH of 7.0 using 1N NaOH before testing. The pH adjusted eluate was stored in the dark prior to use in toxicity assessments. Samples were never stored for more than 48 h prior to testing.
The pooled eluates served as a complex mixture for use in bioassays. Samples were diluted using dechlorinated tap water, the same water used to make the extraction solvent. The dechlorinated tap water served as the control (uncontaminated) test medium in all tests.

IV. Acute Toxicity Assessment

The acute toxicity of eluates from soil samples was evaluated using a *Daphnia magna* bioassay (Peltier and Weber 1985). In each of these 48 h acute toxicity tests, 10 (6 for eluate VAWE1) neonate animals (< 24 h old) were exposed to duplicates of five test dilutions (1, 3, 10, 30, and 100%) of eluate and a control. Test concentrations were sometimes adjusted to account for the volume of eluate available or the suspected toxicity level of the sample. Tests were conducted in 100 ml of test medium in 250 ml beakers covered with plastic wrap. Dissolved oxygen, temperature, and pH were measured at 0 and 48 h. Light was provided by daylight equivalent lights (Duro-Test Vita-Lite®) at an intensity of 50-100 ft-c (500-1000 lux); photoperiod was 16L:8D. Immobilization was recorded at 24 and 48 h. An EC50, the concentration of diluted eluate (%) that would affect 50% of the test population, was estimated using Spearman-Karber analysis (Finney 1968). Concentrations for the microcosm tests were determined based on results of the *Daphnia magna* 48 h tests.

V. Microcosm Toxicity Tests

Microcosm tests were run in 6 L glass aquaria. Tests were conducted under static conditions with one addition of toxic material (eluate). Before use, test containers were cleaned by soaking in 2% Contrad 70®, 5% nitric acid, and rinsing in acetone and air drying. Dechlorinated tap water was used to dilute the eluate to 3 L volumes of a specified concentration in each test container.

Fifteen polyurethane foam (PF) artificial substrates were colonized to approximate species equilibrium in Pandapas Pond (Jefferson National Forest) for 21 days before each test. Ten substrates were used as species sources (epicenters) for establishing microcosms. The colonized PF substrate was secured at one end of the glass tank by attaching a cotton string loop tied around the substrate to a plastic hook cemented to the tank bottom. A second, initially sterile PF substrate was placed at the opposite end of each test container to serve as barren habitat for microbial colonization.

Microbiota colonizing artificial substrates in Pandapas Pond served as a reference complex natural community for comparing the actions of the various eluates. Microbiota (bacteria, algae, protozoa, micrometazoa) are similar among a wide variety of ponds, and the majority of species are considered to be cosmopolitanly distributed.
At the beginning of a test, a single epicenter and island substrate were placed in each of ten test containers (2 replicates of 5 concentrations) filled with eluate and dilution water to a volume of 3 L. The containers were covered with plastic wrap to retard evaporation and placed under daylight equivalent lighting (Duro-Test Vita-Lite®) at 1000 lux on a 16L:8D schedule as in the Daphnia tests. Test concentrations were determined from results of daphnid tests. The highest test concentration used was one-half the lowest effect concentration seen in daphnid tests. The dilution series consisted of four test concentrations, each one-half of the next highest concentration, and a control.

After seven days, all island substrates were harvested and squeezed into plastic collecting jars. After settling, 2-3 subsamples of the settled material were examined at 100-400X for the number and kinds of protozoa present. On day 8 the epicenters from the control and highest concentration test containers were harvested and sampled in a similar manner. If the species richness in the highest concentration epicenters was less than 80% of control epicenters, then the remaining 6 epicenters were collected and sampled. The number of species found on day 8 epicenters was compared to the number of species found on two reference PF substrate samples examined at the beginning of each test.

Dissolved oxygen and pH readings were taken in each test container on day 0 and day 7. In addition, the conductivity, alkalinity and hardness of the eluate and dilution water were measured for each test, although the color of some eluates made it impossible to determine hardness. Initial values for test systems are shown in Table 3.

