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Abstract

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Mass Balance of Metolachlor in a Grassed Phytoremediation System

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Metolachlor is a point-source pollutant at agrochemical dealerships in the Midwest, as well as a non point-source contaminant of surface waters caused by runoff. Prairie grasses have been used in filter strips to control runoff and are also useful for phytoremediation; however, little is known about the fate of metolachlor and its metabolites within a grassed system. Effects of uptake by prairie grasses on the formation and fate of degradation products are not known. In this study, [U-ring-^{14}C]metolachlor was added to enclosed systems to determine the fate of the parent compound and its metabolites in soil and plants. Mineralization and volatilization were monitored over the 97 day study and found to be 1.05 and 0.2%, respectively, for vegetated systems. At the end of the study, soil and plant material was evaluated for the presence of parent metolachlor and selected metabolites, as well as bound residues. Metolachlor ethane sulfonic acid was the dominant metabolite in soil and plant tissue. Over 7% of applied radioactivity was taken up by the grasses, and plant uptake/metabolism appeared to be the main mechanism for phytoremediation of metolachlor. Vegetation significantly reduced the amount of metolachlor in soil by 9%, indicating potential success as a remediation tool.

Introduction

Pesticides are widely used in agricultural settings and are known nonpoint-source contaminants in surface waters, as well as point-source contaminants in soil at agrochemical dealership sites (*1*). Metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(methoxy-1-methylethyl)acetamide), a widely used chloracetanilide herbicide, has been detected in soil at such dealership sites. Metolachlor is commonly used to control broadleaf weeds and annual grasses in row crop agricultural systems in the US. The principal mode of action of metolachlor is the inhibition of very long chain fatty acid biosynthesis, which affects cell plasma membranes and, in turn, shoot elongation (*2*). According to 1992−1995 data from the U.S. Geological Survey, metolachlor ranked third among all pesticides used, based on the amount of active ingredient applied (*3*). Metolachlor ranked in the top ten for pesticide usage according to 2000 data from the U.S. Department of Agriculture. Crops on which metolachlor is used include corn, soybeans, sorghum, peanuts, sweet corn, and cotton. Estimates from 2001 U.S. pesticide sales data indicate metolachlor usage at approximately 10 million kg of active ingredient per year (*4*).

Once applied, metolachlor has an estimated half-life of 16−289 days in soil, depending on soil characteristics, moisture, temperature, and depth, as well as microbial activity (*5*−*7*). Metolachlor and its metabolites are also slightly mobile in soil (*8*); in several leaching studies, approximately 1% of applied metolachlor leached through soil columns, while selected metolachlor degradates appeared to be more mobile in soil (*9*, *10*).

Phytoremediation using prairie grasses has been shown to be effective at enhancing dissipation of metolachlor, as well as other herbicides. Belden et al. (*11*, *12*) report a decreased mobility of herbicides through soil columns with grasses, as compared to non-vegetated controls. This information makes phytoremediation an attractive technology; however, questions remain about the degradation and fate of contaminants and their metabolites within a grassed phytoremediation system. Effects of plant uptake on remediation, fate of the parent chemical and degradation products within the plant, and mass balance of the herbicide within a grassed system are relatively unknown (*13*−*15*).

The goal of this study was to track the fate of metolachlor in soil in a phytoremediation system (i) to obtain a mass balance of the herbicide, (ii) to determine the types and amounts of metolachlor and degradation products present in grasses and in soil, and (iii) to quantify volatility and mineralization rates. To accomplish these objectives, [^{14}C]-metolachlor was applied to soil, and the fate was monitored in a closed system. After 97 days, the soil and the grass tissues were extracted to identify and quantify parent metolachlor and selected metabolites.

