Enhanced degradation of pesticide wastes in soil: Implications for bioremediation of agrochemical dealer sites

by

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I dedicate this dissertation to the many people who have supported me along the way in my efforts to obtain this degree, including my parents Bernice and Bazil Wenzel, other close relatives, friends, coworkers, and my major professors. To my son Adam, I give special thanks for his constant support and love. I also want to dedicate this dissertation to the memory of my closest friend, Marie Rogge, who shared with me all the trials, tribulations, and celebrations of this trek up until this past year.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1.

CHAPTER 2.

CHAPTER 3.

LIST OF TABLES

CHAPTER 1.

CHAPTER 2.

CHAPTER 3.

CHAPTER 4.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

A growing environmental concern in areas of intense pesticide usage is the occurrence at agrochemical dealership sites of soils contaminated with high concentrations of pesticides. Such contamination has resulted from many years of incidental or accidental spillage of agricultural chemicals during loading and mixing (Buzicky et al., 1992). In midwestern states such as Kansas, Iowa, Wisconsin, and Illinois, a strong correlation has been found between pesticide detects in public wells and proximity to fertilizer/agrochemical dealerships (Frieberg, 1991; Gannon, 1992).

Traditional methods for remediation of contaminated soils are costly, and can include excavation, removal, and treatment of the contaminated soil (Autry and Ellis, 1992; Warith et al., 1992; Hildebrandt and Wilson, 1991; Ryan et al., 1991). Such methods are not economically viable for most agrochemical dealerships. Thus, there is a need for more cost-effective, *in situ*, remedial alternatives.

Current remediation technologies result in the transfer of contamination from one part of the environment to another and concentration of contamination which must be disposed of futher (Fiorenza et al., 1991). An *in situ* tactic would eliminate the transfer of contamination in the environment and might involve the use of plants at the site to increase biodegradation of pesticide wastes by soil microorganisms. Plants can be beneficial in stimulating the removal of unwanted compounds by uptake and accumulation, and by increased microbial degradation in the rhizosphere (Shimp et al.,

1993). Movement of pesticides in soil water down through the soil profile may also be reduced by the presence of vegetation (Nair et al., 1993; Shimp et al., 1993).

There is growing interest in the use of plants to increase microbial degradation of hazardous organic chemicals in soil (Walton and Anderson, 1992). This increased degradation is due to intense microbial activity in the rhizosphere of plants. It is not uncommon for the number of microorganisms in this region to be 10 to 100-fold greater than in surrounding bulk soil. This phenomenon is due to the presence of root exudates which provide a carbon and energy source for these organisms. Exudates are natural organic substances such as amino acids, carbohydrates, and polysaccharides, that aid the root in its movement through the soil matrix. The rhizosphere system is an optimal site for degradation of unwanted organic compounds since the associated diverse microbial community can in some cases utilize the compounds as primary carbon and energy sources. This zone is also an optimal site for cometabolism, as enzymes that degrade exudates may also coincidentally degrade other compounds in the soil. The overall degradative capability of communities of microorganisms often results in the complete mineralization of organic compounds to carbon dioxide (McCarty, 1991).

In a preliminary study investigating the potential of plants to enhance the degradation of pesticide wastes in soil at an agrochemical dealership site in Iowa, it was noted that several plant species were able to survive in soil even though herbicide concentrations far exceeded the recommended rate of application. In a laboratory incubation study, enhanced degradation of three major herbicides was observed in soil from the rhizosphere of herbicide-tolerant *Kochia* as compared with nonvegetated soil

(Anderson et al., 1994).

A recent U.S. Environmental Protection Agency (EPA)-requested workshop has identified research priorities in bioremediation technologies (Waste Management News, 1991). High-priority research needs include ways to bring about the biological destruction of weathered pollutants, that is, pollutants that are less bioavailable due to aging, and to determine ways in which to exploit microorganisms having the ability to detoxify unwanted organic contaminants. Also, technologies need to be developed that enhance cometabolism. The proposed research will address the issue of biological destruction of aged residues in soil by exploiting the increased microbial activity in the rhizosphere which will provide an optimal environment for degradation of herbicide wastes.

Biodegradation of pesticide wastes in the root zone of tolerant plants will be investigated in this research. Microbial communities which colonize roots of these plants will potentially degrade the chemicals to a greater extent than in nonvegetated soils. Bacteria have been shown to withstand, and even multiply in, the presence of agrochemicals at higher than field-application rates (Martensson, 1992).

Disappearance of unwanted chemicals in vegetated soils may be due to plant uptake or increased mineralization in the root zone. The use of radiotracers in these studies will provide a means of differentiating the contribution of each mechanism of disappearance, and will also allow for the determination of bound residue formation and subsequent mobilization and biodegradation of herbicide residues. These studies will help provide information about the feasibility of using herbicide-resistant plants to enhance the

degradation of pesticide wastes. If *in situ* microbial degradation can be optimized at the site of contamination in the rhizosphere of herbicide-resistant plants, it is conceivable that aged pesticides could be eliminated through natural biological processes.

Enhanced Degradation of Pesticides

Numerous studies have investigated the fate of pesticides in soil primarily at non-point source concentrations. Enhanced degradation of some pesticides has been noted in soils with previous exposure to the particular chemical (Walker and Welch, 1991; Somasundaram, 1993). It is thought that this increased degradation is due to a microbial population which utilizes the chemical as a primary carbon or nitrogen source (Coats, 1993). Others have observed more rapid degradation of pesticides in soils that are vegetated as compared to nonvegetated soil (Hsu and Bartha, 1979; Reddy and Sethunathan, 1983). The insecticide carbofuran has been shown to be degraded more rapidly in the rhizosphere (root zone) soil of rice (Ramakrishna and Sethunathan, 1982). A microbial community isolated from wheat root systems was found to have the capability of growth on mecoprop and could also degrade 2,4-D and MCPA (Lappin et al., 1985). Several studies have noted decreased persistence of pesticides in the rhizosphere (Table 1). Enhanced degradation of herbicides in soil is of negative consequence if the result of this phenomenon is decreased control of the target pests. Enhanced degradation would be beneficial in soils that are contaminated with pesticides, in that rapid removal of these wastes would reduce risk of mobility and human exposures to such contaminants. Plants can influence removal of wastes through uptake into plant tissue, or by more rapid degradation in the root zone or rhizosphere.

Table 1. Studies indicating decreased persistence of pesticides in plant rhizospheres.

Rhizosphere	Compound	Reference
Corn	Atrazine	Alvey and Crowley, 1996
Wheat	Mecoprop	Lappin et al., 1985
	2,4-D, MCPA	
Sugarcane	2,4-D	Sandmann and Loos, 1984
Bush Bean	Diazinon	Hsu and Bartha, 1979
	Parathion	
Corn	Atrazine	Seibert et al., 1981

Rhizosphere Microbiology

The rhizosphere is an area of intense microbial activity that is under the influence of exudates excreted by plant roots (Curl and Truelove, 1986). The exudates are organic substances such as amino acids, organic acids, and carbohydrates. The presence of these substances in soil results in an optimal environment for large diverse microbial communities which use the substances as carbon and energy sources. The greater density and diversity of microorganisms commonly observed in the rhizosphere, compared with less diverse microbial communities in nonvegetated soils, may result in greater rates of metabolism of xenobiotic compounds. The microbial community also influences the

atmosphere of the soil thereby benefitting the plant. For example, large quantities of CO_2 liberated by the rhizosphere occupants form carbonic acid which causes a solubilization of insoluble, inorganic nutrients, that would not be readily available to the plant. This process effectively increases the supply of assimilable inorganic nutrients such as phosphorus, potassium, magnesium, and calcium, and thus improves crop nutrition (Alexander, 1977).

These phenomena have important implications in biological remediation of waste sites where establishing or cultivating selected plants at these sites could enhance the degradative capability of microorganisms in the soil and accelerate biodegradation of unwanted organics. Plants have been shown to have a positive influence on the degradation of man-made organic chemicals. Numerous studies have investigated the behavior of xenobiotics in plant/soil systems (Ebing and Schuphan, 1979; Kloskowski et al., 1981; Scheunert et al., 1983; Bellin and O'Connor, 1990; Malik and Drennan, 1990; Lee et al., 1991; Nair et al., 1993; Shimp et al., 1993). Enhanced degradation of nonagricultural chemicals has been noted to occur in vegetated soils (Rasolomanana and Balandreau, 1987; Federle and Schwab, 1989; Aprill and Sims, 1990). It has been proposed that plant-microbe systems be utilized in hazardous waste treatment (Walton and Anderson, 1992). In soil contaminated with industrial chemicals, plants can easily survive since they usually are not an intended target. For pesticide-contaminated sites, the problem is complicated due to the presence of herbicides which are designed to kill plants.

Agrochemical Dealerships

Since the early 1950s, production and use of pesticides in agriculture has dramatically increased. Coupled with the increase in pesticide usage has been the rapid growth of retail agrochemical dealerships. An unfortunate situation has developed in many instances as a result of normal operating procedures at these sites, where soil and water have become contaminated. This is a serious issue facing the agrochemical industry (Myrick, 1992; Gannon, 1992; Buzicky et al., 1992). Typically, it is difficult to assess the scope of the contamination at these sites because of the properties of the chemicals and their widespread use in areas nearby. However, it is estimated that most dealerships throughout the Midwest have some type of problem from chemical contamination.

Current technologies available for cleanup of contaminated soil and water are cost prohibitive, preventing their use at most agrochemical dealerships. Another difficulty that dealers face is the acquisition of insurance coverage for cleanup of major spills, especially when some contamination already exists. Although stopping or minimizing additional pollution input has decreased pesticide detections in soil and groundwater at these sites, in most cases additional remediation is warranted (Gannon, 1992). Currently, there is a need to provide dealerships with viable technologies for remediating soils and groundwater at these sites.

Very little information exists in the literature on the degradation of high concentrations of pesticides, especially mixtures of such compounds, in soils that have been exposed over a long period of time. The herbicides chosen for this research are

widely used in agricultural areas and are thus often detected in high concentrations in soils at agrochemical dealership sites. A report summarizing 28 agrochemical dealership sites in Iowa with contaminated soils reveals several major herbicide contaminants (Table 2).

Atrazine and metolachlor are frequently reported herbicide contaminants at thesesites. This is reasonable since they are among the most widely used herbicides in the Midwest. For a portion of this research, a mixture of two chemicals, atrazine and metolachlor, were used. Other common herbicides used in the Midwest include alachlor, pendimethalin, and trifluralin. Important properties and chemical structures of the five herbicides are shown in Table 3 and Figure 1, respectively.

Microbial Degradation of Atrazine and Metolachlor

Pure and mixed populations of microorganisms have been isolated from polluted and nonpolluted soils that have the ability to degrade atrazine. Mirgain et al. (1993) isolated degraders from agricultural soils. Among the degraders they identified were *Acinetobacter calcoaceticus, Pseudomonas alcaligenes* associated with *Agrobacterium* sp., and *Pseudomonas putida/Xanthomonas maltophilia.* A mixture of five bacteria was isolated from a garden soil that had been treated only once with atrazine. Mandelbaum and others (1993) isolated 200 pure cultures from agrochemical dealership sites being considered for bioremediation. The sites have been exposed to repeated spills of atrazine, alachlor, and metolachlor. While pure cultures failed to utilized atrazine as a nitrogen source, a mixture of the cultures was able to degrade atrazine at rates far exceeding reported values in soils, waters, and mixed and pure cultures of bacteria.

Table 2. Occurrence of major herbicides at 28 agrochemical dealership sites in Iowa (Gannon, 1992).

Herbicide	Drinking water standard (µg/L)	Field application rate (µg/g)[A]	Frequency reported[B]	Range of Concentrations	
				Groundwater (µg/L)	Soil (µg/g)
Atrazine	3	0.6	71	0.37 - 4,600	0.11 - 681
Alachlor	2	0.86	75	0.10 - 51,100	0.10 - 5,580
Metolachlor	100	1.26	67	0.10 - 25,000	0.09 - 856
Cyanazine	10	0.96	67	0.16 - 880	0.2 - 248

[A] Calculations from product label information and assuming uniform incorporation at 6-inch soil depth and soil density of 1.8 g/cm^3

[B] Percentage of sites at which the compound was reported

Table 3. Physicochemical properties of major herbicides

	Atrazine	Metolachlor	Alachlor	Pendimethalin	Trifluralin
Chemical name	2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide	2-chloro-*N*-(2',6'-diethylphenyl)-(methoxymethyl)-acetamide	*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine	2,6-dinitro--*N*,*N*-dipropyl-4-(trifluoromethyl) benzenamine
Class	triazine	acetamide	acetamide	dinitroaniline	dinitroaniline
Mode of action	inhibit plant photosynthesis	inhibit cell division, cell elongation, and protein synthesis	inhibit cell division, cell elongation, and protein synthesis	inhibit cell division (results in the inhibition of lateral root formation)	inhibit cell division (results in the inhibition of lateral root formation)
Empirical formula	$C_8H_{14}ClN_5$	$C_{15}H_{22}NO_2Cl$	$C_4H_{20}ClNO_2$	$C_{13}H_{19}N_3O_4$	$C_{13}H_{16}F_3N_3O_4$
Molecular weight	215.7	283.8	269.8	281.3	335
Water solubility (ppm at 20-27°C)	33	530	242	1	1
K_{oc} [A]	100	200	170	5000	8000
$T_{1/2}$ (days)[A]	60	90	15	90	60
Vapor pressure	3.0×10^{-7} mmHg @ 20°C	1.1×10^{-5} mmHg @ 20°C	2.2×10^{-5} mmHg @ 25°C	3.0×10^{-5} mmHg @ 25°C	1.1×10^{-4} mmHg @ 25°C

[A]Values taken from the Soil Conservation Service-Agricultural Research Service C.E.S. Database

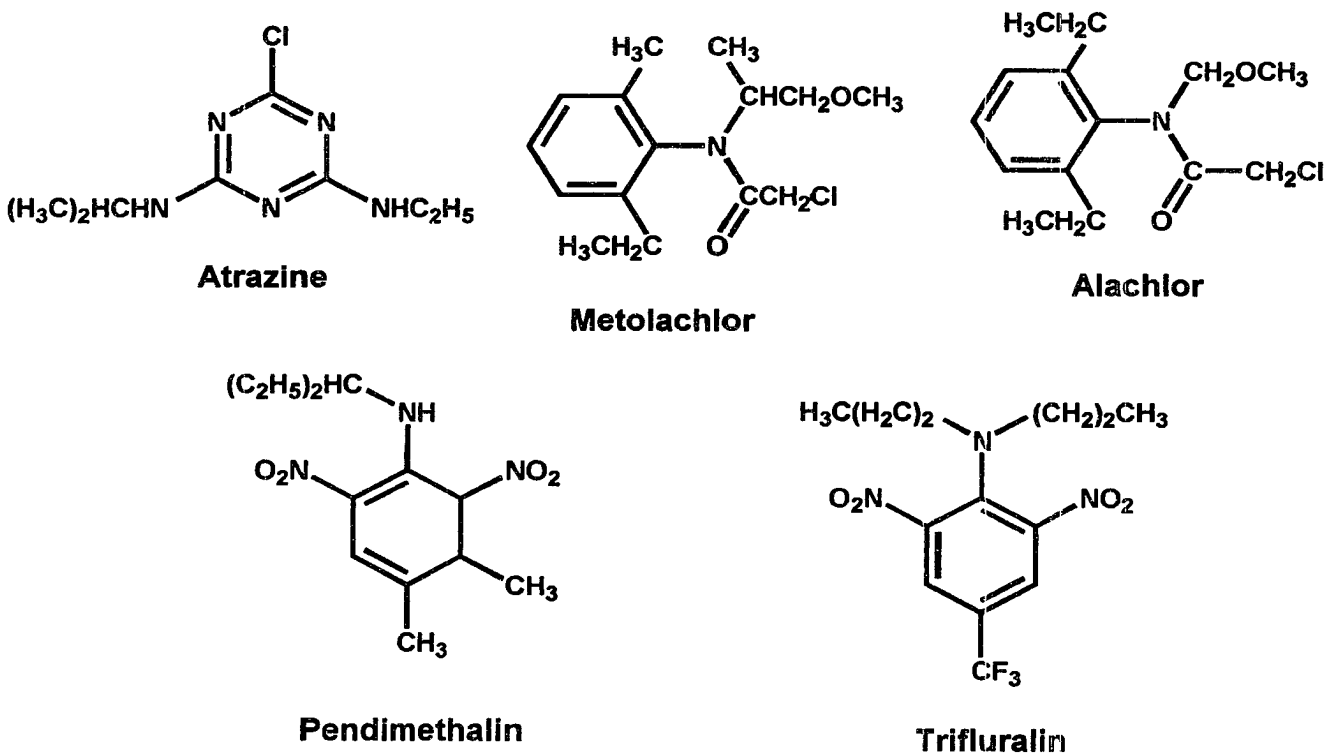

Figure 1. Structures of major herbicides

Seibert and others (1981) monitored the influence of maize rhizosphere on the degradation of atrazine. They noted that the microbial activity was much higher and atrazine concentrations were less in the rhizosphere soil as compared to the nonvegetated soil.

