BIOAVAILABILITY AND DESORPTION CHARACTERISTICS OF AGED, NONEXTRACTABLE ATRAZINE IN SOIL

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Abstract—The bioavailability and desorption characteristics of nonextractable soil-aged atrazine were investigated to examine the significance of this potentially large contaminant fraction in agricultural soils. Radiolabeled atrazine-applied soil was aged for 3 months and then extracted by one of three methods—methanol-water (4:1) at 75°C, acetone, and artificial soil water—representing a range of extraction efficiencies. Viable microcosms were constructed with the dried, extracted soil to compare rates and extents of microbially facilitated release and mineralization with desorption rates in sterile microcosms. The most rigorously extracted soil exhibited slow desorption over a 90-d period, but the other soil treatments showed continued slow sorption after reaching an initial maximum sorption concentration within 1 to 5 d. No microbially facilitated release of the nonextractable atrazine was detected for any of the treatments. Rates of ring- and chain-labeled atrazine mineralization declined with decreasing extractability of the soil-associated atrazine fraction. However, the extent of biodegradation (less than 2% of the total atrazine for all extracted soil treatments) was less than the extent of abiotic desorption. Mass-balance calculations suggest that all biodegradation occurred in the dissolved phase.

Keywords—Atrazine Nonextractable Bound Bioavailability Desorption

INTRODUCTION

Bound-residue formation is an important soil-zone mechanism that affects the environmental fate of the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and many other organic contaminants [1]. Operationally defined, bound (nonextractable) residues are organic chemicals remaining in soil or organic matter after exhaustive sequential extractions. Aging of contaminants in soil has been shown to cause a gradual increase in the bound fraction, resulting in an amazing persistence of aged contaminants in soil. Nine years following field application of 14C-labeled atrazine, Capri et al. [2] found 50% of the 14C still present in the soil as nonextractable residues. Scientists have observed a notable decline in extractability and rates of desorption and biodegradation with increased contaminant aging time in soils [3–5]. Although the processes of aging and bound residue formation are not fully understood, suspected mechanisms include slow diffusion through intraparticle micropores, bonding with soil constituents, sorption at high-energy sites, and entrapment within the molecular structure of organic matter [5,6]. However, these mechanisms imply widely ranging rates from permanent removal to slow rerelease of contaminants into the environment.

Previous research has shown that sorption and desorption of most organic compounds on soils occurs in two stages: fast, on the time scale of hours to days, and slow, over months to years. Slow sorption might account for a large percentage of the total sorbed contaminant. Pignatello and Xing [6] summarized data showing an increase in the apparent sorption distribution coefficient ranging from 30 to 1,000% between short and long equilibration periods. Studies of the desorption characteristics of soil-aged contaminants show increasing resistance to desorption over time within a single experiment, revealing a continuum of mass-transfer coefficients within a single soil sample [7]. The slowly desorbing fraction increases with contaminant aging time [8,9] and has been shown to be more significant at low applied concentrations [10,11]. Some researchers also assert the occurrence of an irreversibly sorbed organic-contaminant fraction on sediments or natural organic matter [12,13], although readers should note the time scales of the experimental work on which such conclusions are drawn.

The decreasing extractability that occurs on contaminant aging in soil is frequently associated with a decline in bioavailability [5,14,15], yet researchers have drawn widely ranging conclusions about the bioavailability of soil-bound contaminant residues [16–18]. Scribner et al. [16] investigated the degradation of fresh and nonextractable simazine residues in soil and detected no degradation of the bound simazine versus 48% degradation of the freshly applied simazine. Research on the bioavailability of sorbed 2,4-D, di-n-butyl phthalate, and chlorophenol has also reported no degradation of the sorbed phase [19,20]. However, other researchers have reported slow but significant biodegradation of aged and sorbed compounds. Roberts and Standen [21] reported 25 to 40% mineralization of nonextractable [14C]cypermethrin during a 26-week incubation with fresh soil. Racke and Lichtenstein [22] conducted a detailed investigation of the degradability of soil-bound [14C] parathion and reported 12.5% mineralization of the bound compound after a 14-d incubation with fresh soil. Guerin and Boyd [18] investigated the biodegradability of soil-sorbed naphthalene using two naphthalene-degrading bacterial strains and observed degradation of the sorbed naphthalene by only one of the bacterial strains; the other strain degraded only the aqueous-phase contaminant.