Materials and Methods

Chamber Construction. Eight enclosed test chambers were constructed for the studies (Figure 1). Each chamber consisted of a polyvinyl chloride (PVC) cleanout sealed to an acrylic tube (10 cm internal diameter, 60 cm length, Aquatic Ecosystems, Apopka, FL) using PVC cement (Oatley, Cleveland, OH). The cleanout was closed and sealed using a non-hardening pipe joint compound, which consists of Teflon, $CaCO_3$, oil, and titanium dioxide (Oatley). The top of each acrylic tube was sealed with a flat piece of acrylic using acrylic cement (Weld-On 16, IDS, Gardena, CA). Chambers were connected to a pressure pump (Gast DDL, Benton Harbor, MI) in an open flow-through design using a manifold with valves for flow adjustment. Traps for collecting CO_2 and volatile organics were connected to each chamber using Nalgene Tygon tubing (3/16 in. i.d.); each trap consisted of a 40 mL glass EPA vial with a rubber Teflon septa (Fisher Scientific, Pittsburgh, PA) with aforementioned tubing pulled through the septa as an inlet and 3.8 cm 18-gauge hypodermic needles piercing the septa as an outlet. The base of the needle was inserted into tubing; this served as inlet to the next trap. Additionally, polyurethane foam volatile traps were added to ensure trapping of volatiles (Figure 1).

Preparation of Prairie Grasses. Several species of grasses were examined for their effects on herbicide degradation and fate, and a mixture of three native prairie grasses was selected because of its ability to increase degradation, specifically by increasing atrazine mineralization and the dissipation of metolachlor (*11*, *16*). Big bluestem (*Andropogon gerardii* var. Pawnee), yellow indiangrass (*Sorghastrum nutans* var. Holt), and switchgrass (*Panicum vergatum* var. Pathfinder) are all perennial warm-season grasses with extensive root systems (>30 cm), as is typical of prairie grasses.

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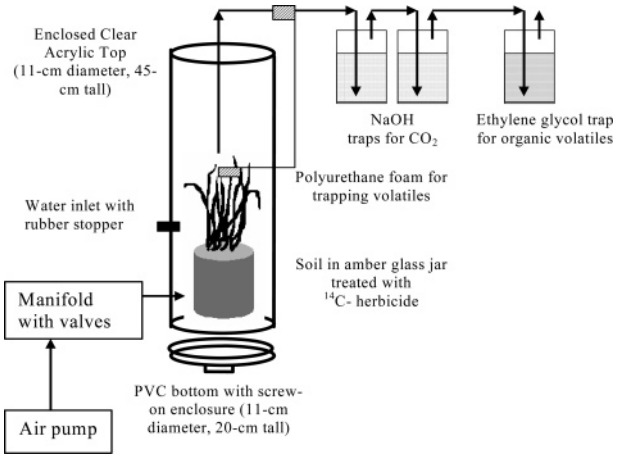

FIGURE 1. Schematic of enclosed chamber system, with arrows indicating direction of air flow through tubing.

Each of these species is native to the U.S. and is known to be relatively tolerant of extremes in soil texture, pH, temperature, and moisture, making them hardy, which is important in phytoremediation settings (*17*).

Growth cones were filled with 32 g dry wt of the same agronomic soil described in the next section and planted with 5 seeds from one of the three species of grasses: big bluestem, yellow indiangrass, and switchgrass. Seeds were obtained from United Seed Company (Omaha, NE); germination rates for all three species were >97%. Twelve growth cones were filled with soil, but were not planted (soil blanks), to be added to non-vegetated control systems ($n = 4$, with three cones per chamber). Grasses and soil blanks were reared in a greenhouse for 35 days at 27 ± 3 °C with a 16:8 light/dark cycle until being transferred to test chambers.

Fortification of Soil. Agronomic topsoil with no recent pesticide history (>20 years) was collected from a field site near Ames, IA (Field 55, ISU Ag Engineering Farm) and was sieved (2.8 mm) to remove rocks and plant material. The texture of the soil was sandy loam with 2.8% organic matter and pH 7.0 (51% sand, 28% silt, and 21% clay; Midwest Laboratories, Omaha, NE). Soil (dry wt) was fortified with a [^{14}C]metolachlor/analytical grade metolachlor solution so that each of the four 1.25 kg portions received 16.36 mL of a 2.14 mg of metolachlor/mL of methanol spiking solution, to bring the final concentration of metolachlor in soil to 28 mg/kg dry wt and 8.38 μCi per jar. Methods for adding a spiking solution to the soil and for mixing the soil were similar to those described by Zhao et al. (*18*). Specific activity was 0.241 μCi/mg. The [U-ring-^{14}C]metolachlor was provided in kind from Syngenta, and analytical grade metolachlor was purchased from ChemService (West Chester, PA). Radiopurity was 97.6%, and chemical purities for the radiolabeled and analytical-grade compound were 96.4 and 99%, respectively. Purity was determined using thin-layer chromatography as described by Rice et al. (*6*).

