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Sorption and Photodegradation Processes Govern Distribution and Fate of Sulfamethazine in Freshwater–Sediment Microcosms

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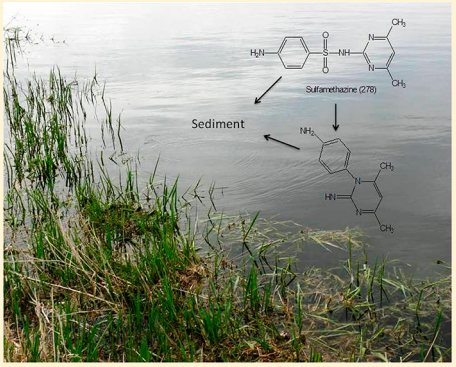
Supporting Information

ABSTRACT: The antibiotic sulfamethazine can be transported from manured fields to surface water bodies. We investigated the degradation and fate of sulfamethazine in pond water using ^{14}C-phenyl-sulfamethazine in small pond water microcosms containing intact sediment and pond water. We found a 2.7-day half-life in pond water and 4.2-day half-life when sulfamethazine was added to the water (5 mg L^{-1} initial concentration) with swine manure diluted to simulate runoff. Sulfamethazine dissipated exponentially from the water column, with the majority of loss occurring via movement into the sediment phase. Extractable sulfamethazine in sediment accounted for 1.9–6.1% of the applied antibiotic within 14 days and then declined thereafter. Sulfamethazine was transformed mainly into nonextractable sediment-bound residue (40–60% of applied radioactivity) and smaller amounts of photoproducts. Biodegradation, as indicated by metabolite formation and $^{14}CO_2$ evolution, was less significant than photodegradation. Two photoproducts accounted for 15–30% of radioactivity in the water column at the end of the 63-day study; the photoproducts were the major degradates in the aqueous and sediment phases. Other unidentified metabolites individually accounted for <7% of radioactivity in the water or sediment. Less than 3% of applied radioactivity was mineralized to $^{14}CO_2$. Manure input significantly increased sorption and binding of sulfamethazine residues to the sediment. These results show concurrent processes of photodegradation and sorption to sediment control aqueous concentrations and establish that sediment is a sink for sulfamethazine and sulfamethazine-related residues. Accumulation of the photoproducts and sulfamethazine in sediment may have important implications for benthic organisms.



INTRODUCTION

Veterinary antibiotics contained in animal manures have the potential to be transported from manured crop fields or pastures to surface waters. Previously, veterinary antibiotics were detected in 48% of 139 stream waters tested in 30 states, according to the U.S. Geological Survey.[1] Sulfamethazine (SMZ) has wide use in livestock production for growth promotion and therapeutic purposes. Manure from treated swine contained over 5 mg kg^{-1} SMZ, and SMZ exceeded 100 μg L^{-1} in swine lagoon water.[2,3] However, lower concentrations are reported in tile drainage water, streamwater, and groundwater.[1,3−7] Kim and Carlson[6,8] detected antibiotic residues up to 0.1 mg kg^{-1} in sediment from an impacted river, but typical mean concentrations were 0.001–0.03 mg kg^{-1}.

Previous studies have indicated that SMZ and related sulfonamide antibiotics are initially weakly sorbed to soils but become more strongly sorbed over time.[9−13] Sulfamethazine is highly mobile in the aqueous portion of runoff, thus being likely to reach streams and farm ponds.[14,15] Sulfonamide antibiotics entering soil or water environments could potentially alter bacterial populations and their activity in soil, sediment, and water, thus affecting biodegradation, nutrient cycling, and water quality.[16−18] Small ponds and wetlands that serve as key breeding sites for amphibians and support invertebrate communities can receive significant amounts of agricultural runoff, which could contain antibiotic residues.[19] An understanding of the degradation and fate in small ponds is important to assessing the ecotoxicological impacts of antibiotic residues entering these habitats.[20] Though its fate has been extensively examined in soil, SMZ fate in surface water, and sediment in particular, has not been extensively studied. The objective of the present study was to investigate the fate of sulfamethazine in microcosms simulating pond water systems. Specific objectives were to determine the effects of sediment and swine manure inputs on persistence of SMZ in a pond water system and to evaluate potential mechanisms of SMZ dissipation in pond water, including sorption to sediment and degradation processes.

