Biodegradation and Bioremediation

Impact of Land Use on Microbial Transformations of Atrazine

C. Marjorie Aelion* and Dana C. Cresci

ABSTRACT

Atrazine has been one of the most heavily applied preemergent herbicides in the USA, and with alachlor, represents more than 45% (4.082×10^6 kg) of total herbicide application in coastal U.S. counties. Several studies using bacterial isolates or mixed cultures have demonstrated that mineralization of atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] is rapid, yet in soil microcosms mimicking in situ conditions, mineralization accounts for <1% of the applied compound. This study examined the impact of land use on atrazine biotransformation in sediments from three coastal sites, all in an historically agricultural area that has undergone extensive development in recent years. Production of $^{14}CO_2$ was monitored in sediment incubated with [U-^{14}C]-atrazine from a forested preserve, a suburban neighborhood and a golf course, all of which were previously impacted by agriculture. Solid phase extraction of both liquid and sediment fractions was carried out over time to determine whether metabolic transformation of atrazine occurred. Results indicated that mineralization represented <1% of the atrazine added, and ring carbons were not assimilated into cellular biomass. Production of ^{14}C-transformation products was a significant portion of the total ^{14}C-atrazine added at all sites, but was greatest in the golf course sediments. This may be attributed to several environmental conditions of land development such as the preexposure of the golf course bacteria to frequent pesticide applications, and 100% sand content of the golf course sediment vs. higher clay and organic C content at the other two sites, causing less sorption and more bioavailability of atrazine.

ATRAZINE, a preemergent herbicide used extensively to control broadleaf weeds and some grasses in corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], sugarcane (*Saccharum officinarum* L.), and conifer crops, has been one of the most heavily applied herbicides in the USA (USEPA, 1994).

Atrazine is considered to be moderately persistent in soils, and because of its slight water solubility (33 μg L^{-1} at 22°C) and small partition coefficients (K_d = 0.19–2.46), transport to surface and subsurface waters is likely (Huber, 1993; Solomon et al., 1996). Degradation of atrazine proceeds through the soil catalyzed chemical hydrolysis of the two position chlorine atom, to hydroxyatrazine (HYA), or through the microbial *N*-dealkylation of the ethyl and isopropyl chain constituents, to deethylatrazine (DEA) and deisopropylatrazine (DIA) (Kaufman and Kearney, 1970; Kruger et al., 1993). Two of these transformation products, DEA and DIA, are phytotoxic, thus monitoring the disappearance of atrazine without accounting for the subsequent degradation to transformation products may be insufficient to determine its ultimate impact on the environment.

Several studies using bacterial isolates or mixed cultures have demonstrated the rapid and complete mineralization (i.e., conversion to CO_2, NH_3, and H_2O) of atrazine (Behki et al., 1993; Mandelbaum et al., 1993; Yanze-Kontchou and Gschwind, 1994; Radosevich et al., 1995). Conversely, in soil microcosms mimicking in situ conditions, complete mineralization of atrazine is usually nonexistent (<1% of the applied compound) (Goswami and Green, 1971; Wolf and Martin, 1975; Dao et al., 1979; Jones et al., 1982; Kruger et al., 1993). Notable exceptions occur in systems that have undergone multiple exposures to atrazine (Topp et al., 1995).

Estuarine sediments can serve as a sink for the majority of the atrazine load entering coastal rivers, and reduce overall primary productivity, energy flow, and nutrient cycling (Caplan et al., 1984). Isensee (1987) calculated that 50 to 60% of atrazine in the headwater regions of coastal rivers should be adsorbed to salt marsh sediments (organic C content of 112 g kg^{-1}). Seasonal contamination of aquatic systems has been documented extensively and ecotoxicological effects become evident when concentrations of atrazine persist above 20 μg L^{-1} (Huber, 1993; Solomon et al., 1996).

Coastal South Carolina is predominantly agricultural with an estimated annual application of 1.474×10^6 kg per year of atrazine in 1988, second only to alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)-acetamide] (Pait et al., 1992). In addition to traditional agriculture and silvaculture, coastal South Carolina is undergoing unprecedented land development for recreational, and housing purposes (Vernberg et al., 1992). This rapid influx of tourists and residents has increased deforestation, channelization of streams, urban-runoff, and pesticide use associated with golf courses and residences (Vernberg et al., 1992; Whitmore et al., 1992; Miles et al., 1992). As a result, elevated levels of nitrate, phosphate, heavy metals, and pesticides including atrazine, have been detected in estuaries (Vernberg et al., 1992; Kucklick and Bidleman, 1994).

Although numerous studies have examined the removal of atrazine from agricultural soils and river waters, there has been little research addressing atrazine removal by microorganisms found in coastal and estuarine sediments and the impact of land use on the biodeg-

C.M. Aelion, Dep. of Environmental Health Sciences and Marine Science Program; and D.C. Cresci, Dep. of Environmental Health Sciences, Univ. of South Carolina, Columbia, SC 29208. Received 6 Feb. 1998. *Corresponding author (aelionm@sc.edu).

Published in J. Environ. Qual. 28:683–691 (1999).

Abbreviations: DEA, deethylatrazine; DIA, deisopropylatrazine; DOC, dissolved organic carbon; DPM, disintegrations per minute; ECD, electron capture detector; GC, gas chromatography; HYA, hydroxyatrazine; K_{oc}, organic carbon partition coefficient; K_d, sediment water partition coefficient; LSC, liquid scintillation counter; PVP, polyvinylpyrrolidone; SPE, solid phase extraction.

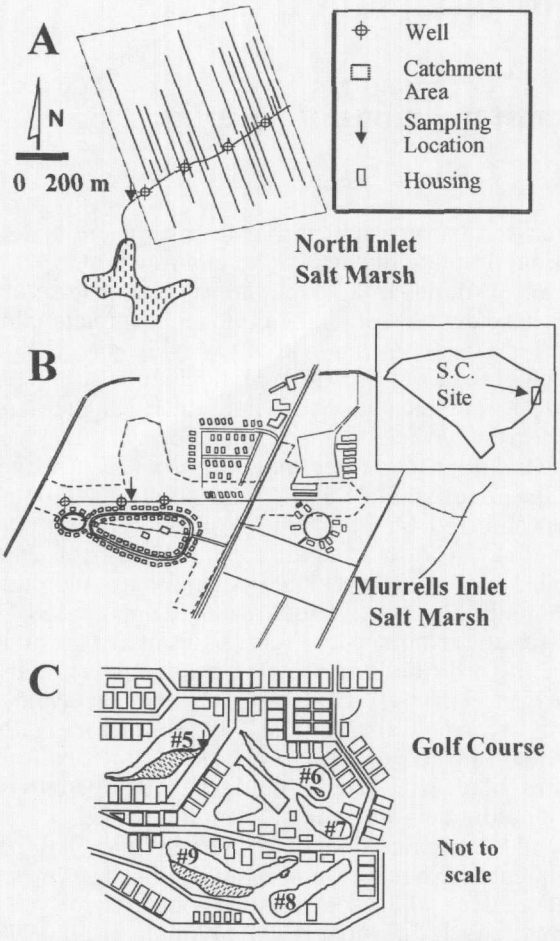

Fig. 1. Stream sediment sampling locations for (*A*) reforested North Inlet site, (*B*) suburban Murrells Inlet site, and (*C*) golf course.

radation of atrazine. The purpose of this study was to determine the extent and rate of atrazine degradation in sediment sampled from a golf course, a suburban residential area, and an undeveloped, forested site on South Carolina's coast that reflects the emerging land use patterns that result in additional inputs of pesticides and fertilizers.

