Remediation of Pesticide Contaminated Soil by a Combination of Compost Addition and Planting

Michael A. Cole,
Liu Zhang,
Xianzhong Liu

University of Illinois
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Michael A. Cole,
Liu Zhang,
Xianzhong Liu
University of Illinois
at Urbana-Champaign

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by

Michael A. Cole,
Liu Zhang,
Xianzhong Liu,
University of Illinois
Department of Agronomy
Urbana, Illinois

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ABSTRACT

Pesticide-contaminated material was obtained from five locations at four agrichemical retail facilities in Illinois. The physical, chemical, and microbial attributes that may limit bioremediation of the materials were identified. Limitations included inappropriate pH values for good plant growth or microbial activity, high soluble salts content, high bulk density, low organic matter content, low plant nutrient availability, low microbial activity, and the presence of phytotoxic organics in addition to identified herbicides. In an effort to improve physical properties and increase microbial activity and plant growth in the contaminated matrix, the material was mixed with uncontaminated soil or with mature yard waste compost to determine the impact of compost compared to soil on plant establishment and growth, rhizosphere populations, development of soil microbial populations and activity, and herbicide degradation. Plants were established and grew well in pesticide-containing soil when consideration was given to compatibility between plant herbicide tolerance and the specific herbicide(s) present. Rhizosphere fungal and bacterial populations developed to values that are typical for uncontaminated soil. Soil bacterial populations were significantly higher in compost-containing mixes when compared to contaminated soil alone, while populations in soil mixes were not affected by any treatment. Fungal populations were significantly higher in planted mixes and in unplanted mixes with compost than they were in contaminated soil alone. Microbial activity, as measured by dehydrogenase activity, was significantly higher in compost-containing mixes than in soil mixes. Planting contaminated material with a herbicide-tolerant plant species or a combination of planting and compost addition resulted in significant increases in herbicide degradation in the contaminated materials. The results strongly suggest that remediation of herbicide contamination at agrichemical retail facilities can be achieved quite rapidly and at moderate cost.
1.0 INTRODUCTION

Contamination of soil and groundwater at numerous agrichemical retail facilities has been reported in Illinois and Wisconsin. A survey of 49 agrichemical facilities in Illinois (Krapac, et al., 1993) showed that soil contamination with the herbicides alachlor, atrazine, metolachlor, trifluralin, pendimethalin, cyanazine, and metribuzin was very common, while contamination with insecticides was much less common. Herbicide concentrations ranged from a few micrograms per kilogram (parts per billion) of soil to several grams per kilogram of soil. Since most herbicides are effective in the mg kg$^{-1}$ range, there were secondary problems at the sites, including off-site erosional transport of contaminated soil by wind and water, which resulted in plant death on adjacent property or pollution of surface water. Similar results were found in a survey of Wisconsin agrichemical dealerships (Habacker, 1989). Taylor (1993) found detectable pesticides in groundwater samples from wells at agrichemical retail sites, including several compounds for which drinking water standards exist. He suggested that agrichemical facilities are primary sources of groundwater contamination in Illinois. The combination of adverse environmental impacts on the earth’s surface and subsurface indicates that remedial activities at these sites would be appropriate.

In most cases, the pesticides detected at the agrichemical facilities were probably not the result of recent spills, but rather were the result of years of accrued contamination. In the case of the materials described in this report, herbicides had most likely been in the materials for at least six months prior to sampling. Since the majority of the compounds found at these sites are reportedly degradable by biotic or abiotic reactions in soil, but were relatively persistent at the facilities, we were interested in identifying the factors that limited the rate of degradation in the contaminated areas.

Felsot and coworkers (1988, 1990) attempted bioremediation of pesticide-contaminated soil from an inactive agrichemical facility and found that pesticide degradation occurred quite slowly, with detectable concentrations of alachlor, atrazine, metolachlor, and trifluralin still present at 380 days after land application of excavated soil. Since these authors demonstrated that freshly-added herbicides were degraded rapidly in comparison to aged materials when applied to agricultural fields, it is likely that the bioavailability of the herbicides from the agrichemical facilities was low, thereby decreasing the degradation rate. Felsot and Dzantor (1990) also found that dehydrogenase activity (a measure of soil microbial activity) increased only slightly in soils amended with corn or soybean stubble, so lack of sufficient stimulation of microbial activity may also have been a contributory factor to the slow degradation they observed.

A variety of chemical and physical factors could account for the accumulation of pesticides at spill sites and for the slow degradation when mixed with uncontaminated soil. Many pesticides are adsorbed by clay, silt, and organic matter. Adsorption processes reduce potential groundwater contamination, but may have a negative impact on microbial access to the compounds (Schröder, et al., 1992; Steinberg, et al., 1987). Highly compacted materials display poor water and gas permeability, both of which will restrict microbial activity by
limiting water, oxygen, and solute movement within the matrix. High nitrate concentrations were found in some samples from agrichemical facilities, a result which suggests that some spills were fertilizer-herbicide mixtures. The resultant high concentrations of soluble salts would adversely affect microbial activity because of osmotic effects (Stanier, et al., 1986). In addition to active ingredients, most pesticide formulations contain various non-pesticidal components such as emulsifiers, by-products of pesticide synthesis, as well as surfactants. Emulsifiers and surfactants are used widely in detergents and disinfectants because of their toxicity to microbes. The presence of these compounds would reduce potential microbial activity and may also change the partitioning of pesticides between aqueous and adsorbed phases. Few microbes are competent degraders of a wide range of pesticides and some compounds require more than one microbial species for full degradation to occur (Senior, et al., 1976); in such cases, high biotic diversity increases the probability that degradation will take place. Disturbed sites with compacted soil, low nutrient supplies, and periodic additions of potentially toxic organic chemicals typically have a limited range of microbial species and may need an external source of organisms if degradation is going to occur. Based on these considerations, the prospect for "natural" remediation of these sites is poor.

Figure 1. Structures of herbicides whose degradation was studied in this research.
The principal contaminants found in the samples used for this investigations were trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine; marketed under the tradename, TREFLAN), metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-
methylethyl)acetamide; marketed under the tradenames DUAL or PENNANT), and pendimethalin (N-(1-ethylpropyl)-3,4,4-dimethyl-2,6-dinitrobenzenamine; marketed under the tradename, PROWL); structures of the compounds are shown in Figure 1. Biodegradation is regarded as the principal mechanism for destruction of trifluralin and metolachlor (Weed Science Society of America, 1989). Trifluralin is partially degraded to yield a wide range of metabolites which are apparently persistent in soil (Golab, et al., 1980); degradation is much more rapid when soil redox potential is low (Willis, et al., 1974) or when the soil is waterlogged (Mazersmith, et al., 1971). Metolachlor is converted to several hydroxylated and/or demethylated metabolites by pure cultures of an actinomycete (Krause, et al., 1985), a fungus (McGahan and Tiedje, 1978), and by mixed microbial cultures and is slowly mineralized in soil (Bollag and Liu, 1991). Its half-life has been estimated to be 11 to 70 days in soil at 30°C (Braverman, et al., 1986). In all cases (pure cultures or soil), the metabolites formed are a mixture of hydroxylated and/or dealkylated compounds. Based on work by Nelson (1979), pendimethalin is apparently degraded by both chemical and biotic reactions, since soil sterilization reduced degradation to about 50% of the values obtained with non-sterile soil.

Because microbial degradation was the major route for environmental destruction of trifluralin and metolachlor, and also contributed to pendimethalin degradation, one objective of this work was to stimulate microbial activity in the contaminated soils with the expectation that such stimulation might accelerate degradation of these pesticides. Two approaches and a combination of both approaches were tried. First, an attempt was made to grow plants in the contaminated mixes, since plant growth increases microbial populations in soil, especially root-associated (rhizosphere) bacterial populations. Second, compost was added as a source of microorganisms and organic matter because addition of compost or other organic materials can stimulate soil microbial activity. Mature compost is the stabilized end-product of microbial degradation of organic-rich materials such as yard waste, sewage sludge, manure, or municipal solid waste in a managed system. It typically has a higher microbial population than found in soil and it often has a stimulatory effect on plant growth. Since plant growth and compost when used individually can have beneficial effects on remediation, a combination of planting and compost addition was also tried.

Growth of plants in contaminated soils has a number of potential benefits for the remediation process. As described by Shann and Boyle (1994), pesticide degradation in the rhizosphere can be rapid, thereby decreasing the time required for remediation. Plant growth has been shown to facilitate degradation of several environmental contaminants in addition to pesticides (Anderson and Coats, 1994; Schwab and Banks, 1995). Second, plant uptake of soil water results in upward movement of water (Hillel, 1980), which may reduce downward flow of contaminated water from the vadose zone to groundwater. Third, successful plant establishment results in decreased erosional transport of contaminated soil to adjacent surface water or property. Since many pesticides are toxic to fish, decreased transport of
contaminated soil to surface water would substantially diminish adverse impacts of contaminated sites. Finally, plant growth improves soil structure and provides organic materials which may stimulate microbial cometabolism of pesticides.