After fortification with the metolachlor spiking solution, the soil was mechanically shaken for three 10 min periods to allow for thorough mixing of metolachlor throughout the soil. Jars were vented between shake cycles to allow for evaporation of the methanol carrier. Treated soil was then incubated for 25 days to simulate an aging period, which could be several years at a dealership site. Aging occurred in four large amber jars (2.5 L) in a dark incubator maintained at 17 °C; jars were shaken for 2 min and vented for 2 min every day during the aging period. Venting was important to prevent development of anaerobic conditions.

Following aging, a 15 g wet wt aliquot of soil was obtained from each jar to determine the extent of degradation and binding that occurred during the aging period. This pre-study soil was extracted and combusted using the same procedures outlined later in this section. The remaining soil from each jar was divided, with one-half receiving the phytoremediation treatment and the other serving as a control. Each 0.62 kg (dry wt) half was fertilized, and the soil moisture was brought up to field capacity (1/3 bar = 15.0%, Midwest Laboratories, Omaha, NE) using 12.5 mL of an aqueous solution containing 0.005% N (total N), 0.005% K (as K_2O), and 0.0075% P (as P_2O_5), plus trace levels of Fe, Mn, and Zn (Schultz Plant Food, St. Louis, MO).

Addition of Phytoremediation Treatment. Soil receiving the phytoremediation treatment ($n = 4$) was amended with one cone of each type of prairie grass (switchgrass, yellow indiangrass, and big bluestem; age 35 days), and four control soils received similar aliquots (3 × 32 g) of the same agronomic soil. Following the paired design, vegetated and non-vegetated systems were randomly assigned chamber positions next to one another.

Chamber Conditions. Forced air was pumped through each 5 L chamber at 23 L/h (SE = 1 L/h). [^{14}C]CO_2 and volatile [^{14}C]organic metabolites were collected using a flow-through system consisting of two 30 mL 2 N KOH traps, one 30 mL ethylene glycol trap, and three polyurethane foam traps, similar to the method described by Rice et al. (*6*) (Figure 1). Throughout the study, the temperature was maintained at 27 ± 3 °C, and the soil moisture was maintained at 15% moisture (field capacity) using a HoldAll moisture meter (AmerTac, Monsey, NY). Beginning at day 15, vegetated systems required an average of 3.72 mL (SE = 0.45) more water than controls every 2 days. Light intensity inside the chambers was 3400 lx.

Monitoring of Mineralization and Volatilization. Contents of CO_2 traps were analyzed every 3 days throughout the 97 day testing period, and volatile organic traps were analyzed every 6 days. Two milliliters of each CO_2 trap solution was counted in 12 mL of a ScintiSafe Plus 50 (Fisher Scientific) scintillation cocktail for quantification of [^{14}C]CO_2 evolved, and a 1 mL aliquot of each volatile organic trap was counted in 12 mL of ScintiSafe Plus 50. The in-line polyurethane traps were exchanged every 6 weeks, extracted with 30 mL of hexane for 72 h, and the radioactivity in the extract was quantified by counting 2 mL of extract with 11 mL of Ultima Gold scintillation cocktail (Fisher Scientific). Extraction efficiency was 94%. Methods for these traps were similar to those described by Rice et al. (*6*). Radioactivity in CO_2 and volatile traps was quantified using a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Inc., Fullerton, CA).

Analysis of Plant Material and Soil. At the end of the testing period, plant material was collected and analyzed according to the type of plant material: root (below-ground biomass) and leaf (above-ground biomass). Leaf material was clipped at the soil surface. Large roots were separated out using forceps, and soil aggregates greater than 2 mm were scraped from the roots; small root hairs were analyzed with the soil. Plant material was homogenized 3 times using a mortar and pestle in 50 mL of methanol, followed by a 30 mL methanol rinse (5 min per extraction), then was shake-extracted 3 times with 30 mL of 95:5 deionized water/methanol (v/v), and rinsed once with 10 mL of 95:5 water/methanol (5 min per shake).

