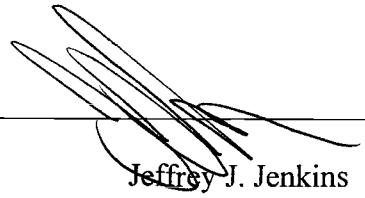
AN ABSTRACT OF THE THESIS OF

Heather B.Runes for the degree of Doctor of Philosophy in Toxicology presented on December 1, 2000. Title: Remediation of Atrazine in Irrigation Runoff by a Constructed Wetland.

Abstract approved: 

Jeffrey J. Jenkins

Due to its frequent use in agriculture and its frequent detection in surface water, atrazine was chosen as a model compound to study the treatment capability of a surface flow wetland used to remediate irrigation runoff at a container nursery near Portland, Oregon. Further evaluation of treatment of atrazine was performed under controlled conditions using static wetland microcosms. The potential to enhance microbial degradation of atrazine in the constructed wetland was investigated using bioaugmentation with an atrazine spill-site soil containing a large population of atrazine-degrading microorganisms. For the first five field experiments conducted in 1998 and 1999, the percent atrazine recovered at the outlet of the constructed wetland during a 7-d period ranged from 16 to 24% and several degradation products (deethylatrazine (DEA), deisopropylatrazine (DIA)) were detected in runoff water. Changes in total flow or frequency and intensity of runoff events did

not affect treatment. However, for a sixth experiment, when runoff events were longer in duration treatment was compromised. Static water-sediment column experiments suggested that sorption is an important mechanism for atrazine loss from water passing through the constructed wetland. Less than 12% of the atrazine applied to static wetland microcosms remained in the water column after 56 d. Atrazine degradates were observed in water and sediment, with hydroxyatrazine (HA) the predominant degradate. The presence of a large population of atrazine-degrading organisms was not observed in wetland sediment, suggesting that microbial degradation of atrazine in the constructed wetland was inconsequential. Wetland sediment bioaugmented with spill-site soil (1:100 w/w) was shown to rapidly degrade 30% of atrazine added, and most probable number (MPN) assays confirmed growth of microorganisms in bioaugmented wetland sediment. Enhanced atrazine degradation using bioaugmentation into static wetland microcosms was successful when atrazine treatment in the water column of microcosms was employed. Results of these studies indicated that this constructed wetland effectively reduced overall atrazine concentration in irrigation runoff by sorption, abiotic degradation, and possibly by plant uptake. Due to the limited presence of atrazine-degrading microorganisms in wetland sediment, bioaugmentation provides the best opportunity for enhancing microbial degradation of atrazine in sediment in this wetland system.

Remediation of Atrazine in Irrigation Runoff by a Constructed Wetland

by

Heather B. Runes

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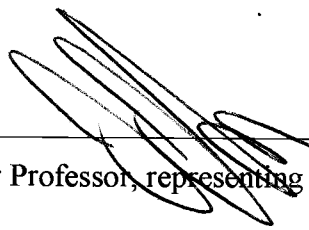

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Heather B. Runes

Heather B. Runes, Author

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CONTRIBUTION OF AUTHORS

The various coauthors of the chapters are listed in alphabetical order together with major contributions to the study.

Bottomley, Peter J.	Technical support; critical review of the experimental design, data, and manuscripts.
Jenkins, Jeffrey J.	Technical support; critical review of the experimental design, data, and manuscripts; guidance; patience.
Lerch, Robert N.	Technical support; critical review of the manuscripts.
Moore, James A.	Technical support; critical review of the manuscripts.
Wilson, Bruce D.	Technical support; critical review of the manuscipts.

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CHAPTER I

INTRODUCTION

There is growing concern that pesticide use practices both in agricultural and urban areas will result in contamination of ground and surface water. Transport of soil-applied pesticides to water bodies is likely to occur through surface transport in runoff water. In order to assess the extent of pesticide contamination in the nation's major hydrologic basins, the National Water Quality Assessment Program (NAWQA) was developed. In the first cycle of NAWQA water-quality data collection (1992-1996), seventy-six pesticides and seven pesticide degradation products in 8,200 samples of ground and surface water were analyzed. More than 95% of all samples collected from streams and rivers contained at least one pesticide and atrazine and its degradation product deethylatrazine were among the most frequently detected pesticides in agricultural areas (U.S. Geological Survey, 1998).

EXISTING CONSTRUCTED WETLAND SYSTEMS FOR THE TREATMENT OF WASTEWATER

The use of constructed wetlands for the treatment of agricultural runoff is gaining in popularity as a relatively inexpensive alternative to traditional treatment

methods (Kirschner, 1995). Constructed wetlands are commonly used in the treatment of agricultural, municipal, industrial, and stormwater waste, and there are currently more than 300 in operation in the United States (Electric Power Research Institute, 1997). Typically, wetlands have been used to successfully treat water contaminated with phosphorus, nitrogen, BOD, hydrocarbons, animal waste, and heavy metals from the aforementioned wastes.

In 1995, the Allegheny Power Service (APS) in Springdale, PA installed a wetland treatment system to bring metal-bearing discharge into compliance with its NPDES permit limits in a cost-effective manner. The system is composed of eight treatment cells and removes 96% of dissolved iron and 92% of total iron. The system also treats other metals including aluminum and manganese (Electric Power Research Institute, 1997). Studies are currently being conducted in cooperation with two other constructed wetland sites to better understand how 26 trace elements are retained in sediments, water, or assimilated into the roots and shoots of vegetation.

The Amoco Oil Company expanded its water treatment facilities due to stringent EPA discharge standards. Amoco constructed 16 ponds planted with wetland species including cattail and brush species. All pollutant concentrations were reduced as they passed through the system. These pollutants included chromium, ammonia, and total suspended solids (Litchfield, 1993).

Although constructed wetlands have successfully treated many types of wastewater, for various reasons, there has been little evaluation of their treatment of

pesticides (Watanabe, 1997). This may be due, in part, to the fact that these organic compounds and their transformation products are difficult to analyze (Watanabe, 1997; Thurman and Meyer, 1996).

DEFINITION AND FUNCTION OF CONSTRUCTED WETLANDS

Constructed wetlands are wetlands created from non-wetland sites for the purpose of treating wastewater (Hammer, 1997). They consist of saturated substrates, emergent and submergent vegetation, invertebrates and vertebrates, aerobic and anaerobic microbial populations, and a water column (Hammer, 1989). There are two predominant types of constructed wetlands--sub-surface and surface flow wetlands. The majority of existing constructed wetlands are surface flow systems in which water flows across and above the substrate surface (Reed, 1990).

Pollutants are removed by a variety of physical, chemical, and biological processes in wetlands. Macrophytes may remove pollutants via direct assimilation into their tissue or by providing surfaces, on which microorganisms live and transform pollutants (Brix, 1993). A significant portion of a constructed wetland's treatment of wastewater is believed to be a result of transformation and detoxification of compounds by microorganisms residing on the rhizomes of wetland vegetation (Kirschner, 1995; Adler et al., 1994; Alvey et al., 1996; Brix, 1987; Anderson et al., 1993). In order for this to occur, wastewater must come in contact with the rhizosphere where it is treated by pollutant binding to soil particulates (Brix, 1987).

PHYTOREMEDIATION

Phytoremediation of organic pollutants may occur in wetlands. It involves three mechanisms: 1) plant bioconcentration and metabolism, 2) release of exudates and enzymes into the soil which stimulates microbial activity and biochemical transformations, and 3) enhanced mineralization at the root-soil interface due to mycorrhizal fungi associated with the root surface (Schnoor et al., 1995; Cunningham and Ow, 1996).

Wetland plants are well adapted to growing in water-saturated substrates due to the aerenchyma found throughout plant tissues. The aerenchyma supply oxygen to the submerged plant material and the roots and rhizomes leak oxygen into the substrate resulting in oxidized zones in an otherwise reduced substrate (Brix, 1987; Hammer, 1989). Root cells exude compounds into the rhizosphere (Uren and Resisenaquer, 1988) and these compounds may serve as substrates for microorganisms (Hale and Moore, 1979; Rice, 1984).

Enhanced mineralization in the rhizosphere is due to a phenomenon known as the rhizosphere effect, which is expressed as the ratio of microorganisms in rhizosphere soil to the number of microorganisms in non-rhizosphere soil (Anderson et al., 1994). Walton et al. (1990) suggested that the microbial communities associated with the rhizosphere may play an important role in the degradation of hazardous organic chemicals in soils due to their increased density and greater diversity in rhizosphere versus non-rhizosphere (non-vegetated) soil. Microbial densities in the plant rhizosphere are an order of magnitude or more

above those in non-rhizosphere soils, which could translate into an increase in the degradation rate for xenobiotics in the rhizosphere (Anderson et al., 1993). These microbial populations are supported by secretions of degradable organic substrates at the root-soil interface (Curl and Truelove, 1986) whose presence may facilitate cometabolic transformation of organic compounds in the rhizosphere. Previous exposure to xenobiotics may be an important factor determining the composition of this microbial community (Sandmann and Loos, 1984) since enrichment of certain microorganisms may occur in response to exposure to a specific xenobiotic. Much of the knowledge regarding the microbial transformations of organic compounds in the rhizosphere is a result of the use of agricultural chemicals. Enhanced degradation of insecticides and herbicides has been reported in crops including, but not limited to rice, wheat, corn, legumes, and tobacco (Anderson et al., 1994). In some cases the increased degradation capacity was correlated with an increase in the population of pesticide-degrading microorganisms.

The uptake and biotransformation of trichloroethylene (TCE) by hybrid poplars has been studied. TCE was taken up and transformed by hybrid poplars without microbial metabolism (Newman et al., 1997). Hybrid poplars have also been shown to remove, hydrolyze, and dealkylate atrazine from soil; atrazine metabolism occurred in roots, stem, and leaves (Burken and Schnoor, 1997).

A study of the hairy root cultures of several aquatic plants was undertaken to evaluate the ability of these plants to transform 2,4,6-trinitrotoluene (TNT). Several of the plants studied were able to transform TNT and subsequent uptake of

transformation products was observed (Hughes et al., 1997). One researcher traced a nitroreductase enzyme as one of several enzymes contributing to the breakdown of TNT. Results demonstrated that the rate of TNT breakdown was proportional to the amount of nitroreductase in the plant (Watanabe, 1997).

Recently, the herbicide simazine was shown to be taken up from an aqueous nutrient media by *Typha latifolia*, or cattail (Wilson et al., 2000). Simazine was detected primarily in the leaves and uptake was correlated with water uptake. These results suggested that cattail may be a good candidate for use in phytoremediation.

The literature reviewed suggests that constructed wetlands are able to treat a variety of wastewater and runoff pollutants. The rhizosphere and associated microorganisms appear to play an important role in treatment processes, but factors that influence the metabolism of xenobiotics by rhizosphere microorganisms are not well understood. Results vary from plant to plant, soil to soil, and from laboratory to laboratory.

SEDIMENT SORPTION

Sediment sorption and subsequent pollutant degradation may play a significant role in dissipation of pollutants from the water column of constructed wetlands, especially for hydrophobic compounds. Studies on adsorption and desorption of pollutants fom agricultural soils may not describe their fate in wetland sediment. In contrast to agricultural soil, wetland sediment is characterized by high organic matter content and a narrow pH range (6.5-7.5) (Mersie and Seybold, 1996). For certain pollutants, especially herbicides, soil organic carbon is

the dominant property influencing the degree of sorption (Green and Karickhoff, 1990). Following pollutant sorption to wetland sediment, biotic or abiotic degradation, desorption, or sequestration (irreversible sorption) may occur (White et al., 1999).

INTENSIVE AGRICULTURE AND WASTEWATER TREATMENT

The nursery industry, an example of intensive agriculture, is one of the fastest growing segments of U.S. agriculture (Berghage et al., 1999). This industry uses insecticides, fungicides, growth regulators, and fertilizers to aid production and high intensity irrigation practices may create large volumes of wastewater. Traditional overhead hand or sprinkler irrigation has been reported to generate 18,000 to 90,000 L of wastewater per hectare daily (Aldrich and Bartok, 1994). Some states require greenhouse and nursery growers to treat and/or recycle wastewater. Regulations such as these are leading to the increased use of water treatment and water recycling systems (Berghage, 1995).

ATRAZINE BACKGROUND INFORMATION AND ENVIRONMENTAL FATE

Atrazine (2-chloro-4-ethylamino-6-ispropylamino-1,3,5-triazine) is a selective triazine herbicide (Figure I-1) used to control broadleaf and grassy weeds in a variety of agricultural commodities with an annual usage of 75 to 85 million pounds in the United States (Aspelin and Grube, 1999).

Agricultural runoff may be a significant source of pesticide contamination in watersheds. Losses of 0.1% to 4% of atrazine applied to agricultural fields have

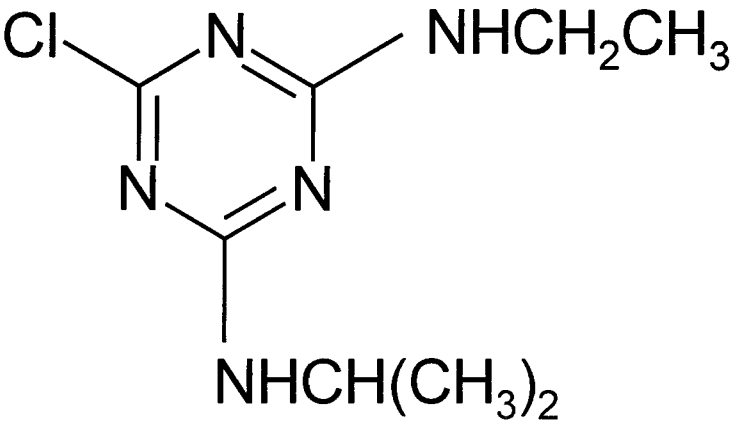

Figure I-1. Structure of atrazine (2-chloro-4-ethylamino-6-ispropylamino-1,3,5-triazine).

been reported, depending on soil type and intensity of the runoff event (Hall et al., 1972; Leonard et al., 1979; Jones et al., 1982; Glotfelty et al., 1984; Squillace and Thurman, 1992). The most important runoff events for triazine herbicides occur within two weeks of application and losses are dominated by movement in the water phase rather than in eroded soil (Glotfelty et al., 1984). Due to its frequent use in agriculture and its high frequency of detection in surface water, atrazine is a model compound to study the efficacy of constructed wetlands as a treatment system for pesticide-contaminated water.

Atrazine is moderately water-soluble and moderately to highly mobile in soils (Tables I-1 and I-2), especially those with low clay or organic matter content (Hornsby et al., 1996; Ma and Selim, 1996). Reported atrazine half-lives in water are variable and range from several days to several months (Glotfelty et al., 1984; Jones et al., 1982; Hamilton et al., 1989). Atrazine has a low K_{oc} value which accounts for its inability to strongly absorb to soil (Hornsby et al., 1996) and it is subsequently susceptible to leaching and runoff.

Atrazine biodegradation in the aqueous environment proceeds slowly (Grover and Cessna, 1991). Dissipation in water under aerobic or low oxygen conditions is attributed to sediment sorption and degradation to hydroxyatrazine and dealkylated products (Jones et al., 1982). Several studies conducted under laboratory conditions have reported atrazine biodegradation by microbial consortiums from liquid media and water samples (Assaf and Turco, 1994; Goux et al., 1998; Mirgain et al., 1995).

Table I-1. Physical and chemical properties of atrazine.

Physical/Chemical Properties[a]	Atrazine
Vapor Pressure (mmHg)	3.00×10^{-7}
K_{oc}	38-1686[b]
Water Solubility (mg/L) at 20°C	30-70
$T_{1/2}$ (days)	18-120

[a]Hornsby et al. 1996; Seybold and Mersie, 1996; Mersie and Seybold, 1996; Roy and Krapac, 1994.
[b]In general, atrazine's K_{oc} does not exceed 200.

Table I-2. Description of the ranking system for physical and chemical properties of compounds.

	RANKING[a]		
	High	Moderate	Low
Volatility (mmHg)	$>10^{-3}$	10^{-3} - 10^{-6}	$<10^{-6}$
Water Solubility (mg/L)	>100	100-10	<10
K_{oc}	>1000	500-1000	<500
$T_{1/2}$	>100 days	30-100 days	<30 days

[a]Jenkins and Thomson, 1999.

Atrazine can be degraded by either biotic or abiotic processes in soil and sediment (Goswami and Green, 1971). Chemical hydrolysis and subsequent degradation by soil microorganisms are believed to account for most of atrazine's breakdown in soil (Jones et al. 1982; Goswami and Green, 1971; Grover and Cessna, 1991). Dechlorination often occurs by a soil-catalyzed chemical hydrolysis process (Armstrong et al., 1967) but may also be microbially-mediated, leading to the production of hydroxyatrazine (HA), the primary hydrolytic product (Behki and Khan, 1986). This process is catalyzed by the soil surface and it is favored by acidic or basic environments, soils high in organic matter, low moisture content, and by high temperature (Burkhard and Guth, 1981; Gamble and Khan, 1988). Hydrolysis rates may increase in sterile sediment or in the presence of dissolved or humic or fulvic acids (Grover and Cessna, 1991).

Studies have reported atrazine degradation by bacteria belonging to *Nocardia* (Giardina et al., 1980), *Pseudomonas* (Behki and Khan, 1986), and *Rhodococcus* species (Behki and Khan 1994); however the degradation products vary and are dependant on the growth stage of the microorganism (Behki and Khan, 1994; Levanon, 1993). Mineralization of the side chains has been attributed to fungi; bacteria are responsible for breaking the heterocyclic ring (Levanon, 1993; Mandelbaum et al., 1993a; Radosevich et al., 1995), and both anaerobic and aerobic soil metabolism may occur (Mandelbaum et al., 1993a; Radosevich et al., 1995).

Atrazine N-dealkylation produces the two major microbial dealkylation products in soil, deisopropylatrazine (DIA) and deethylatrazine (DEA) (Armstrong et al., 1967; Ma and Selim, 1996) and deethylation is preferred over deisopropylation (Adams and Thurman, 1991; Durand and Barcelo, 1992). Following transformation to one of these primary metabolites, further degradation may occur yielding dealkylated and/or di-dealkylated hydroxyatrazine (HA) products. Complete oxidation of the triazine ring results in formation of cyanuric acid and complete mineralization yields ammonia and carbon dioxide (Cook, 1987).

Atrazine exhibits low volatility (Tables I-1 and I-2) and a low Henry's constant. Under normal field conditions volatilization of atrazine from water should not contribute significantly to loss from aqueous systems (Grover and Cessna, 1991). Atrazine is readily taken up by plant roots from nutrient solutions and distributed throughout plants (Raveton et al., 1997; Audus, 1976). In poplar trees it is taken up and translocated to leaves without resulting in phytotoxicity; this is presumably due to metabolism to a detoxified form (Burken and Schnoor, 1996). Atrazine is metabolized by plants to form HA derivatives, especially HA, as well as N-dealkylated metabolites, and peptide conjugates (Audus, 1976; Jensen et al., 1977). Complete oxidation to carbon dioxide or incorporation of one or more residues into tissue may also occur (Audus, 1976).

Under laboratory conditions atrazine is also subject to photodegradation by both direct and indirect photolysis (Pelizzetti et al., 1990; Torrents et al., 1997).

Weak absorption of radiation at wavelengths greater than 290 nm occurs and ultraviolet photolysis of aqueous solutions of atrazine yields HA analogs. In addition, sensitized photolysis may occur, resulting in the production of dealkylated and HA triazines (Grover and Cessna, 1991). Although atrazine photodegradation may occur, the environmental significance of this pathway remains questionable (Lerch et al., 1998).

Atrazine fate and transport in the Des Plaines wetlands and in the laboratory were recently investigated. Cattail (*Typha latifolia*), pond weed (*P. amphibium),* and white water lily (*N. tuberosa*) were present in the wetland. The wetlands delayed, reduced and spread out the peaks, removing 26 to 64% of the inflow atrazine concentration depending on residence time (Alvord and Kadlec, 1996).

McKinlay and Kasperek (1999) recently reported that analyses of water from a subsurface flow wetland microcosm containing marsh plants and 6 ppm of circulating water containing atrazine, reduced the atrazine concentration in water, reaching the limit of detection of the herbicide in 32 days. Upon repeated applications of atrazine, the rate of degradation, which was believed to occur by a microbially-based mechanism, increased.

Constructed wetland cells were recently used to evaluate treatment of atrazine in water from a simulated storm runoff event. Thirty-five days following the application, 34 to 70% of the application was transferred or transformed from the water column. In addition, atrazine was not detected in any plant or sediment samples collected on a weekly basis (Moore et al., 2000).