MATERIALS AND METHODS

Study Locations

Sediments for microcosm incubations were collected from three riparian channels flowing through an undeveloped forested preserve (Oyster Creek at North Inlet), suburban development (Dog Creek at Murrells Inlet), and a golf course (Pawley's Island) all located in coastal Georgetown County, South Carolina (Fig. 1). The undeveloped preserve, the Belle W. Baruch Institute for Marine Biology and Coastal Research, has been protected from development and maintained in its natural state for educational purposes for approximately 30 yr (Fig. 1a). This catchment (37 ha) was formed by a hand dug ditch network and earthen dike more than 100 yr ago for the cultivation of rice and subsequently tree farming. Currently, the watershed consists of a 30-yr-old stand of loblolly pines (*Pinus taeda* L.), live oak (*Quercus virginiana* Miller), water oak (*Q. nigra* L.), sweet gum (*Liquidambar styraciflua*), American elm (*Ulmus americana* L.), and an understory of wax myrtle (*Myrica cerifera*) (Wahl et al., 1997).

The creek alternates between dry and wet periods and normally flows from November to May. It has a low gradient (<0.1%, channel slope), and drains into the North Inlet Salt Marsh (Wahl et al., 1997). The soil of the riparian bed is poorly drained and composed of an organic-rich layer followed by varying textured sands, shell, coarse sand, and mud. The surface water overlying the sediment is typical of blackwater streams of the southeastern USA with high concentrations of DOC, specifically humic acids (average DOC = 26 mg L^{-1}) and large amounts of total dissolved inorganic N discharged to the estuary (14 kg N yr^{-1}) (Wahl et al., 1997).

Dog Creek at Murrells Inlet is a suburban site located approximately 20 miles north of North Inlet, and like North Inlet it discharges into a bar-built estuary system (Vernberg et al., 1992) (Fig. 1b). The 13 km^2 surrounding Murrells Inlet has been extensively developed to accommodate residential growth (330% increase in the last decade) and intense tourist activity. Because of this development and industrial inputs, polycyclic aromatic hydrocarbons (PAHs), heavy metals, nitrate, and phosphate concentrations are elevated compared to the undeveloped preserve (Vernberg et al., 1992). The extent of pesticide usage in the residential area is unknown, although national pesticide surveys have revealed that approximately 63% of households have one to five pesticide products in storage and 22% have greater than five (Whitmore et al., 1992).

The Dog Creek watershed (11 ha) is characterized by housing, and a highly developed infrastructure to support the local residents and influxes of tourists. The stream was originally dug to serve as drainage for a housing development and a now defunct agricultural field. The channel is deeply excavated (2–3 m below water table) and cuts across natural topographic divides to provide maximum drainage. The channel slope is 0.34%. Land cover now consists of 82% turf grasses and impervious surfaces (12% = 1.2 ha roads, 0.5 ha housing, 0.25 ha commercial buildings). The soil is composed of an organic-rich surface layer followed by course and fine sands, a coquina layer, and various gleyed sands (Wahl et al., 1997).

Land development at the golf course consists of 360 condominium units surrounding an 18 hole course (Fig. 1c). The soils in the drainage ditches and stream beds along the fairways are almost 100% sand below a 5-cm layer of organic humus. The banks of the water bodies are cut steeply, with channels running 2 m deep (significantly below water table) to provide maximum drainage. Vegetative cover consists of scattered trees and shrubs on one side and groomed grasses on the other. Maintenance of the greens requires routine applications of pesticide and fertilizer.

Sediment and Field Data Collection

Sediment cores were collected from 0 to 15 cm depths with an ethanol sterilized hand auger (5 cm diam.) from the center of each riparian channel or tidal creek in May, July, and October 1996. Sediments were collected into autoclaved, 1 L, wide-mouthed mason jars that were filled completely with sediment and pore water to minimize headspace, and immediately put on ice and returned to the laboratory. Prior to incubation, 30 g of sediment and associated pore water from each location were extracted through a modified Luke method (Method 302—U.S. Food and Drug Administration, 1996) at the South Carolina Department of Agriculture to assess the background pesticide contamination. Results of these extractions were quantified against standard curves generated for over 70 pesticide standards. The sediment was analyzed for carbamate compounds on a Millipore Waters high pressure liquid chromatograph (Milford, MA). Remaining classes of pesticides were analyzed either on a Perkin Elmer 8500 gas chromatograph (GC) equipped with a flame photometric detector and an electron capture detector (ECD), or a Hewlett

Packard 6890 GC equipped with an ECD and a nitrogen phosphorus detector.

Percent organic C was determined for sediments by drying and weighing a subsample of sediment, burning in the muffle furnace at 450°C for 24 h, and weighing the residue. Samples were then analyzed for sediment particle size by wet sieving according to the standard procedure as described by Lewis (1984). The percentage of silt and clay was determined through hydrometer readings as described by the American Society for Testing and Materials (1985).

At the time of sediment collection, surface water was collected and one sample from each site at each collection time was analyzed for nitrate, dissolved oxygen, redox potential, conductivity, temperature, and pH. Water samples were filtered through a 0.2 μm polysulfone Whatman filter directly into Nalgene autoclaved amber bottles, placed on ice for transport, and frozen until analysis. Nitrate concentrations were measured using an automated chemistry analyzer (Shena Inc., Lithonia, GA) equipped with a cadmium reduction column and automatic data acquisition system or manually as described in Aelion et al. (1997). Temperature, redox, pH, and conductivity were measured in the field using a YSI 3500 water quality monitor (Yellow Springs Instrument Co., Yellow Springs, OH). DO was measured in the field using AccuVac ampules indigo carmine method 8316 (Hach Co., Loveland, CO).

