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Abstract

Soils at agrochemical dealer sites often are contaminated with pesticide residues from decades of accidental and incidental spillage. We have determined that prairie grasses native to the Midwestern U.S. are suitable for phytoremediation because they are tolerant of most herbicides and of climatic extremes, such as heat, cold, drought, and flooding. A mixed stand of big bluestem, switch grass, and yellow indiagrass develops a rhizosphere with microflora that can readily detoxify pesticide residues. Specific atrazine-degrading bacteria or the free enzyme atrazine chlorohydrolase also can enhance the rate of biotransformation of atrazine in soil. Metolachlor degradation can be accelerated significantly by the prairie grass/rhizosphere effect. Several grasses used in filter strips have also been evaluated for their pesticide degradation capabilities. The prairie grasses also have been demonstrated to reduce the rates of leaching of pesticides through intact soil columns, since less water leaches out of vegetated soil columns compared to non-vegetated soil columns. The evaluation of the degree of success of remediation has relied heavily on chemical residue analysis, but recent studies on biological endpoints have shown promise for providing more ecologically relevant indications of the potential exposure of organisms to pesticides in the soil. Earthworm 8-day bioaccumulation assays and root growth assays have shown the value of assessing the bioavailability of the residues. Mass balance experiments have utilized radiolabeled atrazine and metolachlor to ascertain the complete metabolism and binding profile of those two pesticides in phytoremediation studies.

Keywords

Phytoremediation, Pesticides, Bioaugmentation

Disciplines

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Comments

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Phytoremediation of Pesticide Wastes in Soil

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Soils at agrochemical dealer sites often are contaminated with pesticide residues from decades of accidental and incidental spillage. We have determined that prairie grasses native to the Midwestern U.S. are suitable for phytoremediation because they are tolerant of most herbicides and of climatic extremes, such as heat, cold, drought, and flooding. A mixed stand of big bluestem, switch grass, and yellow indiagrass develops a rhizosphere with microflora that can readily detoxify pesticide residues. Specific atrazine-degrading bacteria or the free enzyme atrazine chlorohydrolase also can enhance the rate of biotransformation of atrazine in soil. Metolachlor degradation can be accelerated significantly by the prairie grass/rhizosphere effect. Several grasses used in filter strips have also been evaluated for their pesticide-degradation capabilities. The prairie grasses also have been demonstrated to reduce the rates of leaching of pesticides through intact soil columns, since less water leaches out of vegetated soil columns compared to non-vegetated soil columns. The evaluation of the degree of success of remediation has relied heavily on chemical residue analysis, but recent studies on biological endpoints have shown promise for providing more ecologically relevant indications of the potential exposure of organisms to pesticides in the soil. Earthworm 8-day bioaccumulation assays and root growth assays have shown the value of assessing the bioavailability of the residues. Mass balance experiments have utilized radiolabeled atrazine and metolachlor to ascertain the complete metabolism and binding profile of those two pesticides in phytoremediation studies.

Key words: Phytoremediation, Pesticides, Bioaugmentation

Introduction

Pesticides are commonly used throughout agricultural regions of the United States for the control of fungi, weeds, and insect pests. There is concern over pesticides as major contaminants of U.S. surface and ground water supplies, resulting from runoff or leaching after intentional application or accidental spillage. United States Geological Survey data indicate that over 90% of streams and 50% of wells sampled are contaminated with one or more pesticides (U.S. Geological Survey, 1999). Atrazine (a triazine), metolachlor (a chloracetanilide), and pendimethalin (a dinitroaniline) are three herbicides commonly used to control broad-leaf weeds and annual grasses in the row crop agricultural systems in the U.S. Usage estimates according to the U.S. Environmental Protection Agency list annual usage of atrazine at approx. 35 million kg active ingredient (a.i.), and of metolachlor at approx. 13 million kg a.i., while pendimethalin usage was approx. 8 million kg a.i. (U.S.

EPA, 2000). According to 2001 data by the U.S. Department of Agriculture, atrazine and metolachlor ranked first and third, respectively, for herbicide usage on corn in the United States, and pendimethalin ranked eighth (U.S. Department of Agriculture, 2004).