The soil was mixed thoroughly, and two 20 g aliquots per chamber were shake-extracted 3 times with 60 mL of methanol for 10 min per extraction. The 20 g aliquots of methanol-extracted soil were then shake-extracted for 10 min with 60 mL of 95:5 deionized water/methanol (v/v) and allowed to settle for 12 h in the dark at 4 °C. Extraction efficiencies were >96% for spike recoveries in soil and plant tissues. The sequential extraction technique used the same filter paper for methanol and 95:5 water/methanol extractions. Ten milliliters of 95:5 water/methanol extracts was

TABLE 1. HPLC Separation of Parent Metolachlor and Metabolites, Including Fractionation and Retention Times

	fraction number	time (min)
Blank	1	0−1.8
Ethane sulfonic acid	2	1.8−3.3
Unidentified metabolite(s)	3	3.3−6.0
Morpholinone	4	6.0−7.5
Carbinol	5	7.5−11.5
Blank	6	11.5−12.5
Unidentified metabolite(s)	7	12.5−15.5
Metolachlor	8	15.5−17.5
Unidentified metabolite(s)	9	17.5−20.0

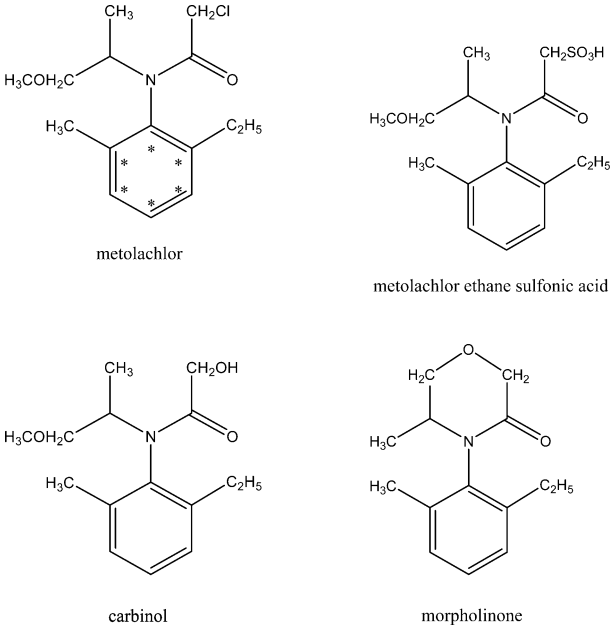

FIGURE 2. Chemical structures of metolachlor and selected metabolites. Asterisk indicates position of the radiolabel.

centrifuged for 40 min, and 4 mL of supernatant was counted in 11 mL of the ScintiSafe Plus 50 scintillation cocktail for measurement of other extracted polar compounds. Methanol extracts were concentrated and analyzed for determination of metabolite and parent compound content using a Hewlett-Packard 1100 series HPLC equipped with an autosampler and UV absorbance detector. An Adsorbosphere C18 5 μm, 4.6 mm × 250 mm HPLC column (Alltech, Deerfield, IL) was used in a 20 min run with a mobile phase consisting of 50:50 acetonitrile/phosphate buffer (pH = 3.5). The flow rate was 1 mL/min, the column temperature was maintained at 30 °C, and the pressure was at 100 bar. A total of 100 μL of each concentrated methanol sample extract was injected, and fractions were collected based on retention times of analytical standards and absorbance at 230 nm (Table 1). Non-labeled reference standards were used to develop the HPLC method described; peaks were separated by greater than 2 min to ensure accurate fraction collection. Analytical standard compounds were provided by Syngenta. Because there are several metabolites of metolachlor, we chose to focus on three of the most commonly reported: morpholinone, carbinol, and ethane sulfonic acid (ESA) (Figure 2). Morpholinone is considered in the tolerance level for metolachlor and metabolites in commodity crops as dictated by the U.S. Environmental Protection Agency. The ESA metabolite is important because of its abundance in the environment at concentrations higher than those of the parent metolachlor, specifically in water monitoring studies (*19*). Radioactivity in each extract fraction was quantified using a Beckman LS 6500 liquid scintillation counter.

Following the sequential extraction technique, homogenized plant material and soil subsamples were formed into pellets. Pellets were combusted using a Packard 307 Sample Oxidizer (Packard Instruments, Downers Grove, IL) to quantify remaining bound residues in both soil and plant tissues.