The number of species observed on initially barren PF substrates was compared among test containers using analysis of variance procedures. The relationship of eluate dose to the number of species sampled was determined by ordinary least-squares regression using both the eluate concentration and the logarithm of the eluate concentration as the independent variable. Dose-response relationships were determined by whichever regression had the greater explained variation ($r^2$). A chronically toxic level of eluate was estimated as the EC20 (the eluate concentration resulting in a 20% reduction in species relative to controls) from the dose-response regression using inverse prediction methods (Sokal and Rohlf 1981).
RESULTS

I. Soil Analysis

Results of soil characterization are given in Table 4. The soils generally had similar characteristics, although three soils had low cation exchange capacity. One of these soils (VAFL1) had low pH and high levels of aluminum. Additionally, organic matter levels were high in VARI1 soil, probably due to high levels of wood preservatives present in the contaminated soil. Similarly, elevated organic matter levels were measured in the other soil sampled from a closed waste lagoon (VAWE1). Bacterial and flagellate numbers were in the normal range of values reported for soils. Numbers of bacteria were generally lower in soils from sites with toxic contaminants (Table 5).

II. Daphnia Tests

*Daphnia* acute tests showed that two of the soil leachates were not toxic, two were moderately toxic, and three were highly toxic (Table 6). Estimates of median effective concentrations (EC50) were used to compare effects. The control soil (VAM01) and VAAL1 were not acutely toxic. Soil from the Virginia Tech coal storage pile (VAM02) and from VARI2 were moderately toxic with EC50 estimates ranging from 50 - 79%. These levels correspond to the proportion of leachate (after dilution) that would be acutely toxic to 50% of a test population. Leachates from the three most toxic soils showed severe acute toxicity such that the EC50 could not be directly estimated. We estimated EC50’s at less than the lowest test concentration (0.5-1%). These leachates were extremely toxic and resulted in rapid death of the test organisms; all mortality occurred within the first few hours of exposure. We did not conduct further acute testing to determine more precise values for acutely toxic levels of these leachates because only a limited volume of leachate was available.

III. Microcosm Tests

Microcosm tests revealed patterns of eluate toxicity similar to those determined using acute toxicity tests. The two sites that showed modest acute toxicity (VAM02 and VARI2) showed no significant chronic toxicity as determined from microcosm tests. Eluate from VAM02 soil showed modest toxicity (Figure 2) but the dose-response relationship for species loss from the microcosms was only marginally significant (Table 7). There was some consistent species loss in microcosms dosed with eluate from this soil, but the effect at the lowest test concentration (16%) did not increase in higher doses, indicating a modest all-or-none response.

Three soils had eluates that were chronically toxic (Figure 3). These three sites also showed strong acute toxicity. There was a strong dose-response relation-
ship and all three soils had estimated chronic effect levels at < 3%. Two soil eluates (from VAWE1 and VAFL1) showed similar toxicity near 2%. The third soil eluate (from VARI1) was extremely toxic (EC20 < 0.05%).

In three tests there was significant loss of species in one of the two control microcosms. Systematic evaluation of cleaning methods and technical procedures failed to account for what appeared to be gross contamination of these experimental units. Previous testing of microcosm systems in our laboratories over several years has never recorded these unexplained problems. The contaminated samples were eliminated from all analyses.
DISCUSSION

I. Soils

Numbers of microbes in waste site soils were not directly related to observed toxicity, although an intensive investigation of soil microbial numbers was not undertaken. In addition to results reported for bacteria and flagellate numbers in soils, we examined soil protozoa using a dilution counting technique (Foissner 1983). The number of protozoa found even in samples from uncontaminated sites was so small that more elaborate measures would have had to be undertaken to recover sufficient cell numbers for analysis. There was some indication of depression of cell numbers in the soils considered most toxic. Numbers were depressed by 50% or more in many cases, but the pattern was not consistent, probably because of the diversity of toxic materials in soils. It is likely that bacterial numbers were enhanced in soils with organic contamination (e.g., VAM02) even though these soils showed some toxicity.

II. Toxicity Evaluation

We used both acute and microcosm toxicity measurements to rank the relative toxicity attributable to extracts from the various soils (Table 8). Acute toxicity was often in the same range as estimated chronic effects determined in microcosm experiments. The exceptions to this pattern were two samples with moderate acute toxicity that showed no chronic toxicity. We postulated that these samples contained organic toxicants that were effectively degraded by the complex microbial community present in the microcosm tests. Toxicity results for each soil studied are discussed below.

VAM01. This control soil and leachate had high bacterial and flagellate numbers and did not show any toxic effects on experimental systems.