Atrazine may be adsorbed to soil by a variety of mechanisms including physical fixation, ionic or covalent bonding, van der Waals forces, and hydrophobic bonding (Barriuso et al., 1994; Wang et al., 1992; Weber, 1993) each of which is dependent on soil composition and pH. For example, atrazine adsorption is higher at lower soil pH and is at a maximum when the surface acidity is equal to the dissociation constant of atrazine (pKa = 1.68) (Kalouskova, 1989; Wang et al., 1992). Organic matter content has been shown to be positively correlated with atrazine's distribution coefficient (K_d) (Brouwer et al., 1990). Therefore, in order to normalize the distribution coefficient for comparison of studies, the organic carbon distribution coefficient (K_{oc}) is often reported instead of the K_d. Although the K_{oc}s for atrazine, HA, DEA, and DIA vary from study to study, their distribution coefficients follow the same general trend (HA>>atrazine>DIA>DEA) (Mersie and Seybold, 1996; Seybold and Mersie, 1996; Moreau and Mouvet, 1997). Results of these sorption and corresponding desorption studies indicate that HA has a very strong affinity for soil and its desorption is hysteretic. Although atrazine, DEA, and DIA also exhibit an affinity for soil, they are more likely to desorb from soil than HA (Mersie and Seybold, 1996; Seybold and Mersie, 1996; Moreau and Mouvet, 1997).

Laboratory soil incubation studies report that 20 to 70% of added ^{14}C-atrazine forms bound residues (Skipper et al., 1967; Gan et al., 1996; Miller et al., 1997; Capriel et al., 1985; Kruger et al., 1993), and hydroxyatrazine has often been

identified as the major degradation product in surface soils (Skipper et al., 1967; Winkelmann and Klaine, 1991; Sorenson et al., 1994; Gan et al., 1996; Miller et al., 1997).

Binding of atrazine and its metabolites to wetland sediment may also be an important mechanism of loss from the water column. In one study of atrazine edge-of-field runoff into an estuary, bottom sediments did not reveal detectable atrazine residues, but metabolite analysis was not performed (Glotfelty et al., 1984). Huckins et al. (1986) studied atrazine fate in 0.5 to 1 L wetland microcosms simulating prairie wetland potholes and found that at the end of a six week study approximately 40% of total atrazine detected was present in the sediment. Chung et al. (1996) also observed atrazine and HA in wetland sediment from a system without wetland plants. These studies provide evidence of the importance that wetland sediment plays as a compartment for accumulation of atrazine residues.

In summary, the main pathways for atrazine degradation in water and soil compartments are as follows: chemical or microbial hydrolysis, followed by microbial degradation in sediments and sorption to sediments. The major metabolites in soil and water are deethylatrazine and hydroxyatrazine, respectively.

RESEARCH OBJECTIVES

The objectives of this research included the following. First, an evaluation of treatment of atrazine contaminated water by a field-scale constructed wetland was performed. The test site was a constructed wetland, located near Portland, Oregon. This evaluation was conducted through simulated atrazine runoff

experiments and subsequent monitoring of wetland water. Second, in order to better understand mechanisms of treatment under laboratory controlled conditions, characterization of the fate of atrazine in the water and sediment of static wetland microcosms containing *Typha latifolia* or cattail, was performed. In addition, the presence of atrazine-degrading microorganisms was investigated to enhance understanding of atrazine's fate in these microcosms. These two objectives require the use of sensitive, robust analytical technology that enable the determination of environmental levels of atrazine and its metabolites. Finally, an investigation into enhancing microbial degradation of atrazine in wetland sediment was conducted. These experiments required the use of microbiological methodology and included the addition of organic amendments to wetland soil and bioaugmentation with soil containing a large population of atrazine-degrading microorganisms.

CHAPTER II

TREATMENT OF ATRAZINE IN NURSERY IRRIGATION RUNOFF BY A CONSTRUCTED WETLAND

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ABSTRACT

To investigate the treatment capability of a surface flow wetland at a container nursery near Portland, Oregon, atrazine was introduced during simulated runoff events. Treatment was evaluated as the percent atrazine recovered in the water column at the wetland's outlet. For the first five experiments, the percent atrazine recovered at the outlet of the constructed wetland during a 7-d period ranged from 18-24% in 1998 and 16-17% in 1999. Changes in total flow or frequency and intensity of runoff events did not affect treatment. During the sixth experiment, when runoff events were longer in duration than previous experiments, treatment was compromised. During experiments 3 and 6, deethylatrazine (DEA) and deisopropylatrazine (DIA) accounted for 13-21% of the initial application, but hydroxyatrazine (HA) was rarely detected in the water. Organic carbon adsorption coefficients (k_{oc}) were determined from batch equilibrium sorption isotherms with wetland sediment and they decreased in the order of HA>DIA>atrazine>DEA. Static water-sediment column experiments indicated that sorption is an important mechanism for atrazine loss from water passing through the constructed wetland. The presence of a large population of atrazine-degrading organisms was not observed in the wetland sediment, suggesting that microbial degradation of atrazine in the constructed wetland was inconsequential.

INTRODUCTION

There is growing concern that pesticide use practices in agricultural and urban areas will result in adverse impacts on ground and surface water. Transport of soil-applied pesticides to water bodies is primarily through surface transport in runoff water. In order to assess the extent of pesticide contamination in the nation's major hydrologic basins, the National Water Quality Assessment Program (NAWQA) was developed. From 1992 to 1996, seventy-six pesticides and seven pesticide degradation products in 8,200 samples of ground and surface water were analyzed. More than 95% of all samples collected from streams and rivers contained at least one pesticide, and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and its degradation product deethylatrazine (DEA) were among the most frequently detected pesticides in agricultural areas (U.S. Geological Survey, 1998).

The nursery industry, an example of intensive agriculture, is one of the fastest growing segments of U.S. agriculture (Berghage et al., 1999). This industry uses insecticides, fungicides, growth regulators, and fertilizers to aid production. In addition, high intensity irrigation practices may create large volumes of wastewater. Traditional overhead hand or sprinkler irrigation may generate 18,000 to 90,000 L of wastewater per hectare daily (Aldrich and Bartok, 1994). Some states require greenhouse and nursery growers to treat and/or recycle wastewater. Regulations

such as these are leading to the increased use of water treatment and water recycling systems (Berghage, 1995).

The most important runoff events for triazine herbicides occur within two weeks of application and losses are dominated by movement in the water phase rather than in eroded soil (Glotfelty et al., 1984). Losses of 0.1% to 4% of atrazine applied to agricultural fields have been reported, depending on soil type and intensity of the runoff event (Hall et al., 1972; Leonard et al., 1979; Jones et al., 1982; Glotfelty et al., 1984; Squillace and Thurman, 1992). The first 65% of a runoff event contains 90% of atrazine discharged (Klaine et al., 1988) and concentrations of 0 to 2500 μg/L atrazine have been reported during storm runoff events (Hall et al., 1974; Triplett et al., 1978; Jones et al., 1982; Klaine et al., 1988; Schottler et al., 1994; Solomon et al., 1996).

Constructed wetlands are commonly used in the treatment of agricultural, municipal, industrial, and stormwater waste. Typically, wetlands have been used to treat water contaminated with phosphorus, nitrogen, hydrocarbons, animal waste, and heavy metals from the aforementioned wastes. There has been little evaluation of their treatment of pesticides (Watanabe 1997) due in part, to the fact that these organic compounds and their transformation products are difficult to analyze (Watanabe 1997; Thurman and Meyer 1996).

The main pathways for atrazine degradation in water and soil compartments are as follows: chemical or microbial hydrolysis, followed by microbial degradation in sediment (Jones et al., 1982) and sorption to sediment. The major

metabolites in soil and water are deethylatrazine (DEA) and hydroxyatrazine (HA), respectively. Atrazine half-lives in water are variable and range from several days to several months (Glotfelty et al., 1984; Jones et al., 1982; Hornsby et al., 1996). Atrazine is moderately water soluble (33 ppm) and exhibits low volatility (approximately 10^{-7} mm Hg), which gives a low Henry's law constant (approximately 10^{-7}) (Hornsby et al., 1996). Direct and indirect atrazine photolysis result in the production of N-dealkylated and hydroxy analogs (Grover and Cessna, 1991).

Due to its frequent use in agriculture and its frequent detection in surface water, atrazine was chosen as a model compound to study the treatment capability of a surface flow wetland used to remediate irrigation runoff at a container nursery near Portland, Oregon. Atrazine was introduced during simulated runoff events and treatment was evaluated by the percent atrazine recovered at the wetland's outlet. In order to investigate the contribution of atrazine sediment sorption to treatment of irrigation water, two types of sorption experiments were conducted; batch equilibrium and static water-sediment column experiments. Atrazine-degrading microorganisms in wetland sediment were enumerated using most probable number (MPN) assays to determine the contribution of microbial degradation to atrazine treatment in the constructed wetland.

MATERIALS AND METHODS

Chemicals

Analytical standards of atrazine, DEA, deisopropylatrazine (DIA), and HA were ≥ 95% pure (Chem Service, West Chester, PA). The following radiolabeled compound was donated by Novartis (Greensboro, NC): [U-ethyl-^{14}C]atrazine (98.5% radiochemical purity). Internal standards were as follows: phenanthrene-d_{10} (Pd_{10}) (98% pure, Chem Service, West Chester, PA) and terbuthylazine-2-hydroxy (HT) (95% pure, Crescent Chemical, Hauppauge, NY) for gas chromatography/mass spectrometry (GC/MS) and high-pressure liquid chromatography (HPLC) analyses, respectively. Stock solutions of HA were made in 0.1 N reagent grade HCl, and working standards were prepared in 2:3 CH_3OH:5 mM KH_2PO_4 (pH 7.5) (v/v) (40% CH_3OH) at concentrations of 100 µg/L to 5000 µg/L. Atrazine, DEA, and DIA stock solutions were prepared in ethyl acetate (EA), and working standards were prepared in EA at concentrations of 63 µg/L to 5000 µg/L. Pd_{10} was prepared in CH_3OH, and HT was prepared in 0.1 N HCl. All solvents and KH_2PO_4 were HPLC or GC grade (Fisher Scientific, Pittsburgh, PA). The KH_2PO_4 solutions were adjusted to the appropriate pH using reagent-grade NaOH (50% w/v solution).

Extraction and QC Procedures

Five hundred ml water samples (containing 1% CH_3OH) for atrazine extraction were filtered through 90-mm GF/F filters (Whatman, Clifton, NJ). Forty-

seven mm C_{18} Empore disks (Varian, Sugarland, TX) connected to solid-phase extraction (SPE) glassware filtration equipment and a manifold (Kontes, Vineland, NJ), were conditioned with 10 ml of ethyl acetate (EA), 10 ml of methanol (CH_3OH), and 10 ml of deionized water (DI). Water samples were passed through the disks which were subsequently dried under vacuum for 15 to 30 min and eluted with 7 ml EA into 15 ml glass test tubes. Pd_{10} was added and EA was evaporated under a stream of nitrogen and a water bath heated to 40 °C using an N-evap sample concentrator (Organomation, Berlin, MA) to a final volume of 1 ml or 200 µl followed by GC/MS analysis. The limit of quantitation (LOQ) and limit of detection (LOD) for atrazine were 50 and 5 ng/L, respectively.

DEA, DIA, and HA water extraction and analysis protocols were previously described (Runes et al., in press). Briefly, extractions were performed using C_{18} SPE cartridges (500 mg, 6 ml polypropylene reservoir; Varian, Sunnyvale, CA) followed by GC/MS or HPLC analysis. Sample volumes were 100 ml for DEA and DIA, and 250 ml for HA. For 100-ml samples concentrated to 1 ml, the limits of quantitation (LOQ) were 1000, and 1000 ng/L, and the limits of detection (LOD) were 40, and 250 ng/L for DEA, and DIA, respectively. The LOQ and LOD for HA were 2.0 and 0.6 µg/L, respectively.

Field Experiments (1998 and 1999)

Field experiments were conducted at a constructed wetland consisting of five sequential cells approximately 3 m × 40 m (Figure II-1). The elevation drop between cells 1 and 5 was approximately 1 m. The constructed wetland is located at

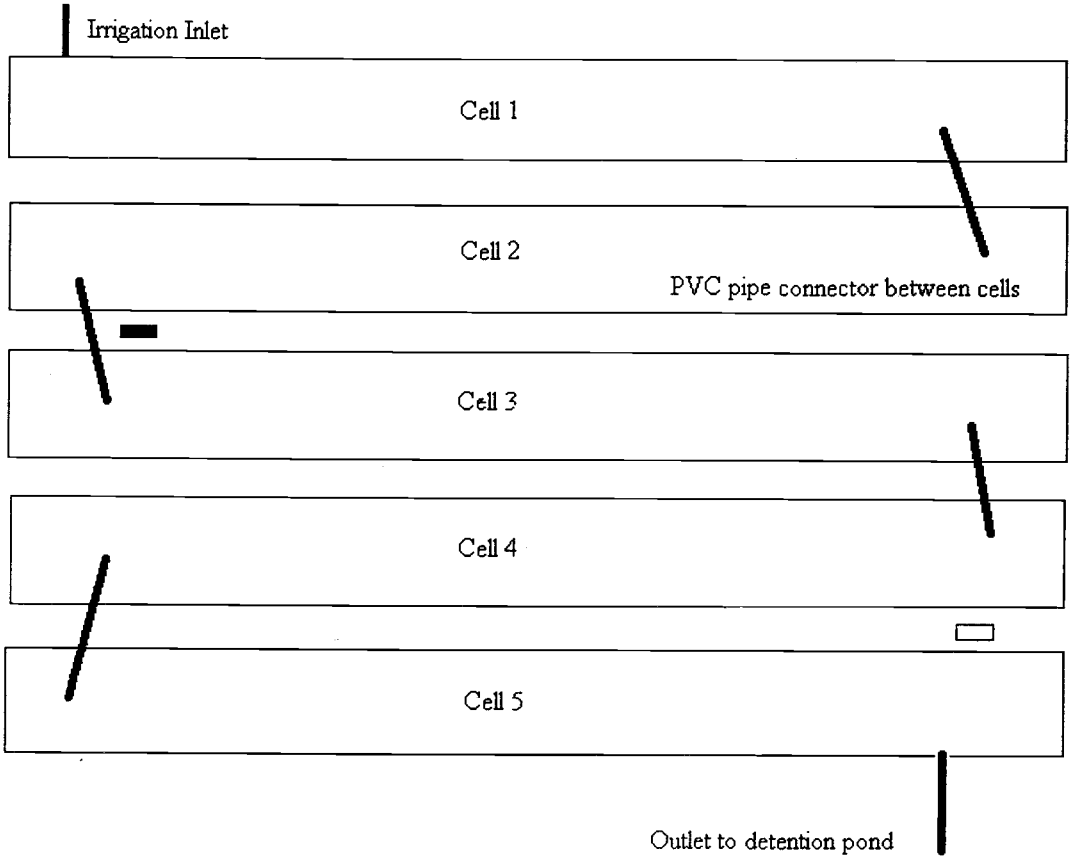


Figure II-1. Schematic of the constructed wetland at the field site. Each cell is approximately 3 m × 40 m. Weather station placement during 1998 (solid rectangle) and 1999 (open rectangle) were different.

a container nursery in the Tualatin watershed near Portland, Oregon, and it receives runoff from 2.4 ha. Irrigation can occur both above cell 1 and spanning the entire right side of cells 1 through 5. For purposes of this research, the wetland was designed so that water entered cell 1 at a single location. Additional runoff water was diverted around the perimeter of the wetland. Experiments were conducted under different flow conditions determined by the amount, frequency, and duration of irrigation events necessary to meet water requirements of nursery plants.

In 1996, each cell was planted with a variety of wetland plants with the predominant species *Typha latifolia*, or cattail. Subsequent plantings were conducted in 1998 and 1999 with *Typha latifolia.*

Field experiments occurred from July through September 1998 (experiments 1 to 3) and 1999 (experiments 4 to 6). For each experiment 6 g (active ingredient (a.i.)) Aatrex (atrazine wettable powder formulation) was transported to the field where it was added to a glass container fitted with a recirculating pump for mixing. Approximately 15.1 L of tap water was added to the container, yielding a concentration of 400 mg/L atrazine. To approximate 2% runoff of the recommended application rate (1100 g/ha a.i.), the solution was metered into the inlet of cell 1 of the constructed wetland at 100 ml/min for approximately 2.5 h during a runoff event at the nursery. Atrazine application was timed to occur either at the peak (high flow) or tail (low flow) of the runoff event. Beginning with experiment 5, a runoff bypass was engineered to divert irrigation runoff around the constructed wetland to reduce flow during high intensity runoff events.

Discrete (1998) or composite (1999) 500 ml water samples were collected at the outlets of cells 1, 2, and 5 every hour during application, every two hours through day 5, and every three hours through day seven. Composite samples consisted of four 125 ml samples taken at 15 min intervals during atrazine application, five 100 ml samples taken at 24 min intervals through day 5, and five 100 ml samples taken at 40 min intervals thereafter. Water samples were collected using Isco Model 2900 samplers (Isco, Lincoln, NE) containing 24, 500 ml plastic sample bottles. Sample bottles were collected every 2 to 3 d and water samples were immediately transferred to 500 ml polypropylene Nalgene bottles (Rochester, NY) and stored at 4 °C until extraction. Twenty percent of samples were matrix spikes (blank matrix spiked with atrazine). Field spikes (DI spiked with atrazine) were transported to the field site with each Isco sampler and collected every 2 to 3 d. Treatment efficiency was evaluated as atrazine recovery, defined as the mass of atrazine recovered during 7 d divided by the mass of atrazine applied. Atrazine recovery was calculated by multiplying each sample concentration by flow by the corresponding period of time (2 to 3 h).

During 1998, flow data was collected using a Stevens Type F Water Level Recorder (Ben Meadows Company, Canton, GA) placed at the outlet of cell 2. Equations used to calculate flow for the 90° V-notch weir were the following:

$$(1)\ Q_w = 1470h^{2.47}$$

where, Q_w is weir flow in liters per second, and h is the head above the bottom of the V-notch in meters (King et al., 1948).

$$(2)\ Q_w = CLH^{3/2}$$

where, Q_w is weir flow in liters per second, C is the weir coefficient = 3.1, L is the weir length in meters (circumference of the pipe), and H is the head above the top of the pipe in meters (King et al., 1948).

$$(3)\ Q_w = 24.5h_t^{0.483}$$

where, Q_w is weir flow in liters per second, and h_t is the depth above the top of the pipe in meters (Humphreys et al., 1970). When head was 0 to 0.102 m, equation (1) was used. For heads from 0.102 to 0.144 m, equations (1) and (2) were used, and for heads from 0.144 to 0.25 m, equations (1), (2), and (3) were used.

During 1999, additional flow data were collected using a Campbell Scientific (Logan, UT) weather station equipped with a CR10 measurement and control module and two Druck model 1830 pressure transducers (New Fairfield, CT). The pressure transducers were placed just above the sediment surface near the outlet of cells 1 and 5. Figures II-2a to c show flow data collected from cells 1 and 5 during experiments 4 through 6. The flow data were recorded as head, and subsequently converted to flow using standard weir and orifice equations (King et al., 1948). The equations were the following:

$$(4)\ Q_w = CLH^{3/2} \text{ (see above) and,}$$

$$(5)\ Q_0 = ca(2gh)^{1/2}$$

where, Q_0 is orifice flow in liters per second, c is orifice coefficient = 0.6, a is area of the riser inlet in square meters and, h is head above the riser inlet in meters. The

equations can be further modified because $L = \pi D$, and $a = (\pi D^2)/4$, for round pipes. The resulting equations are as follows:

$$(6)\ Q_w = 9.73DH^{3/2}$$

$$(7)\ Q_0 = 3.78D^2h^{1/2}$$

The weather station also collected ambient temperature, and solar radiation data at one h (1998) or 10 min (1999) intervals during experiments. One-hundred ml water samples were collected from cells 1, 2, and 5 prior to beginning the experiment and on day 7 of the experiment for analysis of nitrate, ammonium, and ortho phosphate concentrations. Other parameters monitored included pH and water temperature (Orion, Beverly, MA), oxidizing-reducing potential (Sensorex, Stanton, CA), and dissolved oxygen (Hanna Instruments, Bedforshire, UK).

Batch Equilibrium Sorption Experiments

Sorption isotherms for atrazine, DEA, DIA, and HA on a composite sample of wetland sediment cores (18 mm in diameter taken to a depth of 5 cm) were determined using the batch equilibration technique (Mersie and Seybold, 1996; U.S. Environmental Protection Agency, 1998). Wetland conditions were maintained by using fresh samples without drying except where noted. Sediment was not sieved prior to use except as noted where it was air-dried overnight and sieved to <2mm. Atrazine, DEA, DIA, and HA solutions were prepared in 0.01 M $CaCl_2$ from approximately 0.1 to 1.0 mg/L and a 10 ml aliquot of each solution was added to 1 g of sediment (dry-weight equivalent) in a 50 ml centrifuge tube. Each concentration was replicated three times and an analytical control sample for each

Figure II-2. Flow and atrazine concentration in the water column of cells 1 and 5 during experiments four (a), five (b), and six (c).

Figures II-2a and II-2b.

