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Sorption, degradation, and transport of sulfamethazine in soils and manure-amended soils

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Sorption, degradation, and transport of sulfamethazine in soils and manure-amended soils

by

Warisara Lertpaitoonpan

A dissertation submitted to the graduate faculty

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ABSTRACT

Agricultural fields, surface waters and ground water can be contaminated with antibiotics through the application of antibiotic-contaminated swine manure. One of the more common antibiotics used for the swine industry is sulfamethazine (SMZ). The focus of this dissertation is to investigate the fate and transport of SMZ in soils in the presence of manure when applied to the fields. Sorption coefficients of SMZ for five local soils from Iowa with organic carbon (OC) contents ranging from 0.1 % to 3.8 % and solution pHs ranging from 5.5 to 9 were determined using batch sorption experiments. Sorption data fitted well to a linear sorption model but not to a non-linear Freundlich model. The linear sorption coefficients (K_d) were found to decrease with an increase in soil-solution pH. In addition, the K_d values were found to increase with an increase in % OC of soil at a given pH. At pH 5.5, K_d values were 0.58 L kg^{-1} and 3.9 L kg^{-1} for soils with 0.1 % OC and 3.8 % OC, respectively. Hydrophobic sorption was probably involved for pH < 7.4 due to the unionized form of SMZ while some surface sorption was probably involved for pH > 7.4 due to the ionized form of SMZ. A mechanistic model and a linear regression model incorporating soil properties and fractions of ionized SMZ were developed and found to estimate K_d values of other studies.

Inhibitory effects of SMZ on anaerobic microbial respiration were observed at SMZ concentration of 50 mg kg^{-1} while inhibition of aerobic microbial respiration was observed at between 50 and 100 mg kg^{-1}. The availability-adjusted first-order model but not simple first-order kinetics was found to fit the data well. Half-lives of SMZ ranged from 1.2 to 6.6 days and 2.3 to 15.1 days under aerobic and anaerobic conditions, respectively. The fate of ^{14}C-

SMZ in soil showed that 70 to 91 % of ^{14}C-SMZ was bound to soils. Only 0.1 to 1.5 % of ^{14}C-SMZ was completely mineralized to $^{14}CO_2$ with the highest mineralization under aerobic conditions. The metabolites accounted for 5 to 10 % of SMZ and were found to be *N*4-acetylsulfamethazine and desamino sulfamethazine.

Leaching of SMZ from soils and from manure-amended soils were investigated using topsoil mixed with SMZ or with SMZ-contaminated manure to a concentration of 7.25 mg kg^{-1} soil. The first simulated rainfall event applied to soil cores at 1, 4 and 7 days after the application of SMZ was followed by a second rain event, three days after the first rain event. Concentrations of SMZ in leachate were found to be the highest for first day after rainfall with concentration of 432 ± 167 µg L^{-1}. Concentrations of SMZ in the leachate decreased with longer time duration between application of SMZ and the first rain event. The results showed that manure in the soils did not impact the leaching of SMZ and that colloid-facilitated transport of SMZ was unlikely. This study suggested that SMZ is expected to leach from manure-amended soils or manure even though SMZ may be degraded or bound to the soils.

CHAPTER 1. INTRODUCTION

1.1 General introduction

Pharmaceuticals are used in humans, animals, and aquatic farming for disease control and for maintenance of health. Certain activities such as disposal of expired medicine in the sewage system, excretions of unmetabolized pharmaceuticals from humans and animals, discharge of wastewater and surface runoffs to receiving water, land application of biosolids and manure or disposal of biosolids at landfill can result in the dispersion of these compounds in the environment. Due to concerns of pharmaceutical compounds in the water and soil environment, research on these compounds in the environment were initiated in the 1990s in Europe and in the U.S. The risks posed by these compounds are not well understood and many of these compounds do not have regulatory standards for surface waters and drinking waters. However, for some compounds, it has been shown that concentrations as low as in the $\mu g\ L^{-1}$-level may result in serious impacts to aquatic and terrestrial organisms. Of the many pharmaceuticals, antibiotics are one class of compounds which are being closely examined.