Degradation of metolachlor is due primarily to microbial decomposition (Sahid and Wei, 1993). In studies investigating the microbial degradation of metolachlor, it was found that several microbial species could dechlorinate metolachlor and did not require previous acclimation to the herbicide (Liu et al., 1991). Enhanced mineralization of metolachlor has been noted in herbicide-acclimated soils. In a soil perfusion experiment using soils with five years of metolachlor history or no metolachlor history, mineralization accounted for 18.4% in history soil compared with 3.5% in the no-history soil (Liu et al., 1988). Liu et al. (1989) found that a stable bacterial community could absorb and transform about 80% of the ^{14}C-metolachlor (50 μg/ml) added to a liquid medium. Several microbial species could transform metolachlor at high concentrations, including a *Fusarium* sp. that could grow and transform metolachlor up to a conentration of 300 ppm (Saxena et al., 1987).

Bioavailability

As chemicals reside in soil over time, their bioavailability is presumed to decrease (Alexander, 1995). This decreased bioavailability is evidenced by a reduced rate of degradation of the chemicals after a period of time. Scribner et al. (1992) found that aged simazine residues were unavailable to microbial degraders, whereas recently added simazine showed substantial degradation. Nelson and Khan (1990) found that

endomycorrhizal infection increased the bioavailability of soil-bound pesticide residues to plants.

Concentrations of chemicals that are solvent-extractable are quite different than the concentrations which are actually bioavailable to microorganisms or plants (Alexander, 1995). This is an important consideration when designing and conducting experiments for bioremediation of pesticides in soils, and is also important in decisions made about what target concentrations constitute remediation.

Bioremediation

In a bioremediation scenario for clean up of a site contaminated with multiple chemicals, a complex microbial community is required for effective degradation of numerous chemical types (Anderson et al., 1993). It may also be necessary to incorporate more than one plant species in a bioremedial scheme. The microbial composition of the rhizosphere is dependent on root type, plant species, plant age, and soil type (Anderson et al., 1993). Successfully enhancing degradation or removal of pesticide contaminants in soil by using plants would provide a cost-effective bioremedial option for agrochemical dealerships.

Dissertation Objectives

The overall objective of my research is to contribute evidence supporting the following hypotheses: 1) Enhanced degradation of herbicides or degradation products can occur in soils with long-term exposure to the chemical; 2) enhanced degradation of herbicide wastes can occur in rhizosphere soils from contaminated sites; 3) the presence of plants at pesticide-contaminated sites enhances the dissipation of pesticide wastes as a

result of increased degradation by microbial communities in the rhizosphere. Specific objectives my research are:

1. To test pesticide-contaminated soils in radiotracer studies for their degradative capabilities on freshly applied mixtures of herbicides
2. To quantify specific herbicide degraders
3. To compare degradation of an aged mixture of herbicides in vegetated and nonvegetated soil
4. To test for enhanced degradation of a major herbicide metabolite in soils that have had long-term exposure of the parent compound

Dissertation Organization

This dissertation is composed of a general introduction and three journal papers. The first paper addresses the assessment of degradative capabilities of soils taken from nonvegetated and vegetated areas of two pesticide-contaminated sites in Iowa. Additionally, the influence of two plant species on the degradation of a herbicide applied in a mixture was determined. This paper will be submitted to *Environmental Toxicology and Chemistry*. The second paper discusses the results of a screening study that involved assessing the degradative capabilities of soils from four pesticide-contaminated sites on the degradation of four major herbicide contaminants, atrazine, metolachlor, alachlor, and pendimethalin (at 50 μg/g each). In a soil with enhanced atrazine-degradation capabilities, an additional experiment was conducted to determine the influences of high concentrations (200 μg/g) of other herbicides on this enhanced degradative capability. This paper will be submitted to *Journal of Environmental Science and Health*. The third

paper discusses a study to investigate the enhanced degradation of a major metabolite of atrazine, deethylatrazine, in soils that have had long-term exposure of atrazine at field application rates. This paper has been submitted to *Pesticide Science*. A General Conclusion chapter follows the last paper. An additional reference section at the end of the thesis includes citations used in the General Introduction and General Conclusion chapters. An Acknowledgment section follows the additional reference section.

CHAPTER 2. EVALUATION OF THE DEGRADATIVE CAPABILITIES OF SOILS FROM PESTICIDE-CONTAMINATED SITES: INFLUENCE OF TWO PLANT SPECIES ON THE DEGRADATION OF AGED RESIDUES

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

Ellen L. Kruger[1], Brenda S. Perkovich[2], Todd A. Anderson[1], and Joel R. Coats[1].

Abstract The fate of ^{14}C-atrazine and ^{14}C-metolachlor, applied as a mixture (50 μg/g each), in soils taken from two pesticide-contaminated sites in Iowa (Alpha and Bravo) was determined in laboratory studies. In the Bravo site study, degradation of metolachlor was greater in the *Kochia scoparia* rhizosphere soil with a mean of 53% of the applied ^{14}C remaining in the rhizosphere soil compared to 71% in the nonvegetated soils after 60 days of incubation. A significantly greater percentage of applied ^{14}C-atrazine was mineralized in soil taken from the rhizosphere of *Kochia* than from nonvegetated soils. Atrazine was less persistent in the rhizosphere soil than in nonvegetated soils. Soil-bound residues were present in greater quantity in nonvegetated soils compared with the rhizosphere soils. In the Alpha site study, significant differences were seen between the rhizosphere and nonvegetated soils in the amount of CO_2 evolution, atrazine remaining and soil-bound residues. The half-life for atrazine was significantly less in the

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rhizosphere soil than in nonvegetated soil ($p \leq 0.05$), with a half-life of 50 d compared to 193 d in the nonvegetated soils. In plant/soil studies, chemical analyses revealed significant differences between the *Kochia*-vegetated and *Brassica*-vegetated soils (192 d post-herbicide treatment). Combustion of plant material revealed that significantly more ^{14}C was taken up by *Kochia* plants (9.9% of the applied ^{14}C) than by *Brassica* plants (0.8%) ($p = 0.0001$). Significantly less atrazine was extractable from soils vegetated with *Kochia* (4.3% of the applied ^{14}C) than soils vegetated with *Brassica* (9.8%) ($p < 0.05$). At 240 d post-herbicide application, significantly less atrazine was extractable from soils vegetated with *Kochia* (5.3% of the applied ^{14}C) than from nonvegetated soils (8.3%) ($p < 0.01$). Combustion of the plants revealed that 6.5% of the applied ^{14}C-atrazine was taken up into the plant tissue. Low populations of atrazine degraders were seen in soils from Alpha site, ranging from 7 to 326 organisms g^{-1} of soil. Atrazine degraders at the Alpha site were significantly more numerous in *Kochia* rhizosphere soils than in soil from a nonvegetated area. Greater numbers of atrazine degraders were noted at Bravo site, with significantly more in the nonvegetated soil (17,412 organisms g^{-1}) compared with the *Kochia* rhizosphere soil (1,107 organisms g^{-1}). The potential for vegetation and degrader microorganisms to aid in bioremediating pesticide wastes in soil is promising.

Key words phytoremediation, point-source contamination, bioremediation, atrazine, metolachlor

INTRODUCTION

A growing environmental concern in areas of intense pesticide usage is the occurrence of soils contaminated with high concentrations of pesticides. Such contamination can occur, for example, at agrochemical dealership sites as a result of many years of incidental or accidental spillage of agricultural chemicals during loading and mixing (Buzicky et al., 1992). In Midwestern states such as Kansas, Iowa, Wisconsin, and Illinois, a strong correlation has been found between pesticide detects in public wells and proximity to fertilizer/agrochemical dealerships (Frieberg, 1991; Gannon, 1992).

Traditional methods for remediation of contaminated soils including excavation, removal, and treatment of the contaminated soil (Autry and Ellis, 1992; Warith et al., 1992; Hildebrandt and Wilson, 1991; Ryan et al., 1991) are costly and are not economically viable for most agrochemical dealerships. Thus, there is a need for more cost-effective, *in situ*, remedial alternatives.

There is growing interest in the use of plants to increase microbial degradation of hazardous organic chemicals in soil (Anderson et al., 1993). This increased degradation is due to intense microbial activity in the rhizosphere of plants. It is not uncommon for the number of microorganisms in this region to be 10 to 100-fold greater than in surrounding bulk soil. This phenomenon is due to the presence of root exudates which provide a carbon and nutrient source for these organisms. The exudates consist of natural organic substances such as amino acids, carbohydrates, and polysaccharides, that aid the root in its movement through the soil matrix. The rhizosphere system is an

optimal site for degradation of unwanted organic compounds since the associated diverse microbial community can in some cases utilize the compounds as primary carbon and nutrient sources. While pesticide contamination can have negative affects on microbial populations, one study has shown that bacteria can withstand, and even multiply in, the presence of agrochemicals at concentrations higher than field-application rates (Martensson, 1992).

Little is known about the fate of mixtures of chemicals at point-source contamination levels. The objectives of this study were to determine concentrations of herbicides present in pesticide-contaminated sites, to test the soils in radiotracer studies for their degradative capabilities on freshly applied mixtures of herbicides, to quantify the specific herbicide degraders in these soils, and to compare degradation of an aged mixture of herbicides in vegetated and nonvegetated soils.

MATERIALS AND METHODS

Site selection

Two agrochemical dealer sites in Iowa were chosen for this study. A site in Iowa, denoted as Bravo, is an operating agrochemical dealership. A site map of Bravo is shown in Figure 1. A second site in Iowa, denoted as Alpha, is an agrochemical dealership plagued by herbicide contamination in the groundwater since the mid-1980s. Among the herbicides detected at this site were atrazine, metolachlor, alachlor, and trifluralin. The contamination of soil was confined, for the most part, to the top 40 cm. A site map is shown in Figure 2.

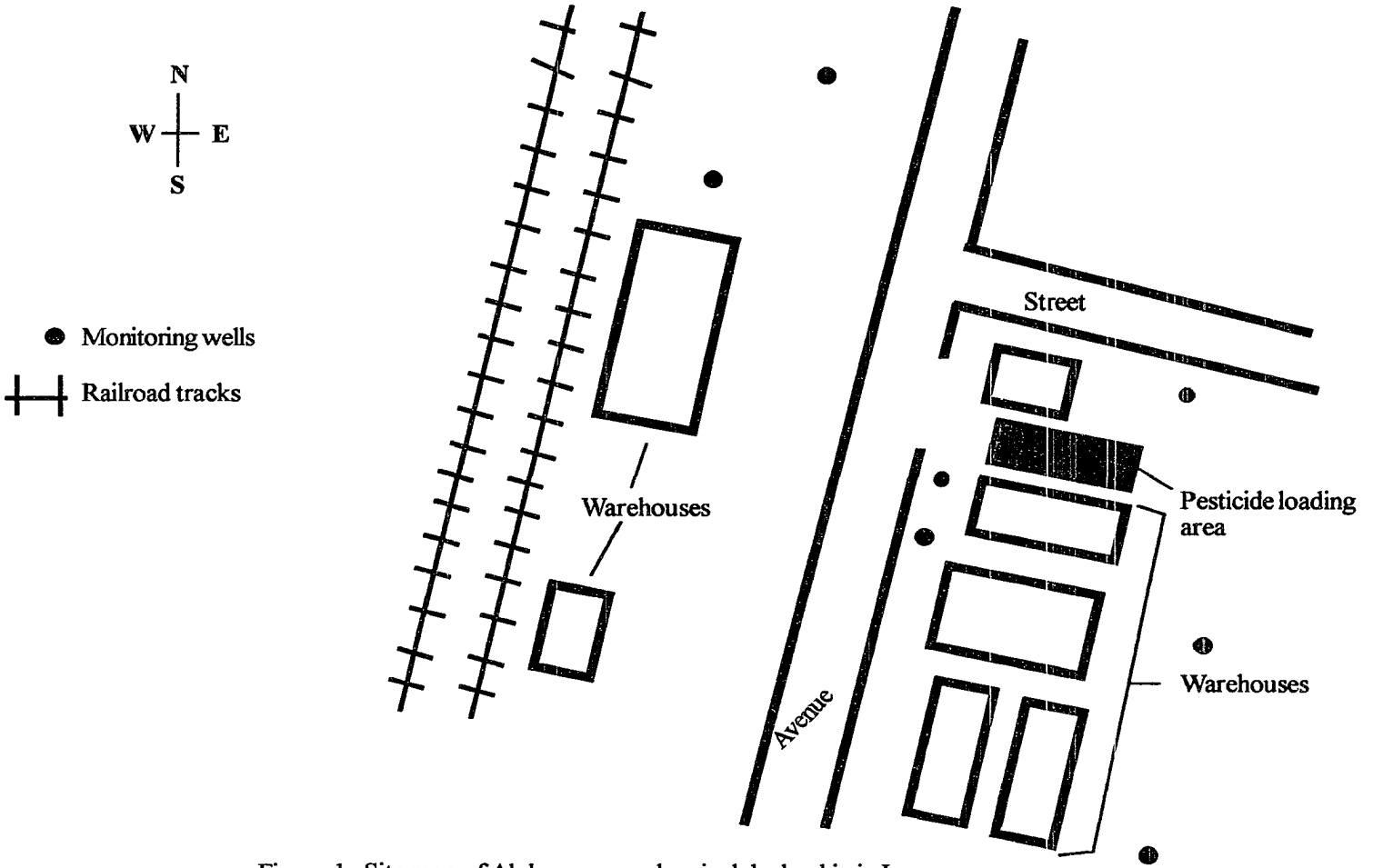

Figure 1. Site map of Alpha, an agrochemical dealership in Iowa.

