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Bioremediating Herbicide-Contaminated Soils

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ABSTRACT

Combinations of landfarming and biostimulation were evaluated for remediating pesticide wastes. Various amounts of soil contaminated with alachlor and trifluralin (≥100 mg/kg each) and metolachlor and atrazine (≥20 mg/kg each) were applied to field plots, and sewage sludge or corn meal was incorporated into designated plots. Plots were also treated with fresh spray mixtures in amounts similar to those applied as contaminated soil. Soil bioactivity and dissipation of parent herbicides were monitored after the treatments. During 100 d, soil dehydrogenase activities were highest in organic-material-amended plots. During the same period, the levels of alachlor had declined by 85–95% in amended, contaminated soil-treated plots and by 75–85% in corresponding unamended plots. In freshly sprayed plots, 95–100% of the initial doses of alachlor had dissipated in amended plots, and 85–95% was lost in corresponding unamended plots. The levels of trifluralin had declined by 70–80% in corn-meal-amended plots and by 60–75% in unamended plots. There were no significant differences between dissipation of trifluralin applied as contaminated soil or fresh sprays.

Index Entries: Bioremediation; biostimulation; land farming; contaminated soil; herbicides.

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INTRODUCTION

Soil contamination with high concentrations of pesticides through improper product handling or waste disposal, or through accidents, is a major concern at many agrichemical facilities. High concentrations of many ordinarily biodegradable pesticides are more persistent and more mobile in soils than low concentrations (*1–5*). The combination of prolonged persistence and greater mobility increases the risk of surface or groundwater contamination by high pesticide concentrations, and emphasizes the need for their expeditious cleanup.

The usual methods of disposing of waste-contaminated soils are excavation, and subsequent landfilling or incineration. These methods are expensive, but they usually represent only waste translocation from one site to another or the conversion of wastes into other forms, without the problem of contaminant detoxification being fully addressed. As more contaminated sites are discovered, it is becoming increasingly important to seek alternative cleanup technologies that result in permanent waste reduction and are duly cognizant of the widespread nature of the waste problems.

Bioremediation—the controlled use of microbiological systems to detoxify waste—is emerging as a cost-effective and environmentally more desirable alternative to traditional cleanup methods. The utility of two common bioremediation strategies, viz. landfarming and biostimulation for decontaminating herbicide wastes in soils, was previously evaluated. These studies revealed a number of limitations when these strategies were applied independently. For example, aged residues in contaminated soil tend to be very persistent even after landfarming. Also, landfarming of material containing mixtures of herbicides may be limited by problems with crop phytotoxicity (*8*). In the laboratory, it was observed that the use of various organic amendments accelerated the cometabolic degradation of alachlor and metolachlor that were freshly added to soil (*4,7,8*). However, the same amendments produced only marginal increases in the degradation of herbicide residues that had aged in soil (*9*). Another study, however, showed that aged alachlor and metolachlor residues degraded rapidly when they were diluted 10-fold with fresh soil and also amended with corn meal. Soil dilution alone, or amending the undiluted, contaminated soils with organic material, produced only small increases in dissipation of the herbicides compared to the dissipation in corresponding undiluted and unamended controls. The results suggested that a combination of landfarming, which the soil dilutions simulated, and biostimulation may provide an effective means for detoxifying certain herbicide contaminants in soil. This article is a report on a study in which the combined strategy of landfarming and biostimulation to bioremediate soils contaminated with a mixture of alachlor, trifluralin, metolachlor, and atrazine was assessed.

METHODOLOGY

Herbicide-Contaminated Soil

During April 1990, water used to fight a fire at a pesticide warehouse in Lexington, IL, flooded soil surrounding the building, and deposited high concentrations of trifluralin and lesser concentrations of atrazine, alachlor, and metolachlor. The soil was excavated and stored at a farm, where it was eventually disposed of by landfarming during August 1990. Analysis of 18 individual cores (5 cm diameter × 10 cm deep) just prior to landfarming showed that trifluralin was the primary constituent (range 3–1003 mg/kg on an oven-dry soil [ods] basis). Approximately 22 t of this waste soil were transported to the University of Illinois Cruse Farm during August. The soil was covered with a thick (6 mil) black plastic sheet and stored through the winter. During March 1991, six cores (5 cm diameter × 10 cm deep) were collected randomly from the pile. The following concentrations were found: 118 ± 58 mg/kg trifluralin, 18 ± 4 mg/kg metolachlor, 1 ± 1 mg/kg atrazine, and 1 ± 1 mg/kg alachlor.