VAM02. This sample was obtained from the site of the Virginia Tech coal storage pile. Leachate from this soil was moderately toxic in acute tests and showed some non-linear toxicity in microcosm tests. It is likely that the soil and leachate contained a variety of complex organics and some moderately toxic inorganics (e.g., several heavy metals) from the stored coal. Bacterial numbers were lower than in control soil but flagellate numbers were somewhat elevated. Toxicity of coal pile runoff has been previously reported in relation to adverse impacts on small streams (Swift 1985). Inconsistent reports on the toxicity of coal leachates (e.g., Carlson et al. 1979; Cox et al. 1979) probably reflect differences in the types of coal used to make toxic leachates. There may be further differences in toxicity related to the length of time the coal has been stored.
VAAL1. This soil was obtained at a gasket manufacturing site where soil was grossly contaminated with solvents such as toluene, methyl ethyl ketone, trichloroethylene, and other wastes. Despite apparently severe contamination at the site, we were not able to detect significant toxicity in leachate from this soil. Bacteria and flagellate numbers were lower than in the control soil.

VARI1. This soil, taken from a solvent storage area at a wood preserving operation, was extremely toxic. Bacterial numbers were comparable to controls but flagellates were somewhat depressed. The percent base saturation of this sandy soil was very low and both hydrogen and aluminum levels were elevated. Estimated acute toxicity was very high and chronic toxic effects (EC20) in microcosms were estimated at < 0.1%. Leachate from this soil was the most toxic of the soils/leachates studied and is probably reflective of the gross, recent contamination in the site where soil was sampled.

VARI2. This soil showed moderate acute toxicity and no measurable chronic toxicity. Soil at the site had known chromium contamination. It is possible that the leaching and pH adjustment radically changed chromium speciation in leachate such that highly toxic chemical species were not present.

VAWE1. Leachates from soil at this site were very toxic. Extreme acute toxicity was detected at 0.5%. Chronic toxicity was found at slightly higher levels. Soil was from a wood preserver’s waste pond that had been closed.

VAFL1. Soil at this site was known to contain several toxic heavy metals. Further, soils were very acidic and had high aluminum levels, although it is unlikely that soluble aluminum was present after pH adjustment of the leachate. Nevertheless, toxicity in leachates was high in both acute and chronic tests.

Leachates from soils were ranked according to their relative toxicity within a particular testing regime (acute or microcosm/chronic). These rankings were added for both tests to obtain a rank sum used to list the soil leachates in order of decreasing toxicity (Table 8). Leachates from three sites (VAWE1, VARI1, and VAFL1) were extremely toxic. Leachate from VARI1 showed extremely high chronic toxicity in microcosm studies. Of the other leachates tested, only VAM02 showed an indication of modest chronic toxicity.

III. Method Evaluation

The methods developed in this project differ in several ways from those developed for evaluating waste site soils (e.g., EP toxicity test, Porcella 1983). The EP procedures utilize acetic or nitric acid, and both of these could affect responses in the microbial test since nitrate ions contribute nitrogen in
nutrient form metabolizable organic substrate. Our method utilizes a slightly larger extraction volume than the volume of soil packed in columns (2000 ml extractant per 1500 cc soil). The extractant used was dechlorinated tap water acidified with hydrochloric acid and, thus, contains a variety of cations and nutrients that percolate through the soil. In terms of low pH (4.5) and slow percolation time, the extraction method is similar to extraction that might occur by rainfall, even though the extraction volume is only ca. 10% of estimated annual rainfall in Virginia and ca. 20% of what infiltrates soil (Chermisinoff and Gigliello 1983).

During preliminary investigations, we found artificial rain water too deficient in calcium even after percolation through the soil column to allow survival of daphnids for toxicity testing. This meant that an additional stress was added to the toxic stress of interest, a fact we considered unacceptable. Because waste site soils differ considerably in their chemical makeup and exchangeable cations, we considered it necessary to provide a source of cations in the elution water. By acidifying dechlorinated tap water (the same water used to rear test organisms and to dilute leachates) background water quality/chemistry was the same for all tests. An alternative would have been to reconstitute leachates to dilution water hardness and alkalinity after leaching.

We considered other methods to remove toxic materials from soils. Recently, surfactants have been used (Ellis et al. 1985) to remove hydrophobic compounds in cleanup efforts. Removal of these compounds by normal rainfall runoff or percolation seems unlikely, however, and we concentrated on water/acid soluble materials that might be more mobile. This may account for the failure to detect toxic materials at grossly contaminated sites such as VAAL1. If hydrophobic materials are not mobile under normal low pH conditions, they may be of lower risk to aquatic biota than more water soluble compounds. Alternatively, hydrophobic organics are often quickly degraded by the microbial milieu. There was no evidence of enhancement of bacterial numbers in VAAL1 soils.