As the research progressed, it was soon evident that all of the materials we obtained from contaminated agrichemical sites had one or more potential impediments to plant growth and microbial activity other than pesticide content. These impediments included relatively high pH values for good plant growth and microbial activity, high soluble salts content, high bulk density, low organic matter content, low nitrogen content, low microbial activity, and the presence of phytotoxic organics such as phthalate esters in addition to identified herbicides. While it would have been possible to deal with impediments individually on a site-by-site basis (which was the original intent of this research), we concluded that developing more generic and less analytically intensive methods for remediation of these sites would be desirable. The use of compost is emphasized in the present work because it has many virtues as a soil amendment resulting from its high organic matter content as well as a large and diversified microbial population. Our choice of compost as an organic amendment is based upon results from several researchers who had reported that pesticides degrade quite rapidly in compost (Lemmon and Pylypiw, 1992; Michel, et al., 1993).

This work was conducted in two stages: First, site-specific factors that were likely to limit bioremediation of contaminated materials were identified. Second, we determined whether or not it was possible to establish plants and microbes in the contaminated material. The result was a system using a combination of planting with a herbicide-tolerant plant species and compost addition to enhance pesticide degradation and reduce phytotoxicity and antimicrobial activity of the materials. The goal of remediation was to reduce herbicide concentrations below the amounts expected if the compounds were applied to soil at rates recommended by the manufacturer. Specific target concentrations are based on values given in the Herbicide Handbook (1989).

In our work, we have taken a broader view of successful remediation than defining success solely as degradation of target compounds. Improving soil conditions to promote plant growth and increase microbial activity were also goals of our research activities. With these considerations in mind, this work was conducted with the objectives of:

1. Identifying potential limitations to microbiALLY-catalyzed pesticide degradation,
2. Determining whether or not healthy plants could be established in soils containing a mixture of herbicides, some of which were present at several times recommended application rates, as well as other unidentified contaminants,
3. Comparing the benefits for plant growth and microbial proliferation and activity of mixing contaminated soil with uncontaminated soil or with compost derived from yard trimmings, and
4. Comparing the rate and extent of herbicide degradation in mixes of contaminated soil with either uncontaminated soil or compost.
2.0 EXPERIMENTAL

2.1 Soil and compost samples.

Pesticide-contaminated soils were obtained during soil samplings at agrichemical retail sites in Illinois as described by Krapac, et al. (1993). The samples were collected by commercial environmental sampling companies. Material was obtained from locations identified by Illinois Department of Agriculture (IDOA) coded as site 14, site 20 (from which material was collected at two distinct contaminated locations, which are identified in this report as site 20A and site 20B), site 27, and site 40. These sites were chosen randomly without respect to the contaminants present (that information was not available at time of sample collection). The specific sampling locations were near loading docks and other areas at which spills were more likely to occur. Cores of 8.1-cm diameter were collected to a depth of 457-cm and a composite sample of all contaminated cores was used for the present work. Contamination was generally restricted to the top 20 to 60 cm of material, most of which was road pack consisting of gravel, silt, clay, and sand. Based on analytical results obtained from IDOA about one year after this project started (Goetsch, unpublished data), a total of 22 pesticides were found in the samples when analyzed by USEPA methods 8080 and 8141 (USEPA, 1986, 1989), but most compounds were present at low concentrations and because of the low concentrations, were not target compounds in our work.

After collection, materials were mixed and placed in 55 gallon steel drums. Sampling was conducted during late November-early December, 1993, and the drums were stored in an unheated shed until February, 1994, when we removed samples from the drums. When we obtained the samples, all of them were solidly frozen. Materials were maintained in a frozen state until used for the experiments described in this report. Two weeks prior to initiating experiments, the samples were transferred to a 10°C incubator to thaw. The samples were passed through a 4 mm screen to remove large gravel and then used without further processing.

Mature compost derived from yard trimmings was obtained from DK Recycling Systems, Inc., Lake Bluff, IL, and from the Yard Waste Reclamation Site, Urbana, IL. The material which passed a 6-mm screen was used. Compost from DK Recycling was produced by a thermophilic process, which resulted in a weed-free product. This compost was used to assess the effects of planting along with compost addition on herbicide degradation in material from site 20A. Compost from the Urbana site was produced by a mesophilic process and the finished product had a high population of viable seeds of grassy and broad-leaf weeds. This compost was used for the studies with Site 14 contaminated material.

Uncontaminated soil was a 50:50 (w/w) mixture of sand with Drummer silty clay loam soil obtained from a local gardener. The sand was added to increase the permeability of the relatively poorly drained Drummer soil. No pesticides had been applied to the soil for four years and no pesticides were detected when the soil was extracted with methylene chloride +
acetone or with ethyl acetate and analyzed by GC/MS procedures. Material which passed a 6 mm screen was used.

2.2 Physical and chemical analysis of samples.

Bulk density, % sand, silt, clay, and gravel, inorganic-N, pH, and electrical conductivity were determined by standard methods (Klute, 1986; Page, et al., 1982).

2.3 Pesticide extraction and analysis.

In preliminary work, samples were extracted with ethyl acetate for 2 X 45 min, as described by Felsot and coworkers (1990) or by Soxhlet extraction with 1:1 (v/v) methylene chloride/acetone (USEPA, 1986, 1989). As described in Results, neither of these methods were particularly satisfactory, and therefore we developed the following procedure. A 25-g sample was ground in a Waring blender for 1 min at high speed and transferred into a rectangular 160-mL glass bottle with a PFTE cap liner. Ten milliliters of 1M sodium chloride solution was added along with sufficient water to make a soil slurry, followed by 50-mL of pesticide- or HPLC-grade ethyl acetate and 2-mL pesticide-grade acetone. The bottle was shaken horizontally on a rotary shaker at 150 rpm for 24 h at 20° C. The ethyl acetate (upper) layer was removed and an additional 25-mL ethyl acetate was added, followed by mixing by inverting the bottle 20 times. The ethyl acetate phase was centrifuged at 4,000 Xg for 10 min to separate the solid, aqueous and ethyl acetate phases. The soil and aqueous phases were discarded and the ethyl acetate was dehydrated by passage through a 10-cm column of anhydrous sodium sulfate, reduced to 1.0-mL final volume in a Kuderna-Danish concentrator, and passed through a 10-cm column of anhydrous sodium sulfate. Samples were transferred into glass vials with PFTE stoppers and stored at 4°C until analyzed. Extracts were brought to room temperature before injection into the GC. All extracts were analyzed within 2 weeks of preparation, beyond which time, pesticide concentrations decreased. Extraction efficiency and instrument performance was evaluated by spiking the various matrices with either single pesticides or pesticide mixtures.

For routine analysis, samples were injected without further purification into a Chrompack CP9000 gas chromatograph equipped with a nitrogen-phosphorus detector. A 50 m X 0.25 mm (i.d.) column of WCOT fused silica with CP-Sil-8 CB stationary phase (Chrompack, Inc.) was used for all analyses. The thermal program is based on USEPA method 507; the initial column temperature was 80°C, with the first ramp at 30°C min⁻¹ to 178°C, a second ramp of 2°C min⁻¹ to 205°C, a third ramp at 30°C min⁻¹ to 310°C, and a final holding period of 4 min at 310°C. Injector and detector temperatures were 290°C.

Initial identification of pesticides in the original samples was done by extracting with ethyl acetate/acetone as described above or by Soxhlet extraction with methylene chloride/acetone (1:1, v/v) (USEPA, 1989, Method 3540) and analyzing the extracts by GC-MS procedures.
2.4 Plant growth procedures.

General experimental design for this work is shown in Figure 2. Blends which contained 0, 1.5, 6, 12.5, 25, and 50% (w/w) contaminated soil and either control soil or compost were prepared and transferred into pots of 15-cm diameter and 15-cm height for greenhouse studies. Four pots of each soil or compost mixture were planted with 5 seeds of sweet corn (Zea mays, cv. 'Golden Beauty') and placed in a greenhouse. Plants were watered weekly with 15:30:15 NPK fertilizer (Miracle-Gro, Inc.). Four unplanted pots were treated in an identical manner. Sweet corn was chosen as the plant for these studies because of its tolerance to the major identified pesticides in the contaminated soil. Since one of the objectives of this work was to assess the impact of roots on pesticide degradation, smaller than optimally sized pots were used to ensure extensive root development in the soil mixes.