Statistical Analysis. Statistical differences between vegetated and non-vegetated treatments and between leaf and root tissue were assessed using paired *t*-tests (two-sided $p < 0.05$). Means are expressed with a standard error. Each test that was performed utilized three degrees of freedom and tested the null hypothesis that the difference between treatments was equal to zero. *p*-Values are expressed as double-sided. Statistical calculations were performed using Statview (*20*).

Results and Discussion

After the 25 day aging period, 90.1% of applied radioactivity was extractable with methanol (SE = 0.6); of that, over 92% remained as the parent metolachlor, which corresponds to 23.3 mg/kg. Approximately 4.6% of the applied [^{14}C]residue was extracted with 95:5 water/methanol, and 5.3 ± 0.2% remained bound after the sequential extraction technique. The results that follow are described as the percentage of initial, which refers to day 0 after the 25 day aging period (av. 23.3 mg/kg; 4.19 μCi).

Soil Residues. After 97 days, the total mass balance was 95.2 ± 0.9% for non-vegetated systems and 92.5 ± 2.0% for vegetated systems. Vegetation treatment significantly decreased the concentrations of aged metolachlor in the soil as compared to controls, with 16.2% of the applied remaining as a parent in vegetated treatments as compared to 25.3% for controls (3.8 and 5.9 mg/kg, respectively) ($t = 3.902$ and $p = 0.0298$). These results are consistent with persistence data previously described by others, who reported half-lives ranging from 23 to 71 days (*5*−*7*), although we will not speculate on an exact half-life in this study. The amount of total radioactivity (metolachlor, metabolites, and bound residues) found in soil was significantly lower in vegetated systems as compared to non-vegetated systems at the end of the study, with 85.1% remaining in soil for grassed systems and 94.8% for non-vegetated systems (Table 2; $t = 9.411$ and $p = 0.0024$).

There were no significant differences between vegetated and non-vegetated soils in the amount of radioactivity extracted with 95:5 water/methanol or in the amount retrieved through combustion. More radioactivity was extracted from soil with methanol in non-vegetated systems than in vegetated systems, which could be an indicator of increased bioavailability of metolachlor and metabolites to soil organisms in untreated soils (Table 2; $t = 6.304$ and $p = 0.0081$); the plant effectively removed some of the residues in the vegetated soil systems, thereby limiting the availability of those residues to soil organisms, which is a goal of a remediation strategy. Solvent extractability is considered to be an indicator of bioavailability (*21*). In addition to lower metolachlor concentrations in grassed systems, the analysis of methanol extracts also revealed lower concentrations of morpholinone and unidentified metabolites as a percentage of the total applied (Figure 3; morpholinone: $t = 3.935$ and $p = 0.0292$; unidentified: $t = 3.657$ and $p = 0.0353$). Previous work by Liu et al. (*22*) indicated significant occurrences of the morpholinone and carbinol metabolites after just 16 days in microbial cultures isolated from metolachlor-perfused soils, with the morpholinone forming from the carbinol. Metolachlor ESA was the dominant metabolite for both the grassed and the control systems. ESA is believed to result from a glutathione conjugation (*23*). Such a conjugation could

TABLE 2. Total Mass Balance of Metolachlor Equivalents in the System

	% of total applied $^{14}C \pm SE$[a]		concentration (mg/kg[b]	
	vegetated	control	vegetated	control
Soil total*[c]	85.1 ± 2.0	94.8 ± 1.0	19.8	22.1
Extracted with methanol*	30.4 ± 1.0	41.5 ± 2.3	7.08	9.67
Extracted with 95:5 water/methanol	33.3 ± 2.1	31.3 ± 2.2	7.76	7.29
Retrieved with combustion	21.3 ± 1.1	22.0 ± 0.9	4.96	5.13
Plant material total	7.23 ± 0.26		155	
Mineralization	1.05 ± 0.02	1.27 ± 0.17		
Volatilization*	0.197 ± 0.015	0.305 ± 0.026		
Total	92.3 ± 2.0	95.2 ± 0.9	21.5	22.2

[a] SE = standard error. [b] The mg/kg of metolachlor equiv based on specific activity = 0.24 μCi/mg of metolachlor applied and dry wt of soil and wet wt of plant material. [c] Asterisk indicates statistically significant differences between vegetated and control systems (two-sided $p < 0.05$).