Only one other study has examined toxicity of extracts from contaminated soils. Thomas et al. (1986) used EP procedures to evaluate toxic material in a variety of contamination sites at the Rocky Mountain Arsenal. They conducted a series of single species tests on extracts and contaminant-spiked clean soils to evaluate the effectiveness of acid extraction and subsequent toxicity testing. Contaminated soils at this site contained heavy metal, pesticide, and other unknown toxicants. Estimated effect levels (EC50) from acute bioassays on freshwater species ranged from 0.002% to 94% (not including several no effect measurements). This corresponds well with the results of our experiments where acute effect concentrations ranged from <0.5% to ca. 80% (with several no effect measurements).
We also evaluated chronic effect levels using microcosm tests. Previous research has shown that effect levels in microcosm tests similar to the one used here are in the range of numerical water quality criteria (e.g., Niederlehner et al. 1985; Cairns et al. 1986). The colonization process integrates effects on source communities and propagules. We seldom observed direct effects on source communities, and these effects generally paralleled toxicity to colonizing species. Effects on source communities are usually less severe (in terms of effect levels) than effects on colonizing communities on the initially barren island substrates. Colonization of new habitat is a functional process in these communities. Other investigations have confirmed effects on soil community processes affected by toxicants (Babich et al. 1983; Killham et al. 1983). However, microcosm tests were only marginally more sensitive to extracted toxicants than *Daphnia* bioassays. In one case, the microcosm test was more sensitive by two orders of magnitude. However, moderate toxicity found in some daphnid tests was not confirmed in microcosm tests. This may have been due to adaptation and resistance in the microbial community, a response that is not observable in the single species tests.
SUMMARY

Contamination of soils can lead to adverse environmental effects due to percolation of toxic materials to groundwater or runoff of contaminants to surface waters. Chemical detection can provide evidence of the presence of toxic materials in waste site soils but cannot predict the eventual toxicity of complex mixtures of contaminants in water. In many cases, the full spectrum of chemical contaminants is not known. Some contaminants are water soluble while others are not. Some contaminants are degradable (and may have toxic breakdown products) while others are more persistent. Our experiments have evaluated the potential transfer of water soluble toxicants out of contaminated soils and have shown that acute and chronic tests can provide quite different estimates of effect levels. This suggests that short-term chronic bioassays should be included in the evaluation of waste site soils. Careful evaluation of the extent of contamination will also require depth and area sampling to chart the extent of contamination. Toxicological evaluations should be used in follow-up studies of cleaned sites to ensure that toxic materials have been effectively removed from the site.
### TABLE 1
Selected Priority Pollutants at Selected Abandoned Hazardous Waste Sites.

Values are ug/L. (Data from Chrermisinoff and Gigliello 1983)

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organics</strong></td>
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</tr>
<tr>
<td>chlorobenzene</td>
<td>10</td>
<td>170</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Heavy Metals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>silver</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>arsenic</td>
<td>7</td>
<td>16</td>
<td>60</td>
<td>91</td>
</tr>
<tr>
<td>beryllium</td>
<td>8.8</td>
<td>12</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>cadmium</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>chromium</td>
<td>33</td>
<td>18</td>
<td>3</td>
<td>220</td>
</tr>
<tr>
<td>copper</td>
<td>95</td>
<td>94</td>
<td>9.4</td>
<td>880</td>
</tr>
<tr>
<td>mercury</td>
<td>0.37</td>
<td>21</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>lead</td>
<td>400</td>
<td>5500</td>
<td>60</td>
<td>5300</td>
</tr>
<tr>
<td>nickel</td>
<td>87</td>
<td>30</td>
<td>30</td>
<td>140</td>
</tr>
<tr>
<td>antimony</td>
<td>40</td>
<td>50</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>selenium</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>thallium</td>
<td>0.4</td>
<td>4</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>zinc</td>
<td>1500</td>
<td>6100</td>
<td>40</td>
<td>7700</td>
</tr>
<tr>
<td>Virginia Class I Substances</td>
<td>Listed in EPA Regulations for Hazardous Wastes&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>yes (P90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creasote/Coal Tar</td>
<td>yes (U051)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinyl halide monomers</td>
<td>yes&lt;sup&gt;b&lt;/sup&gt; (U048)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>yes (U228)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroprene</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>yes&lt;sup&gt;c&lt;/sup&gt; (U021)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzidine</td>
<td>yes (U013)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asbestos</td>
<td>yes (U019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Hazardous/Toxic waste number shown in parentheses (USEPA 1980)

<sup>b</sup> Chloroethene

<sup>c</sup> Several lead salts
### TABLE 3
Results of Chemical Analysis of Dilution Water and Evaluates Used in Microcosm Tests.

