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ATRAZINE BIODEGRADATION TO DEISOPROPYLATRAZINE AND DEETHYLATRAZINE IN COASTAL SEDIMENTS OF DIFFERENT LAND USES

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Abstract—Atrazine, a triazine herbicide widely used in the United States, contributes to surface-water and groundwater contamination, as can deisopropylatrazine (DIA) and deethylatrazine (DEA), two of its microbial degradation products. Production of DIA and DEA by native bacteria in aquatic sediments has not been investigated thoroughly. We assessed atrazine and production of DIA and DEA over time in coastal aquatic sediments associated with different land uses including creeks from an undeveloped preserve and a suburban development, a golf course drainage ditch, and a contaminated commercial harbor. Sediments were incubated in microcosms, spiked with U-^{14}C-atrazine, extracted, and analyzed for ^{14}C in a liquid scintillation counter. Atrazine, DIA, and DEA also were quantified by gas chromatography–mass spectrometry. The amount of ^{14}C recovered varied at each site as a function of the sediment organic carbon content and decreased significantly over time. Both DEA and DIA were measured primarily in the aqueous phase. Transformation was more extensive to DIA than to DEA. The ratio of DIA to atrazine recovered from the undeveloped preserve was as high as 0.13. In contrast, the golf course had limited biotransformation, and had the greatest atrazine recoveries so atrazine, not DEA and DIA, may have a greater impact at this site.

Keywords—Atrazine Deisopropylatrazine Deethylatrazine Biodegradation Pesticides

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) has been widely used in the United States for decades. Atrazine is a preemergent herbicide used to control broadleaf and grassy weeds in the agricultural production of crops such as corn, as well as on fallow and industrial lands [1–4]. Non–point-source effluents contribute to atrazine contamination of groundwater and surface water [5], and atrazine was detected more frequently in shallow groundwater in the United States than any other pesticide [6].

Studies indicate that deisopropylatrazine (DIA; 2-amino-4-chloro-6-isopropylamino-*s*-triazine) and deethylatrazine (DEA; 2-amino-4-chloro-6-ethylamino-*s*-triazine), two of the major biodegradation products of atrazine, are formed from the dealkylation of the isopropyl and ethyl side groups, respectively, and can be subsequently degraded to didealkylatrazine (DDA). Because they remain chlorinated, these degradation products retain their phytotoxic property [7], similar to their parent compound, atrazine [1,5]. Atrazine and its degradation products are suspected to be endocrine disruptors, thus, interest is increasing in the transformation and fate of this pesticide [8]. Hydroxyatrazine (HYA), a less toxic transformation product of atrazine, is formed when atrazine is hydrolyzed at the chlorine atom position. Hydroxyatrazine strongly sorbs and is not considered to be mobile in sediment [9].

Many researchers have measured atrazine and its metabolites in groundwater and surface waters in the United States and found them to be frequently contaminated with atrazine [3] and its microbial metabolites DIA and DEA [4]. Berg et al. [10] found that DEA concentrations in natural waters such as rivers, lakes, and groundwater were present in higher concentrations than atrazine, and Lerch et al. [11] found HYA to be present at greater concentrations than atrazine, DEA, or DIA in midwestern agricultural soils. The majority of published studies measured atrazine and its degradation products in natural waters, and limited research has been carried out on the production of these metabolites by native, unenhanced bacteria in aquatic sediment systems.

The potential of atrazine, HYA, DIA, and DEA to contaminate sediment and groundwater depends on factors such as their half-life in sediment, solubility in water, and sorption to soil particles. Atrazine's long half-life is estimated to be 60 to >100 d in soil [4,12]. In microcosm studies, the half-life of DIA and DEA ranged from a few weeks [1,2] to 100 d [13]. Atrazine, DIA, and DEA can form strong bonds with sediment organic carbon, preventing their movement through the sediment [14]. However, because atrazine is moderately water soluble (33 mg/L) and DIA and DEA are more soluble (670 mg/L and 3,200 mg/L, respectively), all have the potential to contaminate groundwater, particularly in sediment with low clay and low organic carbon content. Both DIA and DEA have lower organic carbon partition coefficients (K_{oc}; 128 L/kg and 80 L/kg, respectively, in sediment with 1.3% organic carbon) than atrazine (140 L/kg in sediment with 1.3% organic carbon) [15], so sediment characteristics are expected to impact the distribution of these chemicals between natural sediment and aqueous systems.

Although atrazine is known to be completely biodegraded in pure culture studies [16], it seems to resist complete mineralization by native microbial communities [17,18], stressing the importance of carrying out experiments with natural sediment and native microbial communities to assess the potential impact of these contaminants on environmental systems. The objectives of this study were to quantify the relative concentrations of DIA and DEA produced in U-^{14}C-atrazine–amended