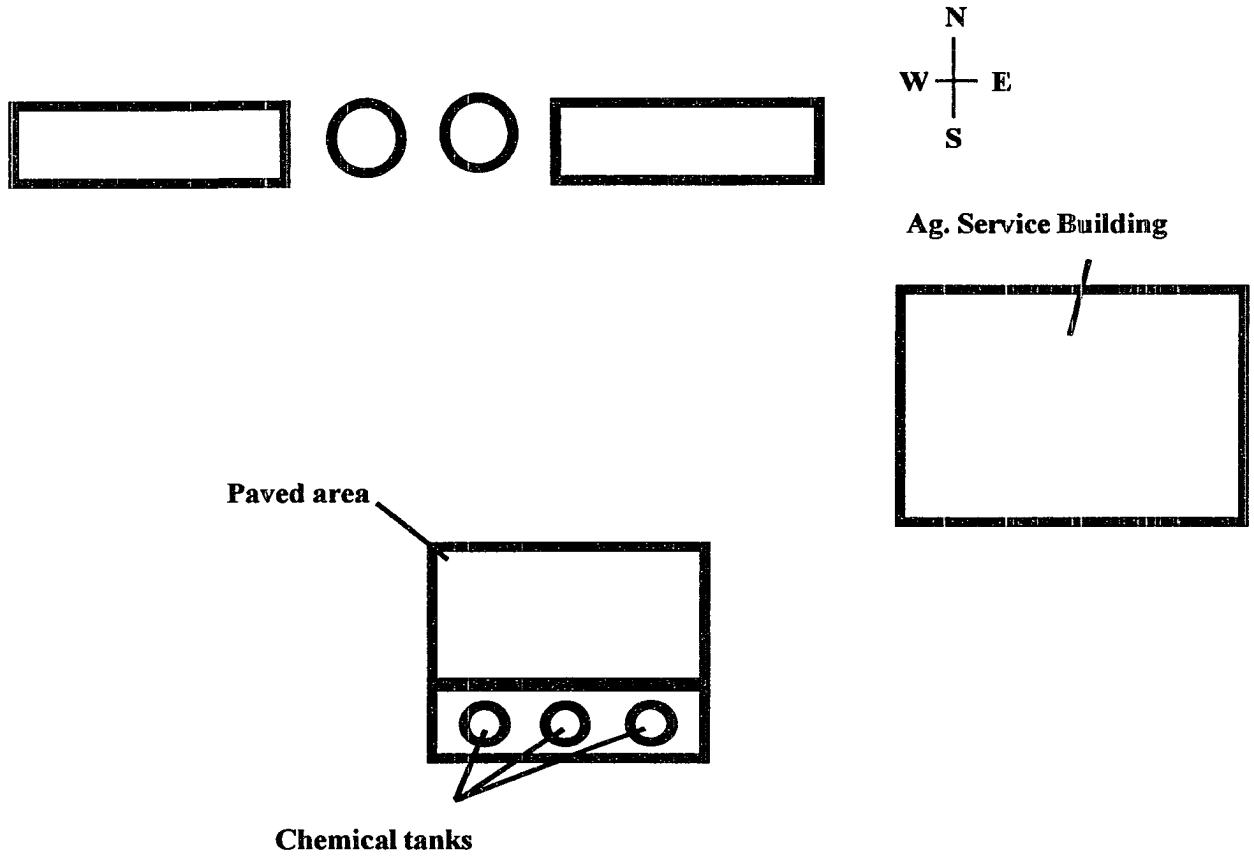

Figure 2. Site map of Bravo, an agrochemical dealership in Iowa.

Soil Sampling

Surface soils (top 15 cm) were taken from various locations at two agrochemical dealership sites in Iowa (Alpha and Bravo). Three independent composite samples were taken from nonvegetated areas. Rhizosphere soils were collected in independent composite samples by shaking soil from the roots of *Kochia scoparia*. Soils were transported to the laboratory on ice. Soils were sieved (2.4 mm) and stored at 4 °C for no more than 14 d prior to the beginning of this experiment. Composite samples of nonvegetated and *Kochia* rhizosphere soil were sent to A & L Laboratory (Omaha, NE) for determination of physicochemical characteristics.

Chemicals

Uniformly ring-labeled ^{14}C-atrazine, ^{14}C-deethylatrazine, ^{14}C-deisopropylatrazine, ^{14}C-didealkylatrazine, and ^{14}C-metolachlor, and technical grade standards for these compounds were obtained from Ciba Geigy Corp. (Greensboro, NC).

Complete Metabolism Study

Prior to the beginning of this study, background herbicide contamination was determined by gas chromatographic analysis (Anderson et al., 1994). Concentrations of herbicides were found to be quite heterogeneous at these sites. Soils from nonvegetated areas and from the rhizosphere of *Kochia scoparia* were treated with a mixture of either ^{14}C-atrazine/unlabeled metolachlor (50 μg/g each) or ^{14}C-metolachlor/unlabeled atrazine (50 μg/g each). Treatment of soil from each soil type and replication was done in a large treating jar, and 100-g subsamples were placed in French square bottles to be incubated for 30, 60, or 135 days. Soil moistures were adjusted and maintained at -33 kPa (near

field capacity) throughout the incubation period at a temperature of 24 °C (in the dark) for 30, 60 or 135 d. The Alpha site study included only the herbicide mixture with radiolabeled atrazine, while the Bravo site study included both combinations.

An initial extraction was carried out on Day 0 for each soil type to determine the actual amount of radioactivity applied. These quantities of radioactivity were used as the basis for the percentage of each component in the mass balances. Subsamples of extracted soils from the Day 0 samples were combusted to determine the extraction efficiencies for the compounds. Extraction efficiencies were 100% for both atrazine and metolachlor.

Plant/Soil Incubations

Additional 100-g subsamples from the complete metabolism study of the Alpha site were incubated at 24 °C in the dark for 165 d to allow ^{14}C-atrazine/unlabeled metolachlor to age in the soil. Samples were opened weekly to change NaOH traps. On day 165, post herbicide application, soils were planted with either *Kochia scoparia*, *Brassica napus,* or left unvegetated and were then placed inside a growth chamber. The growth chamber was located in a temperature-controlled room (24 °C) with a light:dark cycle of 16:8 hours. A flow-through system was used to remove $^{14}CO_2$ and ^{14}C-organic volatiles from the atmosphere of the chamber to prevent incorporation into plant tissue. After 27 additional days (192 d post-herbicide application), nonvegetated and vegetated samples were removed from the growth chamber, plants were removed, and soils were extracted and analyzed. Entire plants and subsamples of soil were combusted to quantify plant uptake and soil-bound residues.

Additionally, nonvegetated or *Kochia*-vegetated samples remained in the growth chamber until 240 d post herbicide application. Once again, samples were removed from the growth chamber and analyzed as discussed previously. The time line for the Alpha site study including the complete metabolism portion and the vegetated/unvegetated portion is shown in Figure 3.

^{14}C-Most-Probable-Number Determination (MPN)

A ^{14}C-MPN procedure was done to determine the number of atrazine or metolachlor degraders present in the Alpha and Bravo soils (Jayachandran et al., 1996). Each soil sample was homogenized and mixed well prior to removing 10 g (dry weight) for preparation of the initial 10^{-1} soil dilution. Serial dilutions from both depths of ATR-history and no-history soils were made up in sterile phosphate buffer solution (0.0125 M). A minimal salts broth (MSB) (Kaufman and Kearney, 1965) was prepared and mixed with 1/100 strength nutrient broth (0.08 g liter^{-1} MSB) and then autoclaved for 20 minutes. A stock solution of trace elements (Zeyer and Kearney, 1982) was prepared, filter sterilized, and added to the MSB/nutrient broth at a concentration of 1 ml liter^{-1} MSB. Two treating solutions, one for atrazine and one for metolachlor, were prepared by adding either analytical grade herbicide and uniformly ring-labeled ^{14}C-herbicide to the mixture of MSB, nutrient broth, and trace elements to give a final herbicide concentration of 1 μg/L with radioactivity of 10,000 disintegrations per minute (dpm) ml^{-1}. In these treating solutions, either atrazine or metolachlor were the only source of N.

Within a laminar flow hood, 100-μL aliquots of each soil dilution and 500 μL of either the ^{14}C-atrazine or ^{14}C-metolachlor treating solution were pipetted to individual

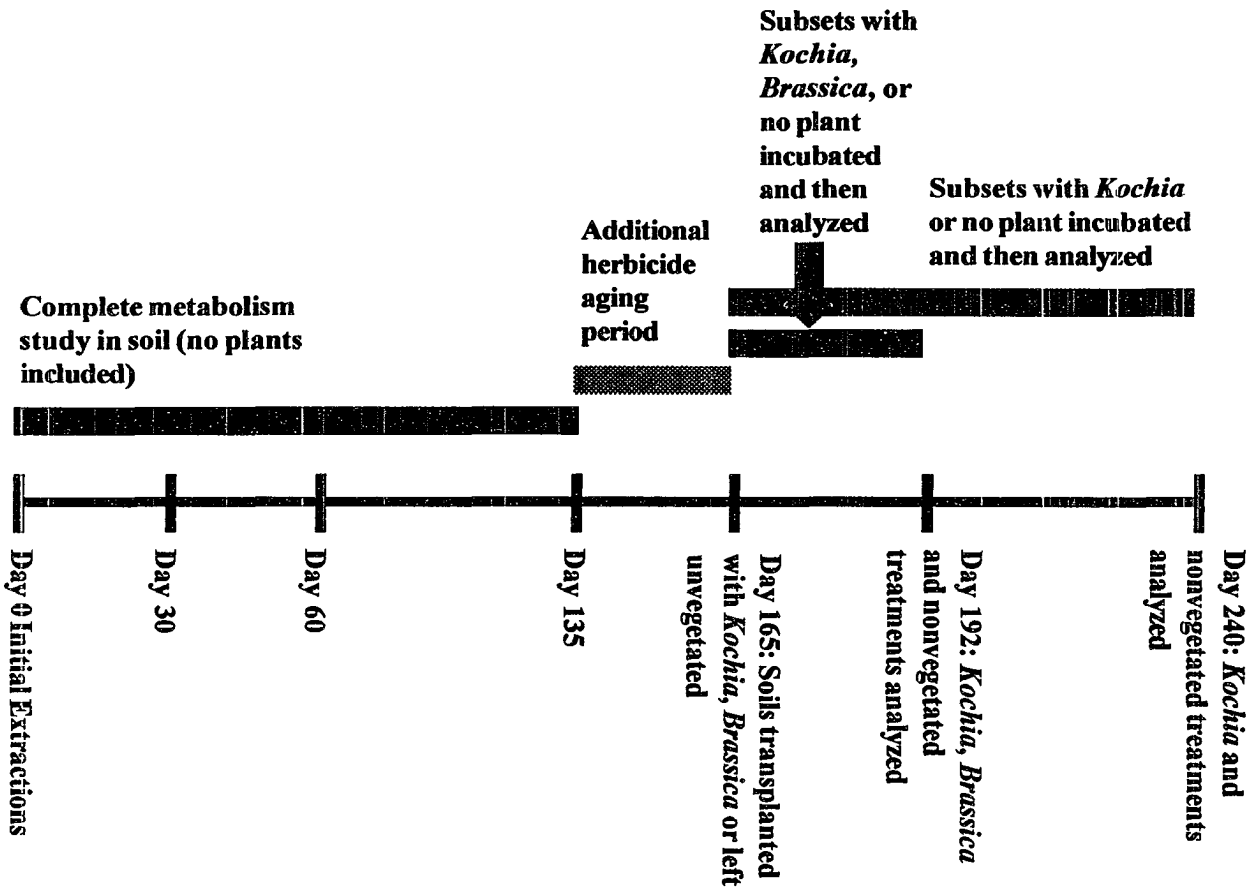

Figure 3. Time line of Alpha site experiment.

sterilized shell vials which were then capped with sterile foam plugs. For each soil dilution, five replicates were made. Twelve sterile buffer control samples were also prepared at intervals throughout this procedure by adding 100 μL of sterile phosphate buffer and 500 μL treating solution to additional shell vials. Capped shell vials were placed inside 20-ml disposable scintillation vials containing 1 ml of 1 N NaOH. Cone-capped lids provided a secure seal for the 20-ml scintillation vials. All vials were incubated in the dark at 20 °C for 50 days. At the end of the incubation period, individual shell vials were rinsed with 1 ml ultrapure water into their respective 20-ml scintillation vials. Ultima Gold (Packard Instrument Co., Downers Grove, IL) scintillation cocktail (12 ml) was added to each scintillation vial. Quantification of radioactivity trapped in the NaOH was determined by liquid scintillation techniques.

In order to determine the presence or absence of atrazine or metolachlor degraders in each of the five replicates from the soil dilutions, the dpm from these vials were compared to the mean dpm from sterile buffer controls. Vials were scored as positive for degraders if dpm were 20% above those of the sterile buffer controls. A MPN determination was calculated according to statistical tables. (Halverson and Ziegler, 1933; Cochran, 1950; Alexander, 1982)

Statistical analysis

An analysis of variance (ANOVA) was performed on data for the mass balance of applied ^{14}C in the complete metabolism study. Degradation rate constants were derived using methods of Walker (1987). Confidence intervals for the MPN procedure were determined using methods of Cochran (1950).

Chemical Analysis

Methods of Kruger et al. (1993) were used for the soil analysis. At the end of incubation periods, soils were extracted 3 times with 150 ml methanol:water (9:1). Extracts were concentrated with a rotary evaporator. Soil extracts were partitioned into dichloromethane. The organic phase was further concentrated and used for thin-layer chromatography using normal phase silica gel plates (Fisher Scientific, Itasca, IL). Radiolabeled standards were spotted on each plate prior to ascending chromatography in solvent systems for either atrazine or metolachlor analysis. Autoradiography was carried out by placing a X-Omat Kodak diagnostic film (Eastman Kodak Co., Rochester, NY) in contact with each plate for three to five weeks. Films were developed at the end of this period. Zones corresponding to the R_f values of standards were scraped and quantified using liquid scintillation techniques. The quantity of ^{14}C in zones not corresponding to standards were categorized as unidentified transformation products.

Extracted soils were subsampled and combusted using a Packard Sample Oxidizer (Packard Instrument Co., Downers Grove, IL). $^{14}CO_2$ was trapped in Carbo-Sorb E and Permafluor V (Packard Instrument Co., Downers Grove, IL.). Plants were also combusted to determine the amount of chemical uptake. Quantification of radioactivity was determined using liquid scintillation techniques with a Rack Beta model 1217 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD) to obtain a mass balance for the amount of ^{14}C-applied to soils.

RESULTS AND DISCUSSION

Soil Characteristics

Soils from Alpha and Bravo sites were of a sandy loam and loam texture, respectively (Table 1). The pH of nonvegetated and *Kochia* rhizosphere soils taken from the pesticide-contaminated sites ranged from 7.0 to 7.9. Other pertinent soil characteristics are shown in Table 1.

Complete metabolism study

Bravo Site A significantly greater percentage of applied ^{14}C-atrazine was mineralized in soil taken from the rhizosphere of *Kochia* than from nonvegetated soils ($P = 0.0163$), with 62 and 49% as CO_2 after 30 d (Table 2). Atrazine was less persistent the rhizosphere soil than in nonvegetated soils ($P = 0.0271$), with only 2% extractable from rhizosphere soils compared with 7.3% in nonvegetated soils. Soil-bound residues were in greater quantity in nonvegetated soils (11% of the applied ^{14}C) compared with the rhizosphere soils (5.8%) ($P = 0.0003$).

Mineralization of metolachlor was minimal after a 30-d incubation with less than 1% mineralized to $^{14}CO_2$ (Perkovich et al., 1996). Degradation of metolachlor was greater in the *Kochia scoparia* rhizosphere soil than in nonvegetated soil ($P = 0.0005$), with a mean of 53% remaining in the rhizosphere soil compared to 71% in the nonvegetated soils after 60 d (Table 3).

Alpha Site For the complete metabolism study that was carried out for 30, 60, or 135 days, significant differences were seen in the amount of CO_2 evolution, atrazine remaining, and soil-bound residues between rhizosphere and nonvegetated soils. An

Table 1. Soil characteristics of Alpha and Bravo soils used in this study.

Site/Soil	Texture	Sand (%)	Silt (%)	Clay (%)	O.M.[a] (%)	N[b] (%)	pH	C.E.C.[c]
Alpha/nonvegetated soil	Sandy loam	75	17	8	2.0	0.07	7.9	9.8
Alpha/rhizosphere soil	Sandy loam	68	21	11	2.5	0.08	7.8	10.0
Bravo/nonvegetated soil	Loam	50	34	16	3.9	0.26	7.0	12.7
Bravo/rhizosphere soil	Loam	32	50	18	3.9	0.22	7.5	14.1

[a] Organic matter content of soil

[b] Total nitrogen content

[c] Cation exchange capacity

Table 2. Degradation of ^{14}C-atrazine (applied with metolachlor at 50 μg/g per herbicide) in *Kochia scoparia* rhizosphere soil and nonvegetated soils taken from Bravo site after a 30-d incubation (reported as % of applied ^{14}C).