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EXPERIMENTAL MATERIALS AND METHODS

Chemicals. ^{14}C-U-Phenyl-sulfamethazine and nonlabeled analytical-grade sulfamethazine (SMZ) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, HCl, and NaOH were purchased from Fisher Scientific (Waltham, MA).

Matrix Collection. Pond water (surface 20 cm) and submerged sediment were collected from the Iowa State University Horticulture Research Station pond (Gilbert, IA); 1−15 cm depth sediment samples were manually collected using a soil auger and stored in the dark at 4 °C. Mixed sediment had a moisture content of 46.2%, a sandy loam (60% sand, 28% silt, 12% clay) texture, with 2.0% organic carbon (OC) and a pH of 8.1. The pH of the pond water was 8.1, the alkalinity was 103 mg mL^{-1}, and the total hardness was 150 mg mL^{-1}.

Fresh manure was obtained from adult female hogs on a corn-based, antibiotic-free diet (>20 days withdrawal) from the Iowa State University Swine Nutrition Facility (Ames, IA). Manure was refrigerated at 4 °C until use (<7 days). Swine excrete 99% of sulfamethazine and metabolites within 8 days after treatment, and the 20-day withdrawal time would result in minimal residues in the manure.[21]

Treatment Preparation. Treatment preparation was briefly described by Henderson et al (2009).[22] The fate of sulfamethazine was examined in four pond water systems: pond water and sediment (PWS), autoclaved pond water and sediment, pond water without sediment (PW), and pond water and sediment with SMZ added with dilute manure (PWS +M) with four replications of each. Microcosms were constructed by adding 73 g of sediment (50 g dry wt) into wide-mouth pint jars (Ball Corp., Broomfield, CO), and topped with 177 mL of pond water to equal 200 mL of water per jar. Each jar served as a replicate. For the autoclaved pond water and sediment treatment (autoclaved PWS), four 1-L samples of pond water were autoclaved for 20 min each and sediment was autoclaved in 1 h cycles three times over the course of 1 week with a day between each cycle. Autoclaved microcosms were constructed as described above. For the autoclaved PWS treatment (sterile system), all work was completed using sterilized equipment in a laminar flow hood.

Sediment was allowed to settle 1 h prior to treatment with ^{14}C-SMZ solution. Stock solutions of labeled and nonlabeled SMZ were prepared to make a final treatment solution to be added to each microcosm. A solution of nonlabeled SMZ and ^{14}C-labeled SMZ was prepared in 10% methanol that contained 0.425 mg mL^{-1} of SMZ, and 0.085 μCi mL^{-1} was prepared. Each replicate jar received 2.35 mL of treatment solution, so the final concentration of SMZ in pond water was 5 mg L^{-1} and 0.2 μCi jar^{-1}. After treatment solution was added, water was gently stirred with a sterile spatula to allow for mixing without disturbing the sediment. This concentration of SMZ is greater than the concentrations typically observed in surface waters but allowed accurate quantification of SMZ in the water and sediment.

For manure treatment (PWS+M), a manure slurry was added using a syringe to obtain 0.1% manure in pond water. This concentration was chosen to represent manure delivered to a pond in storm runoff following land application. The slurry consisted of a 33% w/v solution of fresh manure (33 g wet wt, 22% dry mass, in 100 mL of distilled water). Slurry was stirred for 40 min to break up large chunks of manure, and 0.6 mL of the 33% slurry was added to each replicate. The pond water microcosm consisted of 200 mL of pond water treated with sulfamethazine solution described previously. Microcosms were maintained in environmental chambers at 22 °C in a 12:12 photoperiod for the 63-day incubation; pH was monitored weekly in the pond water of the microcosms and did not significantly change during the test period. Lighting was provided by fluorescent and incandescent lights.

Measurements. Mineralization of ^{14}C-SMZ added to the microcosms was measured using NaOH traps at 3, 7, 14, 21, 28, 35, 42, 49, and 56 days. Traps consisted of a 25-mL high-density polyethylene vial glued to the inner surface of the jar and filled with 10 mL of 0.5 M NaOH. Traps were changed at each time point; 3 mL of NaOH solution were mixed with 12 mL of Ultima Gold XR cocktail and counted for radioactivity using a Packard Tri-Carb 1900 (Perkin-Elmer, Waltham, MA).