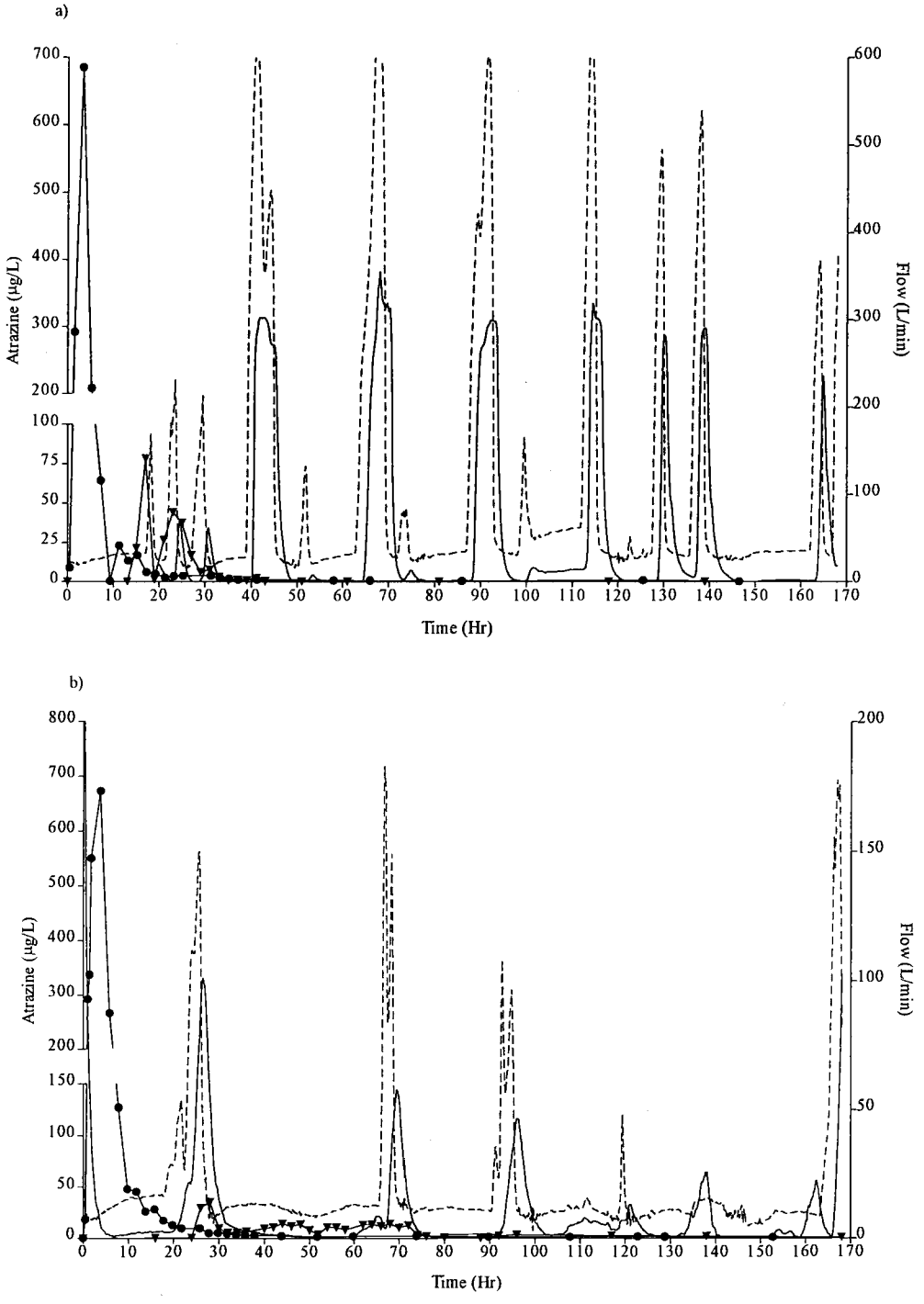
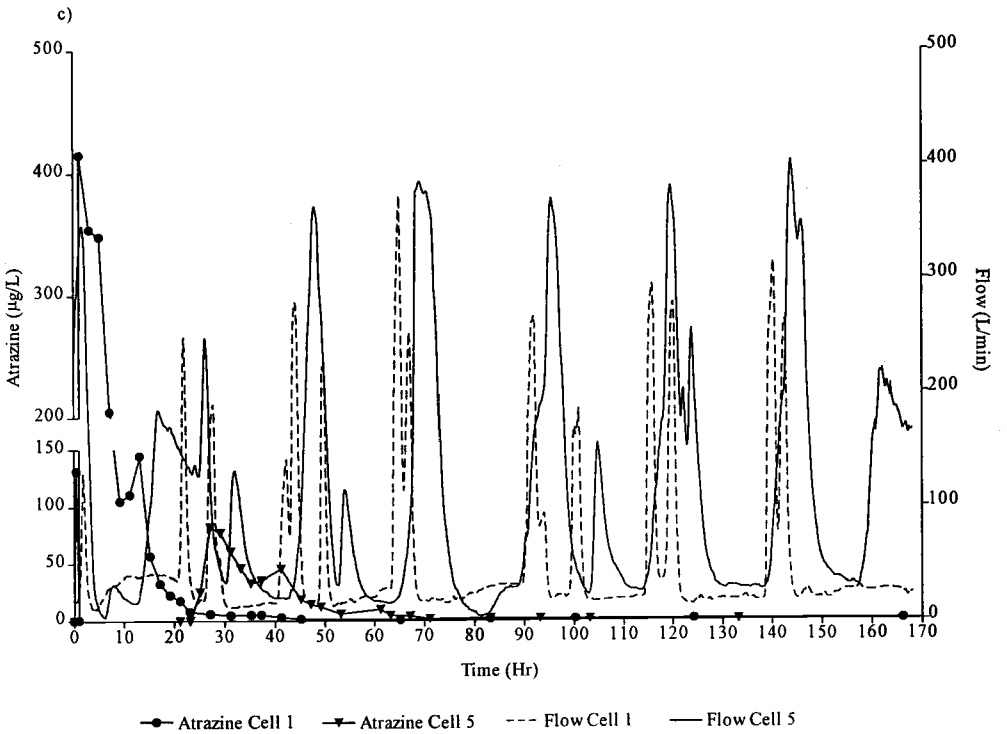


Figure II-2c.


c)
Atrazine (µg/L)
Flow (L/min)
Time (Hr)
Atrazine Cell 1
Atrazine Cell 5
Flow Cell 1
Flow Cell 5

concentration and two $CaCl_2$ blanks were set up. The samples were sealed with Teflon caps and shaken for 24 h on a wrist-action shaker. They were subsequently centrifuged for 15 min at 3000 rpm. The supernatant was collected and analyzed for atrazine, DEA, DIA, or HA by GC/MS or HPLC as previously described (Runes et al., in press). The amount of chemical adsorbed was calculated as the difference between the supernatant concentration and the amount of chemical initially added (determined from the analytical control sample). Adsorption isotherms were calculated using the linearized form of the Freundlich equation:

$$(8)\ \log (x/m) = \log K_f + (1/n) \log C$$

where x/m is the micrograms of atrazine or degradate adsorbed per gram of soil, C is the micrograms of atrazine or degradate per liter of supernatant after equilibrium, and K_f and 1/n are empirical constants. Adsorption distribution coefficients (K_d), calculated by dividing the adsorbed concentration by the solution concentration: $K_d = (x/m)/C$, were determined at each concentration and were averaged across all equilibrium concentrations to obtain a single estimate. The organic carbon adsorption distribution coefficients (K_{oc}) were calculated using the following equation:

(9) $K_{oc} = (K_d \times 100)/ \%OC$, where %OC is percent organic carbon, or 2.13%.

Static Water-Sediment Column Sorption Experiments

In order to better understand the role that sorption to wetland sediment played in atrazine dissipation from the water column of the constructed wetland,

static water-sediment column sorption experiments were designed to simulate wetland conditions. For each of the two experiments, six sediment columns were constructed consisting of three replicate test columns, two sediment blank columns, and one analytical control column in 275 ml glass cylinders (Fisher Scientific, Pittsburgh, PA, USA) packed with 7.6 cm of unsieved wetland sediment (80 g dry weight). Twenty-five cm of 0.01 M $CaCl_2$ (220 ml), containing technical grade atrazine as the wettable powder formulation was added to each column. Column solutions were collected by siphoning them out of columns under vacuum into 1 L side-arm flasks. Samples were stored in 500 ml polypropylene Nalgene bottles at -20 °C until analysis. Two sorption experiments were designed to mimic atrazine concentrations observed in cells 1 and 5 during field experiments. Therefore, each replicate column was sequentially exposed to different atrazine concentrations for various periods of time, and sorption was evaluated (Table II-1).

MPN Assays

Populations of atrazine-degrading microorganisms were enumerated using MPN assays conducted with ^{14}C-ethyl atrazine as previously described (Runes et al., in press). MPN assays were conducted during 1998 using rhizosphere and non-rhizosphere sediment cores taken from the constructed wetland prior to conducting field experiments and following completion of 1998 field experiments. An MPN dilution series was scored positive if mean cumulative counts were greater than the mean and three standard deviations of the sterile soil and mineral salts medium control counts. Atrazine degrading populations were enumerated using the Most

Table II-1. Experimental design for sediment column sorption experiments mimicking cell 1 and cell 5 field results.

Cell 1 Experiment		Cell 5 Experiment	
Exposure Time (h)	Starting Concentration (μg/L)	Exposure Time (h)	Starting Concentration (μg/L)
0-2	200	0-14	0
2-7	1000	14-16	50
7-9	200	16-24	100
9-24	20	24-48	10
24-48	0	48-72	0
48-72	0	72-96	0
72-96	0	96-120	0
96-120	0	120-144	0
120-144	0		

Probable Number Calculator, Version 2.80, program for PCs (U.S. Environmental Protection Agency, Cincinnati, OH).

RESULTS

Water Quality Parameters

A summary of weather station measurements and water quality parameters (cells 1, 2, and 5) during 1998 and 1999 experiments is presented in Table II-2. No rainfall occurred during the experiments. Nutrient values were variable, presumably due to intermittent fertilization at the nursery.

1998 Field Experiments

Three experiments (experiments 1 to 3) were conducted during 1998. Flow events were ranked by intensity (low <250 L/min, medium 250 to 500 L/min, high >500 L/min) and frequency of the events (low <6 events/experiment, medium 6 to 9 events/experiment, high >9 events/experiment) for all experiments. During the first two experiments, atrazine was applied during the peak (high flow) of runoff events. Subsequent flow was characterized by high frequency and low to medium intensity runoff events (Table II-3). During the third experiment, atrazine was applied during the tail (low flow) of the runoff event. Runoff events occurred at medium frequency and were characterized by high intensity. The peak cell 5 atrazine concentration was reached 20 to 26 h following application, and was followed by a rapid decline in which low concentrations persisted for several days

Table II-2. Weather and water quality measurements for 1998 and 1999 field experiments.

	1998 Experiments (Min – Max[†])	1999 Experiments (Min - Max)
Ambient Temperature (°C)	5.65 – 38.3	8.53 – 48.4
Water Temperature (°C)	16.4 – 30.2	14.4 – 30.6
pH	5.8 – 7.9	6.21 – 6.88
Dissolved Oxygen (mg/L)	4.8 – 15.8	5.9 – 12.4
Oxidizing-Reducing Potential (mV)	53 – 403	53.5 – 91.3
Nitrate (mg/L)	7.6 – 17.4	0.06 – 58.3
Ammonium (mg/L)	0.06 – 7.88	0.27 – 21.6
Ortho Phosphate (mg/L)	0.04 – 44.6	0.69 – 6.46

[†]Minimum – Maximum

Table II-3. Description of flow events and atrazine percent recovery for cells 1, 2, and 5 for 1998 experiments.

Experiment	Runoff Frequency	Runoff Intensity	Atrazine Percent Recovery		
			Cell 1	Cell 2	Cell 5
1	High	Low	97	20	18
2	High	Medium	9.4	31	24
3	Medium	High	1.3	6.4	20

for all three experiments. Percent atrazine recovered from cells 1, 2, and 5, based on flow data from a single location at the outlet of cell 2 is reported in Table II-3. Atrazine recovery from cells 1 and 2 varied widely possibly as a result of using discrete sampling which may have missed peak atrazine concentrations, thus introducing error. Additional error may have been introduced by calculating recovery for cells 1 and 5 using cell 2 flow data. Atrazine recovered from cell 5 ranged from 18-24%. Field and matrix spike recoveries ranged from 82 to 105%. Atrazine concentrations were not corrected for spike recoveries. Atrazine application to the wetland at the tail (low flow) of an irrigation event (experiment 3) did not greatly reduce atrazine recovery from cell 5. However, subsequent runoff events, which were high intensity, may have affected treatment.

Samples from cell 5 only were analyzed for degradates during experiment 3 and degradate analyses were performed with less frequency than atrazine. Because degradate concentration was constant for samples analyzed, the mean concentration for samples was used to calculate recovery. An additional 8% and 5% of the atrazine applied during the third experiment was detected as DEA and DIA, respectively. HA was detected at the limit of detection in several samples. For the third experiment, overall percent atrazine recovered from the wetland outlet, including degradates was 33%.

1999 Field Experiments

Three experiments (experiments 4 to 6) were conducted during 1999. The fourth experiment was initiated during high flow and the frequency and intensity of

subsequent runoff events were high (Table II-4). The fifth experiment was conducted during low flow and was followed by moderately frequent, low intensity runoff events. The sixth experiment was initiated during low flow and was characterized by high frequency and moderate intensity runoff events as shown in Figure II-2c. In addition, runoff events were longer in duration than previously observed. Compared to experiment 4, lower total flow (cell 1) was observed in experiments 5 and 6 due to the installation of a runoff bypass above the inlet to cell 1.

Atrazine concentrations in cells 1 and 5, together with flow data for experiments 4 through 6 are shown in Figures II-2a to c. Atrazine recovery from cells 1 and 2 was less variable than during 1998 possibly due to composite sampling and calculation of recovery values using flow data from each cell (cells 1, 2, and 5). As previously observed, the peak atrazine concentration in cell 5 (reached in 17 to 28 hr) was followed by rapidly decreasing concentrations and low-level atrazine detections for several days. Percent atrazine recovered from cells 1, 2, and 5 is reported in Table II-4. Atrazine recovered from cell 5 for experiments 5 and 6 was 17% and 16%, respectively. Field and matrix spike recoveries ranged from 76 to 116%. Atrazine concentrations were not corrected for spike recoveries. During the sixth experiment 106% of applied atrazine was recovered in 7 d. This is most likely a result of the frequent runoff events due to warm ambient temperatures. These runoff events were longer in duration than typically observed, resulting in increased flow. Flow events in cell 5 were broader and taller than in

Table II-4. Description of flow events and atrazine percent recovery for cells 1, 2, and 5 for 1999 experiments.

Experiment	Total Flow (L) [†]		Runoff Frequency	Runoff Intensity	Atrazine Percent Recovery		
	Cell 1	Cell 5			Cell 1	Cell 2	Cell 5
4	8.8×10^5	4.8×10^5	High	High	73	24	17
5	1.4×10^5	9.1×10^4	Medium	Low	45	52	16
6	5.0×10^5	1.1×10^6	High	Medium[‡]	106	70	106[§]

[†]Total flow was determined from the sum of the area under the curve for each runoff event.
[‡]Runoff events were longer in duration than for all other experiments.
[§]Cell 5 recovery may be overestimated due to suspected surface and subsurface flow entering above cell 5.

cell 1, possibly indicating surface or subsurface flow into the wetland at a point below cell 1 (Figure II-2c). The presence of surface or subsurface flow into the wetland is supported by peaks appearing in cell 5, but not cell 1(from 10 to 20 h and from 160 to 170 h). In addition, it is possible that some of the atrazine recovered was carry over (desorbed from sediment) from the previous two experiments. An additional 12% and 9% of the atrazine was recovered as DEA and DIA, respectively. HA was not detected in any samples analyzed. For the sixth experiment overall percent atrazine recovered from the wetland outlet, including degradates was 121%.

Batch Equilibrium Sorption Experiments

Batch equilibrium sorption experiments were conducted on atrazine, DEA, DIA, and HA using sieved and unsieved wetland sediment. Sorption isotherm constants (K_f and 1/n), K_d, and K_{oc} values are presented in Table II-5. Sorption isotherms calculated using the linearized form of the Freundlich equation reasonably described sorption (see r^2 values). The K_{oc} values decreased in the order of HA unsieved > atrazine sieved > DIA unsieved > atrazine unsieved > DEA unsieved. Results from the atrazine sorption experiments indicated that while drying and sieving sediment may create a more homogeneous sample useful in standard tests, it also affects sorption.

The slopes for sorption of atrazine, DEA, and HA in unsieved sediment which were <1, indicated that the percentage of these chemicals sorbed to wetland sediment decreased as the initial concentration increased. However, the percentage

Table II-5. Adsorption coefficients (K_f), slopes (1/n), adsorption distribution coefficients (K_d), and organic carbon partition coefficients (K_{oc}) for atrazine, DEA, DIA, and HA on wetland sediment.

	K_f (L/kg)	1/n	r^2	K_d[†] (L/kg)	K_{oc}[‡] (L/kg)
Atrazine Sieved[§]	1.14	1.25	0.98	8.79 (2.08)	413
Atrazine Unsieved[¶]	0.306	0.878	0.90	2.56 (0.927)	120
DEA Unsieved	0.161	0.956	0.96	1.53 (0.279)	72
DIA Unsieved	0.477	1.01	0.99	3.04 (0.274)	142
HA Unsieved	2.01	0.932	0.99	132 (16)	6197

[†]Numbers in parentheses are the standard deviations of the mean.
[‡]Percent organic matter was 2.13.
[§]Sediment was dried and sieved to <2mm.
[¶]Sediment was not sieved.

of atrazine sorbed to sieved sediment increased as the initial concentration increased (slope >1). Sorption of DIA in unsieved sediment was near unity.

Static Water-Sediment Column Sorption Experiments

Static water-sediment columns were employed to better understand the role sorption played in atrazine dissipation from the water column of the constructed wetland. The general pattern of atrazine concentration in water samples from cells 1 and 5 was used to design the experiment (Table II-1). Figure II-3 shows the mean atrazine concentration in the aqueous phase of static water-sediment columns following contact with the sediment for the first experiment, representing cell 1. Sorption to the sediment appeared to be greatest for the initial 2 h exposure to 200 μg/L atrazine. Some sorption was observed at the subsequent 5 h exposure to 1000 μg/L, and desorption was observed at 20 μg/L and during subsequent desorption periods with $CaCl_2$ solution. The percent of applied atrazine sorbed to the sediment was 89% during the first exposure period. The cumulative percent atrazine sorbed during the second exposure period was 49%, and by the end of the experiment 40% remained sorbed to sediment. No sorption to wetland sediment was observed at any concentration (10, 50, and 100 μg/L) over the time course of the second experiment, representing cell 5.

MPN Assays

MPN assays using ^{14}C-ethyl atrazine indicated the presence of a small community of degraders in non-rhizosphere and rhizosphere sediment at the

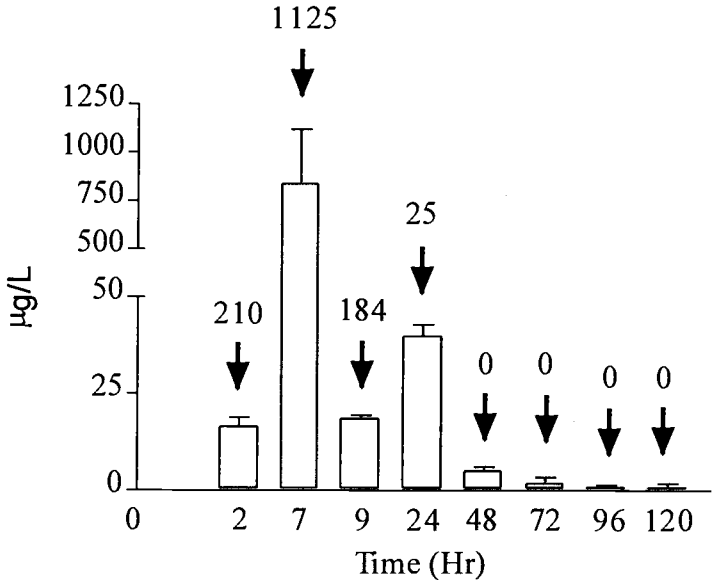


Figure II-3. Mean atrazine concentration in the aqueous phase of static water-sediment columns (n = 3, standard deviation) following contact with the sediment. The numbers above the bars represent atrazine concentration added to each static water-sediment column after removing the $CaCl_2$ solution from the previous exposure period.

beginning of 1998 (time 0) and following completion of 1998 field experiments. The mean number of atrazine-degrading organisms per gram of sediment was 83 cells/g (4-270 cells/g for 95% lower-upper confidence interval (c.i.)) for non-rhizosphere sediment at time 0, 128 cells/g (22-321 cells/g for 95% lower-upper c.i.) for rhizosphere sediment at time 0, 187 cells/g (3-1196 cells/g for 95% lower-upper c.i.) for non-rhizosphere sediment at the end of 1998, and 355 cells/g (34-1699 cells/g for 95% lower-upper c.i.) for rhizosphere sediment at the end of 1998. These numbers represent small population of atrazine-degrading organisms and exposure to atrazine in runoff did not substantially increase population size.