![Diagram]

Combined to give mixes containing 0, 1.5, 6, 12.5, 25, or 50% by weight contaminated soil and distributed into 8 pots, 15 cm diameter. 4 pots were planted with sweet corn in the greenhouse, 40 days, analyzed as described in text.

Figure 2. Summary of experimental design for greenhouse studies.
2.5 Plant analysis.

Plants were harvested at 40 d after planting and separated from soil. Roots and shoots were separated, and the roots were washed in tap water to remove adherent soil. Root samples used for rhizosphere analysis (Section 2.7) were processed within 15 min of collection. Samples for rhizosphere analysis were taken before the roots were dried. Dry weights of roots and shoots were determined by drying at 90° C to a constant mass.

2.6 Microbial culture media and solutions.

Buffer solution used to blend root samples for rhizosphere populations and to dilute samples for plate counts contained 0.28 g L⁻¹ \( \text{KH}_2\text{PO}_4 \), 0.28 g L⁻¹ \( \text{K}_2\text{HPO}_4 \), and 0.18 g L⁻¹ \( \text{MgSO}_4 \).

Bacterial populations of soil and rhizosphere samples were assessed on glucose-tryptone agar (Cole and Turgeon, 1978), and fungal plate counts were determined on rose bengal agar (Martin, 1950). Values are expressed as colony-forming units (cfu) per gram dry weight of soil.

2.7 Determination of rhizosphere microbial populations.

Approximately 1-g wet weight of roots was transferred to 30-mL of buffer and blended for 2 min in a high-speed mixer (Waring blender) operated at maximum speed. Samples were diluted and plated on glucose-tryptone agar or rose bengal agar. Plates were incubated at 30° C until colonies were large enough to count. For fungi, a 5 d incubation period was typical and 7 d incubation was typical for bacteria. Population counts were calculated as colony forming units (cfu) g⁻¹ dry weight of root. The rhizosphere:soil ratio (R:S ratio) of bacterial and fungal populations was calculated as:

\[
\text{R:S} = \frac{\text{Mean population in rhizosphere (cfu g}^{-1}\text{ root)}}{\text{Mean population in soil (cfu g}^{-1}\text{ soil)}}
\]

2.8 Determination of soil microbial populations.

These procedures are based upon previously published work (Cole, 1976). About 2-g wet weight of soil were added to 100-mL sterile buffer and shaken at 100 rpm on a rotary shaker for 10 min. Dilutions were prepared and plated on glucose-tryptone agar (bacteria) or rose bengal agar (fungi) and incubated as described above.

2.9 Soil dehydrogenase activity.

Dehydrogenase activity was determined by a variation of the method described by Benefield, et al. (1977). Two grams wet weight of soil were mixed with 4.5-mL of a 1% (w/v) solution of triphenyltetrazolium chloride (TTC, Sigma Chemical Co.) and incubated at
30° C for 24 h. The red, water-insoluble formazan, which is produced by reduction of TTC, was extracted from soil by shaking with 5-mL n-butanol for 1 h, followed by centrifuging to separate butanol and aqueous phases. The butanol phase was removed and its absorbance at 485 nm was determined. Dehydrogenase activity is expressed as μmol formazan produced g⁻¹ dry soil 24 h⁻¹.

2.10 Glucose utilization in soil slurries.

This procedure is a modification of a previously described method (Cole and Turgeon, 1978). The purpose of this test is to determine whether or not the contaminated soil contains bioavailable inhibitors of microbial activity in uncontaminated soil. If the soil is inhibitory to microbial activity, then the prospects for biological degradation of the pesticides in it are decreased unless toxicity can be diminished by dilution with uncontaminated soil or other materials. Mixtures were prepared that contained 1:3, 1:1, or 3:1 (w/w) proportions of contaminated to uncontaminated soil. Slurries were prepared (in duplicate) by adding 10-g of soil to 100-mL of medium containing 100-μg mL⁻¹ glucose and the inorganic salts in tryptone-glucose agar. Suspensions containing only uncontaminated or contaminated soil were also prepared. Flasks were incubated at 25° C on a rotary shaker at 250 rpm. Aliquots were removed periodically and residual glucose was determined as described by Nelson (1944).

2.11 Statistical analysis.

Data were subjected to appropriate statistical analysis (SAS, 1985). Comparisons between treatments that are referred to as "significantly different" were different at P = 0.05 or better.
3.0 RESULTS

3.1 Chemical and physical properties of contaminated materials.

The fundamental physical and chemical properties of contaminated materials are given in Tables 1 and 2. In all cases, materials were light-colored (an indication of low soil organic matter content) and had no aggregate structure.

Table 1. Physical and chemical characteristics of contaminated matrices.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bulk density (g cm(^{-3}))</th>
<th>pH</th>
<th>Electrical conductivity (dS m(^{-1}))</th>
<th>% moisture</th>
<th>NH(_4^+) - N (mg kg(^{-1}))</th>
<th>NO(_3^-) - N (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>1.34</td>
<td>7.3</td>
<td>2.31</td>
<td>10.1</td>
<td>1.8</td>
<td>0.03</td>
</tr>
<tr>
<td>20A</td>
<td>1.26</td>
<td>8.4</td>
<td>2.08</td>
<td>9.1</td>
<td>2.1</td>
<td>0.09</td>
</tr>
<tr>
<td>27</td>
<td>1.86</td>
<td>6.9</td>
<td>8.74</td>
<td>18.3</td>
<td>3.2</td>
<td>0.17</td>
</tr>
<tr>
<td>40</td>
<td>2.09</td>
<td>7.7</td>
<td>0.57</td>
<td>16.6</td>
<td>2.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Drummer soil + sand</td>
<td>1.43</td>
<td>6.6</td>
<td>0.18</td>
<td>14.5</td>
<td>10.2</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2. Particle size distribution of contaminated matrices.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% gravel (w/w)</th>
<th>% sand (w/w)</th>
<th>% silt (w/w)</th>
<th>% clay (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>11</td>
<td>23</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>20A</td>
<td>22</td>
<td>27</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>23</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>17</td>
<td>48</td>
<td>35</td>
</tr>
<tr>
<td>Drummer soil + sand</td>
<td>0</td>
<td>55</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>
3.2 Glucose utilization in mixtures of contaminated materials with uncontaminated soil.

Rates of glucose utilization by undiluted site 14 and site 20A materials, uncontaminated soil, and mixtures of contaminated and uncontaminated soils are shown in Tables 3 and 4. The same procedure was used with samples obtained from sites 27 and 40; these samples were not inhibitory to glucose utilization (data not shown).

Table 3. Glucose utilization rates in contaminated matrix, control soil, and mixtures of contaminated matrix and control soil of site 14 samples.

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Glucose utilization rate (μg h⁻¹ 10 g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>25</td>
<td>4.8</td>
</tr>
<tr>
<td>50</td>
<td>3.0</td>
</tr>
<tr>
<td>75</td>
<td>1.2</td>
</tr>
<tr>
<td>100</td>
<td>6.0</td>
</tr>
</tbody>
</table>

ᵃ Expected values were calculated by formula: Expected activity = [(mass fraction of control soil X glucose utilization rate of control soil) + (mass fraction of contaminated matrix X glucose utilization rate of contaminated matrix)].

ᵇ ns, not significantly different from expected values based on no interaction between control and contaminated soil; *, significantly less than expected based on no interaction between control and contaminated soil. Values are means of duplicate assays of two replicates of each mixture.
Table 4. Glucose utilization rates in contaminated matrix, control soil, and mixtures of contaminated matrix and control soil of samples from site 20A.

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Glucose utilization rate (µg h⁻¹ 10 g⁻¹ soil)</th>
<th>deviation from exp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>0</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>25</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>75</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>100</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

⁵ Expected values were calculated by formula: Expected activity = [(mass fraction of control soil X glucose utilization rate of control soil) + (mass fraction of contaminated matrix X glucose utilization rate of contaminated matrix)]

ⁿ ns, not significantly different from expected values based on no interaction between control and contaminated soil; *, significantly less than expected based on no interaction between control and contaminated soil. Values are means of duplicate assays of two replicates of each mixture.