Because we were interested in landfarming high concentrations of a greater diversity of herbicides, a simulated spill of alachlor was created on April 7, 1991 by pouring a 9.5-L jug of Lasso 4E (480 g alachlor/L) in 30-cm deep trenches that were dug into the surface of the pile; the trenches were then filled with soil. On April 25, 1991, 1.4 L of Aatrex 4F (480 g atrazine/L) was spilled on the surface of the pile, and the soil was overturned with a spade. On April 26, 1991, a front loader mixed the pile of contaminated soil by completely overturning it in one direction and then overturning it a second time in a direction perpendicular to the first. The soil was then piled about 0.6–0.9 m high within a 7.6 × 3.0 m area. On May 31, 10 cores (5 cm diameter × 15 cm deep) were collected along a diagonal transect laid across the surface of the pile, and 10 cores (15 cm deep) were collected at a depth of 30–40 cm. Herbicide concentrations in individual cores averaged 172 ± 99 mg/kg alachlor, 99 ± 53 mg/kg trifluralin, 18 ± 9 mg/kg metolachlor, and 14 ± 11 mg/kg atrazine.

Experimental Design

A plot measuring approx 4.5 × 30 m was moldboard plowed and harrowed several times to provide a well-prepared bed. The plot was divided into three blocks, and 21 fabricated metal barriers measuring 1 × 1 × 0.15 m were laid out in each block. The metal barriers were pounded into the soil to depths of approx 0.1 m. The 1-m^2 subplots were bordered by buffer zones that measured 0.46 m within and 0.6 m between blocks.

The persistence of herbicides applied as contaminated soil or fresh sprays were monitored in subplots that were either left unamended, or amended with 5% (w/w) corn meal or 2.5% (w/w) sewage sludge. Percentages were based on the weight of 7.6 cm depth of soil. Contaminated soil

was applied to simulate the agronomic rate of alachlor application (1X = 4.49 kg ai/ha), 5X the recommended alachlor rate (22.5 kg ai/ha) and 10X the recommended alachlor rate (45 kg ai/ha). The fresh spray mixture consisted of alachlor, trifluralin, metolachlor, and atrazine in the same proportions as that determined in analysis of the waste contaminated soil (172, 99, 18, and 14 mg ai/kg ods, respectively).

Immediately after application, the contaminated soil or fresh spray mixtures were thoroughly incorporated into 7.6 cm depth of soil by raking. Sewage sludge or corn meal was similarly incorporated into the soil. Within 2–3 h after the treatments, soil samples were taken from the plots to be analyzed for initial herbicide concentrations and soil bioactivity. One week after the treatments, each plot was planted with two rows of corn spaced at approx 18 cm within and 30 cm between rows. The buffer zones were seeded with grass.

Soil Sampling, Preparation, and Storage

Soils were collected from the plots with a 5-cm diameter bucket auger. Three samples of soil from depths of 0–7.6 cm were collected from each plot within a block for analysis. To prevent cross-contamination, the auger was washed with acetone between subsamples taken from different treatments. Soils were sieved through a 3-mm mesh screen. Subsamples of soil from each plot were combined in Whirl-Pak bags and stored at 2°C for analysis for soil dehydrogenase activities. The individual samples were frozen at −20°C until analyzed separately for herbicide residues.

Soil Extraction and Analysis

Thirty grams of soil were slurried with 15 mL of distilled water and extracted three times each by stirring with 60 mL of ethyl acetate on a magnetic stirrer. The extracts were evaporated to dryness on rotoevaporators, and the residues were resuspended in ethyl acetate for analysis. All herbicides were qualitatively analyzed by packed-column gas–liquid chromatography (GLC, Packard Model 328) with nitrogen-phosphorus specific detection. Residues were separated isothermally at 190°C on a 90 cm X 0.2 mm id glass column packed with 5% Apiezon+0.1% DEGS. Residues were quantified by the method of external standards, which was used to calibrate the GLC response each day of analysis.

