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The Use of Vegetation to Enhance Degradation of Ethylene Glycol and Propylene Glycol: Prevention of Runoff and Movement to Surface Waters

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Dr. Joel R. Coats

Pesticide Toxicology Laboratory
Iowa State University
Ames, IA 50011-3140

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Dr. Fred Hedberg and
Dr. Walter Kozumbo
Program Managers
Air Force Office of Scientific Research
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14. ABSTRACT

There is a growing concern about the quantity of aircraft deicing agents (primarily ethylene glycol (EG) or propylene glycol (PG)) that migrate offsite and inadvertently contaminate the soil and water environments. However, it is known that vegetation can enhance the removal of man-made organic compounds and pollutants in soil environments by plant uptake and microbial degradation in the rhizosphere. Therefore, three objectives of this research were to: 1) determine the influence of rhizosphere microorganisms on the degradation of ethylene glycol (EG) and propylene glycol (PG), 2) identify environmental variables affecting the degradation of EG and PG in soil, and 3) determine the influence of vegetation on EG and PG movement in soil. Results from these studies found that degradation of EG and PG was enhanced in rhizosphere soils compared with nonvegetated soils. Also, increased soil temperatures enhanced the mineralization of EG and PG, and aquatic emergent plants significantly enhanced mineralization of EG and PG in surface water systems. Lastly, results from this study indicate vegetation reduced the quantity of PG and EG that moved through the soil profile.

15. SUBJECT TERMS
deicing agents, phytoremediation, bioremediation, ethylene glycol, propylene glycol, leaching, runoff, biodegradation

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EXECUTIVE SUMMARY

INTRODUCTION

There is a growing concern about the quantity of aircraft deicing agents that migrate offsite and inadvertently contaminate the soil and water environments. Propylene glycol (1,2-propanediol) is widely used in aircraft deicing agents and vehicular antifreeze. Type I deicers that are commonly used in North America consist of a minimum of 80% glycol by weight, primarily ethylene glycol (EG) or propylene glycol (PG). Under FAA regulation, deicing agents must be used to remove and prevent ice and frost from accumulating on aircraft and airfield runways. As a result, over 43 million liters of aircraft deicing products are used each year nationwide [1]. During severe storms, large planes may require thousands of gallons of deicing-fluids per deicing event. An estimated 80% of the fluids spill onto the ground, ultimately causing on-site pooling, soil infiltration, runoff, and contamination of soil, surface water and groundwater aquifers [1-3].

Vegetation can enhance the removal of man-made organic compounds and pollutants in soil environments by microbial degradation in the rhizosphere and plant uptake [4,5]. Increased diversity and biomass of microbial communities in the rhizosphere render this zone better for degradation of industrial chemicals (trichloroethylene [6], polycyclic aromatic hydrocarbons [7]), and petroleum [8] in rhizosphere soil compared to root-free soil. Vegetation may play a vital role in reclaiming polluted ecosystems and preventing further contamination by enhancing degradation and uptake into tissues, thereby reducing migration to surface waters and groundwater aquifers.

The three objectives of this research project, as described in the original proposal, were:

1. Determine the influence of rhizosphere microorganisms on the degradation of ethylene glycol (EG) and propylene glycol (PG).
2. Identify environmental variables affecting the degradation of EG and PG in soil.
3. Determine the influence of vegetation on EG and PG movement in soil.

ACCOMPLISHMENTS

Objective 1. Determine the influence of rhizosphere microorganisms on the degradation of ethylene glycol (EG) and propylene glycol (PG).

A. Ethylene glycol (see appendix 1)

Comparisons of the biodegradation of 14C-ethylene glycol ([^{14}C]EG) in rhizosphere soils from five different plant species, nonvegetated soils, and autoclaved control soils at various temperatures (-10°C, 0°C, 20°C) indicate enhanced mineralization ($^{14}CO_2$ production) in the rhizosphere soils. After 28 days at 0°C, 60.4%, 49.6%, and 24.4% of applied [^{14}C]EG degraded to $^{14}CO_2$ in the alfalfa (*Medicago sativa*), Kentucky bluegrass (*Poa pratensis*), and nonvegetated soils, respectively. Ethylene glycol mineralization was also enhanced with increased soil temperatures. Our results provide evidence that plants can enhance the degradation of EG in soil. Vegetation may be a method for reducing the volume of aircraft deicers in the environment and minimizing offsite movement to surface waters.

B. Propylene glycol (see appendix 2)

Root zone soils from several grasses and legumes were tested for their ability to enhance mineralization of propylene glycol (PG). Propylene glycol (1000 ppm) was applied to root zone soils incubated at either 22°C or 7°C under darkened conditions. Root zone soils from legumes showed the highest rate of mineralization, however, soils from various grasses did not show consistently different mineralization rates from each other. Mineralization rates decreased 2.2 to 4.2-fold at the lower temperature; however, treatment soils still mineralized propylene glycol faster than autoclaved controls.

Objective 2. Identify environmental variables affecting the degradation of ethylene glycol (EG) and propylene glycol (PG) in soil (see appendix 3).

The mineralization of [^{14}C]EG and [^{14}C]PG in sterile control soil, nonvegetated soil, and soil containing *Scirpus fluniatilis*, *Scirpus acutus*, and *Scirpus validus* were determined. Elevated levels of $^{14}CO_2$ in whole-plant systems indicate accelerated mineralization in the vegetated treatments compared to the nonvegetated and sterile control soil samples. After a 7-d incubation period, aquatic macrophytes enhanced the mineralization of [^{14}C]PG by 11% to 19% and [^{14}C]EG by 6% to 20%. Less than 8% of applied radiocarbon was detected in the plant tissues, with the majority of the ^{14}C recovered in the roots. Artificial wetland and

shallow storage basins cultured with aquatic macrophytes may be valuable for treating airport and air base runoff, thus reducing the biological oxygen demand and glycol concentrations in receiving waters.

Objective 3. Determine the influence of vegetation on ethylene glycol (EG) and propylene glycol (PG) movement in soil (see appendices 4 and 5).

Undisturbed soil columns were planted with alfalfa (*Medicago sativa*) or rye grass (*Lolium perenne L.*) to assess their potential to reduce the infiltration of ethylene glycol (EG) or propylene glycol (PG). Ethylene glycol and PG were applied to the surface of nonvegetated, *M. sativa*, and *L. perenne* soil columns and leached daily with deionized water. Results from this study indicate vegetation reduced the quantity of PG and EG that moved through the soil profile. Leachates from the vegetated soil columns contained significantly less PG than leachates from the nonvegetated columns. Similar results were for EG. These results suggest that plants can reduce the mobility of glycol-based deicing fluids in the soil and minimize its potential to leach and contaminate groundwater.

PERSONNEL

The co-principal investigator, Dr. Todd Anderson, is now an Associate Professor at Texas Tech University, in their Institute for Human and Environmental Health. Dr. Patricia Rice finished her Ph.D. and worked as a postdoctoral research associate here until she accepted a new position at BASF in Princeton, NJ, in their Environmental Fate (agrichemicals) group. Dr. Ellen Arthur worked as a postdoctoral research associate here until she accepted a new position at Bayer Corporation in Stillwell, KS, in their Environmental Fate (agrichemicals) group.

INTERACTIONS

A. Presentations

1997 Phytoremediation of surface water and soil contaminated with aircraft deicing agents, Annual Meeting of the Society of Environmental Toxicology and Chemistry, Ozark-Prairie Regional Chapter, Columbia, Missouri, May 15-17. (Poster)

1997 Phytoremediation of surface water and soil contaminated with aircraft deicing agents, the 12th Annual Conference on Hazardous Waste Research at Kansas City, Missouri, May 20-22, 1997. (Poster)

1996 The use of plants to enhance microbial degradation of deicing agents in soil and surface water, 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington, D.C., November 17-21. (Platform)

1996 The use of vegetation to enhance biodegradation and reduce off-site movement of aircraft deicers, 212th National Meeting of the American Chemical Society, Orlando, Florida, August 25-29. (Poster)

1996 The fate of methyl bromide, ethylene glycol, and propylene glycol in soil and surface water: Influence of soil variables and vegetation on degradation and off-site movement, Department of Entomology Seminar Series, Iowa State University, December 2, 1996. (Platform)

B. Publications

Arthur, E.L., P.J. Rice, P.J. Rice, T.A. Anderson, and **J.R. Coats**. 1998. Mobility and degradation of pesticides and their degradates in intact soil columns. In F. Fuehr et al. (eds.), Comprehensive Tracer Studies on the Environmental Behavior of Pesticides: the Lysimeter Concept, American Chemical Society, Washington, DC pp. 88-114.

Rice, P.J., T.A. Anderson, and **J.R. Coats**. 1997. Evaluation of the use of vegetation for reducing the environmental impact of deicing agents. In E.L. Kruger, J.R. Coats, and T.A. Anderson, eds., *Phytoremediation of Soil and Water Contaminants*, ACS, Symposium Series. American Chemical Society, Washington, DC pp. 162-176.

Rice, P.J. 1996. *The Fate of Methyl Bromide, Ethylene Glycol, and Propylene Glycol in Soil and Surface Water: Influence of Soil Variables and Vegetation on Degradation and Off-site Movement*. Ph.D. Dissertation, Iowa State University, Ames, IA.

Rice, P.J., T.A. Anderson, and **J.R. Coats**. The use of aquatic plants to remediate surface waters contaminated with aircraft deicing agents. *Environ. Toxicol. Chem.* (to be submitted)

Rice, P.J., T.A. Anderson, and **J.R. Coats**. The influence of vegetation on the mobility of propylene glycol and ethylene glycol through the soil profile. *Environ. Toxicol. Chem.* (to be submitted)

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APPENDIX 1. Evaluation of the use of vegetation for reducing the environmental impact of deicing agents

CHAPTER 3. EVALUATION OF THE USE OF VEGETATION FOR REDUCING THE ENVIRONMENTAL IMPACT OF DEICING AGENTS

A paper to be submitted to *Environmental Toxicology and Chemistry*

Patricia J. Rice,† Todd A. Anderson‡ and Joel R. Coats†

Abstract This research project was conducted to evaluate the use of plants for reducing the environmental impact of aircraft deicers. Significant quantities of ethylene glycol-based deicing fluids spill to the ground and inadvertently contaminate soil and surface water environments. Comparisons of the biodegradation of ^{14}C-ethylene glycol ([^{14}C]EG) in rhizosphere soils from five different plant species, nonvegetated soils, and autoclaved control soils at various temperatures (-10 °C, 0 °C, 20 °C) indicate enhanced mineralization ($^{14}CO_2$ production) in the rhizosphere soils. After 28 days at 0 °C, 60.4%, 49.6%, and 24.4% of applied [^{14}C]EG degraded to $^{14}CO_2$ in the alfalfa (*Medicago sativa*), Kentucky bluegrass (*Poa pratensis*), and nonvegetated soils, respectively. Ethylene glycol mineralization was also enhanced with increased soil temperatures. Our results provide evidence that plants can enhance the degradation of ethylene glycol in soil. Vegetation may be a method for reducing the volume of aircraft deicers in the environment and minimizing offsite movement to surface waters.

Keywords- Ethylene glycol Propylene glycol Phytoremediation Rhizosphere soil

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†Pesticide Toxicology Laboratory, Iowa State University, Ames, Iowa 50011

‡The Institute of Wildlife and Environmental Toxicology, Clemson University, Pendleton, SC 29670

INTRODUCTION

Under FAA regulation, deicing agents must be used to remove and prevent ice and frost from accumulating on aircraft and airfield runways. Aviation deicing-fluids used in North America primarily consist of ethylene glycol (EG) and/or propylene glycol (PG) with a minimal amount of additives [1]. Vast quantities of glycols enter the environment through deicing of aircraft, spills, and improper disposal of used antifreeze. Approximately 43 million L/yr of aircraft deicing products are used nationwide. During severe storms, large planes may require thousands of gallons of deicing-fluid per deicing event [1]. An estimated 80% of the fluids spill onto the ground, which may lead to the contamination of soil, surface water, and groundwater [1-3]. Runoff may also be collected in airport storm-sewer systems and directly released (untreated) into streams, rivers, or on-site retention basins [1,2,4,5]. Airport runoff and storm-sewer discharge have been found to contain concentrations of EG ranging from 70 mg/L to > 5,000 mg/L [1]. Hartwell et al. [3] reported 4,800 mg/L EG in a creek which had received drainage from an airport storage basin. Ethylene glycol has been detected in groundwater at 415 mg/L [1] and 2,100 mg/L [6]. Surface waters contaminated with airport runoff have been shown to be harmful to aquatic communities [1,4,7]. Fisher and co-workers [8] studied the acute impact of airport storm-water discharge on aquatic life and reported a 48-h LC50 of 34.3 and 69.3% effluent for *Pimephales promelas* and *Daphnia magna,* respectively. The primary concern of untreated runoff released into surface waters is the high biological oxygen demand produced by the rapid biodegradation of EG and PG. Even dilute levels of contamination may deplete the available dissolved oxygen, resulting in asphyxiation [1,2,4,7]. Fish kills have been observed in waters with direct discharge of airport runoff and waste [1].

Vegetation can enhance the removal of human-made organic compounds and pollutants in soil environments by microbial degradation in the rhizosphere and plant uptake [9,10]. The rhizosphere is the region of soil influenced by the roots. Plant roots secrete energy rich exudates and mucilages which support large and diverse populations of microorganisms [11-14]. Increased

diversity and biomass of microbial communities in the rhizosphere render this zone better for degradation of organic pollutants. Previous research has shown enhanced degradation of industrial chemicals such as trichloroethylene [15,16], polycyclic aromatic hydrocarbons [17], and petroleum [18] in rhizosphere soil as compared with root-free soil. In addition to enhanced degradation in the rhizosphere, plants may take up contaminants as part of their transpiration stream [9]. Vegetation may play a vital role in remediating polluted ecosystems and preventing further contamination by enhancing degradation and uptake into tissues, thereby reducing migration to surface waters and groundwater aquifers.

Previous research has revealed that microbial degradation of EG can occur in both aerobic and anaerobic environments. Several genera of bacteria that utilize EG as a carbon and energy source have been isolated [19-21]. Only recently has the fate of EG been studied in the soil, despite the widespread use of this compound [5,22]. McGahey and Bouwer [22] studied the biodegradation of EG in simulated subsurface environments, utilizing inocula from soil, groundwater, and wastewater. They concluded that naturally occurring microorganisms were capable of degrading EG and that substrate concentration, soil type, temperature, and quantity of oxygen affect the rate of biodegradation. In addition, Klecka and co-workers [5] measured the biodegradation rates of five different aircraft deicing-fluids in soil collected near an airport runway. Rates of degradation for the deicers ranged from 2.3 to 4.5 mg/kg soil per day and 66.3 to 93.3 mg/kg soil per day for samples at -2 °C and 25 °C, respectively.

Recently, there has been interest in reducing the contamination of glycol-based deicing agents in the environment, because of their widespread use and adverse effects on aquatic ecosystems. The purpose of our research was to evaluate the use of plants to enhance the biodegradation of glycols in soil. In addition, we observed the influence of two potential rate-limiting factors (soil temperature and substrate concentration) on the mineralization rate of EG in the rhizosphere and nonvegetated soils.

MATERIALS AND METHODS

Chemicals

Ethylene glycol (EG) and propylene glycol (PG) were obtained from Fisher Scientific (Fair Lawn, NJ) and Sigma Chemical Company (St. Louis, MO). The radiolabeled compounds ethylene glycol-1,2-^{14}C ([^{14}C]EG) and uniformly labeled propylene glycol ([^{14}C]PG) were purchased from Aldrich Chemical Company (Milwaukee, WI) and New England Nuclear-Dupont (Boston, MS). Upon receipt, the [^{14}C]EG and [^{14}C]PG were diluted with ethylene glycol and propylene glycol to yield a stock solution of 0.277 μCi/μl and 0.247 μCi/μl, respectively.

Soil collection

Pesticide-free soil was collected from the Iowa State University Agronomy and Agricultural Engineering Farm near Ames, (Boone County) Iowa. Ten golf-cup cutter (10.5 cm x 10 cm, Paraide Products Co.) soil samples were randomly removed from the field and combined for each replicate. Samples were sieved (2.0 mm), placed in polyethylene bags, and stored in the dark at 4 °C until needed. Soils were analyzed by A & L Mid West Laboratories (Omaha, NE) to determine physical and chemical properties. The sandy loam soil had a measured pH of 6.6 and consisted of 54% sand, 29% silt, 17% clay, 3.1% organic matter.

In cooperation with the Air Force, soil samples were collected from Offutt Air Force Base (Omaha, NE). The sampling sites were adjacent to airport runways or taxiways where deicing activities had once occurred (Fig. 1). Observations were made on the type of vegetation located in these areas. The top six inches of soil was collected with a shovel. Upon return to the laboratory the soils were sieved, analyzed by A & L Mid West Laboratories (Table 1), and stored as described above. Three 10-g soil (dry weight) subsamples from each site were analyzed to determine if ethylene glycol or propylene glycol was present in the soil. Ten grams of soil (dry weight) were extracted with 30 ml methanol and analyzed on a Varian 3740 gas chromatograph (Varian Associates, Sunnyvale, CA, USA) equipped with a flame ionization

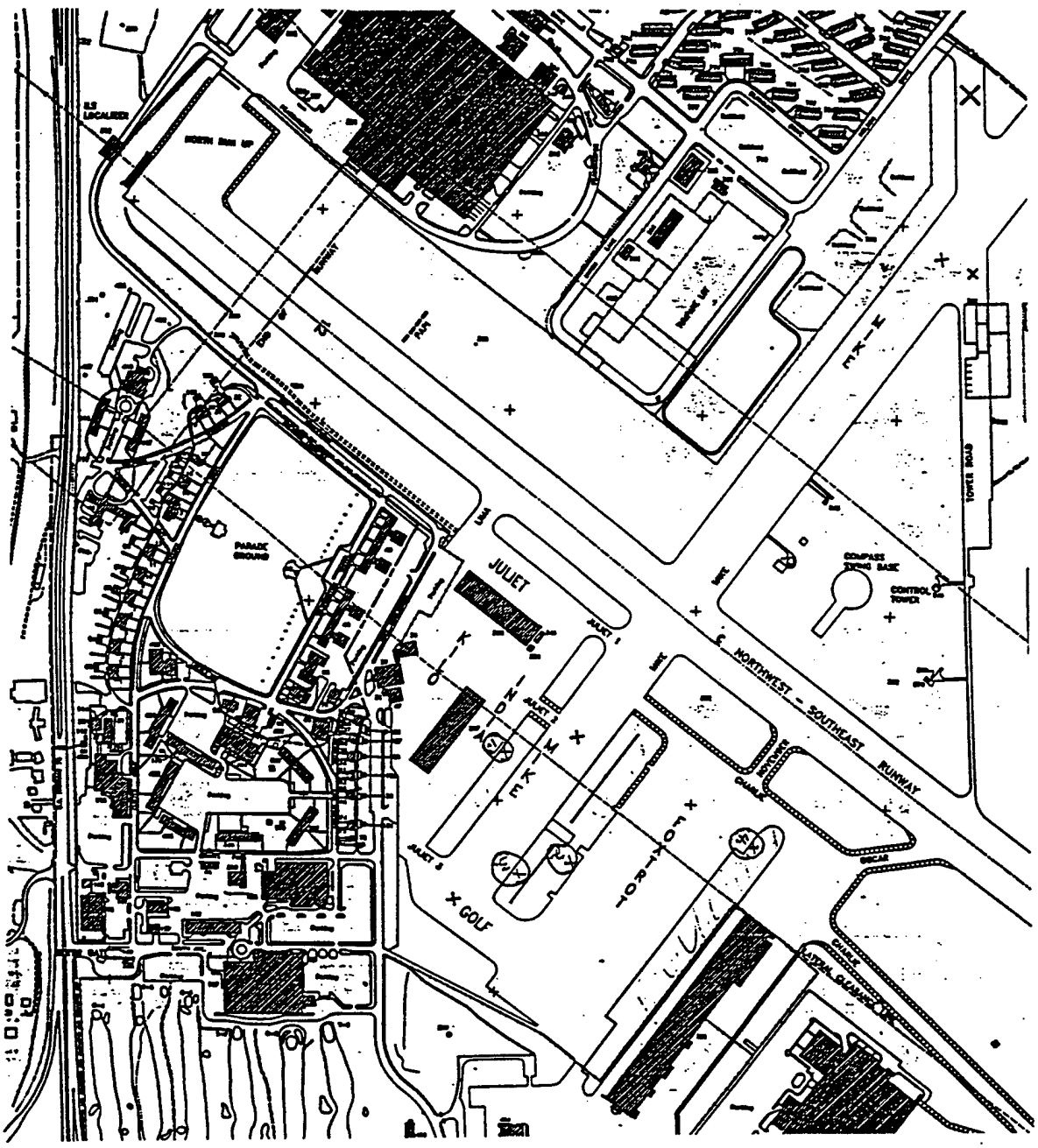

Fig. 1. Sampling sites at Offutt Air Force Base, Omaha, NE.

Table 2. Soil characteristics of surface soil (0-10 cm) from three fields professionally fumigated with methyl bromide

	Texture	Sand (%)	Silt (%)	Clay (%)	O.M.[a] (%)	C.E.C.[b] (meq/100g)	pH[c]
Field 1	Clay loam	42	28	30	3.3	18.4	7.4
Field 2	Sandy clay loam	48	28	24	2.9	14.2	7.2
Field 3	Clay loam	40	32	28	3.4	17.2	7.1

[a]Organic matter.

[b]Cation exchange capacity.

[c]1:1 (soil:distilled water).

detector and a 31 cm x 2 mm (ID) column containing 5% Carbowax 20M coated on Chromosorb WHP (Supelco, Inc., Belefonte, PA, USA) [23]. The soil extracts were analyzed at column temperatures ranging from 120 °C to 160 °C.

Rhizosphere soils from several different grass and legume plant species were used in this study. Plants were grown from seed for 6 to 8 weeks in pesticide-free soil under the same environmental conditions (25 °C, 14:10 light:dark cycle). The different plant species consisted of tall fescue (*Festuca arundinacea*), perennial rye grass (*Lolium perenne L.*), Kentucky blue grass (*Poa pratensis L.*), alfalfa (*Medicago sativa*), and birdsfoot trefoil (*Lotus corniculatus*). These plants were chosen to represent vegetation that may be found adjacent to airport deicing areas, airport runways, and leguminous plants capable of fixing atmospheric nitrogen. Rhizosphere soil was collected from each plant species. Soil that closely adhered to the roots was considered rhizosphere soil. In addition, a mixed rhizosphere soil was studied. Mixed rhizosphere soil was collected from soil that contained the cool season grasses (*F. arundinacea, P. pratensis*), a legume (*M. sativa*), and *L. perenne*. Soils were sieved (2 mm), placed in a polyethylene bag, and stored in the dark at 4 °C for less than 48 h before they were used in the degradation studies.

Degradation study: treatment and incubation

Portions of the [^{14}C]EG stock solution were diluted with acetone and ethylene glycol to make a 100 μg/g (0.5 μCi/0.004 g), 1,000 μg/g (0.5 μCi/0.04 g), and 10,000 μg/g (0.5 μCi/0.4 g) treating solutions. A measured 1,000 μg/g [^{14}C]EG and [^{14}C]PG were applied to rhizosphere soil, Offutt site soil, nonvegetated soil, and autoclaved (autoclaved 3 consecutive d for 1 h) soil. In addition, 100 μg/g and 10,000 μg/g [^{14}C]EG were added to *M. sativa* rhizosphere soil and nonvegetated soil determine the effect of substrate concentration on the rate of EG mineralization. After the acetone evaporated from the soil, four 10- or 20-g (dry weight) subsamples of the treated soils were transferred to individual incubation jars, and the soil moistures were adjusted to 1/3 bar (-33 kPa). One sample from each soil treatment was extracted three times with either

30 ml 9:1 (v/v) $CH_3OH:H_2O$ or 30 ml CH_3OH to determine the actual quantity of ^{14}C applied to the soil. The extraction efficiencies ranged from 95% to 103%. The three remaining samples were the three replicates for each soil treatment. A vial containing 3 ml 2.77 M NaOH was suspended in the headspace of each incubation jar to trap $^{14}CO_2$ evolved from the mineralization of [^{14}C]EG. These traps were replaced every 24 h for the first 3 d, and every 48 h thereafter for the remainder of the study. The quantity of [^{14}C]EG mineralized to $^{14}CO_2$ was determined by radioassaying subsamples of the NaOH on a RackBeta® model 1217 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). Soils were incubated at -10 °C, 0 °C, and 20 °C for 30 d (28 to 30 d).

Mineralization is considered the ultimate degradation of an organic compound. The $^{14}CO_2$ produced during the mineralization of a radiolabeled substrate can be used to determine the degradation rates of that compound [24]. Therefore we calculated the mineralization time 50% (MT50), the estimated time required for 50% of the applied [^{14}C]EG to mineralize, by using formulas previously used for determining degradation rate constants and half-lives [25,26]. Calculations of MT50s were based on the assumption that the dissipation of ethylene glycol from the soil by mineralization followed first-order kinetics. Linear regressions of the natural log of percentage $^{14}CO_2$ (100% of applied ^{14}C - % $^{14}CO_2$ evolved) vs. time were used to determine the MT50 and coefficients of determination (r^2). Data points used to calculate these values include the quantity of $^{14}CO_2$ produced from the initial treatment of the soil through the log or exponential phase of the mineralization curve (Fig. 2). The lag phase was accounted for in the calculations as described by Larson [26]. Lag time in this study was defined as the number of days before $^{14}CO_2$ exceeded 2% of the applied radiocarbon. The MT50 values compared well with the actual time required for 50% of the applied ^{14}C to mineralize (further discussed in the results). These calculated MT50s were only used to compare the differences between the different soil types at -10 °C, 0 °C, and 20 °C, because oversimplification of the actual mineralization rates

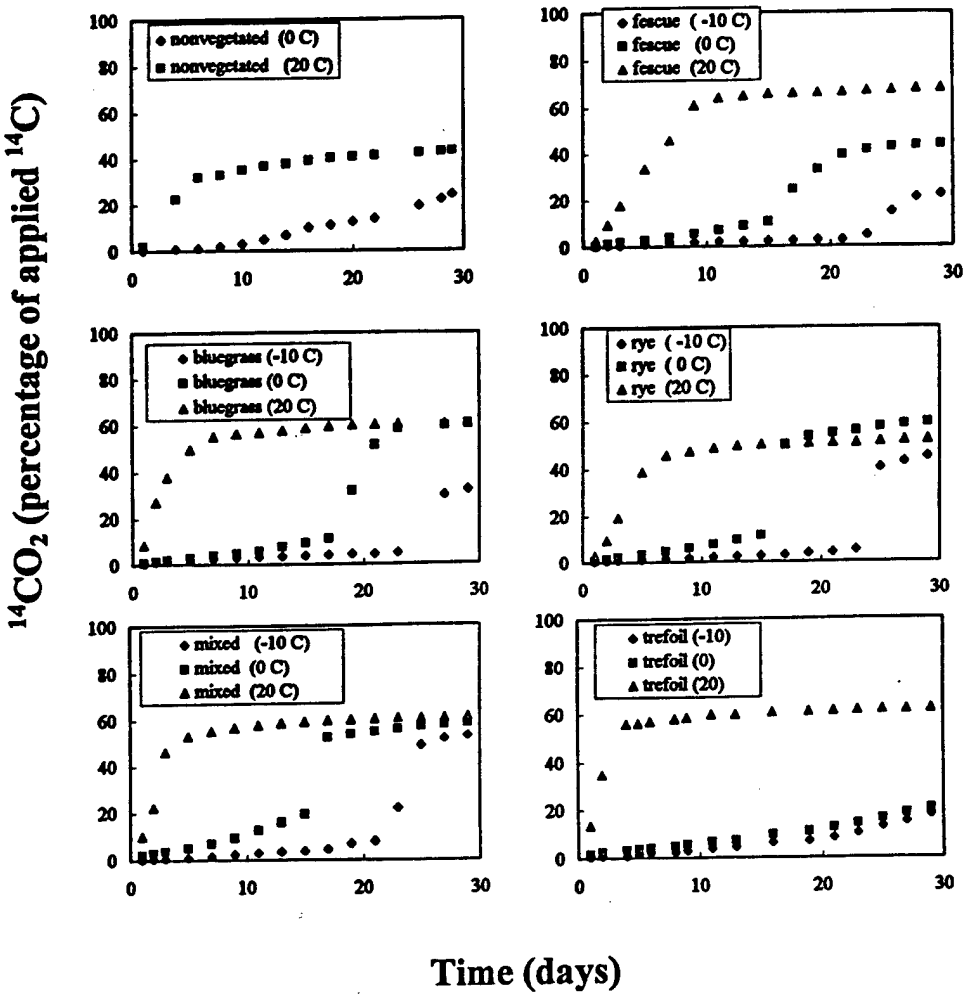

Fig. 2. Mineralization of [^{14}C]ethylene glycol in nonvegetated soils and bluegrass (*P. pratensis*), fescue (*F. arundinacea*), rye (*L. perenne*), trefoil (*L. corniculatus*), and mixed rhizosphere soils at -10 °C, 0 °C, and 20 °C. Mixed rhizosphere soils were collected from soil that contained *M. sativa*, *F. arundinacea*, *L. perenne*, and *P. pratensis*.

may have occurred. Analysis of variance and the least squared means were used to test for significant differences between the different soils at the $p \leq 0.05$ level of significance [27].