3.3 Pesticide analysis of contaminated materials.

3.3.1 Pesticide content of samples.

Unpublished data supplied by Illinois Department of Agriculture (Goetsch, personal communication) indicated that materials from these sites were contaminated with a total of 22 pesticides, most of which were present at concentrations of a few hundred µg kg⁻¹ soil, with the major compounds being metolachlor, pendimethalin, and trifluralin. For our analyses, we initially tried the 2 X 45 min extraction with ethyl acetate described by Felsot, et al. (1990). However, no pesticides were found in extracts prepared in this manner. Soxhlet extraction with methylene chloride + acetone (USEPA, 1986, 1989) was successful, but the procedure was slow and generated large volumes of waste chlorinated solvent. As an alternative to these procedures, a method using ethyl acetate + acetone was developed, as described in the Experimental section. This procedure was not well documented and because we were extracting herbicides from a variety of matrices, including contaminated material mixed with a relatively high organic content soil or compost of high organic content, an evaluation of the procedure was conducted by spiking the various matrices with known compounds and
determining recovery. The results in Tables 5, 6, and 7 indicate that the procedure was effective for recovery of freshly-added herbicides and that the specific matrix had only a small impact on recovery of added herbicides. A high percentage recovery was of special concern because the compounds of interest can be adsorbed by organic matter, and the Drummer soil and compost contained about 6% and 30% organic matter, respectively. For comparative purposes, recoveries attained with the ethyl acetate/acetone extraction procedure fell within the ranges accepted by USEPA for organophosphate insecticides (USEPA, 1989, Method 8141) and for chlorinated herbicides (USEPA, 1989, Method 8150) and were considered to be sufficient for this research.

Table 5. Effect of matrix composition on recovery of trifluralin.

<table>
<thead>
<tr>
<th>Matrix a</th>
<th>Pesticide Concentration (mg kg⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
</tr>
<tr>
<td>50:50 contaminated + uncontaminated soil</td>
<td>0.62</td>
</tr>
<tr>
<td>50:50 compost + soil</td>
<td>1.0</td>
</tr>
<tr>
<td>100% contaminated soil</td>
<td>1.42</td>
</tr>
</tbody>
</table>

a Material from site 14 was the source of contaminated soil. All samples were obtained upon completion of a greenhouse incubation period, and therefore, concentrations are lower than found in the initial material.

b Values are means of duplicate analyses of two samples of each unspiked or spiked matrix.
Table 6. Effect of matrix composition on recovery of metolachlor.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Pesticide Concentration (mg kg\textsuperscript{-1} soil)</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Added</td>
</tr>
<tr>
<td>50:50 contaminated + uncontaminated soil</td>
<td>3.04</td>
<td>1.86</td>
</tr>
<tr>
<td>50:50 compost + soil</td>
<td>nd\textsuperscript{c}</td>
<td>1.86</td>
</tr>
<tr>
<td>100% contaminated soil</td>
<td>1.56</td>
<td>1.86</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Material from site 14 was the source of contaminated soil. All samples were obtained upon completion of a greenhouse incubation period, and therefore, concentrations are lower than found in the initial material.
\textsuperscript{b} Values are means of duplicate analyses of two samples of each unspiked or spiked matrix.
\textsuperscript{c} nd, not detected.

Table 7. Effect of matrix composition on recovery of pendimethalin.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Pesticide Concentration (mg kg\textsuperscript{-1} soil)</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Added</td>
</tr>
<tr>
<td>50:50 contaminated + uncontaminated soil</td>
<td>1.00</td>
<td>14.5</td>
</tr>
<tr>
<td>50:50 compost + soil</td>
<td>0.84</td>
<td>14.5</td>
</tr>
<tr>
<td>100% contaminated soil</td>
<td>1.56</td>
<td>14.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Material from site 14 was the source of contaminated soil. All samples were obtained upon completion of a greenhouse incubation period, and therefore, concentrations are lower than found in the initial material.
\textsuperscript{b} Values are means of duplicate analyses of two samples of each unspiked or spiked matrix.
The major pesticides and their concentrations in site 14 and site 20A materials according to our analyses are shown in Table 8. None of the herbicides listed in Table 8 (nor any others) were found in samples from sites 27 or 40, nor in uncontaminated soil or compost. The analyses must be qualified by our observations that there was good control of both grassy and broadleaf weeds when contaminated soil from both site 14 and site 20A was diluted with uncontaminated soil to give 1.5% (w/w) contaminated soil. Based on the data in Table 8, a mixture with 1.5% contaminated soil was calculated to contain only 0.25 to 0.3 mg kg⁻¹ total concentration of analytically identified pesticides; at this concentration there should have been little weed control. Since there was good weed control, the presence of an unidentified herbicide or other phytotoxic compounds must be suspected.

Table 8. Herbicide content of samples from site 14 and site 20A.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site 14</th>
<th>Site 20A</th>
<th>Typical Application Rate b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>--- mg kg soil⁻¹---</td>
<td></td>
<td>0.25 - 0.50</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>6.9 ± 1.8a</td>
<td>2.2 ± 0.9</td>
<td>0.75 - 2.0</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>4.8 ± 1.0</td>
<td>3.0 ± 0.2</td>
<td>0.25 - 2.0</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>15.6 ± 4.9</td>
<td>11.8 ± 5.1</td>
<td>0.25 - 2.0</td>
</tr>
</tbody>
</table>

a Values are means ± 1 standard deviation of four subsamples from each site.

b Based on manufacturer’s recommendations on product labels and Weed Science Society of America, 1989.

Since the focus of this project was herbicide degradation, no attempt was made to quantify or identify the compounds seen in GC/MS analyses. Based on their extractability by ethyl acetate and the dark color, many of these compounds are likely to be phenols or aromatic acids that could be byproducts of pesticide production or formulation, metabolites formed by degradation of pesticides, or additives to the pesticide formulations.

Because site 27 and site 40 samples did not contain detectable pesticides, and did not inhibit microbial activity in the glucose utilization test (data not shown), no further work was done with these materials. Experiments with samples from site 20B will be reported elsewhere (Liu and Cole, 1995).
3.3.2 Analytical difficulties associated with analysis of contaminated matrix.

Ethyl acetate extracts from Site 14, Site 20A, Site 20B, and Site 27 were dark brown, in contrast to the nearly colorless extracts of uncontaminated soil or Site 40 matrix. Since the uncontaminated soil was a high organic matter soil (a dark prairie soil), yet yielded nearly colorless extracts, the highly pigmented extracts from several sites suggested that extractable organic compounds other than pesticides that are not naturally occurring compounds were present in the samples. Materials obtained from Sites 14, 20A, 20B, and 40, and uncontaminated soil were subjected to GC/MS analysis as shown in Figures 3 through 6. There were relatively few peaks in extracts from uncontaminated soil (Figure 7), but the extracts of agrichemical site materials contained a substantial number of peaks, some of which were identifiable as phthalate esters. In contrast to the numerous peaks found by GC/MS analysis, GC analysis using a nitrogen-phosphorus detector (NPD) revealed only a small number of peaks (Figure 8).

![Image of GC/MS analysis](image)

**Figure 3.** Analysis of ethyl acetate extract of undiluted, contaminated material from site 14 by GC/MS.
Figure 4. Analysis of ethyl acetate extract of undiluted, contaminated material from site 20A by GC/MS.

Figure 5. Analysis of ethyl acetate extract of undiluted, contaminated material from site 20B by GC/MS.
Figure 6. Analysis of ethyl acetate extract of undiluted, contaminated material from site 40 by GC/MS.

Figure 7. Analysis of ethyl acetate extract of uncontaminated soil by GC/MS.
Figure 8. Analysis of ethyl acetate extract of site 20A material spiked with 1 mg kg$^{-1}$ of trifluralin, metolachlor, and pendimethalin by GC/NPD. The pesticide peaks are atrazine (internal standard, 14.1 min), trifluralin (17.4 min), metolachlor (22.6 min), and pendimethalin (23.5 min).

3.4 Effect of diluting contaminated material with soil or compost on plant growth, microbial activity, and pesticide degradation (Site 14 material).

3.4.1 Plant growth in pesticide-contaminated soils.

As shown in Figures 9 and 10, shoot weight and total weight of corn growing in mixtures of 50:50 contaminated + uncontaminated soil (hereafter referred to as soil mixes) was significantly greater than obtained in 50:50 compost-containing mixes (hereafter referred to as compost mixes). Weed growth in all soil mixes was significantly less than growth in compost mixes (Figure 11), with no growth in 100% contaminated soil. Total biomass (corn + weeds) was significantly greater in mixes containing 1.5% and 25% compost than in contaminated soil alone (Figure 12). Total biomass in soil mixes was significantly greater than found with 100% contaminated soil only in the 25% contaminated soil mix (Figure 12).
Figure 9. Corn shoot weight of plants grown in soil and compost mixes of site 14 material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.

Figure 10. Corn shoot + root production of plants grown in soil and compost mixes of site 14 material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.
Figure 11. Weed shoot + root production of plants grown in soil and compost mixes of site 14 material without corn plants. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.

Figure 12. Total plant production (roots + shoots) of corn and weeds grown in soil and compost mixes of site 14 material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.
3.4.2 Dehydrogenase activity.

We used dehydrogenase activity as a broad-spectrum indicator of microbial activity in the mixes. This enzyme has been used by several investigators as an indication of overall heterotrophic activity in soil (Schaffer, 1993). Dehydrogenase activity in all mixes of soil from Site 14 containing compost were significantly higher than activity in mixes without compost (Table 9). No mix containing uncontaminated soil had significantly greater dehydrogenase activity than found in 100% contaminated soil.