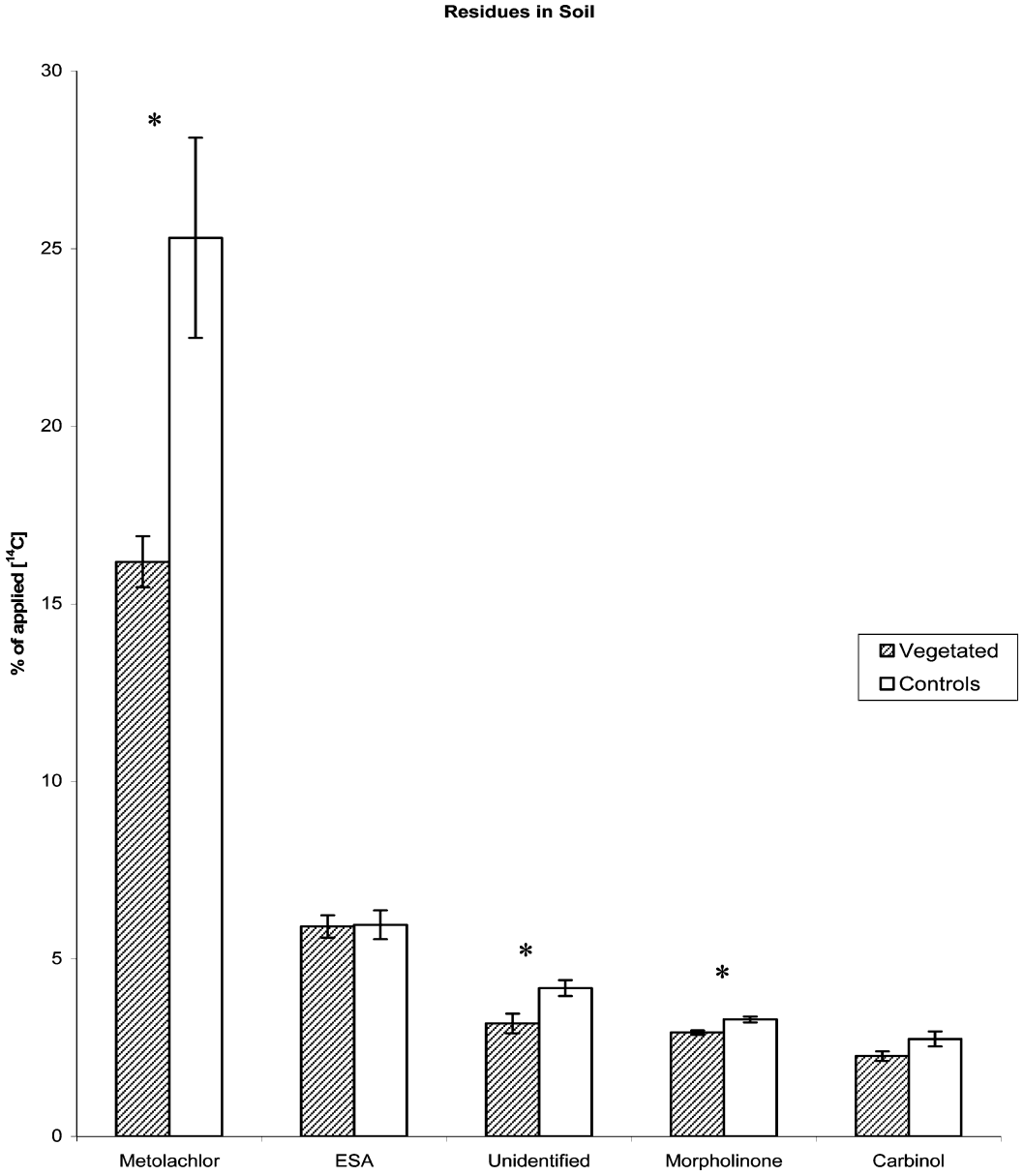

FIGURE 3. Parent metolachlor and metabolite presence in vegetated and control (non-vegetated) soils, expressed as % of applied radioactivity, after 97 days. Asterisk indicates statistically significant differences between vegetated and non-vegetated systems for % of applied radioactivity (two-sided $p < 0.05$).

be conducted by microbes in the soil or, in the case of grassed systems, by the plants and then released back into the soil (*24*).