(NOTE: ND = Not Done; Hardness Determination Affected by Sample Color.)

<table>
<thead>
<tr>
<th>Site</th>
<th>DO</th>
<th>pH</th>
<th>Alk</th>
<th>Hard</th>
<th>Cond</th>
<th>DO</th>
<th>pH</th>
<th>Alk</th>
<th>Hard</th>
<th>Cond</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAM01</td>
<td>6.6</td>
<td>7.0</td>
<td>10</td>
<td>ND</td>
<td>274</td>
<td>6.5</td>
<td>7.3</td>
<td>6.3</td>
<td>ND</td>
<td>153</td>
</tr>
<tr>
<td>VAM02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAAL1</td>
<td>6.4</td>
<td>7.3</td>
<td>143</td>
<td>340</td>
<td>431</td>
<td>8.8</td>
<td>8.0</td>
<td>5.0</td>
<td>60</td>
<td>140</td>
</tr>
<tr>
<td>VAWE1</td>
<td>8.1</td>
<td>7.6</td>
<td>53.8</td>
<td>ND</td>
<td>281</td>
<td>8.3</td>
<td>7.9</td>
<td>5.0</td>
<td>60</td>
<td>136</td>
</tr>
<tr>
<td>VARI1</td>
<td>8.6</td>
<td>7.2</td>
<td>15.0</td>
<td>ND</td>
<td>334</td>
<td>8.1</td>
<td>7.6</td>
<td>6.0</td>
<td>60</td>
<td>136</td>
</tr>
<tr>
<td>VARI2</td>
<td>8.3</td>
<td>7.5</td>
<td>58.8</td>
<td>ND</td>
<td>482</td>
<td>8.4</td>
<td>7.3</td>
<td>6.0</td>
<td>60</td>
<td>147</td>
</tr>
<tr>
<td>VAFL1</td>
<td>7.8</td>
<td>9.3</td>
<td>758</td>
<td>ND</td>
<td>4670</td>
<td>7.6</td>
<td>6.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Site</td>
<td>Organic Matter (%)</td>
<td>pH</td>
<td>Ca (meq/100 g soil)</td>
<td>Mg (meq/100 g soil)</td>
<td>K (meq/100 g soil)</td>
<td>Al (meq/100 g soil)</td>
<td>H (%)</td>
<td>% Base Sat.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>--------------------</td>
<td>------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM01</td>
<td>5.95</td>
<td>3.17</td>
<td>5.30</td>
<td>0.37</td>
<td>0.00</td>
<td>9.00</td>
<td>42.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM02</td>
<td>6.58</td>
<td>1.24</td>
<td>6.91</td>
<td>0.29</td>
<td>0.05</td>
<td>3.20</td>
<td>74.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAAL1</td>
<td>7.65</td>
<td>2.88</td>
<td>22.50</td>
<td>0.50</td>
<td>0.21</td>
<td>0.20</td>
<td>99.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAAL1</td>
<td>5.17</td>
<td>12.14</td>
<td>0.37</td>
<td>0.06</td>
<td>0.05</td>
<td>1.25</td>
<td>74.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAAL1</td>
<td>5.94</td>
<td>1.19</td>
<td>2.83</td>
<td>0.60</td>
<td>0.13</td>
<td>0.15</td>
<td>4.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAFL1</td>
<td>3.27</td>
<td>1.44</td>
<td>8.70</td>
<td>0.26</td>
<td>0.02</td>
<td>12.15</td>
<td>36.60</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 5
Bacteria and Microflagellates in Soil from Study Sites.
Bacterial counts based on DAPI-stained direct counts; flagellate counts based on FITC-stained direct counts.

<table>
<thead>
<tr>
<th>Site</th>
<th>Bacteria x10^7 cells/g DW soil</th>
<th>Microflagellates x10^6 cells/g DW soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAMO1</td>
<td>9.8</td>
<td>8.2</td>
</tr>
<tr>
<td>VAMO2</td>
<td>7.3</td>
<td>10.4</td>
</tr>
<tr>
<td>VAAL1</td>
<td>6.2</td>
<td>3.6</td>
</tr>
<tr>
<td>VARI1</td>
<td>11.3</td>
<td>2.7</td>
</tr>
<tr>
<td>VARI2</td>
<td>1.7</td>
<td>5.7</td>
</tr>
<tr>
<td>VAWE1</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>VAFL1</td>
<td>3.4</td>
<td>4.0</td>
</tr>
</tbody>
</table>
### TABLE 6
Estimated Acute Toxicity Values for Leachates from the Seven Study Sites

Toxicity is expressed as EC50, the concentration of leachate that would result in death of 50% of the test population within 24 h under standard conditions. Values in parentheses are 95% confidence intervals.