With such high usage rates, atrazine, metolachlor, and their metabolites have been found to be important moderately persistent soil and water contaminants (Baluch *et al.*, 1993; Arthur *et al.*, 1998). Pendimethalin is more persistent in soil, with field dissipation half-lives ranging from 47 d (Zimdahl *et al.*, 1984) to 407 d (Walker and Bond, 1977) depending on temperature and soil characteristics. According to results from the National Water Quality Assessment performed by the U.S. Geological Survey, atrazine was detected in over 90% of streams sampled in agricultural areas, and in 75% of streams in urban areas. Metolachlor was detected in over 80% of agricultural streams and in 50% of urban streams (Martin *et al.*, 2003). In addition to contamination of surface waters, there

is concern for these two herbicides as ground water contaminants. In agricultural areas, atrazine and an important metabolite, deethylatrazine (DEA), were detected in 42% of ground water wells sampled; metolachlor was detected in 17% of agricultural land wells. In urban settings, atrazine and DEA were detected in 32% of wells, and metolachlor was present in 9% of sampled urban wells (Kolpin and Martin, 2003). With a relatively high K_{oc} , pendimethalin is generally considered to be a very limited threat to ground and surface water (U.S. Department of Agriculture, 1990).

All three herbicides have been implicated in point-source pollution at agrochemical dealership sites due to accidental spillage prior to field application. Such spillage may result in the contamination of nearby soil and water sources. Current methods of removing such contamination are costly, and often involve the transportation of contaminated soil to other locations for processing or incineration (Gannon, 1992; Arthur and Coats, 1998). Economical, on-site remediation techniques such as phytoremediation may prove more efficient for agrochemical dealerships.

There are several mechanisms by which phytoremediation can occur. Degradation of xenobiotics may occur in the rhizosphere of plants, via release of degrading enzymes from plants, or via microbial degradation. For example, Yoshitomi and Shann (2001) report increased mineralization of pyrene with the addition of corn root exudates. Microorganisms, including those residing in plant rhizospheres, have the enzymatic ability to degrade environmental contaminants. Bacteria isolated from pesticide-contaminated soils have the ability to degrade atrazine, for example (Zhao *et al.*, 2003; Mandelbaum *et al.*, 1995; Radosevich *et al.*, 1996; Struthers *et al.*, 1998). Several studies have shown the plant rhizosphere provides an ideal residence for such microbial communities and suggest the use of plants for pesticide degradation (Arthur and Coats, 1998; McGrath *et al.*, 2001). Other mechanisms of phytoremediation include uptake by the plant, either through the transpiration process or through a more active process, and subsequent sequestration and/or metabolism of the compounds by enzymes within the plant tissues. Plants are able to take up moderately hydrophobic compounds with K_{ow} of 0.5–3.0 rather easily, while more lipophilic compounds may partition into root tissue but are more difficult for the plants to transport (Briggs *et al.*, 1982). Such plant uptake

may prove valuable for controlling the contaminant plume by decreasing leaching of the compound.

Because of their extensive root zone and general hardiness, prairie grasses have been used on roadsides and in vegetative filter strips, and have been suggested as a phytoremediation technique (Arthur and Coats, 1998; Anderson *et al.*, 1993; Comis, 1996). Belden *et al.* (2004, 2004a) have shown decreased mobility and bioavailability of several herbicides in prairie grassed soil columns compared to unvegetated control soil columns.

Previous work has also shown increased dissipation of atrazine and metolachlor residues in soil from an agrochemical dealership site using prairie grasses (Belden *et al.*, 2004b). Questions remain about the degradation and fate of contaminants and their metabolites within a grassed phytoremediation system. Effects of plant uptake on degradation products formed, and on the ultimate fate of such products, are relatively unknown (Mersie *et al.*, 1999; Belden *et al.*, 2004a). It is also unclear if dissipation of the pesticides is due to plant uptake or microbial degradation, or both. Studies incorporating a total mass balance of the pesticide in a phytoremediation setting would provide valuable information for furthering the advancement of phytoremediation science.