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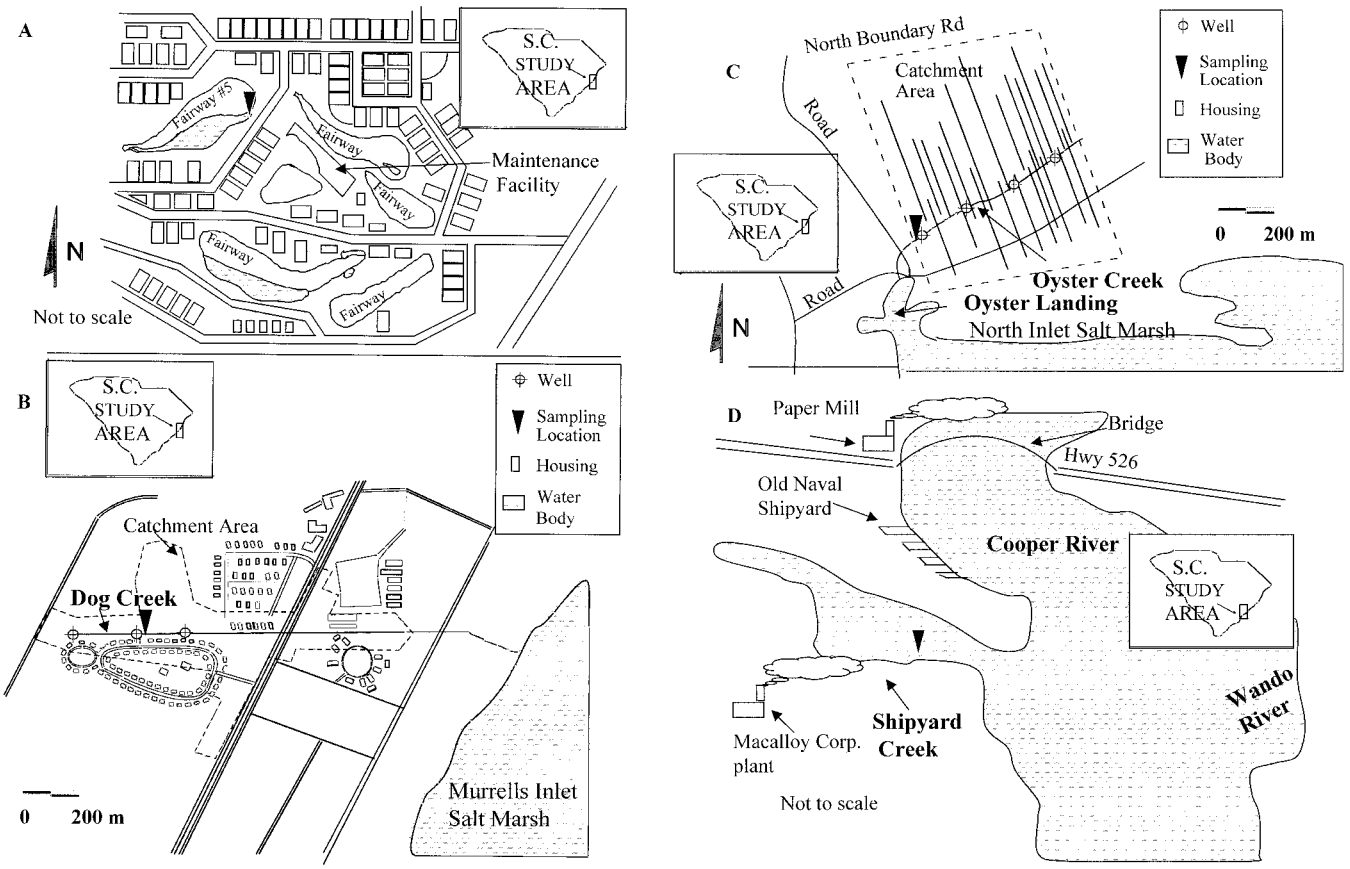

Fig. 1. Coastal sampling locations at (**A**) Oyster Creek and Oyster Landing at North Inlet, (**B**) Dog Creek in Murrells Inlet, (**C**) the golf course at Pawleys Island, and (**D**) Shipyard Creek in Charleston Harbor (SC, USA).

microcosms with natural aquatic sediments of different land uses. Aquatic sediments were collected at four sites in coastal South Carolina, USA, that ranged in land use from an undeveloped, forested nature preserve, to a highly industrialized commercial harbor.

MATERIALS AND METHODS

Site description

Aquatic sediment was collected from four sites with distinct land uses, including a drainage ditch at a golf course located on Pawleys Island; Dog Creek, a small black-water creek located in a suburban residential area in Murrells Inlet; Oyster Creek, a small black-water creek located in the forested uplands of an undeveloped nature preserve in North Inlet; Oyster Landing, located in the estuarine area of North Inlet; and Shipyard Creek, a polluted, industrialized navigable waterway in Charleston Harbor (SC, USA) (Fig. 1).

The golf course was located in a country club resort 16 km north of North Inlet. Housing around the course has increased since its development more than 20 years ago. Pawleys Island has light residential and commercial development and supports a large tourist industry. The 18-hole golf course received fertilizer and pesticide input; however, atrazine was not used at this site. To maintain the greens, they were fertilized once a month and the fairways, tees, and rough were fertilized twice a year. Sediment and surface water samples were collected from a drainage pond that was approximately 2 m deep in the center and 15 m across at its widest section [19].

Dog Creek is a small black-water stream approximately 1.5 m wide that drains into Murrells Inlet salt marsh. Dog Creek contained water all year-round because it was excavated across the natural groundwater divide to provide drainage for the adjacent residential development. Located 30 km north of North Inlet estuary in a suburban watershed with a 95-acre drainage area [20], this area has extensive impervious surfaces (roads), limited agricultural and industrial development [21], and increasing tourism. Residential development in this area has increased 330% in the last decade [19] as have concentrations of polynuclear aromatic hydrocarbons, heavy metals, nitrate, and phosphate [22].

Oyster Creek, also a small black-water creek (3 m wide), drains into the pristine North Inlet, an undeveloped, natural preserve that is part of the Belle W. Baruch Institute for Marine Biology and Coastal Research. Oyster Creek is in a low-gradient watershed (92 acres) characterized by dense stands of pine hardwoods [20], as well as forested wetlands. The forested estuary receives essentially no input from cars, boats, or tourism. Although exogenous input should be limited to atmospheric deposition, chlorinated pesticides (aldrin, 0.05 mg/kg, and captan or DDT, 38.7 mg/kg) were detected in sediment samples from Oyster Creek, probably from agricultural activity in the 1950s, during which time these pesticides were used routinely [17]. Sediments were collected in both the freshwater upland area of Oyster Creek, and Oyster Landing, the saline (32‰) estuarine area.

