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Organic Amendments to Enhance Herbicide Biodegradation in Contaminated Soils

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Abstract
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Keywords
Bioremediation, Pesticides, Atrazine, Metolachlor, Trifluralin

Disciplines
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Introduction

Accidental spills during mixing and loading of pesticides have resulted in the contamination of soil and water at agricultural chemical dealerships in the USA. Approximately 90% of the dealerships in Iowa have some degree of detectable groundwater contamination, with up to half of the dealerships having contamination exceeding the state's cleanup guidelines (Gannon 1992). In several midwestern states and Canadian provinces, the occurrence of herbicide-contaminated wells corresponds to the presence of agricultural chemical dealership sites (Fawcett 1989; Frank et al. 1987a, 1987b). This was also found in a study in Iowa, where 16 of the 18 public water-system wells which were contaminated with pesticides were within 1,000 feet (305 m) of an agricultural chemical dealership (Frieberg 1991). The Iowa Fertilizer and Chemical Association estimated that in Iowa alone, it could cost between $50 and $100 million to assess, monitor, and remediate the contaminated dealerships (Gannon 1992). With these costs increasing in the future, it is important to conduct research on potentially economical and efficient strategies to remediate these sites.

Generally, multiple herbicide residues are present in soils at agricultural chemical dealerships. Previous studies have reported that herbicides are more persistent in high concentrations, and organic amendments often stimulate biodegradation (Schoen and Winterlin 1987; Winterlin et al. 1989; Dzantor et al. 1993). In contrast, some amendments and mineral N have reduced biodegradation (Alvey and Crowley 1995; Gan et al. 1996). Few studies have

focused on the interactive effects of multiple herbicides at high concentrations (>100 mg kg^{-1} soil) on microorganisms and herbicide persistence. Herbicides can also be toxic to the microbial populations in soil contaminated with high levels of pesticides (Dzantor and Felsot 1991).

In this study we determined the effect of organic amendments (compost, cornstalks, sawdust, manure, peat and a fermentation byproduct) on the biodegradation of atrazine [6-chloro-*N*-ethyl-*N'*-(1-methyethyl)-1,3,5-triazine-2,4-diamine], metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide], and trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine] applied either individually or in combination. Atrazine, metolachlor and trifluralin have been widely used in the corn- and soybean-growing area of the United States and elsewhere, and are present as contaminants at agrichemical dealership sites. High concentrations of herbicides are often found on small areas of the dealership sites. The experiments address the bioremediation of these herbicides at relatively high concentrations with simple, low-cost techniques that would be suitable for on-site use. The amendments used in this study were chosen because of their low cost and wide availability.

Materials and methods

Soil taken from the surface 5 cm of an Iowa agricultural chemical dealership (Bravo) contained 78% sand, 18% silt, and 4% clay with 2.4% organic C, 0.05% total N; and pH of 6.5. Grass and garden waste municipal compost, sawdust, aged cattle manure, peat, a corn fermentation byproduct (CFB), and cornstalks were used as amendments. All amendments except the CFB were ground and passed through a 4-mm-mesh sieve prior to use. The CFB was a slurry with the following characteristics: 40% solids; 6.9% total N; 0.52% P_2O_5; a density of 1.19; and a pH of 4.7. In the first biostimulation experiment, aged herbicide residues were treated with amendments added at 0.5% and 5% (w/w). Herbicide-treated soil was aged for 63 days at 25°C in darkness prior to addition of the amendments in the first biostimulation experiment. A solution of NH_4NO_3, $CaSO_4$ and K_2HPO_4 (10:1:1:1 ratio of N:P:K:S) was added to all treatments, providing 50 mg N kg^{-1} soil. The C/N ratios of the amended soil, including inorganic nutrients, were between 10 and 50, except for the fermentation byproduct (C/N of 1.6 at the 5% rate) and the sawdust (C/N of 225 at the 5% rate). Atrazine, 92% purity, and metolachlor, 97% purity, were obtained from Novartis, and trifluralin 99% purity was obtained from Chem Services (West Chester, Pa.). Herbicides were applied as concentrated methanol or acetone solutions to 50 g soil in 0.45-l jars. Solvent was allowed to evaporate for 30 min and the soil was stirred to mix the herbicide. Three replicates were prepared for each treatment. Moisture content was adjusted to 10% and jars were opened periodically during incubations for aeration and moisture adjustment.