Antibiotics are used in concentrated animal feeding operations (CAFOs), (USDA, 2002) for the purposes of treating diseases and promoting growth. Approximately 70 percent of the total pharmaceutical use for livestock in the U.S. is administered to promote growth rate and feed conversion efficiency (Union for Concerned Scientists Press Release, 2001). The swine industries use more antibiotics as food additive than other livestock industries (Giguère et al., 2007). Antibiotics administered to animal are not totally absorbed into body and are excreted through urine and feces (Thiele, 2000;

Tolls, 2001; Thiele-Bruhn and Aust, 2004; Göbel et al., 2005). Antibiotics in animal manure can persist in tanks, pits, or lagoons (Langhammer, 1989; Kolz et al., 2005) and can enter the environment when the stored animal manures are applied to land as agricultural fertilizer. The fate of these antibiotics in soil is dependent on the type of antibiotic compounds, soil characteristics, and environmental conditions (Tolls, 2001; Kumar et al., 2005b; Thiele-Bruhn, 2003). Transport of antibiotics via infiltration and runoff to subsoil, groundwater, and surface water can occur as shown by many studies (Christian et al., 2003; Thiele-Bruhn and Aust, 2004; Batt and Aga, 2005).

As reported by USDA, manure produced in the U.S. at CAFOs was approximately 335 million tons (dry matter) per year (USDA-ARS, 2005). Typical concentrations of antibiotics in manure ranged from 1 to 10 mg kg^{-1}, while concentrations as high as 200 mg kg^{-1} can be found (Kumar et al., 2005b). Even though the antibiotic residues in the environment are at trace levels and below toxic levels to humans, the possibility of chronic adverse effects, for instance, allergy and chronic toxicity cannot be ruled out. In addition to chronic effects to human health, one of the greatest concerns with regards to antibiotics is the development and spread of antibiotic resistant bacteria (Morris and Masterton, 2002; Kumar et al., 2005a; Doyle, 2006).

In order to understand the risks posed by these antibiotics, it is essential to know the environmental parameters that control the migration of antibiotics from manure to soil and to water. Not only sorption, a key process, has a direct effect on the fate and transport of antibiotics, biodegradation of these compounds will also impact their fate. The pH and media properties typically influence the sorption and impact the degradation of these antibiotics as well, due to their bioavailability in soil media. The goal of this research was to determine the fate and transport of SMZ in soils and in manure-amended soils. The specific objectives of this study were to:

1) Investigate the effect of organic carbon content of soils and soil pH on the sorption of SMZ and to develop a simple model to estimate the sorption coefficients of SMZ
2) Investigate the impact of SMZ concentrations on the inhibition of soil microbial activities in soils and to determine the degradation rates of SMZ in soils and manure-amended soils under aerobic conditions and anaerobic conditions
3) Investigate the mineralization of SMZ and conduct mass balances of the distribution of ^{14}C-SMZ during the degradation process
4) Evaluate the mobility of SMZ in soil columns under simulated rain conditions, the impact of the duration of application of contaminated swine manure and the presence of manure itself and the first rain event on the leaching of SMZ through soil columns.

1.2 Dissertation organization

This dissertation is organized into a total of six chapters with three chapters as individual manuscripts to be published in peer-review journals. Chapter 1 introduces the background, the research questions and describes the goal of study. Chapter 2 provides the literature review giving information on usage, risks, concentrations in environment, sorption, and degradation of sulfonamides, especially sulfamethazine. Chapter 3 describes the findings from batch sorption experiments using five different soils. Chapter

4 describes the study on the impact of SMZ concentrations on the soil microbial respiration, the degradation rates of SMZ for aerobic and anaerobic conditions, and the mineralization of ^{14}C-SMZ for aerobic and anaerobic conditions. Chapter 5 details the soil column study to investigate the mobility of SMZ in soils and in manure-amended soils.

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CHAPTER 2. LITERATURE REVIEW

2.1 Antibiotics usage

Use of antibiotics in agricultural business was estimated to be approximately 9 to 13 million kg in the U.S. annually (Shea, 2003). In Korea, the amount of veterinary antibiotics used was approximately 1600 tons in 2001, and 1400 tons in 2004 of which 600 tons were used as feed additives (Korea Food and Drug Administration, 2006). Approximately 100 tons were used in Sweden in 2003 (Johansson and Mollby, 2006), and 700 tons were used in the United Kingdom in 2004 (Veterinary Medicines Directorate, 2005).