Four replicate microcosms for each treatment were used for analysis of sulfamethazine in sediment and water at 7, 14, 28, and 63 days after addition of SMZ. Briefly, total radioactivity in water was determined by liquid scintillation counting. SMZ in the water was extracted with HLB solid-phase extraction cartridges and analyzed by HPLC using both UV and ^{14}C detection (see Supporting Information for details).

Sediment samples were extracted twice with 100 mL of 70% methanol. Methanol in the extracts was evaporated under a stream of N_2 gas. The remaining aqueous fraction was passed through the HLB SPE cartridge and eluted in methanol. Extracts were analyzed for SMZ (UV detection) and degradates (^{14}C detection). Residual nonextractable (bound) ^{14}C was measured by combusting 0.5 g of dried, ground sediment in a OX-600 biological oxidizer (RJ Harvey Instrument Co., Hillsdale, NJ), and measurement of the $^{14}CO_2$ produced.

^{1}H Nuclear Magnetic Resonance (NMR). Photoproducts were detected in the course of the experiment, and ^{1}H NMR was used to verify their identity. SMZ was added at 4 mg L^{-1} to pond water and incubated under the same conditions described previously. Aqueous extracts were concentrated by freeze drying, redissolving in solvent, and partially purifying by collecting HPLC eluant at appropriate retention times (R_T). ^{1}H NMR was performed with a Varian VXR-300 equipped with a wide-bore 7 T magnet. Photoproducts were dissolved in chloroform-*d* with 1% trimethylsilyl trifluoromethanesulfonate (Cambridge Isotope Laboratories Inc., Andover, MA). ^{1}H NMR data were analyzed with XWIN-NMR software (Bruker BioSpin; Billerica, MA).

Statistical Analysis. Statistical analysis of the data included linear models and least-squares means to assess treatment differences among various parameters (e.g., proportion of bound residues, SMZ concentrations in water and sediment, etc.) and time points (SAS V9.1; SAS Institute, Cary, NC). The Tukey method was used for multiple comparisons of treatment means.

First-order exponential decay models were used to describe SMZ dissipation from the pond water for the PWS, autoclaved PWS, and PW treatments (eq 1)

$$C_t = C_0 \times e^{-kt} \quad (1)$$

where C_t is the concentration remaining in pond water at time t, C_0 is the initial concentration of SMZ in the pond water, t is days after spiking, and k is the rate constant.

An exponential decay model with two compartments (fast and slow) fits the data for the PWS+M treatment (eq 2). The two-compartment model provided better fit to the experimental

Table 1. Distribution of ^{14}C Residues in Pond Water and Sediment Microcosms at 7 and 63 Days after Addition of ^{14}C-Sulfamethazine[a]

treatment	total in water (%)	CO_2 (%)	sediment extractable (%)	sediment bound (%)
day 7				
pond water and sediment (PWS)	80.0 ± 0.43	0.21 ± 0.01	4.4 ± 0.03	16.1 ± 0.36
pond water, sediment and manure (PWS+M)	72.7 ± 0.59	0.05 ± 0.003	5.0 ± 0.22	24.8 ± 0.40
autoclaved PWS	80.2 ± 1.85	0.02 ± 0.004	6.8 ± 0.32	15.2 ± 0.48
pond water only (PW)	92.1 ± 0.69	0.07 ± 0.005		
day 63				
pond water and sediment (PWS)	49.2 ± 1.31	0.91 ± 0.15	5.3 ± 0.11	40.4 ± 0.92
pond water, sediment, and manure (PWS+M)	18.5 ± 3.42	0.25 ± 0.03	4.3 ± 0.29	61.2 ± 1.08
autoclaved PWS	52.1 ± 2.04	0.25 ± 0.05	8.3 ± 0.49	38.3 ± 1.89
pond water only (PW)	88.03 ± 0.48	2.45 ± 0.11		

[a]All values are shown as mean % of applied ^{14}C ± standard error (n = 3). Adapted from Henderson et al.[22]

data, particularly at the later sampling times than the first-order single-compartment model

$$C_t = C_1 \times e^{-klt} + C_2 \times e^{-k2t} \quad (2)$$

where initial concentrations in the fast and slow pools are C_1 and C_2, respectively, and $C_1 + C_2 = C_0$. Rate constants $k1$ and $k2$ are for the fast and slow dissipation pools, respectively.