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Microbially mitigated release of nonextractable, aged residues has been observed in several of the biodegradation studies, including some investigations in which no microbial degradation of bound residues was observed. Microbial release of bound-atrazine residues by two Pseudomonas strains was investigated by Khan and Behki [23]. At the end of an 83-d incubation with soil-bound atrazine residues, 30 to 35% of the initially bound 14C was extractable compared to only 3% in the sterile control. Gas chromatographic analysis of the released compounds indicated the presence of the parent compound atrazine, hydroxyatrazine, and their associated dealkylated metabolites. Khan and Behki hypothesized that as the bacteria consume soil organic matter as their primary substrate, the bound-atrazine molecules are freed from their entrapment within the humic macromolecules. This research determined that the atrazine-binding mechanism can be reversed through microbial activity while maintaining the identity of the parent molecule. Wszolek and Alexander [24] conducted similar experiments to determine the effects of microbes on the sorption of n-alkylamines to clays and discovered that microbes facilitated desorption from the clay surfaces.

The relationship between solvent extractability and bioavailability in the soil environment remains poorly defined. Frequently, regulators set hazardous contamination limits at the lowest detection limits without regard to bioavailability of the contaminant. Yet the bioavailability of a contaminant in terms of release by soil microbes or uptake to plants and animals ultimately affects its threat to the environment. Limited research has been conducted on the relationship between extractability and bioavailability [14, 25]. Kelsey et al. [14] aged atrazine and phenanthrene on soils for periods ranging from 7 to 124 d and then extracted the soils by 11 different extraction methods. The results of these extractions were then compared to extents of microbial mineralization or earthworm uptake from the aged (but not extracted) soils. Although no single extraction method was directly correlated with the bioavailability results, several mild, selective extraction methods proved to be reasonable predictors of the aged-contaminant bioavailability. Atrazine extractability was also shown to decline at a slower rate with aging than its bioavailability.

Continued research is necessary to define the role of contaminant aging either as a beneficial outcome of natural soil processes that reduce contaminant transport to deeper groundwater systems or as a long-term reservoir for contaminants awaiting degradation or release by slow desorption, microbial activity, or changing geochemical conditions. In the present microcosm-scale study, the fate of soil-aged, nonextractable atrazine was examined with respect to three natural soil processes: biodegradation, microbially facilitated release, and abiotic desorption. The desorption behavior of the variably extracted soil treatments revealed a complex distribution of sorption sites reflected in the co-occurrence of fast desorption, slow desorption, and the competing process of slow sorption. Microbially facilitated release of the nonextractable atrazine was not detected in any of the treatments. Rates of atrazine mineralization by the natural microbial consortium were exceeded in nearly all cases by desorption rates, suggesting no mass-transfer limitation in the biodegradation of nonextractable atrazine within this study. Mass-balance calculations indicated that no biodegradation of the soil-associated atrazine fraction occurred regardless of extraction procedure.

METHODS

Soil aging and extractions

Soil was collected from the uppermost 10 cm of a silty-clay loam soil (3.8% organic carbon, pH 6.9, <0.015 µg/g extractable atrazine) in an agricultural catchment in the Shenandoah Valley of Virginia. The soil is characterized as Frederick and Lodi silt loams, and the soil mineralogy is dominated by kaolinite, illite, and quartz [26, 27]. Grain size analyses on the soil collected reveal 19% clay, 58% silt, and 23% sand. Ten-gram spoon samples of the air-dried, homogenized soil were weighed into 20-ml amber glass vials and sealed with PTFE-lined silicone septum closures. The soil samples were sterilized by a minimum of 21.9 kGy gamma irradiation from a 60Co source at the University of Virginia (UVa) Reactor Facility.

The atrazine used in these experiments was donated by Ciba (Greensboro, NC, USA). The ring-labeled atrazine [(triazinyl-14C)-atrazine] had a specific activity of 37.8 µCi/mg (chemical purity 94.3%, radiochemical purity 98.4%); the chain-labeled atrazine [(ethyl-14C)-atrazine] had a specific activity of 45.1 µCi/mg (chemical purity 92.4%, radiochemical purity 96.6%). The chemical purity of the unlabeled atrazine as reported by the manufacturer was 92.15%. The radiolabeled and unlabeled atrazine (combined at ratios from all labeled to 1:1 labeled to unlabeled) were dissolved in sterile 0.005 M NaCl. Five milliliters of the aqueous atrazine solution (11.2 mg/L) were injected onto each 10-g irradiated soil sample through the septum, resulting in a soil moisture content of approx. 50% and an atrazine concentration of 5.6 µg/g soil. This concentration is equivalent to 2.2 kg/ha applied over a 6-cm soil depth, assuming a soil bulk density of 1.5 g/cm³. The sealed soil samples were then aged in the dark at room temperature for a minimum of 90 d to allow the formation of bound-atrazine residues.