Sulfonamides, one of antimicrobial pharmaceuticals, have been used extensively in livestock farming, especially in swine production (Bajpai et al., 2000; Lindsey et al., 2001; Tolls, 2001; Grant et al., 2003). In cattle, sheep, and goats, sulfonamides have been used to treat bovine interdigital *necrobacillosis* and *coccidiosis*, and to control *E. streptococcal* infections and atrophic rhinitis in swine as well as used as growth promoting additives (Giguère et al., 2007). Sulfonamide class is the second largest group of antibiotics used in France, Germany, and United Kingdom, between 11 and 23 percent of total antibiotics used (Thiele-Bruhn, 2003). In the U.S., sulfanomides is the fourth largest group of antibacterials sold by Animal Health Institute (AHI) and accounted for 6 percent of total antibiotics sold in 2000 (AHI, 2001). Among sulfonamide drugs, sulfamethazine (SMZ) is nontherapeutically administered for cattle and swine production in the U.S. (Huang et al., 2001), and has been estimated to be approximately 400 tons of SMZ per year (Mellon et al., 2001).

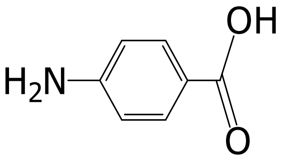
2.2 Environmental and human risks

Some of the concerns with the environmental presence and exposure of pharmaceuticals include anomalous physiological developments, reproductive destructions, increase of cancer, development and spread of antibiotic resistant bacteria (Davis and Bradlow, 1995; Phillips et al., 2004; Kaniou et al., 2005; Pruden et al., 2006). The phytotoxicity of antibiotics to plants has been studied and sulfonamide (sulfadimethoxine) at a concentration of 300 mg L^{-1} was found to interfere the growth of roots, stalks, and leaves in millet, pea, corn, and barley (Migliore et al., 1995; 1996; Jjemba, 2002). Maynard et al. (2003) isolated bacteria from animals that showed resistance to sulfonamides after being treated with sulfonamides for many years. Studies from National Center for Toxicological Research (NCTR) revealed that thyroid tumors in mice and rats were associated with high doses of SMZ at 2,200 ppm in their diet for two years (Littlefield, 1989; Littlefield et al., 1990). The acceptable daily intake (ADI) of SMZ based on an evaluation in 1994 is 0.05 mg kg^{-1} body weight (JEFCA, 2007). The maximum residue level (MRL) for SMZ in animal tissues is 0.1 mg kg^{-1} (JEFCA, 2006) but there is no MRL for plant-based products.

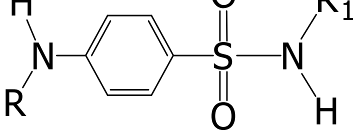
SMZ, in manure-amended soils, can be accumulated in crop-plant tissues, such as corns, lettuces, and potatoes. The amount of SMZ accumulation in plant tissue after 45 days of growth was found to be less than 0.1 percent of the amount of SMZ applied to manure-amended soils and approximately 70 percent of the SMZ applied remained in the soils (Dollivera et al., 2007). The study also found that the concentration of SMZ in plant tissues was directly proportional to the SMZ concentrations in soils. Antibiotic residues in plant products are not regulated, and the risks of contaminated crops to human health are unknown but there is the possibility that chronic effects may take place.

2.3 Characteristics of sulfamethazine

Sulfonamides, known as sulfa drugs, are derivatives of sulfanilamide and are synthetic antimicrobials which inhibit the incorporation of para-aminobenzoic acid (PABA) into the folic (pteroylglutamic) acid molecule by competing with PABA for the enzyme dihydropteroate synthetase. This results in the cessation of folic acid biosynthesis in bacterial cells (Giguère et al., 2007; Katzung, 2007), and therefore restraining bacterial growth and activities. The chemical structures of PABA and sulfonamide group are presented in Figure 1.



para-aminobenzoic acid (PABA)



sulfonamide group

Figure 1 Chemical structures of PABA and Sulfonamide group

Because sulfonamides are wide spectrum antimicrobial agents, they can affect most gram-positive and many gram-negative organisms, and some protozoa (Ophardt, 2003). They are used to treat bacterial infections, and some fungal and protozoan infections. However, in recent years, their antibacterial activities have been found to be not effective due to the development of drug resistance by the microbes after several decades of use of these compounds (Giguère et al., 2007). There are about a hundred different compounds in sulfonamide class in the market (Holm at al., 1995) and they differ in the hydrocarbon (R) at amido group ($-SO_2NHR$) (see Figure 1). The R groups of compounds frequently used in sulfonamides are presented in Table 1. Sulfonamides usually have two dissociation constants (pK_a). They can be positively charged, neutral, and negatively charged depending on the pH of the solution (Ingerslev and Halling-Sørensen, 2000).