Measurement of Soil Biostimulation

Soil dehydrogenase activities were measured as an indicator of soil biostimulation. Dehydrogenase activities in the soils were measured as the amount of triphenylformazan (TPF) formed after incubation of soils with triphenyltetrazolium chloride (*10*). The results are reported as averages of triplicate determinations of combined samples from each plot.

Table 1
Dehydrogenase Activities in Field Plots Following Land Farming and Biostimulation

Herbicide treatment			Soil dehydrogenase activity, μg TPF/g od × 10, after day[b]			
Type	Level[a]	Amendment	0	16	30	60
None	0X	None	7.1 (1.0)	6.9 (0.5)	5.6 (1.9)	9.1 (2.3)
		Sewage sludge	41.0 (29.0)	54.1 (0.8)*	28.1 (0.6)*	53.4 (12.3)*
		Corn meal	25.3 (7.9)	12.5 (7.3)	31.1 (15.7)*	67.6 (12.9)*
Soil	1X	None	5.9 (0.8)	7.3 (0.6)	6.1 (1.9)	9.1 (1.4)
		Sewage sludge	60.1 (5.4)*	50.8 (11.8)*	36.3 (10.2)*	39.3 (6.0)*
		Corn meal	20.2 (3.6)*	12.0 (4.1)	34.0 (12.7)*	60.2 (0.8)*
	10X	None	10.1 (1.5)	11.1 (0.8)	15.4 (6.9)	21.1 (1.7)
		Sewage sludge	37.0 (3.7)*	41.6 (4.7)*	27.6 (4.5)	35.8 (12.9)
		Corn meal	34.8 (7.8)*	9.4 (0.8)	33.5 (9.1)*	49.9 (2.5)*
Spray	1X	None	10.4 (2.7)	0.9 (1.4)	6.3 (3.1)	11.3 (1.9)
		Sewage sludge	65.3 (7.2)*	45.3 (14.8)*	34.1 (9.1)*	43.5 (9.8)*
		Corn meal	20.7 (3.1)	13.4 (3.3)	30.8 (4.0)*	57.4 (15.1)*
	10X	None	8.2 (2.9)	6.2 (3.1)	6.1 (3.0)	23.4 (15.3)
		Sewage sludge	35.3 (13.0)*	47.9 (1.8)*	28.4 (1.8)*	42.6 (20.6)
		Corn meal	22.1 (8.2)	14.4 (5.1)	21.9 (12.5)*	44.5 (2.7)*

[a] *See text* for treatment level designations.
[b] Values are means of nine determinations followed by standard deviation in parentheses; means with asterisks are significantly different from their corresponding control (none) treatments according to Duncan's multiple-range test ($p = 0.05$).

Statistical Analyses

Herbicide residue and soil bioactivity data were statistically analyzed using the SAS General Linear Means (GLM) Procedure (*11*). Differences between means within treatment groups were tested for significance using the Duncan's multiple-range test.

RESULTS AND DISCUSSION

Dehydrogenase Activities During Landfarming and Biostimulation

Soil dehydrogenase activities were measured as the indicator of the effect of herbicide application and organic amendment on soil bioactivity. In several of our field and laboratory studies, the activity of this enzyme was most sensitive to high herbicide concentrations, and its depression was most consistently associated with prolonged persistence of herbicides in soil (*4,5*). Within 16 d after treatment, dehydrogenase activities were significantly higher ($p = 0.05$) in soils from sewage-sludge-amended plots than in soils from corresponding unamended plots. (Table 1: only data for