On completion of the aging period, the aged soil samples were extracted by one of three solvent extraction techniques representing a range of extraction efficiencies: methanol-water (4:1 ratio) at 75°C for 4 h, room-temperature acetone extraction for 24 h, and artificial soil water (ASW; 0.005 M NaCl) extraction at room temperature for 24 h. The hot methanol-water extraction was reported by Huang and Pingmatello [28] to be among the most efficient solvent-extraction methods for atrazine. In all three extraction methods, the 10-g moist soil sample was transferred into a 50-ml Teflon® centrifuge tube, and 30 ml of solvent were added. The ASW- and acetone-extracted soils were shaken in the dark for 24 h on an orbital shaker table at 100 rpm. The methanol-water-extracted soils were shaken for 4 h in a 75°C water bath. The tubes were centrifuged for 10 min at 12,000 g (15 min at 17,400 g for ASW). The extractability was determined by quantifying the 14C in the solvent extract by liquid scintillation counting (LSC). The extracted soil was rinsed vigorously with 25 ml of clean solvent to remove the dissolved atrazine in the pore fluids and immediately centrifuged for 7 min, and then the rinse was repeated.

The extracted soils were transferred to amber glass vials and were placed uncovered in the hood and stirred periodically to allow the solvents to evaporate. Once the soils had dried, all vials containing extracted soil were capped and again irradiated.
Microcosm construction

Three separate experiments using viable and sterile soil microcosms were conducted to investigate three primary processes affecting the fate of bound residues in soil: biodegradation, microbially facilitated release, and desorption. Atrazine biodegradation was examined in terms of mineralization by flushing and trapping \(^{14}\)CO\(_2\) from the microcosm headspace. Both \(^{14}\)C-ring- and chain-labeled atrazine were used in the biodegradation experiments to examine the mineralization rates and extents at two steps along the biodegradation pathway. Microbially facilitated release of \(^{14}\)C-ring- and chain-labeled atrazine was determined by reextracting the soils after incubation in viable and sterile microcosms. Desorption of \(^{14}\)C-ring-labeled atrazine was examined in batch under sterile conditions.

Five microcosm treatments were designed containing soil-associated atrazine along a range of extractability: freshly applied atrazine, no soil extraction; atrazine-applied soils aged 90 d and extracted with artificial soil water; aged atrazine-applied soils extracted with acetone; aged atrazine-applied soils extracted with hot methanol-water; and aged atrazine-applied soils extracted with hot methanol-water that were amended with glucose in the soil microcosm. Triplicate viable microcosms and duplicate sterile controls were prepared for each extraction treatment, except in the chain-labeled release experiment, which used duplicate viable microcosms.

The freshly applied soil treatments, containing 5 ml sterile aqueous atrazine solution plus 10 g sterile soil in a 20-ml amber vial, were prepared immediately before the microcosm construction. To maintain the same quantities of ASW in all microcosms within a single experiment, 5 ml of sterile ASW or glucose-amended ASW were added to the aged and extracted soil treatments for the biodegradation experiment. Because no freshly applied treatment was required for the release experiments, no additional ASW was added to these samples.

To create the viable soil microcosms, the soil treatments were amended with a mixed microbial community. The microbial amendment was prepared from fresh sandy clay loam soil collected from the uppermost soil zone in a cornfield in the Shenandoah Valley of Virginia where atrazine had been applied on an annual basis. The soil was stored at 4°C during transport to the lab. The microbes were added in an 8-ml ASW-based soil slurry containing approx. 30% soil (dry weight). A separate soil slurry was prepared for the glucose-amended microcosms using an ASW solution containing 2,000 ppm glucose. An equivalent liquid volume of autoclaved ASW or glucose-amended ASW was added to the sterile controls.

The sterile desorption microcosms were prepared in 40-ml amber glass vials by adding 20 ml of sterile ASW to the 10-g aged and extracted soil samples. The freshly applied microcosms were prepared by adding 5 ml of sterile aqueous \(^{14}\)C-labeled atrazine 1 to 2 h before the addition of 15 ml sterile ASW.