	Rhizosphere soil[a]	Nonvegetated soil[b]	F-Value	$p > F$
Atrazine remaining	2.0	7.3	11.6	0.0271
Deethylatrazine	1.4	1.5	0.01	0.9170
Deisopropylatrazine	0.6	0.8	1.1	0.3573
Didealkylatrazine	0.5	0.8	10.9	0.0301
Hydroxyatrazine	0.4	1.3	19.2	0.0119
Deethylhydroxyatrazine	0.4	0.6	12.5	0.0241
Deisopropylhyroxyatrazine	0.3	0.6	5.2	0.0842
Unidentified polar degradates[c]	1.6	4.8	87.3	0.0007
CO_2	62	49	15.9	0.0163
Soil bound residues[d]	5.8	11	151.5	0.0003
Other[e]	1.1	1.9	8.0	0.0473
Total	76	79	0.6	0.4834

[a] Soil was taken from the rhizosphere of *Kochia scoparia* at the Bravo site

[b] Soil was taken from nonvegetated areas at the Bravo site

[c] From the percentage of applied ^{14}C remaining in the aqueous phase after partitioning

[d] Unextractable ^{14}C, as determined by soil combustion

[b] Percentage of applied ^{14}C not associated with standards in thin-layer chromatography

Table 3. Degradation of ^{14}C-metolachlor (applied with unlabeled atrazine at 50 μg/g per herbicide) in *Kochia scoparia* rhizosphere soil and nonvegetated soils taken from Bravo site after a 60-d incubation (reported as % of applied ^{14}C).

	Rhizosphere soil[a]	Nonvegetated soil[b]	F-Value	$p>F$
Metolachlor	53	71	103.74	0.0005
Carbinol degradate	0.7	0.9	1.06	0.3607
Morpholino degradate	1.2	0.8	4.88	0.0916
Unidentified polar degradates[c]	3.6	1.4	97.59	0.0006
CO_2	1.53	1.46	7.9	0.0483
Soil bound residues[d]	19	17	4.63	0.0977
Other[e]	2.3	3.7	1.92	0.2379
Total	81	96	61.8	0.0014

[a] Soil was taken from the rhizosphere of *Kochia scoparia* at the Bravo site

[b] Soil was taken from nonvegetated areas at the Bravo site

[c] From the percentage of applied ^{14}C remaining in the aqueous phase after partitioning

[d] Unextractable ^{14}C, as determined by soil combustion

[b] Percentage of applied ^{14}C not associated with standards in thin-layer chromatography

ANOVA was carried out on all incubation periods. Least square means and corresponding significance levels are shown in Table 4.

The half-life for atrazine was significantly less in the rhizosphere soil than in nonvegetated soil ($p \leq 0.05$), with a half-life of 50 d in the rhizosphere soil compared to193 d in the nonvegetated soils. From thin-layer chromatography/ autoradiography, it was determined that the ratio of dealkylated-atrazine-degradates to atrazine ratio ranged from 0.07 to 0.4. Differentiation among the dealkylated degradation products was difficult due to interference of unknown contaminants (from the agrochemical dealer site) present in the organic extract.

Plant/Soil Incubations

192-d Post-Herbicide Application Chemical analyses revealed significant differences between the *Kochia*-vegetated and *Brassica*-vegetated soils. Significantly less atrazine was extractable from soils vegetated with *Kochia* (4.3% of the applied ^{14}C) than soils vegetated with *Brassica* (9.8%) ($p < 0.05$). In all three treatments, atrazine was fairly degradable. The percentage of extractable atrazine for the three treatments are given in Table 5.

Combustion of plant material revealed that significantly more ^{14}C was taken up by *Kochia* plants (9.9% of the applied ^{14}C) than by *Brassica* plants (0.8%) ($p = 0.0001$). The large difference in uptake was likely due to the fact that the *Kochia* plants were thriving in the pesticide-contaminated soils, while the *Brassica* plants were showing signs of herbicide stress. It was for this reason that Day 192 was chosen to end one set of treatments for analysis.

Table 4. Significant differences between *Kochia*-rhizosphere and nonvegetated soils using ANOVA for the three incubation periods (30, 60, and 135 d). Least square means and the corresponding *p*-values are given.

	Kochia-rhizosphere soil	Nonvegetated soil	*p*-value
	--% of the applied ^{14}C--		
Atrazine (extractable)	54	85	0.0062
CO_2	12	0.3	0.0002
Soil bound	23	17	0.0381

240-d Post-Herbicide Application Significantly less atrazine was extractable from soils vegetated with *Kochia* (5.3% of the applied ^{14}C) than from nonvegetated soils (8.3%) ($p < 0.01$). Combustion of the plants revealed that 6.5% of the applied ^{14}C-atrazine was taken up into the plant tissue. No significant differences were seen in the quantities of particular degradates and soil-bound residues between the two treatments.

Table 5. The percentage of applied ^{14}C-atrazine extracted from nonvegetated, *Kochia scoparia*-vegetated, or *Brassica napus*-vegetated soils.

	Non-vegetated	*Kochia scoparia*	*Brassica napus*
	(% of applied ^{14}C extracted from soils)		
Atrazine	9.3[ab]	4.3[a]	9.8[b]

[a-c] Least square differences (LSD); means with same letter are not significantly different ($p < 0.05$).

^{14}C-Most-Probable-Number Determination

Low numbers of atrazine degraders were seen in soils from Alpha site, with numbers ranging from 7 to 326 organisms g^{-1} of soil (Table 6). Atrazine degraders at the Alpha site were significantly more numerous in *Kochia* rhizosphere soils than in soil from

Table 6. Number of specific atrazine or metolachlor degraders in pesticide-contaminated soils obtained from Alpha and Bravo sites, as determined by a ^{14}C-most-probable-number procedure. Results are reported as the number of organisms g^{-1} soil (dry weight) along with the corresponding 95% confidence interval.

	Atrazine degraders	Metolachor degraders
BRAVO SITE:		
Nonvegetated Soil	17,412	
	(6,749 - 44,924)	
Kochia rhizosphere soil	1,107	
	(429 - 2,855)	
ALPHA SITE:		
Nonvegetated Soil	17	70
	(7 - 45)	(27 - 180)
Kochia rhizosphere soil	1,126	281
	(49 - 326)	(78 - 518)

a nonvegetated area. Greater numbers of atrazine degraders were noted at Bravo site, with significantly more in the nonvegetated soil (17,412 organisms g^{-1}) compared with the *Kochia* rhizosphere soil (1,107 organisms g^{-1}).

CONCLUSIONS

Soils obtained from pesticide-contaminated sites showed significant capability to degrade a mixture of the herbicides metolachlor and atrazine. Atrazine was significantly more degraded in *Kochia* rhizosphere soils from the two pesticide-contaminated sites than in soils taken from nonvegetated areas at the site. Specific atrazine and metolachlor degraders were present in soils from the two pesticide-contaminated sites in this study. The presence of *Kochia scoparia* in soils significantly reduced the amount of aged extractable ^{14}C-atrazine compared to nonvegetated soils. The potential for plants to aid in remediation of pesticide wastes in soils is promising.

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CHAPTER 3. SCREENING STUDY FOR ENHANCED HERBICIDE DEGRADATION IN SOILS FROM PESTICIDE-CONTAMINATED SITES

A paper to be submitted to the *Journal of Environmental Science and Health*

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ABSTRACT

A screening study was conducted to determine if soils obtained from several pesticide-contaminated sites had enhanced capability to degrade four herbicides. Uniformly ring-labeled ^{14}C-atrazine, metolachlor, alachlor, or pendimethalin were applied at a concentration of 50 μg/g to soils taken from pesticide-contaminated areas of four agrochemical dealer sites. Soil moistures were adjusted to 60% saturation and incubated under controlled conditions. Complete mineralization of the herbicides, as quantified by trapping $^{14}CO_2$ in NaOH traps and counting by liquid scintillation techniques, was monitored for 9 weeks. Atrazine mineralization was extensive in four of the soils. From a site in Nebraska, 30% of the applied atrazine was mineralized in 9 weeks. Mineralization of atrazine in rhizosphere soil taken from an Iowa agrochemical dealer site, appeared to have a shorter lag phase than did a comparable nonrhizosphere soil from that site. A control soil (uncontaminated) from an agrochemical dealer site in Illinois mineralized approximately 35% of the applied ^{14}C-atrazine. Very low amounts of metolachlor, alachlor, or pendimethalin were mineralized in these soils. The Illinois control soil exhibited the greatest amount of mineralization of alachlor, metolachlor, and

pendimethalin among all the soils tested. To determine if even higher concentrations of herbicide mixtures could inhibit degradation of atrazine in a soil that rapidly mineralized atrazine at 50 μg/g, a study was conducted by using *Kochia* rhizosphere soil from the Iowa site. Atrazine, metolachlor, and trifluralin were applied in all possible combinations at a rate of 200 μg/g each. Only the atrazine was radiolabeled, to allow for monitoring of $^{14}CO_2$ evolution arising from complete degradation of atrazine. Rapid mineralization of atrazine occurred in all soil treatments, with 60 to 80% mineralization after nine weeks.

INTRODUCTION

The problem of pesticide-contaminated soils at agrochemical dealerships in the Midwest is widespread. Contamination of soils often occurs as a result of accidental spillage of chemicals during mixing or loading. Soils containing mixtures of pesticides at concentrations well above field application rates have been reported (Gannon, 1992). The effects of herbicides on ecology and activity of soil microorganisms has been reviewed (Moorman, 1994). Little effect has been noted on total soil biomass as a result to long-term exposures of herbicides at field application rates. Research on microbial responses to large concentrations of herbicides in soil has shown that simulated spills of alachlor, metolachlor, and trifluralin initially inhibited bacteria, but the bacterial numbers recovered to levels similar to the untreated controls after 7 days. In that study, specific enzyme activities were affected by some of the herbicide spill treatments (Dzantor and Felsot, 1991).

The accumulation of pesticide wastes in soils at agrochemical dealership sites has occurred over numerous years in most cases. Long-term exposure of soils to pesticides can result in adaptations for portions of the microbial community. Microorganisms that can quickly adapt to an additional nutrient source such as carbon, nitrogen, or phosphorus will have a selective advantage (Coats, 1993). This can result in rapid degradation of chemicals to which the adaptation has occurred, a phenomenon known as enhanced microbial degradation (EMD). Numerous reports of EMD have been made with pesticides applied at field application rates (Racke and Coats, 1988; Racke and Coats, 1987; Roeth, 1986; Kaufman et al., 1985).

While EMD of herbicides can result in failure to control the intended target pests, it could also be potentially beneficial, in that adapted microorganisms could be used for cleanup of unwanted pesticide wastes, spills or carryover (Coats, 1993). Researchers have recently isolated microorganisms from a spill site that can utilize atrazine as a sole nitrogen source (Mandelbaum et al., 1993) and that exhibit the capability of metabolizing atrazine at very high concentrations (Mandelbaum et al., 1995). Increased degradation of a mixture of high concentrations of atrazine, metolachlor, and trifluralin was noted in rhizosphere soils compared with non-rhizosphere soils from a pesticide-contaminated site (Anderson et al., 1994).

This study was conducted to determine if soils that had been exposed to herbicide contamination, at four different agrochemical dealerships in the Midwest had the ability to rapidly degrade freshly applied herbicide at concentrations well above field application rates. A comparison was made between rhizosphere and nonvegetated soils at two of the

sites. Additionally, it was determined whether concentrated herbicide mixtures could suppress the degradative capability of one of the more active soils.

MATERIALS AND METHODS

Chemicals

Uniformly ring-labeled ^{14}C-atrazine [2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine; 98.2% radiopurity] and ^{14}C-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; 97.5% radiopurity] were obtained from Ciba Crop Protection (Greensboro, NC). Analytical grade and uniformly ring-labeled ^{14}C-alachlor [2-chloro-N-(2,6-diethylphenyl-N-(methoxymethyl)acetamide; 98.2% radiopurity] were obtained from Monsanto (St. Louis, MO). Analytical grade and uniformly ring-labeled ^{14}C-pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; 98.3% radiopurity) was obtained from American Cyanamid Co. (Wayne, NJ). Analytical grade atrazine and metolachlor were purchased from ChemService (West Chester, PA).

Soils and initial chemical concentrations

Surface soils (top 15 cm) were taken from pesticide-contaminated areas at four agrochemical dealer sites in the Midwest (Table 1). Background levels of pesticides were determined in each soil by carrying out solvent extractions. A known dry weight of soil was extracted three times with ethyl acetate (2:1 solvent/soil) by agitating for 20 minutes on a mechanical shaker, followed by filtration. Combined extracts were concentrated with a rotary evaporator, and then rediluted to 10 ml. Gas chromatography was carried out on the soil extracts by using a Shimadzu GC9A (Shimadzu Corporation, Kyoto,

TABLE 1.

Soils used in the mineralization screening study.

Location	Site	State	Additional Information
Alpha	A	Iowa	Rhizosphere soil
Alpha	B	Iowa	Non-rhizosphere soil
Bravo	A	Iowa	Non-rhizosphere soil
Bravo	D	Iowa	Rhizosphere soil
Echo	A	Illinois	Non-rhizosphere soil (site 4)
Echo	B	Illinois	Non-rhizosphere control soil
Echo	C	Illinois	Non-rhizosphere soil (site 2)
Foxtrot	A	Nebraska	Non-rhizosphere soil
Foxtrot	B	Nebraska	Non-rhizosphere soil

Japan) equipped with a flame thermionic detector. Chromatographic conditions were: column, 10% DC 200:2% OV 225 (2.0 mm x 1.8 m); carrier gas, N_2 (35 mL/min); injector temperature, 250 °C; column temperature, 230 °C; detector temperature, 250 °C. Five external pesticide standards (atrazine, metolachlor, alachlor, pendimethalin, and trifluralin) were used in determination of chemical concentrations.

Soil treatment with individual herbicides

Treating solutions were made for individual herbicides by mixing analytical grade herbicide and radiolabeled herbicide in acetone to obtain a final concentration of 3 mg ml^{-1} active ingredient and 1.3 x 10^6 distintegrations per minute (dpm) ml^{-1} of ^{14}C-herbicide. Soils were treated at a concentration of 50 $\mu g/g$, solvent was evaporated, soil moistures were adjusted to 60% saturation, and treated samples were incubated at 25 °C in the dark for 9 weeks. Sample jars were equipped with a NaOH (0.5 N) trap which was changed weekly. Subsamples (1 ml) of the NaOH were mixed with 3 ml of Ultima Gold (Packard Instrument Co., Downers Grove, IL) and then counted on a LKB RackBeta model 1217 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). The percentage of applied $^{14}CO_2$ evolved from degradation of the applied herbicide was assessed.

Soil treatment with mixtures of herbicides

To assess whether a mixture of herbicides at high concentrations could inhibit mineralization of atrazine in rhizosphere soil from Bravo site (a soil that had rapidly mineralized atrazine at 50 $\mu g/g$), this soil was treated with atrazine, individually or in combination with metolachlor and/or trifluralin at a concentration of 200 $\mu g/g$ for each herbicide. Only the atrazine was radiolabeled. Treating solutions were made up in acetone and contained analytical grade herbicide and ^{14}C-atrazine (uniformly ring-labeled) with a final herbicide concentration of 6 mg ml^{-1} of active ingredient per herbicide and 1.3 x 10^6 dpm ml^{-1} of ^{14}C-atrazine. Soil treatment, moisture adjustment, and incubation conditions were identical to the individual herbicide treatments described above.