DISCUSSION

Field experiments 1 to 5 did not indicate an association between atrazine treatment efficiency and timing of application, total flow, or frequency and duration of subsequent runoff events. However, in experiment 6 where amount, frequency, and duration of runoff events exceeded all other experiments, treatment was compromised. Based on a comparison of flow in cells 1 and 5 (Figure II-2c), it appears that there was additional flow into the wetland above cell 5 either through surface or subsurface flow. If flow for cell 5 was the result of multiple sources, atrazine recovery would have been overestimated.

Because atrazine recovery data for 1998 was calculated using a single flow measurement at the outlet of cell 2, recovery may be overestimated as some water

loss due to evapotranspiration and seepage between cells 2 and 5 is expected. Additionally, discrete sampling has a greater likelihood of overestimating or underestimating atrazine concentration as compared to composite sampling in which sampling occurs more frequently.

Others have used wetlands to treat atrazine-contaminated water (McKinlay and Kasperek, 1999; Lee et al., 1995, Huckins et al., 1986; Jones et al., 1982). Atrazine in simulated storm runoff was removed during a 35-d period and residue was not detected in sediment or plant samples (Moore et al., 2000). In studies of a natural wetland, the degree of treatment depended on residence time and prior to peak atrazine arrival, river and wetland outflows had the same low concentrations suggesting little wetland function at low atrazine concentrations (Alvord and Kadlec, 1996). The treatment of atrazine in our constructed wetland was comparable or better than treatment reported for atrazine or contaminants (nitrogen, phosphorus, halogenated disinfection by products) in other constructed wetlands (Moore et al., 2000; Rostad et al., 2000; Kovacic et al., 2000; Huang et al., 2000; Stone and Legg, 1992).

To understand the importance of sediment sorption to atrazine dissipation from the water column of the constructed wetland, sorption was studied. For comparison with other literature values, batch equilibrium sorption was determined for atrazine using sieved sediment. The resulting K_{oc} value of 413 was more than three times the value reported for unsieved sediment, but comparable to reported values ranging from 38 to 1686 (Seybold and Mersie, 1996; Mersie and Seybold,

1996; Roy and Krapac, 1994; Hornsby et al., 1996). Reported K_{oc} values for atrazine and dealkylated degradates in sieved soil are also comparable to values (sieved sediment) reported here (Dousset et. al., 1994; Roy and Krapac, 1994; Seybold and Mersie, 1996; Mersie and Seybold, 1996; Moreau and Mouvet, 1997). However, the K_{oc} value of 6197 for HA in constructed wetland sediment was greater than most previous reports which range from 493 to 6584 (Moreau and Mouvet, 1997; Seybold and Mersie, 1996; Mersie and Seybold, 1996). Atrazine and degradate desorption isotherms were not characterized, but hysteresis has been observed for atrazine, DEA, DIA, and HA in both wetland sediment (Mersie and Seybold, 1996) and agricultural soil (Seybold and Mersie, 1996). In addition, binding of atrazine and degradates was less reversible in wetland sediment than in agricultural soil (Mersie and Seybold, 1996).

Although batch equilibrium soil/sediment sorption studies are used to study the relative potential for chemical sorption, these experiments are not designed to mimic experimental field conditions. In an attempt to estimate sorption under wetland field conditions, static water-sediment columns were exposed to atrazine at concentrations representative of those observed in cells 1 and 5 of the constructed wetland. For the experiment representing cell 1, sorption to the sediment was significant. Although some desorption did occur, the mass desorbed was less than one percent as a result of four, 24 h desorption events. The experiment representing cell 5 did not result in measurable sorption at the concentrations tested (10, 50, and 100 μg/L) indicating that there is a threshold concentration below which sorption is

not significant under experimental field conditions. Results of these static experiments provide a rough estimate of sorption under field conditions.

Dilution of atrazine in runoff may be significant once runoff exits the constructed wetland. However, because atrazine was detected in all samples at the outlet of cell 5 dilution does not appear to play a role in dissipation from the constructed wetland.

Based on results of MPN assays, it does not appear that the population of atrazine-degrading organisms is large in the constructed wetland. Therefore, the contribution of microbial degradation to treatment of atrazine in runoff is probably insignificant. However, the presence of dealkylated degradates suggests that some abiotic degradation occurs. Based on atrazine's physical-chemical properties, volatilization from the water column is assumed to be negligible.

During the spring of 1998 and 1999 additional plants were added to increase plant density. Although others have indicated that either the presence of, or type of wetland vegetation plays a role in atrazine degradation in wetlands (Lee et al., 1995; McKinlay and Kasperek, 1999) additional plants did not further increase treatment in this constructed wetland.

CONCLUSIONS

Results of this study indicated that this constructed wetland effectively reduced overall atrazine concentration in container nursery irrigation runoff. No

difference in treatment effect was observed for experiments 1 to 5, when the frequency and intensity of runoff events was varied or when total flow through the wetland was reduced. However, during experiment 6, when amount, frequency, and duration of runoff events exceeded all other experiments, treatment was compromised. For experiments 3 and 6 several atrazine degradates were observed in cell 5 water samples not exceeding 13% (1998) and 21% (1999) of the atrazine applied. Experiments conducted with wetland sediment columns indicated that sorption was a primary mechanism of atrazine removal from the water column during field experiments. MPN assays suggested that microbial degradation of atrazine in the constructed wetland was inconsequential.

CHAPTER III

ATRAZINE REMEDIATION IN WETLAND MICROCOSMS

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ABSTRACT

Laboratory wetland microcosms were used to study treatment of atrazine in irrigation runoff by a field-scale constructed wetland under controlled conditions. Three experiments, in which one ppm atrazine was added to the water column of three wetland, one soil control, and one water control microcosm, were conducted. Atrazine dissipation from the water column and degradate formation (deethylatrazine (DEA), deisopropylatrazine (DIA), hydroxyatrazine (HA)) were monitored. Atrazine dissipation from the water column of wetland microcosms was biphasic. Less than 12% of the atrazine applied to wetland microcosms remained in the water column on day 56. Atrazine degradates were observed in water and sediment, with HA the predominant degradate. Analysis of day 56 sediment samples indicated that a significant portion of the initial application was detected as the parent compound and HA. Most probable number (MPN) assays demonstrated that atrazine degrader populations were small in wetland sediment. Wetland microcosms were able to reduce atrazine concentration in the water column via sorption and degradation. Based on results from this study, it is hypothesized that plant uptake contributed to atrazine dissipation from the water column.

Keywords—Atrazine, Deethylatrazine, Deisopropylatrazine, Hydroxyatrazine, Wetland

INTRODUCTION

Pesticide use practices, both in agricultural and urban areas, has resulted in contamination of ground and surface water (Anderson et al., 1996; Anderson et al., 1997). In a recent USGS study (Phases I and II) of Willamette Basin (Oregon) surface water, 20 of 47 pesticides analyzed were detected more than once (Anderson et al., 1996). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) was detected most frequently at a median concentration of 0.052 μg/L (Anderson et al., 1996). During Phase III of this study (Anderson et al., 1997), 36 of 86 pesticides analyzed were detected, and atrazine was found in 99% of samples analyzed (0.071μg/L median concentration). A cluster analysis of Phase III data revealed associations between pesticide detections in surface water and land-use patterns. Other important variables included geographical location, intensity of agricultural activities, and watershed size (Qian and Anderson, 1999). A recent atrazine risk assessment conducted in Midwestern watersheds recommended site-specific risk assessments in areas with intensive atrazine use (Solomon et al., 1996). The potential for adverse impacts of pesticide use on water quality vary greatly in scope and severity. Therefore, pollution prevention efforts should focus on areas of intensive farming adjacent to vulnerable water resources.

The use of constructed wetlands for the treatment of agricultural runoff is gaining in popularity as a relatively inexpensive alternative to traditional treatment methods (Kirschner, 1995). There are currently more than 300 constructed wetlands used in the treatment of agricultural, municipal, industrial, and storm water waste in

the United States (Electric Power Research Institute, 1997). Although constructed wetlands have successfully treated many types of wastewater, there has been little evaluation of their treatment of pesticides (Watanabe, 1997). This may be due, in part, to the fact that these organic compounds and their transformation products are often considered hazardous at low levels. In addition, they are difficult to analyze, while other contaminants, such as metals, do not form degradation products and are easier to measure (Watanabe, 1997; Thurman and Meyer, 1996).

Atrazine was chosen to evaluate the treatment of pesticide runoff from a container nursery in a region of intensive agriculture in the Tualatin River Watershed near Portland, Oregon. Atrazine is a selective triazine herbicide used to control broadleaf and grassy weeds in a variety of agricultural commodities with 75 to 85 million lbs applied annually to United States agricultural soils (Aspelin and Grube, 1999). Atrazine half-lives in water are variable and range from several days to several months (Glotfelty et al., 1984; Jones et al., 1982; Hornsby et al., 1996). Atrazine is moderately water soluble (33 ppm), and exhibits low volatility (approximately 10^{-7} mm Hg), which gives a low Henry's law constant (approximately 10^{-7}) (Hornsby et al., 1996). Direct and indirect atrazine photolysis result in the production of N-dealkylated and hydroxy analogs (Grover and Cessna, 1991). Atrazine can be degraded by either biotic or abiotic processes in soil and sediment (Goswami and Green, 1971; Giardina et al., 1980) to produce deethylatrazine (DEA) and deisopropylatrazine (DIA). There is evidence in the literature for the presence of atrazine-degrading microorganisms in rhizosphere soil

(Anderson et al., 1994). Dechlorination may occur abiotically or biotically, leading to the production of hydroxyatrazine (HA), the primary hydrolytic product (Armstrong et al., 1967; Behki and Khan, 1986).

Atrazine fate and transport in the Des Plaines natural wetlands in northeast Illinois, was studied. Results indicated that wetlands delayed and reduced atrazine peaks and removed 26 to 64% of inflow concentration (Alvord and Kadlec, 1996). McKinlay and Kasperek (1999) reported that subsurface flow constructed wetlands containing one of several types of marsh plants were able to decontaminate water polluted with atrazine via a microbially-based mechanism associated with the rhizosphere.

Walton and Anderson (1990) suggested that the microbial communities associated with the rhizosphere may play an important role in the degradation of hazardous organic chemicals in soils due to their increased density and greater diversity in rhizosphere versus non-rhizosphere (non-vegetated) soil. Microbial densities in the plant rhizosphere are an order of magnitude or more above those in non-rhizosphere soils, which could translate into an increase in the degradation rate for xenobiotics in the rhizosphere, via cometabolism (Walton and Anderson, 1990; Curl and Truelove, 1986). The large volume of active rhizosphere per surface area associated with wetland plants suggests that if xenobiotics penetrate the rhizosphere and are susceptible to microbial degradation, constructed wetlands may be useful for reducing xenobiotic concentrations in wastewater (Brix, 1987).

Binding of atrazine and its degradates to wetland sediment may also be an important mechanism of loss from the water column. Glotfelty et al. (1984) examined atrazine edge-of-field runoff into an estuary; bottom sediments did not reveal detectable atrazine residues. Huckins et al. (1986) studied atrazine fate in 0.5 to 1 L wetland microcosms simulating prairie wetland potholes and found that at the end of a 6-week study approximately 40% of total atrazine detected was present in the sediment. Chung et al. (1996) observed atrazine and HA in wetland sediment from a system without wetland plants. These studies provide evidence that wetland sediment is an important sink for atrazine residues.

This study had two objectives: (1) to characterize the fate of atrazine in the water and sediment compartments of wetland microcosms containing *Typha latifolia* (cattail), the dominant wetland plant at our field site, and (2) to investigate the presence of atrazine degrading microorganisms in rhizosphere soil using most probable number (MPN) assays. Static wetland microcosms were used to simulate field-scale atrazine treatment by constructed wetlands. Because of the static nature of the wetland microcosms, atrazine treatment was expected to represent the upper limits of remediation potential for wetland systems.

MATERIALS AND METHODS

Chemicals

Analytical standards of atrazine, DEA, DIA, and HA were ≥95% pure (Chem Service, West Chester, PA, USA). The following radiolabeled compounds were donated by Novartis (Greensboro, NC, USA): [U-ring-^{14}C]atrazine (96.8% radiopurity) and [U-ethyl-^{14}C]atrazine (98.5% radiochemical purity). Internal standards were as follows: phenanthrene-d_{10} (Pd_{10}) (98% pure, Chem Service, USA) and terbuthylazine-2-hydroxy (HT) (95% pure, Crescent Chemical, Hauppauge, NY, USA) for GC/MS and HPLC analyses, respectively. Stock solutions of HA were made in 0.1 N reagent grade HCl, and working standards were prepared in 2:3 CH_3OH:5 mM KH_2PO_4 (pH 7.5) (v/v) (40% CH_3OH) at concentrations of 100 μg/L to 5000 μg/L. Atrazine, DEA, and DIA stock solutions were prepared in ethyl acetate (EA), and working standards were prepared in EA at concentrations of 63 μg/L to 500 μg/L. Pd_{10} was prepared in CH_3OH, and HT was prepared in 0.1 N HCl. All solvents and KH_2PO_4 were HPLC or GC grade (Fisher Scientific, Pittsburgh, PA, USA). The KH_2PO_4 solutions were adjusted to the appropriate pH using reagent-grade NaOH (50% w/v solution).

Wetland Microcosms

The wetland microcosms consisted of 265-L Rubbermaid containers (93 cm × 73 cm × 57 cm, length × width × height) which were oval in shape. Each microcosm was filled with approximately 15 cm or 128 kg of soil (originally a

subsurface agricultural soil) from a constructed wetland near Portland, Oregon. The soil used was classified as a silt loam (10% sand, 70% silt, and 20% clay) and was slightly acidic (pH 6.03) with an organic matter content of 4.7%. Three microcosms were planted with 25 *Typha latifolia* rhizomes (Balance Restoration Nursery, Lorane, OR, USA) and filled with 60 L of dechlorinated tap water to form a distinct water layer above the sediment. Plants were allowed to grow undisturbed for 1 month prior to initiation of experiments. Because spider mites compromised plant health following experiment 2, wetland microcosms were replanted prior to initiation of experiment 3. Two additional microcosms were constructed: a water control containing 60 L of water and a soil control (experiment 3 only) containing 15 cm of soil and 60 L of water. Wetland microcosms were kept in a temperature-controlled greenhouse (22 to 24 °C) receiving only natural light. A constant water volume was maintained in each microcosm by addition of water every 2-3 days, corresponding to a 1 cm or less drop in the water level. Studies were conducted from May to September 1998, and from July to September 1999.

Experimental Design

On day 0, 60 mg of analytical grade atrazine dissolved in 25 ml of acetone were added dropwise from a volumetric pipet to each microcosm in a pattern which evenly distributed the droplets. All stock solution concentrations were confirmed by GC/MS analysis. Three hours prior to sampling, the water level was adjusted to 60 L. Water samples (100 ml for atrazine, DEA, and DIA, and 100 ml for HA) were collected using a 60 cc polypropylene syringe. Samples were collected 1, 3, 5,

7, 10, 14, 21, 28, 35, 42, 49, and 56 d after application. To maintain plant vigor fertilizer (5 ml of 18% N, 2% P) was added to microcosms biweekly. One hundred ml water samples were collected once a week for nutrient analysis (nitrate, ammonia, and ortho phosphate).

Sediment samples were taken from each microcosm prior to atrazine application and on day 56. Five soil core samples were taken from each microcosm using a 18-mm diameter soil-coring device that was inserted to the bottom of the microcosm container. Sediment was air dried overnight in a fume hood, composited, and sieved to <2 mm and stored at -10 °C. All matrix spikes were created and frozen on September 23, 1999. Twenty percent of all water and sediment samples were duplicates or matrix spikes.

Other parameters monitored included pH (Orion, Beverly, MA, USA), oxidizing-reducing potential (Sensorex, Stanton, CA, USA), and dissolved oxygen (Hanna Instruments, Bedforshire, UK). Greenhouse temperature was recorded hourly using a HOBO (Onset Computer Corporation, Cape Cod, MA, USA) temperature data logger.

Extraction Procedures

For atrazine, DEA, and DIA, 100 ml water samples (containing 1% MeOH) were extracted using SPE C_{18} cartridges (500 mg, 6-ml polypropylene reservoir; J.T. Baker, Union City, CA, USA) following filtration through 55-mm GF/F filters (Whatman, Clifton, NJ, USA) (Zaugg et al., 1995). Briefly, SPE cartridges were conditioned, and samples were passed through the SPE cartridges followed by

elution with EA. Pd_{10} was added (2 μg) and samples were evaporated using an N-evap sample concentrator (Organomation, Berlin, MA, USA) to either 1 ml or 200 μl followed by GC/MS analysis. For 100-ml samples concentrated to a final volume of 1 ml, the limits of quantitation (LOQ) were 250, 1000, and 1000 ng/L, and the limits of detection (LOD) were 25, 40, and 250 ng/L for atrazine, DEA, and DIA, respectively.

One hundred-ml water samples for HA analysis were extracted as follows. Water samples were filtered through 55-mm GF/F filters to remove particulates. Following filtration, samples were enriched on C_{18} SPE cartridges (500 mg, 6 ml polypropylene reservoir; Varian, Sunnyvale, CA, USA) conditioned with 8 ml CH_3OH and 16 ml Milli-Q (MQ) water (Labconco, Kansas City, MO, USA). Following enrichment, the cartridges were allowed to dry for 1 hour. HA was eluted from the SPE cartridges with 3.4 ml of 9:1 CH_3OH:5 mM KH_2PO_4, pH 7.5. Subsequently, 2 μg of HT was added, and the eluant was evaporated to dryness using an N-evap. The sample was reconstituted in 1 ml of 40% CH_3OH and filtered through 0.45 μm nylon Acrodisc® syringe filters (Gelman Sciences, Ann Arbor, MI, USA) prior to HPLC/UV analysis. The LOQ and LOD for HA were 2.0 and 0.6 μg/L, respectively.

For atrazine, DEA, and DIA soil samples, extraction was based on a modified method (Lerch et al., 1999). Briefly, 20-g soil samples were extracted twice with 100 ml of 4:1 CH_3OH:MQ water (80% CH_3OH) in 250-ml Teflon-lined screw-cap centrifuge tubes. Samples were centrifuged for 30 min at 4000 rpm and

the supernatants were combined. The methanol was evaporated using a Turbovap II Concentration Workstation (Zymark Corporation, Hopkinton, MA, USA) with a water bath heated to 60 °C. The remaining water was extracted three times with chloroform which was collected and evaporated to dryness. The sample was reconstituted in 1 ml of EA, sonicated and vortexed for 1 min each before GC/MS analysis. The LOQs for atrazine, DEA, and DIA in 20-g soil samples were 1.0, 1.5, and 1.5 ng/g, respectively. The LODs for atrazine, DEA, and DIA in 20-g soil samples were 0. 5, 1.0, and 1.0 ng/g, respectively.

For HA soil samples, extraction was based on a modified mixed-mode extraction method (Lerch and Li, in press). A 20-g soil sample was weighed into a 250-ml centrifuge tube with a cap lined with aluminum foil. The mixed-mode extractant (MME), 3:1 of 0.5 M KH_2PO_4, pH 7.5:CH_3CN (v/v) was heated to 38 °C, and 65 ml was added to each sample. The samples were shaken for 2 hrs on an orbital shaker in a temperature-controlled room maintained at 38 °C. Following the extraction, samples were centrifuged for 30 min at 2000 rpm. The extraction was repeated twice with 40 ml of MME per extraction (2 and 1 hr), and all extracts were combined. The total extract volume was recorded, and the supernatant was transferred to a Turbovap flask (Zymark Corporation, Hopkinton, MA, USA) for evaporation of CH_3CN under a stream of nitrogen and in a water bath heated to 60 °C. Sample cleanup and enrichment were performed using 20-g SAX SPE cartridges (Varian, Sunnyvale, CA, USA) followed by 2-g SCX cartridges (Varian, Sunnyvale, CA, USA) Samples were ultimately re-dissolved in 1 ml of 40%

CH_3OH and analyzed by HPLC/UV. HA's LOQ and LOD for a 20-g soil sample were 5.0 and 3.0 ng/g, respectively.

Twenty percent of all water and soil samples were quality control samples. These included duplicate and matrix spike samples. All soil spikes were stored at – 20 °C for 4.5 to 7 months, and recoveries were in the range of 71-127%. In addition, 20% of water samples extracted were MQ water blanks or spikes.