Microcosm sampling

Biodegradation. Headspace sampling of the biodegradation microcosms occurred nearly every day over the 90-d incubation period. A sterile plastic syringe and disposable syringe needle were used to withdraw 25 ml of headspace gas. After several milliliters of headspace gas were initially removed, CO\(_2\)-free lab air was allowed to replenish the headspace through an air-inflow device consisting of the body of a 20-ml plastic syringe, filled with glass wool and ascarite, connected to an autoclaved 0.1-µm syringe filter (to remove airborne bacteria) and a sterile 25-gauge 1.5-inch needle. The long needle length was used so that the inflow gas could be bubbled into the liquid phase of the microcosm to enhance aeration. Each of the samples of headspace gas were injected into a gas trap consisting of a 40-ml amber vial containing 2 ml 1-M NaOH sealed with PTFE-lined silicone septum closures. The gas traps were evacuated manually before each venting. This sampling procedure was repeated every 24 to 48 h over a period of 6 to 12 d to build up a cumulative amount of \(^{14}\)CO\(_2\) from each individual microcosm, at which time the NaOH was removed for analysis by LSC. The scintillation vials were allowed to sit in the dark for at least 48 h before analysis by LSC to minimize the impact of chemiluminescence.

Additional glucose was added to the glucose-amended biodegradation microcosms at days 57 and 82. To minimize the change in liquid volume, 0.1 ml of concentrated glucose (310 g/L) was injected to each glucose-amended microcosm using a sterile 0.5-µm syringe, and the vials were shaken well.

Mineralization "extents" represented the total amount of atrazine mineralized within the 90-d experiment. To ease comparison of the mineralization results with the linear desorption rates, mineralization rates were calculated by a linear fit to the last 35 to 45 d of mineralization data, when the fastest rates of mineralization occurred.

Microbially facilitated release. At 22, 63, and 90 d of microcosm incubation in the dark at room temperature, a subset of microcosms was sacrificially extracted—each vial by the extraction method used after the initial soil-aging period. Extraction extents were determined by analysis of the extract by LSC. During the incubation period, the release microcosms were vented weekly and shaken to aerate the soils. Additional glucose was added to the glucose-amended release microcosms at days 58 and 83 in the same manner as for the biodegradation vials.

Desorption. Amber glass vials containing aged and extracted soil samples and freshly applied soils at a ratio of 1:2 soil to ASW were placed on an orbital shaker table, rotating at 100 rpm. Approximately weekly, the vials were rotated to remix any soil particles that were too heavy to remain in suspension. At six times during the equilibration period (6 h, 24 h, 5 d, 17 d, 55 d, and 90 d), triplicate soil samples were sacrificially sampled. Each sample was transferred to a 50-ml Teflon centrifuge tube, and the glass vial was rinsed with two 5-ml aliquots of ASW. The samples were centrifuged at 27,000 g for 15 min, and the aqueous phase was pipetted off. Aqueous atrazine concentrations were determined by LSC, and desorption concentrations were adjusted for the rinse volume. This procedure enabled the isolation of kinetic desorption from atrazine-aged soils uninfluenced by equilibrium desorption associated with the influx of fresh water as in column or field conditions.

Postincubation analyses. At the end of the 90-d incubation period, the biodegradation microcosms were sacrificed to determine the aqueous-phase atrazine concentrations. The microcosms were allowed to settle for several days before sampling to ease the filtration procedure. For each microcosm, approx. 4 ml of the liquid phase were removed with a glass syringe, and three sample treatments were analyzed by LSC: unfiltered, filtered, and filtered and acidified. The filtered samples were prefilted through a 1.0-µm glass-fiber syringe filter followed by a 0.1-µm PTFE syringe filter. The difference be-
Fig. 1. Percentage of atrazine desorbed with time from freshly applied soil treatments and soil treatments that were aged for 90 d and then extracted by either hot methanol-water, acetone, or artificial soil water (ASW). Percentage of atrazine desorbed is relative to the amount of freshly applied or nonextractable atrazine at the start of the desorption experiment. Lines traced through means of triplicate data points.

tween the filtered and filtered plus acidified samples were calculated for each sample to quantify the amount of radiolabeled HCO₃⁻ present in solution.

Microbial enumeration

Plate counts of the soil slurry were performed on R2A agar (Becton Dickinson) to enumerate viable bacteria in the initial microbial population. One 8-ml dose of the soil slurry was added to 500 mL of 0.1% sodium pyrophosphate solution and shaken for 30 min to enhance suspension of the soil-associated bacteria prior to enumeration. The colony forming units were counted after 48 h at room temperature. Plate counts were repeated on both the live and the sterile biodegradation microcosms at the end of the incubation.