Shipyard Creek is approximately 103 km south of North Inlet near Charleston, one of the largest cities in South Carolina, which has a large tourist industry, and residential, com-

mercial, and industrial development. Shipyard Creek is a major navigable body of water (salinity 23‰) located in Charleston Harbor, a major commercial port, and drains into the Cooper River. Charleston Harbor is heavily contaminated with metals and organic chemicals such as chromium, nickel, cadmium, and polynuclear aromatic hydrocarbons from industrial sources, naval shipyards, sewage-treatment facilities, and hazardous waste sites. Sediment samples were collected from a research vessel with the assistance of Naval Research Laboratory personnel.

Sample collection

Sediments from the golf course, Dog Creek, Oyster Creek, and Oyster Landing were collected at a depth of 15 to 30 cm (6–12 inches) with a hand auger. The hand auger was rinsed with ethanol and autoclaved deionized water after each collection to avoid cross-contamination. Sediment was placed in autoclaved mason jars with a sterile spatula. Any excess water was decanted and jars were filled completely with sediment to avoid headspace. Sediment samples were placed on ice and transferred to the laboratory where they were stored at 4°C until use. Sediment from Shipyard Creek was collected at 32.8372°N latitude and 79.9462°W longitude from the surface to approximately 30 cm (12 inches) with a sediment grab, and placed in autoclaved mason jars with a sterile spatula.

Surface water was collected at all sites except Shipyard Creek in 250-ml screw-cap Nalgene bottles (Rochester, NY, USA). Surface water was filtered on-site with a 0.2-μm Whatman® (Clifton, NJ, USA) sterile polysulfone membrane syringe filter, placed in sterile 40-ml glass VOA vials (Scientific Products, Willard, OH, USA), and stored on ice before analysis for nitrate, nitrite, ammonium, ferric iron, and phosphate.

Surface water and sediment analyses

Surface water from all sites except Shipyard Creek was analyzed on-site for temperature, pH, conductivity, and dissolved oxygen with a YSI 3500 water quality monitor (YSI, Yellow Springs, OH, USA). On-site and laboratory analyses for nitrate (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N), ferric iron (Fe^{3+}), and phosphate (PO_4^{3-}) were performed with a Hach DR/700 colorimeter (Loveland, CO, USA).

Sediment moisture content was measured to normalize concentrations of atrazine, DIA, and DEA on a sediment dry weight basis for data comparisons. Sediment particle size distribution was determined by separating the sand and silt plus clay fractions with a 63-μm sieve by the standard method as described by Lewis [23]. The sand fractions were further characterized into very fine to very coarse sand. Sediment total carbon and total nitrogen were analyzed by dry combustion with a Perkin-Elmer 2400 elemental analyzer (Norwalk, CT, USA). Homogenized sediment was dried in the oven at 60°C for 72 h and crushed with a mortar and pestle. Four to 6 mg of sediment, and 1 to 2 mg of cystine standard were placed in separate tin weigh boats and folded. The carrier gas was ultra–high-purity helium and the reduction temperature was set at 640°C. The weigh boats were combusted at 930°C, and increased to 1,300 to 1,400°C with the addition of oxygen.

^{14}C-Atrazine mineralization experiments

All experiments were conducted with U-^{14}C-atrazine (Sigma, St. Louis, MO, USA) with a specific activity of 20.1 mCi/mmol and 98.4% purity. Mineralization experiments were carried out with Shipyard Creek sediment only, because mineralization in the other sediments had been examined previously [17]. For Shipyard Creek, approximately 5 g of homogenized sediment was added into a 20-ml screw-cap VOA vial in a 1:1 ratio (w/v) with sterilized deionized water in triplicate. One milliliter of saturated mercuric chloride ($HgCl_2$) was added to all abiotic control tubes for a final concentration of 14 μg/g sediment to inhibit microbial activity. Sediment in experimental and abiotic control tubes was then spiked with U-^{14}C-atrazine and cold atrazine in a ratio of 1:1,000 (v/v) for a final concentration of 5 μg/g wet sediment. The $^{14}CO_2$ recovery efficiency was quantified by adding $NaH^{14}CO_3$ (specific activity 58 mCi/mmol; ICN Biomedicals, Irvine, CA, USA) added to a separate set of vials, which were treated similarly to the experimental vials but with no added atrazine. Duplicates of the abiotic controls and recovery efficiency samples were used. All tubes were incubated at room temperature in the dark for 0, 4, 10, 24, 31, 40, 54, and 61 d. At the end of each incubation time, a hanging base trap with 400 μl of 2 N NaOH was placed in each vial, 2 ml of 4 N H_2SO_4 was added to each vial, and the vials were shaken overnight. Absorbent filter paper was used to collect and transfer the contents of each base trap to scintillation vials. Ten milliliters of EcoLite liquid scintillation cocktail (ICN Biomedicals, Costa Mesa, CA, USA) was added, and the activity of each sample was counted on a Packard Tricarb 460 C liquid scintillation counter (Downers Grove, IL, USA). Experimental values were corrected for abiotic $^{14}CO_2$ production by subtracting the mean abiotic concentration at each time point at each site, and also corrected for $^{14}CO_2$ recovery efficiency.

^{14}C-Atrazine transformation experiments

To quantify the production of DIA and DEA, sediment from each site was homogenized, and 10 g was transferred into a centrifuge tube. Sterilized deionized water was added to the tubes in a 1:1 ratio (w/v) to make a sediment slurry. Two milliliters of saturated $HgCl_2$ was added to all abiotic control tubes for a final concentration of 14 μg/g sediment to inhibit microbial activity. Sediment in experimental and abiotic control tubes was then spiked with U-^{14}C-atrazine and cold atrazine in a ratio of 1:2,000 (v/v) for a final concentration of 50 μg/g wet sediment. Concentrations of atrazine added to experimental vials (50 μg/g sediment wet weight) were greater than background concentrations to allow detection of low levels of metabolites that were expected to be produced. All tubes were incubated at room temperature in the dark for 0, 21, 42, and 70 d. The day 0 sample was processed at approximately 4 to 6 h because of the lengthy setup period for the experimental, abiotic and biotic control vials, and the lengthy analytical procedure. Duplicates of the abiotic controls and triplicates of the experimental samples were used. Experimental values were corrected for abiotic metabolite production by subtracting the mean abiotic concentration at each time point at each site. One biotic control with no added atrazine was used for all sites for the first and last incubation periods to account for the presence of any background atrazine, DIA, and DEA, and to assess production of DIA and DEA by unamended samples.