RESULTS AND DISCUSSION

Background contamination concentrations for the soils used in this study are shown in Table 2. The soil from site C of Echo (in Illinois) had an extremely high concentration of trifluralin (greater than 2600 μg/g). Other sites with concentrations well above field application rate included Foxtrot B with atrazine at 9 μg/g, and Echo A with metolachlor at 3.4 μg/g. Field application rates for atrazine and metolachlor (based on calculations from product label information at 6-inch soil depths and soil density of 1.8g/cm^3) are 0.6 and 1.26 μg/g, respectively.

Atrazine mineralization was extensive in four of the soils: Echo B (control soil), Foxtrot B, Bravo A (nonrhizosphere soil), and Bravo D (rhizosphere soil) (Figures 1 & 2). The control soil from Echo and the rhizosphere soil from Bravo mineralized approximately 35% of the applied ^{14}C-atrazine after 9 weeks. No mineralization of atrazine was seen in the Alpha soils, one Foxtrot soil, and the two contaminated Echo soils. Very low amounts of metolachlor were mineralized (Figures 3 & 4). The control soil from Echo showed the greatest amount of mineralization of this herbicide, with 1.8% of the applied metolachlor as CO_2 after 9 weeks. Less than 1% of the applied alachlor was mineralized in six of the soils tested. Both Bravo soils and the Echo control soils mineralized between 2 and 6% of the applied alachlor after 9 weeks (Figures 5 & 6).

Minimal mineralization of pendimethalin was seen in this study, with all but the Echo control soil mineralizing less than 1% for the 9 week incubation period. In the Echo control soil, 2% of the applied pendimethalin was mineralized (Figures 7 & 8).

With the application of atrazine, alone and in combination with metolachlor and/or

TABLE 2. Background contamination concentrations for soils used in the mineralization screening study. Concentrations reported are in $\mu g\ g^{-1}$ dry soil.

Soil Identification	Atrazine	Metolachlor	Pendimethalin	Alachlor	Trifluralin
Alpha A (*Kochia*-rhizosphere soil)	Trace[A]	0.3	--[B]	--	0.1
Alpha B (non-rhizosphere soil)	Trace	0.3	--	--	--
Bravo A (non-rhizosphere soil)	0.3	0.2	0.3	0.2	0.3
Bravo B (*Kochia* rhizosphere soil)	Trace	0.6	0.4	--	0.9
Echo A (non-rhizosphere soil)	0.1	3.4	0.4	1.4	0.2
Echo B (control soil)	--	--	0.6	--	1.5
Echo C (non-rhizosphere soil)	--	--	--	--	2638
Foxtrot A (non-rhizosphere soil)	--	--	1.4	2.7	--
Foxtrot B (non-rhizosphere soil)	9.0	--	--	--	--

[A]Less than 0.1 $\mu g\ g^{-1}$

[B]Below detection limit

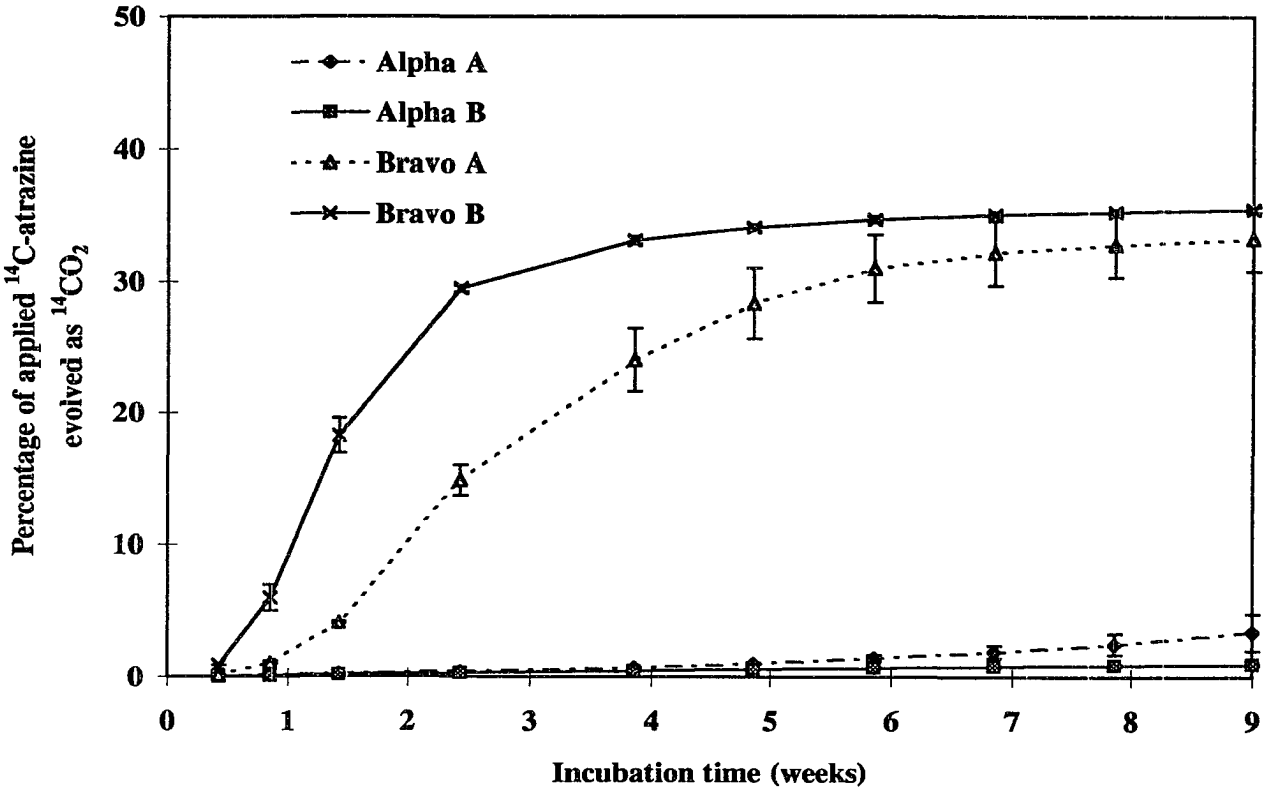

Figure 1. Mineralization of ^{14}C-atrazine in pesticide-contaminated soils from Alpha and Bravo sites. Standard deviations are indicated by the bars.

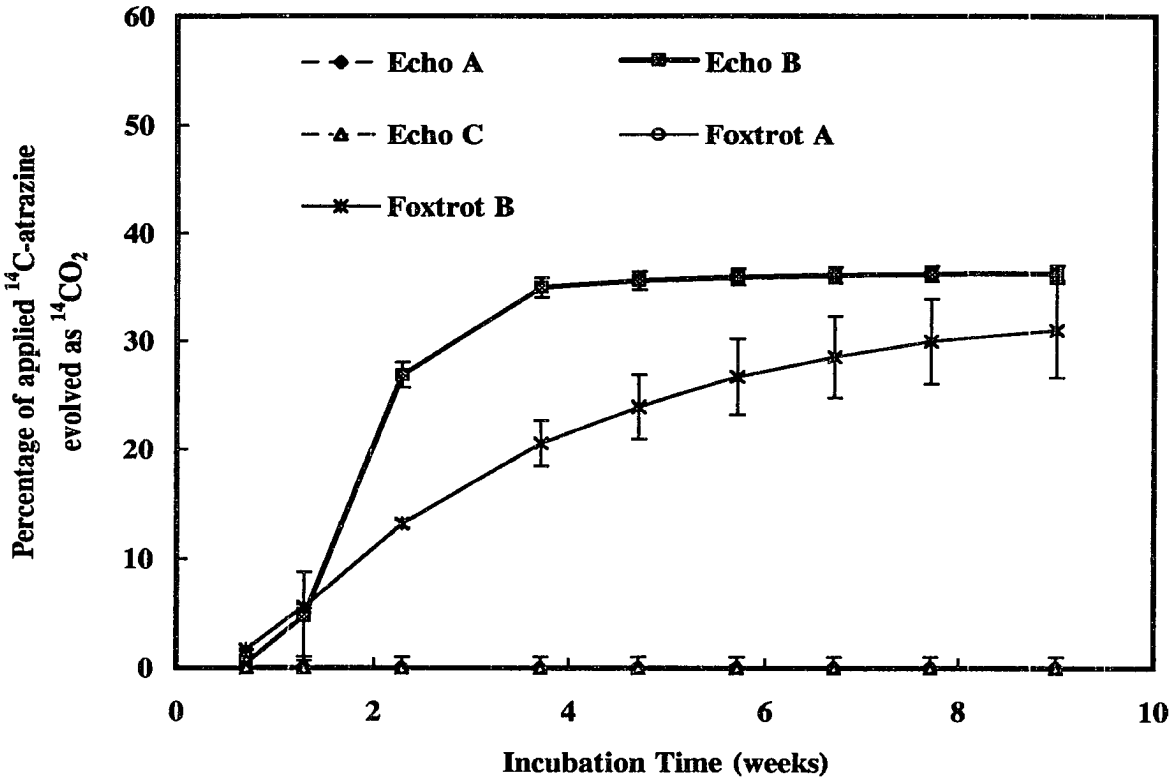

Figure 2. Mineralization of ^{14}C-atrazine in pesticide-contaminated soils from Echo and Foxtrot sites. Standard deviations are indicated by the bars.

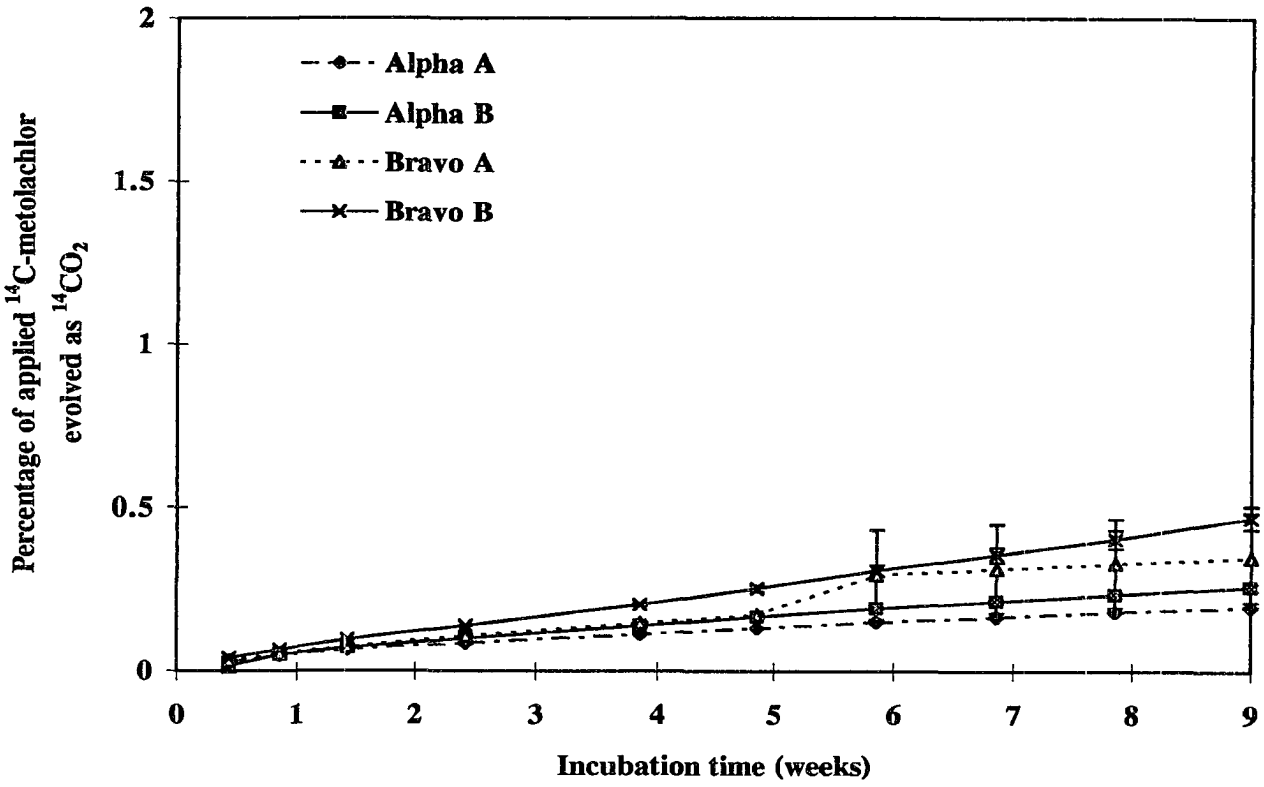

Figure 3. Mineralization of ^{14}C-metolachlor in pesticide-contaminated soils from Alpha and Bravo sites. Standard deviations are indicated by the bars.

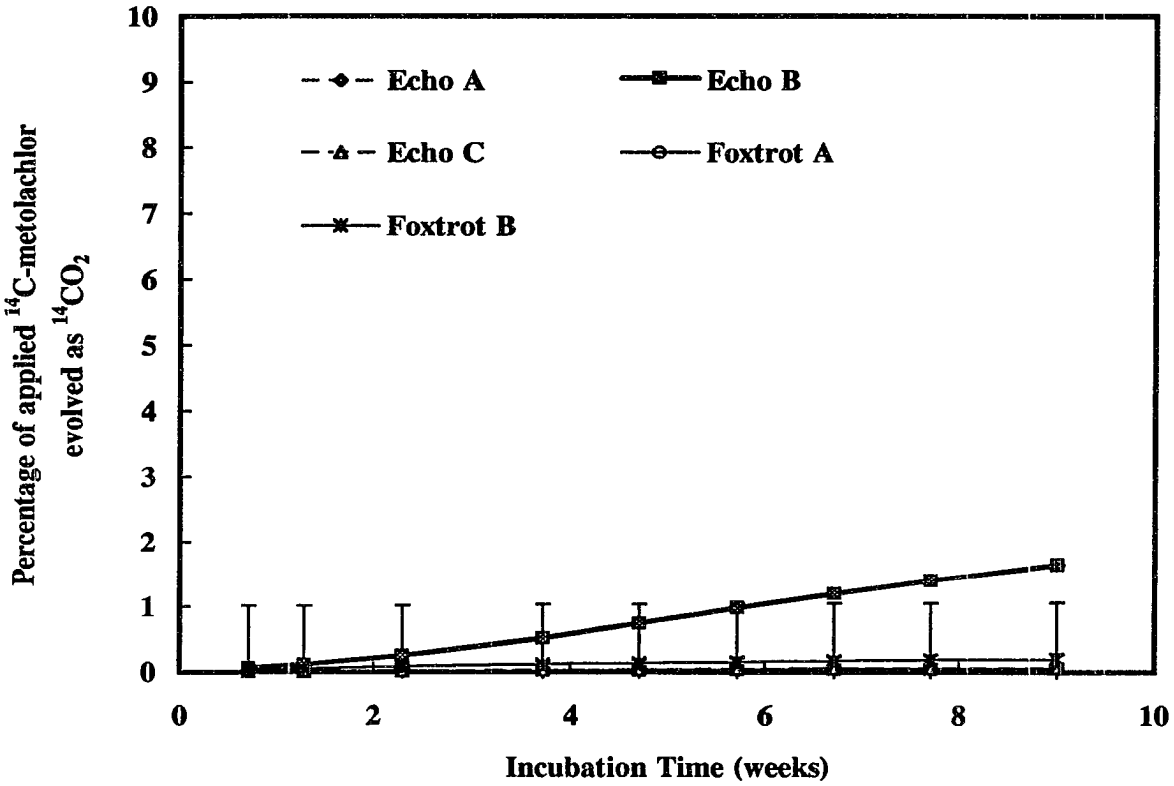

Figure 4. Mineralization of ^{14}C-metolachlor in pesticide-contaminated soils from Echo and Foxtrot sites. Standard deviations are indicated by the bars.