A second biostimulation experiment was conducted to determine the interactive effects of herbicides applied as a mixture compared to the herbicides applied individually. In this herbicide mixture experiment, only sawdust or manure (5% w/w) and inorganic nutrients were added. Soil was treated with either a mixture containing 200 mg kg^{-1} each of atrazine, metolachlor, and trifluralin or 200 mg kg^{-1} of each individual herbicide. The treated soil was mixed and amendments were added immediately.

In both experiments, herbicide degradation was compared to that obtained in nonamended soil. Herbicide residues in 25-g soil samples were measured after extraction with ethyl acetate by gas chromatography as described previously (Anhalt et al. 2000). Bacteria and actinomycetes were enumerated by dilution plating (10-g soil samples) in dilute sterile phosphate buffer on 10% tryptic soy agar containing 50 mg cyclohexamide l^{-1}. Fungi were enumerated on rose bengal agar containing 50 mg streptomycin l^{-1}. Culturable populations were used to provide a direct measure of viable populations in amended soils. Respiration was measured as an instantaneous rate by opening the jars, resealing the jars with a closure connected to a CO_2 analyzer (pump with IR analyzer; CID, Vancouver, WA.), and measuring the CO_2 accumulation over 10 min. Respiration results are expressed as a percent of the nonamended, herbicide-free soil. Dehydrogenase was measured as described by Cambardella et al. (1994).

A third experiment was conducted to determine how high concentrations of herbicide combinations affect the atrazine mineralization in the Bravo soil. Fifty-gram samples of soil were treated with [^{14}C-UL-*ring*]-atrazine at concentrations of 5 mg kg^{-1} soil or 200 mg kg^{-1} soil. Metolachlor and trifluralin were added separately or together at 200 mg kg^{-1} soil (each herbicide) to the atrazine-treated soil. In addition, a treatment consisting of metolachlor and trifluralin at 200 mg kg^{-1} soil (each) and atrazine at 5 mg kg^{-1} soil was prepared. The amounts of ^{14}C used, $^{14}CO_2$ trapping, and liquid scintillation counting procedures were similar to those used previously (Struthers et al. 1998). After aerobic incubation for 63 days, soils were amended with 5% (w/w) cornstalk, and enough water was added to flood the soil and cornstalks. The headspace was sparged with N_2 gas and sealed. On day 98, the water was removed, the soil was stirred, and the incubation was continued for an additional 98 days. Atrazine-degrader populations were measured by the ^{14}C-most probable number method (Jayachandran et al. 1998) and other microbial populations were measured by dilution plating of 10-g soil samples.

Results and discussion

Initial mean concentrations were 175±42 mg atrazine kg^{-1} soil, 182±25 mg metolachlor kg^{-1} soil, and 165±23 mg trifluralin kg^{-1} soil. The 63-day incubation period prior to amendment was designed to produce aged residues. During this time the herbicides presumably became less available to microorganisms through sorption and precipitation processes. Relatively little herbicide degradation occurred prior to amendment addition (data not shown). Herbicide residues declined after amendment addition, but the variability associated with the data and the relatively small amount of degradation preclude meaningful estimates of DT_{50} or half-life. In addition, previous studies have shown that half-lives of alachlor and atrazine were concentration dependent, suggesting deviation from first-order kinetics (Gan et al. 1995, 1996). Therefore, we present only the final herbicide concentrations present in soil for the main treatments.

By the end of the 245-day experiment, including the 182 days after amendment additions, 30% of the atrazine, 33% of the metolachlor, and 44% of the trifluralin was degraded in nonamended soil. Atrazine degradation was enhanced by the 0.5% manure, 5% peat and 5% cornstalk amendments over that in nonamended soils (Fig. 1). Metolachlor degradation was enhanced by the 5% level of sawdust, manure, CFB and cornstalk amendments (Fig. 1), but the 0.5% level of these amendments was less effective. Similar enhancements of alachlor degradation were obtained with corn and soybean stubble