Soil extraction and analyses

At the completion of the study, soils were extracted three times with either 30 ml 9:1 (v/v) $CH_3OH:H_2O$ or 30 ml CH_3OH. The extractable ^{14}C was analyzed on a liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). The extracted soils were air dried then crushed and homogenized in a plastic bag. Subsamples of the soils were made into pellets (0.5 g soil and 0.1g hydrolyzed starch) and combusted in a Packard sample oxidizer (Packard Instrument Co.). The $^{14}CO_2$ produced from the soil combustion was trapped in Permafluor® V and Carbo-Sorb® E. Spec-Chec® ^{14}C standard (9.12×10^5 dpm/ml) was used to determine the trapping efficiency. Three to six soil pellets were combusted for each replicate. The soil-bound radiocarbon was quantified by liquid scintillation. The data were statistically analyzed by analysis of variance and least significant differences at 5% [27].

RESULTS

Mineralization of [^{14}C]EG in rhizosphere and nonvegetated soils

The mineralization of different [^{14}C]EG concentrations in nonvegetated and *M. sativa* rhizosphere soil, incubated at 0 °C, is shown in Figure 3 and Figure 4. An inverse relationship was evident between the concentration of [^{14}C]EG applied to the soils and the percentage of radiocarbon mineralized. Significantly ($p \leq 0.05$) smaller percentages of the applied [^{14}C]EG was transformed to $^{14}CO_2$ as the substrate concentration increased. After 28 days, 55.2%, 20.5%, and 7.14% of applied ^{14}C evolved as $^{14}CO_2$ in the nonvegetated soils treated with 100µg/g, 1,000 µg/g, and 10,000 µg/g [^{14}C]EG, respectively. Comparison of the data in the nonvegetated soils (Fig. 3) and the *M. sativa* rhizosphere soil (Fig. 4) indicated significantly ($p \leq 0.05$) enhanced mineralization in the rhizosphere soil. Within 8 days after treatment, the production of $^{14}CO_2$ in

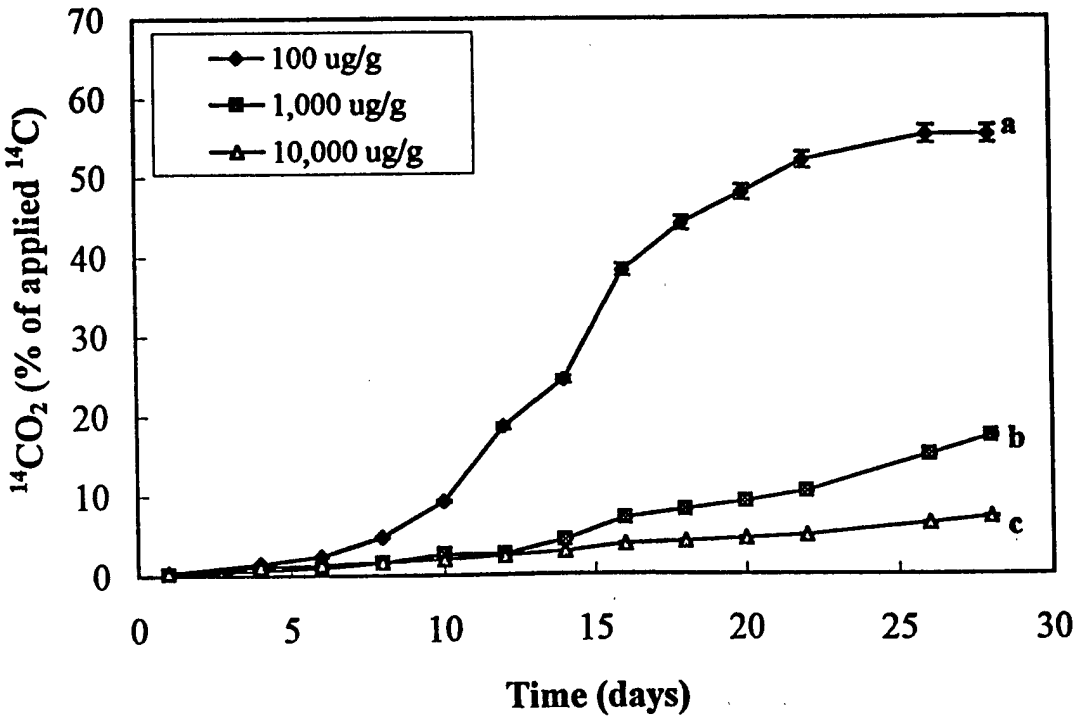

Fig. 3. Mineralization of 100 µg/g, 1,000 µg/g, and 10,000 µg/g [^{14}C]ethylene glycol in nonvegetated soils incubated at 0 °C. Data points are the mean of three replicated ± one standard deviation.

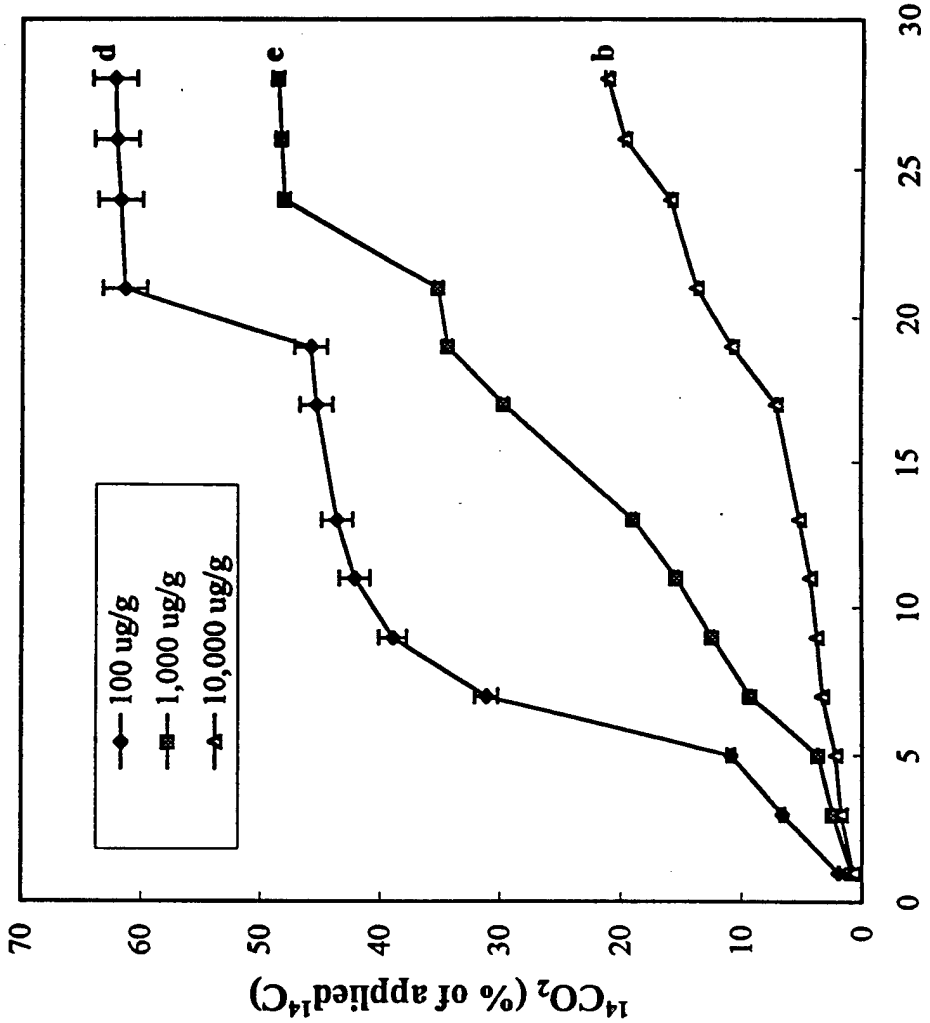

Fig. 4. Mineralization of 100 µg/g, 1,000 µg/g, and 10,000 µg/g [^{14}C]ethylene glycol in *M. sativa* rhizosphere soils incubated at 0 °C. Data points are the mean of three replicated ± one standard deviation.

the 100 μg/g [^{14}C]EG *M. sativa* rhizosphere soils was elevated by 26% compared with the nonvegetated sample at the same concentration. After 28 days, 62.2%, 49.7%, and 21.2% of the added ^{14}C was liberated as $^{14}CO_2$ in the 100 μg/g, 1,000 μg/g, and 10,000 μg/g rhizosphere soils, respectively. Overall, *M. sativa* rhizosphere soils significantly enhanced the mineralization of ethylene glycol by 7% to 29% as compared with the nonvegetated soils with similar [^{14}C]EG concentrations. Furthermore, the total percentage of applied radiocarbon that evolved as $^{14}CO_2$ from the 1,000 μg/g nonvegetated soils and the 10,000 μg/g *M. sativa* rhizosphere soils was not significantly different.

The effect of vegetation and temperature on the degradation of [^{14}C]EG and [^{14}C]PG in the soil was studied by comparing the mineralization of 1,000 μg/g EG and 1,000 μg/g PG in several rhizosphere soils, nonvegetated soils, and sterile soils, and Offutt site soils incubated at -10 °C, 0 °C, and 20 °C (Table 2-4). Examination of $^{14}CO_2$ produced after 15 days showed significantly greater ($p \leq 0.05$) mineralization of [^{14}C]EG as the temperature increased, except for the sterile soils (Fig. 5). A average of 2.7%, 12.2%, and 50.3% of applied radiocarbon was evolved as $^{14}CO_2$ in the *L. perenne* rhizosphere soils incubated at -10 °C, 0 °C, and 20 °C, respectively. *L. corniculatus* rhizosphere soil produced the greatest quantity of $^{14}CO_2$ within the initial 15-day incubation period at -10 °C. No significant differences were observed between the *F. arundinacea, L. perenne,* and *P. pratensis* and the mixed rhizosphere soils. A comparison of the rhizosphere soils, sterile soils, and autoclaved soils at 0 °C and 20 °C indicated that the rhizosphere soils significantly enhanced the mineralization of ethylene glycol. After 15 days, the greatest quantity of $^{14}CO_2$ produced at 0 °C occurred in the mixed and *M. sativa* rhizosphere soils. Over 17.3% and 19.3% of the applied radiocarbon was mineralized in the mixed and *M. sativa* rhizosphere soils compared with 6.73% in the nonvegetated soils. Significant differences were observed between all the soils studied at 20 °C. The transformation of [^{14}C]EG to $^{14}CO_2$ in descending order was *F. arundinacea* rhizosphere>*M. sativa* rhizosphere>*L. corniculatus* rhizosphere>*P. pratensis* rhizosphere>*L. perenne* rhizosphere>mixture rhizosphere

Table 2. Calculated MT50s for [^{14}C]ethylene glycol. MT50s represent the time estimated for 50% of the applied [^{14}C]ethylene glycol to transform to $^{14}CO_2$

Soil sample	Temperature (°C)	MT50 (r^2)[a]
Sterile	-10	>10,000 (r^2=0.81) A
Sterile	0	>10,000 (r^2=0.81) A
Sterile	20	1,523 (r^2=0.99) B
Nonvegetated	0	73 (r^2=0.93) C
Nonvegetated	20	43 (r^2=0.70) D
M. sativa rhizosphere	0	26 (r^2=0.96)E
M. sativa rhizosphere	20	6 (r^2=0.91) F
F. arundinacea rhizosphere	-10	533 (r^2=0.50) G
F. arundinacea rhizosphere	0	28 (r^2=0.69) E
F. arundinacea rhizosphere	20	7 (r^2=0.92) F
L. perenne rhizosphere	-10	40 (r^2=0.56) D
L. perenne rhizosphere	0	20 (r^2=0.83) E,H
L. perenne rhizosphere	20	10 (r^2=0.92) F,H
P. pratensis rhizosphere	-10	59 (r^2=0.56) I
P. pratensis rhizosphere	0	20 (r^2=0.80) E,H
P. pratensis rhizosphere	20	9 (r^2=0.96) F
L. corniculatus rhizosphere	-10	107 (r^2=0.91) J
L. corniculatus rhizosphere	0	103 (r^2=0.95) J
L. corniculatus rhizosphere	20	3 (r^2=0.97) F
mixed rhizosphere[b]	-10	27 (r^2=0.71) E
mixed rhizosphere[b]	0	20 (r^2=0.86) E,H
mixed rhizosphere[b]	20	5 (r^2=0.91) F

[a]Means in each column followed by the same letter are not significantly different ($p = 0.05$).

[b]Samples collected from soils planted with a mixture of *M. sativa*, *F. arundinacea*, *L. perenne*, and *P. pratensis*.

Table 3. Calculated MT50s for [^{14}C]propylene glycol. MT50s represent the time estimated for 50% of the applied [^{14}C]propylene glycol to transform to $^{14}CO_2$

Soil sample	Temperature (°C)	MT50 (r^2)[a]
Sterile	0	>10,000 (r^2=0.90)
Sterile	20	630 (r^2=0.59)
Nonvegetated	0	54 (r^2=0.86)
Nonvegetated	20	13 (r^2=0.64)
M. sativa rhizosphere	0	30 (r^2=0.81)
M. sativa rhizosphere	20	9 (r^2=0.91)
F. arundinacea rhizosphere	-10	
F. arundinacea rhizosphere	0	40 (r^2=0.74)
F. arundinacea rhizosphere	20	5 (r^2=0.85)
L. perenne rhizosphere	-10	
L. perenne rhizosphere	0	18 (r^2=0.85)
L. perenne rhizosphere	20	10 (r^2=0.92)
P. pratensis rhizosphere	-10	
P. pratensis rhizosphere	0	34 (r^2=0.76)
P. pratensis rhizosphere	20	6 (r^2=0.70)
L. corniculatus rhizosphere	-10	
L. corniculatus rhizosphere	0	18 (r^2=0.74)
L. corniculatus rhizosphere	20	5 (r^2=0.55)
mixed rhizosphere[b]	-10	
mixed rhizosphere[b]	0	28 (r2=0.75)
mixed rhizosphere[b]	20	11 (r2=0.54)

[a]A problem with the incubation system at -10 °C caused the temperature to rise above 5 °C, therefore the MT50s and rate constants were not correct for -10 °C and were omitted from the table.

[b]Samples collected from soils planted with a mixture of *M. sativa, F. arundinacea, L. perenne,* and *P. pratensis.*

Table 4. Calculated MT50s for site soils collected at Offutt Air Forc Base.

Soil sample	Temperature (°C)	MT50 (r^2)
Site 1	-10	187 (r^2=0.95)
Site 1	0	95 (r^2=0.93)
Site 1	20	13 (r^2=0.72)
Site 2	-10	128 (r^2=0.85)
Site 2	0	40 (r^2=0.85)
Site 2	20	16 (r^2=0.61)
Site 3	-10	141 (r^2=0.75)
Site 3	0	78 (r^2=0.97)
Site 3	20	17 (r^2=0.768)
Site 4	-10	89 (r^2=0.64)
Site 4	0	34 (r^2=083)
Site 4	20	11 (r^2=0.67)

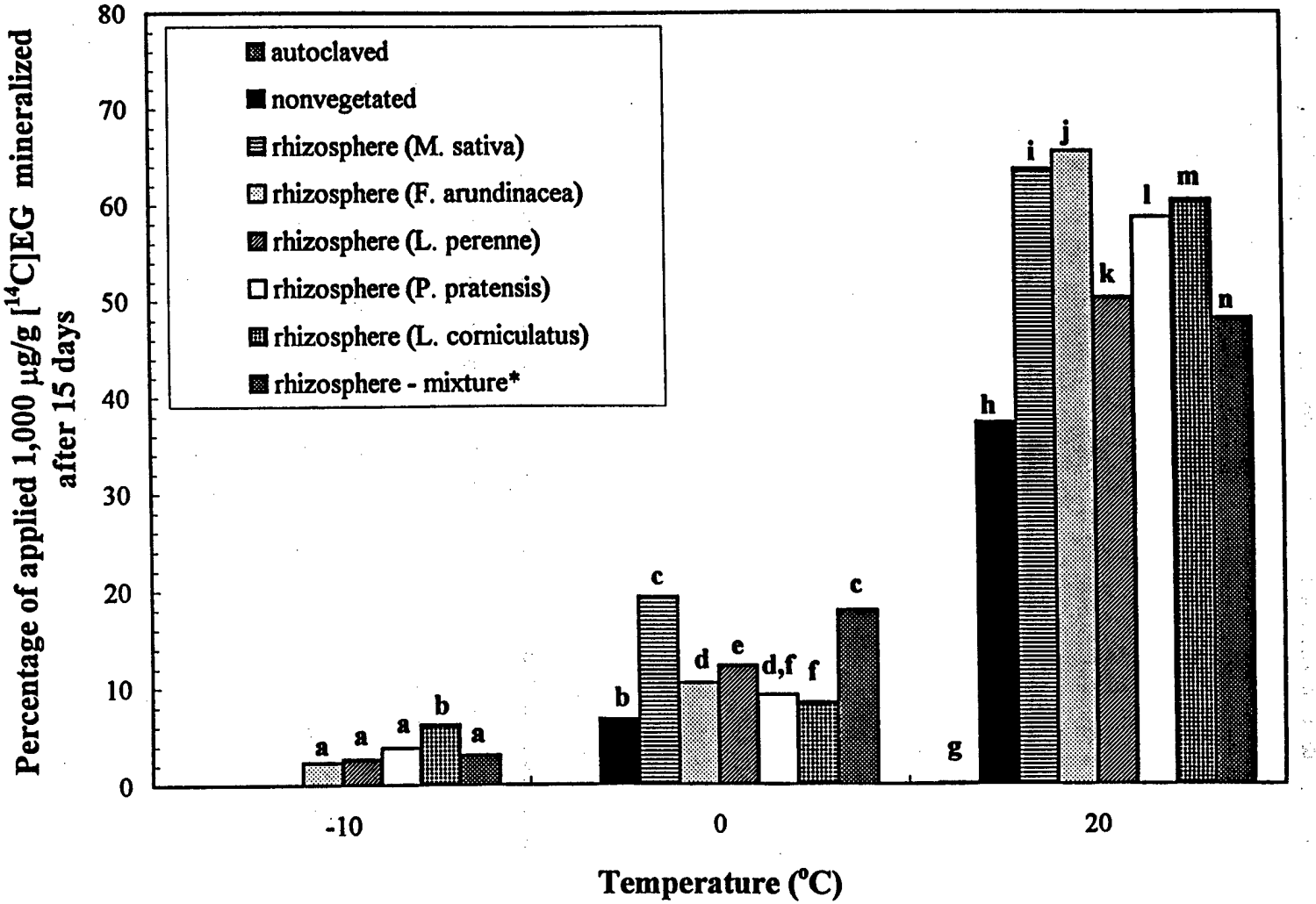

Fig. 5. The effects of vegetation and soil temperature on the mineralization of [^{14}C]ethylene glycol after a 15 d incubation period. Each bar is the mean of three replicates. Bars followed by the same letter are not significantly different (p=0.05).

>nonvegetated>sterile soils. After 15 days, 65.5%, 50.3%, 37.9%, and 0.27% of the applied radiocarbon mineralized in the *F. arundinacea*, *L. perenne*, nonvegetated, and sterile soils, respectively. Comparisons of $^{14}CO_2$ produced after 15 d in [^{14}C]EG and [^{14}C]PG samples indicate [^{14}C]PG mineralized more rapidly in soil than [^{14}C]EG (Fig. 6).

One month (28 d to 30 d) after the application of EG, the different rhizosphere soils continued to enhance the mineralization of [^{14}C]EG by 1.7 to 2.4 times and 1.2 to 1.6 times greater than the nonvegetated soils at 0 °C and 20 °C, respectively (Table 5). Our results showed significantly ($p \leq 0.05$) greater quantities of $^{14}CO_2$ evolved in the soils tested at 20 °C compared with -10 °C, with the exception of the mixed rhizosphere soils. A measured 52.9%, 56.8%, and 53.9% of the applied parent compound was mineralized in the -10 °C, 0 °C, and 20 °C mixed rhizosphere soils, respectively. Further examination of the data at 0 °C and 20 °C (Table 5) revealed no significant differences between the production of CO_2 at 30 days in the *L. perenne*, *P. pratensis*, and mixed rhizosphere soils. After 30 days, the largest quantity of $^{14}CO_2$ that evolved at -10 °C, 0 °C, and 20 °C occurred in the mixed rhizosphere soil, *P. pratensis* and mixed rhizosphere soils, and the *M. sativa* and *F. arundinacea* rhizosphere soils, respectively.

At the completion of the degradation study, the percentage of extractable radiocarbon ranged from 2.4 % to 95.6% (Table 5). Significantly greater quantities of extractable ^{14}C was detected in the sterile soil samples compared with the nonvegetated and rhizosphere soils. Over 93% of the applied radiocarbon was detected in the soil extracts of the autoclaved soils incubated at -10 °C and 0 °C. In addition, extractable ^{14}C was significantly ($p \leq 0.05$) more abundant in the nonvegetated soils incubated at 0 °C than the rhizosphere soils. With the exception of *L. perenne* and mixed rhizosphere soils, significantly greater quantities of extractable radiocarbon were detected in the -10 °C soils compared with the 20 °C soils. The extractable radiocarbon was not significantly different between the biologically active soils at 20 °C.

The quantity of soil-bound residues detected in the soil samples, ranged from 2.7% to 34.0% of the applied radiocarbon (Table 5). Examination of the data in Table 5 indicated that

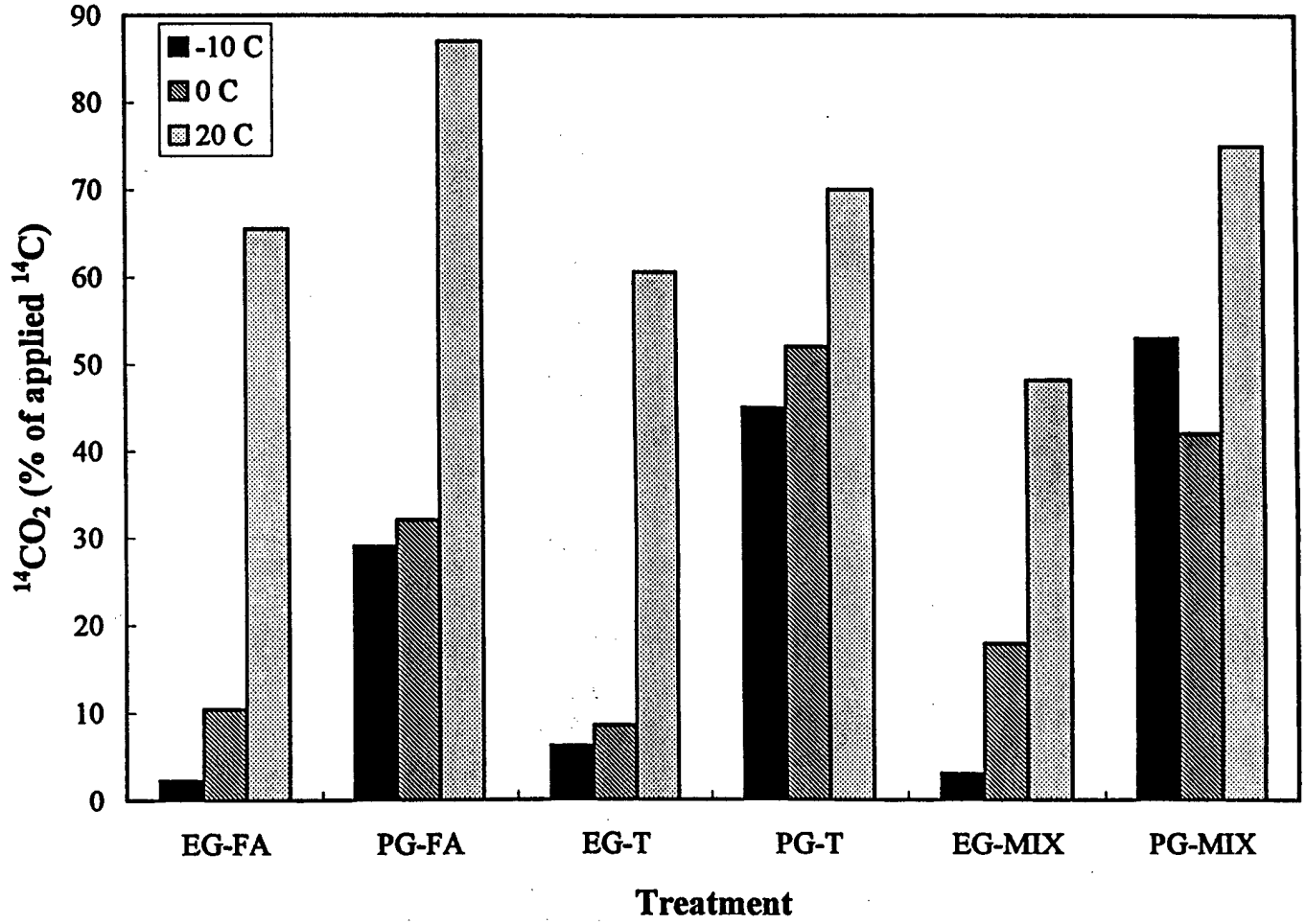

Fig. 6. Mineralization of ethylene glycol and propylene glycol in different rhizosphere soils incubated at -10 °C, 0 °C, and 20 °C. The symbols represent the following treatments EG (ethylene glycol), PG (propylene glycol), FA (*F. arundinacea* rhizosphere soil), T (*L. corniculatus* rhizosphere soil), and M (mixed rhizosphere soil).

Table 5. The effect of vegetation and soil temperature on the degradation of [^{14}C]EG after a 30 d incubation period (reported as percentage of applied ^{14}C)

Soil sample	Temperature (°C)	CO_2[a]	Extractable[a]	Soil-bound residues[a]	Mass balance
Sterile	-10	0.03 A	95.6 A	3.2 AB	98.8
Sterile	0	0.03 A	93.6 A	2.7 A	96.3
Sterile	20	1.7 AB	78.1 B	4.7 B	84.5
Nonvegetated	0	24.4 C	62.8 C	17.5 CD	105
Nonvegetated	20	42.6 D	5.2 D	29.2 E	77.0
M. sativa rhizosphere	0	49.6 EF	3.9 D	34.0 F	87.5
M. sativa rhizosphere	20	71.9 G	4.8 D	26.8 E	104
F. arundinacea rhizosphere	-10	22.2 C	24.8 E	23.3 G	70.3
F. arundinacea rhizosphere	0	43.6 D	5.6 D	22.1 G	71.3
F. arundinacea rhizosphere	20	67.8 G	3.5 D	23.0 G	94.3
L. perenne rhizosphere	-10	45.2 DF	3.8 D	23.5 G	72.5
L. perenne rhizosphere	0	47.1 DFH	3.9 D	17.5 CD	68.5
L. perenne rhizosphere	20	52.4 EHI	3.3 D	18.7 C	74.4
P. pratensis rhizosphere	-10	32.2 J	26.7 E	24.6 G	83.5
P. pratensis rhizosphere	0	60.4 K	4.2 D	23.4 G	88.0
P. pratensis rhizosphere	20	60.7 K	7.5 D	23.1 G	91.3
L. corniculatus rhizosphere	-10	19.5 C	50.2 F	15.5 D	85.2
L. corniculatus rhizosphere	0	20.1 C	42.8 G	12.7 H	75.6
L. corniculatus rhizosphere	20	62.0 K	2.4 D	11.9 H	76.3
mixed rhizosphere[b]	-10	52.9 EI	4.0 H	23.3 G	80.2
mixed rhizosphere[b]	0	56.8 IK	3.7 D	19.3 C	79.8
mixed rhizosphere[b]	20	53.9 I	3.0 D	18.0 C	74.9

[a]Means in each column followed by the same letter are not significantly different ($p = 0.05$).

[b]Samples were collected from soils planted with a mixture of *M. sativa, F. arundinacea, L. perenne,* and *P. pratensis.*

the rhizosphere and nonvegetated soils had significantly ($p \leq 0.05$) greater quantities of bound residues than sterile soils.