Aqueous extraction. At the end of each incubation time point, the entire sample (aqueous and sediment) of the experimental and biotic control tubes was metabolically inhibited with 2 ml of saturated $HgCl_2$ (14 μg/g sediment). All samples (experimental and biotic and abiotic controls tubes) were centrifuged at 1,000 rpm (140 *g*) for 15 min in a IEC B-20A

Table 1. Surface-water characteristics for the golf course, Dog Creek, Oyster Creek, and Oyster Landing (SC, USA) study sites

Site	Temperature (°C)	pH	Conductivity (μS/cm)	DO	NO_3^--N	NO_2^--N	NH_4^+-N	Fe^{3+}	PO_4^{3-}
				(mg/L)					
Golf course	25.9	5.7	15	9.0	1.0	0.01	0.65	0.15	0.09
Dog Creek	23.3	6.8	26	6.0	0.1	0.02	0.30	0.32	0.03
Oyster Creek	24.4	7.4	62	1.3	0.2	0	1.54	5.03	2.46
Oyster Landing	28.0	6.8	1,484	7.0	0.9	0.01	0.67	1.10	0.03

Centrifuge (International Equipment, Needham Heights, MA, USA). After centrifugation, the supernatant was vacuum filtered through a 0.2-μm polyethersulfone (Supor) Gelman-Sciences® membrane filter (Ann Arbor, MI, USA). Each filter paper and filtrate fraction (1-ml aliquot) was measured on the liquid scintillation counter for radioactivity.

Extraction of the filtrate was carried out by solid-phase extraction with Supelco LC-18 (octadecyl, ~10% C, end-capped) reverse-phase cartridges (Supelco Chromatography, Bellefonte, PA, USA) with 500 mg of sorbent and 3-ml tube size. After conditioning the cartridge with two volumes of methanol (two 2-ml washes), it was loaded with 2 ml of the aqueous sample (filtered fraction), and the eluant was collected in a 2-ml crimp-capped glass vial. The solid-phase extraction cartridge was then washed with methanol, and this eluant was collected. These aqueous and methanol eluants were split and analyzed for ^{14}C by liquid scintillation counting, and for parent and metabolite concentrations by gas chromatography–mass spectrometry (GC-MS). For GC-MS analyses, analytical-grade terbuthylazine (Chem Service, West Chester, PA, USA ~99% purity) was added to all aqueous and methanol fractions as an internal standard to give a final concentration of 50 mg/L.

The eluant samples were evaporated to near dryness under a gentle stream of N_2 gas, reconstituted in 600 μl of methylene chloride, and analyzed on an Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) equipped with a Restek® RTX-5 ((5% phenyl)-methylpolysiloxane; 25-μm film thickness) column (30 m × 0.25-mm internal diameter; Bellefonte, PA, USA), and a VG70SQ double-focusing magnetic sector mass spectrometer (Micromass, Beverly, MA, USA) for high mass resolution. To quantify atrazine, DIA, and DEA in each fraction, peak area counts were integrated and corrected for recovery efficiencies with the internal standard terbuthylazine based on the calculated response factor, and concentrations of atrazine, DIA, and DEA were calculated with a standard curve for each compound with analytical grade standards (Chem Service; ~purity from 95 to 98%).

Sediment extraction. After the supernatant was decanted from the aqueous extractions described above, the readily extractable ^{14}C radioactivity associated with the sediment was analyzed by adding 10 ml of a 80:20 methanol:water (v/v) mixture to the sediment in the centrifuge tubes. The sediment was sonicated at room temperature for 30 min in a Bransonic 5200 Ultrasonic Cleaner (Danbury, CT, USA). After sonication, the samples were centrifuged and the supernatant was collected and loaded onto the solid-phase extraction cartridge. The eluant was counted on the liquid scintillation counter, and analyzed by GC-MS according to the protocol described above for the aqueous fraction. After extraction, 0.1 g of the remaining sediment was diluted in 1 ml of autoclaved deionized water, added to 1 ml of EcoLite scintillation cocktail, and measured on the liquid scintillation counter for sediment-associated ^{14}C radioactivity. Negligible radioactivity was measured in this fraction (data not shown).

Statistical analyses

An alpha level of 0.05 was used for all statistical analyses. Three-way analysis of variance by means of SAS® [24] was performed, comparing the mean ^{14}C radioactivity recovered in the aqueous and sediment extractable fractions of the live and abiotic control samples. The differences in the radioactivity measured in the aqueous and sediment-extractable fractions (dependent variable) was compared across the five sampling locations for the two treatment types over four time points (the three independent variables). Significant interactions of the variables, sites, and treatment type with the variable time were found. To account for these interactions, further analyses were performed to look for significant differences among sites and treatment type at each time point with the Tukey multiple comparison procedure [24].

RESULTS

Surface water characteristics did not vary greatly by site, except at Oyster Creek, which had greater concentrations of Fe^{3+} and PO_4^{3-} than the other sites (Table 1). The dissolved oxygen varied by site, with the lowest concentrations at Oyster Creek and highest concentrations at the golf course. In previous studies at these sites dissolved oxygen varied by season with less than 1 mg/L at all sites measured in August and September, and redox values of 1, 5, and 8 mV in surface water at Oyster Creek, Dog Creek, and the golf course, respectively [19]. Conductivity values were similar for all sites except Oyster Landing, which had approximately two orders of magnitude greater conductivity than the other sites. Surface water NO_3^- and NO_2^- concentrations were low at all sites (1.0 mg/L or less). Sediment organic carbon was less than 1% at the golf course and Dog Creek, and was slightly higher at Oyster Landing (Table 2). Much higher sediment organic carbon was measured at Oyster Creek (8.6%) and Shipyard Creek (4.3%). Total sediment nitrogen concentrations were similar at all sites, despite the frequent nitrogen-based fertilizer application at the golf course. Oyster Creek and Shipyard Creek sediment contained higher moisture levels and also higher silt plus clay content (27 and 95%, respectively) than the other sites (Table 2).