Table 9. Effect of mix composition and planting on dehydrogenase activity in mixes of site 14 material.

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Soil mix, Corn + Weeds</th>
<th>Compost mix, Corn + Weeds</th>
<th>Soil Mix, Weeds Only</th>
<th>Compost Mix, Weeds Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>umol formazan g⁻¹ mix 24 h⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>79 ± 6 a</td>
<td>762 ± 33</td>
<td>36 ± 9</td>
<td>678 ± 56</td>
</tr>
<tr>
<td>1.5</td>
<td>65 ± 9</td>
<td>680 ± 140</td>
<td>45 ± 23</td>
<td>616 ± 73</td>
</tr>
<tr>
<td>6</td>
<td>72 ± 25</td>
<td>827 ± 161</td>
<td>43 ± 14</td>
<td>665 ± 68</td>
</tr>
<tr>
<td>12.5</td>
<td>37 ± 8</td>
<td>747 ± 123</td>
<td>34 ± 11</td>
<td>649 ± 93</td>
</tr>
<tr>
<td>25</td>
<td>80 ± 16</td>
<td>586 ± 31</td>
<td>33 ± 17</td>
<td>381 ± 39</td>
</tr>
<tr>
<td>50</td>
<td>40 ± 10</td>
<td>337 ± 42</td>
<td>35 ± 18</td>
<td>317 ± 30</td>
</tr>
<tr>
<td>100</td>
<td>25 ± 4</td>
<td>25 ± 4</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
</tbody>
</table>

a Values are the means ± 1 standard deviation. Values are means of duplicate assays of four replicate pots for each treatment.

3.4.3 Pesticide degradation.

Trifluralin concentrations in Site 14 material were significantly reduced in only one soil mix (100% contaminated soil planted with corn) (Table 10). The best results were obtained with 50:50 compost mixes, where residual concentrations were only 6 to 8% of initial values. Metolachlor was degraded in 100% contaminated soil without corn, but not in 100% contaminated soil with corn. Significant metolachlor degradation did not occur in soil mixes, regardless of plant species. Metolachlor was degraded to low or non-detectable levels in compost mixes and there was no major effect of plant species. Pendiemethalin was degraded to a significant extent in all treatments. As seen with metolachlor, the best results
were obtained with 50:50 compost mixes, where residual concentrations were 1% or less of initial concentrations.

Table 10. Effects of mix composition and plant species on pesticide degradation in site 14 material or mixtures thereof, following 40 d of plant growth.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Plants</th>
<th>Trifluralin (mg kg(^{-1}) soil)</th>
<th>Metolachlor (mg kg(^{-1}) soil)</th>
<th>Pendimethalin (mg kg(^{-1}) soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Concentration</td>
<td>None</td>
<td>6.90 ± 1.76</td>
<td>4.81 ± 1.03</td>
<td>15.6 ± 4.93</td>
</tr>
<tr>
<td></td>
<td>Corn + weeds</td>
<td>5.31 ± 2.44 (0.15)</td>
<td>2.60 ± 2.95 (0.07)</td>
<td>2.37 ± 2.86 (0.01)</td>
</tr>
<tr>
<td>100% contaminated</td>
<td>Weeds only</td>
<td>1.71 ± 0.55 (0.02)</td>
<td>0.83 ± 0.48 (0.002)</td>
<td>1.96 ± 0.99 (0.02)</td>
</tr>
<tr>
<td>50:50 soil</td>
<td>Corn + weeds</td>
<td>3.27 ± 2.39 (0.45)</td>
<td>3.07 ± 2.07 (0.07)</td>
<td>1.77 ± 1.67 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Weeds only</td>
<td>3.74 ± 2.65 (0.49)</td>
<td>0.82 ± 1.25 (0.07)</td>
<td>2.37 ± 2.29 (0.02)</td>
</tr>
<tr>
<td>50:50 compost</td>
<td>Corn + weeds</td>
<td>0.38 ± 0.28 (0.01)</td>
<td>0.08 ± 0.03 (0.008)</td>
<td>0.20 ± 0.01 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Weeds only</td>
<td>0.53 ± 0.16 (0.01)</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± standard deviations of duplicate extractions of four replications per treatment. Values in parentheses indicate the probability that the values are less than expected from dilution alone (based on a one-tailed T-test for means of unequal variance).

\(^b\) nd, not detected.

The results demonstrated that plant growth, microbial activity, and herbicide degradation were increased in compost-containing mixtures, but the fact that there were substantial differences in corn and weed growth in the various mixes, prevented making a distinction between the effects of plant growth and compost addition on microbial activity and pesticide degradation. Therefore, weed-free compost was obtained so that the distinction could be made between the effects of compost versus the effects of plant growth with respect to stimulating microbial activity and pesticide degradation. Since one premise of this work was that rhizosphere microorganisms could contribute to pesticide degradation, the population
of rhizosphere bacteria and fungi in the mixes were examined. We were also interested in whether or not the large increases in dehydrogenase activity seen in compost mixes were paralleled by corresponding increases in the populations of soil bacteria and fungi.

3.5 Effect of diluting contaminated material with soil or compost on plant growth, microbial activity, and pesticide degradation (Site 20A material).

3.5.1 Plant growth.

The roots of corn plants were morphologically normal-looking in all soil and compost mixes of Site 20A. In contrast, roots of plants from 100% contaminated soil did not fully occupy the soil volume, were non-fibrous and displayed cortical hypertrophy. As the data in Table 11 indicate, there were no significant differences in root mass among treatments.

Table 11. Root production in soil and compost mixes of site 20A material.\(^a\)

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Soil Mixes</th>
<th>Compost Mixes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g dry weight pot(^{-1})</td>
<td>g dry weight pot(^{-1})</td>
</tr>
<tr>
<td>0</td>
<td>1.54 (x)(^{b,c})</td>
<td>1.12 (x)</td>
</tr>
<tr>
<td>1.5</td>
<td>1.17 (x)</td>
<td>1.80 (x)</td>
</tr>
<tr>
<td>6</td>
<td>1.66 (x)</td>
<td>1.03 (x)</td>
</tr>
<tr>
<td>12.5</td>
<td>1.52 (x)</td>
<td>1.34 (x)</td>
</tr>
<tr>
<td>25</td>
<td>1.40 (x)</td>
<td>1.43 (x)</td>
</tr>
<tr>
<td>50</td>
<td>1.39 (x)</td>
<td>1.34 (x)</td>
</tr>
<tr>
<td>100</td>
<td>1.97 (x)</td>
<td>1.97 (x)</td>
</tr>
</tbody>
</table>

\(^a\) Reproduced from Cole, et al., 1994, with permission.

\(^b\) Values are means of four pots per treatment.

\(^c\) Values in columns followed by different letters \((x,y,z)\) are significantly different (\(P=0.05\)).

Shoot growth was normal-appearing in all cases. Shoot production was not significantly different among soil mixes, as shown in Figure 13. Shoot production was significantly greater in compost mixes containing 1.5, 25, and 50% compost. These values were also significantly greater than shoot production in 100% control soil.
Figure 13. Corn shoot production of plants grown in soil and compost mixes of site 20A material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.

Figure 14. Corn shoot + root production of plants grown in soil and compost mixes of site 20A material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.
Total plant production (roots + shoots) was not significantly different among soil mixes, (Figure 14), but total plant production was significantly greater in compost mix containing 50% compost.

Weed growth was absent in all compost mixes and there was also very little weed growth in soil mixes, except for 100% uncontaminated soil.

3.5.2 Microbial populations in the rhizosphere.

There were no significant differences among treatments in fungal or bacterial populations of the rhizosphere (Table 12).

Table 12. Microbial populations in the rhizosphere of plants grown in soil or compost mixes of site 20A material. a

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Soil Mixes, Fungi</th>
<th>Compost Mixes, Fungi</th>
<th>Soil Mixes, Bacteria</th>
<th>Compost Mixes, Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cfu g⁻¹ mix (X 10⁴) b</td>
<td>cfu g⁻¹ mix (X 10⁸)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20.4 x c</td>
<td>56.2</td>
<td>31.6</td>
<td>12.6 x</td>
</tr>
<tr>
<td>1.5</td>
<td>33.9 x</td>
<td>26.3</td>
<td>7.9 x</td>
<td>12.6 x</td>
</tr>
<tr>
<td>6</td>
<td>30.2 x</td>
<td>14.1 x</td>
<td>15.8 x</td>
<td>25.1 x</td>
</tr>
<tr>
<td>12.5</td>
<td>17.0 x</td>
<td>29.5 x</td>
<td>15.8 x</td>
<td>25.1 x</td>
</tr>
<tr>
<td>25</td>
<td>7.2 x</td>
<td>23.4 x</td>
<td>63.1 x</td>
<td>10.0 x</td>
</tr>
<tr>
<td>50</td>
<td>11.8 x</td>
<td>28.8 x</td>
<td>12.6 x</td>
<td>20.0 x</td>
</tr>
<tr>
<td>100</td>
<td>15.8 x</td>
<td>15.8 x</td>
<td>12.6 x</td>
<td>20.0 x</td>
</tr>
</tbody>
</table>

a Reproduced from Cole, et al., 1994, with permission.
b Values are means of four pots per treatment.
c Values in columns followed by different letters (x,y,z) are significantly different (P = 0.05).