The objectives of the work presented here were 1.) to evaluate the potential of prairie grasses and/or bacteria inoculant to remediate pesticide-contaminated soil through increased degradation and through decreased bioavailability and mobility of pesticide residues, 2.) to determine the metabolites formed in a remediation system, and 3.) to work toward an understanding of the mechanism of phytoremediation of pesticide-contaminated soils.

Methods

Closed system studies

Enclosed test chambers were constructed for study of phytodegradation and mass balance (Fig. 1). Agronomic soil was fortified with a [^{14}C] analytical grade solution of a pesticide to bring the content of herbicide to 25 mg/kg in the soil. Residues were aged (5 d for atrazine, 25 d for metolachlor and 160 d for pendimethalin) to simulate aging periods at a field site. Following the aging period, soils receiving phytoremediation treatment were planted with plugs of a mixture of three-week-old prairie grasses: big bluestem (*Andropo-*

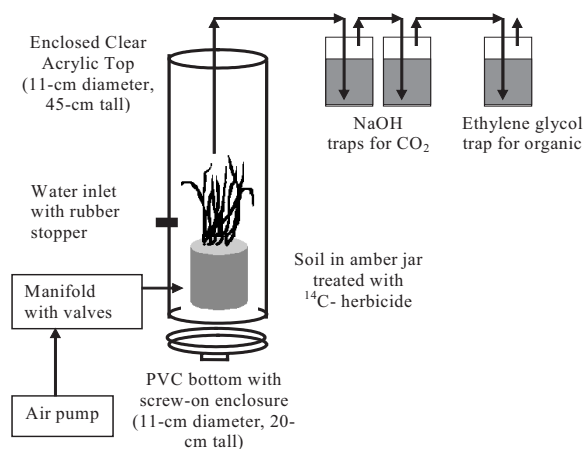


Fig. 1. Schematic of enclosed chamber system, with arrows indicating direction of air flow through tubing.

gon gerardii var. Pawnee), yellow indiagrass (*Sorghastrum nutans* var. Holt), and switchgrass (*Panicum vergatum* var. Pathfinder).

Forced air was pumped through each 5-liter chamber. [^{14}C]CO₂ and volatile [^{14}C]organic metabolites were collected using a flow-through system consisting of two NaOH traps and one ethylene glycol trap, respectively (Fig. 1). At the end of the testing period, leaf and root material and soil were evaluated for radioactivity using a sequential extraction technique (100% methanol or ethyl acetate, 95% water:5% methanol) followed by combustion of samples to derive a mass balance of the pesticide in the entire system. Identity of parent herbicide and related metabolites was determined using HPLC to correlate retention times of analytical standards with sample extracts; fractions were collected and analyzed for radioactivity using liquid scintillation counting (Belden and Coats, 2004). Atrazine and metolachlor metabolites are depicted in Fig. 2. Pendimethalin metabolites were characterized based on mobility in a thin-layer chromatography analysis; a 9:1 hexane/acetone mobile phase was used on silica gel-coated plates (0.25 μm film thickness, Whatman, Clifton, NJ, USA).

Soil column study

Belden *et al.* (2004a) examined the effect of phytoremediation on dissipation, movement, and bioavailability of herbicide residues in soil columns. Naïve agronomic soil was collected and hand-

packed into columns, with the bottom 7 cm of the 21-cm columns left untreated; the top 14 cm of soil was fortified to 25 mg/kg each of atrazine, alachlor, metolachlor, and pendimethalin. After an aging period, a mixture of the three previously described prairie grasses was added as the phytoremediation treatment; controls received similar aliquots of soil as was added with the grass plantings. Columns were kept moist on a regular schedule in a greenhouse for 150 and 240 d (two time points). At the end of the time point, columns were flooded with a 7.5-cm "rainfall", and leachate was collected and analyzed for presence of the herbicides using GC-NPD. After a short drying period, aliquots of soil from the top, middle, and bottom sections of the soil columns were extracted for analysis of herbicide. To determine if the phytoremediation treatment was successful in remediating toxicity of fortified soil, the remaining soil in each section was used to evaluate bioavailability of the herbicides through uptake by earthworms, and inhibition of lettuce growth. Leachate aliquots remaining after solid-phase extraction were used for algal toxicity testing (Belden *et al.*, 2004a).