^{14}C-Atrazine mineralization and transformation experiments

For a total ^{14}C mass balance, radioactivity in the filter papers, solid-phase extraction cartridges, aqueous fraction, and sediments was measured at all sites and time points. Less than 1% of the ^{14}C radioactivity was associated with the filter papers and solid-phase extraction cartridges (data not shown). No mineralization or $^{14}CO_2$ was measured with Shipyard Creek sediments (data not shown), and <1% was measured in pre-

Table 2. Sediment characteristics for the golf course, Dog Creek, Oyster Creek, Oyster Landing, and Shipyard Creek (SC, USA) study sites

Site	Depth (cm)	Temperature (°C)	Organic C[a] (%)	Total N[a] (%)	Moisture[b] (%)	Sand (%)	Silt + clay (%)
Golf course	15–30	20.7	0.82	0.13	16.6	83.8	16.2
Dog Creek	15–30	20.1	0.99	0.07	18.1	82.3	17.7
Oyster Creek	15–30	NA[c]	8.60	0.44	62.9	73.2	26.8
Oyster Landing	15–30	26.3	1.90	0.15	23.8	82.2	17.8
Shipyard Creek	10–20	19.0	4.25	0.44	78.1	5.1	94.9

[a] $n = 2$.
[b] Water content of saturated sediment.
[c] NA = not available.

vious atrazine experiments with sediment from Dog Creek, Oyster Creek, and the golf course [17]. Total ^{14}C radioactivity in the aqueous and sediment-extractable fractions of the abiotic control tubes was generally higher than that in the live tubes at all sites and time points (Table 3). At the 0-d incubation (4–6 h), total aqueous and sediment-extractable recoveries in the experimental tubes ranged from 51 to 71% at all sites, and ^{14}C activity in the aqueous portion of the experimental tubes was the greatest at the golf course (58 ± 4%). At the 21-d incubation, a general decline occurred in total ^{14}C recoveries at all sites except for the golf course, which can be largely attributed to the decline of radioactivity in the aqueous fractions. Mean ^{14}C activity at 21 d in the aqueous fraction was significantly greater at the golf course than at the other sites ($p < 0.0089$). In the sediment-extractable fraction at 21 d, mean radioactivity was not different by site except that it was significantly less at Oyster Creek than at Dog Creek ($p < 0.036$).

Mean ^{14}C activity in the aqueous fraction was again significantly greater at the golf course than at the other sites at 42 d (56 ± 12%; $p < 0.0002$) and at 70 d (33 ± 8%; $p < 0.005$), and was the lowest at Oyster Creek (2 and 1 ± 0.4%, respectively). Mean ^{14}C radioactivity at 42 d in the sediment-extractable fraction was significantly greater at the golf course than at Oyster Creek ($p < 0.0107$), and at 70 d was significantly lower at Oyster Creek than at all other sites ($p < 0.0001$). Overall, total ^{14}C recovery in the experimental tubes was the greatest at the golf course and the least at Oyster Creek, and the bioavailable fraction decreased with chemical aging.

GC-MS analyses. Background concentrations of atrazine in the biotic control tubes (treated similarly to experimental tubes but with no added atrazine) in the aqueous and sediment fractions at day 0 were 0 at all sites except Shipyard Creek (0.2 μg/ml and 1.6 μg/g sediment dry weight; data not shown). No background DIA or DEA was measured in the aqueous or sediment fractions in any of the biotic control samples at day 0, and no DEA was measured at day 70. Minor concentrations of DIA were measured at day 70 only at the golf course (0.07 μg/ml and 0.01 μg/g sediment dry weight), Dog Creek (0.02 μg/ml aqueous), and Shipyard Creek (0.02 μg/ml; data not shown).

In the experimental vials, a decline in atrazine concentration was measured at all sites over time (Table 4), and the mild extraction did not remove the ^{14}C from the sediment, partic-

Table 3. Mean (± standard deviation) ^{14}C-atrazine recoveries (% of initial ^{14}C-atrazine added) in aqueous and sediment-extractable fractions at the five study sites for the experimental and abiotic control vials

		Experimental[a]			Abiotic[b]		
Site	Time (d)	Aqu.[c] (%)	Sed.[d] (%)	Total (%)	Aqu.[c] (%)	Sed.[d] (%)	Total (%)
Golf course	0	58 (4)	13 (2)	71	72	11	83
	21	60 (13)	18 (3)	78	45 (0)	16 (3)	61
	42	56 (12)	26 (9)	82	63 (20)	18 (1)	82
	70	33 (8)	11 (4)	44	37 (17)	17 (11)	54
Dog Creek	0	44 (13)	17 (5)	61	69 (3)	18	87
	21	16 (1)	19 (2)	35	12 (6)	22 (4)	34
	42	14 (3)	23 (3)	37	11 (1)	25 (7)	36
	70	8 (3)	13 (3)	21	9 (1)	18 (6)	27
Oyster Creek	0	43 (11)	18 (4)	61	63 (6)	14 (1)	77
	21	9 (2)	8 (2)	17	13 (1)	11 (2)	25
	42	2 (0)	3 (0)	5	5 (0)	7 (0)	12
	70	1 (0)	1 (1)	2	4 (0)	6 (1)	10
Oyster Landing	0	33 (3)	18 (7)	51	40 (5)	19 (7)	58
	21	17 (3)	17 (3)	34	18 (2)	15 (3)	33
	42	10 (1)	9 (1)	19	14 (1)	11 (0)	25
	70	8 (3)	25 (1)	33	9 (0)	32 (4)	41
Shipyard Creek	0	36 (5)	16 (0)	52	47 (3)	17 (4)	64
	21	17 (3)	13 (1)	30	22 (5)	17 (10)	39
	42	12 (3)	12 (1)	24	19 (2)	14 (0)	32
	70	8 (5)	9 (1)	17	21 (3)	13 (5)	34

[a] $n = 3$.
[b] $n = 2$.
[c] Aqueous fraction.
[d] Sediment-extractable fraction.