Calculated MT50 and mineralization rate of [^{14}C]EG mineralization

Ethylene glycol was mineralized at a faster rate in rhizosphere soils than nonvegetated or sterile soils (Table 2 and 3). The MT50s were determined for all the different soil types studied at the various temperatures. Smaller MT50 values represent faster mineralization rates. The MT50 for [^{14}C]EG in the sterile soils, nonvegetated soils, and *F. arundinacea* rhizosphere soils incubated at 20 °C was 1523 d, 43 d, and 7 d, respectively. Calculated MT50 values compared well with the actual time required for 50% of ethylene glycol to mineralize in the soil. Approximately 50 % of the ethylene glycol applied to *P. pratensis* and *F. arundinace* rhizosphere soils at 0 °C and 20 °C was mineralized in 20 d to 21 d and 7 d to 8 d compared with 20 d and 7 d for the calculated MT50s, respectively. Among the soils evaluated at -10 °C, the rate of ethylene glycol mineralization was greatest to least for mixed rhizosphere>*L. perenne* rhizosphere>*P. pratensis* rhizosphere>*L. corniculatus* rhizosphere>*F. arundinacea* rhizosphere>nonvegetated>sterile soils. Except for the *L. corniculatus* rhizosphere soils, the MT50s were not significantly different between the rhizosphere soils incubated at 0 °C. Based on the MT50s (Table 2), mixed rhizosphere soils mineralized ethylene glycol approximately 1.5 times to 19.7 times faster than the other rhizosphere soils at the same temperature and 1.6 times faster than the nonvegetated soils at 20 °C.

Furthermore, the data (Table 2 and 3) indicate the MT50s significantly ($p \leq 0.05$) decreased with increased temperatures. The MT50 for *F. arundinacea* rhizosphere soil at -10 °C, 0 °C, and 20 °C were 533 d, 28 d, and 7 d, respectively. Increasing the temperature from -10 °C to 20 °C for *F. arundinacea* rhizosphere soils enhanced the mineralization rate by a factor of 76. Generally, a 15 d to 21 d and a 21 d to 27 d lag phase was observed in the 0 °C and -10 °C soil samples, respectively (Fig. 2). Low quantities of $^{14}CO_2$ (<6% of applied ^{14}C) were produced during the

lag phase. Nonvegetated and rhizosphere soils incubated at 20 °C showed no lag phase and consistently mineralized >45% of the applied radiocarbon within 9 d after treatment.

DISCUSSION

Results obtained from our investigation indicate that vegetation can enhance the mineralization rate of [^{14}C]EG and [^{14}C]PG in the soil. Significantly ($p \leq 0.05$) greater quantities of $^{14}CO_2$ were consistently produced in the *M. sativa, F. arundinacea, L. perenne, P. pratensis, L. corniculatus*, and mixed rhizosphere soils than the amount of $^{14}CO_2$ produced in both the sterile control and nonvegetated soils. A comparison of rhizosphere soils and nonvegetated soils showed a two- to four-fold increase in the transformation of [^{14}C]EG to $^{14}CO_2$. The accelerated mineralization rate observed in these soils may be a result of greater microbial biomass and activity generally found in rhizosphere soils [11-14]. Previous research has shown enhanced biodegradation of industrial chemicals [16,17] and pesticides [19-22,24] in rhizosphere soils compared with nonvegetated soils. In addition, microorganisms that utilize ethylene glycol as a carbon and energy source have been previously isolated [20,21].

Results from this study provide strong evidence that mineralization was the predominant factor involved in the dissipation and reduction of ethylene glycol in the soil. Within 30 days, 42.6% to 71.9% of the applied radiocarbon evolved as $^{14}CO_2$ from the biologically active soils at 20 °C. Ethylene glycol mineralization at 0 °C in the sterile soils was minimal (0.03%) compared with the nonvegetated (24.4%) and rhizosphere soils (≥43.6%) indicating that transformation of this aircraft deicer was a microbiological process. Several genera of bacteria have been shown to utilize ethylene glycol as a source of carbon and energy for growth [20,21]. Our results indicate significantly ($p \leq 0.05$) greater quantities of radiocarbon were detected in the soil-bound residues of the nonvegetated and rhizosphere soils compared with the autoclaved soils. Previous research has shown ethylene glycol does not adsorb to soil [23]. Lokke [23] observed the mobility of ethylene glycol through an anaerobic soil column and reported that very little to no

ethylene glycol adsorbed onto the subhorizon of melt water sand, sandy till, and clayey soils. Therefore, we conclude that the increased quantity of ^{14}C soil-bound residues in the biologically active soil was a result of [^{14}C]EG mineralization and, thus, portions of the radiocarbon were incorporated into the cell constituents.

Substrate concentration significantly influenced the mineralization of ethylene glycol in the soil. Our results showed an increase in [^{14}C]EG concentration significantly reduced the percentage of applied radiocarbon that evolved as $^{14}CO_2$ in both the nonvegetated and rhizosphere soils. McGahey and Bouwer [22] noted an increase in the time required for 95% of the applied ethylene glycol to be removed from the samples with increased substrate concentrations. Comparisons of the various [^{14}C]EG concentrations in nonvegetated and *M. sativa* rhizosphere soils clearly indicate that the rhizosphere soil significantly enhanced the mineralization of EG compared with the nonvegetated soils.

A positive relationship occurred between the soil temperature and the ethylene glycol mineralization rate. Increasing the temperature from -10 °C to 20 °C in the biologically active soils resulted in enhanced mineralization rates that were approximately 6 to 7 times faster than the rates noted in the -10 °C soils. Klecka et al. [5] also noted an increase in the biodegradation rate of ethylene glycol from the soil with increased temperatures. Temperature has been shown to greatly effect the enzyme activity and the growth rate of microorganisms [24]. Generally a 10 °C increase approximately doubles the rate of biological reactions [13,14,24]. Examination of our data also indicates that the nonvegetated and rhizosphere soils had significantly ($p \leq 0.05$) greater mineralization rates at -10 °C than the autoclaved soils at 20 °C. These results indicate the microbial communities were able to survive and mineralize ethylene glycol at this cold temperature. Microorganisms are capable of growing and metabolizing organic compounds at low temperatures as long as water continues to exist as a liquid [13,14,24]. The presence of ethylene glycol contamination in the soil may have reduced the freezing point of the water within the soil. Thus, psychrophilic bacteria may have been able to metabolize ethylene glycol at the

subzero temperature. Lag phases were observed in the soils incubated at the two cooler temperatures (-10 °C and 0 °C). This may be due to lower enzyme and biological activity at the cooler temperature and therefore acclimation time was needed. No lag phase was observed in the soils incubated at 20 °C. Rather a large evolution of $^{14}CO_2$ occurred within the first few days after [^{14}C]EG application. Comparisons of the rhizosphere soils and nonvegetated soils at various temperatures indicate that rhizosphere soils significantly ($p \leq 0.05$) enhanced the mineralization of ethylene glycol in the soil.

Rhizosphere soils of different plant species were studied to determine their effect on the mineralization rate of ethylene glycol. Soils were collected from the root zone of various grasses (*F. arundinacea, L. perenne, P. pratensis*), legumes (*M. sativa, L. corniculatus*), and a mixture of these plant species. The mixed rhizosphere soil had the shortest MT50 of the soils incubated at -10 °C. A comparison of the MT50s in the soils incubated at 0 °C indicates the mixed, *P. pratensis*, and *L. perenne* rhizosphere soils had significantly faster mineralization rates than the *M. sativa* and *F. arundinacea* rhizosphere soils, but they were not significantly different from each other. No particular rhizosphere soil collected from an individual plant species was predominately the most efficient at mineralizing ethylene glycol at all three temperatures. The rate of [^{14}C]EG transformation to $^{14}CO_2$ in the mixed rhizosphere soils was unsurpassed at the cooler temperatures (-10 °C and 0 °C) with the most significant difference noted at -10 °C. Approximately, 7% and 30% more $^{14}CO_2$ was produced at -10 °C in mixed rhizosphere soils compared with other rhizosphere soils from individual plant species. In addition, the mineralization rate of [^{14}C]EG in the mixed rhizosphere soils incubated at -10 °C was 1.6 times faster than the mineralization rate in the nonvegetated soils incubated at 20 °C. These results suggest that a mixed culture of plant species would enhance the degradation of aircraft deicers more than a monoculture. Bachmann and Kinzed [28] studied the rhizosphere soils of six different plant species and noted the metabolic activity of the soils were variable depending on the species. The mixed rhizospheres in our study probably had more diverse exudates secreted into the soil from

the mixed plant culture than the monocultures. The mixed rhizosphere soils may have contained more diverse and abundant microbial communities that resulted in greater degradation of ethylene glycol at -10 °C.

The enhanced mineralization of ethylene glycol observed in the rhizosphere soils from these studies may be underestimated in comparison to rhizosphere soils in the natural environment. Plant-soil interactions are responsible for maintaining the increased microbial biomass and activity in the rhizosphere soil. Therefore, by removing the soil from the roots, we may have lost some of the beneficial rhizosphere properties by the end of the experiment [29]. Additional studies are needed that include the intact plant.

CONCLUSION

Our results provide evidence that vegetation may be an effective method for remediating soils contaminated with aircraft deicing fluids. Rhizosphere soils consistently enhanced the degradation of ethylene glycol compared with the nonvegetated soils, regardless of changes in the soil temperature and substrate concentration. In addition, mixed rhizosphere soils were the most prominent ($p \leq 0.05$) soil type for mineralizing ethylene glycol at subzero temperatures. Therefore, a mixed culture of cold-tolerant plant species could be planted alongside airport deicing areas and runways to help enhance the biodegradation of glycol-based deicers that inadvertently contaminate the soil. Facilitating the biodegradation of these deicers in the soil will reduce the offsite migration and minimize the concentration of glycol-based deicers that reach the surface waters, thus reducing their environmental impact.

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APPENDIX 2. Mineralization of propylene glycol in root zone soil

MINERALIZATION OF PROPYLENE GLYCOL IN ROOT ZONE SOIL

Deborah P. Shupack[1] and Todd A. Anderson*[2]

[1]Department of Environmental Toxicology, Clemson University, Clemson, SC 29670

[2]The Institute of Environmental and Human Health, Texas Tech University/Texas Tech University Health Sciences Center, Lubbock, TX 79416

Abstract. Propylene glycol is a common component in aircraft deicing fluids. Runoff from airports may contain high concentrations of propylene glycol, leading to a high biological oxygen demand (BOD) in the runoff. Root zone soils support a diverse microbial community and have been shown to enhance biodegradation of numerous compounds. Root zone soils from several grasses and legumes were tested for their ability to enhance mineralization of propylene glycol. Propylene glycol (1000 ppm) was applied to root zone soils incubated at either 22 °C or 7 °C under darkened conditions. Root zone soils from legumes showed the highest rate of mineralization, however, soils from various grasses did not show consistently different mineralization rates from each other. Mineralization rates decreased 2.2 to 4.2-fold at the lower temperature; however, treatment soils still mineralized propylene glycol faster than autoclaved controls.

Keywords: degradation, root zone, phytoremediation, deicing fluid

*Corresponding author

1. INTRODUCTION

Propylene glycol is a simple organic compound used frequently in pharmaceuticals, food products, and cosmetics (U.S. Department of Health and Human Services, 1997). It is also the primary component in many deicing and anti-icing fluids used in airports around the world. (Deicing and anti-icing fluids will be referred to as simply deicing fluids for the remainder of the paper.) Although it has a low acute toxicity to humans and other animals, it can be hazardous to the environment if it reaches surface water in high concentrations. The rapid biodegradation of propylene glycol in water can lower dissolved oxygen to levels that may be hazardous to the aquatic community (Sills and Blakeslee, 1992). Ethylene or propylene glycol-based deicers are the only type currently permitted by the Federal Aviation Administration (FAA).

Many large airports, and airports that face severe weather conditions frequently through the winter months, are striving to reduce glycol-laden wastewater runoff. Better weather forecasting and ice sensing instrumentation can reduce the amount of deicing fluid needed to keep aircraft safe (Kiernan, 1995). Systems are also being designed to catch spent deicing fluids or divert runoff to treatment or recycling plants (Baker et al., 1994). While these practices can help reduce the environmental impact of using glycol-based deicers, they are cumbersome changes to make in a large established airport and may be too expensive for smaller airports.

Its simple structure makes propylene glycol readily metabolizable by numerous ubiquitous microorganisms in soil and water. This makes biodegradation an attractive option for glycol cleanup. Research has shown that glycols can be degraded by microorganisms in soil (Klecka et al., 1993; Davis-Hoover and Vesper, 1995; Kawai,

1995; Strong-Gunderson et al., 1995; Bausmith and Neufeld, 1996), water (McGahey and Bouwer, 1992), and sewage sludge (Kaplan et al., 1982; Dwyer and Tiedje, 1983; Raja et al., 1991; Kilroy and Gray, 1992; Sabeh and Narasiah, 1992; Nitschke et al., 1996).

A technique available to most airports as a means of glycol control is phytoremediation. Typically, grasses dominate the area surrounding airport runways. While these plants may be useful in reducing runoff, they may not be the most appropriate plants for stimulating microbial degradation of deicing fluid in soil. Management of the indigenous vegetation, and/or incorporation of other plant types may be a low-cost method of reducing deicing fluid in runoff. By reducing the amount of propylene glycol reaching surface waters through reduction of runoff or degradation by rhizosphere microorganisms, the environmental impact of deicing fluids can be minimized. This research investigated the influence of vegetation on the mineralization of propylene glycol in soil.

2. MATERIALS AND METHODS

Root Zone Soils

Soil was collected from the Clemson University Simpson Experiment Station near Pendleton, Anderson County, South Carolina and transported to the greenhouse where it was sieved using a 10 mesh sieve (2 mm). Several plant species and combinations were chosen for this study including common turf grasses, prairie grasses, and legumes (Table I). Plants were grown from seed in plastic cylindrical growth containers. Containers were kept in the greenhouse where they were watered 3

times a day by an automatic sprinkler. Plants were maintained in the greenhouse for 5 weeks after seeding until soils were collected.

The soil was removed from the container and root and plant matter was removed as completely as possible. The soil was homogenized by hand and a 20-g sample was placed in a 125-mL Erlenmeyer flask. Moisture levels varied between samples based on type of plant and root mass. Drier samples were moistened with autoclaved Milli-Q® water (18 mΩ resistance, Bedford, MA) to maintain fairly uniform moisture levels near field capacity.

Sterile (autoclaved) controls were used to monitor $^{14}CO_2$ production from soil by chemical degradation. The control consisted of nonvegetated soil (20 g) placed in a 125-mL Erlenmeyer flask and autoclaved for 45 minutes at 121 °C and 25 psi on 3 consecutive days.

Mineralization Studies

Propylene glycol was obtained from Fisher Scientific (Fair Lawn, NJ). Uniformly labeled ^{14}C-propylene glycol was provided by the Pesticide Toxicology Laboratory at Iowa State University in Ames, Iowa who purchased it from New England Nuclear-DuPont (Boston, MA). A 20,000 ppm (μg/mL) propylene glycol (100,000 DPM/mL) spike solution was made in autoclaved Milli-Q® water (18 mΩ resistance, Bedford, MA). One mL was added to each flask with 20 g of soil to give a final concentration of 1000 ppm (μg/g) propylene glycol in the soil (100,000 DPM per sample). Flasks were incubated at room temperature (22 °C) or in a refrigerator (7 °C) for the duration of each test. Flasks were kept dark and received light only during sampling.

Plastic 7-mL scintillation vials (Fisher Scientific, Fair Lawn, NJ) were used for CO_2 traps. Each vial contained 2 mL of 2.4 M NaOH made with autoclaved Milli-Q® water. The vials were suspended in the flask by a 25 gage wire. Traps were replaced daily for the first 10 days of each experiment, and then daily to once a week depending on the rate of $^{14}CO_2$ production. Experiments continued for a minimum of 24 days and a maximum of 64 days. For the flasks incubated at 22 °C, individual flasks were removed from the experiment when their daily mineralization rates dropped to less than 0.4% of the added radioactivity. For the flasks incubated at 7 °C, the experiment was ended on day 64.

Each day that the traps were changed, 250 µL of the NaOH was removed from the used trap and mixed with 4 mL of ScintiVerse® BD scintillation cocktail (Fisher Scientific, Fair Lawn, NJ). Each sample was counted for 2 minutes on a Beckman LS 6500 Multi-Purpose Scintillation Counter (Beckman Instruments, Fullerton, CA). Counts from a blank of 250 µL NaOH in 4 mL of scintillation cocktail were subtracted from all samples. This method of aliquoting from a larger sample and counting under these conditions was used throughout the study.

During an initial experiment, polyurethane foam traps were also placed in the flasks to trap any volatile organics (^{14}C-propylene glycol or ^{14}C-metabolites) and extracted at the conclusion of the experiment. Since no radioactivity was found in these traps, they were not used in subsequent experiments.

To confirm that the radioactivity measured in the NaOH traps was from $^{14}CO_2$ and not an organic acid, a few milligrams of $Ba(OH)_2$ were added to the trap after an

aliquot was taken for counting. The solution was filtered through a 0.45 µm acrodisk filter (Gelman Science, Ann Arbor, MI) and 250 µL of the filtrate was counted as described above. There was no radioactivity detected in the filtrate, indicating that all the radioactivity was in the form of $^{14}CO_2$.

Mass Balance

At the conclusion of the experiment, soils were analyzed for any extractable ^{14}C. Methanol (20 mL) was added to the flasks and they were shaken at 250 rpm for 1 hour. An aliquot of the extract was filtered through a 0.45 µm acrodisk filter and 250 µL of the filtrate was counted as described above.

The remaining CH_3OH was removed as thoroughly as possible without removing any soil. The flasks were then placed in a fume hood to allow remaining CH_3OH to volatilize. Soil was transferred to a 20-mL scintillation vial and stored at -20 °C until further analysis.

Soils were oxidized using a Biological Material Oxidizer OX-500 (R. J. Harvey Instrument Corporation, Hillsdale, NJ) to determine the amount of unextractable ^{14}C remaining as a bound residue. Soils were oxidized for 2 minutes at 925 °C and the $^{14}CO_2$ was trapped in Carbon 14 Cocktail (R. J. Harvey Instrument Corporation, Hillsdale, NJ). Samples were analyzed on a liquid scintillation spectrophotometer as described above.

Calculations

The cumulative percent of ^{14}C recovered as $^{14}CO_2$ was plotted against time. A rate of mineralization was determined by taking the slope of the best fit line over the

first 8 days. This time period was chosen as it was long enough to include the slight lag period seen in a few samples and short enough to capture only the zero-order mineralization. Rates of mineralization were compared between and within experiments on SigmaStat® using a one way ANOVA and Fisher Least Significance Difference Test with an $\alpha = 0.05$ (Jandel Scientific Software, San Rafael, CA). SigmaStat® verified equal variances and normal distribution. Log transformations were performed on the data in order to conduct this verification.

3. RESULTS

Mass Balance

Mass balance was determined by totaling the DPM recovered from CO_2 traps, extraction, and oxidation. In the autoclaved treatments, recoveries averaged 137% and ranged from 125% to 157%. In all other treatments, recoveries averaged 95% and ranged from 82% to 133%. Within each experiment, there were no significant differences in mass balance among treatments excluding the autoclaved treatments.

Incubation at 22 °C

Mineralization

Root zone soil from alfalfa mineralized propylene glycol faster than soils from all other treatments except nonvegetated soil. The nonvegetated soil had a higher mineralization rate than soil from all other treatments except for the legumes. Soil from trefoil had a higher mineralization rate than soil from all treatments except alfalfa, nonvegetated, and tall fescue. Of the prairie grasses tested, tall fescue root zone soil

had the highest mineralization rate. All treatments mineralized propylene glycol faster than the autoclaved soil.

Mineralization was monitored for 28 to 51 days until daily mineralization rates dropped to 0.4% of the added radioactivity. At this time, 69 to 80% of the radioactivity had been recovered as $^{14}CO_2$ in all treatments except the autoclaved soils where less than 1.5% of the added radioactivity was mineralized. The first eight days of $^{14}CO_2$ production for selected treatments are shown in Figure 1.

Extractable Products

Between 94 and 99% of the applied ^{14}C was detected in the extract of the autoclaved soils. For all other treatments, 0 to 3.2% of the applied ^{14}C remained in the extract. The final distribution of the radioactivity is shown in Table II.

Bound Residues

The autoclaved soils contained more radioactivity as oxidizable ^{14}C than any other treatment (27 to 36% of the applied radioactivity). Among the other treatments, between 5 and 15% of the applied ^{14}C was recovered as bound residue. Few treatments had statistically different amounts of radioactivity as bound residues. However, in general, treatments that had initially higher mineralization rates, such as the legumes and the nonvegetated soil, had lower recoveries as bound residues.

Incubation at 7 °C

Mineralization

Root zone soil from alfalfa had a higher mineralization rate than any other treatment. Trefoil soil had the next highest mineralization rate and was statistically

greater than all other treatments except the nonvegetated soil. The nonvegetated soil had a higher mineralization rate than soils from any of the grasses, but was not statistically higher than the mixed grass + alfalfa soil. There were few differences between the mineralization rates of the soils from the remaining grasses. The first eight days of $^{14}CO_2$ production for selected treatments are shown in Figure 2.

When this experiment was terminated on day 64, several treatments were still mineralizing propylene glycol at rates several times higher than 0.4% per day. By that time, 26 to 85% of the radioactivity had been recovered as $^{14}CO_2$ in all treatments except the autoclaved soils. In the autoclaved soil, less than 1% of the added ^{14}C-propylene glycol was mineralized.

Extractable Products

Several of the treatments were still actively mineralizing ^{14}C-propylene glycol when they were extracted. More ^{14}C was recovered in the extracts from these samples. In the autoclaved soil, 120% of the added ^{14}C was detected in the extract. For other treatments, between 1 and 56% of the added ^{14}C was detected in the extracts. This variability is likely due to the difference in mineralizing capacity of the samples at the time of termination. The final distribution of the radioactivity is shown in Table III.

Bound Residues

In the autoclaved sample, 36% of the added ^{14}C was recovered in the extract. In the remaining treatments, between 6 and 35% of the added ^{14}C remained as bound residue. Again, there was an inverse relationship between the mineralization rates and the amount of ^{14}C recovered as bound residue which was even more pronounced due to the early termination of the experiment.

4. DISCUSSION

Plant matter was removed from the soil before propylene glycol was applied, so there was no vegetation in the experimental flask. Therefore, differences in mineralization rates, extractable ^{14}C-propylene glycol, and bound residues are due to the differences in the microbial communities that had inhabited the root zone soils of the plants.

Autoclaved soils mineralized less than 1.5% of the added radioactivity. This shows that the evolved $^{14}CO_2$ observed in the other treatments is due to biotic activity, not chemical degradation. In these treatments, 52% to 80% of the added radioactivity was captured as $^{14}CO_2$ indicating that mineralization is the primary route for degradation of propylene glycol in soil. This is further supported by the soil extract data where, in the autoclaved treatments, > 95% of the added radioactivity was recovered in the soil extract. In all the other treatments, almost no radioactivity (< 2.5%) was detected in the extract. This indicates that most of the ^{14}C-propylene glycol was consumed and either mineralized to $^{14}CO_2$ or incorporated into the soil or microbial biomass in the treatments, but remained unchanged in the autoclaved soils.

Soil bound residues accounted for 7-13% of the added radioactivity in the treatments. In the autoclaved soils, 32 to 64% was found. After the extraction process, not all of the CH_3OH could be removed. Some of the radioactivity detected during the soil bound residue analysis may have been from combustion of residual CH_3OH extract. This may explain why the soil bound residue values are so high for the autoclaved soils.

Influence of Vegetation

Soils from the legumes had higher propylene glycol mineralization rates than soils from the grasses. These rates were not always statistically greater, but there were no other soils that mineralized propylene glycol significantly faster than soils from the legume treatments. The nonvegetated soil also mineralized propylene glycol faster than soils from the grasses. There were few differences in mineralization rates between the various grass treatments.

While some types of vegetation enhanced initial mineralization rates, they did not effect the overall extent of mineralization as has been observed by Knaebel and Vestal (1992). Rice (1996) compared the MT_{50} (time required for 50% of the applied ^{14}C-propylene glycol to be transformed to $^{14}CO_2$) in various rhizosphere soils and also showed that legumes enhanced mineralization. Rice found enhanced mineralization from all rhizosphere soils over the nonvegetated soil; however, the nonvegetated soil in that study had slower mineralization rates than the nonvegetated soil in the current study.

Influence of Temperature

The temperature of incubation had a definite effect on mineralization rates. Mineralization rates were higher at 22 °C versus at 7 °C. Increases ranged from 2.2 to 4.2 times greater at the warmer temperatures. This follows general metabolic principles of higher metabolic activity at higher temperatures within a biological range. Enzyme activity generally increases approximately two fold with a 10 °C increase in temperature (Atlas and Bartha, 1993). This does not imply a directly proportional increase in metabolism as other factors will influence overall metabolism rates; but for this 15 °C

rise in temperature, the 2.2 to 4.2-fold increase in CO_2 production rates is reasonable. In addition, microbial communities will vary in composition and size with varying temperatures (Davis-Hoover and Vesper, 1995).

The influence of temperature is of particular interest in the proposed application of vegetation for reducing runoff of propylene glycol in deicing fluids. Deicing fluids may need to be applied at temperatures above freezing as supercooling may occur on the surface of the aircraft (ARCO Chemical Company, 1996). Even chemicals that are applied at subfreezing temperatures may not be released to the environment until warmer temperatures and rainfall flush spent deicing fluids from the runways. While airports in northern regions will experience cold weather for many months in a row, airports in warmer regions may only need to apply deicers for a few days at a time, or only for evening flights. Thus, the glycol runoff would enter the environment under temperatures well above freezing. Deicing fluid runoff is significant in the fall and spring when water temperatures and microbial metabolic rates are higher than during winter (Sills and Blakeslee, 1992).

Lower temperatures will slow metabolism, but extremely low temperatures may inhibit microbial survival. Liquid water and fluid cell contents are required for metabolic processes, so survival below the freezing point of water is rare (Atlas and Bartha, 1993). However, glycols are antifreeze agents and their presence may allow microbial communities to persist and be active at sub-zero temperatures. Rice (1996) found that rhizosphere soils incubated at -10 °C were still able to mineralize ethylene glycol at significantly greater rates than autoclaved soils at 20 °C.

Rhizosphere Soils

Previous research has shown that rhizosphere soil from several plant species increased the rate of mineralization of microbial degradation of organic contaminants (Anderson et al., 1993; Shimp et al., 1993), including propylene glycol (Rice et al., 1997), compared to nonvegetated soil. There are factors that may explain why similar results were not seen here. For example, rhizosphere soil is defined as the thin layer of soil that clings to the roots after the bulk soil has been shaken loose (Atlas and Bartha, 1993), but in this study, a larger section of the soil was investigated. This root zone soil includes some rhizosphere soil, but also contains (depending on root growth) some bulk soil. This additional bulk soil may have diluted the rhizosphere microbial populations. Some of the treatment soils may have had a large percentage of essentially nonvegetated soil which would explain why few differences were seen between treatment root zone soils and nonvegetated soils and between the different root zone soils.

The enhanced mineralization by root zone microbial communities is due to root-soil interactions. Walton et al. (1994) noted that the roots influence the soil physically and chemically and that soil removed from the root system will not behave the same as true rhizosphere soil from an intact system. In these experiments, the roots were removed from the soil so these interactions no longer existed. This may be part of the reason why mineralization slows down over time. In a real world application, chemicals would be introduced to plant soil systems and the roots would continue to enhance the microbial community.

The vegetation already surrounding most airports may serve to slow water and chemical movement towards surface waters. This concept is not unlike vegetated buffer strips, or filter strips, which are plots of vegetated land downstream from chemical application sites that intercept runoff before it reaches surface waters. They have been shown to effectively reduce runoff of organic pollutants (Barton and Davies, 1993; Arora et al., 1996).

5. CONCLUSIONS

Root zone soils from legumes were found to enhance mineralization of propylene glycol over root zone soils from grasses and nonvegetated soils. All treatments mineralized propylene glycol faster than autoclaved soils which mineralized almost none of the applied ^{14}C. Much of the applied ^{14}C was recovered as $^{14}CO_2$ in all treatments indicating that mineralization is the primary route for propylene glycol degradation. Few differences in mineralization rates between root zone soils from grasses and nonvegetated soils or between soils from various grasses were observed. Even nonvegetated soils may be effective at mineralizing propylene glycol and reducing the environmental impact of deicing fluids.

Many airports utilize grasses as ground cover around runways. Given the potential of vegetation to slow water and chemical movement after a rain event, this strategy should be encouraged at all airports. Some types of vegetation may enhance mineralization more than others, but all rhizosphere soils tested in this study were capable of mineralizing propylene glycol, even at near freezing temperatures. Legumes can enhance propylene glycol mineralization in the soil, so interspersing indigenous

grasses with legumes may further reduce propylene glycol concentrations in runoff waters, thus further reducing the environmental impact of deicing fluids.