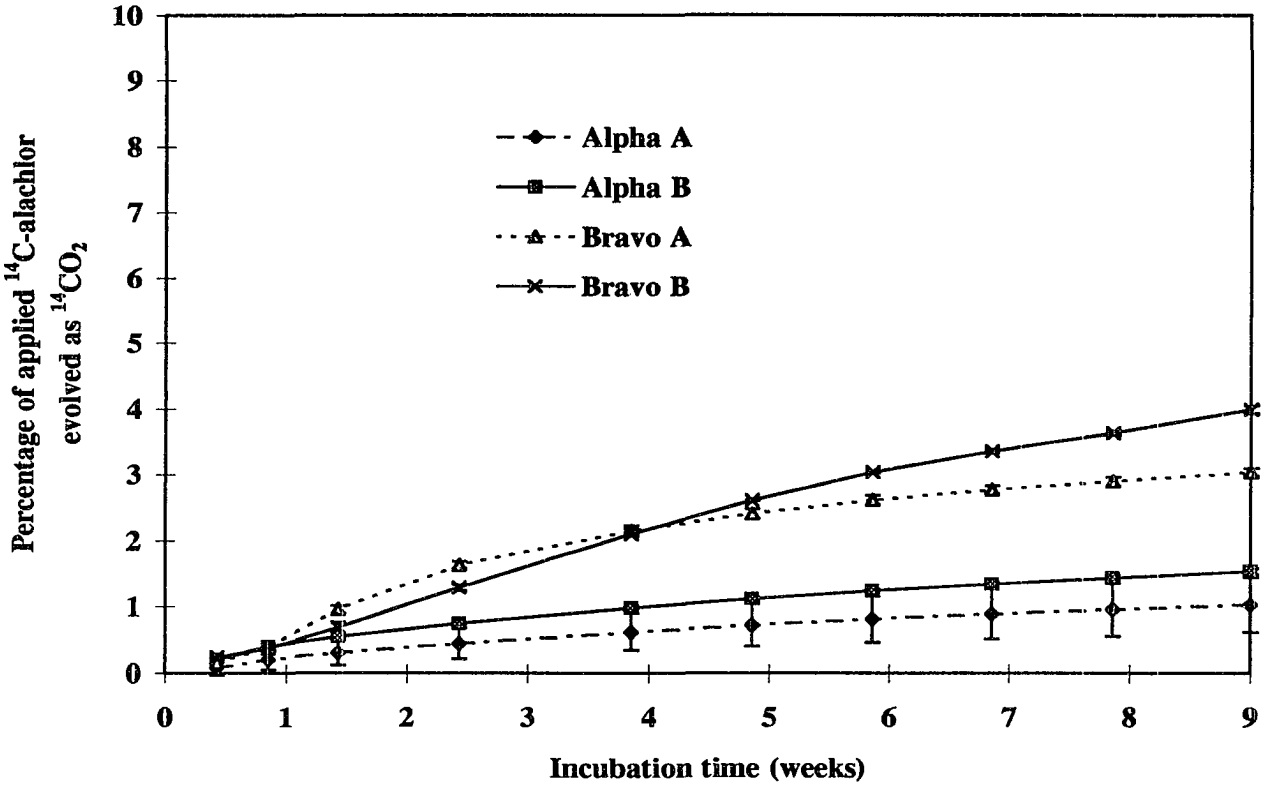

Figure 5. Mineralization of ^{14}C-alachlor in pesticide-contaminated soils from Alpha and Bravo sites. Standard deviations are indicated by the bars.

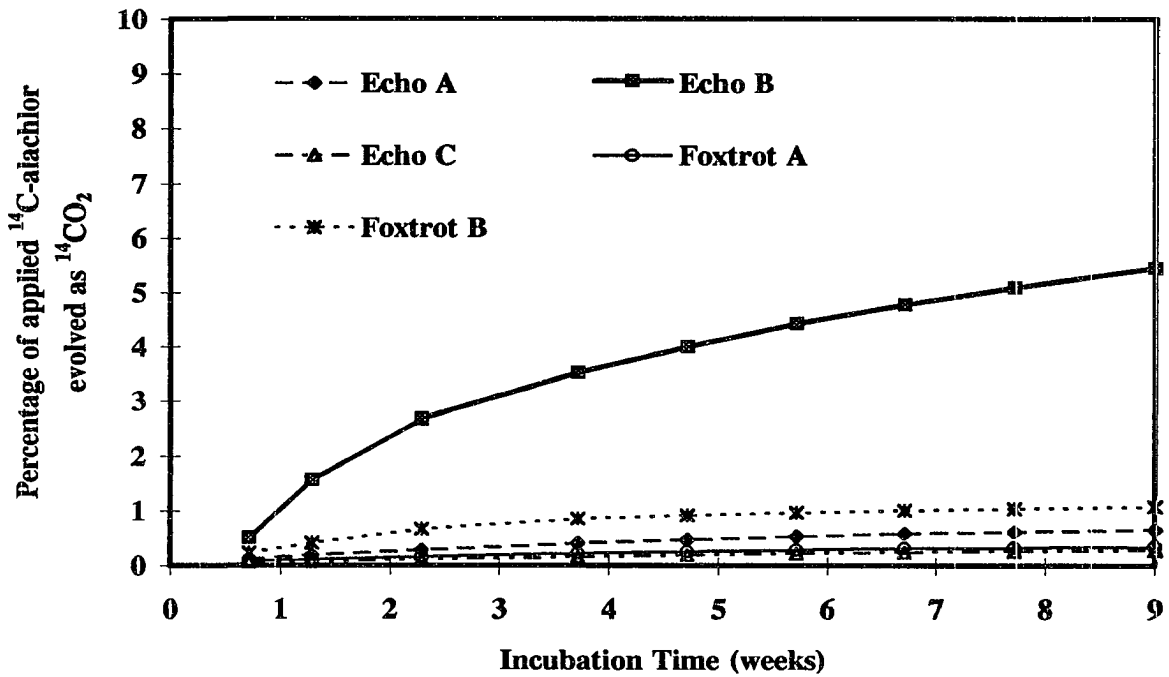

Figure 6. Mineralization of ^{14}C-alachlor in pesticide-contaminated soils from Echo and Foxtrot sites. Standard deviations are indicated by the bars.

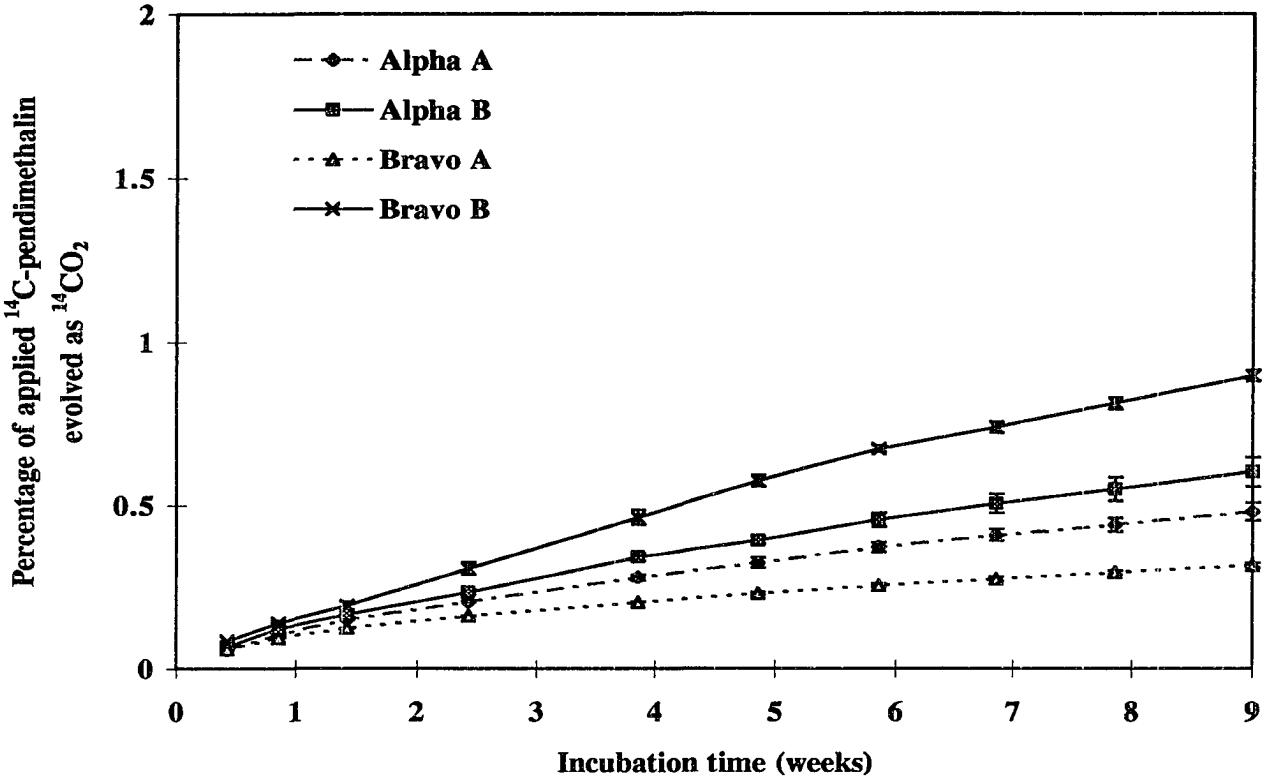

Figure 7. Mineralization of ^{14}C-pendimethalin in pesticide-contaminated soils from Alpha and Bravo sites. Standard deviations are indicated by the bars.

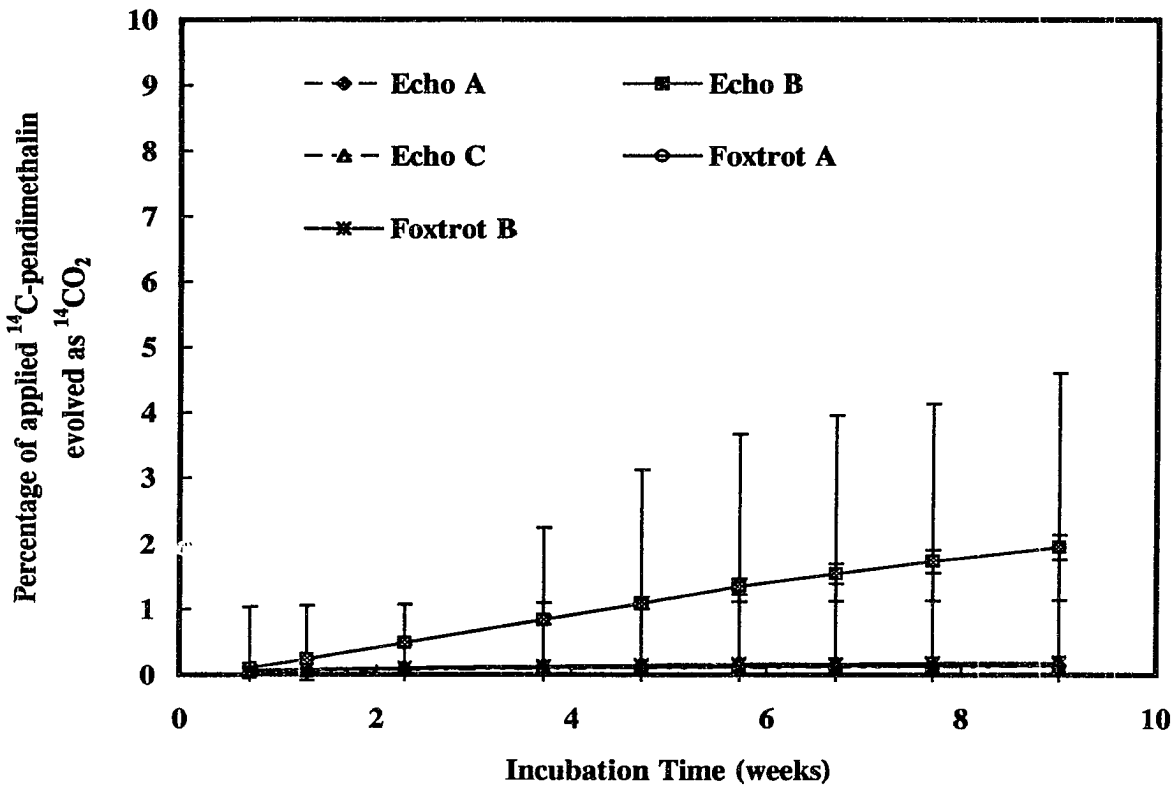

Figure 8. Mineralization of ^{14}C-pendimethalin in pesticide-contaminated soils from Echo and Foxtrot sites. Standard deviations are indicated by the bars.

trifluralin (at 200 μg/g for each herbicide), no inhibition of atrazine mineralization was seen. Rapid mineralization of atrazine occurred in all soil treatments (Figure 9), with 60 to 80% mineralization after 9 weeks. This is an even greater percentage mineralized than that observed in soils treated individually with ^{14}C-atrazine at 50 μg/g.

Rapid mineralization of atrazine has been noted in several studies using various microbial cultures. Mandelbaum et al. (1993) exposed enrichment cultures of bacteria (isolated from soils that were exposed to repeated spills of atrazine, alachlor, and metolachlor) to atrazine concentrations of 100 μg ml^{-1}. Atrazine was the sole source of nitrogen for the bacteria in this study, and half-lives for atrazine ranged from 4 to 8 days by the sixth subculture. Additionally, they have isolated a *Pseudomonas* sp. capable of rapidly mineralizing uniformly ring-labeled ^{14}C-atrazine (Mandelbaum, 1995). Enhanced atrazine mineralization in soils from a pesticide-contaminated site have been reported (Perkovich et al., 1996), with greater than 46% of the applied ^{14}C mineralized after 36 d.

Soils showing enhanced atrazine mineralization would be of significant interest for possible isolation of specific atrazine-degrading microbial populations. Enhanced mineralization of metolachlor has been noted in metolachlor-acclimated soils at field application rate. In a soil perfusion experiment using soils with five years of metolachlor history or no metolachlor history, mineralization accounted for 18.4% in history soil compared with 3.5% in the no-history soil (Liu et al., 1988). Screening pesticide-contaminated soils that have had long-term exposures to various herbicides might lead to isolation of degraders for pesticides other than atrazine. Further studies are being carried out to assess whether addition of the enhanced atrazine-degrading soils to soils that did

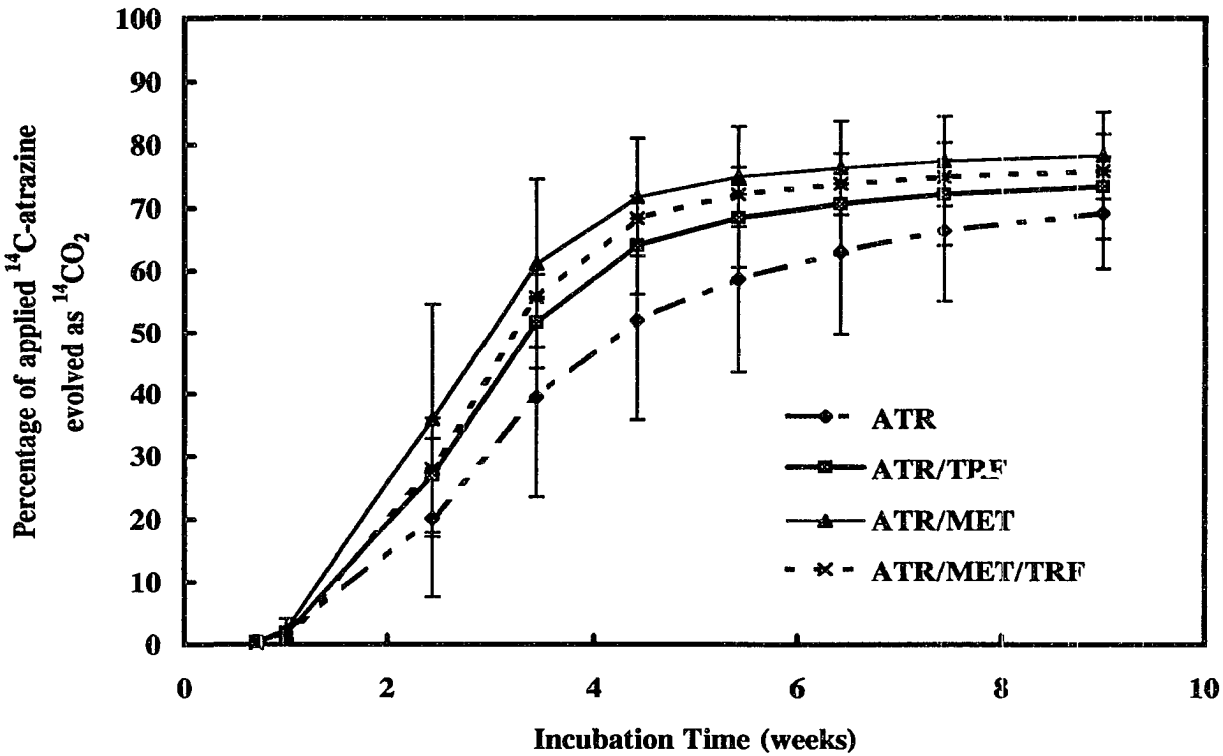

Figure 9. Mineralization of ^{14}C-atrazine in rhizosphere soil from Bravo site as influenced by the presence of metolachlor and/or trifluralin.

not show such activity could increase atrazine degradation in the non-active soils. Endpoints other than CO_2 evolution, such as decreased parent compound, increased degradate formation, and increased soil-bound residues, will be assessed for the herbicides that were not rapidly mineralized.