Grass species evaluation

To evaluate the effect of grass type on persistence and mobility of herbicides, Belden and Coats (2004) utilized a microcosm approach in which intact soil columns were collected and planted with five different grass types. Treatments consisted of no vegetation (control), brome grass, big bluestem, fescue, switchgrass, or a mixture of prairie grasses (big bluestem, yellow indiagrass, and switchgrass). Grasses were grown for 360 d, which included one senescence period. Artificial runoff water containing known concentrations of herbicide was applied weekly for a four-week period. During the first runoff event, 840 ml of water containing 1.5 mg/l atrazine and metolachlor, and 0.3 mg/l pendimethalin were added dropwise to the top of the soil columns. Week 2 consisted of 0.75 mg/l atrazine and metolachlor, and 0.15 mg/l pendimethalin, and week 3 and 4 were 840 ml of water only (no herbicide). During each rain event, infiltration rate was recorded. After columns had drained, leachate was analyzed for atrazine, metolachlor, pendimethalin, and two major atrazine metabolites; at the end of the study, soil was also extracted and analyzed. Additionally, degradation

potential of the soil was assessed using microbial techniques (Belden and Coats, 2004).

Bacteria inoculation study

In a series of three experiments, Zhao *et al.* (2003) examined the effect of the addition of the soil bacterium *Pseudomonas* sp. strain ADP, the enzyme atrazine chlorohydrolase, and/or vegetation on the degradation of atrazine and metolachlor in soil. Soils from an agrochemical dealership with naturally aged residues of atrazine and metolachlor were collected from a site in Iowa. In the first two experiments, *Pseudomonas* sp. strain ADP and atrazine chlorohydrolase were added to the dealership soil, which was treated with fresh or 50-day-aged residues of atrazine and metolachlor (approx. 100 mg/kg atrazine and 25 mg/kg for metolachlor experiments). Contents of herbicide were measured at 1, 2, 3, 7, 14, and 28 d post-inoculation. The third experiment examined the influence of prairie grasses and inoculation in a factorial design: vegetation only, enzyme only, bacteria

only, vegetation plus enzyme or bacteria, and control. Contents of remaining atrazine or metolachlor were determined at 28 d. In all three experiments, soil was extracted three times with ethyl acetate and analyzed by GC-NPD (Zhao *et al.*, 2003).

Results

Closed system experiments

Atrazine

Plant uptake of atrazine (Fig. 2) was evaluated in a 21-day test. During that time, less than 0.5% of applied radioactivity was taken up by the plant. Only 0.18% of applied [¹⁴C]residue was detected in root tissue, and 0.18% of applied was found in leaf tissue. Table I outlines the contents of atrazine parent, metabolites, and unknown components identified in the enclosed chamber system. The major metabolites in leaf tissue were didealkylatrazine (DDA) and hydroxyatrazine (HYA). Parent atrazine, deethylatrazine (DEA), and deiso-