GC/MS analysis

Analysis of atrazine, DEA, and DIA was performed using a Hewlett-Packard 6890 GC and a 5972 mass-selective detector (GC/MS) that was run in selected ion monitoring mode (SIM). Operating conditions were as follows: ionization voltage (70 ev); electron multiplier 2100 V; capillary interface at 280 °C. Separation of the compounds was accomplished with a fused-silica capillary column of (50%-Phenyl) methylpolysiloxane (DB-17) of 0.25-μm film thickness, 30 m × 0.25 mm internal diameter (i.d.) (J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1 ml/min. The column temperature was set to 90 °C for 2 min and then ramped at 30 °C/min to 215 °C where it was held for 5 min. The injector temperature was 225 °C. Quantification of the base peak of each compound was based on the response of the 188 ion of the internal standard, Pd_{10}, whose retention time (RT) was 9.13 min. The ions monitored, and RTs for the compounds were as follows: 187, 172, 145, and RT 8.08 min for DEA; 173, 158, 145, and RT 8.22 min for DIA; 215, 200, 173, and RT 8.45 for atrazine.

HPLC/UV Analysis

Analysis of HA was by octyl (C_8) reverse-phase HPLC using a Hewlett Packard 1050 series HPLC equipped with a UV detector (Wilmington, DE, USA). The HPLC conditions were as follows: column, 150 mm × 4.6 mm i.d. octyl 5μ (Phenomenex, Torrance, CA, USA); mobile phase 2:3 CH_3OH: 5 mM KH_2PO_4, pH 7.5; flow rate 1 ml/min; UV detection, 220 nm; injection volume, 40 μl. The RTs for HA and HT were 7.60 and 17.9 min, respectively.

MPN Assays

Populations of atrazine-degrading microorganisms were enumerated using MPN assays conducted with [^{14}C] ring-labeled or with [^{14}C] ethyl-labeled atrazine (Woomer, 1994) and sediment samples taken prior to experiment 1 (pre-exposure) and upon termination of experiment 2 (post-exposure). A 5-g (dry weight) composite soil sample from the three wetland microcosms and one composite sample from the soil control microcosm were suspended in mineral salts medium (MSM), creating either a 1/5 or 1/10 dilution followed by four to five additional dilutions. The MSM contained the following ingredients: 6.8 g/L KH_2PO_4 (anhydrous), 0.5 g/L NH_4NO_3, 0.2 g/L $MgSO_4 \cdot 7\ H_2O$, 0.05 g/L $CaCl_2$, 0.05 g/L yeast extract, 10 ml of trace elements solution, 3.3 ml sodium EDTA solution (0.3%), and 1 ml of 0.4% $FeCl_3$ in 0.1 N HCl. Ten ml of each dilution was transferred to an incubation vial containing 1.0 mg/L atrazine (0.1 μCi ethyl-labeled atrazine + cold atrazine). There were four replicates per dilution plus a sterile soil control (sterilized for 30 min at 121 °C) and a MSM control (contained

no soil). Incubation vials were sealed with rubber stoppers fitted with center wells (Kontes, Redmond, WA, USA). The center wells contained a filter-paper wick soaked with 100 µl of 1 M NaOH to trap $^{14}CO_2$ and the vials were incubated in the dark for 115 d at room temperature. The filter-paper wicks were removed periodically and placed in a 20 ml plastic scintillation vial containing 10 ml of scintillation fluid (Scinti Safe Econo 2; Fisher Scientific, Pittsburgh, PA, USA). Samples were counted (background and quench corrected) using a Beckman 3801 Liquid Scintillation Spectrophotometer (Fullerton, CA, USA). An MPN dilution series was scored positive if mean cumulative counts were greater than the mean of the sterile soil and MSM control counts. Atrazine degrading populations were enumerated using the Most Probable Number Calculator, Version 2.80, program for PCs (U.S. Environmental Protection Agency, Cincinnati, OH, USA).

RESULTS

Water Quality Measurements

Water quality parameters were monitored during all three experiments. The water pH ranged from 5.8 to 8.9, but was generally neutral to slightly basic for all microcosms. The dissolved oxygen water content ranged from 1.4 to 10.7 mg/L but was generally near 6.0 mg/L. The oxidizing-reducing potential was also variable (49 – 394 mV). Nutrient data varied due to biweekly fertilization events. The range of values for ortho phosphate, nitrate, and ammonium were from <LOD (0.01) to

20.6, <LOD (0.02) to 26.9, and <LOD (0.02) to 31.2 mg/L for all experiments, respectively.

Atrazine Dissipation from Microcosm Water

Experiment 1

The initial water concentration in wetland and water control microcosms was 0.82 mg/L and 0.64 mg/L, respectively. Based on mean values (n = 3), atrazine dissipation from the water column of wetland microcosms for all experiments was biphasic (Figure III-1a, 2a, 3a). The first phase showed rapid dissipation for 10 d following application (Figure III-1a). The data point for day 14 was not included in Figure 1a or in the calculation of k and half-life (Table III-1), due to an abnormally large amount of evapotranspiration between days 10 and 14. This required the addition of a large volume of water to the wetland microcosms prior to sampling, possibly resulting in desorption from the sediment. The second phase exhibited a slower rate of dissipation than the first phase (Figure III-1a). Only 7% of the atrazine applied remained in the water on day 56. In the water control microcosm, dissipation was not biphasic, and 62% of the atrazine applied remained in the water on day 56 (Figure III-1a).

Both DEA (Figure III-1b) and DIA (Figure III-1c) were found in wetland microcosm water. The maximum concentrations for DEA and DIA in wetland microcosms were 8 μg/L and 17 μg/L, respectively; DEA and DIA were formed in

Table III-1. Atrazine first-order rate constant (k) and half-life values for dissipation from the water column of wetland microcosms for experiments 1-3.

Table III-1.

Microcosm[1]	Phase I			Phase II			Overall		
	k (day^{-1})	Half-life (days)	Period (days)	k (day^{-1})	Half-life (days)	Period (days)	k (day^{-1})	Half-life (days)	Period (days)
Experiment 1									
Wetland	0.14	5.0	1-10	0.024	29	21-56	0.036	19	1-56
Water Control	NC[2]	NC	N/A[3]	NC	NC	N/A	NC	NC	N/A
Experiment 2									
Wetland	0.11	6.3	1-10	0.033	21	14-56	0.030	23	1-56
Water Control	NC	NC	N/A	NC	NC	N/A	NC	NC	N/A
Experiment 3									
Wetland	0.18	3.9	1-10	0.077	9.0	14-56	0.072	9.7	1-56
Water Control	NC	NC	N/A	NC	NC	N/A	0.0061	115	1-56
Soil Control	NC	NC	N/A	NC	NC	N/A	0.016	44	1-56

Table III-1 (Continued).

[1]Wetland = with plants, soil control = without plants, water control = water only
[2]No correlation at p = 0.05
[3]Not applicable

Figures III-1. Atrazine (a), DEA (b), and DIA (c) in the water column of wetland (n = 3) and water control microcosms (n = 1) for experiment 1.

Figures III-1a and III-b.

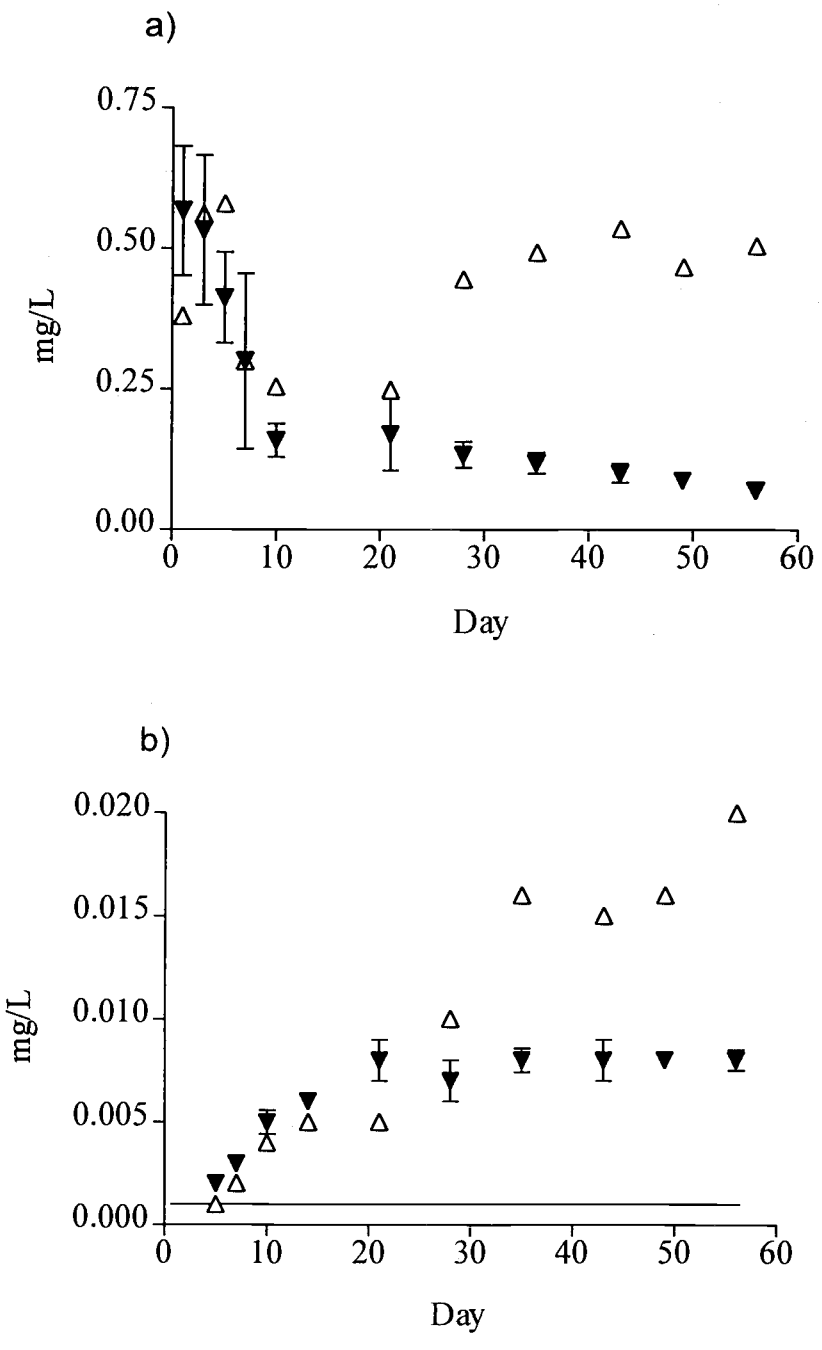
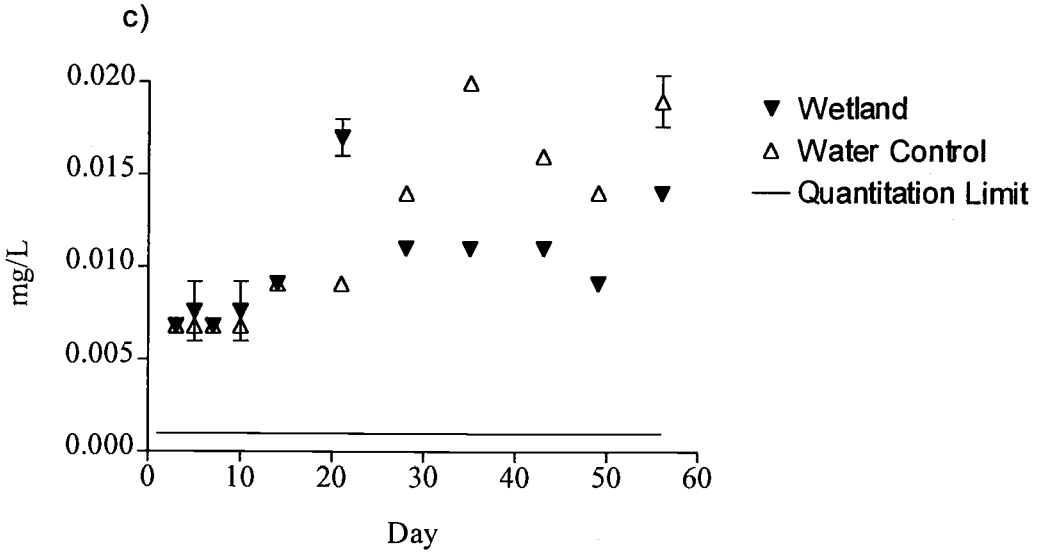


Figure III-1c.



the water control with maximum concentrations of 18 μg/L and 20 μg/L, respectively. HA was detected in wetland microcosms and in the water control microcosm at or below the LOQ of 2 μg/L.

Wetland sediment samples for all experiments indicated that atrazine, DEA, DIA, and HA accumulated in this compartment (Table III-2). The analysis of sediment samples collected on day 67 showed that atrazine was the most prevalent residue followed by HA, DIA, and DEA, respectively.

A mass balance for atrazine and degradates in both the water and sediment compartments of all microcosms was performed (Table III-3). The majority of residues (67%) were detected in the sediment compartment of wetland microcosms (128 kg of sediment/microcosm) in the form of atrazine and HA. The water compartment of wetland microcosms contained significantly less residue than the water control microcosm on day 56.

Experiment 2

Atrazine application was repeated two weeks following termination of the first experiment. The initial water concentration in wetland and water control microcosms was 0.78 mg/L and 0.64 mg/L, respectively. Biphasic atrazine dissipation was observed (Figure III-2a) and half-lives were comparable to experiment 1 (Table III-1). Approximately 12% of the atrazine remained in the wetland microcosms on day 56, while 54% remained in the water control

Table III-2. Sediment atrazine, DEA, DIA, and HA concentration (μg/g) in wetland and soil control microcosms for experiments 1-3.

			Mean ± SD (μg/g)			
Experiment	Day	Microcosm[1]	Atrazine	DEA	DIA	HA
1	0	Wetland[2]	<dl[4]	<dl	<dl	<dl
1	67	Wetland	0.13 ± 0.015	0.012 ± 0.0094	0.030 ± 0.0014	0.099 ± 0.024
2	0	Wetland	0.13 ± 0.015	0.012 ± 0.0094	0.030 ± 0.0014	0.099 ± 0.024
2	56	Wetland	0.15 ± 0.054	0.014 ± 0.024	0.020 ± 0.022	0.19 ± 0.14
3	0	Wetland	0.0094 ± 0.0021	<dl	0.012 ± 0.0018	0.21 ± 0.069
3	0	Soil Control[3]	0.026	0.0063	0.0080	0.18
3	56	Wetland	0.032 ± 0.0090	0.0054 ± 0.0002	0.0085 ± 0.0007	0.28 ± 0.050
3	56	Soil Control	0.14	0.011	0.0069	0.23 ± 0.0023

[1]Wetland = with plants, soil control = without plants
[2]n = 4
[3]n = 1 or 2
[4]<detection limit

Table III-3. Mass balance (% of initial application) for wetland, water control, and soil control microcosms after 56 days for experiments 1-3.

		Mass Balance (% of initial application)				
Microcosm[1]	Compartment	Atrazine	DEA	DIA	HA	Total
Experiment 1						
Wetland	Water	7.0	0.80	1.4	0.17	11
Wetland	Sediment	34	2.7	6.3	24	67
Wetland	*Water + Sediment*					*78*
Water Control	Water	62	2.6	2.4	0.23	67
Experiment 2						
Wetland	Water	12	1.7	1.5	0.59	16
Wetland	Sediment	5.5	0.051	ND[2]	23	29
Wetland	*Water + Sediment*					*44*
Water Control	Water	54	3.4	3	1.3	62
Experiment 3						
Wetland	Water	1	0.18	0.16	0.067	1.4
Wetland	Sediment	16	0.91	ND	12	29
Wetland	*Water + Sediment*					*30*
Water Control	Water	75	0.59	0.4	3.3	79
Soil Control	Water	36	1.8	0.65	0.36	39
Soil Control	Sediment	21	0.79	ND	8.8	31
Soil Control	*Water + Sediment*					*70*

[1]Wetland = with plants, soil control = without plants, water control = water only
[2]None detected

(Table III-3). The maximum wetland microcosm concentration for DEA was 20 μg/L, and the maximum DIA concentration was 14 μg/L (Figures III-2b and III-2c). The maximum DEA and DIA water concentrations for the water control microcosm were equivalent, 25 μg/L. HA was detected often at or below the LOQ in both the wetland and the water control microcosms. The maximum HA concentration for wetland and water control microcosm water were observed on days 21 (4.0 μg/L) and 56 (9.1 μg/L), respectively.

On day 0, atrazine, DEA, DIA, and HA residues were detected in wetland sediment samples (Table III-2). Additional atrazine, HA, and DEA accumulated in sediment samples, but DIA concentration decreased from day 0 to 56. Atrazine and DEA accumulation was less than observed during experiment 1, but HA accumulation was comparable to that observed during experiment 1.

Residue data for the mass balance calculation for this, and subsequent experiments were corrected for carryover. Again, the sediment compartment of wetland microcosms contained the majority of residues which were largely atrazine and HA. Less total residue was accounted for in the wetland microcosms during this experiment compared to experiment 1 (Table III-3).

Figure III-2. Atrazine (a), DEA (b), and DIA (c) in the water column of wetland (n = 3) and water control microcosms (n = 1) for experiment 2.

Figures III-2a and III-b.

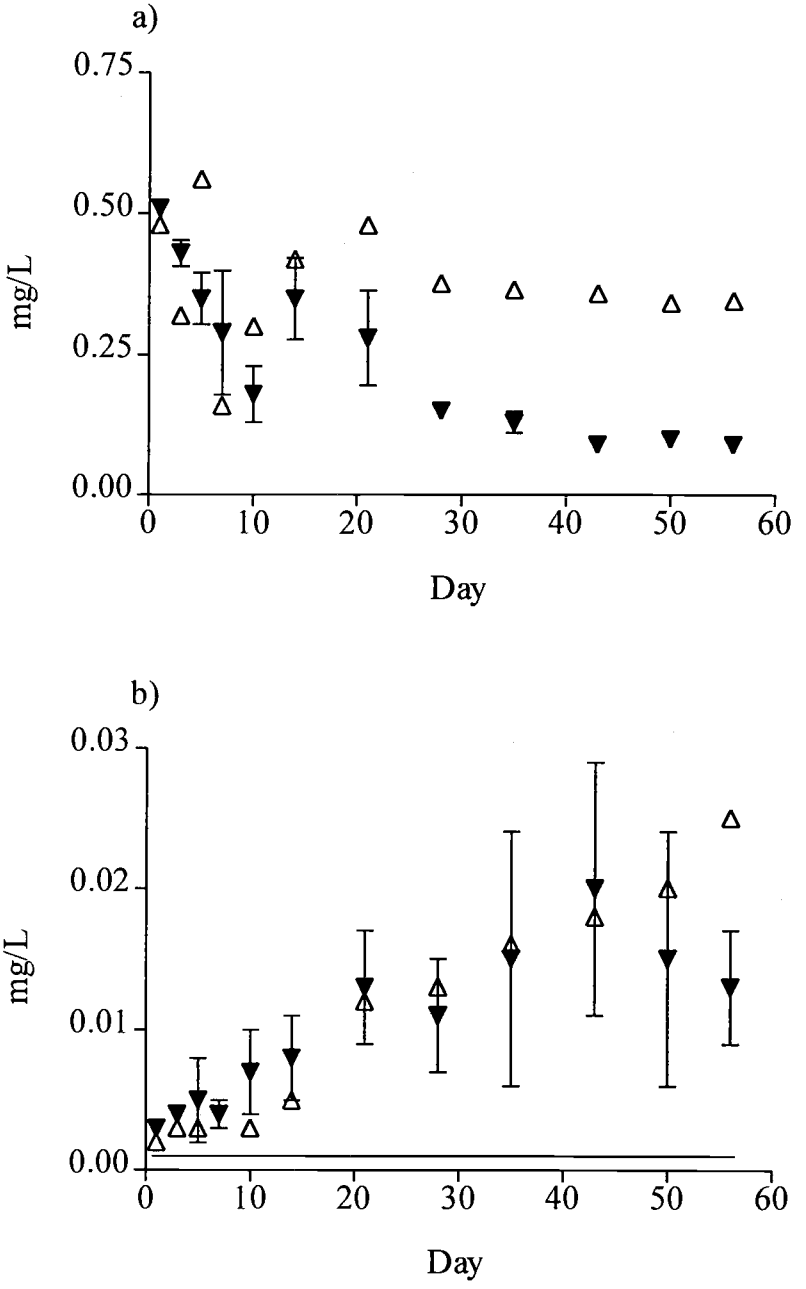
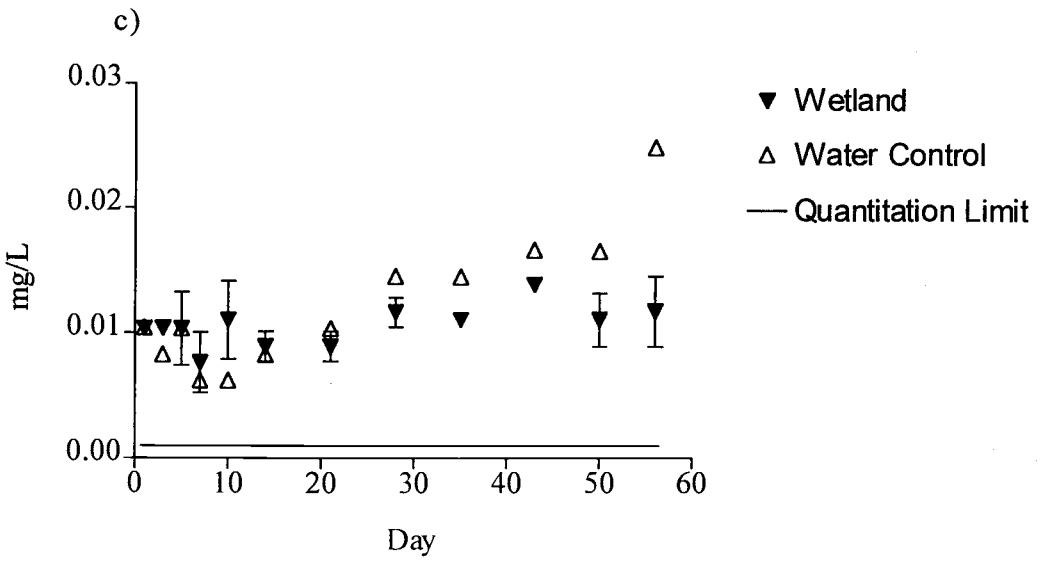


Figure III-2c.