First-order single-compartment accumulation models were used to estimate ^{14}C-bound residues (P_m, % of applied) from the measured percent bound at time (t) where a is the accumulation rate constant (eq 3) and P_{max} is the maximum accumulation of bound residue. Analyses and plots were created using SigmaPlot 10.0 (SyStat Software, Inc., San Jose, CA).

$$P_m = P_{max}(1 - e^{-\alpha t}) \quad (3)$$

Partition coefficients were calculated for SMZ at day 63. Day 63 was chosen because the amount of bound residues had become relatively stable within the system. K_d (L kg^{-1}) was calculated as $K_d = C_s/C_w$, where C_s is the extractable concentration of SMZ in sediment (mg kg^{-1}) and C_w is the concentration of SMZ in the pond water (mg L^{-1}). K_{oc} was calculated as $K_{oc} = K_d$/foc, where foc is the organic carbon fraction of the sediment (2% OC for PWS and autoclaved PWS, 2.16% OC for PWS+M).

RESULTS AND DISCUSSION

Mass Balance. Mean ^{14}C balances for pond water (PW) microcosms were >90% for each sampling time (Table 1 and Table S1, Supporting Information). Total ^{14}C recovery from all sediment-containing systems (PWS, PWS+M, and Autoclaved PWS) exceeded 95%, with the exception of the PWS+M treatment at day 63, with a mean ^{14}C mass balance of 84 ± 2%. Clear differences existed between day 7 and day 63 for sediment binding and amount of ^{14}C remaining in water (p < 0.001 for all treatments).

Dissipation Kinetics. Sulfamethazine dissipated from pond water in all treatments, with the most rapid loss occurring in the sediment-containing microcosms (Figure 1, Table 2). Loss of SMZ was slowest in the autoclaved sediment−water treatment, which had a half-life of 17.8 days. The greater persistence of SMZ in the autoclaved-PWS treatment shows that biological activity played an important role in SMZ removal from the water. Similarly, a comparison of the PW treatment with the PWS and PWS+M treatments showed the effect of sediment on removal of SMZ from the water.

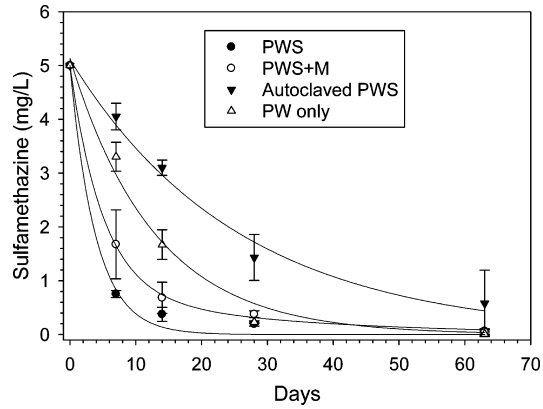

Figure 1. Dissipation of parent sulfamethazine in pond water microcosms. Manure-containing treatment (PWS+M) treatment followed a two-compartment exponential decay model. Pond water plus sediment (PWS), autoclaved PWS, and pond water only (PW) treatments followed a single-exponential decay model. Standard error bars are shown.

The PWS+M treatments followed a two-compartment exponential decay model, while the PWS, autoclaved PWS, and PW treatments followed a single-compartment exponential decay model. The input of manure organic matter into the system appeared to add a second phase of dissipation to SMZ decay; this may be due to alteration of the microbial communities or sorption of SMZ to manure which could decrease the availability of SMZ for photo- or biodegradation.

Sulfamethazine Residues in Sediment. Sulfamethazine moved from the water column into the sediment within the first 14 days of the study (Figure 2). Extractable SMZ concentrations in the sediment rose rapidly in the first 7 days, peaked at 7−14 days, and then showed a slow decline, which corresponded with an increase in bound residue detected in all sediment-containing treatments. Extractable SMZ in sediment accounted for 1.9%, 4.1%, and 6.1% of the added SMZ after 14 days incubation in the PWS, PWS+M, and autoclaved PWS treatments, respectively. The rapid rise in sediment SMZ concentrations in the first 7 days after addition corresponded to the decrease in SMZ in the water phase. Autoclaving, which reduced the rate of SMZ loss in water, increased the concentration of SMZ in the sediment.