3.5.3 Bacterial populations in soil and compost mixes.

There were no significant differences in bacterial populations among any of the soil mix treatments (Table 13). Populations in planted compost mixes were not significantly greater than 100% contaminated soil. Values for compost mixes that were unplanted were
significantly greater than 100% contaminated soil. Bacterial populations were significantly higher in nearly all compost mixes when compared to soil mixes. What is not evident from the population values is that microbial diversity, as indicated by the variety of colony types appearing on dilution plates, was much higher in all mixes than it was in the contaminated soil. Contaminated soil had a very large population of a single colony type for bacteria, in contrast to the mixes with 10 to 20 colony types.

Table 13. Bacterial populations in soil and compost mixes of site 20A material with or without corn plants.a

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Soil mix, Planted</th>
<th>Compost mix, Planted</th>
<th>Soil Mix, Not Planted</th>
<th>Compost Mix, Not Planted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log₁₀ cfu</td>
<td>g⁻¹ dry weight of mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.96 ± 0.24 b</td>
<td>8.25 ± 0.29</td>
<td>7.30 ± 0.3</td>
<td>8.62 ± 0.08</td>
</tr>
<tr>
<td>1.5</td>
<td>7.88 ± 0.11</td>
<td>8.39 ± 0.51</td>
<td>7.74 ± 0.19</td>
<td>8.42 ± 0.17</td>
</tr>
<tr>
<td>6</td>
<td>7.63 ± 0.12</td>
<td>8.02 ± 0.28</td>
<td>7.32 ± 0.63</td>
<td>8.55 ± 0.48</td>
</tr>
<tr>
<td>12.5</td>
<td>7.75 ± 0.28</td>
<td>7.85 ± 0.13</td>
<td>7.60 ± 0.18</td>
<td>8.15 ± 0.75</td>
</tr>
<tr>
<td>25</td>
<td>7.75 ± 0.02</td>
<td>8.21 ± 0.34</td>
<td>7.63 ± 0.08</td>
<td>8.53 ± 0.07</td>
</tr>
<tr>
<td>50</td>
<td>7.52 ± 0.13</td>
<td>7.93 ± 0.17</td>
<td>7.61 ± 0.05</td>
<td>8.06 ± 0.28</td>
</tr>
<tr>
<td>100</td>
<td>7.71 ± 0.19</td>
<td>---</td>
<td>7.27 ± 0.17</td>
<td>---</td>
</tr>
</tbody>
</table>

a Reproduced from Cole, et al., 1994, with permission.
b Values are means ± 1 standard deviation, based upon four replicate pots per treatment.

Between-sample variability was very high in soil mixes and in planted treatments of compost mixes. This variability is probably was the result of incomplete mixing of the compost with soil. In such a situation, a single sample might contain a larger amount of compost with a very high bacterial population and relatively little soil with a smaller population, whereas the next subsample might contain more soil and less compost and have a smaller overall population. We have encountered similar variability in microbial populations and activity when sampling recently reconstructed soils following surface mining (unpublished results).
3.5.4 Fungal populations in soil and compost mixes.

There were no significant differences in fungal populations in most soil mixes with or without plants (Figures 15 and 16). However, fungal populations in 100% contaminated soil with plants were significantly greater than observed in unplanted soil. The results demonstrated that plant growth in contaminated soil mixes had a beneficial effect on fungal proliferation, probably due to release of plant root exudates that supported fungal growth.

![Graph showing fungal populations in soil and compost mixes](image)

Figure 15. Fungal populations in unplanted soil and compost mixes of site 20A material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.

Compost mixes which had not been planted had significantly higher fungal populations than 100% contaminated soil (Figure 15). There were no significant differences among unplanted compost mixes, although populations in all compost mixes were significantly greater than those in uncontaminated soil. Planted compost mixes containing 25 or 50% compost (Figure 16) had significantly lower fungal populations than unplanted mixes with the same percentage of compost (Figure 15).

Comparison of fungal populations in unplanted soil mixes with unplanted compost mixes indicates that compost addition (Figures 15 and 16) resulted in an approximately ten-fold greater fungal population than seen with soil mixes. Populations were also significantly greater in planted compost mixes when compared to planted soil mixes. Based on colony morphology, fungal diversity was also low in contaminated soil with only two species growing on the dilution plates, in contrast to 10 to 15 recognizably different fungal species in mixes containing control soil or compost.
Figure 16. Fungal populations in planted soil and compost mixes of site 20A material. Values are the means of four replicates of each treatment. Vertical lines at the top of each bar indicate one standard deviation.

3.5.5 Dehydrogenase activity.

There were no significant differences among treatments in dehydrogenase activity in planted or unplanted soil mixes, although differences among treatments were substantial (Table 14). Data variability within treatments was relatively large with this assay, probably because of incomplete mixing of rather heterogeneous materials. Other researchers who conducted soil restoration activities following surface mining have encountered similar variability problems (Lindemann, et al., 1984). Dehydrogenase activity was significantly greater in all compost-containing mixes when compared to 100% contaminated soil and all soil mixes. Activity was significantly lower than expected in planted and unplanted mixes containing 25% or 50% contaminated soil.
Table 14. Effect of mix composition and planting on dehydrogenase activity in mixes of site 20A material.a

<table>
<thead>
<tr>
<th>% contaminated soil</th>
<th>Soil mix, Planted</th>
<th>Compost mix, Planted</th>
<th>Soil Mix, Not Planted</th>
<th>Compost Mix, Not Planted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>umol formazan g(^{-1}) mix 24 h(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>68 ± 20 (^b)</td>
<td>1464 ± 161</td>
<td>40 ± 19</td>
<td>1299 ± 112</td>
</tr>
<tr>
<td>1.5</td>
<td>67 ± 27</td>
<td>1080 ± 422</td>
<td>43 ± 40</td>
<td>703 ± 289</td>
</tr>
<tr>
<td>6</td>
<td>50 ± 17</td>
<td>1075 ± 50</td>
<td>24 ± 10</td>
<td>972 ± 138</td>
</tr>
<tr>
<td>12.5</td>
<td>54 ± 16</td>
<td>1052 ± 168</td>
<td>33 ± 13</td>
<td>825 ± 96</td>
</tr>
<tr>
<td>25</td>
<td>59 ± 26</td>
<td>575 ± 40</td>
<td>25 ± 10</td>
<td>613 ± 111</td>
</tr>
<tr>
<td>50</td>
<td>32 ± 14</td>
<td>370 ± 91</td>
<td>25 ± 6</td>
<td>336 ± 32</td>
</tr>
<tr>
<td>100</td>
<td>18 ± 6</td>
<td>18 ± 6</td>
<td>16 ± 6</td>
<td>16 ± 6</td>
</tr>
</tbody>
</table>

\(^a\) Reproduced from Cole, et al., 1994, with permission.
\(^b\) Values are the means ± 1 standard deviation. Values are means of duplicate assays of four replicate pots for each treatment.