Residues in Grasses. Grasses grew well during the 97 day study; root growth was observed throughout all of the metolachlor-treated soil, thereby ensuring the plants' exposure to the [^{14}C]residue. Leaf tissue (above-ground biomass) averaged 2.19 g (wet wt; SE = 0.56), and root tissue (below-ground biomass) averaged 6.06 ± 0.91 g (wet wt). Dry mass was found to be 40.1% of wet wt. Concentrations

TABLE 3. Mass Balance of Metolachlor Equivalents in Plant Tissue

	% of total applied $^{14}C \pm SE$[a]	concentration (mg/kg)[b]
Leaf total	2.40 ± 0.11	200
Extracted with methanol	1.06 ± 0.05	91.0*[c]
Extracted with 95:5 water/methanol	0.39 ± 0.0.02*	31.7*
Retrieved with combustion	0.95 ± *0.11**	77.2
Root total	4.83 ± 0.24	141
Extracted with methanol	1.11 ± 0.10	32.5*
Extracted with 95:5 water/methanol	0.81 ± 0.05*	23.6*
Retrieved with combustion	2.90 ± 0.22*	84.6
Plant material total	7.23 ± 0.26	155

[a] SE = standard error. [b] The mg/kg of metolachlor equiv based on specific activity = 0.24 μCi/mg of metolachlor applied and wet wt of plant material. [c] Asterisk indicates statistically significant differences between leaf and root tissues (two-sided $p < 0.05$).

are listed as mg of metolachlor equiv/kg of wet wt plant tissue.

Plant uptake of the [^{14}C]residue totaled 7.2 ± 0.3% of the total applied radioactivity over the 97 day period. This amount accounts for the majority of the difference in recovery from soil between vegetated and control systems, possibly pointing to uptake as the primary mechanism of phytoremediation of metolachlor. Leaf tissue had significantly greater mg/kg of metolachlor equiv as compared to root tissue in both the methanol and the 95:5 water/methanol extractions, which may be important for bioavailability of the chemicals to a herbivore (e.g., cattle), based on the concept of bioavailability being correlated with chemical extractability (*21*) (Table 3; $p = 0.0153$ and 0.0328, respectively). The trend was similar for total mg/kg of metolachlor equiv, although not significant ($p = 0.0719$). However, the total radioactivity (μCi) from root tissue was greater than in leaf tissue, as was the combustion component.

In a comparable study of metolachlor uptake into corn from soil, Al-Khatib et al. (*24*) reported rapid absorption and metabolism of metolachlor in a corn−soil system, including rapid metabolism within the plant. In that study, corn absorbed 55% of the applied radioactivity in 15 days, and 75% of that absorbed was found in the foliage of the corn, not in the root. This is different from our study, in which the majority of the radioactivity (μCi) was present in the root tissue. However, it should be noted that there were differences between the methods in the two studies, specifically with the amount of metolachlor applied and the method of application, the length of study, and the type of plant. Arthur et al. (*25*) performed a long term study on remediation and uptake using a mixture of [^{14}C]atrazine and unlabeled metolachlor; in that 192 day study, they described the uptake of a [^{14}C]residue at 9.9% by *Kochia scoparia* and 0.8% by *Brassica napus*, thus further highlighting the differences between plant species (*25*). The relatively small mass of leaf tissue as compared to root tissue in our study may account for this observation, or differences in plant physiology may also explain the observations. Additionally, the greater amount of absorption by other plants as compared to prairie grasses could also be due to plant physiology differences or to the differences in study length and methodology previously described.

The metabolite profile of leaf and root tissue is described in Table 4. Leaf tissue contained significantly less parent metolachlor and less of the carbinol metabolite than root tissue (Table 4; $t = 17.4$ and $p = 0.0004$). The ESA metabolite is prominent in both leaf and root tissues. It has been shown that plants such as corn, soybeans, and sorghum are tolerant of some herbicides through the utilization of a glutathione conjugation detoxification pathway (*23*). It is possible that these prairie grasses contain similar detoxification pathways, thus explaining the relatively high concentrations of the ESA metabolite. In a comparable study on propachlor (another chloracetanilide herbicide) metabolism in soybeans, Lamoureux and Rusness identified propachlor ESA as a predominant metabolite in soybean foliar tissue from plants grown in soil (*26*).