Microcosm Incubations

Sediments were homogenized in a commercial blender with sufficient autoclaved surface water to bring the water content to 40% (w/w). The slurry was then transferred in 10 g aliquots to 100 mL serum bottles (Wheaton, Millville, NJ) and spiked with a mixture of [U-ring-^{14}C]atrazine (Sigma Chemical Company, St. Louis, MO, specific activity of 18.6 mCi mmol^{-1}, 99.8% purity), and unlabeled (cold) atrazine (1:80) bringing the final average concentration in each vial to approximately 5 mg atrazine kg^{-1} slurry (7.64 mg atrazine kg^{-1} dry soil). Hanging base traps containing 0.4 mL 2 *N* NaOH were sealed in each vial to collect $^{14}CO_2$ evolved. Biological activity was inhibited in control vials with 26.7 mg mercuric chloride kg^{-1} dry soil and 7.6 mg sodium azide kg^{-1} dry soil. Replicates containing ^{14}C-sodium bicarbonate ($NaH^{14}CO_3$) at 5.0 mg $NaH^{14}CO_3$ kg^{-1} dry soil (ICN Biomedicals, Irvine, CA; specific activity 58 mCi mmol^{-1}) were incubated simultaneously to assess $^{14}CO_2$ recovery efficiency. Disintegrations per minute (dpm) in live samples were corrected for abiotic activity and $^{14}CO_2$ recovery efficiencies. For most experiments unless noted otherwise, four to six live samples and three inhibited controls were used per time point for each treatment.

At selected times, vials were injected with 1 mL 4 *N* H_2SO_4 to drive $^{14}CO_2$ trapped in the sediment to the receiving base trap. The contents of each base trap were transferred to 10 mL of scintillation cocktail and analyzed for $^{14}CO_2$ activity on a Packard Tricarb 460 C (Downers Grove, IL) Liquid Scintillation System (LSC). In addition to incubation with ^{14}C-atrazine, sediments were spiked with an easily metabolized L-[U-^{14}C] amino acids mixture containing 15 L-amino acids (Amersham Corporation, Arlington Heights, IL; purity ≥97%) at 2.29 mg kg^{-1} dry soil to compare the general microbial activity at each site.

The golf course sediment received significantly more nitrogenous fertilizers than the other sites. To determine what effect this may have upon the mineralization of atrazine (inhibition or enhancement), vials from each location were amended with sodium nitrate at four concentrations (0, 6, 13, and 26 × 10^3 mg NO_3 kg^{-1} dry soil) and mineralization was compared.

Similarly, because the three study locations received different exposure to pesticides, the effect of pre-exposure on atrazine mineralization was examined by pretreating sediments with cold atrazine before serum bottle incubation (three live and three control vials per treatment per location per time point). Sediment from the suburban neighborhood was dosed once with cold atrazine (16 mg kg^{-1} dry soil) 2 mo prior to incubation while sediments from the golf course and the forested preserve were dosed twice with cold atrazine; once 4 mo prior to incubation (25 mg kg^{-1} dry soil) and once again 2 mo prior to incubation (16 mg kg^{-1} dry soil) bringing the final concentration of atrazine added in these sediments to 41 mg kg^{-1} dry soil. During the acclimation period, sediments were kept in the dark at room temperature while unexposed sediments collected at the same time were kept refrigerated at 4°C.

At the beginning of the incubation, 5 mg atrazine kg^{-1} slurry (1:80/hot:cold (7.64 mg atrazine kg^{-1} dry soil)) was added to the unacclimated sediments, and 0.061 μg ^{14}C-atrazine was added to the pre-exposed sediments resulting in a final hot to cold ratio in the acclimated sediments from the undeveloped preserve, the golf course, and the suburban neighborhood of 1:673, 1:673, and 1:404, respectively. In addition, all vials received 100 mg of D-glucose on Day 22 and Day 53 to stimulate microbial activity during the experimental incubations.

Mass Balance

A mass balance was calculated at selected times during the incubation to determine which fraction of ^{14}C activity was associated with liquid vs. sediment, and to estimate the extent of metabolite formation. Following $^{14}CO_2$ recovery, 20 mL of a sodium pyrophosphate (PPi)/polyvinylpyrrolidone (PVP) (0.6%/6.0%) solution were added to three live and three control vials and the contents transferred to respective 35-mL glass centrifuge tubes, modified from Dobbins and Pfaender (1988). Tubes were laid flat and shaken on a Lab-Line Orbit Shaker (Lab-Line Instruments, Melrose Park, IL) at 180 rpm overnight and subsequently spun down at 120 × *g* for 20 min on an IEC B-20A Centrifuge (International Equipment Co., Needham Hts., MA) to separate the sediment fraction (pellet) from the bacterial cells and water soluble portion of ^{14}C-atrazine (supernatant).

The supernatant was passed through a preconditioned 0.2 μm cellulose filter (Whatman, Kent, England) and tested for ^{14}C activity. The filter represented bacterial cells and liquid-phase colloidal material. This filtrate was passed through a Super Clean Sep-Pak LC-18 Solid Phase Extraction (SPE) cartridge (Supelco Chromatography Products, Bellefonte, PA) to estimate the extent of metabolite formation. The SPE cartridge was then flushed with 2 mL of deionized water and ^{14}C polar compounds were collected. This was followed by a methanol wash to collect moderately polar ^{14}C compounds and a final hexane rinse to collect nonpolar ^{14}C compounds. One milliliter of the original centrifuge supernatant was counted on the LSC prior to all manipulations to ensure ^{14}C activity was not lost during the segregation of the liquid fraction. The sediment pellet remaining in the centrifuge tube was extracted stepwise with methanol and hexane (extractable ^{14}C compounds) and finally resuspended with water and tested directly for ^{14}C activity (unextractable ^{14}C compounds).

Statistics

A sample size of six live replicates and four inhibited controls resulted in a statistical power >90 (0.75 SD). After satisfactory duplication of results, this number was reduced to five live and three inhibited controls in the N-amended experiments, and three live and three inhibited controls in the acclimated sediment experiments and the mass balance extractions.

Table 1. Environmental parameters measured in sediment and surface water from the undeveloped preserve, suburban neighborhood, and golf course.

Site sampled	Sample type	H_2O Level	DO	Temp. °C	Cond.	pH	Redox	Org. C	Porosity	Silt & clay	Sand
		cm	mg/L		mmhoh/cm		mv	%			
Undeveloped	Soil			27.7		6.2‡	−31	5.0	61.9	3.9	96.1
	H_2O	0	ND†	28.7	ND						
Suburban	Soil			25.1				4.4	58.4	3.9	96.1
	H_2O	10	5.0	24.1	0.17	6.3	−17				
Golf course	Soil			26.6				1.6	47.3	0.0	100.0
	H_2O	10	8.1	32.8	0.19	7.6	−45				

† ND = Not detected.
‡ No water present at site, pH taken from pore water.

Average inhibited control $^{14}CO_2$ recovery was subtracted from that of live treatments, adjusted for recovery efficiency, and then compared across treatments in a two factor analysis of variance. Multiple comparisons were made when necessary using the Bonferroni approach to preserve the integrity of alpha (Neter et al., 1990). Values also were compared to direct injections of ^{14}C-atrazine made during the experimental setup to calculate percentage recovery of initial ^{14}C-activity added. In the sediment and SPE extractions, similar comparisons were made across all treatments between a subset of two live and two inhibited controls. Degradation rates were estimated over time by the slope of a regression line fit to a plot of the percentage of initial label respired after adjusting for inhibited controls and $^{14}CO_2$ recovery efficiencies. All statistical calculations were conducted using SAS (PROC GLM) or Sigma Stat (SAS Institute, 1989; Jandel Scientific, 1994).