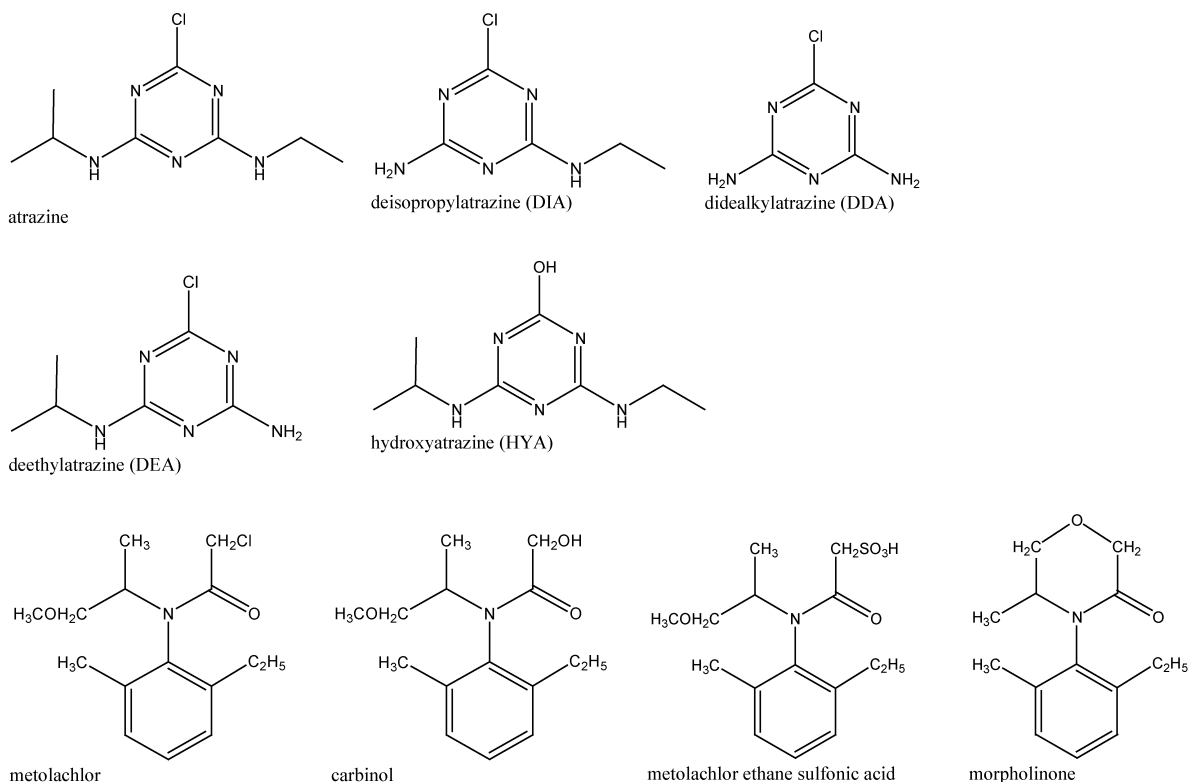


Fig. 2. Chemical structures for atrazine, metolachlor, and selected metabolites.

	Content*			
	Vegetated soil	Control soil	Leaf tissue	Root tissue
Extracted with methanol	7.4 (0.4)	7.4 (1.3)	220 (34)	43 (3.8)
Atrazine	4.5 (0.8)	3.5 (0.5)	32 (11)	15 (4.3)
DEA	0.8 (0.2)	1.3 (0.5)	21 (7.8)	3.9 (1.1)
DIA	0.2 (0.05)	0.3 (0.1)	26 (5.6)	1.3 (0.8)
DDA	1.1 (0.2)	1.1 (0.2)	56 (5.3)	10 (1.8)
HYA	0.3 (0.1)	0.6 (0.1)	66 (9.0)	12.1 (2.1)
Unknowns	0.5 (0.1)	0.5 (0.1)	20 (15)	0.7 (0.6)
Extracted with 95:5 water/methanol	0.9 (0.1)	0.8 (0.3)	32.4 (4.1)	7.3 (0.7)
Retrieved with combustion	10.1 (0.7)	9.6 (1.6)	65.6 (6.5)	14 (2.1)
Total	18.4 (0.7)	17.9 (1.4)	319 (41)	65 (6.4)

Table I. Contents of atrazine and metabolites (as atrazine equivalents) in soil and plant tissues. Standard errors are shown in parenthesis.