Experiment 3

In July of 1999, the last microcosm study was initiated. The initial water concentration in wetland, water control, and soil control microcosms was 1.1 mg/L, 1.1 mg/L, and 1.0 mg/L, respectively. Data points for atrazine water concentration on day 21 in the water control microcosm, and day 35 in the soil control microcosm were not included in Figures 3a, 3b, or 3c, due to an analysis error (day 21 sample) and loss of the day 35 sample. Phase I (Figure III-3a), Phase II, and overall half-lives were shorter than observed for the previous experiments (Table III-1) and biphasic dissipation was not as pronounced as previously observed. Atrazine half-lives in the water column of water and soil control microcosms were longer than observed in wetland microcosms, with the longest half-life observed in the water control microcosm (Table III-1). On day 56, 1%, 36%, and 75% of the original atrazine remained in the wetland, soil control, and water control microcosms, respectively (Table III-3). The maximum wetland microcosm DEA and DIA concentrations were 7.5 and 4.8 μg/L (Figures III-3a and III-3b), respectively. The maximum DEA soil control concentration was twice that of the water control microcosm, but maximum DIA concentrations were similar (Figures III-3b and III-3c). The maximum HA water concentrations were 6.6 μg/L for wetland microcosms on day 21, 13 μg/L for the soil control microcosms on day 35, and 3.6 μg/L for the water control microcosm on day 56. By day 56, HA was below the limit of quantitation in the wetland microcosms.

Figure III-3. Atrazine (a), DEA (b), and DIA (c) in the water column of wetland (n = 3), water control (n = 1), and soil control microcosms (n =1) for experiment 3 (<QL_{we} = less than quantitation limit for wetland microcosms; ND_w = no detection for water control microcosm).

Figures III-3a and III-b.

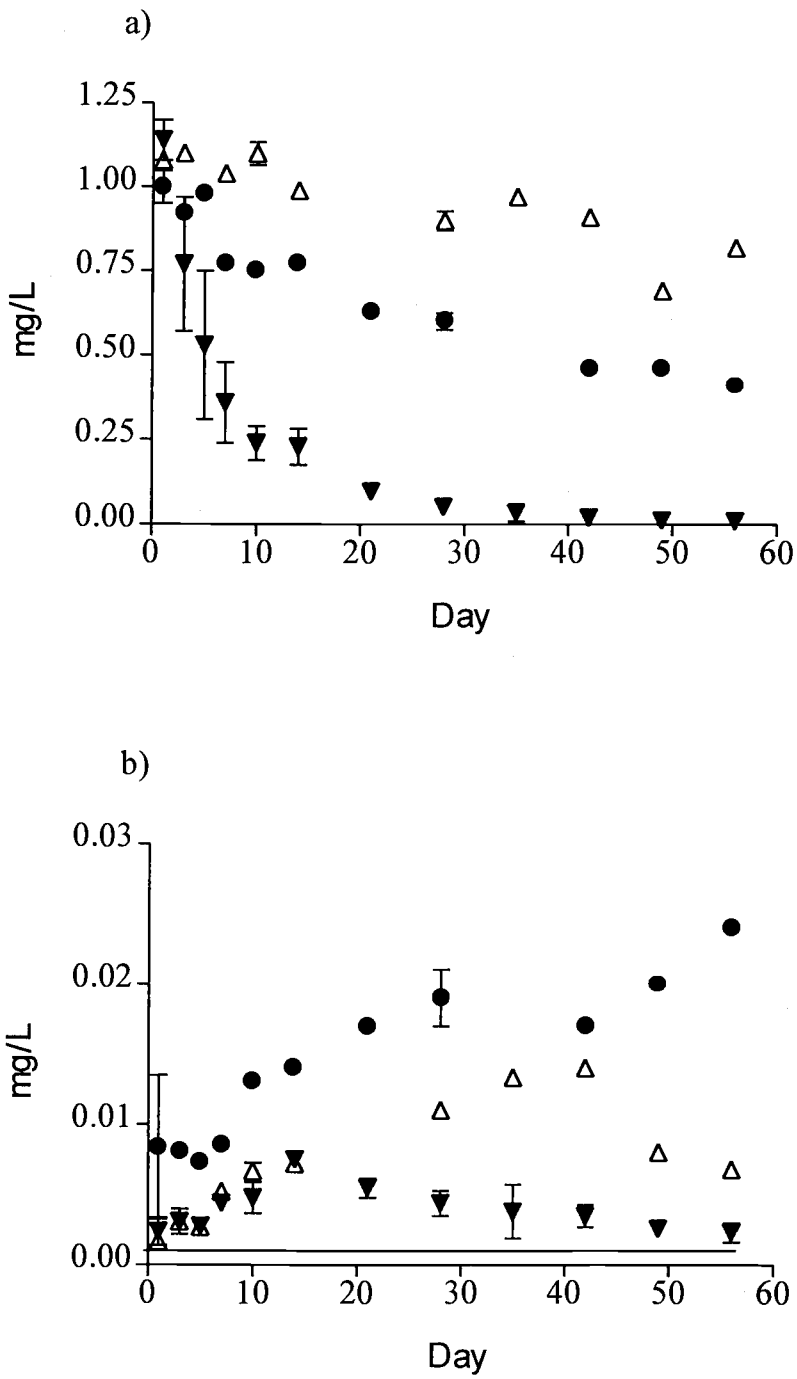
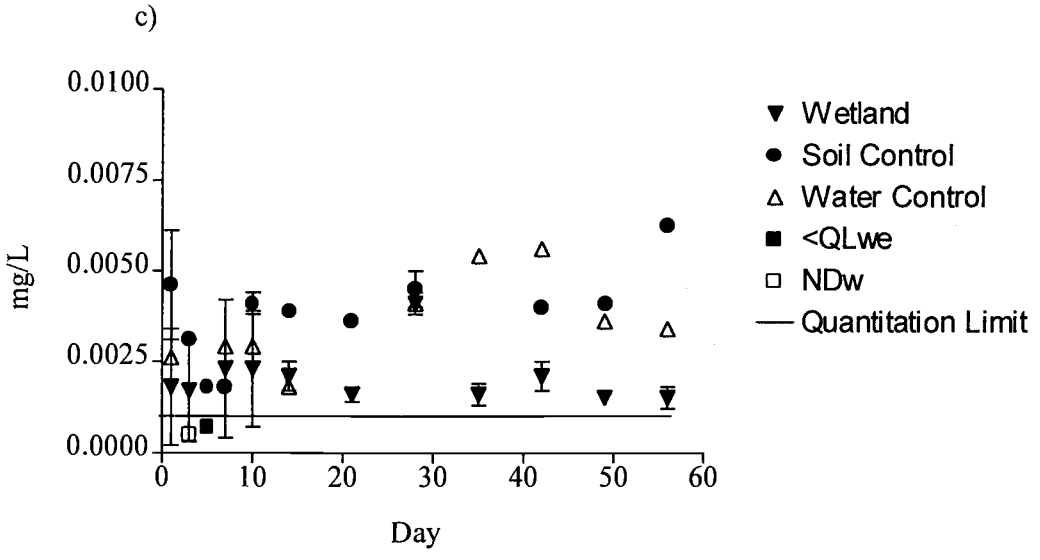


Figure III-3c.



Atrazine and HA were present in greater concentrations than DEA and DIA in sediment samples of wetland microcosms (Table III-2). The accumulation of atrazine and HA concentrations in wetland microcosms was similar to that observed during experiment 2. Atrazine and all degradates accumulated in the soil control microcosm (Table III-2). Day 0 soil control microcosm sediment samples contained detectable atrazine and degradate residues from prior atrazine exposure. When accounting for carryover, there was a net decrease in DIA concentration in soil control microcosm sediment over the course of the experiment.

Mass balance data from this experiment revealed less total residue present in wetland microcosms than in control microcosms (Table III-3). The sediment compartment of wetland and soil control microcosms accumulated the majority of residues in the form of atrazine and HA. In addition, the wetland and soil control microcosms accumulated similar levels of total residues (29% versus 31%, respectively).

MPN Assays

MPN assays were conducted using ^{14}C ring-labeled atrazine and did not indicate the presence of microorganisms able to mineralize atrazine. Because no activity was observed with the ^{14}C ring-labeled atrazine, we tested whether or not microorganisms capable of mineralizing the ethyl side chain, which is preferentially mineralized over the isopropyl side chain (Muir and Baker, 1976), were present. MPN assays using ^{14}C ethyl-labeled atrazine indicated the presence

of a small community of degraders in wetland and soil control microcosms (199 cells/g, 59-502 cells/g for 95% lower-upper confidence interval). Sample variation for replicates (n = 4) was high, presumably due to the small population size.

DISCUSSION

Atrazine Dissipation from Microcosm Water Columns

Our results using static wetland microcosms showed a reduction in atrazine concentration in the water column with time. Abiotic degradation, sorption, and possibly plant uptake, appear to be the primary pathways responsible for atrazine dissipation from the water column. Biphasic atrazine dissipation from the water column of wetland microcosms was observed during microcosm experiments. Biphasic dissipation due to sorption has been observed previously in a wetland microcosm study simulating edge-of-field runoff of atrazine (Huckins et al., 1986). It has been suggested that the rapid dissipation observed in Phase I may be due to a rapid soil sorption phase involving external sites, and Phase II may be influenced by a slower-diffusion-limited sorption phase including diffusion through intraparticle micropores (Karickhoff and Morris, 1985; Pignatello and Xing, 1996; Karickhoff, 1980), which may occur over a period of weeks to months.

On day 56 of experiment 3, atrazine concentrations in the water column of wetland microcosm and the soil control microcosm were less than in a water control microcosm, and residual atrazine in the wetland microcosms was

measurably less than in the soil control microcosm. In addition, overall atrazine half-life in the water column of wetland microcosms for experiment 3 was less than during the previous experiments. A significant increase in plant biomass and associated rhizosphere compared to previous experiments was noted. This suggests that the presence of wetland plants facilitated atrazine dissipation. The mechanism(s) is hypothesized to be either plant uptake (Rice et al., 1997; Burken and Schnoor, 1997) or rhizosphere associated soil microbial degradation (Anderson et al., 1994; Armstrong et al., 1967; McKinlay and Kasperek, 1999). Because MPN results did not indicate a large population of atrazine degrading microorganisms, plant uptake may account for the decrease in sediment residues.

The formation of three degradation products, DEA, DIA, and HA was followed throughout the experiments. DEA and DIA formation in the water column of wetland microcosms were nearly equivalent. This result contradicts several recent studies of dealkylated degradate formation in surface water reporting greater formation of DEA than DIA (Thurman et al., 1994; Kruger et al., 1993). It is widely accepted that the initial step in the microbial degradation of atrazine in soil is removal of the alkyl side chains, forming DEA or DIA (Giardina et al, 1980; Behki and Khan, 1994). The formation of HA, which occurs by either biotic or abiotic processes, may be the rate-limiting step to further degradation (Mandelbaum et al., 1995). Accumulation of DEA and DIA in wetland microcosms (experiments 1 and 2) suggests that the rates of formation were greater than the rates of degradation, sorption, or plant uptake, which might remove them from the

water column. During experiment 3, DEA and DIA accumulation in the water column was not observed possibly due to plant uptake. HA was present at quantifiable levels only once during experiment 1. In subsequent experiments, HA was detected with greater frequency and at higher concentrations possibly due to microbial degradation. If the formation of HA were a result of microbial degradation, it would not have been indicated by the MPN assays performed as they only enumerated microorganisms capable of mineralizing atrazine's ethyl side chain. The infrequency and low levels at which HA was detected in the water column is not surprising given its high affinity for sorption to sediment, and, therefore, we would expect most HA to be found in this compartment (Lerch et al., 1997).

In addition to sorption, degradation, and plant uptake, atrazine dissipation from the water column may be due to volatilization. However, based on atrazine's physico-chemical properties, volatilization from the water column is assumed to be negligible.

Sediment Residues

Atrazine sorption and degradation in the sediment compartment were significant mechanisms of atrazine loss from the water column of wetland and soil control microcosms as indicated by mass balance data (Table III-3). These findings are in agreement with Huckins et al. (1986) in which the distribution of atrazine in simulated wetland microcosms was investigated and the water column contained the largest portion of residues (48.9%), followed by sediment (38%) (Huckins et

al., 1986). The majority of sediment residues (86%) were in the form of bound residues (not extractable by sequential solvent extraction), and sediment samples were not broken down into degradate composition (Huckins et al., 1986).

In another study of extractability and degradation of atrazine in a submerged sediment over 336 d, HA was the primary degradate, accounting for up to 70% of the identified degradates (Mersie et al., 1998). DEA was detected as well, but to a lesser extent than HA; DIA was not detected. Between 30% and 60% of the residue remained bound to sediment incubated for 336 d at 5 and 24 °C (Mersie et al., 1998), and the proportion of nonextractable residue increased with exposure time. This is consistent with other reported data (Lerch et al., 1997). Following the first microcosm experiment, atrazine, DEA, and DIA sediment residues decreased. We do not believe this was a result of bound residue formation because all analytes were quantitatively recovered from aged soil spikes. It is possible however, that further degradation occurred. Our results are consistent with other findings suggesting that HA is the primary atrazine degradate found in sediment and soil systems (Huckins et al., 1986; Chung et al., 1996; Zaugg et al., 1995; Pignatello and Huan, 1991).

CONCLUSIONS

Wetland microcosms may be used to remove atrazine in water simulating irrigation runoff. Biphasic dissipation from the water column of wetland

microcosms was observed. This observation in addition to atrazine and degradate sediment residues indicates that the sediment is a significant compartment for atrazine fate in these wetlands. One hundred percent of the atrazine application was not accounted for, indicating the possibility of further degradation or other mechanisms of loss. Although others have eluded to the importance that microbial degradation plays in the fate of atrazine in the environment (Anderson et al., 1994; Behki and Khan, 1986), a substantial population of atrazine degrading microorganisms was not observed in these wetland microcosms. However, we can not rule out the possibility of the presence of microorganisms capable of degrading atrazine to HA, a non-phytotoxic degradate. Future studies should focus on finding ways to increase the role microorganisms play in atrazine loss from these microcosms. In addition, investigations into plant uptake could be useful.

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CHAPTER IV

CHARACTERISTICS OF ATRAZINE DEGRADATION IN BIOAUGMENTED WETLAND SEDIMENT

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ABSTRACT

The potential to enhance microbial degradation of atrazine in a constructed wetland was investigated using bioaugmentation with an atrazine spill-site soil. The addition of cattail mulch, an organic amendment, resulted in increased atrazine degradation in unamended wetland sediment. Bioaugmentation of sediment with spill site soil (1:100 w/w) resulted in mineralization of 30% of the ^{14}C applied after 62 d. By comparison, spill site soil alone mineralized 25% of the label in 2 d. Most probable number (MPN) assays of the bioaugmented sediment confirmed that a substantial increase in size of an atrazine-degrading population occurred in response to 10 μg/g of atrazine (9.4×10^3 to 6.0×10^5/g). Enhanced rates of atrazine mineralization developed in bioaugmented sediment maintained in wetland or soil microcosms and supplemented with 3.2 mg/L of atrazine for 30 d prior to sediment sampling. Similar percentages of ^{14}C-ethyl and ^{14}C-isopropyl side chain carbon were mineralized over a 27-d incubation (22 to 24%), whereas about twice as much ^{14}C-ring carbon was mineralized (46%) in 25 d. Bioaugmented sediment not exposed to atrazine in the microcosms mineralized only 2.3% of ^{14}C-ethyl atrazine despite MPN estimates indicating that a population of side chain mineralizers had persisted in the microcosms after bioaugmentation.

Abbreviations: DEA, deethylatrazine; DIA, deisopropylatrazine; HA, hydroxyatrazine; MPN, most probable number; WC, water content

INTRODUCTION

The nursery industry is one of the fastest growing segments of U.S. agriculture (Berghage et al., 1999). High pesticide use and high-intensity irrigation practices associated with the nursery industry create large volumes of wastewater that must be treated. Constructed wetlands consisting of saturated substrates, emergent and submergent vegetation, invertebrates and vertebrates, an aerobic and anaerobic microbial population, and a water column are commonly used to treat wastewater (Hammer, 1989) and may be used to treat wastewater from the nursery industry.

A significant portion of a constructed wetland's treatment of wastewater is believed to be a result of transformation and detoxification of compounds by microorganisms associated with the rhizomes of wetland vegetation (Kirschner, 1995; Adler et al., 1994; Alvey and Crowley, 1996; Brix, 1987; Anderson et al., 1993). However, constructed wetland cells are often created by excavation and removal of top soil. Because the majority of soil microbial communities inhabit the top soil, wetland construction may create situations where microbial populations with pesticide-degrading ability are absent from soil. Conceptually, inoculation or bioaugmentation of soil with microorganisms may be used to enhance xenobiotic degradation in such situations. Newcombe and Crowley (1999) reported that repeated inoculation of an atrazine-degrading bacterial consortium was required to clean up contaminated soil. Others have reported that microbial consortia or

specific microbial strains are able to degrade, and in some cases, accelerate atrazine biodegradation (Assaf and Turco, 1994; Radosevich et al., 1995; Mandelbaum et al., 1995).

The purpose of this study was to enhance microbial degradation of atrazine in a constructed wetland treating irrigation runoff from a container nursery near Portland, Oregon. We attempted to establish atrazine-degrading ability in wetland sediment using bioaugmentation with an atrazine-degrading microbial population. Atrazine was used as a model compound because microbial degradation is well characterized and its metabolites are known.

MATERIALS AND METHODS

Chemicals

Analytical standards of atrazine, deethylatrazine (DEA), deisopropylatrazine (DIA), and hydroxyatrazine (HA) were ≥ 95% pure (Chem Service, West Chester, PA, USA). [U-ethyl-^{14}C]atrazine (44 μCi/mg, 98.5% radiochemical purity), [U-isopropyl-^{14}C]atrazine (49.8 μCi/mg, 96% radiochemical purity), and [U-ring-^{14}C]atrazine (56.3 μCi/mg, 96.8% radiochemical purity) were donated by Novartis (Greensboro, NC). Internal standards were as follows: phenanthrene-d_{10} (Pd_{10}) (98% pure, Chem Service,West Chester, PA) and terbuthylazine-2-hydroxy (HT) (95% pure, Crescent Chemical, Hauppauge, NY)

for gas chromatography/mass spectrometry (GC/MS) and high-pressure liquid chromatography (HPLC) analyses, respectively.

Sediment Extraction Procedures, GC/MS, and HPLC Analysis

Soil/sediment samples analyzed for atrazine and degradate residues were taken from a duplicate series of treatments containing unlabeled atrazine that were incubated simultaneously with the radiolabeled treatments. Sediment extraction and subsequent GC/MS or HPLC analysis of atrazine, DEA, DIA, and HA were carried out as previously described (Runes et al., in press; Lerch and Li, in press; Lerch et al., 1999).

Briefly, 20-g soil samples and 100 ml of 4:1 methanol (CH_3OH):MQ water (80% CH_3OH) were combined in a 250 ml centrifuge bottle for extraction on an orbital shaker. Samples were centrifuged, the extraction was repeated, and CH_3OH was evaporated. The remaining water was extracted with chloroform which was collected and evaporated. The sample was reconstituted in 1 ml of EA and analysis of atrazine, DEA, and DIA was performed using a Hewlett-Packard 6890 GC and a 5972 mass-selective detector (GC/MS).

For HA, a 20-g soil sample was weighed into a 250-ml centrifuge bottle and 65 ml of the extractant, 3:1 of 0.5 M KH_2PO_4, pH 7.5:CH_3CN (v/v) was added to each sample. The samples were shaken for 2 h on an orbital shaker in a temperature-controlled room maintained at 38 °C. Samples were centrifuged, the supernatant was collected, and the extraction was repeated twice. The supernatants were transferred to a Turbovap flask (Zymark Corporation, Hopkinton, MA) for

evaporation of CH_3CN under a stream of nitrogen. Sample cleanup and extraction were performed using SAX and SCX solid-phase extraction cartridges (Varian, Sunnyvale, CA) and the final extract was concentrated to 1 ml in 2:3 CH_3OH: 5 mM KH_2PO_4, the HPLC mobile phase. HA was analyzed using a Hewlett Packard 1050 series HPLC equipped with a UV detector (Wilmington, DE).