RESULTS

Site Characterization

Sediments from the undeveloped and suburban locations contained a larger percentage of organic C, silt, and clay fractions than the golf course sediments that were comprised almost entirely of sand (Table 1). Sediment extractions indicated that the golf course also contained lower concentrations of pesticides and/or fewer pesticides than the other sites despite the greater pesticide inputs received by the golf course (Table 2). The undeveloped preserve contained two or three compounds. Captan and/or a DDT metabolite was present at an elevated level, despite this area's status as an undeveloped preserve. Because these compounds co-elute, it was not possible to positively identify which of the two pesticides was present. To ensure the validity of this detection, sediment collection and extraction were performed on two separate occasions with similar results. Finally, nutrient analyses revealed that the golf course is periodically exposed to widely varying amounts of N (Fig. 2a,b). Concentrations in surface water ranged from approximately 15 to 1200 μM NO_3–N compared to below detection limit to 20 μM NO_3–N at the other two sites.

Table 2. Concentrations of pesticides extracted from sediment samples collected from the undeveloped preserve, suburban neighborhood, and golf course.

Pesticide	Undeveloped	Suburban	Golf course
	mg/kg		
Aldrin	0.053	ND	ND
Atrazine	ND†	ND	ND
Benz. hexachloride	ND	0.007	0.003
Captan/DDT	38.72	0.887	0.287
Dicloran	ND	0.006	ND
Endo sulfate	ND	0.002	0.001
Endosulfan I	ND	0.02	ND
Endrin	ND	0.011	ND
Hexachlorobenzene	ND	0.016	0.002

† ND = Not detected.

Mineralization of Amino Acids and Atrazine

Amino acid mineralization was used to assess whether there were any differences in the overall bacterial activity levels of the three sites. Bacteria from all sites were expected to degrade amino acids, and did use ^{14}C amino acids with the majority of mineralization occurring within the first 6 h (Fig. 3a) after which the rate of ^{14}C amino acids mineralization decreased in all sediments. Assuming mineralization of amino acids by bacteria accounts for approximately 50% or less of the breakdown

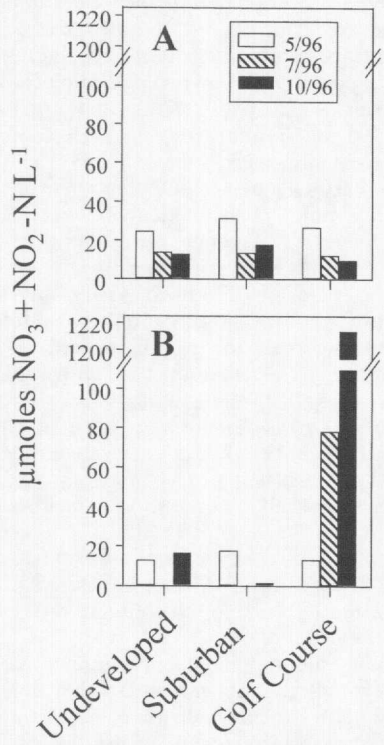

Fig. 2. Nitrate + nitrite–N concentrations (μM) for (*A*) pore water and (*B*) surface water collected from the undeveloped, suburban, and golf course sites.

of amino acids, with cellular incorporation accounting for the remainder (Swindoll et al., 1988), complete utilization of the ^{14}C amino acids occurred at all sites. The recovery of $NaH^{14}CO_3$ ranged from 108.2 to 98.5% over time. The golf course sediments exhibited a higher maximum potential rate estimated from a linear regression of the respiration curve from 0 to 6 h (5.4% h^{-1}) than the undeveloped and suburban sites (4.6 and 4.1% h^{-1}, respectively) while the suburban area had a significantly lower maximum percent mineralization than the golf course and forested site (30% vs. 35 and 45%, respectively).

The maximum potential rate of atrazine mineralization calculated from Day 0 to Day 9 was greatest in the golf course sediments at 0.04% d^{-1} (Fig. 3b). Mean $^{14}CO_2$ produced in all three sediments was significantly different (P-value < 0.0125) with the maximum $^{14}CO_2$ value occurring in the golf course sediment after 69 d (0.41% ± 0.04) compared to 0.2 and 0.35% in the suburban and undeveloped sites, respectively. Regardless of the greater $^{14}CO_2$ produced at the golf course sediments, <1% of the atrazine added was mineralized in all sediments. No significant differences among rates of atrazine mineralization by site were found after Day 9. The $^{14}CO_2$ recovery efficiency rates ranged from 116.6 to 93.8% over time.

Nitrogen Amendments

Four levels of $NaNO_3$ (0, 6, 13, 26 g NO_3 kg^{-1} dry soil) were added to sediments to normalize for the large discrepancy in surface water N concentrations found between sites. The $^{14}CO_2$ recovery efficiencies ranged from 79.2 to 40.9% over time. There were no differences in atrazine mineralization rates at each individual site over the four levels of nitrate amendments when compared to an alpha of 0.0028 as dictated by the Bonferroni approach to multiple comparisons (Fig. 4a,b,c). The golf course sediments again exhibited the greatest amount of ^{14}C-atrazine mineralization (approximately 0.8 vs. 0.3% for the two other study sites).

Repeated Exposures and Glucose Amendments

Mean $^{14}CO_2$ production in acclimated (Oyster and golf course, 41 mg kg^{-1} dry soil; or Dog, 16 mg kg^{-1} dry soil) and unacclimated sediments (all sites, 5 mg kg^{-1} dry soil) from the undeveloped preserve was not significantly different by Day 22 and indicated minimal degradation (Fig. 5a). Microbes in preexposed sediments began to degrade atrazine after Day 22 subsequent to the glucose supplement with the maximum amount of atrazine removed equaling 0.19 mg kg^{-1} dry soil

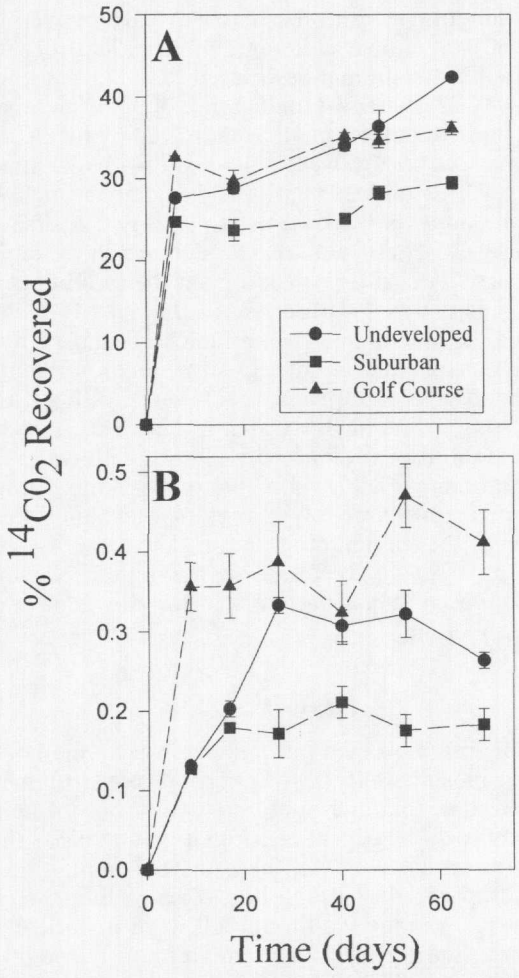

Fig. 3. Mineralization of (*A*) ^{14}C-amino acids and (*B*) ^{14}C-atrazine in sediments collected from the undeveloped, suburban, and golf course sites (± SE of the mean).