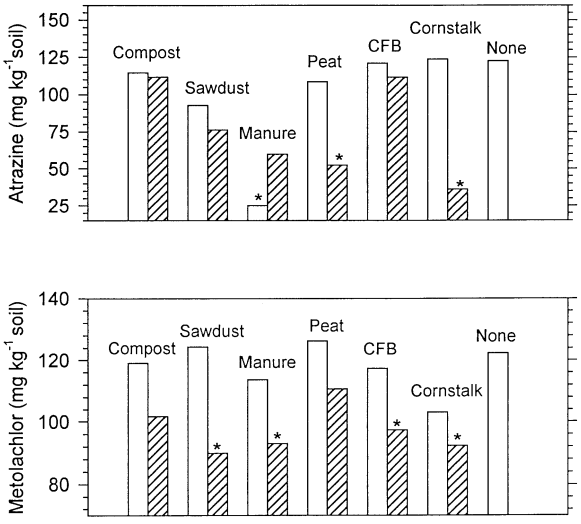

Fig. 1 Degradation of herbicides applied as a mixture and incubated for 245 days, including 182 days after amendment addition. *Shaded bars* indicate 5% (w/w) amendment and *open bars* the 0.5% rate. *Atrazine* 6-Chloro-*N*-ethyl-*N'*-(1-methyethyl)-1,3,5-triazine-2,4-diamine, *metolachlor* 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide, *CFB* corn fermentation byproduct. *Significant difference from the nonamended concentration ($P \leq 0.05$)

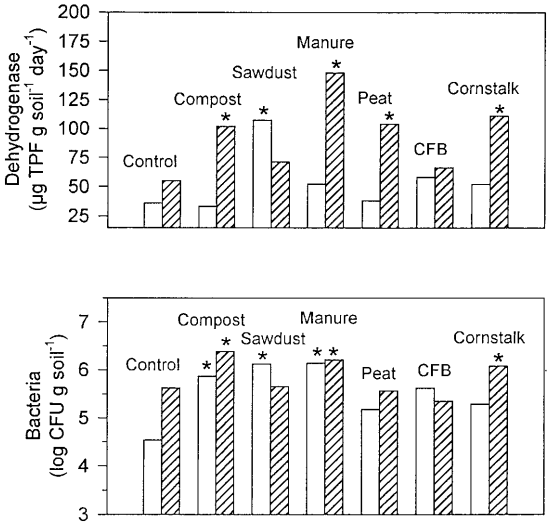

Fig. 2 Effect of organic amendments on microbial dehydrogenase activity and bacterial populations averaged over 8 sampling times. *Shaded bars* indicate 5% (w/w) amendment and *open bars* the 0.5% rate, except for the controls (no amendments). The *open bar control* indicates soil without herbicide treatment and the *shaded bar* indicates soil treated with the herbicide mixture. *TPF* Triphenylformazan, *CFU* colony forming units. *Significant difference from the untreated control ($P \leq 0.05$)

(Felsot and Dzantor 1990). None of the amendments enhanced trifluralin degradation. Inorganic nutrients alone did not stimulate degradation over that in the nonamended treatment (data not shown).

Microbial populations and activity were measured at 8 sampling times during the first experiment. Amendment effects are compared to controls: soil alone or herbicide-treated soil without amendments (Fig. 2). These two controls were not significantly different and there were no effects of the inorganic nutrients on microbial populations or activity. Amendments did not affect fungal or actinomycete populations (data not shown). The 5% rate of compost, manure and cornstalks increased both bacterial populations and stimulated dehydrogenase activity. The 0.5% compost, manure, and sawdust amendments increased bacterial populations, but compost and manure did not increase dehydrogenase activity. The 0.5% sawdust treatment stimulated dehydrogenase activity. The 5% rate of amendments generally increased respiration, but the differences between amended and nonamended soils were not statistically significant (data not shown). The 5% level of sawdust was less stimulatory to microorganisms than the 0.5% level, which may reflect the influence of the higher C/N ratio at the 5% rate of amendment. Microbial responses to the manure and cornstalks appear to coincide with the enhanced degradation of metolachlor produced by these amendments, which is consistent with cometabolic degradation of metolachlor by a wide variety of microorganisms. Compost appeared to stimulate microorganisms, but did not enhance the degradation of herbicides.

The second biostimulation experiment examined the persistence of atrazine, metolachlor, and trifluralin alone, or in combination, in Bravo site soil. Extraction efficiencies of 89%, 68%, and 84% were measured for atrazine, metolachlor, and trifluralin, respectively, immediately after addition to the soil. Atrazine degradation was nearly complete in the nonamended soil (Table 1), which is consistent with the existence of a competent atrazine-degrading community in the Bravo site soil (Struthers et al. 1998; Anhalt et al. 2000). Manure stimulated atrazine degradation in the soil treated with the herbicide mixture, but not to the same extent as in the first experiment. Sawdust repressed degradation of atrazine applied alone (Table 1). The amounts of trifluralin and metola-

Table 1 Effect of organic amendments on herbicide concentrations (mg kg^{-1} soil) at 189 days after addition of individual herbicides or as a mixture. Means followed by *different letters within columns* are significantly different ($P \leq 0.05$). *Atrazine* 6-Chloro-*N*-ethyl-*N'*-(1-methyethyl)-1,3,5-triazine-2,4-diamine, *metolachlor* 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide, *trifluralin* 2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine

Herbicides[a]	Amendment[b]	Atrazine	Metolachlor	Trifluralin
Single herbicide	None	12 c	120 a	84 a
	Sawdust	75 b	89 b	60 a
	Manure	5 c	70 b	87 a
Mixture	None	154 a	137 a	90 a
	Sawdust	137 a	87 b	88 a
	Manure	78 b	105 a	89 a

[a] Initial mean concentrations were 178 mg atrazine kg^{-1} soil, 135 mg metolachlor kg^{-1} soil and 168 mg trifluralin kg^{-1} soil. The mixture consisted of all three herbicides at these concentrations. Background concentrations in the Bravo site soil were ≤5 mg kg^{-1} soil

[b] All amendments were applied at the 5% rate, 7 days after herbicide addition

Table 2 Effect of organic amendments on microbial populations and activity at 189 days after addition of individual herbicides or as a mixture. Means of 6 sampling times over 182 days. Means followed by *different letters within columns* are significantly different ($P \leq 0.05$). *CFU* Colony forming units, *TPF* triphenylformazan

Herbicides[a]	Amendment[b]	Bacteria (log CFU g^{-1} soil)	Dehydrogenase activity (µg TPF g $soil^{-1}$ day^{-1})	Respiration (µg CO_2 g^{-1} soil min^{-1})
Soil	None	6.2 a	78 c	4 a
Soil (plus solvent)	None	6.9 a	40 b	6 a
Atrazine	None	6.2 a	72 c	3 a
	Sawdust	7.6 b	35 b	12 a
	Manure	8.0 b	152 e	28 b
Metolachlor	None	6.6 a	129 d	8 a
	Sawdust	7.6 b	169 e	24 b
	Manure	7.3 b	146 e	8 a
Trifluralin	None	6.5 a	83 c	5 a
	Sawdust	8.2 b	114 d	23 b
	Manure	7.2 a	178 e	17 b
Mixture	None	6.7 a	45 b	5 a
	Sawdust	6.3 a	10 a	11 a
	Manure	7.7 b	116 d	21 b

[a] Herbicides applied as described in Table 1
[b] All amendments were applied at the 5% rate, 7 days after herbicide addition

Table 3 Microbial populations at 63 days after herbicide addition to Bravo soil. *BD* Population below detection limit of dilution plating procedure

Treatment	Atrazine degraders (Cells g^{-1} soil)	Bacteria (Log CFU g^{-1})	Actinomycetes (Log CFU g^{-1})	Fungi (Log CFU g^{-1})
Soil alone	1,950	6.4	6.1	4.8
Atrazine[a]	35,000	7.4	7.6	3.8
Atrazine plus metolachlor	340	8.3	7.1	2.3
Atrazine plus trifluralin	280,000	7.9	7.8	2.9
Atrazine, metolachlor and trifluralin	78	5.4	5.1	BD
Atrazine (5 mg kg^{-1} soil), metolachlor and trifluralin	370	8.1	7.3	3.1

[a] Unless otherwise stated, all of the herbicides were applied at a rate of 200 mg kg^{-1} soil

chlor remaining in soil after 182 days were similar to the amounts remaining at the end of the first experiment. Both sawdust and manure enhanced degradation of metolachlor when metolachlor was applied alone. Less atrazine degradation resulted ($P \leq 0.05$) when atrazine was applied in the mixture. A combined average (amended and nonamended soils) of 123 mg atrazine kg^{-1} soil remained in soil treated with the herbicide mixture while only 31 mg atrazine kg^{-1} soil remained in the soil treated with atrazine only.

In soil with a single herbicide, the sawdust and manure amendments consistently increased microbial populations and activities, but sawdust was not stimulatory to microorganisms exposed to the herbicide mixture (Table 2). These populations are shown in comparison to those in soil receiving no treatment and in soil treated with solvents, but without herbicides. The amendments were not as effective in stimulating herbicide degradation in soils receiving the herbicide mixture, but microbial populations were not significantly reduced by the herbicide mixture in comparison to the controls. Metolachlor appeared to selectively stimulate dehydrogenase activity, but not when in combination with trifluralin and atrazine.