3.5.6 Pesticide degradation.

Trifluralin concentrations in Site 20A material were significantly reduced in planted mixes containing either soil or compost, but not in unplanted mixes (Table 15). The planted 50:50 soil mix was the only treatment in which trifluralin concentrations were reduced to non-detectable levels (0.2 mg kg\(^{-1}\)). Metolachlor was not degraded in planted mixes containing only contaminated soil, nor in unplanted 50:50 compost mixes, while degradation to non-detectable levels (0.1 mg kg\(^{-1}\)) was achieved in planted and unplanted 50:50 soil mixes and in planted 50:50 compost mixes. Pendimethalin was degraded in all mixes except unplanted 50:50 compost. However, degradation to non-detectable levels (0.2 mg kg\(^{-1}\)) was not obtained with any treatment. The presence of corn plants had a marked effect in several cases, apparently stimulating trifluralin degradation in 50:50 soil mixes, as well as metolachlor and pendimethalin degradation in both soil- and compost-containing mixes. Among the treatments, the best results were obtained in planted 50:50 mixes containing either soil or compost.
Table 15. Effects of mix composition and plant species on pesticide degradation in site 20A material or mixtures thereof, following 40 d of plant growth.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Treatment</th>
<th>Trifluralin</th>
<th>Metolachlor</th>
<th>Pendimethalin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg kg(^{-1}) soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Concentration</td>
<td>None</td>
<td>2.2 ± 0.9</td>
<td>3.0 ± 0.2</td>
<td>11.8 ± 5.1</td>
</tr>
<tr>
<td>100% contaminated</td>
<td>Planted</td>
<td>0.80 ± 0.82 (0.27)(^a)</td>
<td>3.4 ± 5.0 (0.25)</td>
<td>1.6 ± 0.4 (0.02)</td>
</tr>
<tr>
<td>100% contaminated</td>
<td>Not Planted</td>
<td>0.48 ± 0.77 (0.77)</td>
<td>0.99 ± 1.4 (0.25)</td>
<td>1.8 ± 0.4 (0.02)</td>
</tr>
<tr>
<td>50:50 soil</td>
<td>Planted</td>
<td>nd(^b)</td>
<td>nd</td>
<td>0.5 ±0.6 (0.01)</td>
</tr>
<tr>
<td>50:50 soil</td>
<td>Not Planted</td>
<td>0.52 ± 0.53 (0.07)</td>
<td>0.18 ± 0.16 (&lt;0.001)</td>
<td>1.0 ± 0.2 (0.02)</td>
</tr>
<tr>
<td>50:50 compost</td>
<td>Planted</td>
<td>0.36 ± 0.33 (0.02)</td>
<td>nd</td>
<td>1.5 ± 0.6 (0.02)</td>
</tr>
<tr>
<td>50:50 compost</td>
<td>Not Planted</td>
<td>0.44 ± 0.69 (0.08)</td>
<td>2.8 ± 3.4 (0.29)</td>
<td>2.6 ± 3.4 (0.12)</td>
</tr>
</tbody>
</table>

\(^a\) Values are means ± standard deviations of duplicate extractions of four replications per treatment. Values in parentheses indicate the probability that the values are less than expected from dilution alone (based on a one-tailed T-test for means of unequal variance).

\(^b\) nd, not detected.
4.0 DISCUSSION

4.1 Chemical and physical properties of contaminated materials.

The bulk density of site 27 and 40 samples exceeded values that would permit good root growth (Table 1). Russell (1973) suggested a limit of 1.5 to 1.6 as a maximum value into which roots will grow in a heavy-textured soil and a value of 1.7 for a light-textured soil. Low porosity is a collateral property of high-density soils, and therefore, it can be assumed that these materials would also be unable to transmit solutes and gases at a sufficient rate to allow high microbial activity. The pH values of 7.7 and 8.4 for site 20A and site 40 samples (Table 1) are sufficiently high to limit bioavailability of the trace elements iron and zinc, and the major nutrients, phosphorus, nitrogen, and potassium (Troeh and Thompson, 1993). The soluble salts content of all materials except site 40 were substantially higher than encountered in productive soils such as Drummer (Table 1). The conductivity value of 8.74 in site 27 material would constitute a severe impediment to plant growth. High soluble salts and reduced nutrient availability would also have a negative impact on some, but not all, microbial groups. The near absence of readily available nitrogen (ammonium and nitrate) would be a severe limitation to plant and microbial growth. A possible limiting factor for degradation in material from sites 14 and 20A is the presence of unidentified toxic compounds that restrict microbial activity when measured by glucose degradation (Tables 3 and 4). There was no indication of inhibition of activity in site 27 or site 40 materials (data not shown), even though site 40 samples had a wide variety of ethyl acetate-extractable organic compounds (Figure 6), just as site 14 (Figure 3) and site 20A samples (Figures 4 and 5) did.

4.2 Pesticide analysis.

There were minor analytical problems that resulted from the presence of high concentrations of phthalate esters in all samples. It was not possible to remove the phthalate esters during post-extraction clean-up without loss of some of the pesticides as well. Therefore, extracts were analyzed without extensive clean-up. The presence of high-boiling contaminants, which did not elute readily from the column, made it necessary to ensure that column and detector performance were not compromised, which we did by running a standard mixture and a solvent blank after each set of six sample injections. Phthalate esters have been reported to be significant interferences with other environmental analyses (Leung and Giang, 1993). In our case, the high-boiling contaminants eluted after the pesticides of interest; these contaminants were a nuisance, and decreased instrument sensitivity, but they did not pose a major problem when GC/NPD analysis was used. Since several phthalate esters are RCRA-regulated compounds (Federal Register, 1993), their presence may preclude land application of some contaminated materials from agrichemical facilities.
4.3 Plant growth.

Considering all the data as a unit, a 50:50 mixture of contaminated soil and compost was the most satisfactory from the standpoints of maximizing plant growth while minimizing dilution of the contaminated soil. The extensive weed growth in compost-containing mixes (Figure 11) could be attributed to either rapid herbicide degradation or extensive adsorption of the herbicides by the compost matrix, thereby reducing bioavailability to sensitive plants. In several cases, residual herbicide content was high enough at the end of the experimental period (Table 10) that inhibition of weed growth would be expected. Therefore, adsorption of the herbicides is the more likely explanation for the good weed growth observed in compost mixes.

The differences in the effects of compost on corn growth between Site 14 (Figures 9 and 10) and Site 20A (Figures 13 and 14, Table 11) materials is probably a reflection of the lack of weed competition in Site 20A mixes in comparison to Site 14 mixes and differences in texture and water-holding capacity of the two composts used. In Site 14 mixes, corn growth was reduced when weed biomass was high, which was probably the result of a greater tolerance of the weed species to the very wet conditions that one finds in high percentage compost mixes. Inbar et al. (1994) found that compost had a much greater tendency to retain moisture than volcanic ash when used in potting mixes. Commercial horticultural growers usually use no more than 10% compost (w/w) in their mixes to avoid water-logging problems. In our studies, plants were watered on a timed schedule by greenhouse staff, irrespective of the water content of the medium. Because of the high water retention of the compost, overwatering and resultant decreases in corn growth in the finer-textured and more moisture retentive compost obtained in Urbana when compared to the DK Recycling compost probably gave a competitive advantage to the weeds in mixes with a high percentage of compost.

4.4 Rhizosphere populations.

Fungal populations in the range of $10^5$ cfu g$^{-1}$ dry weight of root and bacterial populations of $10^9$ cfu g$^{-1}$ soil (Table 12) were similar to values reported by other researchers (Rovira and Davey, 1974; Moorman, 1992; Moorman and Dowler, 1991) for plants growing in un-contaminated soils. The rhizosphere:soil (R:S) ratio was calculated from the data in Table 12. The ratio for fungi was 1.17:1 (soil mixes) and 1.03:1 (compost mixes). The corresponding ratios for bacteria were 45.4:1 (soil mixes) and 14.3:1 (compost mixes). These ratios are consistent with previously published values of 20:1 to 50:1 R:S ratios for bacteria and values around 1.0 for fungi (Rovira and Davey, 1974; Moorman, 1992; Moorman and Dowler, 1991). There was no significant difference between soil and compost mixes in the R:S ratio for fungi. The R:S ratio for bacteria was significantly lower for compost mixes than for soil mixes. The difference was the result of the much higher bacterial populations in compost mixes compared to soil mixes (Table 13).

Taken together, the actual rhizosphere populations and the relationship between rhizosphere and soil populations in the mixes indicate that corn roots growing in soil
containing herbicide concentrations above label recommendations can still produce roots which sustain typical rhizosphere populations.

4.5 Microbial populations in soil and compost mixes.

Fungal populations were significantly higher in all compost mixes when compared with mixes of contaminated and uncontaminated soil (Figures 15 and 16). Plant growth in the soil mixes had a significant stimulatory effect on fungal populations only in the 100% contaminated soil, but not in other soil-containing mixes. Bacterial populations in compost-containing mixes were significantly greater than populations in soil mixes, with the exception of planted mixes containing 25% contaminated soil (Table 13). Plant growth had a significant effect on populations in soil mixes in only three cases (0, 6, and 12.5% contaminated soil). In most cases, bacterial populations were significantly greater in unplanted mixes containing compost than they were in soil mixes.

The generally lower microbial populations seen in planted mixes when compared to unplanted mixes is probably the result of competition for nutrients and water in the densely-rooted matrix. Whether or not similar results would be found under field conditions is unknown.