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Table I. Plants tested for ^{14}C-propylene glycol mineralization in root zone soils.

Common Name	Latin Name
Legumes	
O alfalfa	*Medicago sativa*
O birdsfoot trefoil	*Lotus corniculatus*
Grasses	
O switchgrass	*Panicum virgatum*
O Kentucky bluegrass	*Poa pratensis L.*
O rye grass	*Lolium perenne L.*
O sand dropseed	*Sporobolus crytandrus*
O tall fescue	*Festuca arundinacea*
Mixtures	
O grass mix : fescue, rye grass, and Kentucky bluegrass	
O grass mix + alfalfa : fescue, rye grass, Kentucky bluegrass, and alfalfa	

Table II. Mineralization rates and distribution of ^{14}C derived from ^{14}C-propylene glycol after incubation at 22 °C. Distribution is expressed as a percent of the added ^{14}C recovered. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

Treatment	Mineralization Rate $^{14}CO_2$	Extractable	Soil-Bound	Mass Balance
alfalfa	6.5 [a]	76.2	1.2	7.7	85.1
nonvegetated	5.2 [ab]	76.6	2.3	9.5	88.4
trefoil	4.1 [bc]	74.1	1.5	10.3	85.8
tall fescue	3.7 [cd]	77.8	1.5	8.8	88.2
grass mix	2.5 [d]	74.6	0.4	12.9	87.9
mix + alfalfa	2.9 [d]	75.3	1.4	13.1	89.8
switchgrass	2.7 [d]	72.8	1.8	13.2	87.8
bluegrass	2.2 [d]	73.4	2.1	11.0	86.5
rye grass	2.3 [d]	73.2	0.8	11.8	85.8
sand dropseed	2.4 [d]	73.0	2.0	11.3	86.3
autoclaved	0.03 [e]	1.3	96.4	32.6	130.3

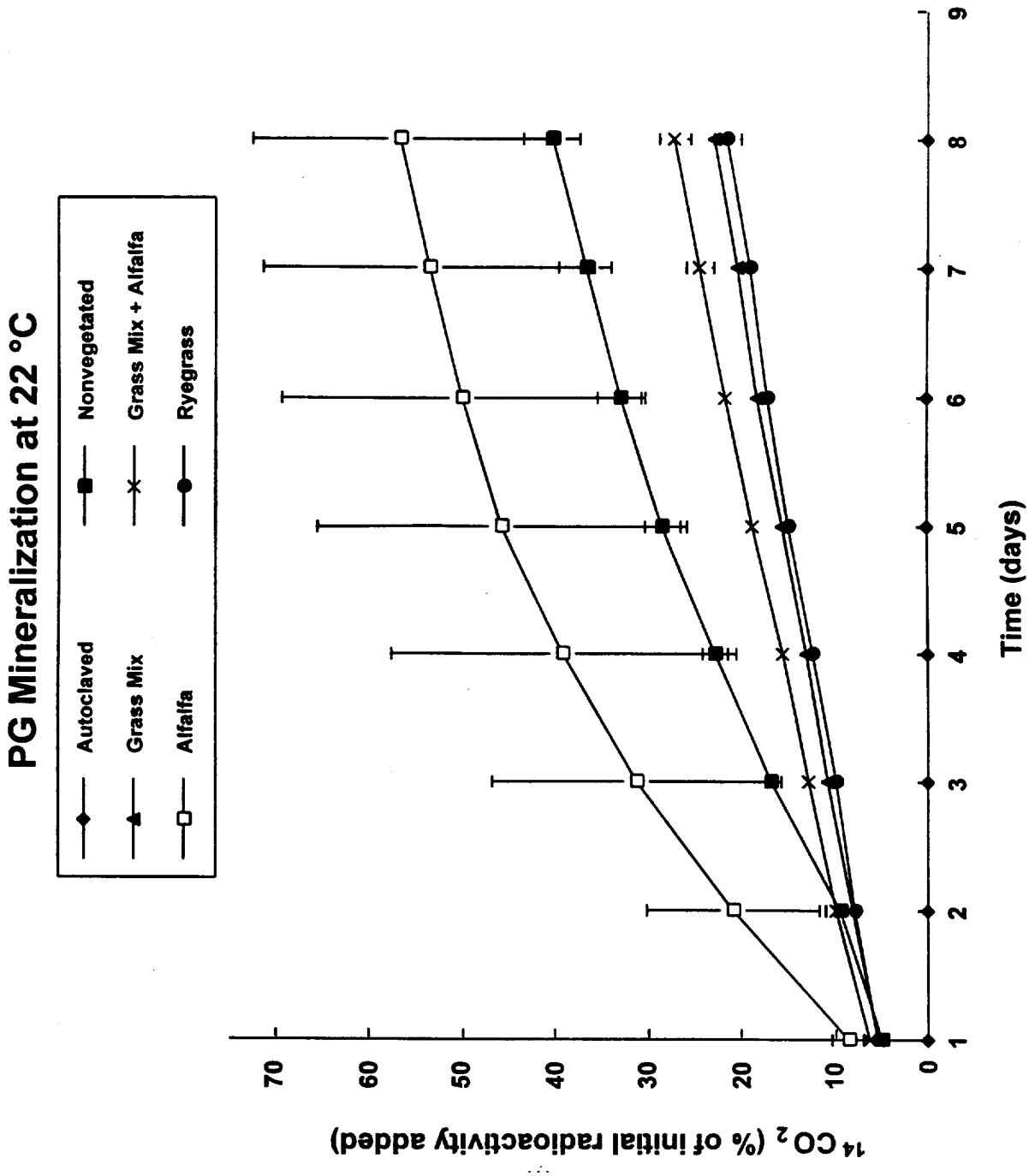
Table III. Mineralization rates and distribution of ^{14}C derived from ^{14}C-propylene glycol after incubation at 7 °C. Distribution is expressed as a percent of the added ^{14}C recovered. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

Treatment	Mineralization Rate	$^{14}CO_2$	Extractable	Soil-Bound	Mass Balance
alfalfa	3.0 [a]	78.8	1.5	8.4	88.6
nonvegetated	1.3 [c]	76.9	6.4	9.3	92.6
trefoil	1.4 [bc]	66.4	15.5	22.1	104.0
tall fescue	0.9 [ef]	55.9	25.4	32.5	113.8
grass mix	0.7 [fg]	42.0	44.5	23.1	109.7
mix + alfalfa	0.9 [de]	52.7	34.1	20.6	107.4
switchgrass	0.8 [efg]	58.2	21.6	14.9	94.7
bluegrass	0.7 [fg]	46.5	35.6	19.0	101.1
rye grass	0.7 [g]	45.0	43.2	22.4	110.6
sand dropseed	0.6 [g]	39.7	45.3	30.2	115.1
autoclaved	0.01 [h]	0.7	119.9	36.3	156.9

Figure Captions

Figure 1. Mineralization of ^{14}C-propylene glycol in selected treatment soils incubated at 22 °C. Error bars indicate one standard deviation of the mean.

Figure 2. Mineralization of ^{14}C-propylene glycol in selected treatment soils incubated at 7 °C. Error bars indicate one standard deviation of the mean.



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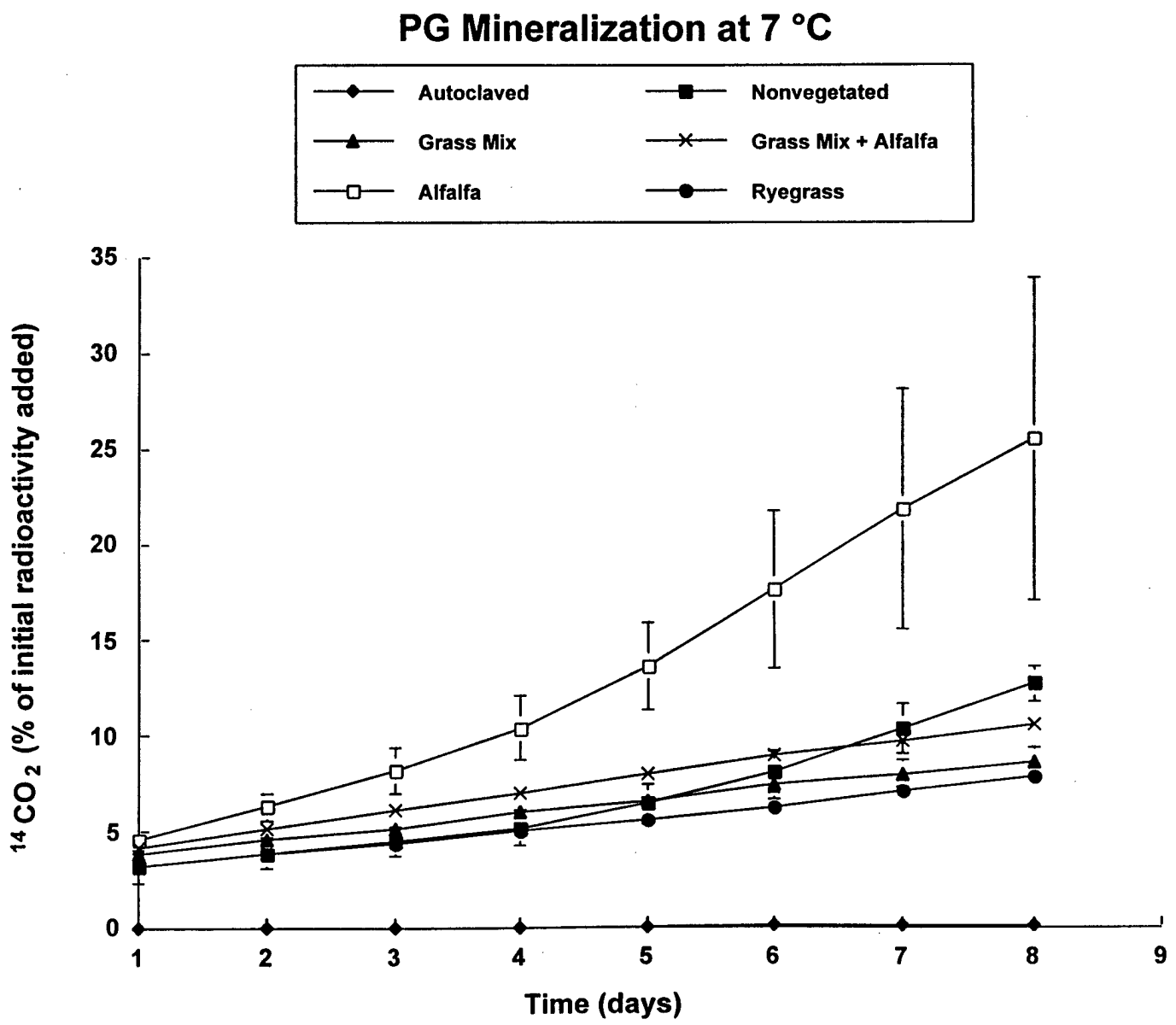
PG Mineralization at 7 °C
Autoclaved
Nonvegetated
Grass Mix
Grass Mix + Alfalfa
Alfalfa
Ryegrass
$^{14}CO_2$ (% of initial radioactivity added)
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Time (days)

APPENDIX 3. The use of aquatic plants to remediate surface waters contaminated with aircraft deicing agents

THE USE OF AQUATIC PLANTS TO REMEDIATE SURFACE WATERS CONTAMINATED WITH AIRCRAFT DEICING AGENTS

A paper to be submitted to *Environmental Toxicology and Chemistry*

Patricia J. Rice†, Todd A. Anderson‡ and Joel R. Coats†

Abstract The purpose of our research was to evaluate the use of aquatic vegetation to enhance the transformation of ethylene glycol and propylene glycol in contaminated surface waters. The mineralization of [^{14}C]EG and [^{14}C]PG in sterile control soil, nonvegetated soil, and soil containing *Scirpus fluniatilis, Scirpus acutus*, and *Scirpus validus* were determined. Elevated levels of $^{14}CO_2$ in whole-plant systems indicate accelerated mineralization in the vegetated treatments compared to the nonvegetated and sterile control soil samples. After a 7-d incubation period, aquatic macrophytes enhanced the mineralization of [^{14}C]PG by 11% to 19% and [^{14}C]EG by 6% to 20%. Less than 8% of applied radiocarbon was detected in the plant tissues, with the majority of the ^{14}C recovered in the roots. Artificial wetland and shallow storage basins cultured with aquatic macrophytes may be valuable for treating airport and air base runoff, thus reducing the biological oxygen demand and glycol concentrations in receiving waters.

Keywords- Phytoremediation Aircraft deicers Aquatic emergent plants Ethylene glycol Propylene glycol

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†Pesticide Toxicology Laboratory, Iowa State University, Ames, IA 50011

‡The Institute of Wildlife and Environmental Toxicology, Clemson University, Pendleton, SC 29670

INTRODUCTION

Over 43 million L/yr of aircraft deicing-agents are used nationwide to remove ice and snow that accumulate on aircraft and airfield runways. Aviation deicing-fluids used in North America primarily consist of ethylene glycol (EG) and/or propylene glycol (PG) with a minimal amount of additives [1]. During a deicing event, the majority (>80%) of the fluid spills to the ground, ultimately causing on-site pooling, soil infiltration, runoff, and contamination of soil, surface water and groundwater aquifers [1-3]. Airport storm-sewer systems may collect runoff and directly release untreated wastewater into streams and rivers [1-3]. Sills and Blakeslee [1] reported airport runoff and storm-sewer discharge to contain concentrations of EG ranging from 70 mg/L to > 5,000 mg/L . Ethylene glycol and propylene glycol contamination of surface waters creates a high biological oxygen demand (BOD) that can adversely impact aquatic communities. Depletion of available oxygen in surface waters has resulted in asphyxiation and death in aquatic organisms [1,2].

Wetland plants may also be utilized to remediate contaminated water and soil. Like terrestrial plants, aquatic macrophytes are capable of taking contaminants up in their tissues and of enhancing biodegradation in the rhizosphere. Aquatic plants have an adaptation that enables efficient translocation of oxygen from the shoots to the roots, thereby forming oxidized microzones in a saturated anaerobic environment [4,5]. The rhizosphere of an emergent aquatic macrophyte is more conducive for microbial growth (aerobes and facultative anaerobes) and activity than saturated root-free soil, thus creating a better environment for enhance biodegradation. Within the past fifteen years, aquatic macrophytes have been utilized for wastewater treatment. Wetland plants have been shown to reduce nutrients, organic contaminants and BOD from industrial, municipal, and agricultural wastewater [5-10]. Gersber et al. [7] observed that aquatic emergent macrophytes, bulrush (*Scirpus validus*), common reed (*Phragmites communis*), and cattail (*Typha latifola*) reduced the BOD and ammonia levels in primary effluents. The artificial wetland beds cultured with *S. validus* were

superior to the other vegetated and nonvegetated beds. *S. validus* had reduced the BOD level in the primary wastewater inflow from 118 mg/L to 5.3 mg/L. Reddy et al. [6] also noted emergent and floating aquatic macrophytes were able to improve sewage effluent by decreasing the BOD and increasing the concentration of dissolved oxygen. The purpose of our research was to evaluate the use of aquatic vegetation to enhance the transformation of ethylene glycol and propylene glycol in contaminated surface waters.

MATERIALS AND METHODS

Chemicals

Ethylene glycol (EG) and propylene glycol (PG) were obtained from Fisher Scientific (Fair Lawn, NJ) and Sigma Chemical Company (St. Louis, MO). The radiolabeled compounds ethylene glycol-1,2-^{14}C ([^{14}C]EG) and uniformly labeled propylene glycol ([^{14}C]PG) were purchased from Aldrich Chemical Company (Milwaukee, WI) and New England Nuclear-Dupont (Boston, MS). Upon receipt, the [^{14}C]EG and [^{14}C]PG were diluted with ethylene glycol and propylene glycol to yield a stock solution of 0.277 μCi/μl and 0.247 μCi/μl, respectively.

Plants and soil

Pesticide-free soil used in this investigation was collected from the Iowa State University Agronomy and Agricultural Engineering Farm near Ames, (Boone County) Iowa. The soil was randomly removed from the field with a golf-cup cutter (10.5 cm x 10 cm, Paraide Products Co.). Ten samples were combined for each replicate. Each of the three soil replicates were sieved (2.0 mm) and analyzed (A&L Mid West Laboratories, Omaha, NE) to determine the physical and chemical properties. The soils were stored in polyurethane bags in the dark at 4 °C until needed.

Roots of aquatic emergent plants were purchased from V & J Seed Farms (Woodstock, IL), and some plants were collected from a small lake and shallow ditch located

in Story County, Iowa. The three plant species utilized in this study were hard-stem bulrush (*Scirpus acutus*), soft-stem bulrush (*Scirpus validus*), and river bulrush (*Scirpus fluniatilis*). Upon arrival, the roots were separated by species and planted in glass aquaria containing pesticide-free soil. These plants were grown in saturated soils and maintained in a greenhouse at 25 °C ± 2 °C with a 16:8 light:dark diurnal cycle. Several months later, after the plants had developed healthy root systems, small root masses or rhizomes from each plant species were individually planted into 250-ml glass jars with pesticide-free soil corresponding to 100 g of dry weight. Each jar was covered with tape to eliminate light in an attempt to deter algal growth and photodegradation of the [^{14}C]ethylene glycol and [^{14}C]propylene glycol in the saturated soils. Nonvegetated control samples were set up identically to the vegetated soil and were maintained under the same environmental conditions. After six weeks, the vegetated and nonvegetated samples were placed in the exposure chamber (described below) and acclimated for 48 h. Often more than one plant emerged from a rhizome. When this occurred, the healthiest plant under 17 cm in length was chosen and the remaining shoots were cut below the water surface. Sterile control soils were autoclaved (1 h on 3 consecutive days) no more than three days prior to the treatment.

Soil-plant systems

Special incubation flasks were used to monitor the fate of [^{14}C]EG and [^{14}C]PG in the aquatic macrophyte whole-plant system (Fig. 1). The apparatus for this system was modified from Anderson and Walton [11] and Federle and Schwab [12]. Just prior to the glycol treatment, water was removed from each incubation flask to adjust the water level to 1 cm above the soil surface. A 3-ml plastic vial, containing 2 ml 0.01 M NaOH for trapping $^{14}CO_2$, was suspended inside each jar. After application of either 1,000 µg/g [^{14}C]EG or 1,000 µg/g [^{14}C] PG to the water layer, the soil-plant systems were sealed around the aquatic macrophytes by using split rubber stoppers and RTV sealant [11]. Each stopper contained

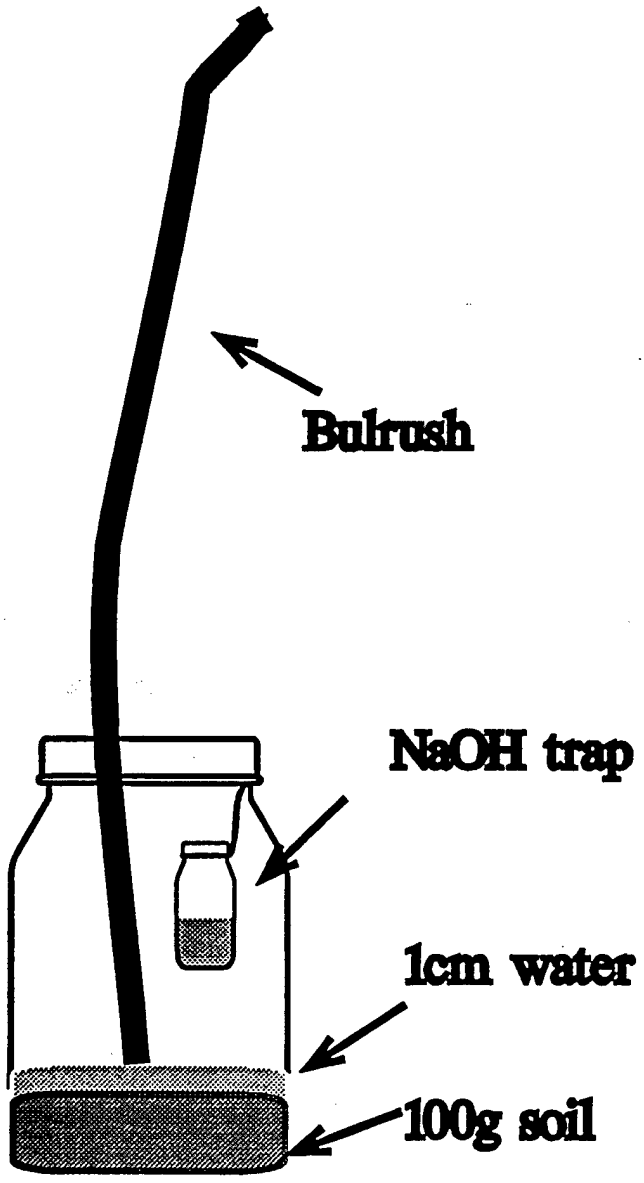

Fig. 1. Apparatus used to measure the fate of [^{14}C]ethylene glycol and [^{14}C]propylene glycol in the aquatic emergent whole-plant system.

two additional openings from which the $^{14}CO_2$ trap was changed and sterile water was added to replace the moisture lost through transpiration. These openings were closed with two smaller rubber stoppers. Deionized water (100 g), in a similar apparatus, was also treated with 1,000 μg/g [^{14}C]EG or 1,000 μg/g [^{14}C] PG. Between three and five replicates of each soil treatment (water, sterile control soil, nonvegetated soil, and vegetated soils) was included in the [^{14}C]EG and [^{14}C]PG experiments. Both the [^{14}C]EG and [^{14}C]PG studies were conducted for 7 d.

After every 24-h interval, the $^{14}CO_2$ traps were changed to prevent saturation of the 0.01 M NaOH and to maintain aerobic conditions within the soil-plant system. Each vegetated incubation jar was weighed to determine if water (sterile) was need to replace moisture lost through transpiration. The full content of each $^{14}CO_2$ trap was radioassayed on a RackBeta model 1217 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD).

Exposure chamber

All the incubation flasks were placed in a glass exposure chamber that was modified from Anderson and Walton [11] (Fig. 2). Air within the glass chamber was constantly replaced by two pumps located on either side of the chamber. Each pump was set on alternating 15-min. cycles. Air evacuated from the chamber was bubbled through a 100ml 0.1 N NaOH trap and a 100-ml Ultima Gold scintillation cocktail trap (Packard Instrument Co., Downer's Grove, IL). The 0.1 N NaOH and scintillation cocktail were used to capture any $^{14}CO_2$ and volatile ^{14}C-glycol or ^{14}C-metabolites that were released into the chamber with the evapotranspiration stream. The glass exposure chamber was contained in an environmentally controlled room at 25 °C ± 1 °C with a 14:10 light:dark cycle. The temperature within the chamber was maintained at 26 °C ± 1 °C. At the completion of the

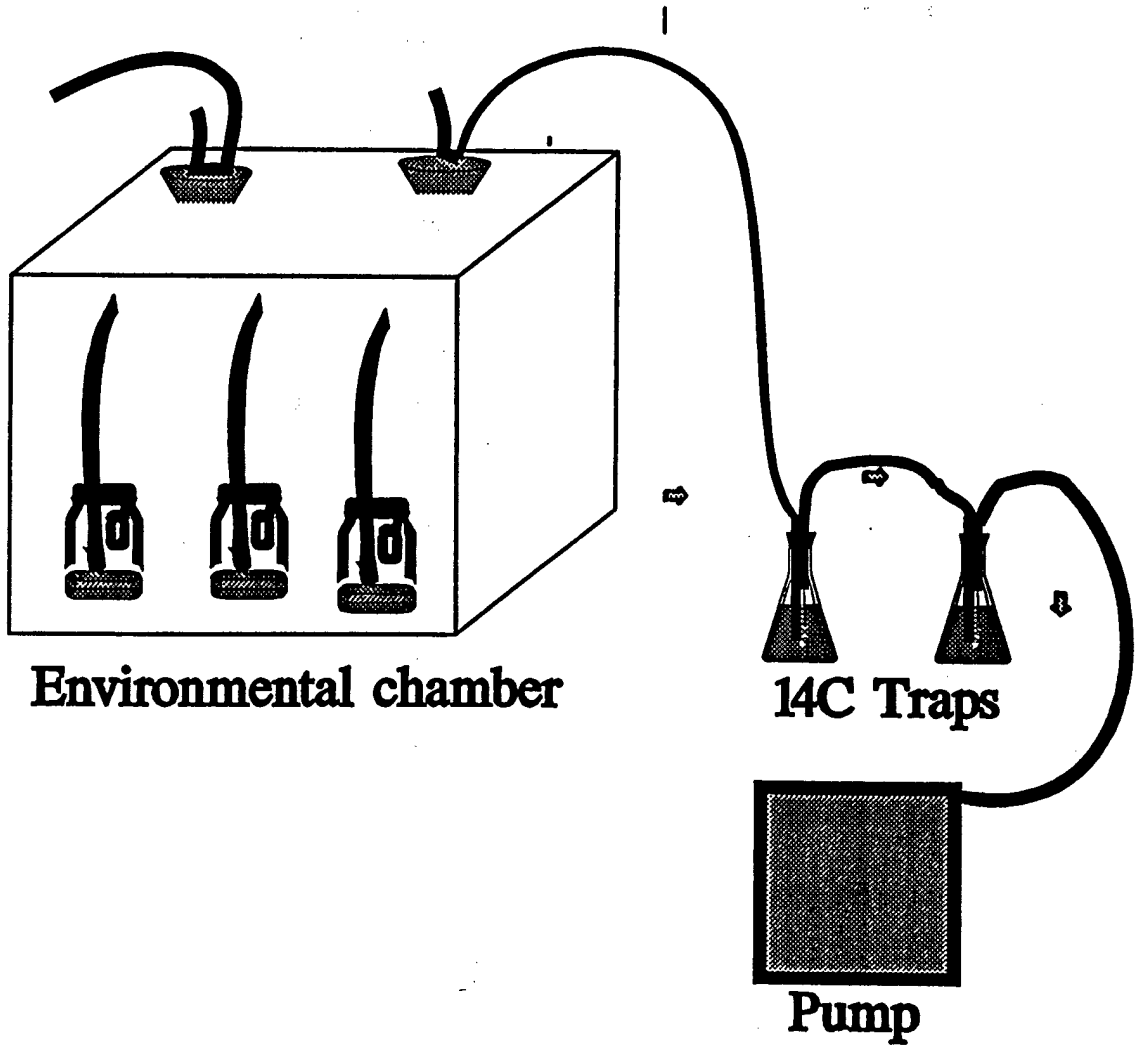

Fig. 2. Glass exposure chamber used to collect radiocarbon released by the plants.

study, subsamples of the 0.1 N NaOH and scintillation cocktail traps were radioassayed on a liquid scintillation counter.

Soil and plant tissue analysis for ^{14}C

Upon completion of the 7-day study, the soil was extracted 3 times with 30 ml methanol. Subsamples of the soil extracts were analyzed on a liquid scintillation counter to determine the percent of extractable ^{14}C remaining in the soil. Subsamples of crushed and homogenized air-dried extracted-soils were combusted using a Packard sample oxidizer. Radiolabeled carbon dioxide from the combusted soils was trapped in Carbo-Sorb E and Permafluor V (Packard) and radioassayed on a liquid scintillation counter to determine the amount of ^{14}C-soil bound residue. Roots and shoots were analyzed separately.

Plant tissues were combusted on a Packard sample oxidizer (Packard Instrument Co.) and radioassayed on a liquid scintillation counter (Pharmacia LKB biotechnology, Inc., Githersburg, MD) to determine the quantity of ^{14}C associated with the plants. The mass balance for the soil-plant system ($^{14}CO_2$, ^{14}C-extractable organics, ^{14}C-soil-bound organics, and ^{14}C in the plant tissues) was determined for each sample (Table 1). Analysis of variance and LSD (5%) were used to determine the significant differences between the treatments [13].

RESULTS

Mineralization of [^{14}C]EG and [^{14}C]PG

Analysis of the $^{14}CO_2$ traps from the aquatic emergent whole-plant degradation studies indicate significantly ($p \leq 0.05$) greater quantities of $^{14}CO_2$ was evolved from the vegetated soils compared to either the sterile control or the nonvegetated soils (Fig. 3 and Fig. 3). After a 7-d incubation period, 45.6%, 32.6%, and 32.3% of applied [^{14}C]EG mineralized in the *S. validus, S. acutus, and S. fluviatilis* soil-plant systems (Fig. 3). Production of $^{14}CO_2$ was elevated by approximately 6% to 19% in the vegetated soil. Significantly ($p \leq 0.05$) greater

Table 1. Distribution of ^{14}C in the [^{14}C]ethylene glycol and [^{14}C]propylene glycol soil-plant systems.