ACKNOWLEDGMENTS

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CHAPTER 4. ENHANCED DEGRADATION OF DEETHYLATRAZINE IN AN ATRAZINE-HISTORY SOIL OF IOWA

A paper which has been submitted to *Pesticide Science* (May 16, 1996)

Ellen L. Kruger, Jennifer C. Anhalt, Todd A. Anderson, Joel R. Coats

Abstract: The degradation of deethylatrazine, a major metabolite of atrazine, was studied by using radiotracers in soils with two different atrazine histories. Deethylatrazine degradation was enhanced in soils which had received long-term exposure to atrazine (atrazine-history soil) compared with soils that had not received long-term atrazine exposure (no-history soil). After 60 d of incubation, mineralization of deethylatrazine to $^{14}CO_2$ in the atrazine-history surface soil was twice that in the no-history surface soils. In surface soils, 25% of the applied ^{14}C remained as deethylatrazine in the atrazine-history soil, compared with 35% in the no-history soil. Microbial plate counts indicated an increase in numbers of bacteria and fungi in soils incubated with deethylatrazine compared to control soils. No significant difference in total microbial respiration was seen among atrazine-history and no-history soils incubated with deethylatrazine, but treated soils had greater microbial respiration than control soils after 6 days. Enumeration of specific deethylatrazine degraders, by using a ^{14}C-most-probable-number procedure, indicated a greater number of deethylatrazine degraders in atrazine-history subsurface soil compared with all other soils in this study ($p < 0.05$). From this study, it appears that an increase in microbial activity contributes to decreased persistence and

increased degradation of deethylatrazine in soils that have had long-term exposure to atrazine at field application rates, compared to soils with no long-term exposure. Decreased persistence of this major metabolite of atrazine in atrazine-history soils is important in that there will be less available for movement in surface runoff and to groundwater.

Key words atrazine, deethylatrazine, deethylhydroxyatrazine, persistence, degradation, metabolites, enhanced degradation

1 INTRODUCTION

With the widespread use of atrazine (ATR: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) on agricultural soil and the concern for movement of this chemical to surface and groundwater, comes an equal concern for potential persistence and movement of chlorinated degradation products of atrazine. Deethylatrazine (DEA: 2-chloro-4-amino-6-isopropylamino-*s*-triazine) is a major metabolite of ATR.[1-5] In monitoring efforts of surface water and groundwater, DEA is frequently detected along with ATR, in some cases at concentrations exceeding the maximum contaminant level for ATR in drinking water (3 μg/L) set by the U. S. Environmental Protection Agency (USEPA).[6,7] DEA is important from a toxicological standpoint in that it has been shown to modify the pituitary activity of offspring from injected pregnant female rats.[8] DEA, along with ATR, can also inhibit pituitary gland activity in male rats.[9]

While the fate of DEA has been studied in soils with no previous pesticide history,[10,11] no studies have been examined the persistence of this metabolite in soils with a previous, long-term exposure to ATR. With recurrent exposures of a microbial community to a chemical, enhanced biodegradation can occur. This is a phenomenon that results in more rapid degradation of a chemical that has been previously applied to a soil. This enhanced degradation is the result of adaptation of a microbial community which has been exposed to a chemical, and the subsequent use of this chemical as a primary energy or nutrient source.[12] This phenomenon has been found to occur with certain insecticides such as carbofuran, isofenphos and isazophos.[13-15]

It was the goal of this study to determine if DEA degradation is enhanced in soils with long-term exposure to ATR. Additionally, levels of total soil respiration, microbial plate counts, and ^{14}C-most-probable-number of DEA degraders were determined for soils in this experiment.

2 MATERIALS AND METHODS

2.1 Chemicals

The following radiolabeled chemicals and analytical standards were obtained from Ciba Corp., Greensboro, NC: [U-ring-^{14}C]DEA (94.8% radiopurity); [U-ring-^{14}C]didealkyl-

atrazine (2-chloro-4,6[diamino]-*s*-triazine; DDA) (98.8% radiopurity). Analytical standards: DEA (99% purity), DDA (97% purity), and deethylhydroxyatrazine (DEHYA; 2-hydroxy-4[amino]-6[isopropylamino]-*s*-triazine).

2.2 Soil sampling

Surface (0- to 30-cm) and subsurface (65- to 90-cm) soil samples were taken from two adjacent, no-till field plots at the Iowa State University Water Quality Site near Nashua, Chickasaw County, Iowa. At the time of soil sampling this farm had been in the possession of Iowa State University for 15 years. The history of plots prior to this time period was not documented. One plot had received 15 consecutive years of ATR applications (ATR-history soil), while the other plot had not received atrazine applications for that time period (for our study, considered a no-history soil).

Three independent composite soil probe samples were collected from each plot and prepared in the laboratory for complete metabolism studies as described by Kruger et al.[4] Soil pH, organic matter, nitrogen, sand, silt, and clay content, and cation exchange capacity were determined for each field plot (A & L Midwest Laboratory, Omaha, NE).

2.3 Complete metabolism studies

2.3.1 Treatments and soil incubation

A treating solution consisting of ^{14}C-DEA and analytical-grade DEA was prepared in acetone and applied to soils at a concentration of 1 μg/g (~0.37 kg/ha). There were three replications per depth and history. For determination of the specific amount of radioactivity applied to soil in each treating jar, one 50-g aliquot was immediately extracted three times with 150 ml of methanol:water (9:1). The mean extraction efficiency was 100%. Two other 50-g (dry weight) aliquots were each placed in French square bottles, and the acetone from the treating solution was evaporated. The soil moisture was adjusted to the gravimetric water content at -33 kPa. Each jar was randomly assigned to either a 30-d or 60-d incubation length. The sample jars were incubated in the dark at 24° C. $^{14}CO_2$ produced from mineralization of DEA was trapped in NaOH traps (0.1 N) which were changed weekly. Polyurethane foam was suspended in each jar to trap ^{14}C-organic volatiles. The foam was changed weekly and extracted with 20 ml hexane for 24 h. Radioactivity in the NaOH and hexane extracts were quantified using liquid scintillation spectroscopy.[4]

2.3.2 Soil extraction and analyses

A detailed description of soil extraction and analyses has previously been described.[4] Briefly, at the end of the incubation periods, soils were extracted three times with 150 ml of 9:1 methanol:water, partitioned into dichloromethane, and analyzed by using thin-layer

chromatography and autoradiography. Soil-bound residues were determined by combusting subsamples of extracted soil which had been dried and homogenized.

2.4 Microbial respiration

Successful microbial degradation studies require that the test chemical(s) be present at concentrations that are not toxic to the microorganisms. Carbon dioxide evolution from incubated soils was monitored as a simple and quick indication of chemical toxicity to soil microorganisms.[16,17] The respiration rates of treated, untreated, and acetone-treated soils were measured using an infrared gas analyzer (IRGA) (LIRA Model 3000, Mine Safety Appliances Co., Pittsburgh, PA).

Twenty-gram aliquots of ATR-history and no-history soils from each depth were placed in 250-cm^3 jars. Soils were either treated with 500 μl of DEA treating solution (1 ml = 0.04 mg DEA), 500 μl of acetone, or left untreated. Soils were adjusted to the gravimetric moisture content at -33 kPa with ultrapure water, and this moisture level was maintained throughout the study. Sample jars were incubated in the dark at 24 °C. The headspace of each jar was monitored for CO_2 at 24-h intervals[18] for 40 d.

2.5 Microbial plate counts

2.5.1 Total microbial enumeration

Bacterial and fungal plate counts were determined on soils before and after the IRGA study. Soil dilutions were made from surface and subsurface soils of both the ATR-history and no-history soils. Aliquots (100 μl) of soil dilutions were pipetted to trypticase soy agar plates to quantify bacterial colonies g^{-1} soil. Plates containing rose bengal agar spiked with streptomycin were used to quantify fungal colonies g^{-1} soil.

2.5.2 DEA as C and/or N source

For phenotypic characterization tests of utilizable substrates (sole carbon or sole nitrogen sources), DEA was added to a basal medium (mineral salts and noble agar). The solid growth medium was similar to that described by Mandelbaum et al.[19] with slight modifications to include the following 4 treatments: (1) DEA as the sole source of carbon, (2) DEA as the sole source of nitrogen, (3) DEA as the sole source of carbon and nitrogen, and (4) DEA present but the sole source of neither carbon or nitrogen. When DEA was the sole source of carbon in the medium, nitrogen was supplied by ammonium sulfate. When DEA was the sole source of nitrogen in the medium, carbon was supplied by glucose. Glucose (5 mg/L) and ammonium sulfate were added to the basal medium when DEA was neither the sole carbon or nitrogen source. Plates were inoculated with different dilutions and incubated at 24 °C for 48 h.

2.6 ^{14}C-Most-Probable-Number (MPN) determination

A ^{14}C-MPN procedure was done to determine the number of DEA degraders present in the soils [20,21] after exposure to freshly applied DEA (1 μg/g). Soils from the IRGA study were used in this procedure. At the end of the 40-d IRGA study, DEA degraders were quantified in the DEA-treated soils and in control (untreated) soils. A composite sample was made from soils of the three replicates for each depth. Each composite sample was homogenized and mixed well prior to removing 10 g (dry weight) for preparation of the initial 10^{-1} soil dilution. Serial dilutions from both depths of ATR-history and no-history soils were made up in sterile phosphate buffer solution (0.0125 M). A minimal salts broth (MSB)[22] was prepared and mixed with 1/100 strength nutrient broth (0.08 g liter^{-1} MSB) and then autoclaved for 20 minutes. A stock solution of trace elements[23] was prepared, filter sterilized, and added to the MSB/nutrient broth at a concentration of 1 ml liter^{-1} MSB. A ^{14}C-DEA treating solution was prepared by adding analytical-grade DEA and uniformly ring-labeled ^{14}C-DEA to the mixture of MSB, nutrient broth, and trace elements to give a final DEA concentration of 1 μg/L with radioactivity of 10,000 disintegrations per minute (dpm) ml^{-1}. In this treating solution, DEA was the only source of N.

Within a laminar flow hood, 100-μL aliquots of each soil dilution and 500 μL of the ^{14}C-DEA treating solution were pipetted to individual sterilized shell vials which were then capped with sterile foam plugs. For each soil dilution, five replicates were made. Twelve sterile buffer control samples were also prepared at intervals throughout this

procedure by adding 100 μL of sterile phosphate buffer and 500 μL treating solution to additional shell vials. Capped shell vials were placed inside 20-ml disposable scintillation vials containing 1 ml of 1 N NaOH. Cone-capped lids provided a secure seal for the 20-ml scintillation vials. All vials were incubated in the dark at 20 °C for 50 days. At the end of the incubation period, individual shell vials were rinsed with 1 ml ultrapure water into their respective 20-ml scintillation vials. Ultima Gold (Packard Instrument Co., Downers Grove, IL) scintillation cocktail (12 ml) was added to each scintillation vial. Quantification of radioactivity trapped in the NaOH was determined by liquid scintillation techniques.

In order to determine the presence or absence of DEA degraders in each of the five replicates from the soil dilutions, the dpm from these vials were compared to the mean dpm from sterile buffer controls. Vials were scored as positive for degraders if dpm were 20% above those of the sterile buffer controls. A MPN determination was calculated according to statistical tables.[24-26]

2.7 Statistical analysis

An analysis of variance (ANOVA) was performed on data for the mass balance of applied ^{14}C in the complete metabolism study. Degradation rate constants were derived using methods of Walker.[27] An ANOVA was conducted on the rate constants from each replication and pairwise comparisons were made among ATR-history and no-history

surface and subsurface soils. Standard errors were determined for CO_2-efflux data from the IRGA study. Confidence intervals for the MPN procedure were determined using methods of Cochran.[25]

3 RESULTS

3.1 Soil characteristics

Soils were mapped as the Kenyon-Readlyn association. The texture of the ATR-history and no-history surface soils had a loam and clay loam, respectively. ATR-history and no-history subsurface soils both had a sandy clay loam texture. The organic matter content of both surface soils was 2.9% and for both subsurface soils 0.7%. The nitrogen content of the ATR-history soils (surface and subsurface) was significantly greater than nitrogen content in the corresponding no-history soils. Other pertinent soil characteristics are given in Table 1.

3.2 Complete metabolism studies

A mass balance of the applied ^{14}C-DEA was determined for soils in this study. DEA was significantly less persistent in ATR-history surface soil compared with no-history surface soil (Table 2). Mineralization of DEA in ATR-history surface soil was twice that of no-history surface soil (34% and 17%, respectively). Soil-bound residues were formed to a

TABLE 1. Soil characteristics of ATR-history[a] and no-history soils[b].

Plot	Depth	Texture	Sand (%)	Silt (%)	Clay (%)	O.M.[c] (%)	N[d] (%)	pH	C.E.C.[e]
ATR-history	0-30	Loam	42	34	24	2.9	0.35	5.5	12.2
ATR-history	65-90	Sandy clay loam	54	18	28	0.7	0.24	6.3	11.8
No-history	0-30	Clay loam	38	34	28	2.9	0.16	5.9	14.0
No-history	65-90	Sandy clay loam	48	22	30	0.7	0.04	6.5	12.2

[a] Soil with 15 consecutive years of atrazine application

[b] Soils with no atrazine application over the past 15 years

[c] Organic matter content of soil

[d] Total nitrogen content

[e] Cation exchange capacity

TABLE 2. Degradation of ^{14}C-deethylatrazine in no-history[a] and ATR-history[b] soils after 60-d incubation (least square means as determined by ANOVA are reported as % of applied ^{14}C).

	No-History[a]		ATR-History[b]	
	Depth (cm)			
	0-30	65-90	0-30	65-90
	----------(% of applied ^{14}C)----------			
Deethylatrazine (remaining)	35A[c]	66B	25C	60B
Didealkylatrazine	0.5AB	0.6B	0.3AC	0.1C
Deethylhydroxyatrazine	0.1A	0.04A	0.2A	0.4A
Polar degradates[d]	5.0A	5.6A	2.8B	4.3A
CO_2	17A	9.8B	34C	13B
Soil bound	32A	16B	26C	16B
Other[e]	1.8A	1.2B	1.0B	1.1B
Total	91A	99A	89A	95A

[a] Soil having no atrazine application over the past 15 years

[b] Soil that has had 15 consecutive years of atrazine application

[c] A-C; least square means (as determined by ANOVA) in each line with the same letter are not statistically different ($p<0.05$)

[d] As quantified by radioactivity in the aqueous phase after partitioning soil extract

[e] As quantified by thin-layer chromatography; radioactivity not corresponding to standards

lesser extent in ATR-history surface soil compared to no-history surface soil despite no differences in their organic matter contents.