After addition of 200 mg [^{14}C-*ring*]-atrazine kg^{-1} to Bravo soil, approximately 37% of the atrazine was metabolized to $^{14}CO_2$ after 63 days (Fig. 3). Atrazine applied in combination with trifluralin produced similar results. However, addition of 200 mg metolachlor kg^{-1} soil in combination with [^{14}C]-atrazine completely repressed atrazine mineralization (Fig. 3). The same effect was observed when all three herbicides were applied to the soil. After 14 days' incubation, 40% of the atrazine applied alone at 5 mg kg^{-1} soil was mineralized (data not shown) compared to <1% of atrazine mineralized at

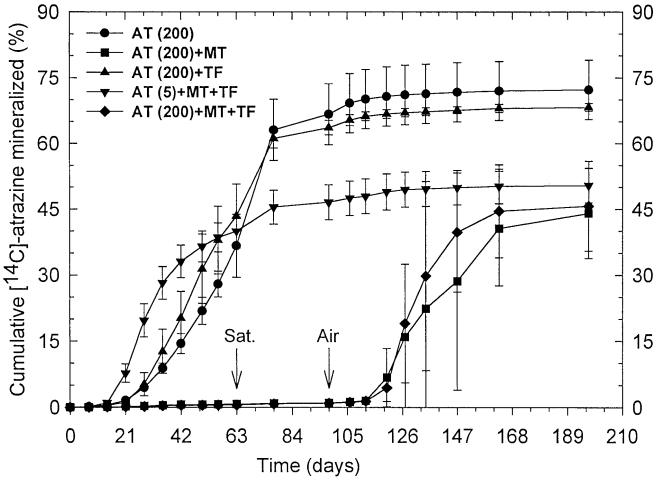

Fig. 3 Mineralization of atrazine (*AT*, 5 mg kg^{-1} soil or 200 mg kg^{-1} soil) alone or the presence of metolachlor (*MT*) and/or trifluralin (*TF*), each applied at 200 mg kg^{-1} soil expressed as means and SDs (n=3). Soil was saturated and amended with cornstalks on day 63. On day 98 the water was removed and soils were aerated

14 days in the presence of trifluralin and metolachlor, although atrazine mineralization increased subsequently. Only 1.1 mg kg^{-1} of the 200 mg atrazine kg^{-1} soil applied in the atrazine plus trifluralin and metolachlor treatment was mineralized after 63 days. In the treatment with 5 mg atrazine kg^{-1} soil (plus 200 mg trifluralin and metolachlor kg^{-1} soil), the 40% mineralization at day 63 corresponded to 2 mg atrazine kg^{-1} soil mineralized. This shows that the inhibitory effect is not dependent upon the atrazine concentration. Atrazine-degrading microorganisms increased in soil treated with 200 mg atrazine kg^{-1}, but did not increase in the soils treated with metolachlor (Table 3). Water saturation and amendment of the soil with cornstalks from day 63 to day 98 subsequently increased mineralization rates in soils treated with atrazine and metolachlor. Approximately 4% of the applied metolachlor was removed with the water; thus, removal of the water is not the primary mechanism of herbicide loss. The anaerobic conditions induced by addition of the water and cornstalks appeared to reduce the metolachlor-induced repression of atrazine degradation.

Our results show the complexity of bioremediating herbicide mixtures. Cornstalks and manure were the amendments that were most consistent in the stimulation of microorganisms and in enhancing herbicide degradation, but remediation was incomplete in the time-frame of the study. Longer incubations would likely have resulted in additional degradation, although the rate of degradation is likely to decline over time. The fact that some amendments (e.g., compost, sawdust) stimulated general microbial populations and activity without concurrent increases in herbicide degradation suggests that the specific microbial populations responsible for degrading the contaminants were not stimulated. The failure to stimulate trifluralin degradation is consistent with our observation of high dinitroaniline concentrations at pesticide distribution sites, and suggests that other factors, such as solubility or adsorption, may limit dinitroaniline degradation. Trifluralin has a water solubility of 0.3 mg l^{-1} compared to the 32 mg l^{-1} and 540 mg l^{-1} solubilities of atrazine and metolachlor, respectively. Our results with trifluralin are in contrast to those observed by Dzantor et al. (1993), who observed enhanced degradation of trifluralin (5% w/w) in corn-meal amended soil. Oat plant root bioassays (data not shown) show that these residues are sufficiently bioavailable to limit establishment of sensitive plants.

High concentrations of metolachlor selectively inhibited atrazine biodegradation, but the exact mechanism of this inhibition is unknown. Concentrations of atrazine and metolachlor applied at 50 mg kg^{-1} soil as a mixture did not have an inhibitory effect on atrazine degradation (Anhalt et al. 2000). The effect of metolachlor appears to be directed at the atrazine-degrading microorganisms primarily, rather than at general microbial populations.

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