		Percentage of total ^{14}C[a]				
Treatment	Compound	$^{14}CO_2$	Extractable	Soil-bound	Plant uptake[b]	Total recovery
Sterile control	EG	15.7 a	98.2 a	9.59 a	na	123
Nonvegetated	EG	25.9 b	10.0 b	27.4 b	na	63.3
S. fluniatilis	EG	32.3 c	10.3 b	20.4 c	7.08 a	70.1
S. acutus	EG	32.7 c	20.2 b	19.2 c	4.58 b,c	76.6
S. validus	EG	45.6 d	15.0 b	19.3 c	5.46 a,b	85.6
Sterile control	PG	14.6 a	78.0 c	6.00 d	na	98.6
Nonvegetated	PG	43.0 d	10.3 b	22.5 e	na	75.8
S. fluniatilis	PG	61.7 e	9.76 b	13.6 f	6.09 a,c	85.1
S. acutus	PG	53.8 f	12.0 b	12.5 f,g	3.61 b	81.9
S. validus	PG	43.0 d	12.1 b	11.4 g	3.72 b	70.3

[a]Means in each column followed by the same letter are not significantly different ($p = 0.05$).
[b]na = not applicable.

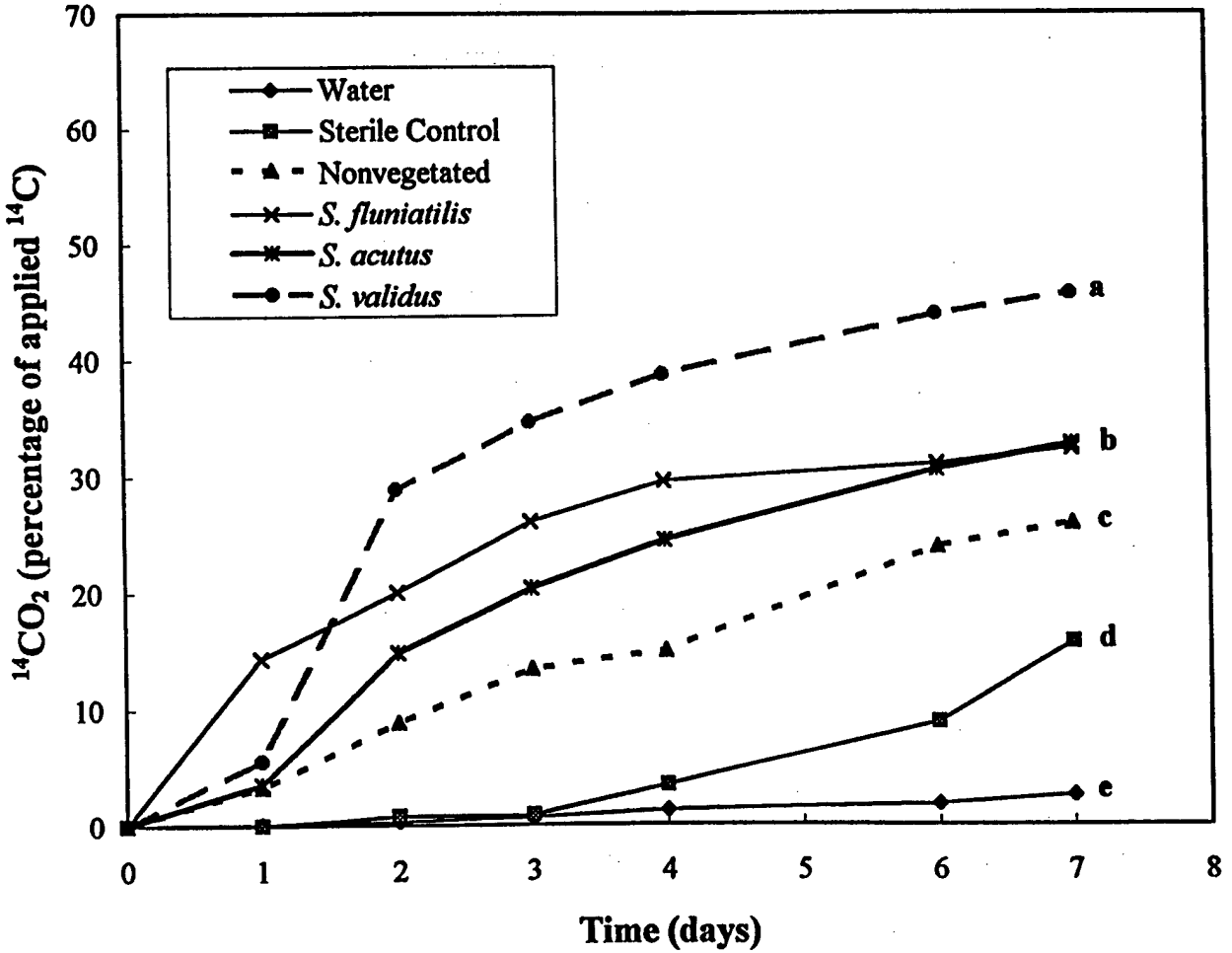

Fig. 3. Mineralization of [^{14}C]ethylene glycol in nonvegetated soil, sterile soil, and soil that contained either *Scirpus fluniatilis*, *Scirpus acutus*, or *Scirpus validus*. Data points (cumulative $^{14}CO_2$) followed by the same letter are not significantly different (p=0.05).

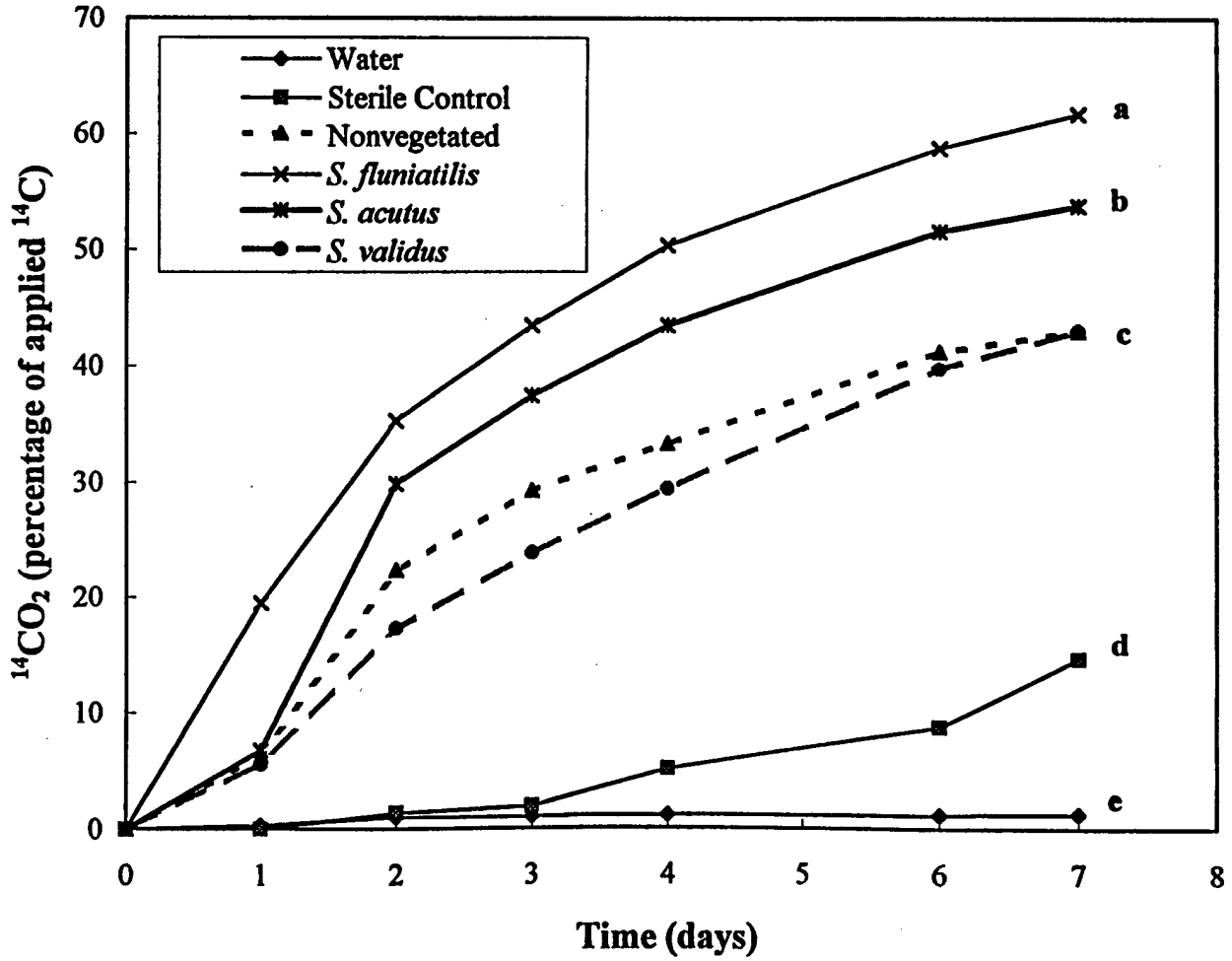

Fig. 4. Mineralization of [^{14}C]propylene glycol in nonvegetated soil, sterile soil, and soil that contained either *Scirpus fluniatilis*, *Scirpus acutus*, or *Scirpus validus*. Data points (cumulative $^{14}CO_2$) followed by the same letter are not significantly different (p=0.05).

mineralization was also noted in the nonvegetated soil compared to the sterile control soil. Minimal quantities of $^{14}CO_2$ was evolved from the water samples. Biologically active soils transformed 3% to 14% of the applied [^{14}C]EG to $^{14}CO_2$ within the first 24 h.

Enhanced mineralization was also observed in the vegetated soils treated with propylene glycol (Fig. 4). Significantly ($p \leq 0.05$) greater quantities of [^{14}C]PG was transformed to $^{14}CO_2$ in soil containing *S. fluniatilis* and *S. acutus* than in either the nonvegetated and or the sterile control soil. $^{14}CO_2$ production was elevated 10.8% to 18.7% in these vegetated soils compared to nonvegetated soil. Comparable amounts of $^{14}CO_2$ was evolved from the *S. validus* and nonvegetated soil samples. A comparison of [^{14}C]PG and [^{14}C]EG mineralization in identical soil-plant systems indicate increased ($p \leq 0.05$) production of $^{14}CO_2$ in the nonvegetated, the *S. acutus*, and the *S. fluniatilis* soil samples treated with [^{14}C]PG compared to [^{14}C]EG (Table 1). Transformation of [^{14}C]PG and [^{14}C]EG to $^{14}CO_2$ was comparable in the sterile control and *S. validus* soil.

Soil analysis for ^{14}C

Analysis of soil from each [^{14}C]EG and [^{14}C]PG whole-plant study indicates significantly ($p \leq 0.05$) greater quantities of soil-bound ^{14}C was detected in the biologically active soil than the sterile control soil (Table 1). In sterile soil, 6.0% and 9.6% of the added [^{14}C]PG and [^{14}C]EG were bound, compared to 20.4% and 13.6% ^{14}C bound in *S. fluniatilis* soil, respectively. Nonvegetated soils were also observed to contain increased levels of bound radiocarbon. Among the five [^{14}C]EG soil treatments evaluated, apparent detection of ^{14}C soil residues was greatest to least for nonvegetated>*S. fluniatilis*=*S. acutus*=*S. validus*>sterile control soil. In contrast, significantly ($p \leq 0.05$) larger quantities of extractable ^{14}C were observed in the sterile control soils; approximately 98% and 77% of applied [^{14}C]EG and [^{14}C]PG was detected in the methanol soil extracts, respectively. No significant difference was

observed between the [^{14}C]EG or [^{14}C]PG nonvegetated and vegetated soil extracts. Less than 21% of applied [^{14}C]EG was detected in the extractable portions in the vegetated soils.

Uptake of ^{14}C into plant tissue

Recovery of applied radiocarbon in the three plant tissues ranged from 4.58% to 7.08% for EG and 3.61% to 6.09% for PG in the tested soil-plant systems (Table 1). Plant roots consistently contained more ^{14}C than plant shoots (Fig. 5). Greater than 70% of the recovered radiocarbon from [^{14}C]EG was detected in association with the roots. Air evacuated from the test chamber contained small quantities of radiocarbon. A comparison of plant species in the [^{14}C]EG and [^{14}C]PG studies indicated significantly ($p \leq 0.05$) greater quantities of ^{14}C in the tissues of *S. fluniatilis* than *S. acutus*. Percentages of radiocarbon recovered in plant tissues of the [^{14}C]EG whole-plant studies were elevated, but not significantly different than in the [^{14}C]PG treated samples. The form or identity of ^{14}C within the plant tissue was not determined.

DISCUSSION

Our results clearly indicate aquatic emergent plants significantly ($p<0.05$) enhanced mineralization of aircraft deicers (EG and PG) in surface water systems. Soils containing *S. fluniatilis, S. acutus,* and *S. validus* increased ($p \leq 0.05$) the transformation of [^{14}C]EG to $^{14}CO_2$ compared to nonvegetated soils. Enhanced degradation of [^{14}C]PG also occurred in the *S. fluniatilis* and *S. acutus* soil samples, but $^{14}CO_2$ production was comparable in the *S. validus* and nonvegetated soils.

Dissipation of ethylene glycol and propylene glycol from surface water was primarily a result of mineralization rather than uptake into plant tissues (Table 1). At the completion of the studies, 43% to 61% of applied [^{14}C]PG mineralized from the soil-plant surface water system as compared to less than 7% of applied radiocarbon taken up into plant tissues.

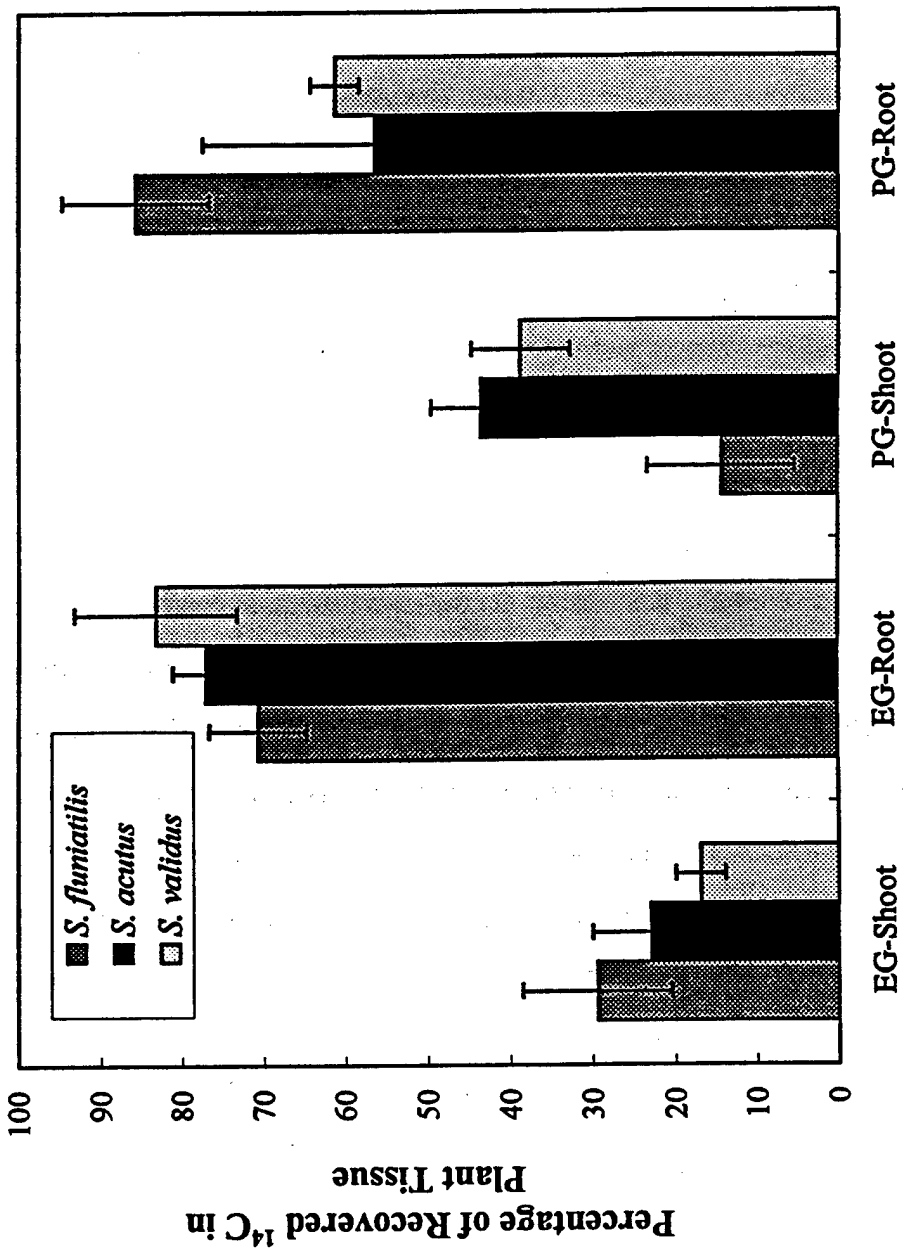

Fig. 5. The distribution of recovered ^{14}C in the plant shoots and roots. The total quantity of applied ^{14}C detected in the plant tissues was less than 8% of the radiocarbon applied.

Aquatic emergent plants can enhance microbial remediation of contaminated water and soil by enhancing degradation in the rhizosphere soil and taking contaminants up in their tissues. In addition, these aquatic macrophytes create a more conducive environment for microbial growth and activity than saturated root-free soil, due to the translocation of oxygen from the shoots to the roots by aerechyma cells [4,5]. Previous research has shown that artificial wetlands containing *S. validus* and *S. acutus* significantly reduced BOD, NH_3-N, and NO_3-N [7,14]

The majority of ^{14}C recovered in the plant tissues was associated with the roots. Radiocarbon detected in plant roots may be a result of uptake or adsorption of ^{14}C to the roots. We believe ^{14}C was primarily due to uptake since EG and PG are small polar compounds, and ^{14}C was detected in shoots and evacuated air from the test chamber, indicating that radiocarbon was translocated from the roots to the shoots.

In addition, significantly greater ($p<0.05$) quantities of ^{14}C soil-bound residues were found in the biologically active soils compared to the sterile control soils. This increased radiocarbon was a result of EG and PG mineralization. Lokke [15] reported ethylene glycol will not adsorb to soil. He observed very little to no adsorption of this glycol in saturated soil columns that contained subhorizons of clayey till, sandy till, and melt water sand. Therefore, glycol-based deicers in surface water and soil water are more bioavailable for microbial degradation and plant uptake. Several genera of bacteria have been reported to utilize ethylene glycol as a carbon and energy source [16,17]. As microorganisms metabolize ethylene glycol and propylene glycol, they incorporate a portion of the ^{14}C into their cell constituents. Therefore, significantly lower levels of soil-bound radiocarbon in the sterile soils were a result of decreased microbial activity.

CONCLUSION

Results from this study clearly indicate aquatic emergent plants enhanced the mineralization of glycol-based deicers in surface waters. Artificial wetland and shallow storage basins cultured with aquatic macrophytes may be useful for treating airport runoff, thus reducing the BOD and glycol concentrations in receiving waters. In addition this management approach is beneficial due to the low cost and easy maintenance of the system. Remediating airport wastewaters prior to its discharge into nearby surface waters will reduce the environmental impact of deicers on aquatic ecosystems.

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APPENDIX 4. The influence of vegetation on the mobility of propylene glycol through the soil profile

APPENDIX. THE INFLUENCE OF VEGETATION ON THE MOBILITY OF PROPYLENE GLYCOL THROUGH THE SOIL PROFILE

A paper to be submitted to *Environmental Toxicology and Chemistry*

Patricia J. Rice,† Todd A. Anderson,‡ and Joel R. Coats†

Abstract - The purpose of this investigations was to evaluate the influence of vegetation on the mobility of aircraft deicing-fluids through the soil profile and their potential to leach to groundwater. Undisturbed soil columns were planted with alfalfa (*Medicago sativa*) or rye grass (*Lolium perenne L.)* to assess their potential to reduce the infiltration of propylene glycol (PG). Propylene glycol was applied to the surface of the nonvegetated, *M. sativa*, and *L. perenne* soil column and leached daily with deionized water. Leachates were collected at the bottom of the columns and analyzed to determine the quantity of propylene glycol and potential degradation products that leached through the soil. The vegetated soil columns reduced the infiltration of propylene glycol through the soil profile. These results suggest plants can reduce the mobility of glycol-based deicing fluids in the soil and minimize its potential to leach and contaminate groundwater.

Keywords - Propylene glycol Vegetation Infiltration

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†Pesticide Toxicology Laboratory, Iowa State University, Ames, IA 50011

‡The Institute of Wildlife and Environmental Toxicology, Clemson University, Pendleton, SC 29670

INTRODUCTION

There is a growing concern about the quantity of aircraft deicing agents that migrate offsite and inadvertently contaminate the soil and water environments. Propylene glycol (1,2-propanediol) is widely used in aircraft deicing agents and vehicular antifreeze. Type I deicers that are commonly used in North America consist of a minimum of 80% glycol by weight, primarily ethylene glycol (EG) or propylene glycol (PG). Under FAA regulation, deicing agents must be used to remove and prevent ice and frost from accumulating on aircraft and airfield runways. As a result, over 43 million liters of aircraft deicing products are used each year nationwide [1]. During severe storms, large planes may require thousands of gallons of deicing-fluids per deicing event. An estimated 80% of the fluids spill onto the ground, ultimately causing on-site pooling, soil infiltration, runoff, and contamination of soil, surface water and groundwater aquifers [1-3]. Ethylene glycol has been detected in groundwater at 415 mg/L [1] and 2,100 mg/L [4].

Vegetation can enhance the removal of man-made organic compounds and pollutants in soil environments by microbial degradation in the rhizosphere and plant uptake [5,6]. Increased diversity and biomass of microbial communities in the rhizosphere render this zone better for degradation of organic pollutants. Previous research has shown enhanced degradation of industrial chemicals (trichloroethylene [7], polycyclic aromatic hydrocarbons [8]), and petroleum [9] in rhizosphere soil compared to root-free soil. Anderson and Walton [10] studied the fate of [^{14}C]TCE in soil-plant systems collected from a contaminated site. They reported 1 to 21% of the recovered radiocarbon (depending on the plant species) was detected in the plant tissues, particularly in the roots. Vegetation may play a vital role in reclaiming polluted ecosystems and preventing further contamination by enhancing degradation and uptake into tissues, thereby reducing migration to surface waters and groundwater aquifers.

The purpose of this research was to evaluate the influence of vegetation on the movement and leaching potential of propylene glycol through the soil profile. High concentrations of propylene glycol were applied to nonvegetated and vegetated undisturbed soil columns to mimic the quantities of glycols detected in airport runoff.

MATERIALS AND METHODS

Column study

Undisturbed soil columns (15 cm diameter x 38 cm length) were obtained from an agricultural field site (no pesticide history) near Ames, IA. The procedures for collection and removal of the columns were previously described by Singh and Kanwar [11]. Columns were stored in the dark at 4 °C until needed. Additional soil samples were collected at the same depths as the column, and soil physical and chemical properties were determined (Table 1).

Soil columns were prepared for laboratory studies as described by Singh and Kanwar [11]. Four soil columns (2 each) were planted with alfalfa (*Medicago sativa*) or rye grass (*Lolium perenne L.*) (Fig. 1). Nonvegetated and vegetated columns were maintained in a greenhouse (25 °C, 16:8 light:dark) for 4 months to allow sufficient growth of the plants. Water was added to the columns as needed. Roots of *M. sativa* and *L. perenne* were observed through the clear perforated plexiglass bottom of the columns. This indicates *M. sativa* and *L. perenne* roots were established through the length of the columns. Following the four month growth period, soil columns were saturated with 0.005 M $CaSO_4$ [11] then drained to field capacity. Two 200-mL quantities of deionized water were leached through the columns and analyzed on a gas chromatograph equipped with a flame ionization detector (GC-FID). These leachates were considered to be background samples. A KBr tracer was applied to the soil surface and leached through the soil columns with deionized water. Breakthrough curves were determined for each column by analyzing the quantity of bromide ion with a bromide-specific electrode.

Table 1. Soil characteristics of the undisturbed soil columns

Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	pH[a]	O.M.[b] (%)	C.E.C[c] (meq/100g)
0	Sandy clay loam	52	26	22	5.3	2.3	12.5
15	Loam	54	24	22	5.5	3.0	12.0
45	Sandy clay loam	42	34	24	5.9	2.5	13.8
60	Sandy clay loam	44	30	26	6.3	1.8	13.2

[a]1:1 (soil:distilled water).

[b]Organic matter.

[c]Cation exchange capacity.

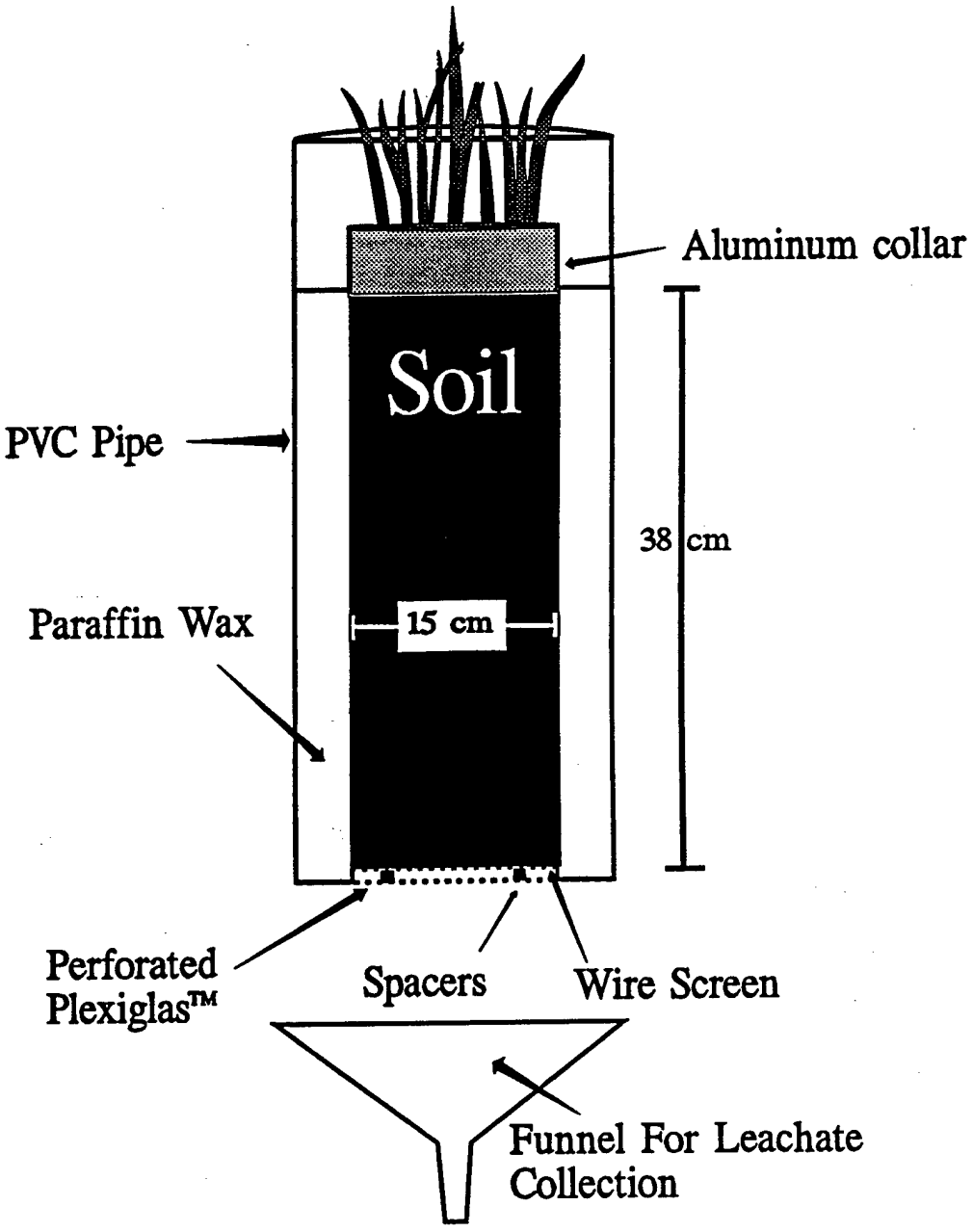

Fig. 1. Vegetated undisturbed soil column used to study the influence of plants on the mobility of aircraft deicers through the soil profile.

Soil columns were moved to an incubator set at 25 °C, and the temperature was slowly decreased (approximately 3 °C/24h) to 10 °C to represent spring conditions. Soil columns were maintained at 10 °C with a 16:8 light:dark cycle for 96 h prior to the treatment to ensure the plants had acclimated to this temperature. During this 96 h time period, soil columns were leached with 400 ml deionized water.