Among the compounds of sulfonamide, sulfamethoxazole is the most popular sulfonamide used in human therapy, while sulfamethazine is the most frequently used in livestock (Huang et al., 2001). Other sulfonamide compounds of interest based on their usage, or their presence in animal manure and water are sulfapyridine, sulfathiazole and sulfadiazine.

Table 1. List of sulfonamides (Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2001)

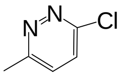
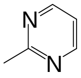
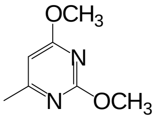
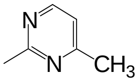
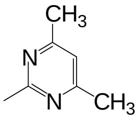
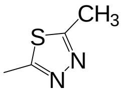
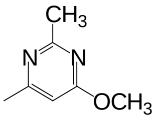
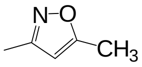
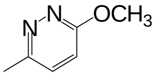
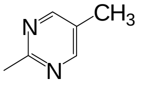
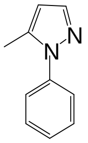
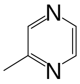
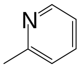
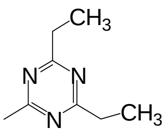
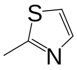
Chemical	R group	Formula	CAS number	Mol. Wt.	Solubility
Sulfachlorpyridazine		$C_{10}H_9ClN_4O_2S$	80-32-0	284.73	na
Sulfadiazine		$C_{10}H_{10}N_4O_2S$	68-35-9	250.28	200 mg/100 mL at pH 7.5 at 37ºC
Sulfadimethoxine		$C_{12}H_{14}N_4O_4S$	122-11-2	310.33	5170 mg/100 mL at pH 8.71 at 37ºC
Sulfamerazine		$C_{11}H_{12}N_4O_2S$	127-79-7	264.31	170 mg/100 mL at pH 7.5 at 37ºC
Sulfamethazine		$C_{12}H_{14}N_4O_2S$	57-68-1	278.34	150 mg/100 mL at 29ºC
Sulfamethizole		$C_9H_{10}N_4O_2S_2$	144-82-1	270.34	1 g/4000 mL at pH 6.5
Sulfamethomidine		$C_{12}H_{14}N_4O_3S$	3772-76-7	294.33	na
Sulfamethoxazole		$C_{10}H_{11}N_3O_3S$	723-46-6	253.28	na

Table 1. List of sulfonamides (Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2001)

Chemical	R group	Formula	CAS number	Mol. Wt.	Solubility
Sulfamethoxypyridazine		$C_{11}H_{12}N_4O_3S$	80-35-3	280.31	147 mg/100 mL at pH 6.5 at 37ºC
Sulfanilamide	H	$C_6H_8N_2O_2S$	63-74-1	172.21	1 g/2 mL boiling water
Sulfaperine		$C_{11}H_{12}N_4O_2S$	599-88-2	264.31	very sparingly soluble in water
Sulfaphenazole		$C_{15}H_{14}N_4O_2S$	526-08-9	314.37	0.15 g/100 mL at pH 7 at 37ºC
Sulfapyrazine		$C_{10}H_{10}N_4O_2S$	116-44-9	250.28	5.2 mg/ 100 mL at 37ºC
Sulfapyridine		$C_{11}H_{11}N_3O_2S$	144-83-2	249.29	1 g/3500 mL
Sulfasymazine		$C_{13}H_{17}N_5O_2S$	1984-94-7	307.38	1 mg/mL in acetate buffer pH 5.9
Sulfathaiazole		$C_9H_9N_3O_2S_2$	72-14-0	255.32	60 mg/100 mL at pH 6.03 at 26ºC

As indicated earlier, SMZ is the most frequently used compound within the sulfonamide group for livestocks. The chemical structure of sulfamethazine and its dissociated forms are presented in Figure 2. The physical and chemical properties of sulfamethazine (4-amino-N-[4, 6-dimethyl-2-pyrimidinyl]-benzenesulfonamide) are as follows:

CAS number	57-68-1
Formula	$C_{12}H_{14}N_4O_2S$
Molecular Weight	278.34
log Kow	0.89
$pK_{a,1}$	2.65 ± 0.2
$pK_{a,2}$	7.4 ± 0.2
Solubility	150 mg/ 100 mL at 29ºC