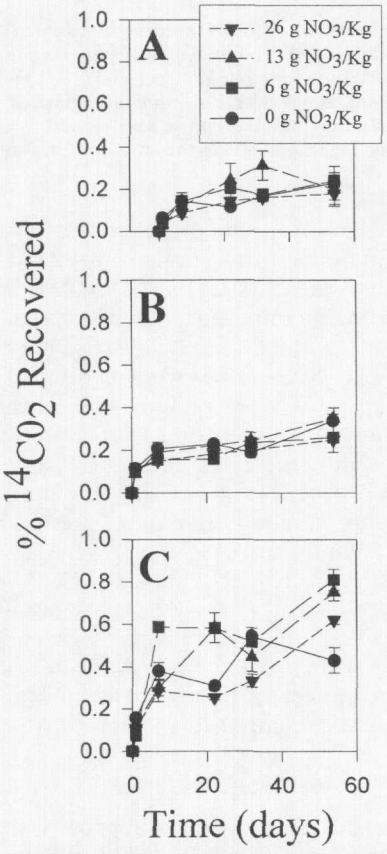

Fig. 4. Mineralization of ^{14}C-atrazine in $NaNO_3$-amended sediment collected from (*A*) the undeveloped, (*B*) suburban, and (*C*) golf course sites (± SE of the mean).

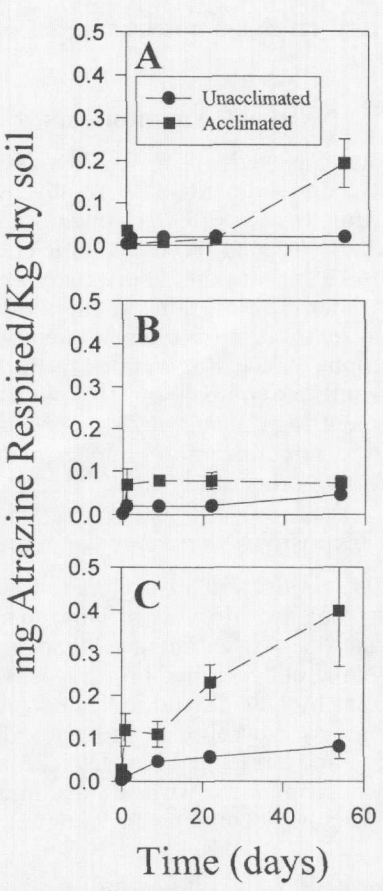

Fig. 5. Mineralization of ^{14}C-URL atrazine in sediment collected from (*A*) the undeveloped (acclimated = 41 mg atrazine kg^{-1} dry soil; unacclimated 5 mg atrazine kg^{-1} dry soil), (*B*) suburban (acclimated = 25 mg atrazine kg^{-1} dry soil; unacclimated 5 mg atrazine kg^{-1} dry soil), and (*C*) golf course (acclimated = 41 mg atrazine kg^{-1} dry soil; unacclimated 5 mg atrazine kg^{-1} dry soil) sites (± SE of the mean).

(± 0.033). Microbes in the pre-exposed sediments from the suburban neighborhood mineralized 0.068 mg atrazine kg^{-1} dry soil (±0.021) by Day 1 (Fig. 5b). The amount of atrazine mineralized in unexposed sediments did not increase until after Day 22 (0.045 mg kg^{-1} dry soil ± 0.005). Mean $^{14}CO_2$ values were significantly different between the pre-exposed and unexposed sediments.

Mean values for $^{14}CO_2$ production in pre-exposed and unexposed sediments from the golf course also were significantly different (Fig. 5c). In acclimated sediments, rates were approximately five times greater than in unacclimated sediments (6.23×10^{-3} vs. 1.32×10^{-3} mg kg^{-1} dry soil d^{-1}, respectively). By Day 58, 0.39 mg atrazine kg^{-1} dry soil (±0.076) was mineralized in the pre-exposed sediments. Recovery efficiencies ranged from 118 to 44.8% over time. As found in previous experiments, mineralization of atrazine was greatest at the golf course compared to the other two locations.

Mass Balance

Over time, the relative percentage of ^{14}C in liquid and sediment fractions was measured. Time 0 represented an initial distribution of parent compound prior to any biological transformations. The ^{14}C trapped by the 2 μm filter representing ^{14}C in bacterial cells or associated with colloids, was identical in both live and inhibited treatments and <1% of the initial ^{14}C-atrazine added suggesting this activity may be associated with colloidal matter and not cellular uptake (Tables 3a,b,c,d).

On Day 1, the liquid fraction accounted for approximately 18 to 39% of the initial atrazine indicating a considerable portion of the activity had already become associated with the sediment fraction (Table 3b). In contrast, controls that were sacrificed immediately (time = 0) had 60 to 75% of the ^{14}C activity still associated with the liquid fraction (Table 3a).

The liquid fraction was then passed through a SPE cartridge. Based on these preliminary experiments, at time 0 it is expected if no transformation of atrazine occurs, then approximately 54 to 70% of the parent compound found in the liquid fraction should elute with MeOH while only 3% should elute with water (Table 3a). The remaining 30 to 40% was expected to be associated with the sediment fraction. In experimental microcosms, a lack of significant activity in the water eluate indicated that significant metabolite production (more polar compounds) had not yet occurred. A large amount of activity was readily extracted from all sediments indicating that the majority of the radiolabel associated with the sediment fraction was still easily extractable (Table 3b). The golf course sediment, with the least organic C had the least sediment-associated ^{14}C.

By Day 22, there was an increase of polar metabolites in the golf course sediment as indicated by the ^{14}C activity measured in the water eluate (Table 3c), approximately 7, 12, and 30% of the ^{14}C activity recovered from the undeveloped, suburban, and golf course sites, respectively. There was also a decrease in the amount of ^{14}C activity easily extracted from the sediments in all sites. This coupled with a rise in the activity tested in the final sediment suspension indicated that with time, an increasing amount of ^{14}C-atrazine or unidentified metabolites became progressively more difficult to extract from sediment. By Day 54, there was a decrease in the amount of radioactivity eluting with water in all live treatments (Table 3d). The total sediment fraction contained approximately three to four times the ^{14}C activity of the total liquid fraction. The total ^{14}C activity recovered (as a percentage of the initial ^{14}C activity added) was reduced particularly for the undeveloped site at this time.