* mg/kg equivalents based on specific activity = 1.89×10^4 Bq/mg atrazine applied, and dry wt. of soil and plant material.

propylatrazine (DIA) were less prominent in the leaf extract. Total [^{14}C]residue detected in leaf tissue was found to be approx. 319 mg/kg dry wt. in the grasses; leaf tissues contained higher total contents of [^{14}C]residue than roots. Leaf tissue contained significantly higher contents of the more polar metabolites, including DIA, DDA, and HYA, compared to root tissue. In root tissue, [^{14}C]atrazine was the dominant compound, followed by metabolites DDA and HYA; DEA and DIA were less prominent. Parent atrazine was detected at approx. 15 mg/kg dry wt. in root tissue, and at 32 mg/kg dry wt. in leaf tissue. The difference between leaf and root tissue contents of DIA, DDA, and HYA is likely due to the fact that DIA, DDA, and HYA are more mobile, and may be more readily taken up into the plant. It is also possible that leaves do more biotransformation of atrazine to the more polar metabolites than roots do, thus explaining the higher contents of polar compounds in leaf tissue. In studies using plant cell suspensions of carrot, corn cockle, foxglove, jimsonweed, soybean, and wheat, only soybean cells had the ability to perform the *N*-dealkylations to form DDA (Schmidt *et al.*, 1997). Based on the presence of DDA in our results, the prairie grasses may have unique transformation potential. Hydroponic studies are in progress to help delineate transformation potential of the plants.

Contents of parent atrazine and metabolites in the grasses were different than those described in other model plant systems, which may indicate different metabolism and/or uptake of atrazine or metabolites by the grasses used in this study. Raveton *et al.* (1997) found HYA was the dominant compound in both roots and shoots (51.5%

and 73% of applied, respectively); DEA, DIA, and DDA were all present at < 1% of applied in corn roots and shoots. Other hydroxy-metabolites were more prevalent; this could be due to the hydroponic nature of their study.

Metolachlor

Phytodegradation of metolachlor (Fig. 2) was evaluated in a 97-day test. Over 7% of applied radioactivity was taken up by the grasses during that time; plant uptake appeared to be the major contributing factor to phytoremediation of metolachlor in the soil during the study. The amount of metolachlor detected in leaf tissue was significantly less than in root tissue. Leaf tissue also contained less of the carbinol metabolite, but more of the ethane sulfonic acid (ESA) (Fig. 3); this is not surprising, considering the relative polarities of the compounds. It has been shown that higher plants, such as corn, soybeans, and sorghum, have developed tolerance for some herbicides through utilization of a glutathione conjugation detoxification pathway (Al-Khatib *et al.*, 2002). It is possible that these grasses contain similar detoxification pathways; leaf tissue in these grasses may contain more biochemical transformation capability than root tissue, thus explaining the higher amount of the ESA metabolite in leaves than roots. Metolachlor ESA was the major metabolite identified in the soil of both control and grassed systems, suggesting the possibility of transformation of metolachlor by other organisms in the rhizosphere. Further studies are underway to determine the potential for the grasses to perform the metabolism to the ESA.

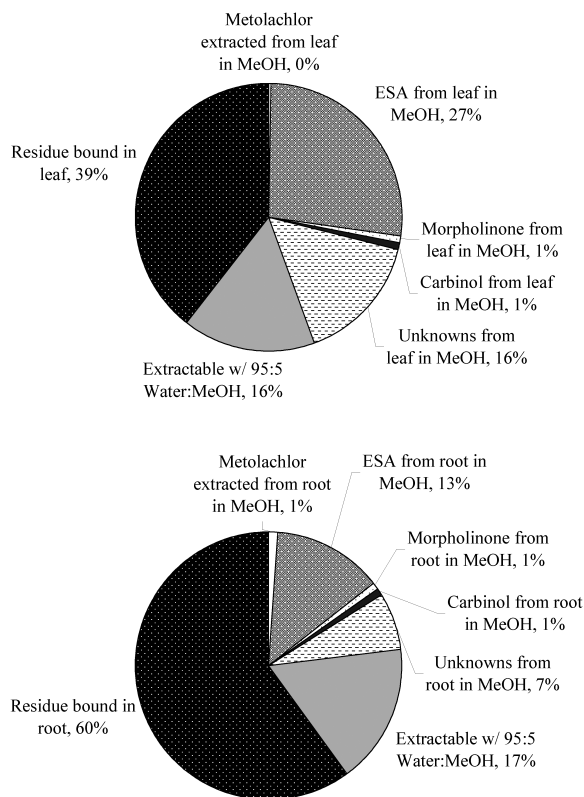


Fig. 3. Distribution of radioactivity in leaf (top) and root (bottom) tissues, including parent metolachlor, selected metabolites, and unidentified components, as percent of total ^{14}C residue in leaf or root.