Propylene glycol (Fisher Scientific, Fair Lawn, NJ) solution (1.76 ml PG/ 364 ml water) was applied to the soil surface. Twenty-four hours after the treatment, soil columns were leached with 400 ml deionized water daily. Water was applied to the columns in four 100-ml increments. This caused a temporary pooling of water each time, which was meant to represent a runoff situation. Leachates were collected at the bottom of the columns and analyzed on a GC-FID[12]. Soil columns were leached daily until the level of PG was below the detection limit. Peak heights were used to construct a calibration curve and quantitate the samples. The data will be statistically analyzed by using analysis of variance and least significant differences at 5% [13].

Analysis of Br^- with a bromide-specific electrode

Following the addition of the KBr tracer, columns were leached with deionized water and leachates were collected and measured for Br^- using a bromide-specific electrode attached to a pH meter (Fisher Scientific, Pittsburgh, PA). Br^- standards were prepared with KBr, deionized water, and 5 M $NaNO_3$ (ionic strength buffer). Calibration curves were constructed from the standards and used to determine the sample concentrations.

RESULTS AND DISCUSSION

Propylene glycol was detected in the leachates of all the soil columns studied (Fig. 2). The greatest PG concentrations occurred within the first two leaching events then gradually decreased until PG was not detectable after 18 d. Approximately 51 to 71% of the recovered

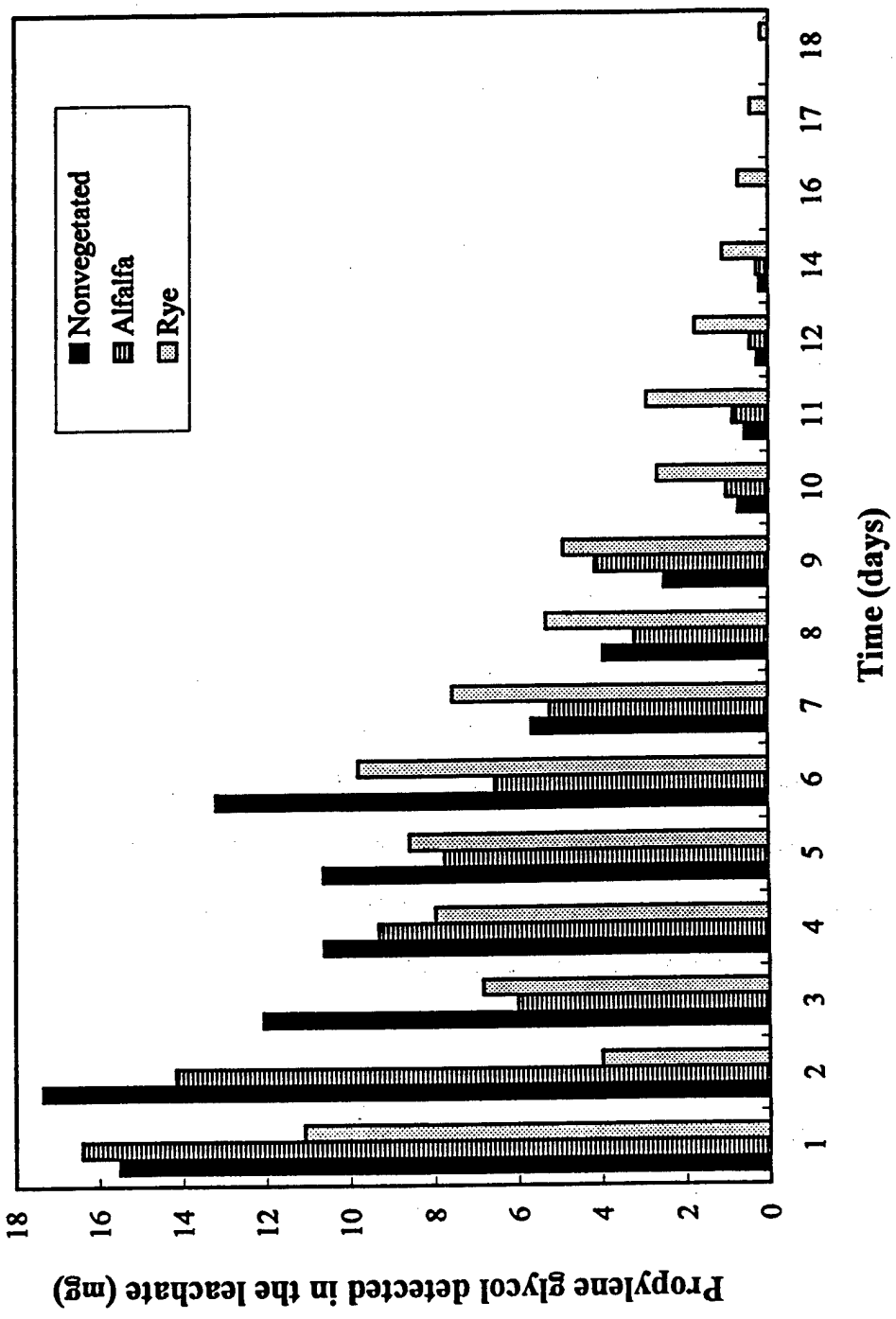

Fig. 2. Concentration of propylene glycol detected in the leachate of vegetated and nonvegetated soil columns.

PG was detected in the leachates within 5 d. Movement of PG through the soil profile depends on its properties and adsorptive characteristics, soil characteristics, soil temperature, and the quantity and frequency of runoff or precipitation [14]. Ethylene glycol, a compound similar in structure to propylene glycol, does not adsorb to soil. Lokke [12] reported little or no adsorption of EG to sandy till, muddy till, or clayey soils. Propylene glycol is water soluble and appears to be mobile within the 38 cm soil profile. Our results indicate vegetation reduced the quantity of propylene glycol that moved through the soil profile (Fig. 3). Measured quantities of 93.3 mg, 75.4 mg, and 75.6 mg of propylene glycol were detected in leachates of nonvegetated, *M. sativa*, and *L. perenne* soil columns, respectively. Plants can decrease the concentration of PG in soil and reduce its movement through the soil profile to groundwater by plant uptake, enhanced degradation in root-associated soils, and reducing the soil water status [5]. Our results from previous studies (Chapter 2 of the thesis) have shown enhanced degradation of PG in the *M. sativa* and *L. perenne* rhizosphere soils compared to nonvegetated soil. Results from the current investigation showed a 4 to 8% decrease in the quantity of water that leached through vegetated soil columns relative to the nonvegetated soil column. Overall, vegetation can reduce the downward movement and leaching of propylene glycol through the soil profile. This implies vegetation can be planted alongside deicing areas and runways to help minimize the quantity of aircraft deicing agents that reach the groundwater.

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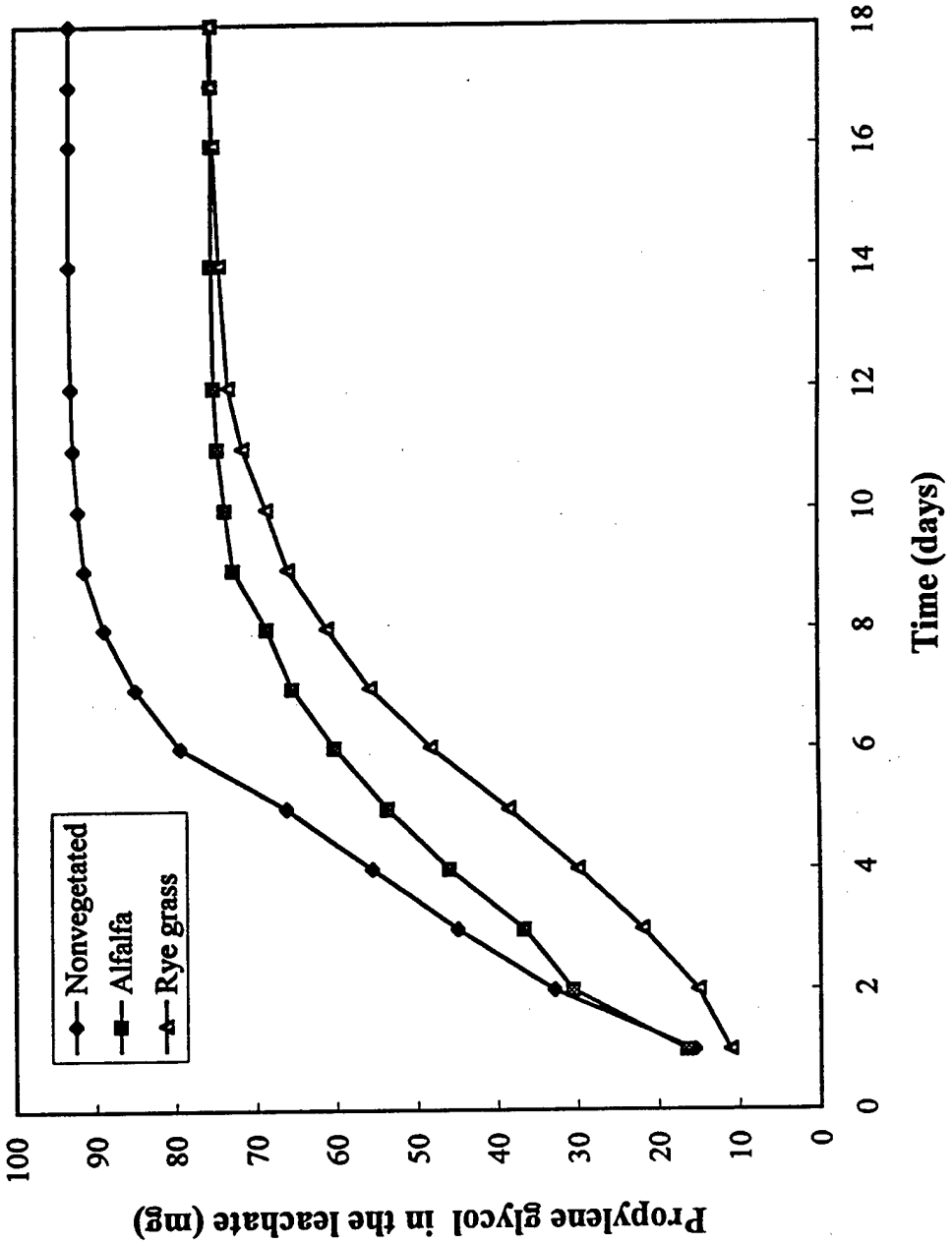

Fig. 3. Cumulative concentration of propylene glycol detected in the leachate of vegetated and nonvegetated soil columns.

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APPENDIX 5. The lysimeter concept: Environmental behavior of pesticides

ACS SYMPOSIUM SERIES 699

The Lysimeter Concept

Environmental Behavior of Pesticides

F. Führ, EDITOR
Forschungszentrum Jülich GmbH

R. J. Hance, EDITOR
Joint FAO/IAEA Division

J. R. Plimmer, EDITOR
Consultant

J. O. Nelson, EDITOR
University of Maryland

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Chapter 7

Mobility and Degradation of Pesticides and Their Degradates in Intact Soil Columns

Ellen L. Arthur, Patricia J. Rice[1], Pamela J. Rice[2], Todd A. Anderson[3], and Joel R. Coats

Pesticide Toxicology Laboratory, Department of Entomology, Iowa State University, Ames, IA 50011–3140

Laboratory studies were conducted to determine the mobility of parent pesticides and degradation products through the use of large undisturbed soil columns. The influence of vegetation on the mobility of pesticide adjuvants was also investigated. Modifications to the laboratory setup of soil columns for studying various pesticides, degradation products, and adjuvants were done to fit the needs of the particular compound being studied. To improve mass balances of volatile parent compounds, such as methyl bromide, as well as biodegradable (mineralizable) pesticide degradation products such as deethylatrazine, modifications of columns to accommodate isolation of volatile degradation products were accomplished by enclosure of the column head space and use of flow-through systems. Evidence of preferential flow of atrazine, deethylatrazine, metolachlor, and methyl bromide were indicated by the presence of either the ^{14}C-compound or Br- (in the case of methyl bromide-applied soil columns) after the first leaching event. Diffusion through the soil matrix was also evident with a peak of ^{14}C in the leachate several weeks after pesticide (or degradate) application to the soil column. Deethylatrazine, a major degradate of atrazine, was more mobile than the parent compound. Vegetation had a significant positive effect on reducing the mobility of the adjuvants propylene glycol and ethylene glycol.

The fate of any pesticide in the environment is important to understand because of any potential detrimental effect; thus the environmental chemistry and environmental toxicology of a pesticide are inextricably linked. Similarly, the environmental fate of a pesticide encompasses both the

[1]Current address: American Cyanamid Company, P.O. Box 400, Princeton, NJ 08543–0400.
[2]Current address: U.S. Department of Agriculture, ARS–NRI–ECL, Beltsville, MD 20705.
[3]Current address: Texas Tech University, Lubbock, TX 79409.

movement and transformation of the compound. There is an interdependent linkage between these two processes: (1) the transformation products have mobilities that differ from the parent molecule, and (2) conversely, the mobility of a compound can have great impact on the transformation processes since the location of a molecule influences the types and rates of transformations that occur.

Depending on their structure and physicochemical properties, soil-applied pesticides can move through runoff, leaching, volatilization, uptake into plants or microbes, or adsorption to the soil matrix. The conditions at each site are different, and the various agents that act upon the pesticide molecule can effect different types of transformations. The transformation products are typically degradates, although occasionally other types of transformations occur, such as conjugations, rearrangements, dimerizations, or isomerizations. The toxicological significance of any transformation product obviously needs to be considered for each compound. Recently there has been a realization that pesticide degradates are sometimes as important or more important than the parent pesticide in environmental settings. Although only a small minority of transformation products are of toxicological significance, serious consideration must be given to their formation mechanisms, quantities present, persistence, and mobility in the environment.

Investigations that focus on soil incubations, plant metabolism, or other component-based experiments have several distinct advantages, such as closely controlled environmental variables, low space requirements, and low cost per experimental unit which allows for more treatments and more replication. On the other hand, there are several shortcomings of very specific laboratory experiments, especially the lack of realistic environmental conditions variability and the scale. Research on larger scales, including field plots, fields, watersheds, or regions allows ample realism in conditions, variability, and scale, but such research has obvious limitations in investigators' capacity to control the variables such as climate; also, the costs of research are much higher at the larger scales.

Lysimeter research, as presented in this volume, represents a concept that optimizes the process of evaluating the environmental fate of pesticides. It incorporates many of the advantages of the laboratory approach, but retains essential elements of the realistic conditions encountered in the field. The scale is midway between the lab and the field, allowing for adequate replication, excellent sensitivity through the use of radiotracers, and manageable costs, but it also takes advantage of an intact soil monolith and natural weather conditions.

Utilization of intact soil columns for studying environmental fate of pesticides has some of the same advantages as the lysimeter concept. These are field-collected but studied under laboratory conditions. There are, of course, trade-offs once again, but they have one important advantage in common with the lysimeters. They provide an *integrated evaluation* of a compound's persistence, binding, degradation, and mobility in one experimental unit.

Laboratory mobility studies are often carried out using soil columns that have been created by packing sieved soils from various soil profiles into a PVC pipe or other cylindrical container. Pesticides applied at the surface move through the column with simulated rainfall. Pesticides or degradation products that are able to move by diffusion through the soil matrix along with water are retrieved at the bottom of the column. Concentrations are often measured by either gas chromatography (GC) or radiotracer techniques. The use of packed soil columns does not take into consideration the natural formation of macropores such as those created by plant roots or earthworm channels, and, thus, the contribution of preferential flow

from rapid movement of the pesticides through such channels can not be determined. Czapar et al. (1) compared mobilities of alachlor, cyanazine, and pendimethalin in soil columns with and without artificial macropores. These herbicides were detected in leachates from only those columns with continuous macropores, and they state that leaching studies that use packed soil columns may underestimate herbicide mobility. With the use of undisturbed soil columns in laboratory studies, one can obtain a more realistic understanding of parent pesticide and degradation product mobility under less variable conditions than in the field. Maintaining the integrity of naturally occurring macropores allows for not only measurement of the mobility of compounds due to diffusion through the soil matrix, but also mobility due to preferential flow of compounds with water.

Methods for acquiring and setting up large undisturbed soil columns for studies conducted in this laboratory were modified from (2), who had used such methods to investigate solute transport through macropores in large undisturbed soil columns. The earliest studies in our laboratory using this method were conducted to determined the mobility of atrazine and major degradation products (3). In earlier fate studies of atrazine, volatility and evolution of CO_2 from its degradation were reported to be minimal (4,5), thus completely enclosing the headspace of the columns for this study was not necessary.

For a deethylatrazine mobility study (6), the soil column set up was modified to enable trapping of $^{14}CO_2$ in order to improve the mass balance of ^{14}C-deethylatrazine applied to the column. Evolution of the modification of the soil column continued with the study of metolachlor mobility (7). A preliminary study indicated a poor mass balance, and, thus, modification to make the system completely enclosed was carried out. A flow-through system was incorporated into the study so that the column was never opened to the atmosphere. Aerobicity was maintained, however, through a port that contained a charcoal trap to allow for air exchange while trapping organic ^{14}C that was generated from metolachlor degradation. In the mobility study of highly volatile methyl bromide (8), additional modifications to soil columns were undertaken. Instead of adding another separate section to the top of the soil column, a continuous PVC pipe was used, thus eliminating the seam between the column and the head space. Additionally, a charcoal trap was suspended in the column to trap methyl bromide that volatilized above the soil.

The influence of vegetation on the mobility of pesticide adjuvants was also investigated. For these studies, undisturbed soil columns were either seeded with alfalfa or rye grass or left unvegetated. During the establishment of vegetation, soil columns were maintained under controlled temperature and lighting conditions for 4 months, with water added to the columns as needed. After sufficient growth of plants had been achieved, as noted by the observation of roots at the bottom of the columns, adjuvants were applied to the soil, and a leaching study was begun.

Fate Studies in Intact Soil Columns

Mobility and Degradation of Atrazine. The fate of atrazine was determined in a laboratory study using large undisturbed soil columns taken from a field with no previous pesticide history (3). Intact soil columns were obtained from a field with no previous pesticide history at the Iowa State University Agronomy and Agricultural Engineering Farm, Till Hydrology Site, near Ames, IA. In order to obtain the undisturbed soil columns, a circular trench 70 cm deep was

dug by using shovels, leaving a soil pedestal of approximately 40 cm in diameter in the middle of the trench. A furnace pipe measuring 15 cm in diameter and 60 cm tall was pressed gently into the top 2 to 3 cm of the soil pedestal, and soil was carved away at a depth of 5 to 10 cm in the same diameter as the furnace pipe before pushing the pipe further into the soil. In this way, compaction was avoided within the soil column. Physical and chemical characteristics of soils throughout the profile were determined (A & L Midwest Laboratory, Omaha) on subsamples of soils taken during column extraction.

Laboratory Preparation. In the laboratory, soil columns were prepared for laboratory experiments (Figure 1A). A polyvinyl chloride (PVC) pipe, measuring 20 cm in diameter and 60 cm in length, was centered around the soil column and the space was filled with molten parafin wax to prevent boundary flow along the outer edges of the columns during the leaching study (9). Prior to this step, the vertical surfaces of the soil columns were sealed with Plasti-Dip® spray (P.D.I., Inc., Circle Pines, MN) to prevent parafin from penetrating the soil column. An aluminum collar (15 cm tall) was fixed around the top of the soil column to prevent leachate from spreading over the wax during the leaching study. Once the wax was cooled and hardened, the bottom 1 cm of soil was removed, and a wire screen was placed in contact with the bottom of the soil column. A perforated Plexiglas™ plate (20-cm diameter) with six metal washers glued to it was mounted on the bottom of the PVC pipe. The washers served as spacers between the screen and the plate to prevent air locks and to assure continuous flow of leachate during the leaching study.

In order for the soil moisture of replicate columns to be equivalent at the beginning of the study, columns were saturated with 0.005 M $CaSO_4$ (2). This solution was chosen for soil saturation as its characteristics more closely resemble those of soil pore water than would ultrapure water. Each column was placed in a large metal garbage can, and $CaSO_4$ solution was added until columns were completely submerged. This submersion was done at a slow rate so that no air would be trapped within the soil column, with complete saturation accomplished over a 48-h period. Soil columns were then mounted in stands in a temperature-controlled room held at 25 °C and allowed to drain to field capacity. Ultrapure water was added to the top of each soil column and was then collected at the bottom to obtain a background leachate sample. A chloride tracer was applied to the top of the soil columns, which were then leached with ultrapure water to verify their performance (9). A qualitative comparison of the precipitate, arising from the drop-wise addition of 1 M $AgNO_3$ to the leachate, was made with background samples to ensure that the amount of chloride in the leachate was above the background level found normally in soil.

Soil Treatment. A solution was prepared with a mixture of Aatrex Nine-0® and [U-ring-^{14}C]ATR (98.2% radiopurity; Novartis Crop Protection, Greensboro, NC) dissolved in deionized water. Each column was applied with ATR at a soil concentration of 2.24 kg (active ingredient) per hectare and radioactivity level of 15 μCi of ^{14}C. The atrazine treatment was incorporated into the top 2 cm of soil. To minimize evaporation, the top of each soil column was covered loosely with aluminum foil. No attempt was made to trap for CO_2, since a previous soil metabolism study in this laboratory indicated minimal mineralization of ATR (< 1%) in soil the same field plot (4).

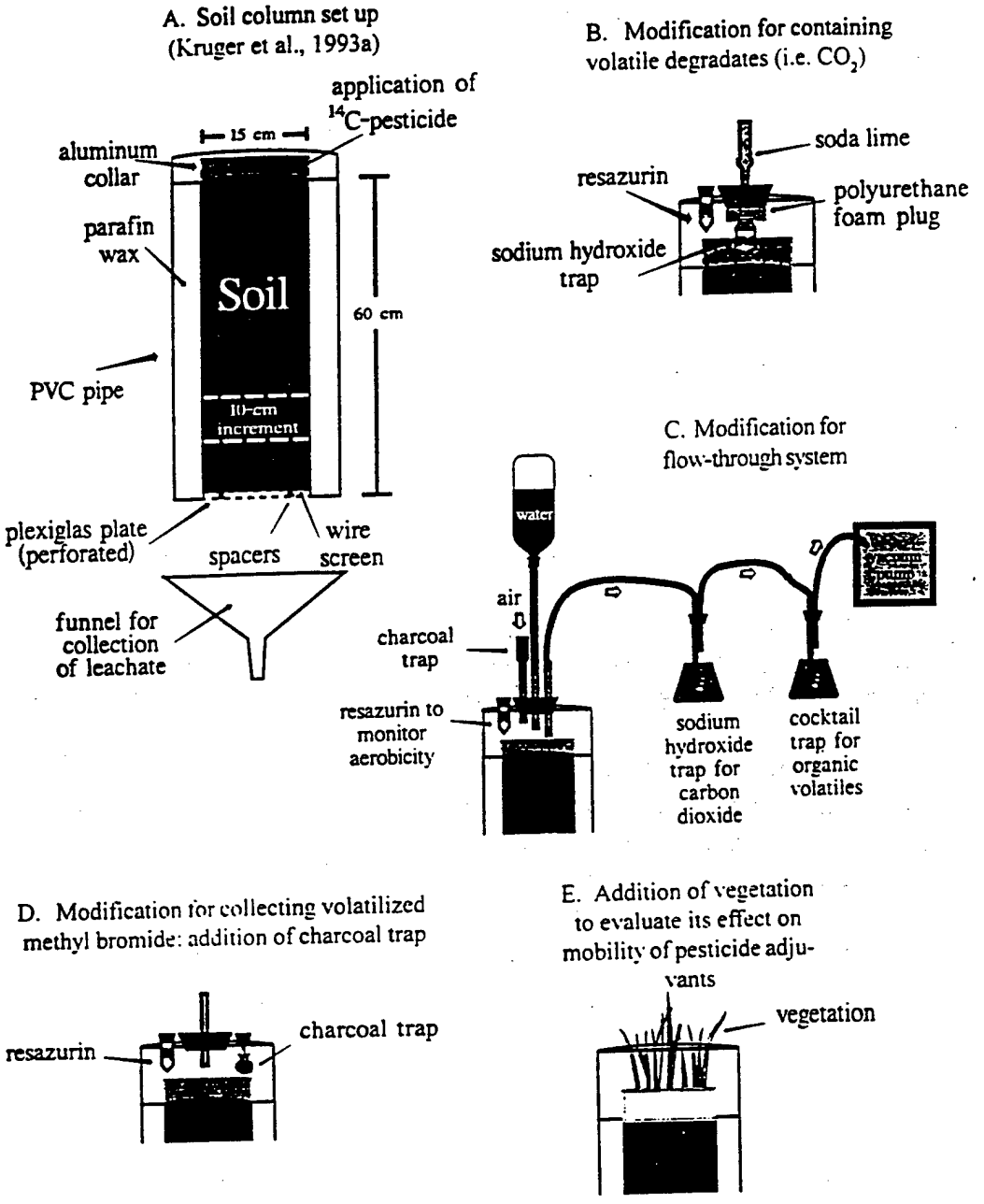

Figure 1. Preparation and subsequent modifications of intact soil columns for laboratory experiments

Leaching Study. So that mobility of not only the parent compound but also degradation products could be determined, a leaching study was not begun until three weeks after ATR treatment to allow for degradate formation from ATR. Soil columns were then leached weekly for 12 weeks with 3.8 cm of simulated rain (a quantity chosen to represent comparable rainfall amounts received during the spring in Iowa). The quantity of radioactivity recovered in leachate each week was determined by radioassaying subsamples of leachate by using liquid scintillation techniques.

Soil Extractions and Analyses. Following the leaching study, soil columns were cut into 10-cm increments. Subsamples from each depth (50-g dry weight) were extracted three times with 150 ml methanol/water (9:1), with an extraction efficiency for ATR of 100%. The extract was partitioned with dichloromethane, and subsamples of the concentrated organic fraction were radioassayed and used for thin-layer chromatography and autoradiography to determine the proportions of ATR and degradates. To determine the quantities of unextractable ^{14}C-residues, subsamples of extracted soils were combusted in a Packard sample oxidizer (Packard Instrument Co.).

Mobility and Degradation of Atrazine. In the leaching study, radioactivity was recovered in the leachate with the first rain event, indicating preferential (macropore) flow. Each week, approximately 0.1% of the applied radioactivity was detected in the leachate and, cumulatively, at the end of the 12-week period, 1.2% of the applied ^{14}C-ATR was recovered. With such a low amount of ^{14}C recovered in the leachate, no attempt was made to characterize the radioactivity (parent versus degradation products). Based on the proportion of ^{14}C to active ingredient in the treating solution, the cumulative concentration of ATR in the leachate was 7.6 µg/L.

The majority of the radioactivity remained in the top 10 cm of soil (77% of the applied ^{14}C), with the greatest proportion of ^{14}C as soil-bound (unextractable) residues (57%) (Figure 2). Deethylatrazine (DEA) was the most predominant degradate, with 3.6% of the applied ^{14}C characterized as DEA in the top 10 cm of soil. Deisopropylatrazine (DIA) was the second most predominant identified degradate. Polar degradation products (from the aqueous fraction after partitioning of the soil extracts) were greater in the top 10 cm of soil than in other depths.

In this study, ATR, along with the two degradation products DEA and DIA, exhibited the greatest mobility as they were detected at all depths. Their presence in soils of all depths of the column might also be attributed to degradation of ATR after reaching these depths.

Mobility and Degradation of Deethylatrazine. A study was conducted to determine the fate of deethylatrazine (DEA), a major metabolite of ATR, in large undisturbed soil columns (6). Soil columns (15-cm diameter x 60-cm length) were obtained from a field with no previous ATR history, as described in the ATR section. Two soil columns were prepared for laboratory experiments by using a modification of the method described by (3) (Figure 1B). In order to obtain a mass balance of the applied ^{14}C, the top of the column was sealed with an additional section of PVC pipe (20-cm diameter by 20-cm length), and the top of this section was capped with a plexiglas plate and sealed with silicon rubber adhesive sealant (General Electric Co., Waterford, NY) (6). Within the Plexiglas™ plate, three holes were cut. A large central

hole, or port, was sealed with a neoprene stopper and used to access the top of the soil column during the leaching experiment. The stopper had a glass rod through the center which served as an attachment site for a polyurethane foam trap for trapping ^{14}C-organic volatiles. In order to trap $^{14}CO_2$ arising from complete mineralization of ^{14}C-DEA, a sodium hydroxide (NaOH) trap was suspended from a neoprene stopper in a second port. In order to assure that aerobic conditions were maintained, a perforated plastic centrifuge tube (capped with a neoprene stopper) containing 5 ml ultrapure water and two drops of a 4% resazurin solution was inserted to serve as a monitor for the aerobicity of the headspace over the soil column. All neoprene stoppers were wrapped with Teflon® tape.