4.6 Dehydrogenase activity.

Observed dehydrogenase activity in site 14 compost mixes (Table 9) was not significantly different than expected based upon no interaction between the contaminated soil and compost, a result that indicates that the soil was not toxic to microbes. This result, obtained at the end of the incubation period, contrasts with the inhibition of microbial activity seen in the glucose utilization tests (Table 3). In contrast, dehydrogenase values were lower in Site 20A compost mixes than would be predicted on the basis of no interaction between contaminated soil and compost. For example, the predicted activity in mixes containing 25% contaminated soil + 75% compost would be about 1100 µmol product g⁻¹ mix. This value was obtained by formula:

\[
\text{Total activity} = (\text{Activity of soil} \times \text{fraction of soil}) + (\text{Activity of compost} \times \text{fraction of compost}).
\]

The expected value for 25% contaminated soil with plants would be:

\[
\text{Total activity} = (18 \times 0.25) + (1464 \times 0.75) = 1102 \mu\text{mol product.}
\]

The observed activity was approximately one-half of the predicted activity, which indicates that the contaminated soil had significant inhibitory effects on microbial activity, even though substantial degradation of analyzed pesticides was demonstrated. In spite of this inhibition, microbial activity was still 10- to 20- fold higher in compost-containing mixes than in soil mixes (Table 14). These results in combination with the microbial population studies with
site 20A material indicated that compost had a dramatic, positive impact on microbial activity in mixes containing contaminated soils.

It is not likely that the observed inhibition was due to the herbicides detected in the samples, since published work (Moorman, 1992; Moorman and Dowler, 1991; Schaffer, 1993) indicated that none of the compounds known to be present are inhibitory to microbial activity at the concentrations present in the samples. Therefore, the presence of unknown antimicrobial compounds must be suspected.

4.7 Pesticide degradation.

No single treatment was effective for stimulating degradation of all the herbicides in sites 14 and 20A material. Degradation of metolachlor was much more rapid than Felsic, et al. (1990) had found when diluting contaminated soil with uncontaminated soil; they reported only limited metolachlor degradation after 42 d of laboratory incubation, whereas we found >95% (Table 10) and about 99% degradation (Table 15) in 40 days of greenhouse incubation. Trifluralin degradation was slower in our experiments than they had reported.

It should be noted that compost also stimulated degradation of pendimethalin, whose degradation is presumed to be the result of both biotic and chemical reactions. Based on these data, compost is apparently a chemically active matrix that can accelerate abiotic degradation of some compounds.
5.0 CONCLUSIONS

Addition of compost to pesticide-contaminated soil significantly increased plant dry matter production. No attempt was made to identify specific reasons for plant growth stimulation by the compost; this phenomenon is commonly reported (Chanyasak, et al., 1983). Compost contains water-soluble trace elements, including copper, manganese, and iron (Cole, unpublished data), which may have been unavailable in the contaminated soil and it also improved soil physical properties by reducing bulk density. Adsorption of pesticides by the organic matter in compost may have reduced phytotoxicity as well. Stimulation of plant growth was observed in several treatments, primarily those containing compost. From a practical standpoint, the advantage of good growth on site is that an area that was an erosive, barren eyesore can be stabilized and rendered inconspicuous.

Microbial populations and activity are often reduced in xenobiotic-impacted soil and introduction of exogenous organisms produced in the laboratory has not been very successful. In contrast, survival of bacteria and fungi added in compost was very good. Bacterial and fungal populations in compost mixes were several-fold higher than populations in soil mixes. Although bacterial populations were lower in unplanted mixes (Table 13) when compared to 4°C storage (data not shown), a result that indicates that population declines occurred in the potted mixes, they were still higher than seen when uncontaminated soil was used as an inoculum. Fungal populations in unplanted soil (Figure 15) were similar to values for 4°C storage (data not shown), which suggests that compost is a particularly good inoculum for introduction of fungi into soil.

There was no evidence for inhibition of dehydrogenase activity with increasing content of contaminated soil in compost-containing mixes from site 14, as was seen with Site 20A material.

The results of Site 20A and Site 14 experiments were consistent in that the treatments other than compost addition did not result in significant increases in microbial activity, whereas compost addition in all cases dramatically stimulated microbial activity.

Several treatments were effective in stimulating degradation of pesticides from both Site 20A and Site 14 materials. The decrease in pesticide content is probably not a result of matrix-dependent differences in extractability, since recovery of added herbicides was quite similar among different matrices (Tables 5, 6, and 7). Since the starting material for our research was obtained from actual contaminated sites, the results strongly suggest that rapid and effective decontamination of the material can be achieved. Remediation of "aged" soils containing pesticides is expected to be more difficult than remediation of fresh spills because the bioavailability of the compounds tends to decrease with age (Schröbner, et al., 1992). The fact that herbicides of two different chemical groups (the nitroanilines, trifluralin and pendimethalin; and an acetamide, metolachlor) were degraded raises the possibility that the described procedures can be used effectively for a wide variety of pesticide contaminants.
Fungal and bacterial populations in the compost mixes were two to 20 times greater than seen in soil mixes, a result which indicates that the microorganisms added with the compost manage to successfully compete in the soil environment. This result is in contrast to results obtained with numerous microbial inoculants, the organisms from which are usually poor competitors when introduced into unsterilized soil. The larger populations in compost-amended soils strongly suggest that compost can be an effective and inexpensive microbial inoculant. Successful establishment of biocontrol fungi using compost as a inoculum has been demonstrated (Hoitink, et al., 1993) and some success with inoculants of xenobiotic-degrading white-rot fungi has been reported (Majcherczyk, et al., 1994; Morgan, et al., 1993). Use of common yard waste compost as an inoculant avoids the potential regulatory problems associated with introduction of exogenous microorganisms; there is a long history of safe use of compost as an amendment of field soil and a substantial literature exists which indicates that compost addition is beneficial to plant growth, as well as enhancing soil invertebrate populations.

The large increases in microbial activity, as measured by dehydrogenase, strongly suggest that the organisms added in the compost do not simply persist in an inactive state when added to soil, but are metabolically active and able to contribute to biodegradation processes. The increased pesticide degradation in many of the compost-containing mixes is consistent with this interpretation.

Considering all the data as a group, the most effective treatment for increasing microbial activity and accelerating pesticide degradation in samples contaminated with moderate levels of several pesticides is a combination of compost addition and planting. The use of compost has the further advantage in these cases of increasing plant biomass production, which should help to more rapidly return the formerly contaminated material to a productive state. We are currently determining the minimum amount of compost which must be added to achieve significant improvements in microbial activity, plant growth, and pesticide degradation.

It should be noted that, as a result of potential inhibition of microbial activity by contaminated soil, addition of the relatively small amounts of compost and other organic materials that are more typically used as a soil amendment (about 20 to 40 tons hectare\(^{-1}\)) is not likely to have the large beneficial effect on microbial populations and activity that addition of high rates of compost have.

Overall, the results indicate that remediation of herbicide-contaminated materials can be achieved by a mixture of compost addition and planting. Although the specific mechanisms for accelerated degradation have not been identified, the results are sufficiently encouraging to suggest that remediation of contaminated materials from agrichemical retail sites can be achieved quite rapidly and at relatively modest cost.

Considering both technical and legal issues together, remediation activities that don’t require extensive dilution or off-site transport of contaminated materials are attractive, but the
costs of many of the currently available methods exceeds the property owner’s willingness or ability to pay for clean-up. Therefore, we believe that we have developed a possible method by which the contaminated soils could be remediated quickly, inexpensively, and without removing contaminated materials from the facility.
6.0 RECOMMENDATIONS

Land-farming of pesticide-contaminated soils has been advocated as a viable and inexpensive option in cases where the total pesticide content is not too high and where a herbicide-tolerant crop can be identified (Felsot, et al., 1990; Goetsch and Kirbach, 1993). When appropriate, this method is the least expensive remedial option. However, highly contaminated soils require a large land application area to adequately dilute the pesticide to normal application rates and such areas may be difficult to find. If the soils are contaminated with a herbicide mixture that is effective against both broad-leaf and grassy plant species, land application is not feasible. In situations where direct land application is not feasible, treatment of the contaminated material to promote biodegradation should be considered. Felsot and coworkers (1990) demonstrated that atrazine, alachlor, and trifluralin were degraded when herbicide-contaminated waste pile material was diluted with uncontaminated soil. In this report, we demonstrated that trifluralin, metolachlor and pendimethalin are degraded when contaminated material is treated by a combination of planting and compost addition. Typical costs for methods involving dilution with soil or compost would be about $50.00 per ton of material treated, which is more costly than land application, but much less expensive than other remedial options. Treatment of herbicide-contaminated soil on site and without extensive dilution (as occurs with land-farming) also has the advantage of minimizing potential legal liability resulting from inadvertent release of soil contaminated with RCRA-regulated compounds such as phthalate esters.

Because of variations in bioavailability and extent of degradation of compounds in complex mixtures, conducting a laboratory- or greenhouse-scale treatability study is highly recommended prior to using biodegradation under field conditions.
7.0 REFERENCES