<table>
<thead>
<tr>
<th>Site</th>
<th>EC50 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAM01</td>
<td>&gt; 100%</td>
</tr>
<tr>
<td>VAM02</td>
<td>50.1% (39.5-63.5)</td>
</tr>
<tr>
<td>VAFL1</td>
<td>&lt; 1.0%</td>
</tr>
<tr>
<td>VAAL1</td>
<td>&gt; 100%</td>
</tr>
<tr>
<td>VARI1</td>
<td>&lt; 1.0%</td>
</tr>
<tr>
<td>VARI2</td>
<td>79.4% (57.2-100)</td>
</tr>
<tr>
<td>VAWE1</td>
<td>&lt; 0.5%</td>
</tr>
</tbody>
</table>
TABLE 7
Summary of Microcosm Experiment Results for Eluates from Toxic Waste Sites

The estimated dose-response relationship is shown along with significance values, explained variation ($r^2$), and EC 20.

The EC20 is the estimated level of eluate that would result in a 20% reduction of species (S) in microcosms, an estimate of chronic toxicity.

Values in parentheses are 95% confidence intervals.

<table>
<thead>
<tr>
<th>Site</th>
<th>Regression</th>
<th>F</th>
<th>p</th>
<th>$r^2$</th>
<th>EC20 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAMO2</td>
<td>$S = 16.6 - 0.10\text{conc}$</td>
<td>3.79</td>
<td>0.099</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td>VAWE1</td>
<td>$S = 24.0 - 2.9 \text{conc}$</td>
<td>16.94</td>
<td>0.004</td>
<td>0.708</td>
<td>2.2% (0 - 9.3)</td>
</tr>
<tr>
<td>VAFL1</td>
<td>$S = 19.3 - 0.91\text{conc}$</td>
<td>26.24</td>
<td>0.001</td>
<td>0.766</td>
<td>2.7% (0-13.7)</td>
</tr>
<tr>
<td>VARI1</td>
<td>$S = 6.60 - 3\log(\text{conc})$</td>
<td>168.2</td>
<td>&lt;0.001</td>
<td>0.96</td>
<td>0.035% (0.013-0.080)</td>
</tr>
</tbody>
</table>
### TABLE 8
Summary of Toxicity of Leachates from Waste Site Soils

Midranks were used for tied values. NT = not toxic.

<table>
<thead>
<tr>
<th>Site</th>
<th>Wastes</th>
<th>Acute Tests</th>
<th></th>
<th>Chronic Tests</th>
<th></th>
<th>Rank Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC50</td>
<td>Rank</td>
<td>EC20</td>
<td>Rank</td>
<td></td>
</tr>
<tr>
<td>VAWE1</td>
<td>wood preservatives</td>
<td>&lt;0.5%</td>
<td>1.0</td>
<td>2.2%</td>
<td>2</td>
<td>3.0</td>
</tr>
<tr>
<td>VARI1</td>
<td>wood preservatives</td>
<td>&lt;1.0%</td>
<td>2.5</td>
<td>0.035</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>VAFL1</td>
<td>heavy metals</td>
<td>&lt;1.0%</td>
<td>2.5</td>
<td>2.7</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>VAMO2</td>
<td>coal storage</td>
<td>50.1%</td>
<td>4.0</td>
<td>——*</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>VARI2</td>
<td>heavy metals</td>
<td>79.4%</td>
<td>5.0</td>
<td>NT</td>
<td>6</td>
<td>11.0</td>
</tr>
<tr>
<td>VAAL1</td>
<td>solvents</td>
<td>NT</td>
<td>6.5</td>
<td>NT</td>
<td>6</td>
<td>12.5</td>
</tr>
<tr>
<td>VAMO1</td>
<td>control</td>
<td>NT</td>
<td>6.5</td>
<td>NT</td>
<td>6</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Toxicity shown but dose-response relationship not significant.
LITERATURE CITED


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- It sponsors, coordinates, and administers research investigations of these problems.
- It collects and disseminates information about water resources and water resources research.
- It provides training opportunities in research for future water scientists enrolled at the state’s colleges and universities.
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