Soil Treatment and Leaching. Each column received an application of DEA equivalent to 0.5 lb a.i./acre and approximately 20 µCi of [^{14}C]DEA by applying a treating solution prepared with a mixture of analytical grade DEA and [U-ring-^{14}C]DEA (94.8% radiopurity) [^{14}C]DEA dissolved in ultrapure water. The DEA treatment was incorporated into the top 2 cm of soil to minimize volatilization of DEA. Three days after treatment, a leaching study was initiated with an equivalent of 3.8 cm of rainfall (675 ml ultrapure water) applied slowly to the top of each column per week for 13 weeks. Rainfall applications usually took between 40 and 60 min. Leachate from each rain event was collected at the bottom of columns in 100-ml aliquots which were analyzed for radioactivity by liquid scintillation counting techniques (LSC).

Solid-phase Extraction (SPE) of Leachate. A modified SPE method was used to isolate DEA and degradates from the leachate (10). After filtering the leachates through a glass-microfiber filter, the pH was adjusted within the range of 7.0 to 7.5 by drop-wise addition of aqueous ammonia or phosphoric acid. For this procedure, Bond Elut® (Varian, Harbor City. CA) cyclohexyl SPE cartridges were used. After conditioning the cartridges with methanol and ultrapure water, leachates were passed through the SPE cartridges at a rate of approximately 5 ml/min, and then the cartridges were air-dried. DEA and degradates were eluted from the cartridges with 10 ml of acetonitrile. Effluent volumes were taken, and subsamples of the effluent and eluate were counted by using liquid scintillation spectroscopy, with radioactivity in the effluent categorized as unidentified polar degradates. DEA and degradates in the acetonitrile eluate were characterized by thin-layer chromatography on normal phase silica gel plates in a solvent system of chloroform: methanol: formic acid: water (100:20:4:2) (Novartis Crop Protection). Autoradiography was used to visualize the radioactive spots associated with ^{14}C-standards. TLC plates were scraped and counted using LSC techniques.

Soil Extractions and Analyses. At the conclusion of the leaching experiment, soil columns were cut into 10-cm increments, and subsamples were extracted and analyzed as described in the ATR section and in (3).

Statistical Analysis. For components of the leachate, an analysis of variance (ANOVA) was performed on the repeated measures design. Orthogonal contrasts were also determined for specific comparisons of leaching events. To determine the effect of soil depth, an ANOVA which used soil columns as a blocking variable was conducted on the components determined in soil extractions and analyses.

Mass Balance of DEA in Leachate and Soil. For this experiment, the overall mean recovery of radioactivity was 97%, with 89% of the applied radioactivity distributed throughout soil columns at the end of the leaching study. Less than 0.2% of the applied ^{14}C was recovered as $^{14}CO_2$, and no ^{14}C-organic volatiles were detected.

Preferential flow was noted during the first leaching event, with a significantly greater percentage of ^{14}C being leached in this rain event (2.3% of the applied ^{14}C) compared with all other rain events ($p = 0.0002$) (Figure 3). Of this amount, 1.3% was a characterized as DEA. There were no significant differences in the quantities of DEA leached from the columns for rain events 2 through 13. Unidentified polar degradates made up 1% of the radioactivity from the first rain event. After the sixth rain event, the concentration of polar degradates exceeded that of DEA in the leachate. Trace amounts (< 0.01%) of didealkylatrazine (DDA) and deethylhydroxyatrazine (DEHYA) occurred in the leachate throughout the leaching study. With the eleventh rain event, significantly greater DDA and DEHYA concentrations were noted, compared with all of the other rain events ($p = 0.002$ and $p = 0.004$, respectively).

Cumulatively, 7.5% of the radioactivity applied to the top of soil columns was recovered in the leachate over the course of the 13-wk leaching experiment. In consideration of the unlabeled analytical grade DEA associated with this quantity of radioactivity in the treating solution and taking into account the total volume of the leachate, this corresponds to a total DEA/degradate concentration of 10 μg/L (in DEA equivalents). With 3.6% as DEA, this corresponds to a concentration of 4.9 mg/L. The percentages of DDA and DEHYA in the leachate over the 13-wk study were less than 0.02% of the applied, while unidentified polar degradates accounted for 3.8% of the applied radioactivity. The unidentified polar degradates may have included DDA and DEHYA since the SPE method used may not have been as efficient for polar degradates including DDA and DEHYA, although it was efficient for isolating DEA. Recent research by (11) has focused on methods for isolation of polar degradates. In comparing the ATR-applied and DEA-applied soil column studies, it was noted that DEA was more mobile than ATR. After 12 weeks, 6% of the applied DEA was leached (DEA and degradates), compared with only 1% in ATR-applied soil columns (Figure 4).

Distribution of ^{14}C-DEA and Degradates in the Soil Profile. The top 10 cm of soil columns retained the majority of the applied ^{14}C (67%). The percentage of DEA was significantly greater in this depth (5.5%) than in the remaining depths (≤ 1.2%) ($p = 0.0001$). There were no significant differences in the quantities of DDA and DEHYA extracted from all depths. Significantly larger quantities of unidentified polar degradates were formed in the top 10 cm (12%) compared with deeper soils (≤ 2.6%) ($p = 0.001$). Fifty-seven percent of the applied radioactivity was unextractable (soil bound) from soil columns (sum of all depths). In the top 10 cm, 48% of the applied radioactivity was unextractable, and this quantity was significantly greater than in soils deeper than 10 cm (≤ 4.8%) ($p = 0.0001$). The quantities of bound residues in the 10 to 20-cm depth were an order of magnitude below those formed in the top 10 cm (4.8%), and there were no significant differences in bound residue quantities among soils below 20-cm depth.

Mobility and Degradation of Metolachlor. Metolachlor (2-chlor-*N*-(2-ethyl-6-methylphenyl)-*N*-(methoxy-1-methylethyl)acetamide) is one of the most widely used herbicides in the Midwestern United States (12). This moderately soluble (530 mg/L at 20°C) nonionizable

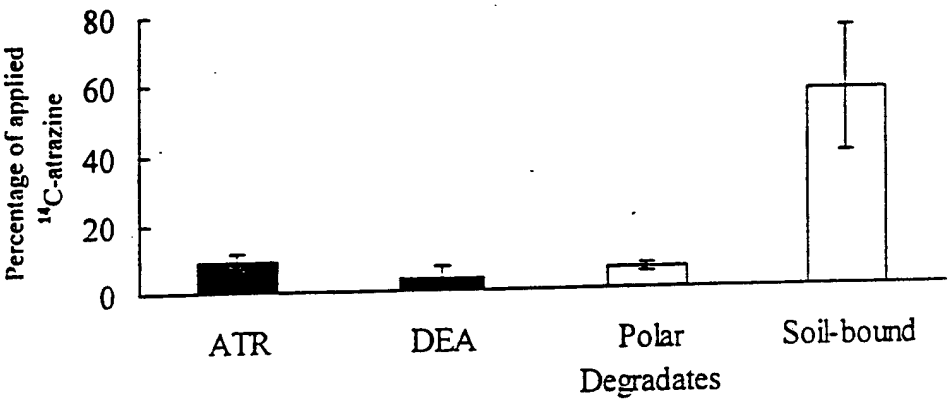

Figure 2. Percentages of applied ^{14}C as ATR, DEA, polar degradates, and soil-bound residues in the top 10 cm of intact soil columns treated with ^{14}C-ATR. Bars represent standard errors (n = 2).

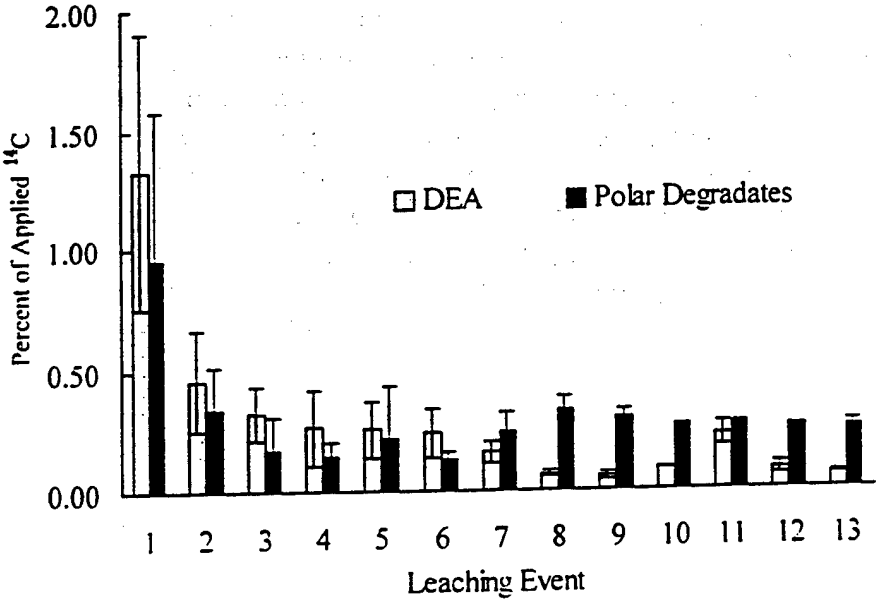

Figure 3. Percentages of applied ^{14}C-DEA recovered in leachate as DEA or polar degradates. Bars represent standard deviations of the mean (n = 2).

herbicide is primarily transported in the water phase and is more mobile and persistent than other chloroacetamide herbicides (13-16). Researchers have detected metolachlor in subsurface soils (2.28 m deep) (14,17), subsurface tile drain water, and groundwater (18-20). Based on the water solubility, sorption behavior, and moderate persistence of this herbicide, the United States Environmental Protection Agency (USEPA) has listed metolachlor as having a potential to leach through soil and contaminate groundwater (21). The following undisturbed soil column studies were conducted to evaluate the fate of radiolabeled metolachlor in the soil profile. The depth to which metolachlor leached in the soil and the quantity of metolachlor and metolachlor degradation products in the soil column and the leachate were determined.

Laboratory Preparation. Undisturbed soil columns were extracted from a pesticide-free field , prepared in the laboratory, and saturated with 0.005 M $CaSO_4$ as previously described (Figure 1A). The performance of each column and the reproducibility between the replicate soil columns were evaluated with a bromide ion tracer. A potassium bromide solution containing 17.5 mg of potassium bromide (9.903 kg/ha) was applied to the surface of each column. The soil columns were leached with 675 ml of ultrapure water to simulate 3.8 cm of rainfall. Leachates were collected in fractions, and the concentration of bromide ion in each fraction was measured using an ion-selective electrode.

Soil Treatment and Modification of the Soil Column for a Flow-through System. Technical grade metolachlor and [U- ring -^{14}C]metolachlor (100 μCi) were dissolved in water and uniformly applied to the surface of each soil column at the rate of 3.36 kg ai/ha. The treating solution was incorporated into the top 2 cm with a spatula, and glass wool was place over the top of each soil column to maintain the integrity of the surface. The top of the PVC pipe was sealed with a Plexiglas™ plate containing a polytetrafluoroethylene-covered neoprene stopper (#13) with three glass tube ports (Figure 1C). The center port was connected to a separatory funnel that allowed ultrapure water to be applied to the soil column weekly. The second port contained a charcoal trap that allowed air into the column and trapped organic volatiles from the headspace of the column. The final port led to a 0.1 N sodium hydroxide trap, followed by an Ultima Gold™ trap. A vacuum pump was used to create a suction that bubbled the contents of each column headspace through 0.1 N sodium hydroxide and Ultima Gold™ traps to absorb $^{14}CO_2$ and ^{14}C-organic volatiles, respectively. The radioactivity of the trapping solutions was measured with a liquid scintillation spectrometer. A vial containing a resazurin solution (several drops of a 4% resazurin solution in ethanol + water) was used to determine when the enclosed headspace of the soil column became anaerobic. When the column headspace became anaerobic, the headspace of the column was exchanged more frequently (22).

Leaching Study. Beginning 3 weeks after the treatment of the soil columns, each column was leached weekly for 12 weeks. The initial leaching of the columns was begun 3 weeks after the herbicide treatment to allow metolachlor to begin to degrade in order to observe the mobility of metolachlor and metolachlor degradation products through the soil profile. The leachates were collected, and the radioactivity in each leachate was determined by LSC.

Solid-phase Extraction (SPE) and Analyses of the Leachate. A portion of each leachate was vacuum filtered (glass fiber filter paper) and drawn through a solid phase extraction cartridge (Supelclean Envi-18™). The quantity of [^{14}C]metolachlor and [^{14}C]metolachlor degradation products in the methanol eluates were characterized by thin-layer chromatography (250-mm silica gel 60 F-254; hexane/methylene chloride/ethyl acetate (6:1:3, v/v/v) solvent system) (23) and autoradiography (X-Omat™ Kodak diagnostic film). The location of the non-radiolabeled standards were identified with an ultraviolet lamp (254 nm), and the percent of radioactivity characterized as metolachlor or metolachlor degradation products was measured by LSC.

Soil Extraction and Analysis. At the completion of the leaching study, soil columns were disassembled and divided into 10-cm sections. Three 50-g subsamples were taken from each 10-cm section and extracted three times with 150 ml of methanol/water (9:1 v/v). The quantity of metolachlor and metolachlor degradation products in the soil extracts were determined by thin-layer chromatography and autoradiography as described in the analysis of the leachates. Extracted soils were combusted with hydrolyzed starch in a Packard sample oxidizer (Packard Instrument Co., Downer's Grove, IL.) to determine the quantity of [^{14}C]soil bound residues. Radiolabeled CO_2 resulting from the combustion of the soil was trapped in Carbo-Sorb® E and Permafluor® V (Packard Instruments Co.) and the radioactivity in each sample was quantified using LSC. Percentages of [^{14}C]metolachlor mineralized to $^{14}CO_2$, leached through the soil column, and the amount remaining in the soil (bound and extractable) was calculated. Analysis of variance (ANOVA) was used to determine significant differences between metolachlor and metolachlor degradation products in the soil extracts and significant differences between soil-bound residues in the 10-cm sections of the extracted soil column.

Mobility and Degradation of Metolachlor. The initial metolachlor mobility studies were conducted with undisturbed soil columns similar to the deethylatrazine-treated columns (Figure 1B). At the completion of the analysis only 42% of the applied radioactivity had been recovered. Additional soil columns were treated with [^{14}C]metolachlor, and modifications were made to the soil columns to reduce chemical loss and improve the final mass balance. Despite the addition of the flow-through system (Figure 1C) and attempts to account for all radioactivity, the recovery of the applied ^{14}C in the modified soil columns was 44%. The results of this study are reported in percentage of recovered radioactivity.

Twenty-five percent of the recovered ^{14}C leached through the soil profile of the undisturbed soil columns. The quantity of radioactivity detected in the leachate gradually declined with the leaching events from 3.34% in the first leachate to 1.09% in the final leachate (Figure 5). Only trace amounts (<1%) of the recovered radioactivity were characterized as the parent compound, [^{14}C]metolachlor, in each of the leachates. At the completion of the leaching study, greater than 6,500 ml of leachate had been collected at the bottom of each column. The calculated concentration of metolachlor in the total leachate was 4.5 µg/L.

Metolachlor was degraded in the soil to a number of degradation products. Between six and eleven degradation products were detected in each leachate. Our findings are in agreement with those of (24) who detected metolachlor and six unidentified metolachlor metabolites in the leachate of greenhouse lysimeters. The presence of eight degradation products in the first leaching event indicates that some of the degradation products of metolachlor were as mobile or more mobile than the parent compound.

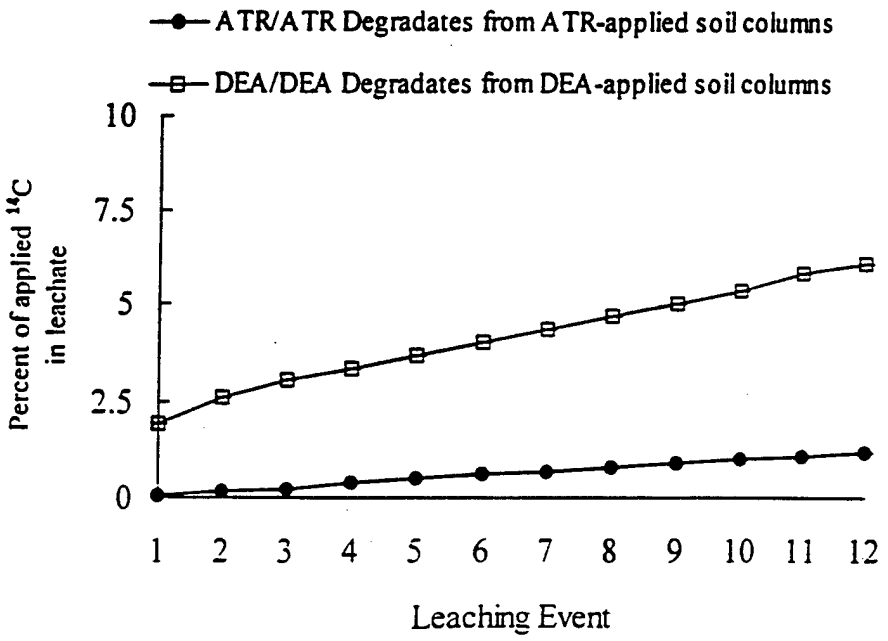

Figure 4. Radioactivity recovered in leachate from atrazine- or deethylatrazine-treated soil columns. Bars represent standard deviations of the mean (n = 2).

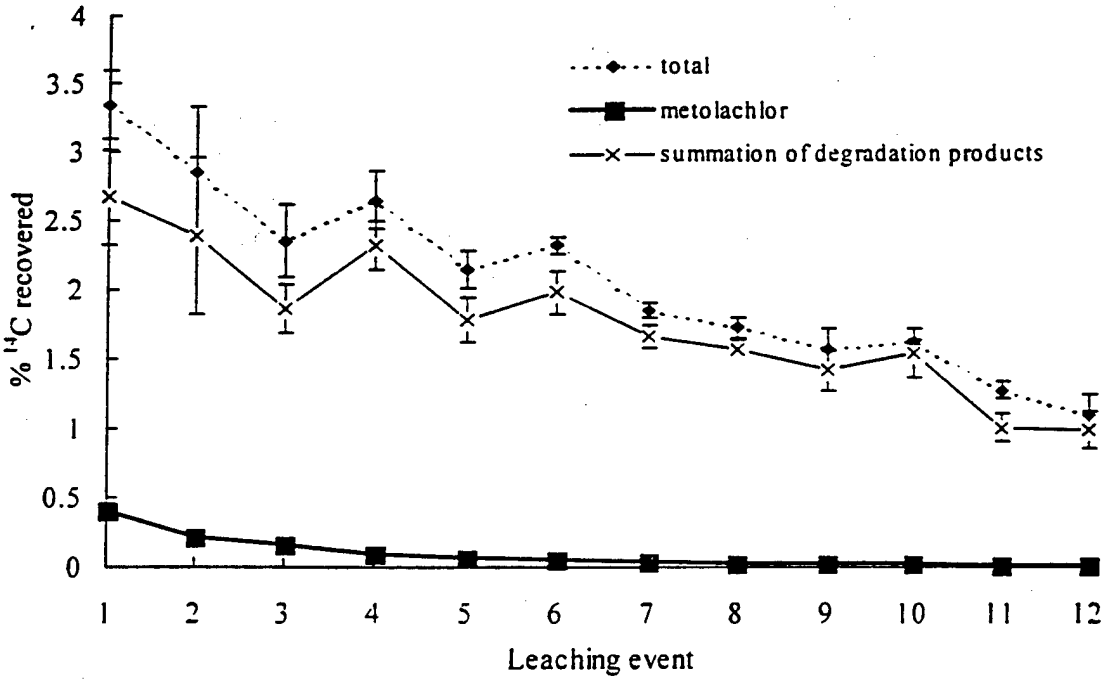

Figure 5. Metolachlor and metolachlor degradation products in the leachate of the undisturbed soil columns.

Distribution of Metolachlor and Metolachlor Degradation Products in the Soil Profile. Seventy-five percent of the recovered ^{14}C remained in the soil column. Surface soils (0-10 cm) contained more than five times the amount of radioactivity detected in any of the subsurface soils (10-60 cm) (Table I). The significantly greater ($p < 0.05$) percentage of radioactivity detected in the surface soils and the significant decline in the quantity of ^{14}C in the subsurface soils are similar to the findings of in the observations of (24,25) the fate of [^{14}C]metolachlor in greenhouse lysimeters and field lysimeters, respectively.

Soil-bound residues accounted for the largest percentage of radioactivity detected in the surface soils (Table I). Subsurface soils contained significantly less ($p < 0.05$) nonextractable residues than surface soils. The quantity of radioactivity bound to the surface soil was between eight and twenty-five times the amount found in the subsurface sections. The fate of agricultural chemicals in the soil and their potential to leach through the soil profile and contaminate groundwater is dependent on the persistence of the compounds and their sorption to the soil (26). Adsorption of metolachlor to soil is correlated with increasing organic carbon content ($r = 0.72$), percent clay ($r = 0.80$), and cation exchange capacity ($r = 0.94$) of the soil (9, 13, 27-29). Surface soils often contain greater quantities of organic matter than subsurface soils. Laboratory and greenhouse studies have reported metolachlor is weakly adsorbed and highly mobile in low-organic matter soils (<1% organic matter) (27). Examination of this Iowa soil reveals a 2.3% to 3.0% organic matter content in the soil from 0 to 45 cm. The presence of 48.7% of the recovered ^{14}C (31.9% nonextractable bound residues) in the top ten centimeters of our columns is believed to be the result, in part, of metolachlor and metolachlor degradation products adsorption to the humic fraction of this soil (30).

The greatest quantity ($p \leq 0.05$) of extractable [^{14}C]metolachlor and [^{14}C]metolachlor degradates were detected in the top 10 cm of the soil column (Table I). Less than 2% of the recovered ^{14}C was identified as [^{14}C]metolachlor in the soil extract of the surface soils, while 14% was identified as extractable metolachlor degradation products. Negligible quantities of metolachlor were measured in the soil extracts of the subsurface soils ($\leq 0.07\%$). Extractable metolachlor degradation products ranged from 0.88% in the 40-50 cm section to 3.49% in the 10-20 cm section.

Mineralization and Volatilization of Metolachlor. Ultima Gold™ traps and sodium hydroxide traps were used to collect organic volatiles and $^{14}CO_2$ produced from the mineralization of [^{14}C]metolachlor. Mineralization of [^{14}C]metolachlor to $^{14}CO_2$ was minimal. Less than one percent of the recovered ^{14}C was detected in the NaOH traps. Organic volatiles were not detected in the headspace of the columns.

Volatility and Mobility of Methyl Bromide. Undisturbed soil columns were used to study the volatility, mobility, and degradation of methyl bromide (MeBr) in soil (7,31). Two undisturbed soil columns (15-cm diameter x 38-cm length) were obtained from an agricultural field site (no previous pesticide history) near Ames, IA. Procedures for the collection, removal, and storage of the columns were previously described by (2) and in this chapter. Additional soil samples were collected at the same depths as the column to determine the soil physicochemical properties. A composite of these soil samples consisted of sandy clay loam soil with a pH of 5.4 and 54% sand, 25% silt, 21% clay, and 2.5% organic matter.

Table I. Distribution of [^{14}C]metolachlor and [^{14}C]metolachlor degradation products in undisturbed soil columns

	Percent of recovered ^{14}C (± SE)[a]					
Soil depth:	0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm	50-60cm
Extractable residues						
Metolachlor	1.30 A (0.102)	0.02 B(0.007)	0.06 B (0.003)	0.06 B (0.009)	0.07 B(0.016)	0.05 B(0.011)
Degradation products	14.0 A (0.76)	3.49 B(0.19)	2.38 C (0.078)	1.55 D (0.055)	0.88 D(0.098)	0.96 D(0.086)
Soil-bound residues	31.9 A (2.1)	3.71 B(0.12)	2.86 B (0.16)	1.84 B (0.088)	1.32 B(0.044)	1.25 B(0.039)
Total[b]	48.7 A (2.07)	8.35 B(0.73)	6.09 B,D(0.25)	5.22 C,D(0.43)	3.62 C,D(0.69)	3.07 C(0.51)

[a]Means in each row followed by the same letter are not statistically different (p=0.05).
[b]Summation of metolachlor, metolachlor degradation products, and soil-bound residues.

Undisturbed soil columns were prepared for laboratory studies as described by (3,6) and in this chapter (Fig. 1A-1B). Modifications were made to collect the MeBr that volatilized from the soil (Fig. 1D) (7). The PVC pipe on the exterior of the column was longer than the soil column to ensure sufficient headspace for the addition of an activated carbon trap. A Plexiglas™ plate with three openings was mounted to the top of each PVC pipe and sealed with silicon rubber adhesive sealant. These openings were sealed with polytetrafluoroethylene-covered neoprene stoppers containing either a granular-activated carbon trap, resazurin trap, or glass tube sealed with a septum (for addition of water). Granular-activated carbon traps were suspended in the headspace of each column to adsorb MeBr that volatilized from the soil. Each trap consisted of 8 g activated charcoal wrapped in 5 x 5-cm, 100% cotton net (1-mm mesh). Resazurin traps (0.5 ml 4% resazurin in ethanol, with 4.95 ml deionized water) were used to indicate if the column headspace was becoming anaerobic. Soil columns were initially saturated with 0.005 M $CaSO_4$ then drained to field capacity as previously described in this chapter. Four 500-mL increments of deionized water were leached through the columns to determine the naturally occurring bromide ion (Br^-) background concentration.

Soil Treatment and Leaching. Liquid MeBr (at 0.57 kg/m3, or 1 lb/yd^3) was applied to the soil surface, and the columns were immediately sealed. Soil columns were incubated at 24 ± 1 °C for 48 h to allow this fumigant to penetrate the soil and reach an equilibrium between the air/soil/water. After the 48-h equilibration period, MeBr-fumigated columns were place in column stands, maintained at 24 ±1 °C, and leached weekly (for 23 weeks) with 500 mL deionized water to represent 2.5 cm of rainfall (7). Leachates were collected at the bottom of the columns and analyzed for MeBr and its degradation product, Br^-.

Collection and Analysis of Volatilized Methyl Bromide. Forty-eight hours after the application of MeBr, activated carbon and resazurin traps were suspended in the headspace of each soil column. Carbon traps were replaced periodically and used to determine the amount of MeBr in the headspace of the column. Upon removal, these traps were placed in 45-mL glass bottles equipped with screw caps and polytetrafluoroethylene-lined septa and stored at -60 °C until analysis. The headspace of these bottles was analyzed prior to the desorption of MeBr from the carbon. Procedures used to desorb MeBr from the carbon traps were modified from (32). Two gram-carbon subsamples from each trap were placed in 7-mL glass vials and sealed with a polytetrafluoroethylene-lined septa. Three mL of air was evacuated from the vials with a gas-tight syringe and replaced with 3 mL benzyl alcohol (Fisher Scientific, Pittsburgh, PA). Samples were warmed to 110 °C for 15 minutes and the headspace was analyzed by gas chromatography (GC). The quantities of MeBr detected in the headspace of the bottles and desorbed off the carbon were considered in the final calculation of MeBr that volatilized from the soil.

Procedures for the analytical standards and analysis of sample and standard headspace were modified from (32). Methyl bromide standards were made in benzyl alcohol, stored at -60 °C, and replaced every 2 weeks. Samples were analyzed on a Varian 3740 gas chromatograph equipped with a ^{63}Ni electron-capture detector. The glass column (0.912 m x 2.0 mm i.d.) was packed with 100/120 mesh Porapak Q (Supelco Inc., Bellefonte, PA) on Carbopack with a carrier gas consisting of ultrapure nitrogen (26 mL/min). Injector, column,

and detector temperatures were 170 °C, 140 °C, and 350 °C, respectively. Peak heights were used to construct a calibration curve and quantitate the samples.

Analysis of Leachate. Soil column leachates were analyzed for MeBr and Br^- by using GC headspace analysis as described above and a bromide-specific electrode attached to pH meter (Fisher Scientific, Pittsburgh, PA). Br^- standards were prepared with NaBr. deionized water, and 5 M $NaNO_3$ (ionic strength buffer). Calibration curves were constructed from the standards and used to determine the sample concentrations.

Soil Extractions and Analysis. At the completion of the study, undisturbed soil columns were cut into 5-cm increments and analyzed for MeBr and Br^- residues. Three 10-g subsamples from each soil profile were placed in 45-mL glass vials, and the headspace was analyzed on a GC as described above. These soil samples were then extracted with 20 mL deionized water by mechanical agitation and centrifugation. The supernatant was removed and analyzed for Br^- using a bromide-specific electrode.