Partitioning of SMZ into sediment from the water phase was consistent with sorption processes. K_d and K_{oc} were calculated for each treatment based on the mean concentration of SMZ in water and extracted from sediment at day 63, 2% OC in

Table 2. Dissipation Kinetics for Sulfamethazine in Pond Water Phase

treatment	dissipation model[a]	k^b (days^{-1})	r^2	p value	half-life (days)
PWS	$C = C_0 \times e^{-kt}$	0.26 ± 0.03	0.9941	0.0002	2.7
PWS+M	$C_t = C_1 \times e^{-k1t} + C_2 \times e^{-k2t}$	k_1: 0.20 ± 0.04	0.9994	0.0307	4.2[c]
	C_1: 4.21 ± 0.67	k_2: 0.03 ± 0.028			
	C_2: 0.79 ± 0.66				
autoclaved PWS	$C = C_0 \times e^{-kt}$	0.04 ± 0.004	0.9881	0.0006	17.8
PW	$C = C_0 \times e^{-kt}$	0.08 ± 0.009	0.9859	0.0007	8.9

[a] C_1 and C_2 represent initial SMZ concentrations in fast and slow pools, respectively. [b] Rate constant ± standard error. [c] Half-life calculated as DT_{50}, time required for 50% disappearance.

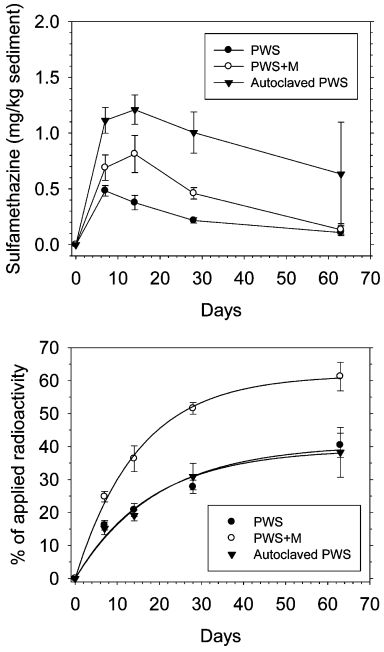

Figure 2. Movement of parent sulfamethazine (SMZ) into sediment (top), and exponential increase in bound ^{14}C-SMZ residues in sediment (bottom) over time. Standard error bars are shown.

sediment, and 42% OC in manure, corresponding to addition of 0.08 g of OC in the manure-containing test systems. Manure organic carbon content was estimated to be 42%.[23] Although the manure contribution of organic carbon in the present study was <8% of the total organic C in the test system, increased particulate surface area or qualitative differences in manure derived C might lead to increased binding. It was assumed that the fraction of SMZ in the sediment pore water would be minimal compared to the adsorbed fraction. For the PWS systems K_d was 1.68 L kg^{-1} and K_{oc} was 83.8, for PWS+M K_d was 2.87 L kg^{-1} and K_{oc} was 133.0, and for the autoclaved PWS K_d was 1.1 L kg^{-1} and K_{oc} was 54.7. The magnitude of sorption observed in this study is relatively consistent with sulfonamide K_d values.[10,11,24,25] Sulfamethazine sorption is governed by pH, clay content, and organic C content, but SMZ would be in neutral or anionic forms in these sediments at pH 8.1, indicating that sorption to organic C could be the dominant sorption mechanism.[10,25−27]

Sulfamethazine adsorption to swine manure and sediment was significantly greater compared to sediment, but swine manure amendments to soils caused a range of increases in K_d.[24,25] It is possible that SMZ sorbed to manure in the pond water phase and then settled onto the sediment. This would also be consistent with the slower initial loss of SMZ in PWS +M water compared to that in the PWS treatment (Figure 1). We did not differentiate between manure and sediment in our extractions and calculations.

In the manure-containing microcosms, >60% of applied ^{14}C was strongly bound (nonextractable) to sediment at the end of the study, nearly twice the amount of binding as the other two treatments (Table 1, Figure 2). Greater bound residue formation in the PWS+M was similar to the difference in K_d between PWS+M and the other treatments. In all microcosms with sediment, the ^{14}C-bound (unextractable) residues increased exponentially and appeared to plateau toward the end of the 63-day study (Figure 2). First-order rate constants (a) for accumulation of bound residues were 0.052, 0.067, and 0.055 days^{-1}, and P_{max} (maximum accumulation of bound residue) values were 40.5%, 61.7%, and 39.2% of applied ^{14}C for the PWS, PWS+M, and autoclaved PWS treatments, respectively. Beginning at day 7, PWS+M had significantly more bound residues than PWS or the autoclaved treatment; mean differences between these treatments were 8.6% and 9.6% of applied radioactivity (p = 0.0428 and 0.0161, respectively). At all other time points (Figure 2) PWS+M had significantly more bound residues than PWS or the autoclaved treatment (p < 0.0001), ending with 61.2% bound residue at day 63 (95% CI 57.7−64.7). Bound residues may result from covalent binding of SMZ or SMZ degradation products with humic substances present in soil or diffusion into micropores in the soil matrix, and these mechanisms are also likely applicable to sediments.[12,28] Batch desorption resulted in <1% of bound residues being desorbed (data not shown), demonstrating the strong degree of binding of these residues to the sediment. Similar amounts of bound/unextractable residue have been reported for sulfonamides in soil.[29,30]