DISCUSSION

Biodegradation of a pesticide in soil is regulated by the size and capability of the microbial consortium present as well as the environmental conditions influencing the activity of the degrader population and bioavailability of the compound. All these factors can be affected by land use. In this study, three sites with distinct land uses were examined, although all were historically agricultural. Sediments from all sites demonstrated preliminary mineralization of atrazine followed by a much slower rate in the suburban and undeveloped sediments that have relatively higher clay and organic C contents.

Table 3. Mass balance and metabolite separation from liquid and sediment following microcosm incubations with ^{14}C-atrazine. Solid phase extractions were carried out at (A) time 0, (B) Day 1, (C) Day 22 and (D) Day 54 following ^{14}C-atrazine addition.

A	Undeveloped	Suburban	Golf course
	Live	Live	Live
Respiration	ND†	ND	ND
Cellular uptake	4	4	5
H_2O eluate‡	3.0	3.0	3.0
MeOH eluate‡	68.0	54.0	70.0
Hexane eluate‡	2.0	3.0	2.0
Total liquid fraction	73.0	60.0	75.0
% of total recovery‡	70.2	57.7	76.5
Extractable§	22.0	33.0	17.0
Unextractable§	5.0	8.0	2.0
Total sediment fraction	27.0	41.0	19.0
% of total recovery§	26.0	39.4	19.4
Total ^{14}C recovery	104	104	98

B	Undeveloped		Suburban		Golf course	
	Live	Dead	Live	Dead	Live	Dead
Respiration	0.05	0.02	0.06	0.03	0.07	0.02
Cellular uptake	0.6	0.7	0.5	0.4	1.1	0.87
H_2O eluate	7.1	4.2	6.6	3.8	0.5	10.6
MeOH eluate	14.5	16.3	10.5	10.8	36.6	26.1
Hexane eluate	0.8	0.9	0.6	0.6	2.4	1.0
Total liquid fraction	22.4	21.4	17.7	15.2	39.4	37.7
% of total recovery	28.2	27.3	20.7	17.7	46.9	52.9
Extractable	40.9	48.3	55.3	56.6	34.6	24.4
Unextractable	15.4	8.1	12.3	13.3	8.9	8.3
Total sediment fraction	56.3	56.4	67.5	70.0	43.5	32.7
% of total recovery	70.9	71.8	78.7	81.8	51.7	45.8
Total ^{14}C recovery	79	78	86	86	84	71

C	Undeveloped		Suburban		Golf course	
	Live	Dead	Live	Dead	Live	Dead
Respiration	0.22	0.1	0.28	0.04	0.41	0.09
Cellular uptake	0.5	0.6	0.4	0.5	0.8	0.8
H_2O eluate	7.2	0.3	12.2	0.3	30.5	14.6
MeOH eluate	6.7	22.3	6.8	16.9	11.1	25.2
Hexane eluate	0.6	0.9	0.6	0.6	0.9	1.7
Total liquid fraction	14.5	23.5	19.6	17.8	42.5	41.5
% of total recovery	21.2	30.2	25.5	20.1	46.8	42.9
Extractable	24.1	36.6	28.6	47.2	24.5	28.7
Unextractable	28.9	17.0	28.0	22.8	22.7	25.6
Total sediment fraction	53.0	53.6	56.6	70.0	47.2	54.3
% of total recovery	77.7	68.9	73.6	79.2	51.9	56.2
Total ^{14}C recovery	68	78	77	88	91	97

D	Undeveloped		Suburban		Golf course	
	Live	Dead	Live	Dead	Live	Dead
Respiration	0.42	0.26	0.38	0.1	0.7	0.12
Cellular uptake	0.4	0.9	0.5	0.6	0.7	1.0
H_2O eluate	4.7	11.1	9.9	6.8	15.5	13.8
MeOH eluate	4.1	19.8	5.3	11.4	8.1	20.3
Hexane eluate	0.0	0.9	0.2	0.6	0.5	0.9
Total liquid fraction	8.8	31.9	15.4	18.8	24.1	34.9
% of total recovery	18.7	39.3	21.8	22.7	26.4	36.1
Extractable	18.0	39.3	28.2	46.6	22.7	25.3
Unextractable	19.5	8.7	26.5	16.7	43.3	35.5
Total sediment fraction	37.6	47.9	54.7	63.3	65.9	60.8
% of total recovery	79.6	59.2	77.1	76.5	72.1	62.8
Total ^{14}C recovery	47	81	71	83	91	97

† ND = Not detected.
‡ = Liquid fraction.
§ = Sediment fraction.

Conversely, the golf course sediments were 100% sand with limited sorption and greater mineralization. Regardless, atrazine mineralization in all sediments was minimal (<1%). Ring carbons were not assimilated by bacteria as evidenced by the lack of ^{14}C activity incorporated into cellular biomass. This is consistent with other research, and is most likely due to the high oxidation state of the ring carbons causing mineralization to be energetically unfavorable for bacteria (Goswami and Green, 1971; Wolf and Martin, 1975; Jones et al., 1982; Kruger et al., 1993). The presence of N in the ring may provide a nutrient source for bacteria but may only encourage ring mineralization in systems where N is limiting. Feakin et al. (1994) found the presence of N in concentrations >1.0 mg L^{-1} inhibited the microbial degradation of atrazine in surface waters as did Chung et al. (1995) in soils treated with 2.0 g L^{-1} NH_4NO_3.

Atrazine mineralization in the suburban and undeveloped sediments was not enhanced after the addition of up to 26 g NO_3 kg^{-1} dry soil and the increased mineralization seen in the golf course sediments could not be attributed to the frequent inputs of nitrogenous fertilizers. The lack of ammonium production, a side product of atrazine mineralization, further suggested that the ring nitrogens were not used by bacteria in these systems. Conversely, the sediment from the undeveloped preserve exhibited an increase in atrazine mineralization after the addition of glucose. This demonstrated that the C atoms were not available to the bacteria. Other researchers have shown that the addition of simple C sources can stimulate the mineralization of atrazine (Wolf and Martin, 1975; Kruger et al., 1993).

Preexposure of sediments by the addition of atrazine enhanced the mineralization of subsequent atrazine exposures in the undeveloped and golf course sediments. While other researchers have found rapid mineralization of atrazine after pretreating soil once with atrazine (Mirgain et al., 1993; Topp et al., 1995) we observed little difference in atrazine mineralization in suburban sediments. In all cases, the addition of a relatively high concentration of atrazine did not inhibit the activity of atrazine degrading microorganisms in any of these systems.