Table 4. Mean atrazine (± standard deviation) concentration, and deisopropylatrazine (DIA), and deethylatrazine (DEA) concentrations produced (corrected for abiotic values) in aqueous (μg/ml) and sediment-extractable fractions (μg/g dry wt) of atrazine-amended sediments at the five study sites

		Atrazine[a]		DIA[a]		DEA[a]	
Site	Time (d)	Aqu.[b] (μg/ml)	Sed.[c] (μg/g)	Aqu. (ng/ml)	Sed. (ng/g)	Aqu. (ng/ml)	Sed. (ng/g)
Golf course	0	35.8	0.4	9	0	12	0
	42	20.1 (1.4)	1.7 (0.2)	8	0.1	36	1
	70	17.9 (6.5)	0.9 (0.2)	10	0	1	0
Dog Creek	0	14.7	0.0	0	0	0	0
	42	9.5 (1.8)	3.0 (0.3)	39	6	19	1
	70	1.6 (0.5)	0.3 (0.1)	14	0	2	0
Oyster Creek	0	8.3	1.2	1,590	0	7	0
	42	0.1 (0.2)	0.1 (0.1)	113	2	1	0.3
	70	0.0 (0.0)	0.2 (0.1)	0	0	0	0
Oyster Landing	0	12.1	0.2	2,667	7	27	0.5
	42	1.6 (0.5)	1.2 (0.1)	1,946	19	34	2
	70	1.0 (0.4)	0.6 (0.1)	0	0	0	0
Shipyard Creek	0	8.2 (4.5)	24.6 (5.5)	0	0	0	0
	42	4.2 (1.5)	3.2 (0.8)	0	0	9	0
	70	2.4 (1.0)	1.6 (1.4)	0	0	16	3

[a] $n = 3$ (except day 0, $n = 1$).
[b] Aqueous fraction.
[c] Sediment-extractable fraction.

ularly at the Oyster Creek and Oyster Landing sites after the initial time point. At the later time points, atrazine was found to be more evenly distributed between the aqueous phase and the sediment-extractable fractions because of the decline in the aqueous concentrations, except in the golf course sediments. This site had the greatest atrazine concentrations at each time point, and retained greater atrazine concentrations in the aqueous fraction than any of the other sites.

Because DIA and DEA were detected at all sites but not in every sample at all time points (with the exception of Shipyard Creek), statistically differentiating them between the study sites was not possible with analysis of variance. At day 0 (~6 h), greater DIA concentrations were detected at Oyster Creek and Oyster Landing than at the other sites. Concentrations of DIA in the aqueous fraction were orders of magnitude greater than those in the sediment-extractable fraction. The golf course and Dog Creek produced lesser amounts of DIA at all time points than the nature preserve, and no DIA was detected at Shipyard Creek.

In general, DEA concentrations were less than those of DIA in aqueous and sediment fractions at all sites except Shipyard Creek. The DEA was present at greater concentrations in the aqueous fractions than the sediment-extractable fractions at all sites and time points. Sediment-extractable concentrations of DEA did not exceed 3 ng/g at any site. In general, DIA and DEA were produced, and absent from, the same fractions at each site and time point, with the exception of Shipyard Creek. Apparently, neither metabolite persisted because concentrations were reduced substantially by day 70.

Metabolite to parent compound ratios were calculated to compare the metabolites measured relative to the atrazine measured in the sediment and aqueous fractions because recovery efficiencies and the amount of atrazine added to the sediment after correcting for sediment moisture content varied by site (atrazine concentrations were 60 μg/g at the golf course, 61 μg/g at Dog Creek, 135 μg/g at Oyster Creek, 66 μg/g at Oyster Landing, and 228 μg/g at Shipyard Creek [sediment dry weight]). Ratios were calculated as the amount of metabolite recovered divided by the amount of atrazine recovered in aqueous plus sediment fractions at each time point. The DIA to atrazine ratios for Oyster Creek and Oyster Landing were high, approximately 0.6 and 0.13, respectively, whereas those at the other sites were negligible (Fig. 2A). The DEA to atrazine ratios ranged from 0 to 0.003 at all sites and time points (Fig. 2B). Overall, metabolite to atrazine ratios were 20 to 30 times greater for DIA than for DEA, but the detection frequency, expressed as the percentage of samples analyzed with a positive presence of the metabolite, was essentially

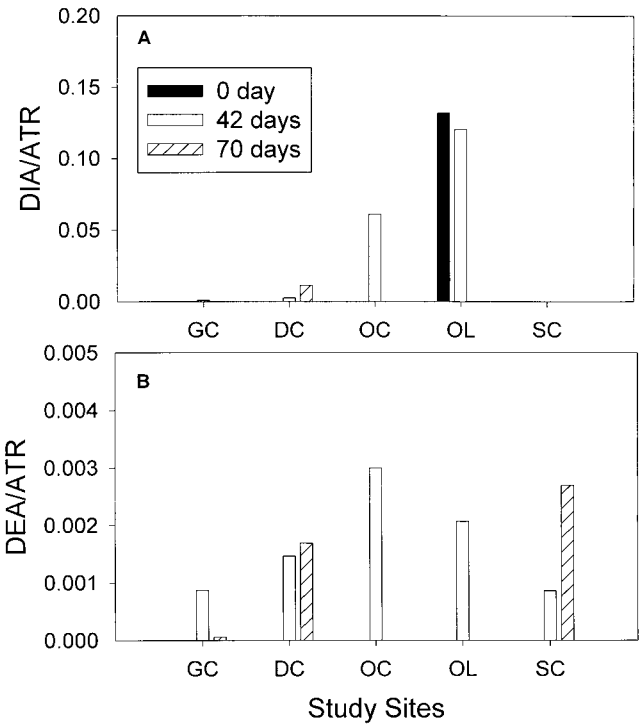

Fig. 2. (**A**) Deisopropylatrazine (DIA) to atrazine and (**B**) deethylatrazine (DEA) to atrazine ratios at 0, 42, and 70 d for the golf course (GC), Dog Creek (DC), Oyster Creek (OC), Oyster Landing (OL), and Shipyard Creek (SC) (SC, USA).