TABLE 4. Concentrations of Metolachlor and Metabolites in Plant Tissues

	concentration (mg/kg)[a]				
	metolachlor	morpholinone	carbinol	ESA	unidentified
Leaf	0.7*[b]	3.6	3.6*	71.8	42.5
Root	0.9*	3.0	2.8*	78.7	39.5

[a] The mg/kg of metolachlor equiv based on specific activity = 0.24 μCi/mg of metolachlor applied and wet wt of plant material. [b] Asterisk indicates statistically significant differences between leaf and root tissues (two-sided $p < 0.05$).

The U.S. EPA lists the tolerance limit for metolachlor and two key metabolites (2-(2-ethyl-6 methylphenyl)amino-1-propanol and 4-(2-ethyl-6-methylphenyl)-2-hydroxy-5-methyl-3-morpholinone) in forage grass as 10 mg/kg (*19*). The morpholinone of concern to the EPA is a hydroxylated metabolite of the morpholinone that we analyzed. The sum of metolachlor and the morpholinone metabolite residues detected in leaf tissue in this study were 4.3 mg/kg. Since the bound residues in the plant tissue are not characterized, and we were not able to identify all of the metabolites in our extracts, it is possible that leaf tissue may contain quantities of relevant compounds greater than the 4.3 mg/kg detected. Any utilization of these grass species in the field for phytoremediation will require some monitoring of the above-ground portion for residues that are included in the EPA tolerance, especially in the case of grazing by livestock or wildlife.

Volatilization and Mineralization. Although less than 0.4% of the applied [^{14}C]residue was detected as volatiles in both treatment and control groups, there were statistically significant differences between grassed and control systems. Non-vegetated control soils (mean = 0.30 ± 0.03% of initial radioactivity) generated more volatiles than vegetated systems, with an average increase of 0.11 ± 0.03% of total applied [^{14}C]residue ($t = 3.353$ and $p = 0.044$). Volatile organic [^{14}C]-compounds accounted for just 0.20% of the initial portion in systems treated with prairie grasses (SE = 0.01%). The [^{14}C]residue from the polyurethane foam traps was minimal; those results were added to the ethylene glycol trap data and are collectively described as volatiles.

No significant differences were noted between vegetated and non-vegetated systems for mineralization at any point during the study. A total of 1.05 ± 0.02% of applied [^{14}C]-residue was mineralized in vegetated systems and 1.27 ± 0.17% in non-vegetated systems. Toward the end of the testing period (around day 70), there appeared to be a trend toward greater mineralization rates in non-vegetated systems (data

not shown). Previous work has shown similar mineralization rates as those observed in this study (*6*, *25*).

The mixed prairie grass system lowered the levels of parent metolachlor in soil, indicating success as a remediation tool. Additionally, metolachlor and metabolites appeared to be less bioavailable (based on extractability) in the vegetated soils, indicating decreased potential for toxicity of the soil or leaching of the residues, which are other key components for successful remediation. Although rhizosphere microbes may be important in biodegradation, plant uptake appears to be the main mechanism of phytoremediation of metolachlor in this prairie grass system. Levels of volatilization, mineralization, and metabolites in soil were comparable to those described by Rice et al. (*6*), and metolachlor ESA was the major metabolite in soil and in grass tissue. Levels of metolachlor and key metabolites in grasses were slightly below the tolerance limits set by the U.S. EPA. The study presented here provides new information on the fate of metolachlor in a grassed−soil phytoremediation system. These data are important for elucidating the principal mechanism of phytoremediation of metolachlor, which appears to be via plant uptake and probable biotransformation. The study also provides important degradation data in a mass balance format. It is important, for implementation of phytoremediation strategies, that we understand the mass balance of the pesticides and their uptake/detoxification by the plants being used. Further studies are needed to determine the biochemical role of the grasses in relation to uptake and metabolism of metolachlor, or uptake of selected metabolites, as well as the fate of residues in senescing and decaying plant tissues.

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