Table 2
Recovery of Alachlor 100 D after Land Farming and Biostimulation

Treatment level[a]	Amendment	Percent of initial recovery from plots treated with[b]	
		Contaminated soil	Spray mixture
1X	None	13.8 (5.6)	4.7 (2.4)
	Sewage sludge	13.3 (2.2)	0.0 (0.0)*
	Corn meal	14.9 (7.8)	0.0 (0.0)*
5X	None	27.7 (10.2)	10.2 (5.1)
	Sewage sludge	16.8 (5.2)	1.2 (0.7)*
	Corn meal	5.1 (0.1)*	1.5 (0.9)*
10X	None	20.6 (2.0)	16.8 (11.0)
	Sewage sludge	11.4 (2.8)	6.9 (4.4)
	Corn meal	10.8 (6.6)	0.9 (0.5)

[a] *See text* for treatment level designations.
[b] Values are means of nine determinations per treatment followed by standard deviations in parentheses; means with asterisks are significantly different from their corresponding control (none) treatments according to Duncan's multiple-range test ($p = 0.05$).

1X and 10X treatments are shown). During the same period, dehydrogenase activities in corn meal-amended soils were generally higher than those in corresponding unamended soils, but they were lower than those in sewage sludge-amended soils. By day 30, dehydrogenase activities in sewage sludge-amended plots had declined slightly, but they had increased significantly in corn-meal-amended plots. Bioactivity in the amended plots remained elevated over the following 70 d (data for 100 d not shown). Neither the type nor level of herbicide application had any consistent influence on the levels of dehydrogenase activities in the treatments (Table 1).

Herbicide Recoveries from Land Farmed, Biostimulated Plots

Herbicide recoveries from soil during 100 d after various treatments are shown in Tables 2–4. The results are presented as means of nine determinations per treatment expressed as percentages of zero day recoveries. Dissipation of alachlor and metolachlor, which are both acetanilide herbicides, followed a similar pattern, so, for brevity, only results of alachlor dissipation are shown. After 100 d, 13–27% of the initial doses of alachlor were recovered from contaminated soil-treated plots that did not receive organic amendments, and about 5–17% of the initial doses was recovered from corresponding freshly sprayed plots (Table 2). Mean alachlor recoveries from freshly sprayed, amended plots were consistently lower than the recoveries from corresponding unamended soils; the differences at the 1X and 5X application levels were statistically significant ($p = 0.05$).

Table 3
Recovery of Trifluralin 100 D after Land Farming and Biostimulation

Treatment level[a]	Amendment	Percent of initial recovery from plots treated with[b]	
		Contaminated soil	Spray mixture
1X	None	38.1 (9.8)	40.5 (24.8)
	Sewage sludge	98.5 (86.9)	62.5 (39.8)
	Corn meal	11.4 (4.6)	12.9 (6.7)
5X	None	37.2 (18.0)	25.3 (12.5)
	Sewage sludge	67.7 (28.6)	48.0 (30.7)
	Corn meal	12.0 (2.18)	13.8 (0.6)
10X	None	36.7 (11.3)	33.2 (16.2)
	Sewage sludge	57.5 (15.9)	38.8 (7.9)
	Corn meal	28.0 (21.1)	30.3 (4.0)

[a] *See text* for treatment level designations.
[b] Values are means of nine determinations per treatment followed by standard deviations in parentheses; means within treatment levels are not statistically different from their corresponding control (none) treatments according to Duncan's multiple-range test ($p = 0.05$).

Table 4
Recovery of Atrazine 100 D after Land Farming and Biostimulation

Treatment level[a]	Amendment	Percent of initial recovery from plots treated with[b]	
		Contaminated soil	Spray mixture
1X	None	72.9 (63.6)	44.2 (2.4)
	Sewage sludge	35.5 (36.5)	15.1 (8.4)*
	Corn meal	75.4 (48.4)	7.1 (10.1)*
5X	None	38.0 (7.4)	19.7 (8.6)
	Sewage sludge	35.9 (20.3)	16.6 (3.0)
	Corn meal	41.1 (20.1)	9.3 (1.0)
10X	None	43.3 (14.1)	14.4 (0.8)
	Sewage sludge	20.4 (12.4)	11.9 (5.7)
	Corn meal	16.8 (5.3)	6.9 (0.9)

[a] *See text* for treatment level designations.
[b] Values are means of nine determinations per treatment followed by standard deviations in parentheses; means with asterisks are significantly different from their corresponding control (none) treatments according to Duncan's multiple-range test ($p = 0.05$).