Pendimethalin

Pendimethalin dissipation was greater in vegetated systems than in unvegetated controls; 44.2% of applied radioactivity remained as parent pen-

dimethalin in controls at the end of the 106-day study, whereas 37.0% remained as pendimethalin in vegetated systems ($t = 4.30$; $p = 0.023$). 7–9% of applied radioactivity was mineralized to $[^{14}\text{C}]\text{CO}_2$, which is similar to the previously reported values of 3–5% in lysimeter studies (Schroll *et al.*, 1999) and 16–24% in composted soils (Barriuso *et al.*, 1997). Plant uptake accounted for 3.1% of total radioactivity, with 60% of that amount remaining in the roots. More parent pendimethalin was present in root tissue than in leaf tissue, and the majority of the metabolites detected were of low mobility (Table II). Other studies have reported greater degradation of pendimethalin (Belden *et al.*, 2004a); the lesser degree of degradation in this study could be due to shorter growing periods for the grasses, or limited space availability for grass development from the enclosed chamber system used.

In addition to these mass balance systems, other researchers have reported uptake and conjugation of other contaminants and their metabolites, including sequestration of the compounds within plant tissues. Bhadra *et al.* (1999) reported reductive and oxidative metabolism of TNT in an aquatic plant species, as well as accumulation of the compound throughout the plant. In studies with the explosives 1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), Bhadra *et al.* (2001) found uptake, metabolism, and sequestration of RDX, with a small fraction (< 10%) of RDX being “bound” in plant tissue after 65 d. For HMX, differences in the ability of aquatic plant species to remove HMX were seen. These findings illustrate the need

Table II. Distribution of ^{14}C -pendimethalin and transformation products in pretest soil as compared to the distribution in test chambers with and without vegetation treatments. Values are based on percent of applied radioactivity. Standard errors are shown in parenthesis.

	Pretest ^a	Percent of applied Unvegetated	Vegetated
Soil	100	75.6 (2.1)	78.0 (1.6)
Pendimethalin	40.0 (1.0)	14.7 (0.7)	12.9 (0.8)
Extractable metabolites	14.6 (0.3)	9.8 (0.4)	10.1 (0.7)
Bound ^b	45.4 (1.0)	51.1 (1.3)	55.0 (0.5)
Plant tissue	–	–	2.7 (0.1)
Mineralized	–	7.4 (0.5)	6.3 (0.3)
Volatile organic	–	0.39 (0.05)	0.25 (0.03)
Mass balance	100	83.4 (2.3)	87.3 (1.8)

^a Pretest refers to the status of radioactive residues in the soil after the 160-day aging period and before the addition of the phytoremediation treatment.

^b Bound refers to residual radioactivity after extractions, as determined by combustion.

for further research on absorption and metabolism of different compounds in different plant species.

Soil column study

Belden *et al.* (2004a) found that vegetation was important for the degradation of metolachlor and pendimethalin; the grasses also decreased mobility of metolachlor in the soil columns. In vegetated columns, metolachlor residues were 78% lower than metolachlor levels in unvegetated control columns. Additionally, less metolachlor was found in leachate from columns receiving the phytoremediation treatment than those without, and metolachlor was less mobile through the soil of vegetated columns than control columns. Vegetated columns also contained 39% less pendimethalin than control columns at the end of the study. For both metolachlor and pendimethalin, much more degradation had occurred by 250 d compared to the 160-day time point. Atrazine and alachlor degraded rapidly in the soils, regardless of treatment.