Volatility, Mobility, and Degradation of Methyl Bromide. MeBr volatilized rapidly from the soil. The flux of MeBr from the undisturbed soil columns is shown in Figure 6. Greater than 75% of the MeBr flux occurred within 48 h after the fumigation period. After 7 days, MeBr was not detected in the soil column headspace. The volatilization of MeBr from our undisturbed soil column study was comparable to the MeBr field study results reported by (31,33-34). Anderson et al., (31), also observed greater than 75% of the MeBr flux occurred within 4 days after MeBr application. Negligible quantities of soil gas MeBr were detected after 7 d (31,33).

Soil column leachates from each rain event were analyzed for Br^- and MeBr. Within the first rain event following the MeBr fumigation, Br^- increased from a background level of 0.01 μg/g to 0.4 μg/g (Figure 7) The concentration of Br^- in the leachate continued to increase, peaked at 3 weeks (4.3 μg/g), and gradually decreased with subsequent rain events. A total of 28.8 μg/g Br^- leached through the soil column, which represents > 5% of the MeBr initially applied. MeBr was not detected in any of the soil column leachates throughout the 23-week study. Wegman et al. (35) detected MeBr and Br^- in drainage water from fumigated glasshouse soils. They observed a sharp increase in the concentration of Br^- during the initial irrigation of greenhouse soils, followed by a steady decrease. The increase of Br^- and the absence of MeBr in the soil column leachates indicate MeBr will degrade in the soil and will not leach through this soil profile.

After the final rain event, soil columns were divided into 5-cm fractions and analyzed for MeBr and Br^-. Residuals of this fumigant and the metabolite were not detected in the soil profile. Levels of Br^- were similar to control (untreated) soil samples. Persistence of MeBr in soil appears to be low, primarily due to its rapid volatilization, as well as biological and chemical degradation. Based on these results MeBr would not be expected to contaminate groundwater unless preferential flow was involved.

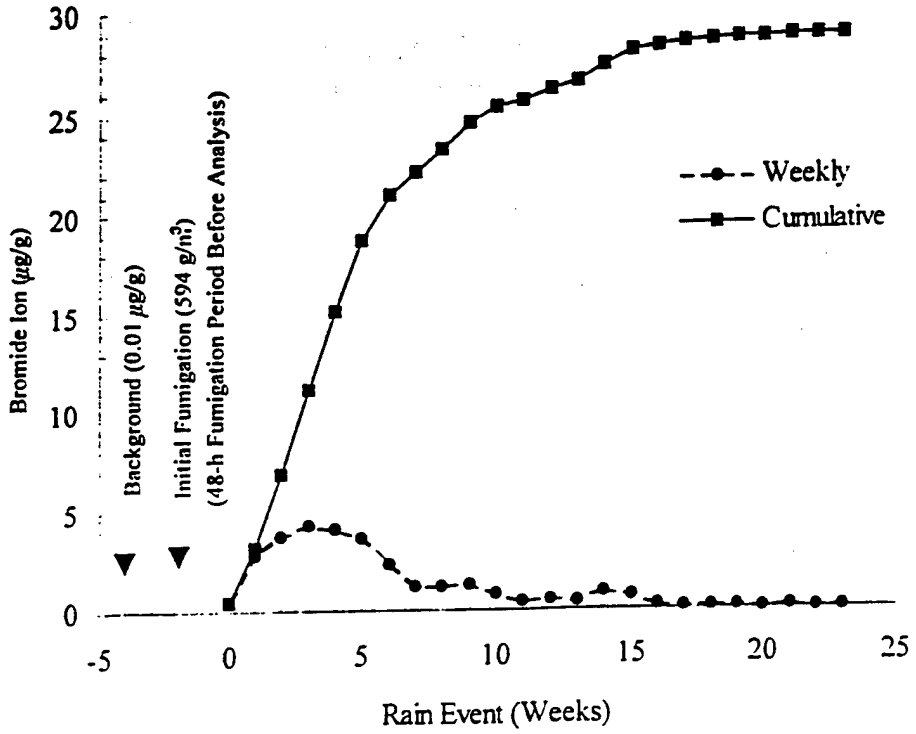

Figure 6. Volatility of methyl bromide in undisturbed soil columns following a 48-h fumigation period. Data points are the mean ± one standard deviation.

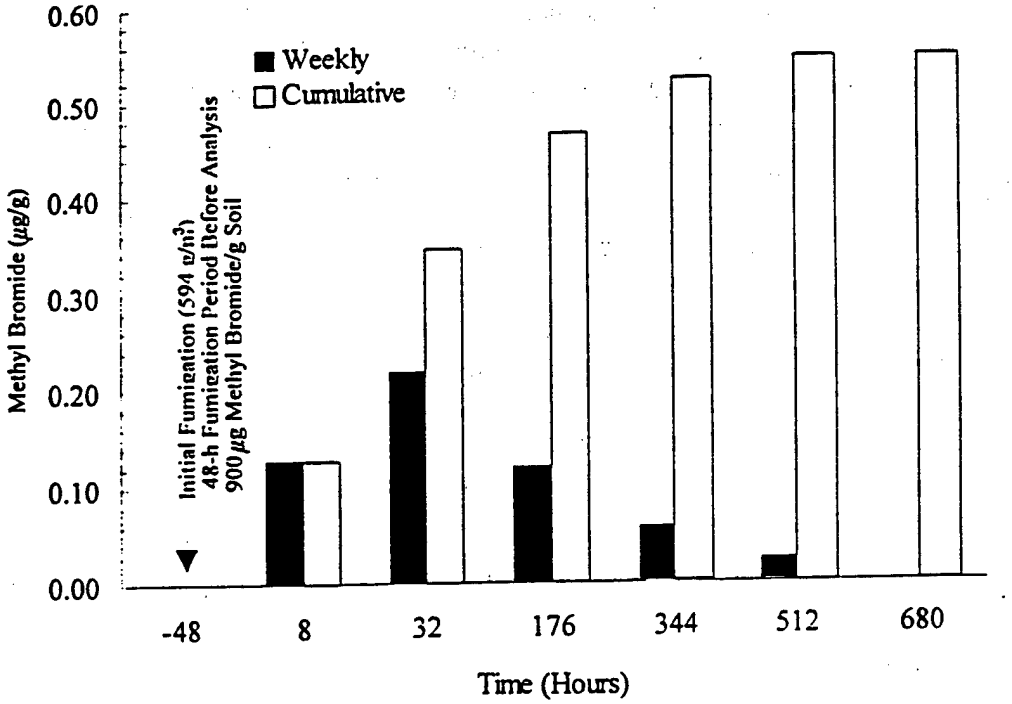

Figure 7. Bromide ion breakthrough from an undisturbed soil column treated with methyl bromide. Soil columns were leached weekly after the 48-h fumigation period.

Pesticide Adjuvants.

Pesticide applications invariably include the use of various adjuvants in the commercial formulations. This broad category can include solvents, emulsifiers, sticking agents (adhesives), UV-light protectants and dyes, among others. They are mostly considered to be inert ingredients, but their environmental fate should also be addressed. Most of them have other industrial uses and, hence, other environmental inputs.

Ethylene glycol and propylene glycol have been used as solvents in pesticide formulations as well as antifreeze in vehicles and deicing agents for airplanes and runways. Their environmental impact is often expressed as an excess nutrient input into bodies of water, leading to a eutrophic anoxic system in which fish and other species with high oxygen requirements die. Their degradation and mobility have not been previously investigated in a soil column experiment.

Effects of Plants on the Degradation of Pesticide Adjuvants in the Intact Soil Column. Rhizosphere is the region of soil directly influenced by the roots. Plant roots secrete energy-rich exudates (sugars, amino acids, vitamins, and keto acids) and mucilages (polysaccharides) that support large and diverse populations of microorganisms. Root-influenced soils have a greater microbial biomass (10 to 100 times) and activity than bulk soils; therefore, enhanced degradation of organic compounds may occur in the rhizosphere (36-39). In addition, the interaction between plants and their associated microbial communities is mutually beneficial for both types of organisms. Soil microorganisms have a positive influence on plants by 1) solubilizing inorganic nutrients and secreting organic compounds (gibberellins, auxins, amino acids, and vitamins) that stimulate plant growth and 2) potentially deterring plant pathogens through competition and production of antibiotics (36, 38, 40).

Vegetation can enhance the removal of human-made organic compounds and pollutants in soil environments by microbial degradation in the rhizosphere and plant uptake (41,42). Previous research has shown enhanced degradation of industrial chemicals such as trichloroethylene (43) polycyclic aromatic hydrocarbons (44), and petroleum (45) in the rhizosphere soil as compared to root-free soil. Increased mineralization of the pesticides parathion (46) and carbofuran (47) has been reported in the rhizosphere of rice plants. Hsu and Bartha (48) noted similar results for parathion in the bean rhizosphere. Accelerated mineralization of pesticides has also been found in the rhizosphere of plants from pesticide-contaminated sites. Anderson et al. (49) observed greater microbial biomass and enhanced degradation of atrazine, trifluralin, and metolachlor (after 14 d) in the rhizosphere soil of herbicide-resistant *Kochia sp.* in comparison to nonrhizosphere and sterile soils, respectively. In addition to enhanced degradation in the rhizosphere, plants may take up contaminants as part of their transpiration stream (41). Lee and Kyung (47) monitored the uptake of fresh and aged carbofuran residues by rice plants. Approximately 60 to 70 % of the ^{14}C detected in the shoots was the intact parent compound in both the freshly applied and aged soils. Anderson and Walton (50) studied the fate of [^{14}C]TCE in soil-plant systems collected from a contaminated site. They reported that 1 to 21% of the recovered radiocarbon (depending on the plant species) was detected in the plant tissues, particularly in the roots. Vegetation may play a vital role in reclaiming polluted ecosystems and preventing further contamination by enhancing degradation and uptake into tissues, thereby reducing migration to surface waters and groundwater aquifers.

Effect of Vegetation on Mobility of Pesticide Adjuvants. Vegetated undisturbed soil columns were used to study the influence of plants on the mobility of propylene glycol (PG) and ethylene glycol (EG) through the soil profile. High concentrations were applied to nonvegetated and vegetated undisturbed soil columns to simulate spills of these solvents used as antifreezes, airplane deicing agents, and pesticide adjuvants.

Undisturbed soil columns (15-cm x 38-cm length) were collected from an agricultural field site and prepared for laboratory studies as previously described in this chapter (Figure 1A) and by (2,3).

Eight soil columns (4 each) were planted with alfalfa (*Medicago sativa*) or rye grass (*Lolium perenne L.*) (Figure 1E). Nonvegetated and vegetated columns were maintained in a greenhouse (25 °C, 16:8 light:dark) for 4 months to allow sufficient growth of the plants. Water was added to the columns as needed. Roots of *M. sativa* and *L. perenne* were observed through the clear perforated Plexiglas™ bottom of the columns. This indicated that *M. sativa* and *L. perenne* roots were established through the length of the columns. Following the four-month growth period, soil columns were saturated with 0.005 M $CaSO_4$ (2), then drained to field capacity.

Soil columns were moved to an incubator set at 25 °C, and the temperature was slowly decreased (approximately 3 °C/24h) to 10 °C to represent spring conditions. Soil columns were maintained at 10 °C with a 16:8 light:dark cycle for 96 h prior to the treatment to acclimate plants to this temperature. During this 96-h time period, soil columns were leached with 400 ml deionized water. These leachates were analyzed on a gas chromatograph equipped with a flame ionization detector (GC-FID) and with a bromide-specific electrode attached to a pH meter (Fisher Scientific, Pittsburgh, PA) to determine background levels of propylene glycol and Br^-, respectively. A KBr tracer was applied to the soil surface and leached through the soil columns with deionized water. Breakthrough curves were determined for each column by analyzing the quantity of Br^- in the leachate. Br^- standards were prepared with NaBr, deionized water, and 5M $NaNO_3$ (ionic strength buffer). Calibration curves were constructed from the standards and used to determine the sample concentrations (8).

Soil Treatment and Leaching. Propylene glycol (Fisher Scientific, Fair Lawn, NJ) solution (1.76 ml PG/ 364 ml water) was applied to the soil surface. Twenty-four hours after the treatment, soil columns were leached with 400 ml deionized water daily. Water was applied to the columns in four 100-ml increments. This application caused a temporary pooling of water each time. Soil columns were leached repeatedly throughout the studies (see Figures 8 & 9). Leachates were collected at the bottom of each column and analyzed on a gas chromatograph (GC) to determine the quantity of PG and EG that moved through the soil profile. Samples were stored in a freezer until the analysis.

Analysis of Leachates. Undisturbed soil column leachates were analyzed following procedures modified from (51). Propylene glycol and ethylene glycol standards were made every two weeks in deionized water and stored in a freezer. Leachate samples were analyzed on a Varian model 3740 GC (Varian Associates, Sunnyvale, CA, USA), equipped with a flame ionization detector (FID) and 2.7 m x 2 mm (i.d.) glass column containing 5% Carbowax 20M on 100/120 mesh Supelcoport^R (Supelco Inc., Bellefonte, PA). Ultrapure nitrogen (99.9%) was used as the carrier gas at a flow rate of 20 ml/min. The injector and oven

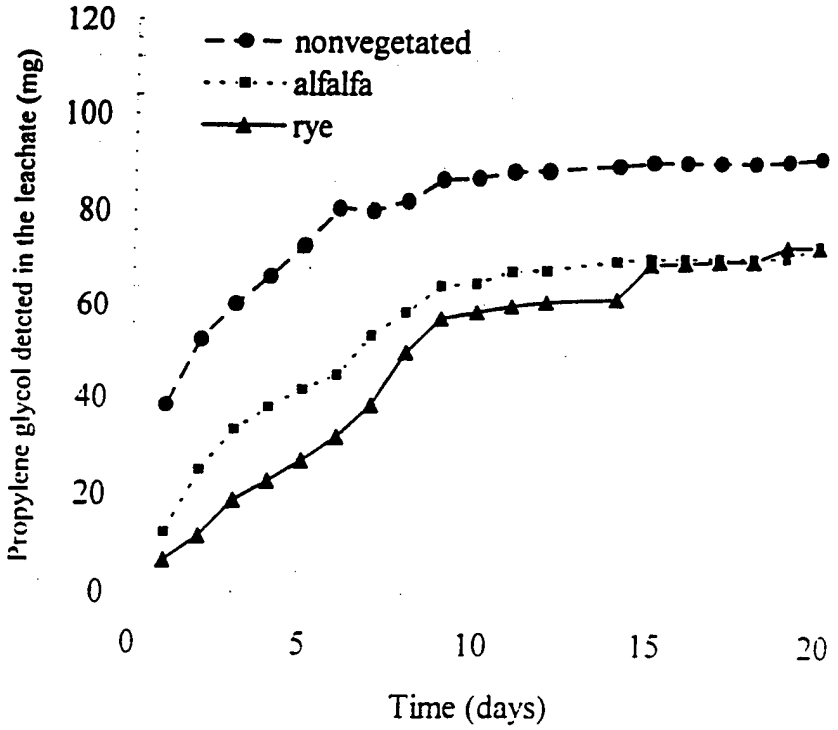

Figure 8. Concentration of propylene glycol detected in the leachate of vegetated and nonvegetated soil columns.

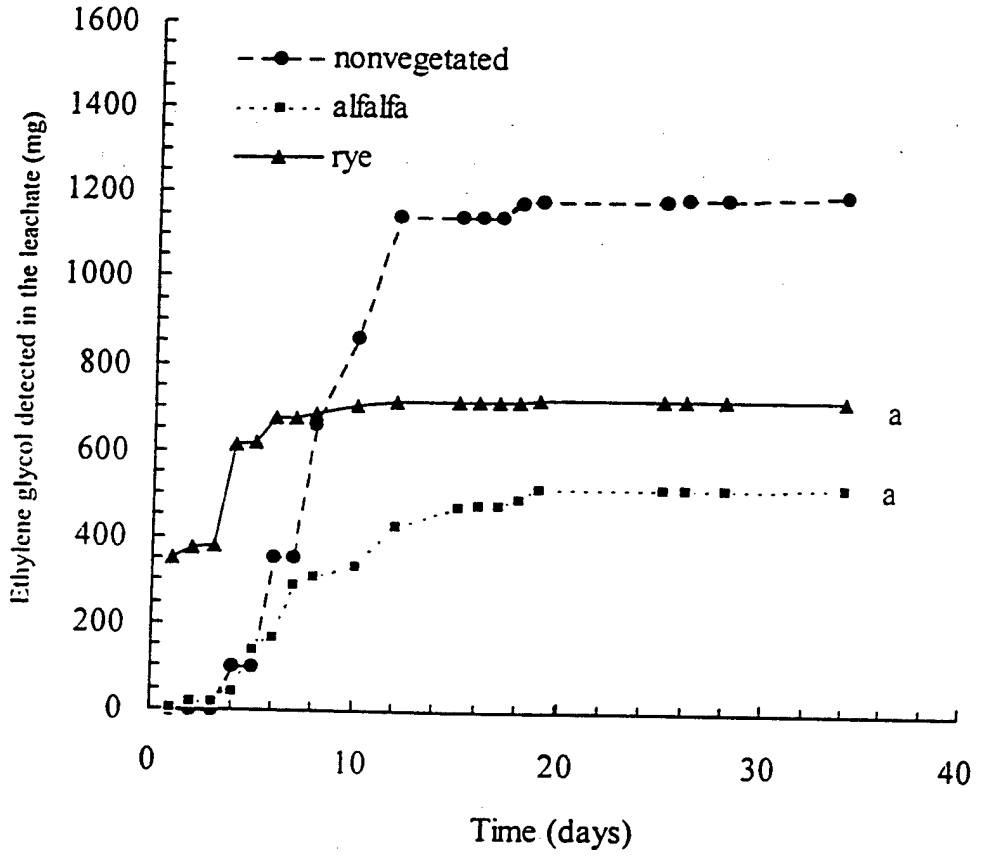

Figure 9. Cumulative concentration of propylene glycol detected in the leachate of vegetated and nonvegetated soil columns.

temperatures were 250 °C and 160 or 200 °C. Propylene glycol samples were also analyzed on a Shimadzu GC-9A GC-FID (Shimadzu Corp., Kyoto, Japan) equipped with a 5% Carbowax 20M packed column (1.2 m x 3 mm i.d.). The carrier gas was helium, and the injector and oven temperatures were 300 °C and 160 °C, respectively. Peak heights were used to construct each calibration curve and to quantitate the glycol in the samples. All the standard curves had correlation coefficients exceeding 0.990. One-way analysis of variance (ANOVA) and the least squared means were used to test for significant differences among the treatments (52).

Influence of Vegetation on the Mobility of Glycols in Soil Columns. Propylene glycol and ethylene glycol were detected in the leachates of all the soil columns studied (Figure 8 and 9). The greatest PG concentrations occurred within the first four leaching events and continued to decrease with time. Approximately 53 to 86% of the recovered PG was detected in the leachates within 7 d.

Greater than 500, 300, and 200 μg/ml EG was noted in leachates of the nonvegetated, *M. sativa* and *L. perenne* soil columns. After 10 days, 64 to 92% of the recovered EG had leached through the soil columns. Movement of PG and EG through the soil profile depends on its properties and adsorptive characteristics, soil characteristics, soil temperature, and the quantity and frequency of runoff or precipitation (53).

Previous research has shown EG does not adsorb to soil (51). Lokke (51) reported little or no adsorption of EG to sandy till, muddy till, or clayey soils. Propylene glycol and ethylene glycol are water soluble and appear to be mobile within the 38-cm soil profile.

Results from this study indicate vegetation reduced the quantity of PG and EG that moved through the soil profile. Leachates from the vegetated soil columns contained significantly ($p = 0.05$) less PG than leachates from the nonvegetated columns (Figure 8). Measured quantities of 91.5, 73.0, and 73.0 mg of PG were detected in leachates of nonvegetated, *M. sativa*, and *L. perenne* soil columns, respectively. Similar results were noted with the EG-treated soil columns (Fig. 9). The total quantity of EG that infiltrated through nonvegetated, *M. sativa*, and *L. perenne*, and soil columns was 1195, 519, and 722 mg, respectively. The results of the nonvegetated column shown in Figure 9 contain only one replication due to the loss of the second nonvegetated soil column. Less EG was detected in the leachate from *M. sativa* soil columns than *L. perenne*, but they were not significantly ($p = 0.05$) different. Plants can decrease the concentration of PG and EG in soil and reduce their movement through the soil profile to groundwater by plant uptake, enhanced degradation in root-associated soils, and reduction of the soil water status (41). Results from previous research show enhanced degradation of EG and PG in the *M. sativa* and *L. perenne* rhizosphere soils compared to nonvegetated soil (54). In the current study, we observed a 9 to 12% decrease in the quantity of water that leached through vegetated soil columns relative to the nonvegetated soil columns, together with even greater reductions in the percentages of PG and EG that leached in the nonvegetated columns. Overall, vegetation can clearly reduce the leaching of PG and EG through the soil profile.

Comparisons With Other Methods

Incubation Studies. While soil column studies provide important information on the mobility of parent compounds and degradation products, comparisons of data from pesticide-applied columns to that of controlled soil metabolism studies gives a better understanding of the fate of the compounds. In studies conducted using intact soil columns, such as described in this chapter, the presence of degradation products at various depths may be due either to movement of degradation products formed in upper layers or to the degradation of the parent compound once it has reached a particular depth. This is not the case in controlled soil metabolism studies using contained soils from various depths, where the presence of degradation products cannot be due to movement from another depth. Soil metabolism studies with a time series of analyses can also give information on half-lives for applied pesticides.

From the ATR-applied soil column study (3), it was noted that DEA was present in subsurface soils which could be due not only to degradation of ATR in all depths, but also to movement after formation in upper layers. DEA was more mobile than ATR in the soil column studies (3, 6). The comparative fate of ATR and DEA in surface (0 to 30 cm) and subsurface (65 to 90 cm) soils was studied in the laboratory (55). The concentration of DEA arising from ATR degradation in subsurface soil increased 3-fold from a 60- to 120-d incubation period. The half lives of DEA and ATR were significantly greater in subsurface soils than in the surface soils. However, in soil from the 90- to 120-cm depth (held at -33 kPa soil moisture tension), the half-lives for DEA and ATR were 178 and 161 d, respectively (55, 56).

Soil Thin-layer Chromatography. Studies on the mobility of pesticides have been carried out by using a method of thin-layer chromatography (TLC) that incorporates a thin film of soil onto a glass plate. Soil TLC (STLC) plates applied with radiolabeled pesticides are then submitted to ascending chromatography by placing the plates in developing chambers containing water as a mobile phase. The differential affinity of a pesticide for soils of various characteristics and water can easily and economically be determined. While the assessment of pesticide mobility in large, intact soil columns is more true to field conditions, space requirements, time, and cost are considerations for running multiple columns of various soil characteristics. Concerns, however, with using STLC are that no indication of preferential flow can be obtained, and in the process of making STLC plates, pulverization of soil can alter soil characteristics such that care must be taken with inferences to the real world.

In comparing the ATR- and DEA-applied soil column studies with an STLC study, all conducted in this laboratory, it was noted that relative mobilities of ATR and DEA were consistent. The relative mobilities of ATR, DEA, other pesticides, and degradates were determined in an STLC experiment that used soils from the surface (0 to 30 cm) and subsurface (65 to 90 cm) from ten soil types (2 depths, 5 locations) of Iowa (57). In this study, DEA was the most mobile compound in 8 of the 10 soils. These results agree with the soil column studies in which DEA was recovered in greater quantities in leachate than was ATR (Figure 4).

Field Box Lysimeters. With the use of field box lysimeters, it is possible to study the fate of pesticides under field conditions, while maintaining a somewhat contained system. Box-type lysimeters (Figure 10) had been constructed using polyethylene sheets for sides and bottom that were assembled by using stainless-steel bolts to secure aluminum angle-iron corners onto

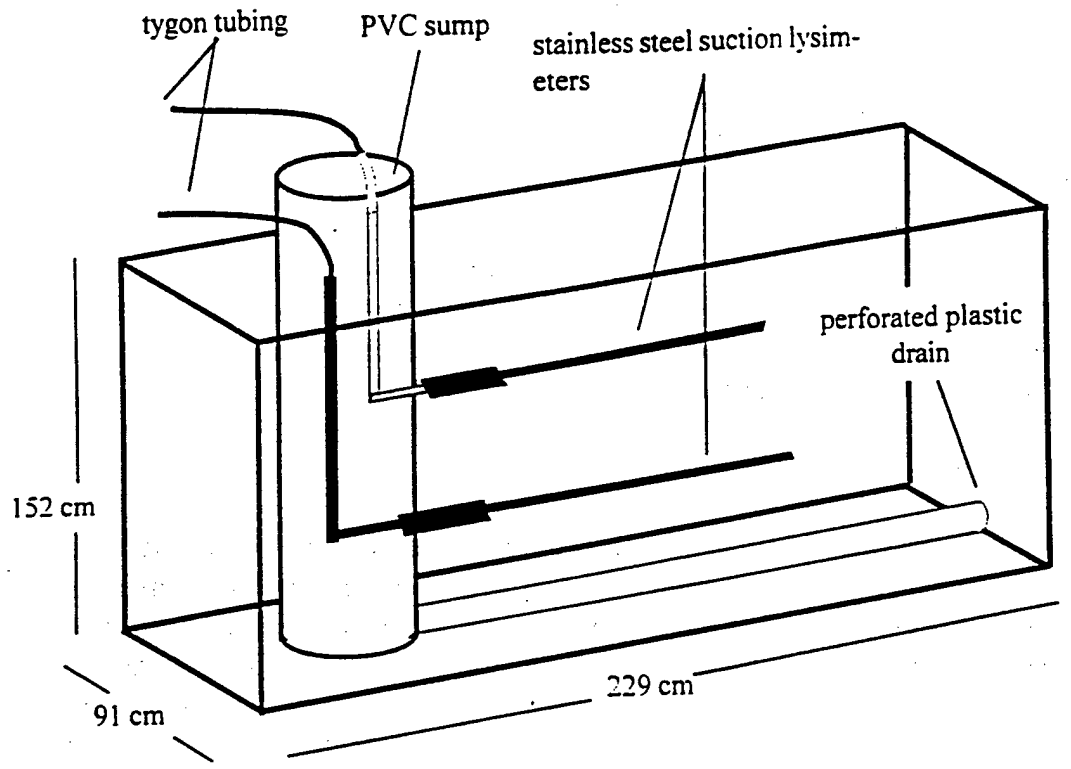

Figure 10. Field box lysimeter used to study pesticide concentrations in leachate and in soil water.

the sheets (58). The corner seams were treated with silicone sealant to make them waterproof, and styrofoam sheets (2.5-cm thick) were placed against each side. Heavy-duty duct tape was used to join each foam sheet, and a 0.5 mm-thick box-shaped, impermeable plastic liner, with the same shape and dimensions as the lysimeter box, was placed inside each lysimeter, pulled tightly over the styrofoam sheets, and attached to the outside of the lysimeter with duct tape. The sides of the lysimeter extended above the soil surface. Through the use of a grave-digging machine, the soil profile was excavated to make a hole that measured 234 cm by 92 cm by 137 cm. For the top 60 cm, soils were separated into 15-cm layers, and for soils between 60 and 150-cm depth, soils were separated into 30-cm layers. A bentonite layer (5-cm thick) was placed at the bottom of each excavated area. The lysimeter box was lowered into the hole, and the gaps between the lysimeter and the soil were fill with bentonite. A drainage-tile sump apparatus was installed, and the soil was replaced in the lysimeter layer by layer, according to the original vertical soil profile. Two stainless steel suction lysimeter tubes were installed, one on each side of the sump. One suction tube was installed at a depth of 60 cm and the other at 90 cm A two-year study of atrazine mobility and degradation in box lysimeters showed that rate of application had an influence on the detection of residues in water collected in the soil profile (59, 60).

This approach to studying the mobility of pesticides is advantageous in that one can conduct rain simulations over the lysimeters, use various cropping systems, monitor pesticide movement to tile drains, and determine concentrations in soil water by using the suction lysimeters. With the excavation of the soil profile, however, it takes some time to reestablish soil structure, and the box lysimeters are too large to remove to analyze.

Conclusion

It is clear that several of the advantages of the lysimeter as an experimental unit are also expressed in the intact soil column. The focus on intact soil columns in this chapter provides some degree of comparison in the techniques. Both maintain the crucial capacity to *integrate the fate of the pesticide*, measuring both degradation and mobility of the parent compound and transformation products. As analytical methods become more sophisticated, as public concern over pesticides grows, and as requirements for pesticide registration data expand, it is important that our understanding of pesticide degradation and movement in the environment continues to advance. The advancement of our knowledge is, to a large degree, limited by the tools we possess to make the assessments of the behavior of agrochemicals in our environment. It is therefore imperative that we constantly strive to develop and improve our methodology, such as the lysimeter approach, to aid in the production and protection of a safe and bountiful food supply, while affording protection of the environment.

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