Although atrazine was not mineralized to a significant extent, it did undergo microbially-mediated transformations. Extractions of both liquid and sediment fractions performed over time indicated major differences in ^{14}C-activity between live and poisoned vials from all sediments. Recoveries in live vials were significantly lower than in inhibited vials as time progressed. The fraction of ^{14}C activity extracted with water and methanol decreased with time while the nonextractable fraction sorbed to the sediment increased, perhaps indicating both polar and nonpolar compound sorption to the sediments. The greatest formation of polar compounds occurred in the golf course further suggesting that atrazine may be more bioavailable in this sandy system.

Knaebel et al. (1994) reported that the environmental matrix has a profound effect upon the mineralization of chemicals in soils in addition to the chemical characteristics and mode of application of the compound. Sand and kaolinite, having primarily external binding sites, exhibited the highest mineralization rates while expanding clays with interstitial binding surfaces exhibited

the lowest. Furthermore chemicals with a high measured K_d or that were already adsorbed prior to inoculation with a bacterial consortium did not degrade as rapidly or as extensively as less sorptive chemicals. Gordon and Millero (1985) suggested that sorbed organic material is less available for bacterial degradation than that in a dissolved state and the decrease in bioavailability is correlated with the degree of adsorption and clays.

Differences in ^{14}C-recovery efficiencies between sites also suggested the importance of sorption. It was suspected that the loss in recovery efficiency over time was not distributed equally among the various extractions but was a function of the inefficiency in measuring the percentage of activity irreversibly sorbed to the sediment, specifically activity associated with the organic and clay fractions. This would explain recovery efficiencies of >90% after 54 d in the golf course (0% clay and silt, 16 g kg^{-1} organic matter) while efficiencies in the undeveloped (3.87% silt and clay, 49 g kg^{-1} organic matter) and suburban sediments (3.94% silt and clay, 44 g kg^{-1} organic matter) after 54 d were 47 and 71%, respectively. Physical sorption and chemisorption both contribute to retention of atrazine by soil organic matter (Laird et al., 1994). Moreau and Mouvet (1997) carried out detailed sorption/desorption studies with atrazine and several daughter products and found that HYA had greater affinity for organic matter and higher K_{oc} values than atrazine. Lerch et al. (1998) measured hydroxyatrazine in northern Missouri streams at a prevalence of 87 to 100% and hypothesized that dissolved-phase transport and sediment desorption controlled HA concentrations in streams.

Biological transformation of atrazine in our study appeared to produce daughter products with higher K_d or K_{oc} values than the parent compound. The identity of the compounds that resisted extraction was not assessed in this study. If the nonextractable compounds were comprised of the undegraded parent compound and the *N*-dealkylated metabolites (DIA and DEA), then there remains a risk to submerged aquatic vegetation. Conversely, if the nonextractable fraction were comprised primarily of the nonphytotoxic HYA polar compounds, then the risk to aquatic vegetation in these estuarine systems may be negligible. Because DIA and DEA metabolites were not present to a large extent in the extractable fractions in studies by Dao et al. (1979) and Jones et al. (1982), it is likely that the nonextractable compounds present in the sediment were in fact the *N*-dealkylated metabolites or the undegraded parent compound. This assumption was further supported by the fact that the amount of activity associated with the sediment suspensions was significantly lower in inhibited controls as would be the formation of DEA and DIA which are microbially-mediated transformation products.

Because atrazine has proven to be extremely resistant to mineralization based on field studies of natural microbial communities, and is in fact sorbed to estuarine sediments, the accumulation of residues and transformation products in estuarine systems appears likely. The effects atrazine will have may depend on the identity and bioavailability of this sorbed fraction that represents both the parent and the transformation products and can be elucidated using more sophisticated separation and identification techniques. Accounting for sorption, diffusion, and microbial growth, is critical for estimating pesticide bioavailability and biodegradation in the environment (Shelton and Doherty, 1997a,b).

Bacterial communities in our three study areas historically have been impacted by agriculture but currently are impacted by land use changes. These include channelization of coastal streams to provide maximum drainage for surrounding development that has changed intermittent low-flow black water steams into continually flowing systems (Wahl et al., 1997). Deforestation around the streams has reduced the amount of organic matter being recycled. In coastal golf course development extensive wetland areas and shallow water tables necessitate deforestation, channelization, and application of enormous amounts of sand which greatly reduces the clay and organic fractions of the soils. The suburban area may be particularly at risk as chemical applications increase with increased development, and biodegradation appears to be suppressed. Conversely, although other environmental impacts are associated with golf courses (Robinson and Snyder, 1991), the accumulation of atrazine or chemically-similar pesticides may be less likely as biodegradation is greater and less sorption occurs due to lack of organic C content in the sandy sediments. However, these same properties make transport to groundwater more likely in the golf course area, in agreement with the chemical studies of Moreau and Mouvet (1997).

ACKNOWLEDGMENTS

This research was supported by a grant from the National Oceanic and Atmospheric Administration, Office of Oceanic Research Programs (F.J. Vernberg and W.B. Vernberg, co-directors) and the National Science Foundation (93-50314). We greatly appreciate the facilities at the Belle W. Baruch Institute for Marine Biology and Coastal Research, and thank Mr. William Dahne for his interest and access to the golf course.

REFERENCES

Aelion, C.M., J.N. Shaw, and M.H. Wahl. 1997. Impact of suburbanization on ground water quality and denitrification in coastal aquifer sediments. J. Exp. Mar. Biol. Ecol. 213:31–51.

American Society for Testing and Materials. 1985. Standard test method for particle-size analysis of soils. D 422-453 (1972). 1985 Annual Book of ASTM Standards 04.08:117–127. ASTM, Philadelphia, PA.

Behki, R.M., E. Topp, W. Dick, and P. Germon. 1993. Metabolism of the herbicide atrazine by Rhodococcus strains. Appl. Environ. Microbiol. 59:1955–1959.

Caplan, J.A., A.R. Isensee, and J.O. Nelson. 1984. Fate and effect of [14C] fenvalerate in a tidal marsh sediment ecosystem model. J. Agric. Food Chem. 32:166–171.

Chung, K.H., K.S. Ro, and D. Roy. 1995. Atrazine biotransformation in wetland sediment under different nutrient conditions. I: Anaerobic. J. Environ. Sci. Health A30:109–120.

Dao, T.H., T.L. Lavy, and R.C. Sorenson. 1979. Atrazine degradation and residue distribution in soil. Soil Sci. Soc. Am. J. 43:1129–1133.

Dobbins, D.C., and F.K. Pfaender. 1988. Methodology for assessing respiration and cellular incorporation of radiolabeled substrates by soil microbial communities. Microb. Ecol. 15:257–273.

Feakin, S.J., E. Blackburn, and R.G. Burns. 1994. Biodegradation of *s*-triazine herbicides at low concentrations in surface waters. Water Res. 28:2289–2296.