RESULTS

Extraction of soil-aged atrazine

Soil samples extracted by the hot methanol-water method showed a significant reduction in atrazine extractability with aging time. After aging 3 months, atrazine extractability with hot methanol-water decreased to 66% (SD = 5.5), compared to 96% (SD = 1.8) atrazine extraction efficiency for soils aged less than 1 h. At the end of the 3-month aging period, acetone and artificial soil water extracted 51% (SD = 5.9) and 29% (SD = 3.1) of the aged atrazine, respectively [29].

Desorption

The desorption of atrazine from the aged and extracted soils displays three phases that are distinguished by the slopes of the kinetic desorption data: an initial fast desorption during the first 1 to 5 d; a slow desorption rate beyond 5 d, most notable in the methanol/water-extracted soils; and slow sorption, which produces a negative slope on the desorption plot after the fast desorption period (Fig. 1). The freshly applied atrazine treatment, in which atrazine was applied to the soil 1 h before the start of the desorption experiment, best displays the process of slow sorption. The maximum desorption concentration (1.36 mg/L, equivalent to 55% of the applied atrazine occurring in the aqueous phase) for the freshly applied treatment is achieved within 24 h. This desorption concentration steadily declines to 0.86 mg/L (35% in the aqueous phase) because of the slow sorption processes associated with contaminant aging. The extracted soil treatments all exhibit fast initial rates of desorption within the first 5 d. Afterward in the ASW-extracted soil treatment, any slow desorption is exceeded by the rate of slow sorption (aging).

The acetone- and hot methanol-water-extracted treatments exhibited trends of slow desorption after 5 d, whereas the other treatments showed slow sorption (Fig. 1). The slow desorption trend is most clearly displayed by the methanol-water-extracted treatment in terms of percentage of atrazine desorbed. A linear fit to the aqueous concentrations reveals similar rates of slow desorption in micrograms per day for both the methanol-water- and the acetone-extracted soils: 0.0046 and 0.0044 μg/d, respectively (Table 1). However, the methanol-water-extracted treatment exhibited a much faster rate of desorption in terms of percent of nonextractable atrazine desorbed: 0.036%/d for the methanol-water-extracted treatment compared to 0.011%/d for the acetone-extracted soils. This difference between percentage and milligrams atrazine desorbed resulted from variability among the individual extractions that led to different starting concentrations of soil-associated atrazine. A one-way analysis of variance (ANOVA) on the methanol-water-extracted treatment showed significant differences among the sample means between 5 and 90 d for the percentage of atrazine desorbed. All tests of significance reported here were conducted at α = 0.05.

Mineralization

Rates and extents of ring- and chain-labeled atrazine mineralization were examined to approximate atrazine biodegradation at two steps along the degradation pathway. Mineralization of the atrazine chain label, detected by flushing and trapping headspace ¹⁴CO₂, reflects an early step in atrazine biodegradation, side-chain cleavage. Evidence of ring-labeled atrazine mineralization requires cleavage of the triazine ring and is among the last steps of atrazine degradation. Results of the mineralization experiments showed low extents of ring-

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Table 1. Comparison of atrazine desorption and mineralization rates for freshly applied soil treatments and soil treatments that were aged for 90 d and then extracted by either hot methanol-water, acetone, or artificial soil water (ASW).
Bioavailability and desorption of nonextractable atrazine and chain-labeled atrazine mineralization for all treatments over the 90-d incubation period, although the mineralization rates were either steady or increasing when the experiment was halted.

The maximum extent of ring-labeled atrazine mineralization for all soil treatments occurred in the freshly applied treatment from which a mean of 2.2% of the applied atrazine, equivalent to 1.2 μg atrazine, was collected as CO₂ after 90 d (Fig. 2a). The freshly applied atrazine mineralization rate increased notably with time. Mineralization rates and extents for the aged and extracted ring-labeled atrazine treatments declined with decreasing extractability of the soil-associated atrazine fraction (Fig. 2a). For example, the ASW-extracted soils that contained the most extractable atrazine, having undergone the least rigorous extraction technique, showed both the highest rates and the highest extents of mineralization of the three extracted soil treatments. The hot methanol-water-extracted soils, which contained the least extractable atrazine, showed the lowest mineralization rates and extents. The extracted soil treatments showed a range in mean extents of mineralization from 0.074 to 0.25 μg atrazine, representing a range in percentage degraded from 0.41 to 0.61% of the total atrazine in the microcosms. The extracted soil treatments show a slight increase in mineralization rate after day 34, but the rate change is minor compared to the dramatic rate increase in the freshly applied treatment (Table 1). The glucose-amended methanol-water-extracted treatment exhibited less mineralization than the nonamended methanol-water-extracted soil (Fig. 2a). A t test assuming equal variances showed that the extent of mineralization for the glucose-amended treatment was significantly less than that in the nonamended treatment.