Photoproducts and Metabolites. In addition to sulfamethazine, two unidentified compounds with HPLC retention times of 5.3 and 7.6 min were detected using a diode array detector at 254 nm. Further confirmation of these compounds as SMZ breakdown products was obtained by collecting fractions of the HPLC effluent corresponding to the retention times and counting for radioactivity. Detection of ^{14}C indicated that at least a portion of the phenyl ring of SMZ was present. Interestingly, neither compound was retained on the Oasis HLB cartridge during the solid-phase extraction step, pointing to the polarity of the compounds; both compounds (R_T = 5.3 and 7.8 min) were seen in all treatments, indicating that they were likely products of a physical or chemical degradation process, such as hydrolysis or photodegradation. Correspondingly, a separate nonradiolabeled light/dark study was performed (Figure S1, Supporting Information) in sterile conditions using the same environmental chamber conditions described in the Experimental Materials and Methods section. Deionized water was filter sterilized and spiked to 5 mg L^{-1}

SMZ; dark treatment was achieved by wrapping the sealed vessels in aluminum foil (n = 4). Replicates were extracted and analyzed using HPLC/MS. After 14 days exposure to light, a photoproduct (R_T = 5.3 min) with a mass (M + H) of 215 was produced, but it was not produced in the dark treatment. Boreen et al.[31] describe SMZ photodegradation with 1.2−7.5 day half-life, which corresponds well with half-lives observed in this study; however, their conditions were quite different from those described here. The compound 4-(2-imino-4,6-dimethyl-pyrimidin-1-(2*H*)-yl) aniline (compound IV, Figure 3) was

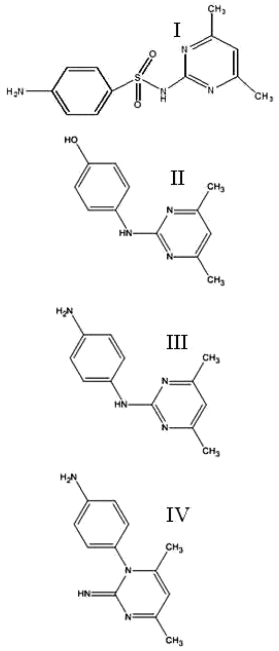

Figure 3. Possible identities of sulfamethazine photoproducts with estimated mass units of 215 (M + H): sulfamethazine (I), 4-[(4,6-dimethylpyrimidin-2-yl)amino]phenol (II); *N*-(4,6-dimethylpyrimidin-2-yl)benzene-1,4-diamine (III); 4-(2-imino-4,6-dimethylpyrimidin-1-(2*H*)-yl)analine (IV).

identified as a possible photoproduct that has a molecular weight of 215 as determined by mass spectrometry, ^{1}H NMR, and ^{13}C NMR.[31] We subsequently prepared additional photoproduct and after purification by HPLC performed ^{1}H NMR analysis on two photoproducts that have the same molecular mass but different retention times on HPLC. The photoproduct having a retention time of 5.3 min had a similar ^{1}H NMR to that reported by Boreen et al.,[31] but peaks were shifted upfield. Chemical shifts from ^{1}H NMR ($CDCl_3$): δ 1.18 (s, 3H), 1.71 (s, 3H), 4.59 (s, 3H), 4.88 (m, 2H), 5.81 (d, 2H). ^{1}H NMR analysis was also performed with the second photoproduct that had a retention time of 7.8; however, these results were inconclusive. Figure 3 suggests possible photoproducts that have a mass of 215, and compound III was produced from sulfamethazine in a photoreaction experiment.[32]