Table 5. Frequency of detection of atrazine metabolites deisopropylatrazine (DIA) and deethylatrazine (DEA) produced in experimental vials (corrected for abiotic values) in combined aqueous and sediment extractable fractions at 0 and 70 d for the golf course, Dog Creek, Oyster Creek, Oyster Landing, and Shipyard Creek (SC, USA) study sites

Site	Time (d)	DIA[a] (%)	DEA[a] (%)
Golf course	0	100	100
	42	100	100
	70	33	33
Dog Creek	0	0	0
	42	100	100
	70	33	33
Oyster Creek	0	100	100
	42	100	67
	70	0	0
Oyster Landing	0	100	100
	42	100	100
	70	0	0
Shipyard Creek	0	0	0
	42	0	100
	70	0	100

[a] $n = 3$.

equivalent for DEA and DIA with the exception of Shipyard Creek (Table 5).

DISCUSSION

Atrazine, DIA, and DEA form strong chemical bonds with sediment organic carbon and become physically bound within the internal sediment binding surfaces and irreversibly sorbed to sediment over time [1]. In addition to irreversible sorption of the parent, decreased ^{14}C recoveries also may be due to other physicochemical processes such as volatilization. Finally, biological and chemical transformation of atrazine to products that were not analyzed in this study, such as HYA, or further degradation of the metabolites produced could have occurred, which may have been reflected in the decrease of DIA concentrations over time at three of the five sampling locations. However, atrazine is not considered to be highly volatile, with a vapor pressure of 3×10^{-7} mm Hg at 20°C (Chem Service), and the decrease in DIA was small compared to nonrecoverable atrazine concentrations.

Assuming that sorption was responsible for the majority of the unrecovered ^{14}C in this study, physicochemical processes and not biological processes accounted for the majority of removal of the readily extractable ^{14}C because recoveries were reduced in the abiotic controls and the experimental vials. Organic carbon seemed to be more important than silt plus clay content for sorption of the ^{14}C. Oyster Creek, the site with the most organic carbon, had the lowest total ^{14}C recoveries compared to the other sites at all the incubation periods. Although Shipyard Creek sediment had much higher silt plus clay content compared to that of Oyster Creek sediment, its sediment recoveries were greater and it had lower organic carbon content. Finally, the golf course, the site with minimal organic carbon content and silt and clay, had the highest total recoveries of ^{14}C radioactivity compared to the other sites at all time periods. Novak [25] used South Carolina Coastal Plain soils to examine the relation of soil organic carbon to atrazine sorption and found that the atrazine sorption equilibration partition coefficient (K_d) for soils increased with increasing soil organic carbon for sorption experiments carried out for 72 h. The magnitude of atrazine sorption was strongly and positively correlated with soil organic carbon.

Regardless of the organic carbon content, all sites exhibited a decline in the recoverable ^{14}C-atrazine activity over time in our study. Reduced recoveries primarily were a function of a reduction in the ^{14}C activity of the aqueous fraction, with no subsequent increase in the sediment fraction. The mild extraction solvent, a methanol–water mixture, was not capable of removing strongly sorbed ^{14}C, but only the readily bioavailable fraction as described by Kelsey and Alexander [26] and Tang and Alexander [27]. This approach is distinct from chemical equilibrium experiments or those attempting complete recovery of the chemicals, such as that of Gan et al. [28], who used harsh chemical extractions with accelerated solvent extraction at elevated temperature and pressure and recovered approximately 50% of the atrazine initially applied to sediments after eight weeks of incubation. Studies in our laboratory examining degradation of phenanthrene in these same sediments showed that up to 20% of the added phenanthrene was found to be biologically transformed to products that were not extracted by methanol–water and hexane, but only by a harsh alkaline hydrolysis that attacked the humic materials of the sediment (unpublished data). Although harsher extractions provide greater recovery, the mild extractions may be useful for predicting genotoxicity [29] as well as bioavailability.

Research with pure bacterial cultures and atrazine-degrading consortia have reported nearly complete atrazine removal by mineralization. Shati et al. [16] reported 90% atrazine reduction in 64 h by *Pseudomonas* sp. strain ADP based on a multilayer sampler in which bacteria were confined in dialysis cells in contact with groundwater. A 75% reduction in atrazine was reported by Topp et al. [30] in 180 min with resting cell suspensions of a *Pseudoaminobacter*, strain C147, although 6 of the 14 isolates did not mineralize atrazine, but accumulated HYA. Newcombe and Crowley [31] reported up to 70% mineralization in soil inoculated multiple times with an atrazine-degrading bacterial consortium.

In native sediment with only the bacteria present, much less mineralization of atrazine has been measured. Hoyle and Arthur [18] summarized 14 atrazine degradation studies that used groundwater or sediment in the United States and Europe, and the majority did not mineralize atrazine. Most of these studies did not examine degradation products, but measured reductions in atrazine concentrations and production of $^{14}CO_2$. In the current study, reduced ^{14}C recoveries were unlikely to be due to mineralization of atrazine because conversion of ^{14}C-atrazine to $^{14}CO_2$ was not observed with Shipyard Creek sediment, nor in previous atrazine studies carried out in our laboratory with sediment from the three other sites [17]. Similarly, Larsen et al. [32] measured limited mineralization of atrazine added to microcosms containing sandy sediments from Denmark from 0.06- and 7.68-m depths. Only 3% of the atrazine was recovered as $^{14}CO_2$ after 119 d and no mineralization of atrazine was measured below 0.7 m.