Lower amounts of alachlor were recovered from several amended, contaminated soil-treated plots than from unamended plots, but the differences were generally not statistically significant (Table 2). Within the range tested, level of application did not significantly affect the rates of dissipation in unamended plots after 100 d.

The above observations were similar to those previously reported (*4–9*). First, herbicide recoveries from soil were quite variable, which may be because of sampling error, compounded by herbicide "segregation" (*12*). The high variabilities prevented detection of significant differences between several treatment comparisons. Second, consistently higher amounts of alachlor (and metolachlor) were recovered from contaminated soil-treated plots than in freshly sprayed plots. This suggested that the residues had "aged" in the contaminated soil, and had become less desorbable (*13–15*) and consequently less readily available for biodegradation (*14–16*) than the fresh applications. Finally, soil amendment with organic material greatly stimulated the dissipation of fresh applications of alachlor (and metolachlor), but had only limited effects on aged residues.

Results of trifluralin dissipation from field plots are shown in Table 3. About 38% of the initial doses of trifluralin was recovered in soil from unamended, contaminated soil-treated plots; corresponding recoveries from freshly sprayed plots ranged between 25–40% of the initial doses. Numerically lower amounts of trifluralin were recovered from corn meal-amended plots than from unamended ones, but average recoveries from sewage sludge-amended plots were consistently higher than recoveries from unamended plots. In a recent unpublished laboratory study, retardation of trifluralin dissipation from unsaturated, sewage sludge-amended soil was observed, whereas in saturated soil, sewage sludge (and other organic amendments) greatly stimulated biotransformation of the herbicide. It has not been determined whether the slower dissipation of trifluralin in unsaturated soil was owing to sorption of the herbicide to sewage sludge, which in turn slowed down its loss by other mechanisms. Those initial results, however, suggested that appropriate amendment and soil moisture management may provide a means for rapid biotransformation of trifluralin in contaminated soils.

Atrazine dissipation from soil during land farming and biostimulation followed a pattern that was somewhat similar to that of alachlor (Table 4). Higher amounts of atrazine were recovered from contaminated soil-treated plots than from corresponding freshly sprayed plots. Also, soil amendment with organic material, especially corn meal in this case, appeared to stimulate the dissipation of freshly sprayed atrazine, whereas the amendments did not significantly affect the dissipation of aged residues. Like all the other herbicides, the variabilities in atrazine recoveries were quite high, thereby preventing detection of statistically significant differences between several treatment means.

CONCLUSIONS

The results of our study suggest that combinations of land farming and biostimulation with organic amendments may be used to decontaminate certain herbicide wastes in soil effectively. During 100 d in the field,

the levels of alachlor, which is one of the major contaminants examined, had declined by 75–85% in unamended, contaminated soil-treated plots and by 85–95% in corresponding plots that were amended with organic material. Freshly sprayed alachlor dissipated faster than applications as contaminated soil, especially in amended plots that received 1X and 5X herbicide treatments. This underscores the need for expeditiousness in implementing bioremedial action, while contaminants are still "fresh" in soil. The levels of trifluralin, which is the other major contaminant examined, had declined by 60% in unamended plots and by 70–85% in corn meal-amended plots during 100 d of land farming and biostimulation. In the studies reported here and in unpublished laboratory results, it was observed that trifluralin dissipation appeared to be retarded in unsaturated, sewage sludge-amended soils. The laboratory studies also showed, however, that the same amendment greatly stimulated dissipation of the herbicide under soil saturation conditions. This suggested that landfarming and biostimulation to detoxify wastes should be accompanied by aggressive management practices to create physicochemical conditions as may be necessary to enhance the biodegradation of different groups of chemicals in contaminated soil. Several different factors must interact in specific ways for biodegradation of organic contaminants to proceed at desired rates. This emphasizes the need for laboratory studies to understand biodegradation factors and how they may be manipulated to maximize bioremediation.

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