No statistically significant differences in concentrations of ^{14}C-photoproducts in water were noted among treatments until day 28 of the study (Figure 4). At day 28, the PW treatment had significantly higher amounts of photoproducts in the water compared to PWS+M and autoclaved PWS treatments (p = 0.0257 and 0.0083). The water in the PWS+M treatment was visibly cloudy, thus inhibiting light penetration of the water. The higher levels of photoproducts in PW compared to

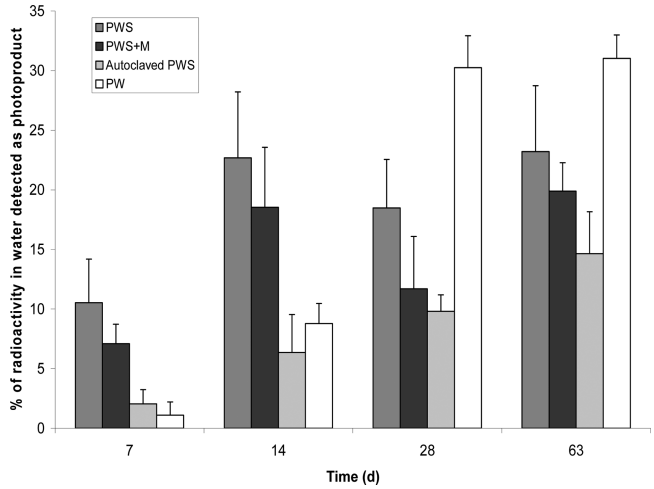

Figure 4. Amount of photoproduct(s) in water over time, displayed as percent of radioactivity in the water column detected as photoproduct. Standard error bars are shown.

sediment-containing treatments at day 28 may be due to increased light penetration. Alternatively, adsorption of SMZ residues to sediment could limit the amount of SMZ available in the water column for photodegradation. Sediments would also reduce photoproduct concentrations through adsorption. The lower amount of photoproducts in the autoclaved PWS microcosm than the PWS microcosm (Figure 4) suggested an interaction between microbial activity and the photodegradation process. A similar interaction was reported for sulfonamides previously, but no mechanism was established.[33]

By day 63, photoproducts were 31% (95% CI 24−37%) of total radioactivity in the PW treatment and the dominant degradates. For the PWS and PWS+M treatments, the photoproducts accounted for 23% of applied radioactivity (95% CI 16−30%) and 20% (95% CI 13−27%) of detected compounds in the aqueous phase at day 63, respectively. The autoclaved PWS treatment had slightly less photoproduct at day 63 compared to the other treatments, with a mean 14.6% (95% CI 7.8−21.5%; p = 0.0832).

To evaluate the timeline for photoproduct formation, comparisons of amount of photoproduct at each time point were made within individual treatments. Comparisons within the sediment-containing treatments (PWS, PWS+M, and autoclaved PWS) revealed no significant differences in photoproduct concentrations across time (Figure 4). However, the PW treatment showed significant increases in amount of photoproduct when comparing days 7 and 14 to days 28 and 63 (p < 0.005), indicating that the majority of photoproduct formation occurred between days 14 and 28 in the study. Additionally, a relative plateau in concentration is visible when comparing data for days 28 and 63 for the PW treatment (Figure 4); no differences were detected between concentrations at days 28 and 63 for the PW treatment.

Analysis of sediment extracts revealed that photoproducts were the major breakdown products in all treatments, accounting for approximately 15% of radioactivity in the extracts. At day 63, the mean amount of photoproducts in sediment extracts was 17% of extractable radioactivity in sediment for PWS (95% CI 11−22%), 16% for PWS+M (95% CI 10−21%), and 13% (95% CI 8−19%) for the autoclaved treatment. No differences were noted between treatments; however, an overall time effect was noted (p = 0.0054),

indicating an increase in the photoproducts partitioning from the water column into the sediment over time. The photoproducts were the major degradates detected in both the aqueous and the sediment fractions in our microcosm study.