Soil/Sediment Incubation Setup

Soil/sediment used for these experiments was obtained from a constructed wetland used to treat irrigation runoff at a container nursery near Portland, Oregon. Specific soil characteristics are shown in Table IV-1. Prior to use, the soil/sediment was air-dried overnight in a hood and sieved to <2mm. Sediment used in bioaugmentation experiments was maintained under wetland conditions and it was not sieved. Incubations were conducted in 60-ml glass serum bottles containing either 10 or 20 g of sediment. Each experimental treatment consisted of four replicates, while control treatment groups (sterile soil and deionized water) contained two replicates. Serum bottles were sealed with rubber stoppers fitted with center wells (Kontes, Redmond, WA) containing a 1 cm × 1 cm filter paper wick soaked with 100 µl of 5 M NaOH to trap $^{14}CO_2$. Bottles were incubated in the dark at room temperature. Filter paper wicks were removed periodically, placed in 7 ml plastic scintillation vials along with 1 ml of deionized water (DI), and 5 ml of scintillation fluid (Scinti Safe Econo 2, Fisher Scientific, Pittsburgh, PA). Samples were counted using a Beckman 6500 Liquid Scintillation Counter (Fullerton, CA),

Table IV-1. Physical and chemical characteristics of constructed wetland sediment.

Sand (%)	Silt (%)	Clay (%)	O. C. † (%)	pH	C.E.C.‡ (meq/100 g)
10	70	20	2.1	6.03	20.3

†Organic Carbon
‡Cation Exchange Capacity

and counts were corrected for background and quenching. Bottles were aerated at 3 to 4 d intervals for 30 min in a laminar flow hood, and sediment/soil WC was adjusted every two weeks. All samples received 3.7×10^9 Bq of ^{14}C-ethyl, ^{14}C-isopropyl, or ^{14}C-ring atrazine plus an additional amount of cold atrazine to produce a final atrazine concentration of 1 or 10 μg/g.

Effect of organic amendment and water content on atrazine mineralization in wetland sediment

An experiment was conducted to examine the interaction between water content and organic amendment on atrazine degradation. Twenty g of wetland sediment received 1 μg/g atrazine, and water content was adjusted to either 30% (unsaturated) or 39% (saturated) WC (w/w). Because a previous report showed that a cellulose amendment increased atrazine degradation (Yassir et al., 1998), sediment was amended with cellulose (2.2 mg/g) (TLC grade, Whatman, Fairfield, NJ) and ammonium sulfate, resulting in a carbon to nitrogen ratio of 10:1 (Yassir et al., 1998) (Fisher Scientific, Pittsburgh, PA). An additional organic amendment treatment (2.2 mg/g) consisted of dried, ground, cattail leaves containing 48% C and 1.1% N, respectively (Central Analytical Laboratory, Oregon State University, Corvallis, OR) and ammonium sulfate, resulting in a carbon to nitrogen ratio of 10:1 (Yassir et al., 1998). The $^{14}CO_2$ evolution was monitored for 78 d. Two unamended controls containing radiolabeled atrazine and either gamma irradiated soil (Radiation Safety Center, Oregon State University, Corvallis, OR) or 10 ml of sterilized deionized water (DI) were included in this and subsequent experiments.

Both the gamma irradiated soil, and the DI control treatments did not result in significant atrazine degradation in soil incubations. All statistical analyses were performed using SAS Version 7 computer program (SAS Institute, Cary, NC).

Effect of atrazine concentration on mineralization by wetland sediment and spill-site soil

Two soils (10 g per sample), wetland field sediment, and a soil from an atrazine spill-site (spill site soil) containing a large population of atrazine-degrading organisms (9.4×10^5 cells/g), were used. Each soil/sediment was amended with cattail and either 1 or 10 μg/g atrazine to investigate the effect of concentration on atrazine degradation. The $^{14}CO_2$ released was monitored for 54 d.

Effect of soil bioaugmentation on atrazine mineralization by wetland sediment

Wetland sediment (10 g per sample) was inoculated with spill site soil to determine if atrazine degradation in wetland field sediment could be bioaugmented and if cattail residue influenced the kinetics of degradation. One part spill site soil was thoroughly mixed into 99 parts of wetland field sediment (1:100 inoculation w/w). The $^{14}CO_2$ evolved was monitored for 62 d.

A most probable number (MPN) assay was performed to evaluate whether or not the population of atrazine degraders increased during the course of the experiment. The assay was conducted with ^{14}C-ethyl atrazine and with 10 μg/g atrazine as previously described (Runes et al., in press). Following a one-month incubation, MPN dilutions were scored positive if mean cumulative counts recovered were greater than the mean plus three times the standard deviation of the

gamma irradiated soil and mineral salts medium (no soil) control counts. Atrazine-degrading populations were enumerated using the Most Probable Number Calculator, Version 2.80, program for PCs (U.S. Environmental Protection Agency, Cincinnati, OH).

In order to determine whether or not bioaugmentation would be successful under wetland conditions, the experiment was repeated with saturated sediment (39% WC) amended with either 1 or 10 μg/g atrazine.

Static wetland microcosm bioaugmentation

To evaluate if the presence of wetland plants might influence the success of bioaugmentation and the development of an atrazine-degrading population, an experiment was conducted in static wetland microcosms. Approximately 60-kg quantities of wetland sediment were added to each of four, 120-L plastic tubs housed in a temperature-controlled greenhouse. The sediment was inoculated with a soil enrichment of atrazine-degrading microorganisms. The soil enrichment was produced by mixing one part of spill site soil with 99 parts of wetland sediment (1:100 w/w) and incubating the sediment with 10 μg/g atrazine for 21 d. An MPN assay was conducted on a composite subsample of the enrichment to quantify the growth of atrazine degraders. A suspension of the soil inoculum was prepared by suspending 600 g of the enriched soil (dry weight) in 12 L of dechlorinated tap water and adding it to microcosms. Following inoculation, 25 *Typha latifolia* (cattail) rhizomes were planted in two microcosms (wetland microcosms), and two microcosms were left unplanted (soil microcosms). The water level was adjusted to

16 L and maintained for 54 d under greenhouse conditions to permit plant growth. On day 54, atrazine (3.2 mg/L) was added to one wetland (planted) and one soil (unplanted) microcosm; the remaining microcosms did not receive atrazine. Dechlorinated tap water was added to the wetlands to achieve a final volume of 30 L, and the water level was adjusted every 2 to 3 d. Following 30 d of atrazine exposure (84 d after bioaugmentation), 30 sediment cores (1.8 cm diameter × 2 cm deep) were taken from each microcosm, composited, and thoroughly mixed prior to use. Incubations were set up with 1 μg/g atrazine (^{14}C-ethyl) under saturated conditions (39% WC) as previously described. Sediment from wetland (planted) and soil (unplanted) microcosms with and without atrazine treatment were also incubated with ^{14}C-isopropyl and ^{14}C-ring atrazine. The $^{14}CO_2$ evolved was monitored for up to 48 d. MPN assays were conducted on sediment from each of the four bioaugmented microcosms using
^{14}C-ethyl atrazine as described above.

RESULTS

Effect of organic amendment and water content on atrazine mineralization in wetland sediment

Figure IV-1 shows cumulative $^{14}CO_2$ evolved from unamended, cattail-amended, and cellulose-amended wetland sediment incubated with ^{14}C-ethyl atrazine under both unsaturated (30%) and saturated (39%) water content (WC). An extremely low rate of $^{14}CO_2$ release was observed. Cattail amendment stimulated

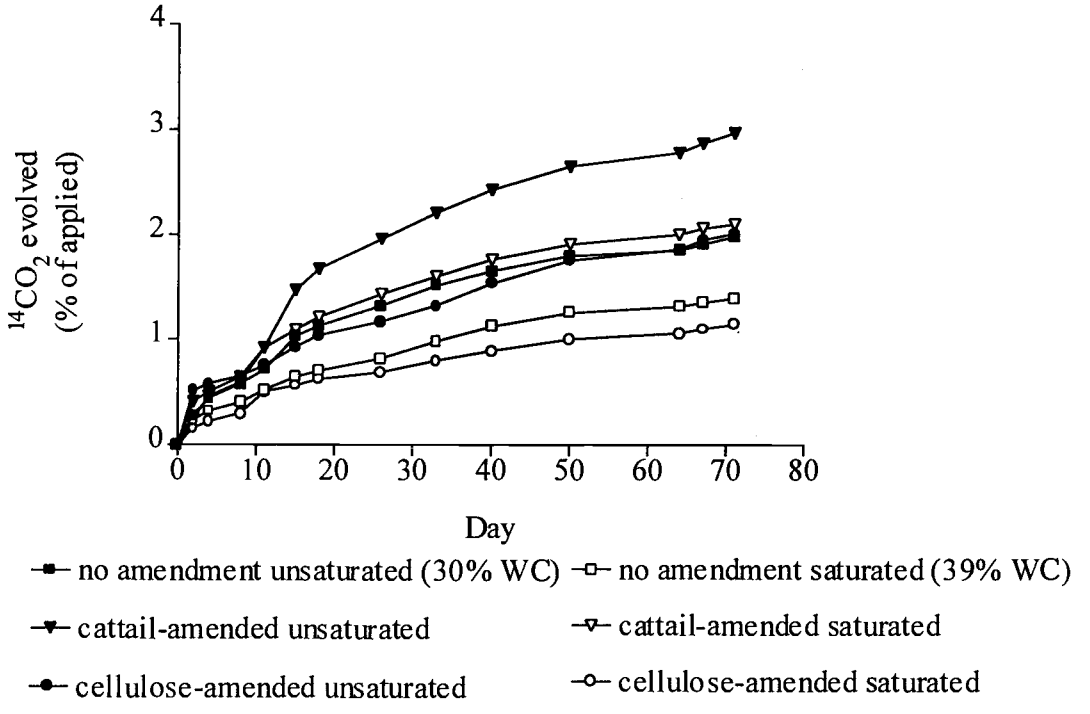


Figure IV-1. Influence of amendment and water content (WC) on percent $^{14}CO_2$ evolved from soil incubations (n = 4).

atrazine degradation at both WCs (ANOVA, $P < 0.05$). The cattail treatment was statistically different from the cellulose and unamended treatments (Bonferroni t-test, $P < 0.05$), but the cellulose and unamended treatments were not statistically different.

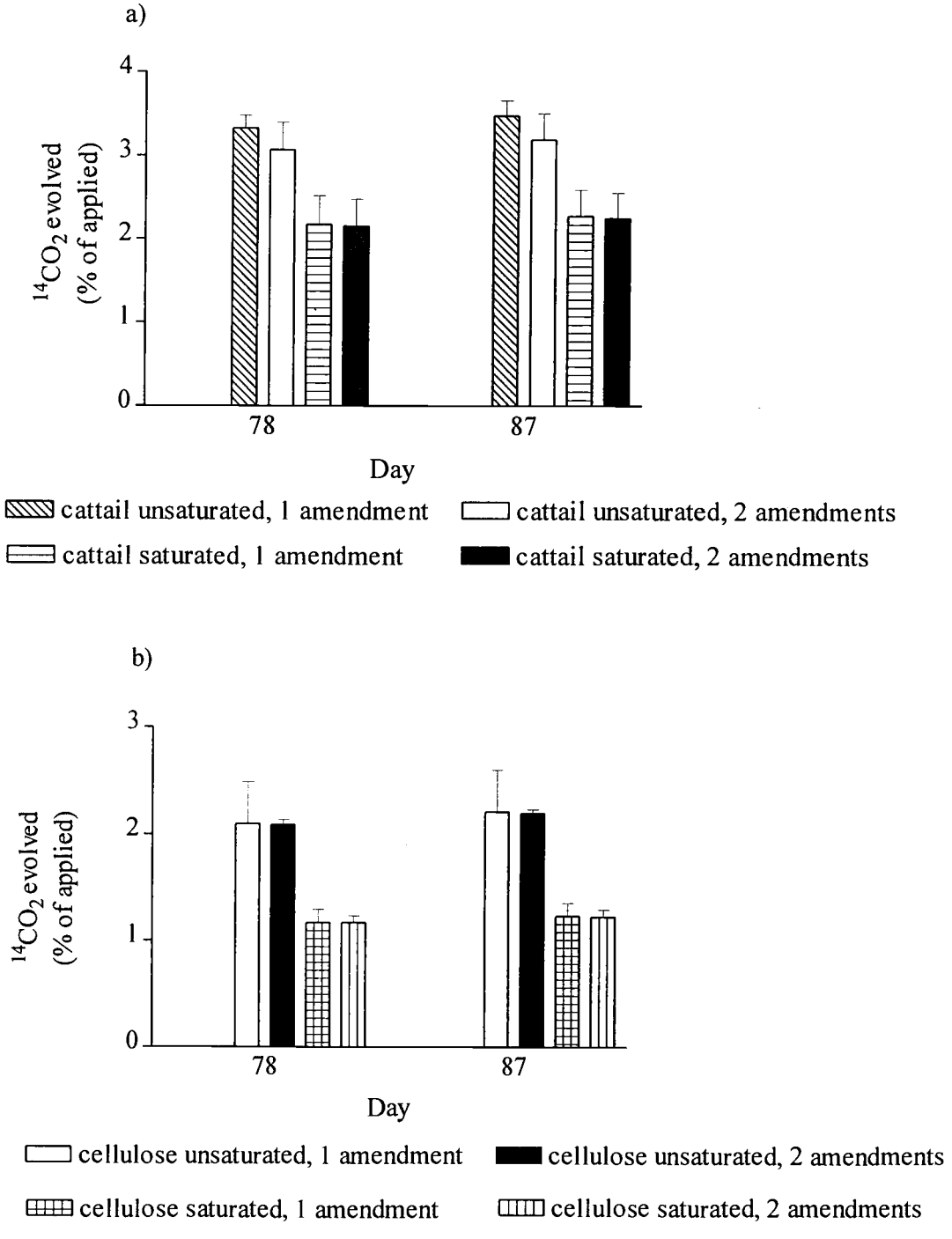
On day 71, two of the four replicates of the cellulose and cattail-amended treatments at both WCs received an additional organic amendment to determine if further mineralization could be stimulated. Release of $^{14}CO_2$ was followed to day 87 (Figures IV-2a and IV-2b) and no significant difference was observed between the mean percent $^{14}CO_2$ released from treatments receiving one or two organic amendments (Bonferroni t-test, $P > 0.05$).

Effect of atrazine concentration on mineralization by wetland sediment and spill-site soil

Atrazine mineralization by spill site soil (30% WC) occurred rapidly. Mineralization of ^{14}C-ethyl side chain was essentially complete within 8 d and approximately 30% of the label was mineralized to CO_2 (Figure IV-3). Atrazine degradation in the wetland sediment was slow and followed a linear trend. A similar percentage of added atrazine was mineralized at both concentrations (1 and 10 μg/g).

Figure IV-2. Cumulative percent $^{14}CO_2$ evolved from sediment incubations (n = 2, mean ± standard deviation) with either one or two cattail (a) or cellulose (b) amendments.

Figures IV-2a and IV-2b.



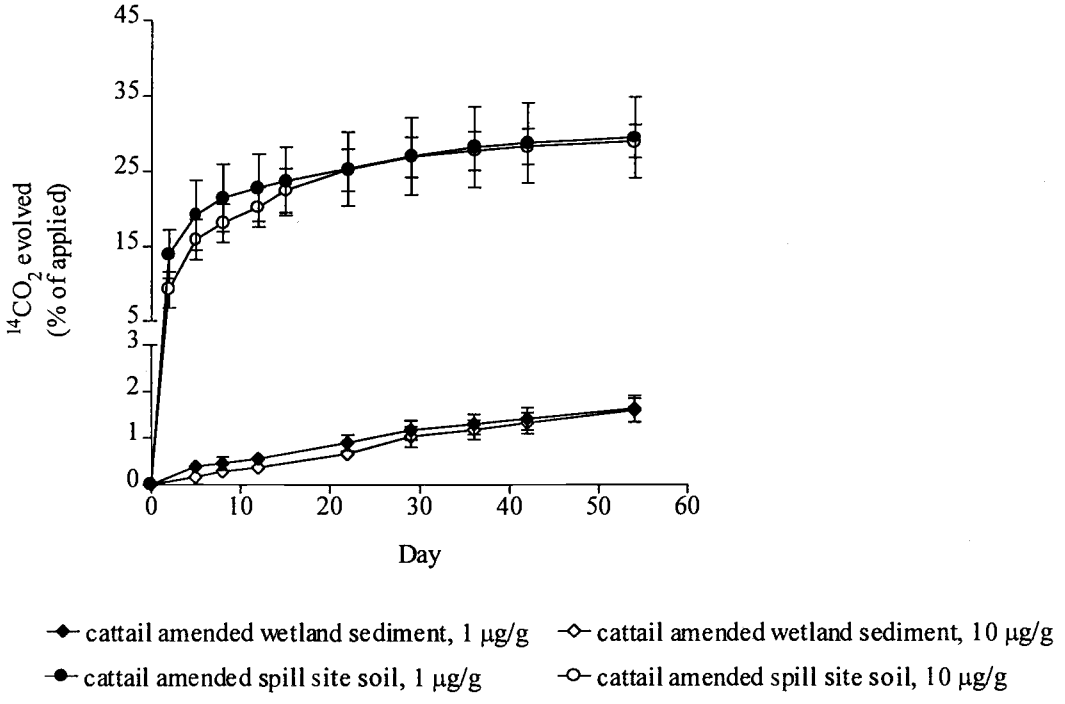


Figure IV-3. Cumulative percent $^{14}CO_2$ evolved in unsaturated (30% water content) sediment incubations (n = 4, mean ± standard deviation) with 1 or 10 µg/g atrazine.

Effect of soil bioaugmentation on atrazine mineralization by wetland sediment

Although the cumulative percentage $^{14}CO_2$ evolved from the spill site soil was greater than from the bioaugmented wetland sediment (Figures IV-4a and IV-4b), total degradation by wetland sediment was considerably improved as a result of bioaugmentation. By day 3 of the experiment the majority of $^{14}CO_2$ was released from the spill site soil (27.0-28.3%), and by day 30, the majority of $^{14}CO_2$ was released from the inoculated wetland sediment (24.3-27.5%). There was no significant effect of cattail amendment on atrazine degradation (ANOVA, $P > 0.05$). Growth of atrazine degraders in the bioaugmented wetland sediment was confirmed by comparing MPN estimates of the population sizes of atrazine degraders in bioaugmented wetland sediment before and after incubation. The bioaugmented wetland sediment contained 9400 cells/g (3100-23000 cells/g lower-upper 95% confidence interval (c.i.)) and 6.0×10^5 cells/g (2.1×10^5–1.5×10^6 cells/g lower-upper 95% c.i.) on days 0 and 62, respectively.

Bioaugmented wetland sediment was extracted and atrazine, DEA, DIA, and HA were quantified in both day 0 and day 62 samples. On day 0, there were 33 μg/g and 0.26 μg/g of atrazine and HA, respectively, in the sediment. At the end of the experiment, 0.23 μg/g and 0.75 μg/g of atrazine and HA were detected, and less than 0.020 μg/g of DEA and DIA were detected. In total, less than 5% of applied atrazine was detected as HA and less than 1% was detected as atrazine, DEA, and DIA on day 62. This result implies that the remaining atrazine-carbon is probably associated with biomass of the atrazine degraders.

Figure IV-4. Influence of cattail amendment on $^{14}CO_2$ released from 10 μg/g atrazine applied to unsaturated (30% water content) spill site soil (a) or bioaugmented wetland sediment (b), and $^{14}CO_2$ released from 1 or 10 μg/g atrazine applied to saturated (39% water content) bioaugmented wetland sediment (c) (n = 4, mean ± standard deviation).

Figures IV-4a and IV-4b.