Like the ring-labeled samples, chain-labeled atrazine mineralization declined with decreasing extractability of the soil-associated atrazine fraction. However, differences are observed in both the shapes of the mineralization versus time plots and the extent mineralized for each treatment (Fig. 2b). The fastest mineralization rate again occurred in the freshly applied atrazine treatment, in which a mean extent of 1.3 μg atrazine (2.2% of atrazine applied) was mineralized in 90 d, the same percentage as that degraded in the ring-labeled treatment. The extracted soil treatments showed a range in mean mineralization extents from 0.19 to 0.69 μg chain-labeled atrazine in 90 d, representing a range in percentage degraded from 0.94 to 1.8% of the total radiolabeled atrazine in the microcosms (Fig. 2b). These extents of mineralization are nearly three times greater than the corresponding extents in the ring-labeled extracted soil treatments. The extents of mineralization over 90 d for the glucose-amended and nonamended methanol-water-extracted treatments were not significantly different.

Microbial growth of one to two orders of magnitude occurred in the microcosms during the experiment. Initial plate counts showed 1.3 × 10⁷ colony-forming units (CFU) per gram of soil. At the end of the experiment, plate counts ranged from 8.8 × 10⁷ to 1.4 × 10⁸ CFU/g soil.

Analysis of the liquid phase in the biodegradation microcosms at the end of the 90-d incubation showed a small but significant difference between the filtered and unfiltered fractions of both the ring- and the chain-labeled treatments according to a one-tailed paired t test. A significant difference was also observed between the pairs of filtered and filtered/acidified aqueous samples. The results indicate that the maximum amount of HCO₃⁻ detected represented only 3.3% of the total radioactivity in the vial and was associated with the live, freshly applied, ring-labeled treatment. Most of the ring- and chain-labeled treatments contained less than 1% of the ¹⁴C in solution as HCO₃⁻.

**Microbiologically facilitated release**

No microbially facilitated release of nonextractable atrazine was detected in the live microcosms compared to the sterile controls for any of the treatments at any of the three sampling times (22, 60, and 90 d) (Fig. 3). An ANOVA of the atrazine extractabilities (relative to the amount in each vial at the beginning of the incubation) showed no significant difference between the live and the control vials in either the acetone- or the methanol-water-extracted treatments. The only treatments that did show a significant difference between the live and sterile vials were the ASW-extracted treatments (both chain labeled and ring labeled). The results were opposite from that expected; the live microcosms showed significantly lower extraction extents than the sterile controls.
ment ultimately represented only a small fraction of the total soil-associated atrazine, yet these batch experiments underestimate the total desorbable atrazine that would occur in a column or field setting. The experimental design did not enable the distinction between irreversible sorption and extremely slow desorption.

The freshly applied atrazine treatment best displays the process of slow sorption or aging (Fig. 1). Aging is also exhibited in the ASW-extracted soils, in which after 5 d any kinetic desorption is exceeded by a slow sorption trend. This continued sorption trend in the ASW treatment coincides with the results from the freshly applied atrazine treatment that suggest equilibrium is not achieved within the length of the present experiment. After the first 90-d aging period, more sorption sites were still available for contaminant aging.

In the abiotic desorption experiments, sorption and desorption reactions occur simultaneously according to rates that are influenced by aqueous and sorbed atrazine concentrations. Under conditions of large aqueous atrazine concentrations, sorption mechanisms dominate with both ASW-extracted and nonextracted soils. Aged solvent-extracted soils, which were associated with low aqueous atrazine concentrations and high sorbed concentrations, exhibited slow desorption. Unfortunately, the experimental design based on multiple extraction methods is directly linked to the resulting aqueous atrazine concentrations. As a result, it is difficult to distinguish concentration effects from extraction effects.