Vegetation significantly decreased the toxicity of the herbicide residues to lettuce. At the 160-day time point, soil from columns receiving the phytoremediation treatment had less inhibition of lettuce seedling growth than the control soils. Additionally, leachate from vegetated columns was significantly less toxic to algae than leachate from unvegetated columns. This study found that aged pesticide residues were substantially degraded and stabilized by the addition of prairie grasses as a remediation technique. Additionally, toxicity and bioavailability of the pesticides were reduced in the vegetated columns; it is important to examine toxicity and bioavailability when evaluating the success of a remediation strategy. In this case, phytoremediation using prairie grasses was important for reducing the impact of the pesticide residues in soil columns (Belden *et al.*, 2004a).

Grass species evaluation

Belden and Coats (2004) found that presence of grasses significantly increased infiltration rate of the runoff water, but decreased the volume of leachate collected compared to unvegetated columns. The mixture of prairie grasses allowed less leachate than fescue. In addition to increasing infiltration rate, vegetated columns held the amount of herbicide leached to the same or lower amounts compared to unvegetated columns. During the final two rain events, which had no additional herbi-

cide added, all grasses were better than unvegetated control columns when comparing amount of atrazine and metolachlor in the leachate. More specifically, mixed prairie grasses were significantly better than the rest of the grass types, by reducing the amount of atrazine and metolachlor leaching by 43 and 44%, respectively.

Soil from columns with mixed prairie grasses and with brome had greater atrazine mineralization capacities than all other treatments. Additionally, bound metolachlor residues were higher in soil from these two treatments, potentially indicating greater formation of metabolites.

Bacteria inoculation study

Zhao *et al.* (2003) found that addition of the enzyme atrazine chlorohydrolase significantly lowered fresh and aged atrazine levels in soil. The enzyme atrazine chlorohydrolase transforms parent atrazine to the hydroxyatrazine metabolite, which in turn may be more readily degradable by other mechanisms. Inoculation of the pesticide-treated soil with *Pseudomonas* sp. strain ADP also significantly lowered aged and fresh atrazine contents. There was no difference found when comparing atrazine degradation ability of the enzyme with that of the bacteria. Both degradations were rapid; the estimated half-lives were all less than or equal to 7 d for aged and fresh residues. However, for both experiments, atrazine was more persistent in the aged residues compared to freshly applied residues. Aging of the residues affected the bioavailability, and therefore the ability of the enzyme or bacteria to degrade atrazine; atrazine may adsorb to soil rapidly, thus decreasing bioavailability.

In a separate experiment, vegetation was added to determine the effect on degradation with and without enzyme or bacteria inoculation. Addition of prairie grass as vegetation significantly decreased levels of metolachlor in the 28-day study, and significant interaction was seen in the treatment that included vegetation and enzyme, although the enzyme alone had no effect on metolachlor degradation. Addition of vegetation did not significantly reduce atrazine contents in soil in the 28-day study. Other work has shown it takes longer than 28 d to degrade atrazine with the use of vegetation; significant atrazine degradation was seen in a 71-day study with prairie grasses (Zhao *et al.*, 2005). *Pseudomonas* sp. strain ADP is believed to mineralize atrazine as a nitrogen source

when sufficient carbon is available in the soil (Mandelbaum *et al.*, 1995; Shapir and Mandelbaum, 1997). Addition of vegetation may help stimulate the remediation process if root exudates provide a carbon source.

Prairie grasses are capable of absorbing pesticide residues and incorporating the residues into their tissue. Such plant uptake may be an important mechanism for remediation of pesticide wastes in soil. Metabolites of atrazine, metolachlor, and pendimethalin were detected in the grasses, as well as in the rhizospheric soil, which indicates the important biotransformation capability of the grasses and/or microorganisms in the rhizosphere, which is also a crucial mechanism in bioremediation strategies. The compilation of all of these studies highlights the complexity of interac-

tions among organisms in a phytoremediation system. Additional studies are needed to investigate the interactions among rhizospheric bacteria, both native and introduced, and the plants providing the rhizosphere, and subsequently how such interactions affect remediation of pesticide wastes.

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