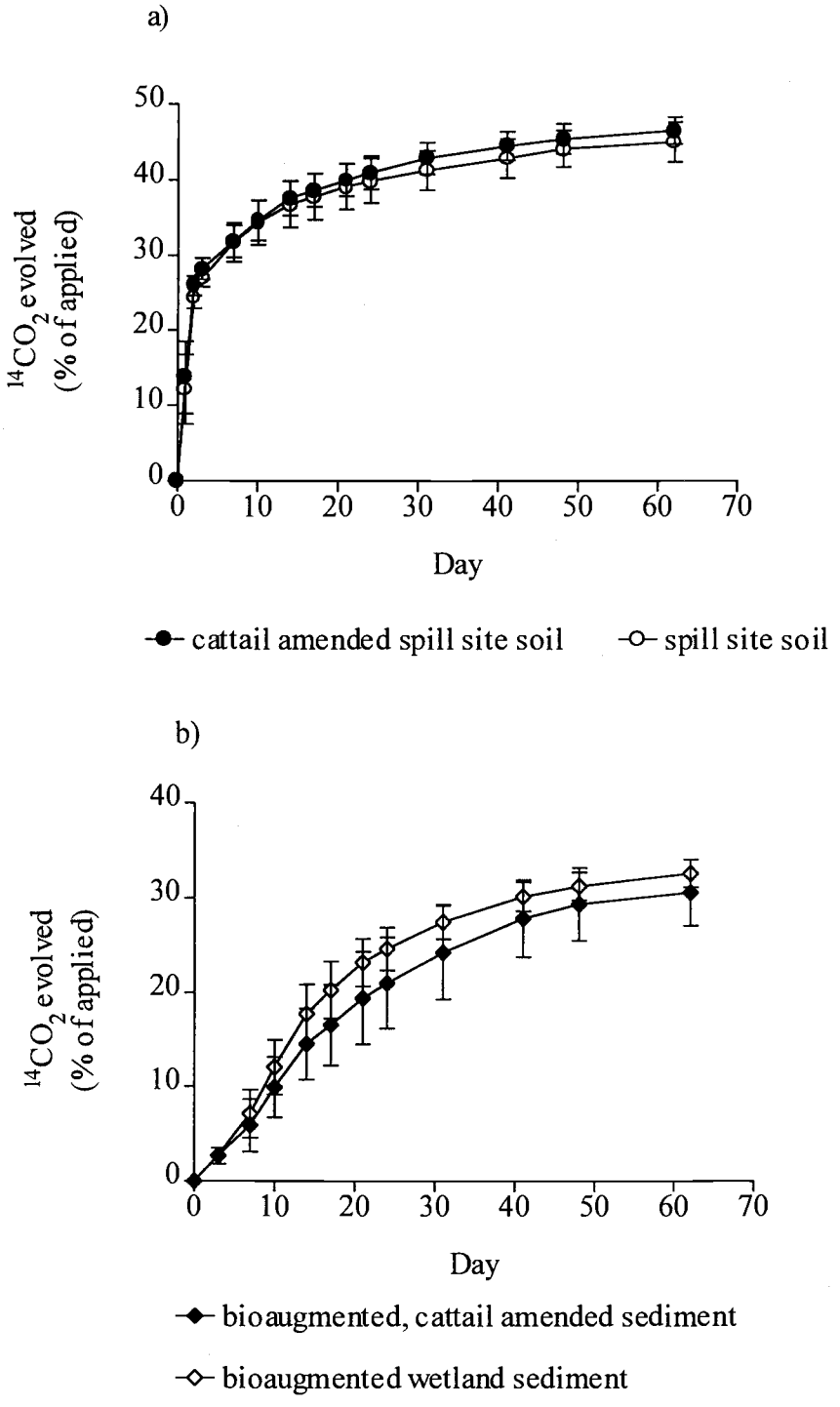
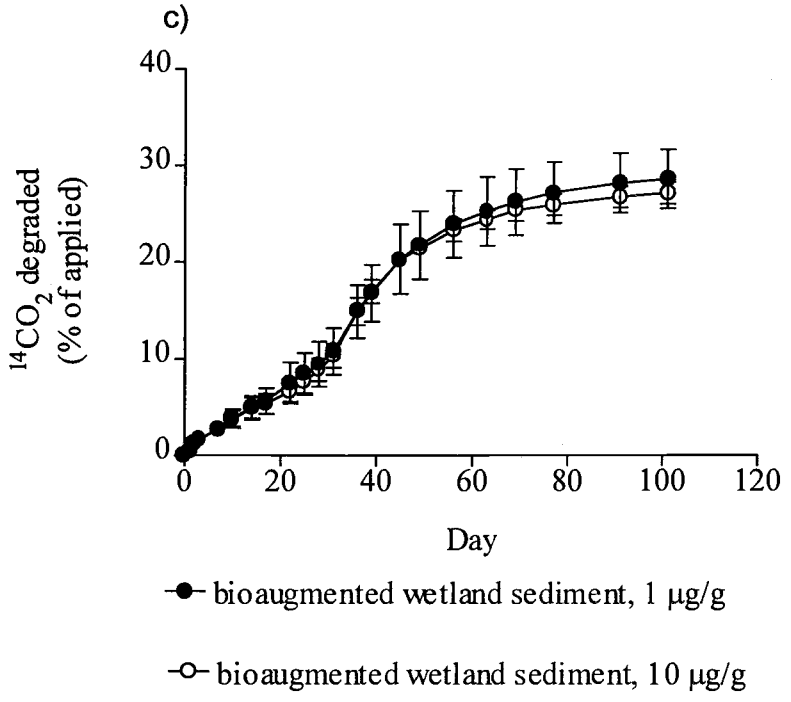


Figure IV-4c.


c)
40
30
20
10
0
$^{14}CO_2$ degraded
(% of applied)
0
20
40
60
80
100
120
Day
bioaugmented wetland sediment, 1 µg/g
bioaugmented wetland sediment, 10 µg/g

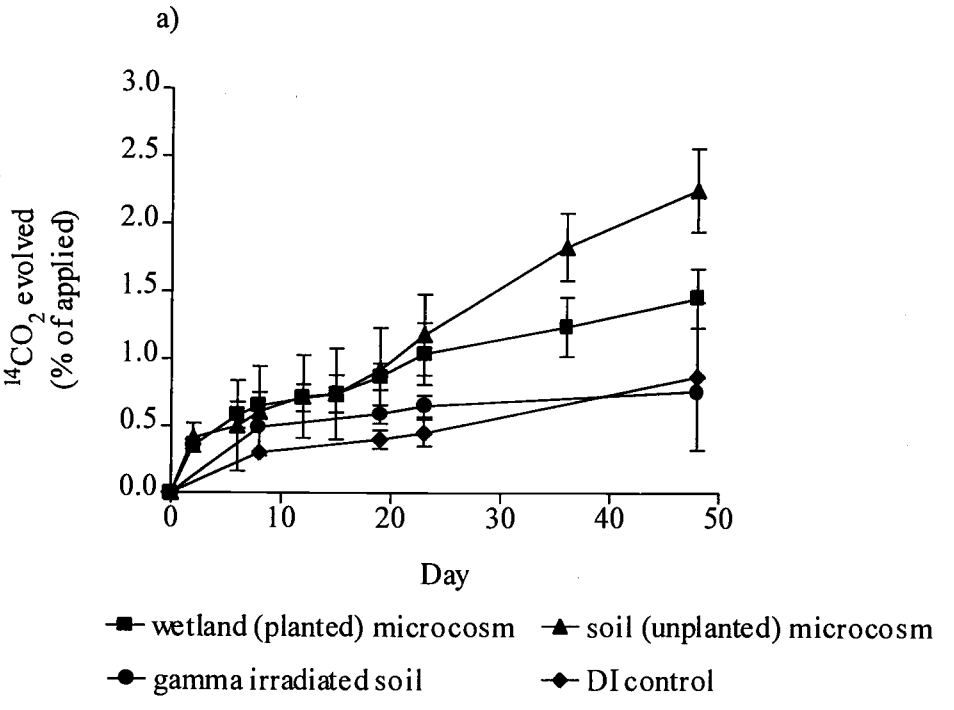
The rate of atrazine degradation by bioaugmented sediment was sensitive to water content. The initial rate of mineralization was about three times slower under saturated than unsaturated conditions. Nonetheless, a similar maximum percent ^{14}C-ethyl side chain was mineralized after 60 d in saturated incubations versus 30 d under unsaturated conditions. The maximum percent of ^{14}C-ethyl side chain mineralized was similar at either 1 or 10 μg/g (Figure IV-4c) (Bonferroni t-test, $P > 0.05$).

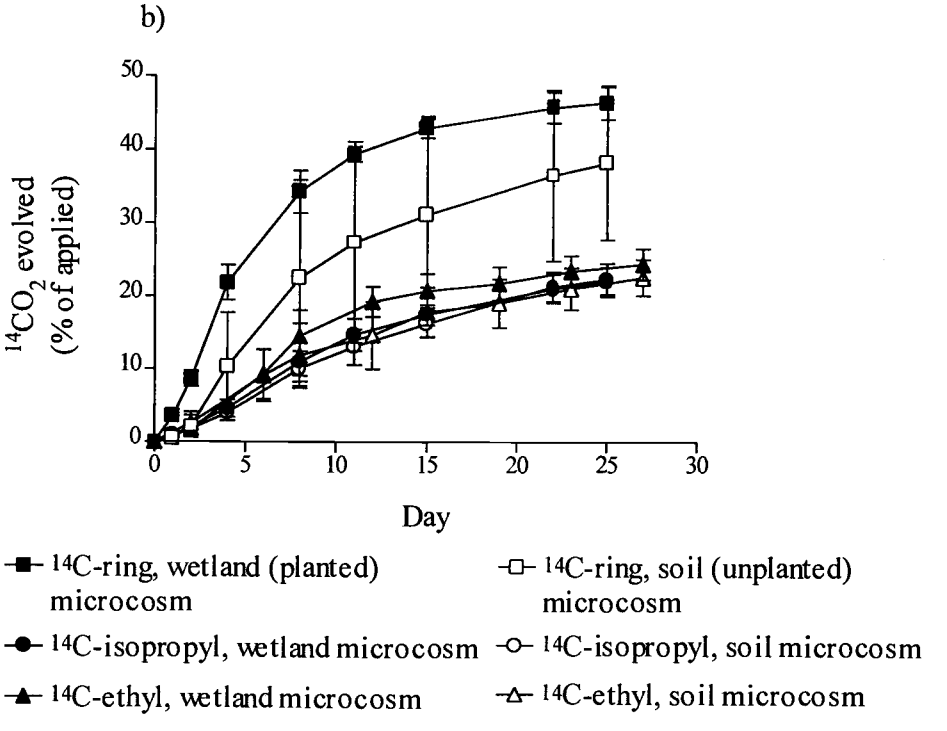
Static wetland microcosm bioaugmentation

Prior to microcosm bioaugmentation an MPN estimate was made of the population size of atrazine degraders that developed in atrazine-enriched wetland sediment inoculated with spill site soil. From this value we calculated that initial bioaugmentation would provide approximately 7.0×10^3 cells/g. Results of mineralization of ^{14}C-ethyl atrazine from incubations conducted on sediment from bioaugmented wetland microcosms are shown Figures IV-5a and IV-b. Mineralization of ^{14}C-ethyl atrazine in bioaugmented wetland (planted) and soil (unplanted) microcosms that received no atrazine treatment was slow and did not exceed 2.3% in 48 d (Figure IV-5a). When bioaugmented sediment from microcosms treated with atrazine was incubated with atrazine labeled in either the ethyl or isopropyl side chains, or in the triazine ring, rapid degradation resulted. The pattern of isopropyl side chain mineralization followed the same trend observed with ^{14}C-ethyl atrazine incubations and was essentially complete by day 12 regardless of the presence or absence of plants (Figure IV-5b). In the case of

Figure IV-5. Cumulative $^{14}CO_2$ evolved from sediment incubations with sediment from wetland and soil microcosms receiving no atrazine treatment and incubated with ^{14}C-ethyl atrazine or, sediment from wetland and soil microcosms receiving atrazine treatment (3.2 mg/L) and incubated with ^{14}C-ethyl, ^{14}C-isopropyl, or ^{14}C-ring atrazine (b) (n = 4, mean ± standard deviation). All sediment incubations were with 1 μg/g atrazine.

Figures IV-5a and IV-5b.





ring label, however, the rate of mineralization was two to four times more rapid than side chain mineralization. By day 25 about 22% of ^{14}C-isopropyl atrazine was mineralized, whereas 46% of ^{14}C-ring atrazine was mineralized by day 27. Although the presence of plants had no statistically significant effect on the total atrazine mineralized in microcosms, the initial rate of mineralization of ring carbon was more rapid in the wetland (planted) than in the soil (unplanted) microcosms.

Despite the low level of atrazine mineralization in the microcosms that did not receive atrazine during the incubation, MPN estimates of atrazine degraders in bioaugmented sediment samples taken from wetland and soil microcosms confirmed that atrazine mineralizers with the ability to mineralize atrazine's ethyl side chain persisted throughout the 54 d incubation regardless of atrazine presence. Two widely variable population estimates were obtained from the atrazine treated microcosms and prevented us from concluding that population growth had occurred in response to atrazine exposure.

DISCUSSION

A low rate of atrazine degradation observed in the unamended wetland sediment may be due to the fact that the constructed wetland was created in subsurface soil which had not been previously exposed to atrazine. In addition, the population of organisms with atrazine-degrading ability was very low and incapable of growth on atrazine. The extent of atrazine degradation in soil ranges

widely and is often related to history of soil treatment, sorption, or other soil properties (Yassir et al., 1999; Giardina et al., 1979; Radosevich et al., 1996; Singleton, 1994). For example, as little as 6 to 14% of ^{14}C-ethyl atrazine may be mineralized in unamended, uninoculated soil (Yassir et al., 1998; Doyle et al., 1978; Jayachandran et al., 1998), but reports of 60 to 85% of initial radioactivity mineralized exist in the literature (Jayachandran et al., 1998; Yassir et al., 1999).

Mixed success has been reported for stimulating pesticide degradation in soil with organic amendments (Doyle et al., 1978; Alvey and Crowley, 1995; Felsot and Dzantor, 1997). Although cellulose was shown to stimulate atrazine N-dealkylation in soil (Yassir et al., 1998), we observed that the addition of cattail, not cellulose, stimulated degradation in wetland sediment. The absence of a lag phase prior to degradation indicated that degradation might be driven by a cometabolic process (Giardi et al., 1985). Nonetheless, the addition of another quantity of cellulose or cattail (day 71), did not significantly increase atrazine degradation, indicating that organic substrate availability did not limit further degradation. The possibility cannot be overlooked, however, that increasing atrazine sorption to sediment over time may explain the inability of additional amendments to stimulate further atrazine degradation (Alexander, 1994).

Soil water content affected total atrazine degradation in both unamended and bioaugmented wetland sediment. Degradation rate was decreased in bioaugmented saturated sediment, but overall degradation was comparable to unsaturated conditions, suggesting that microbial growth was adversely affected by

limited oxygen availability (Sethunathan, 1973; Singleton, 1994). Water content has been reported to retard atrazine degradation in saturated surface soil (Wolf and Martin, 1975; Goswami and Green, 1971). Although our saturated incubations were not carried out anaerobically, conditions were presumably more anoxic than in the unsaturated incubations, resulting in less oxygen availability, and potentially hindering degradation. In wetlands, oxygenation of sediment by plant roots might play a critical role in enhancing aerobic degradation of compounds.

Atrazine degradation in bioaugmented wetland sediment was much greater than in uninoculated wetland sediment. Various studies have reported significant atrazine degradation in soils inoculated with cultures of soil microorganisms or soil consortia (Mandelbaum et al., 1993; Struthers et al., 1998; Assaf and Turco, 1994). Microbial growth was observed concurrently with atrazine degradation (Mandelbaum et al., 1993b; Struthers et al., 1998). In contrast to our observations, Newcombe and Crowley (1999) found that repeated applications of atrazine-degrading bacteria were required for successful atrazine degradation implying that growth did not occur. In a field experiment, 38 to 72% of an atrazine application (100 mg/kg) was degraded after 11 weeks when repeated applications of bacteria were made. In contrast, no measurable degradation occurred in soil inoculated once.

MPN estimates of population densities before and after incubation showed that the inoculum in the bioaugmented sediment grew approximately 64-fold in response to 10 μg/g atrazine. The fact that only 24% of the ethyl and isopropyl

label was captured as $^{14}CO_2$ in contrast to 46% of ring label, implies that a higher percentage of side chain label was assimilated into microbial biomass carbon than ring label. Atrazine and degradate soil residue did not account for the remaining atrazine application in bioaugmented incubations. Not surprisingly, HA was the primary degradate present (Runes et al., in press; Mersie et al., 1998; Chung et al., 1996; Alvey and Crowley, 1995; Assaf and Turco, 1994), and dealkylated degradate residues were insignificant. It is possible that a portion of the atrazine was degraded and incorporated into biomass, therefore precluding the release of $^{14}CO_2$ or its detection during residue analysis. Incorporation of substantial quantities (30 to 70%) of ^{14}C-ethyl atrazine has been reported in bacterial strains used in bioremediation of contaminated soils (Struthers et al., 1998) and in soil treated with ^{14}C-ring or ethyl atrazine (Yassir et al., 1999) and this may have occurred in our bioaugmented microcosms. Other degradates which could have been formed without the release of $^{14}CO_2$, and thereby account for some of the remaining application, include deisopropylhydroxyatrazine (DIHA). Formation of DIHA is thought, however, to be a minor degradation product in soil (Kruger et al., 1993). In addition, the formation of bound (unextractable) residues may account for some of the atrazine (Gan et al., 1996).

Bioaugmentation of wetland and soil microcosms was successful for those treatments receiving a treatment with 3.2 mg/L of atrazine in the water column. Physiological adaptation (e.g. enzyme induction) may have been induced in cells following contact with atrazine present in the soil (Giardina et al., 1979). However,

sediment from microcosms not receiving atrazine did not exhibit significant atrazine degradation despite our ability to measure the existence of 10^3 to 10^4 atrazine degraders/g. It is unclear why a population of degraders could be detected by MPN assay in bioaugmented sediment not treated with atrazine, and yet fail to mineralize atrazine. One possibility is that the atrazine population in the spill site soil exists as a consortium and that in the absence of atrazine some members died out. The absence of critical members of the consortium may have prevented efficient atrazine mineralization.

CONCLUSIONS

The addition of an easily-degradable carbon source to uninoculated wetland sediment weakly stimulated atrazine degradation. Bioaugmentation of wetland soil with a spill site soil significantly increased atrazine degradation, and growth of atrazine degraders was observed. Water content had an effect on total atrazine degradation in uninoculated wetland sediment and bioaugmented wetland sediment. Enhancement of atrazine degradation by bioaugmentation was successful when atrazine was added to the water column of microcosms. The presence of plants in microcosms did not influence the size of the atrazine-degrading population. Future studies should focus on finding the lower limit of atrazine that may be added to microcosms to induce microbial growth and determining whether or not bioaugmentation of a field-scale constructed wetland can be achieved.

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CHAPTER V

CONCLUSIONS

Results of field studies indicated that this constructed wetland effectively reduced overall atrazine concentration in container nursery irrigation runoff in experiments 1 through 5. No difference in treatment effect was observed when the frequency and intensity of runoff events was varied or when total flow through the wetland was reduced. However, in experiment 6 when amount, frequency, and duration of runoff events exceeded all other experiments, treatment was compromised. Experiments conducted with static water-sediment columns indicated that sorption was a primary mechanism of atrazine removal from the water column during field experiments.

Biphasic dissipation from the water column of wetland microcosms simulating the constructed wetland at the field site was observed. This observation, in addition to accumulation of atrazine and degradates in sediment confirmed that the sediment is a significant compartment for atrazine fate in these wetlands. A substantial population of atrazine-degrading microorganisms was not observed in wetland microcosms indicating that microbial degradation of atrazine was inconsequential.

The potential to stimulate the atrazine-degrading microbial population of the constructed wetland was investigated. Bioaugmentation of wetland sediment

with an atrazine spill-site soil significantly increased atrazine degradation, and microbial growth was observed. Enhanced atrazine degradation was observed using bioaugmentation into wetland (planted) and soil (unplanted) microcosms pretreated with atrazine. Future studies should focus on finding the lower limit of atrazine pretreatment required to induce microbial growth. Identification of the microorganism(s) responsible for atrazine degradation may be useful in optimizing conditions (e.g. carbon to nitrogen ratio) for maximum degradation and microorganism survival. An assessment of whether or not bioaugmentation of a field-scale constructed wetland can be achieved is needed.

There is a need to optimize all pathways for pesticide degradation in constructed wetlands. Depending on the compound, sorption and plant uptake may provide the best opportunities for remediation where large populations of pesticide-degrading microorganisms are absent. When sorption is a significant pathway for pesticide loss from runoff, bioaugmentation with soil from either a pesticide spill site, or an agricultural site where repeated pesticide applications have been made may improve treatment.

In general, there was significant treatment of atrazine measured as percent recovery from cells 1 to 5. Treatment may be further increased by recirculating water exiting cell 5. This would increase atrazine's residence time possibly resulting in increased abiotic degradation and sorption. Recirculation of water would be most practical between runoff events. In addition, investigations into

plant uptake and degradation of atrazine should be conducted. Further research should attempt to quantify atrazine and degradation products in wetland plants.

It was our experience that constructed wetlands require significant maintenance to maximize their treatment capabilities. Proper maintenance included repair of diversion ditches and soil islands between wetland cells. Weir pipes were kept free of debris and additional wetland plants were added to wetland cells on occasion.

Constructed wetlands treating pesticide-contaminated runoff may be most useful for intensive agriculture, rather than for large-scale agricultural operations due to the amount of maintenance required. Several studies indicate that the use of natural wetlands to treat river water containing atrazine is possible. The use of these natural wetlands, or restored wetlands, may be better suited to treat water of larger volumes than constructed wetlands.

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