**Biodegradation**

The low extent of ring-labeled atrazine mineralization detected in this present study (2.2% of the freshly applied atrazine in 90 d) agrees with others reported in the literature for mixed microbial communities collected from the soil environment. Kruger et al. [30] reported less than 1% ring-labeled atrazine mineralization in 180 d. Alvey and Crowley [31] detected 2.4% mineralization of freshly applied ring-labeled atrazine in 5 weeks. The freshly applied ring-labeled atrazine mineralization rate in the present study increased notably with time, especially after day 34 (Fig. 2a). This rate increase probably reflects the exponential population growth of atrazine degraders. Although plate counts were made at the beginning and end of the experiment, it is impossible to determine the growth rate of the atrazine-degrading population, which probably represented only a small fraction of the mixed microbial community. Mineralization rates also increased over time in the extracted soil treatments, although much more gradually than in the freshly applied treatment.

Glucose was added in an attempt to stimulate the microbial population and perhaps create nitrogen-limited conditions, under which atrazine degradation is thought to be enhanced [32]. However, the presence of glucose as an additional carbon source reduced the ring-labeled atrazine mineralization rate (Fig. 2a). On the basis of the low mineralization rates, it is suspected that nitrogen-limiting conditions were not met within the time scale of this experiment. It is possible that enhanced population growth of non-atriazine-degrading microbes would outcompete the degrading organisms for space and required nutrients. Plate counts of the live microcosms at the end of the biodegradation experiment showed a significant difference in CFUs by an ANOVA among the five treatments. Higher CFUs were detected in the live glucose-amended microcosms than the other live treatments, suggesting that total microbial viability was not decreased but altered by the unusually rich

![Graph showing atrazine extractabilities](image)

**DISCUSSION**

**Desorption**

The desorption results reflect three processes that occur simultaneously: an initial fast desorption that reaches a maximum desorption concentration within 1 to 5 d, a slow desorption noticeable only in the methanol-water- and less clearly in the acetone-extracted soils, and the competing process of aging (slow sorption), which produces a negative desorption rate. The large extent of desorption that occurs in the first few days is at least partially an artifact of the original soil extraction procedure in which the soils were extracted once only and then rinsed to remove the extracted atrazine remaining in the pore fluid. Had the soils been extracted repeatedly with fresh solvent until no further atrazine was removed, this initial extent of desorption would be less, although not entirely absent. In the methanol-water- and ASW-extracted treatments, fast desorption contributes 80 to 85% of the maximum desorption concentration attained over 90 d (Fig. 1).

The linear slow desorption rate exhibited by the methanol-water extracted soil (0.036%/d) suggests that even "nonextractable" atrazine shows moderate desorption over time (Table 1). The rate of desorption is much slower than the original sorption rate during the aging process (0.23%/sorbed per day in the freshly applied treatment). This reduced desorption rate might reflect the competition between continued aging and kinetic desorption. The acetone-extracted soil also reveals a slow desorption trend, but the scatter in the data requires more experimentation to assert its significance. The atrazine desorbed from the solvent-extracted soils over the 90-d experi-
Bioavailability and desorption of nonextractable atrazine

Carbon source. Published research has reported both increases and declines in atrazine mineralization rates with glucose amendments [32,33].

The fastest mineralization rate for the chain-labeled soils in the present study occurred in the freshly applied atrazine treatment, in which a mean of 1.3 μg atrazine (2.2% of atrazine applied) was mineralized in 90 d (Fig. 2b). This percentage mineralized is the same as that degraded in the freshly applied ring-labeled treatment despite the fact that ring cleavage has been suggested to occur at much slower rates than side-chain cleavage [31]. Side-chain cleavage might represent a rate-limiting step in this system based on the microbes present in the population, although this explanation seems unlikely. Another hypothesis is that uptake into biomass is greater in side-chain degradation; thus, mineralization rates would be lower. However, analyses of the aqueous phase of the microcosms at the end of the 90-d incubation show only slightly increased radioactivity in unfiltered samples than in filtered samples, suggesting that biomass is not a sink for large quantities of 14C. Despite the low extents of chain-labeled atrazine mineralization in the freshly applied treatment, the chain-labeled extracted-soil treatments exhibited much larger mineralization extents than the corresponding ring-labeled treatments.

Microbiologically facilitated release

Although several researchers have demonstrated the occurrence of microbiologically facilitated release of nonextractable atrazine, no facilitated release was detected in these experiments for any of the soil treatments. Contrary to expectations, the live microcosms showed significantly lower extraction extents than the sterile controls, probably because of biodegradation losses in the aqueous phase, and microbiologically facilitated release was not enhanced by glucose amendments (Fig. 3). These findings contradict the conclusions of Khan and Behki [23] and Rake and Lichtenstein [22], who noted significant release of initially bound residues after incubation in live microcosms. However, the required soil microbes might not have been present in the soil selected to release the nonextractable atrazine.