For comparative purposes, first-order degradation rate constants and half-lives were calculated for each of the treatments. In the subsurface soils, there was no difference in DEA half-life (Table 3). In surface soils, the half-life for DEA was significantly shorter in ATR-history soil (29 d) compared with no-history soil (39 d).

3.3 Microbial respiration

The respiration rates of DEA-treated and control (acetone-treated) soils were suppressed for 6 days after treatment (Figure 1) compared with untreated soils, suggesting an initial toxic effect. Soils appeared to recover after the sixth day, with comparable efflux rates for both DEA- and acetone-treated soils. The slightly higher respiration rates in the DEA and acetone-treated soils from day 8 on compared to untreated soils, seems to indicate utilization of the acetone and possibly the DEA by soil microorganisms. The untreated soils were more active, with higher respiration rates at the beginning of the study. However, activity subsided after the sixth day, returning to what we termed as a basal level of soil respiration. Respiration rates in subsurface soils were similar for untreated control, DEA- and acetone-treated soils.

TABLE 3. Degradation rate constants (k) and half-lives ($T_{1/2}$) of deethylatrazine (DEA) in No-history[a] and ATR-history[b] soils.

Soil history	Depth (cm)	k[c]	$T_{1/2}$[d] (days)
No-history	0-30	0.018A[e]	39
	65-90	0.007B	99
ATR-history	0-30	0.024C	29
	65-90	0.009B	77

[a] No-history soil had received no atrazine application for the past 15 years

[b] ATR-history soil received 15 consecutive years of atrazine (ATR) applications

[c] Degradation rate constant (Walker, 1987)

[d] Half-life equals time required for 50% of DEA to be degraded

[e] A-C; rate constants (presented as least square means as determined by ANOVA) with the same letter are not statistically different ($p < 0.01$)

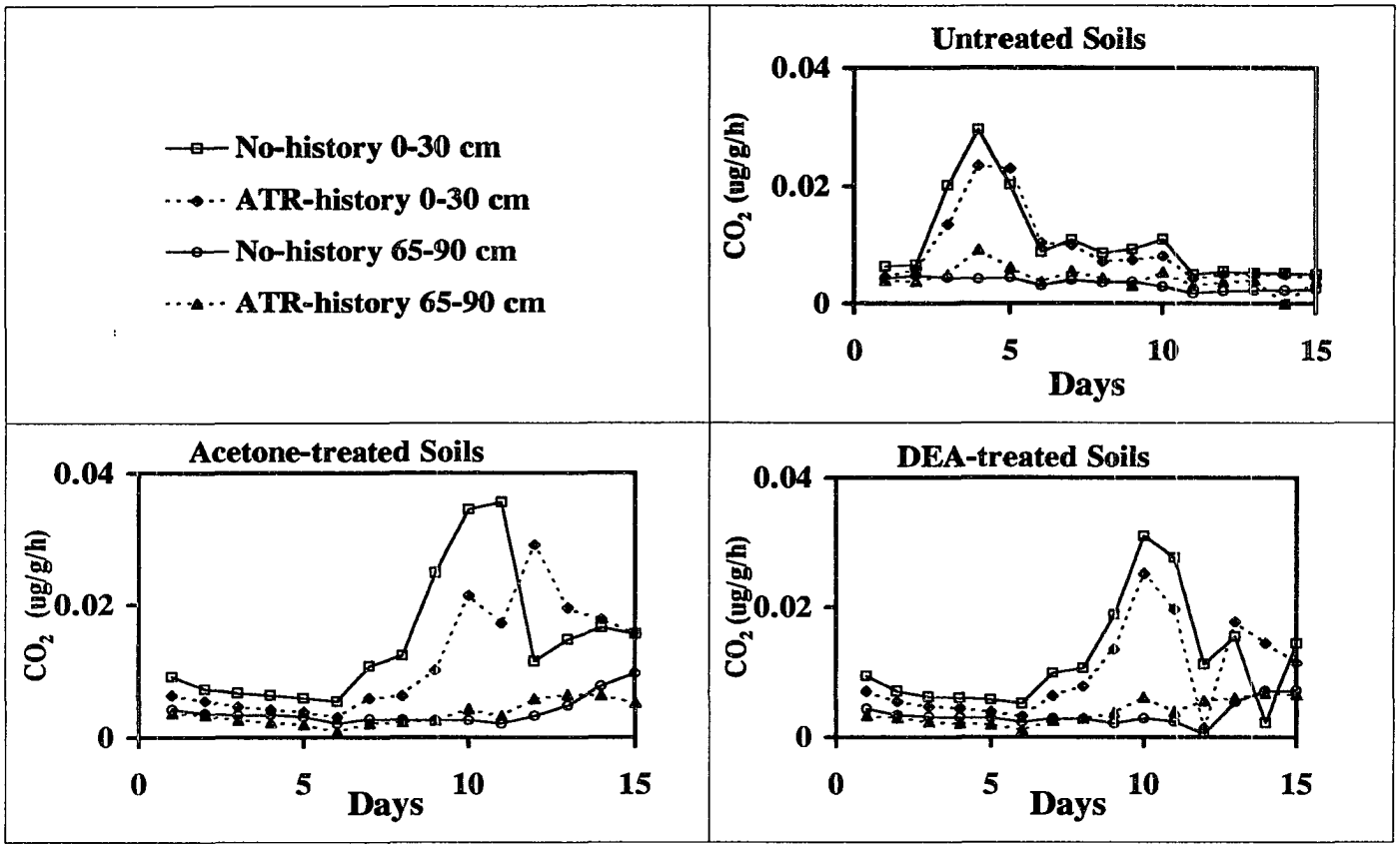

Figure 1. Soil respiration (CO_2) of DEA-treated (1 ug/g), acetone-treated, and untreated soils incubated in the dark at 24 °C. Standard deviations are indicated by the bars.

3.4 Microbial plate counts

3.4.1 Total microbial enumeration

Microbial counts (colonies g^{-1} soil) in untreated soils prior to the IRGA study are given in Table 4. For bacteria, the counts ranged from 1.9×10^7 in no-history subsurface soil to 1.2×10^8 in no-history surface soil. Fungal counts ranged from 9.1×10^5 to 1.5×10^6. At the end of the IRGA study, bacterial counts were greater in most DEA-treated soils compared with the pre-IRGA study plate counts. The greatest increase in bacterial colonies g^{-1} was seen in the ATR-history subsurface soil, with 1.2×10^{10} compared to 4.7×10^7 in untreated soils prior to the IRGA study. Bacterial counts in no-history surface control soils were similar to pre-IRGA counts. In no-history subsurface and ATR-history surface soils, an increase was seen in control soils after the IRGA study compared with pre-IRGA counts. Bacterial counts decreased in ATR-history subsurface control soils compared to pre-IRGA counts.

Fungal populations in DEA-treated soils increased an order of magnitude in ATR-history and no-history surface soils, and two orders of magnitude in the ATR-history subsurface soil. In control soils, increases in fungal populations were seen for subsurface soils. In ATR-history surface soil, fungal populations decreased after the IRGA study, compared with pre-IRGA counts. Due to a contamination problem, the fungal counts for no-history surface soil were unavailable.

TABLE 4. Microbial plate counts of ATR-history and no-history soils.

	Bacterial Plate Counts[a]				Fungal Plate Counts[b]			
	No-history soil		ATR-history soil		No-history soil		ATR-history soil	
	(# organisms g^{-1} soil (dry weight))							
	0-30	90-120	0-30	90-120	0-30	90-120	0-30	90-120
Pre-IRGA study:								
Untreated soil	1.2×10^8	1.9×10^7	3.7×10^7	4.2×10^7	9.4×10^5	1.5×10^6	9.1×10^5	3.8×10^5
Post-IRGA study:								
DEA-treated soil	6.6×10^9	1.8×10^7	4.5×10^9	1.2×10^{10}	3.3×10^6	2.8×10^5	3.5×10^6	3.5×10^7
Control (untreated) soil	1.5×10^8	1.4×10^8	9.4×10^8	3.8×10^6	----------[c]	4.7×10^7	8.4×10^5	3.5×10^6

[a] Serial dilutions plated on typticase soy agar

[b] Serial dilutions plated on rose bengal agar spiked with streptomycin

[c] Due to contamination, counts were unavailable

3.4.2 DEA as C or N source

Results of phenotypic characterization tests of utilizable substrates for microorganisms in ATR-history and no-history soils were inconclusive in identifying any differences. For both surface and subsurface soils, nearly confluent growth was observed at 48 h in all plates: (1) DEA as the sole source of carbon, (2) DEA as the sole source of nitrogen, (3) DEA as the sole source of carbon and nitrogen, and (4) DEA present but neither the sole source of carbon or nitrogen. It is possible that the microbial growth noted in this procedure is due to oligotrophs which are able to scavenge trace amounts of carbon or N.

3.5 ^{14}C-Most-Probable-Number (MPN) determination

The most-probable numbers of DEA degraders, using DEA as a source of nitrogen, are given in Table 5. The mean number of DEA-degraders in DEA-treated soil from the IRGA study ranged from 272 to 4,651 organisms g^{-1}. In control soils, the range was from 116 to 511 organisms g^{-1}. With the wide confidence intervals typical of this type of procedure, the only significant difference in numbers of DEA-degraders was seen in the ATR-history subsurface soil ($p \leq 0.05$).

4 DISCUSSION

Decreased persistence of DEA in ATR-history soils may be due to enhanced microbial degradation of DEA by soil microorganisms which have become adapted over

TABLE 5. Enumeration of deethylatrazine degraders in atrazine-history and no-history soils as determined by ^{14}C-most-probable-number technique (reported as number of organisms per g soil).

	No-history soil		Atrazine-history soil	
	Depth (cm)			
	0-30	65-90	0-30	65-90
	(# organisms/g soil (95% confidence interval))			
DEA-treated soil	366 (104, 1293)	374 (106, 1320)	272 (77, 961)	*4651 (1318, 16418)
Control soil	116 (33, 410)	201 (57, 710)	511 (144,1804)	200 (57, 708)

* Significant at α=0.05

the course of repeated ATR (and thus DEA) exposures. The increase in soil respiration in soils treated with DEA and acetone may have been due to soil microorganisms using either chemical as a source of carbon. In addition, it is clear from the ^{14}C-MPN study that DEA can be utilized as a source of N by soil microorganisms. Mandelbaum et al.[19] has reported that ATR can be used as a N source by bacterial mixed cultures. Bacterial plate counts and ^{14}C-MPN results indicated greater numbers in ATR-history subsurface soils compared with other soils. However, results from the complete metabolism study did not show a significant difference in DEA dissipation between ATR-history subsurface soil and no-history subsurface soil. Other studies have noted an increase in DEA persistence in subsurface soil compared with surface soil.[10,11] In this subsurface soil, limiting factors may exist that do not allow for microbial proliferation and DEA degradation. In the MPN technique, aliquots from soil dilutions are incubated with a minimal salts media and trace elements, thus essentials for growth were present. This suggests that if pertinent growth factors, nutrients, and energy sources are provided, microbial proliferation might lead to enhanced degradation of DEA in the subsurface soil. Assaf and Turco[28] have reported that microbial populations appeared to use ATR metabolites as N sources when provided with a significant carbon enrichment, suggesting that degradation of metabolites was limited by a lack of carbon. Another characteristic of the MPN procedure is that soil dilutions are incubated in a somewhat more aerobic environment than would be subsurface soil. The headspace of the 20-ml scintillation vial containing aliquots of each soil dilution and ^{14}C-DEA solution is aerobic. Nair and Schnoor[29] have reported that oxygen limitation at deeper soil depths will retard ATR

transformation and mineralization as the soil environment becomes more anoxic. It is also possible that mineralization of DEA may be the result of a consortium of organisms, some of which were not enumerated by the MPN procedure.

DEA was readily degraded to CO_2 in surface soils. Winkelmann and Klaine[3] found that 25% of the applied ^{14}C-DEA was mineralized in soil microcosm studies after 180 d in a Falaya silt loam surface soil. In a study investigating degradation of ^{14}C-DEA in 5 soils of Iowa with no previous ATR exposure, Baluch et al.[10] reported that the mineralization of DEA in surface soils ranged from 2 to 18% of the applied ^{14}C after 60 d. In the current study, greater mineralization of DEA in ATR-history surface soil compared to no-history surface soil indicates that, within the ATR-history surface soil, there exists a microbial population more capable of completely degrading the DEA molecule. Further tests of DEA degradation in other ATR-history soils would be needed to determine if increased DEA degradation is a function of repeated ATR exposure. Decreased persistence of this major phytotoxic metabolite of ATR in ATR-history soils is important in that there will be less available for movement in surface runoff and to groundwater, leading to decreased human exposure.

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GENERAL CONCLUSIONS

Soils obtained from pesticide-contaminated sites showed a significant capability for degradation of a mixture of the herbicides metolachlor and atrazine. Atrazine was significantly more degraded in *Kochia* rhizosphere soils from the two pesticide-contaminated sites than in soils taken from nonvegetated areas at the site. Specific atrazine and metolachlor degraders were present in soils from the two pesticide-contaminated sites in this study. The presence of *Kochia scoparia* in soils significantly reduced the amount of aged extractable ^{14}C-atrazine compared to nonvegetated soils. The potential for plants and degrader microorganisms to aid in remediation of pesticide wastes in soils is promising.

A screening study was conducted to determine if soils obtained from several pesticide-contaminated sites had enhanced capability to degrade four freshly applied herbicides (at concentrations of 50 μg/g). Mineralization of atrazine was extensive in soils from several pesticide-contamination sites. Very low amounts of metolachlor, alachlor, or pendimethalin were mineralized in these soils. To determine if even higher concentrations of herbicide mixtures could inhibit degradation of atrazine in a soil that rapidly mineralized atrazine at 50 μg/g, a study was conducted by using *Kochia* rhizosphere soil from Bravo site. Atrazine, metolachlor, and trifluralin were applied in all possible combinations at a rate of 200 μg/g each. Only the atrazine was radiolabeled to allow for monitoring of $^{14}CO_2$ evolution arising from complete degradation of atrazine. Rapid mineralization of atrazine occurred in all soil treatments, with 60 to 80%

mineralization after 9 weeks.

Decreased persistence of deethylatrazine (a major phytotoxic metabolite of atrazine) was noted in an Iowa soil that had received long-term exposure to atrazine compared to a comparable soil that had no long-term exposure to atrazine. Decreased persistence of this major phytotoxic metabolite of ATR in ATR-history soils is important in that there will be less available for movement in surface runoff and to groundwater, leading to decreased human exposure.

Enhanced degradation of atrazine in pesticide-contaminated soils may indicate that microbial adaptation for use of this herbicide as a carbon or nitrogen source has occurred as a result of long-term exposure. Atrazine has not been shown to undergo enhanced degradation at field application rates in agricultural soils. Perhaps selection pressure is low, due to the availability of alternate C, N, or energy sources, and there has not been sufficient to cause such microorganismal adaptation by organisms under normal field conditions, compared to pressures at agrochemical dealer sites where soils are often organically deficient. Enhanced degradation of deethylatrazine, a major metabolite of atrazine, in an Iowa atrazine-history soil is a positive consequence, in that less of this degradate would be available for movement to ground water. The presence of a herbicide-tolerant plant significantly reduced the amount of soil-extractable atrazine than in nonvegetated soils, indicating that the potential for phytoremediation of pesticide-contaminated soils, at least for atrazine, looks promising.

Phytoremediation of pesticide wastes would provide an affordable alternative to

agrochemical dealers facing required remediation of pesticide-contaminated soils. Further research is needed to identify optimal plant species, plant densities, management practices and factors affecting the success of such an approach.

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