Gordon, A.S., and F.J. Milero. 1985. Adsorption mediated decrease in the biodegradation rate of organic compounds. Microb. Ecol. 11:289–298.

Goswami, K.P., and R.E. Green. 1971. Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. Environ. Sci. Technol. 5:426–429.

Huber, W. 1993. Ecotoxicological relevance of atrazine in aquatic systems. Environ. Toxicol. Chem. 12:1865–1881.

Isensee, A.R. 1987. Persistence and movement of atrazine in a salt marsh sediment microecosystem. Bull. Environ. Contam. Toxicol. 39:516–523.

Jandel Scientific. 1994. SigmaStat™ statistical software user's manual. Jandel Sci., San Rafael, CA.

Jones, T.W., W.M. Kemp, J.C. Stevenson, and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632–638.

Kaufman, D.D., and P.C. Kearney. 1970. Microbial degradation of *s*-triazine herbicides. Residue Rev. 32:235–265.

Knaebel, D.B., T.W. Federle, D.C. McAvoy, and J.R. Vestal. 1994. Effect of mineral and soil constituents on microbial mineralization of organic compounds in a natural soil. Appl. Environ. Microbiol. 60:4500–4508.

Kruger, E.L., L. Somasundaram, R.S. Kanwar, and J.R. Coats. 1993. Persistence and degradation of [^{14}C] atrazine and [^{14}C] deisopropyl-atrazine as affected by soil depth and moisture conditions. Environ. Chem. 12:1959–1967.

Kucklick, J.R., and T.F. Bidleman. 1994. Organic contaminants in Winyah Bay, South Carolina I: Pesticides and polycyclic aromatic hydrocarbons in subsurface and microlayer waters. Mar. Environ. Res. 37:63–78.

Laird, D.A., P.Y. Yen, W.C. Koskinen, T.R. Steinheimer, and R.H. Dowdy. 1994. Sorption of atrazine on soil clay components. Environ. Sci. Technol. 28:1054–1061.

Lerch, R.N., P.E. Blanchard, and E.M. Thurman. 1998. Contribution of hydroxylated atrazine degradation products to the total atrazine load in Midwestern streams. Environ. Sci. Technol. 32:40–48.

Lewis, D.W. 1984. Practical sedimentology. Hutchison & Ross, New York.

Mandelbaum, R.T., L.P. Wackett, and D.L. Allen. 1993. Rapid hydrolysis of atrazine to hydroxyatrazine by soil bacteria. Environ. Sci. Technol. 27:1943–1946.

Miles, C.J., G. Leong, and S. Dollar. 1992. Pesticides in marine sediments associated with golf course runoff. Bull. Environ. Contam. Toxicol. 49:179–185.

Mirgain, I., G.A. Green, and H. Monteil. 1993. Degradation of atrazine in laboratory microcosms: Isolation and identification of biodegrading bacteria. Environ. Toxicol. Chem. 12:1627–1643.

Moreau, C., and C. Mouvet. 1997. Sorption and desorption of atrazine, deethylatrazine, and hydroxyatrazine by soil and aquifer solids. J. Environ. Qual. 26:416–424.

Neter, J., W. Wasserman, and M. Kutner. 1990. Applied linear statistical models. Irwin, Homewood, IL.

Pait, A.S., A.E. De Souza, and D.R.G. Farrow. 1992. Agricultural pesticide use in coastal areas: A national summary. Strategic Environmental Assessments Div., National Oceanic and Atmospheric Administration, Rockville, MD.

Radosevich, M., S.J. Traina, Y. Hao, and O.H. Tuovinen. 1995. Degradation and mineralization of atrazine by a soil bacterial isolate. Appl. Environ. Microbiol. 61:297–302.

Robinson, J.H., and H.S. Snyder. 1991. Golf course development concerns in coastal zone management. Coastal Zone '91. p. 431–443. *In* O.T. Magoon et al. (ed.) Proc. of the 7th Symp. on Coastal and Ocean Management. Am. Soc. of Civil Eng., New York.

SAS Institute. 1989. SAS/STAT® user's guide. Version 6. 4th ed. Vol. 1. SAS Inst., Cary, NC.

Shelton, D.R., and M.A. Doherty. 1997a. A model describing pesticide bioavailability and biodegradation in soil. Soil Sci. Soc. Am. J. 61:1078–1084.

Shelton, D.R., and M.A. Doherty. 1997b. Estimating losses of efficiency due to pesticide biodegradation in soil: Model simulations. Soil Sci. Soc. Am. J. 61:1085–1090.

Solomon, K.R., D.B. Baker, R.P. Richards, K.R. Dixon, S.J. Klaine, T.W. La Point, R.J. Kendall, C.P. Weisskopf, J.M. Giddings, J.P. Giesy, L.W. Hall, Jr., and W.M. Williams. 1996. Ecological risk assessment of atrazine in North American surface waters. Environ. Toxicol. Chem. 15:31–76.

Swindoll, C.M., C.M. Aelion, D.C. Dobbins, O. Jiang, S.C. Long, and F.K. Pfaender. 1988. Aerobic biodegradation of natural and xenobiotic organic compounds by subsurface microbial communities. Environ. Toxicol. Chem. 7:291–299.

Topp, E., D.W. Gutzman, B. Bourgoin, J. Millette, and D.S. Gamble. 1995. Rapid mineralization of the herbicide atrazine in alluvial sediments and enrichment cultures. Environ. Toxicol. Chem. 14:743–747.

U.S. Environmental Protection Agency. 1994. Atrazine, simazine, and cyanazine, notice of initiation of special review. Fed. Reg. 59:60412–60443.

U.S. Food and Drug Administration. 1996. Pesticide analytical manual. Vol. I. B.M. McMahon and R.F. Wagner (ed.) U.S. Food and Drug Admin., Washington, DC.

Vernberg, F.J., W.B. Vernberg, E.R. Blood, A. Fortner, M. Fulton, H.N. McKeller, W.K. Michener, G. Scott, T. Siewicki, and K. El Figi. 1992. Impact of urbanization on high-salinity estuaries in the southeastern United States. Neth. J. Sea Res. 30:239–248.

Wahl, M.H., H.N. McKeller, and T.M. Williams. 1997. Patterns of nutrient loading in forested and urbanized coastal streams. J. Exp. Mar. Biol. Ecol. 213:111–131.

Whitmore, R.W., J.E. Kelly, and P.L. Reading. 1992. National home and garden pesticide use survey: Final report: Executive summary. p. 1–22. *In* RTI/5100/17-03F. Research Triangle Inst., Research Triangle Park, NC.

Wolf, D.C., and J.P. Martin. 1975. Microbial decomposition of ring ^{14}C-atrazine, cyanuric acid, and 2-chloro-4,6-diamino-*s*-triazine. J. Environ. Qual. 4:134–139.

Yanze-Kontchou, C., and N. Gschwind. 1994. Mineralization of the herbicide atrazine as a carbon source by a pseudomonas strain. Appl. Environ. Microbiol. 60:4297–4302.