Bioavailability of nonextractable atrazine

Mass-balance calculations were performed to determine whether the aqueous phase alone could have supplied all the atrazine lost to both mineralization and sorption or whether atrazine in the nonextractable phase was also required. The mass-balance calculations utilized the aqueous phase atrazine concentrations (filtered and acidified) in the viable and sterile control mineralization microcosms at the end of the mineralization experiment. A mass-balance error was calculated as follows:

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\text{Mass balance error} = (\text{Final mg aqueous atrazine in mineralization control}) - (\text{Final mg aqueous atrazine in viable mineralization microcosm}) - (\text{mg atrazine mineralized})
\]

An additional sorption term is not required because sorption should affect both the viable and sterile control microcosms equally. If a large amount of nonextractable atrazine had been mineralized, the mass-balance error result would have been negative because additional mineralization would have occurred that the aqueous phase could not supply. The mass-balance error would also have been negative if microbiologically facilitated release had occurred, as the final aqueous concentration in the viable microcosms would have increased because of additions of nonextractable atrazine. However, the mass-balance error results were entirely positive, suggesting that measurements of atrazine mineralization underestimated the biodegradation losses of aqueous atrazine. Additional aqueous atrazine losses probably occurred through uptake into microbial biomass and incorporation into [14C]HCO3-. No nonextractable atrazine was required to account for the mineralization or sorption losses. Biodegradation could have occurred entirely in the aqueous phase.

Comparisons of rates of desorption and mineralization can also be useful to examine future potential mass-transfer limitations (Table 1). The sorption trends exhibited by the freshly applied and ASW-extracted treatments would probably be reversed under extremely low aqueous concentrations; thus, this analysis was limited to the solvent-extracted treatments that exhibited desorption. The ring-labeled mineralization rates for the acetone- and methanol-water-extracted soils are lower than the rates of desorption in terms of both micrograms and percentage. Thus, a future mass-transfer limitation of atrazine mineralization would not be expected to occur unless the mineralization rates increased significantly or the desorption rates declined over time. The chain-labeled mineralization rates were also lower than the rates of desorption in the methanol-water-extracted treatments. Although the linear fits to the acetone-extracted desorption data were poor, the acetone-extracted treatment exhibited chain-labeled mineralization rates that exceeded the linear rates of desorption in terms of both micrograms and percentage and represented the only treatment that suggested a future mass-transfer limitation.

CONCLUSIONS

An understanding of nonextractable soil-aged contaminants is necessary to assess the long-term fate and significance of this potential contaminant reservoir. This research investigated the impacts of three natural soil processes—desorption, biodegradation, and microbiologically facilitated release—on the fate of soil-aged atrazine extracted by a range of extraction methods. The desorption experiments revealed a co-occurrence of fast desorption, slow desorption, and the competing process of slow sorption. Even the most rigorously extracted soil treatment showed significant slow desorption over 90 d, suggesting a need for further research on the desorption rates of aged contaminants. No microbiologically facilitated release of the nonextractable atrazine was detected for any of the soil treatments. The slow rate of mineralization of both ring- and chain-labeled atrazine limited the ability to draw conclusions on the biodegradability of nonextractable atrazine in the absence of a moderate supply of aqueous atrazine, thus restricting new insights into the relationship between extraction method and bioavailability. However, mass-balance calculations indicated that no biodegradation of the soil-associated atrazine fraction occurred within the time scale of this study regardless of extraction procedure. Rates of desorption in nearly all cases exceeded atrazine mineralization rates, suggesting no mass-transfer limitation in the biodegradation of nonextractable atrazine in this study.

This research demonstrates that at least a portion of soil-aged, nonextractable atrazine, also termed bound residues, remains available to slow desorption over a period of 90 d, although no enhanced release or degradation of the soil-associated atrazine was detected with microbial activity. The term bound residues is often misinterpreted in the literature.
to imply contaminants that are irreversibly bonded to soil particles, yet the definition of bound residues [34] is an operational one based on solvent extraction procedures. Even a soil remediated to the level of zero extractable atrazine might continue to leach atrazine slowly into soil water and ultimately into groundwater. The concentrations that result from slow leaching of a contaminant-aged soil are undoubtedly small, but these low concentrations might prove significant for highly toxic contaminants. More research is needed to understand the rate and extent of slow desorption from contaminant-aged soils over long time periods.

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