Biodegradation was also important in the environmental fate of SMZ: the half-life of sulfamethazine in autoclaved pond water and sediment was 17.8 days, compared to the 2.7-day half-life in pond water and sediment without autoclaving (Table 1). Unidentified radiolabeled metabolites other than previously described photoproducts were detected in pond water and sediment extracts. Individual unidentified metabolites accounted for less than 8% of radioactivity in pond water or sediment extracts, while photoproducts often exceeded 15%. These unidentified compounds may be products of incomplete biodegradation or chemical degradation processes. Other possible chemical degradation routes for sulfonamides have been suggested, including free-radical-mediated reactions or microbially-mediated reductions by Fe−II or goethite.[34,35] Sulfamethazine biodegradation products are diverse, with the major metabolite being N^4-acetyl-sulfamethazine, but N-methylation and hydroxylations also occur.[36] Less than 3% of the applied ^{14}C-SMZ was mineralized to CO_2, but differences in mineralization were noted among the treatments (Figure 5).

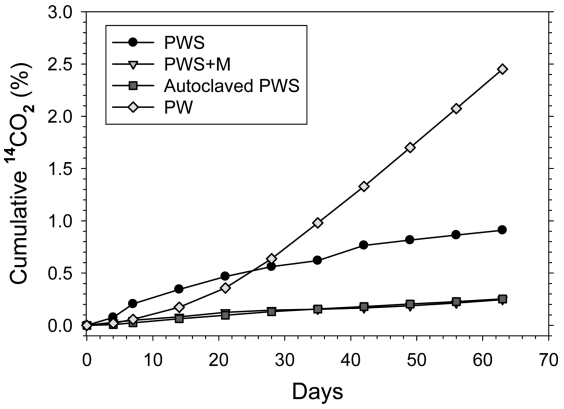

Figure 5. Mineralization of ^{14}C-sulfamethazine in pond water−water microcosms. Mineralization data for treatments autoclaved PWS and MWS+M overlap.

Interestingly, the treatment containing pond water alone had the highest mineralization rate, while the manure-containing and autoclaved treatments had nearly identical mineralization rates. This could be due to differences in availability of the SMZ and related residues for complete degradation by microorganisms in the systems. Sulfamethazine in the pond water is removed by competing processes, photodegradation, biological degradation, and adsorption to sediments, and the predominance of one mechanism over another is likely governed by site-specific conditions.

CONCLUSIONS

Current monitoring data reveal that sulfonamides are frequently and widely detected in surface water bodies throughout the world; sources of these residues include human and livestock origins. Although detection of sulfonamide residues in the water is important, sediment serves as a sink for these residues. For example, Kim and Carlson[8] detected SMZ in 25% of river sediment samples, with a mean of 4.7 μg kg^{-1} and maximum concentration of 13.7 μg kg^{-1}; overlying water samples from that study were typically < 0.1 μg L^{-1}, pointing to the accumulation of sulfamethazine in sediment. These experiments directly show the rapid movement of sulfamethazine from water into sediment. Futhermore, we show the simultaneous degradation of sulfamethazine by photolysis and biological means. Finally, sulfamethazine, photoproducts, and metabolites became strongly bound (nonextractable) to the pond sediment over time, accounting for 40−60% of the applied ^{14}C. Heise et al.[29] reported similar sulfonamide affinity for soil, with nonextractable residues exceeding 90% of the applied antibiotic.

Given the extent of bound residue formation, the bioavailability of these residues is of ecotoxicological interest. SMZ adsorbed or bound to nonhumified organic matter in soil might desorb or become bioavailable during humification or mineralization of the nonhumified fraction.[22] Uptake of bound sulfonamide antibiotics by *Brassica rapa* or *Lumbricus terrestris* was only 1% or less of applied ^{14}C in soil,[29] but bioavailability of bound resides in aquatic sediments has not been evalutated. Potential implications of sediment-associated residues for benthic-dwelling organisms need to be further explored.

Photoproducts were the major extractable degradates detected in the microcosms throughout the entire study. Photoproducts partitioned into sediment, similarly to SMZ, and were detected as the most prevalent degradate in sediment as well. Sediment is a sink for SMZ and SMZ-related residues which may have implications for benthic organisms, such as sediment-dwelling invertebrates or bacteria.[22] The toxicity of these photoproducts remains to be fully determined, and the uncharacterized bound residues may contain SMZ or toxicologically significant metabolites that could become bioavailable over time.

ASSOCIATED CONTENT

Supporting Information

Additional details of experimental methods, data on the distribution of ^{14}C residues in pond water and sediment microcosms, and photoproduct formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS

SMZ	sulfamethazine
OC	organic carbon

PWS pond water and sediment
PW pond water without sediment
PWS+M pond water and sediment plus diluted swine manure
95% CI 95% confidence interval
HPLC high-performance liquid chromatography

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