Despite the fact that recoveries of ^{14}C were low, suggesting extensive sorption, and varied by site, metabolite production occurred at all sites. Greater concentrations of DIA and DEA were measured in the aqueous fraction compared to the sediment fraction. This is expected because DIA and DEA have greater water solubilities compared to atrazine and lower organic carbon partition coefficients. Metabolite production was expected to be greater in Dog Creek, the golf course, and Shipyard Creek sediments because of greater exposure of mi-

crobes to multiple chemicals, including higher background concentrations of atrazine and contamination by polynuclear aromatic hydrocarbons and metals at Shipyard Creek. Yet, Dog Creek and golf course sediments produced minimal amounts of DIA and DEA, and Shipyard Creek sediment produced relatively little transformation of atrazine, and it was entirely to DEA. Conversely, the pristine sites Oyster Creek and Oyster Landing had greater atrazine transformation, particularly to DIA. Oyster Landing was the most saline site, and in a study conducted in the Chesapeake Bay region, a shorter atrazine half-life was found in estuarine sediments compared to agricultural sediment [33]. But salinity did not seem to favor metabolite production at our sites because the freshwater site, Oyster Creek, also had enhanced metabolite production. Also, although Shipyard Creek was saline and DEA was detected at higher concentrations at Shipyard Creek than at the other sites, DEA to atrazine ratios for Shipyard Creek were not greater than those at the other sites.

Atrazine has also been shown to be a source of nitrogen for bacteria [34], and this may have impacted the degradation of atrazine at the contaminated sites, which have more impact by anthropogenic contaminants. However, although previous research at the sites has shown that surface water concentrations of nitrate at the golf course varied by three orders of magnitude, the nitrate did not persist. Surface water and sediment nitrate, nitrite, and ammonia concentrations measured during sediment collection for this study were in similar ranges at all the sites so degradation of atrazine seems unlikely to have been impacted or suppressed at the more developed sites because of anthropogenic nitrate additions, except potentially episodically at the golf course.

In our study, DEA was detected as frequently as DIA, but at lower concentrations. The low DEA to parent compound ratios suggested that little of the added atrazine was transformed to DEA even at high atrazine concentrations. Conversely, DIA to parent compound ratios indicated that potentially a larger fraction of the atrazine was transformed to DIA at Oyster Landing and Oyster Creek, than was transformed to DEA at all sites. The DEA is considered to be the most commonly detected metabolite of atrazine [35], and was produced in greater concentrations in a sandy atrazine-contaminated agricultural soil in Wisconsin over 270 d [4] and in agricultural soil in Iowa after 60 d [36]. But relative concentrations of these metabolites may change in situ, as was reported over a two-year period by Panshin et al. [37]. They measured concentrations of atrazine DEA, DIA, DDA, and HYA in water in lysimeters in Indiana agricultural fields with organic carbon content of 1 to 3% and found the following order of concentrations: DEA $>$ DDA $>$ DIA $>$ HYA in the first year, and DDA $>$ DEA $>$ DIA $>$ HYA in the second year. However, maximum concentrations were in a similar range for DEA and DIA, 0.76 to 1.48 μg/L and 0.11 to 0.78 μg/L, respectively. They also found that atrazine concentrations decreased rapidly in the first year and by the second year atrazine did not persist, although the metabolites were present in most of the lysimeters. They suggested that a source such as a reservoir of atrazine sequestered in the top several centimeters of the soil column may have been the cause of metabolite production at that time. Our maximum concentrations of DEA and DIA were greater than those of Panshin et al. [37], but our initial concentration of atrazine also was greater. Our metabolites did not persist at longer time points, nor to a greater extent than atrazine. Our sites were not agricultural fields and atrazine was added only once, so no reservoir was available.

Black et al. [38] identified a significant relation between organochlorine pesticides in fish tissue and percentage urban and agricultural land use. However, they did not find a similar significant relation with land use and streambed sediment pesticide concentrations. The reasons suggested included the continual scouring and resorting of streambed sediments and possible dilution of the more contaminated sediments with clean sediments. Microbial degradation was not examined in their study. In our study, land use seemed to affect the production of metabolites in streambed sediments, although not in the anticipated fashion. Greater metabolite production occurred at the undeveloped nature preserve compared to any of the suburban and industrialized areas. Because these transformation products are water soluble and mobile than atrazine, they are more likely to contribute to DIA groundwater and surface-water contamination than at the other sites. Atrazine may also be a potential problem, although the majority of atrazine seemed to be strongly sorbed to Oyster Creek and Oyster Landing sediments. Kucklick and Bidleman [39] detected low levels of atrazine (1–104 ng/L) in the surface waters of North Inlet estuary, suggesting that atmospheric, groundwater, or surface-water transport of these chemicals from upland sources may have occurred, but they did not measure atrazine degradation products. Kruger and Coats [36] determined that DEA was more mobile than atrazine in Iowan agricultural soils.

The extensive golf-course developments in this coastal region often use sandy, well-drained soils, and it was not surprising that limited sorption occurred at the sandy golf course site. However, in contrast to Oyster Creek and Oyster Landing, limited metabolite production occurred, and in combination with limited sorption resulted in greater atrazine concentrations at this site than at any of the other sites. So atrazine rather than its metabolites may have a higher potential to contaminate groundwater at this low carbon site. Thus, distinct responses to atrazine by sediment microbial communities combined with sediment manipulations associated with varying land uses may impact the ultimate potential of the parent compound versus the metabolites to contaminate sediment, surface water, and groundwater, and the coastal inlets into which they drain.

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