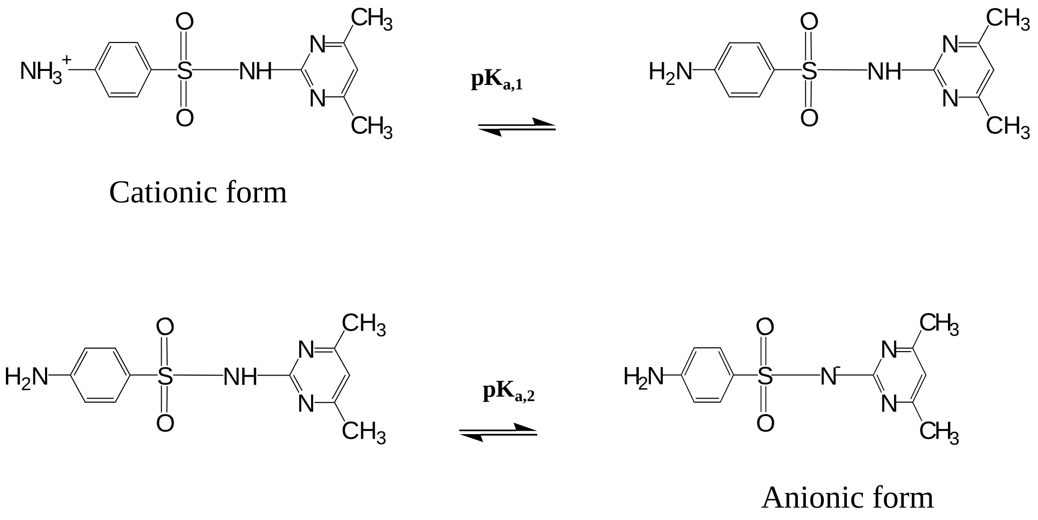


Figure 2 Chemical structures of sulfamethazine and its protonated and deprotonated form

2.4 Environmental concentrations

Concentrations of SMZ have been found to be as high as 15 μg kg^{-1} in field soils in Germany after several months of manure application (Christian et al., 2003). SMZ and sulfamethoxazole have been found in groundwater samples in Germany and in the U.S. at concentrations up to 0.47 μg L^{-1} (Hartig et al., 1999; Hirsch et al., 1999; Sacher et al. 2001; Lindsey et al., 2001). Surface water samples analyzed for sulfonamides showed the presence of sulfamethazine, sulfamethoxazole, sulfadimethoxine, and sulfamethizole with concentrations ranging from 0.05 to 15 μg L^{-1} (Hirsch et al., 1999; Lindsey et al., 2001; Kolpin et al., 2002). Due to their high solubility, low octanol-water distribution coefficients (K_{ow}), and low chelating ability (Lindsey at al., 2001), the movement of sulfonamides in groundwater may be as fast as the groundwater. As reported by Holm et al. (1995), the concentrations of some sulfonamides in groundwater at a distant sampling point were 50 times less than the concentrations in groundwater under the landfill. Since the use of a chloride tracer confirmed that it was not due to dilution, the author suggested that the sulfonamide probably moved with the same velocity as the groundwater in the aquifer.

The presence of SMZ in the groundwater of agricultural area can be assumed to be transported from livestock operations since SMZ is not used in humans. The effluents from sewage treatment plants and surface waters were analyzed and sulfamethoxazole was found instead of SMZ (Hirsch et al., 1999). Even though sulfamethoxazole is used mostly in humans, its presence in groundwater was assumed to be transported from contaminated soils of sewage irrigation fields. The concentrations of sulfamethoxazole in groundwater at the site far away from sewage irrigation fields was found to be 10 times lower than the concentrations in ground water samples from the sewage irrigation fields (0.47 $\mu g\ L^{-1}$) (Hirsch et al., 1999). According to Table 2, sulfonamide compounds such as sulfamethazine, sulfamethoxazole, sulfadimethoxine, and sulfathiazole have been found at concentrations up to 2 $\mu g\ L^{-1}$ in sewage effluent and surface water samples. This indicate that sulfonamides are not totally removed by sewage treatment processes, and can be transported to surface water through the discharge of waste water effluents (Hirsch et al., 1999; Kaniou et al., 2005). Even though sulfonamides are removed in the range of 0 % to 90 % in regular wastewater treatment processes, Halling-Sørensen et al. (1998) reported that sulfonamides are resistant to biodegradation. Besides biodegradation, photolytic degradation is one of the processes whereby sulfonamides are removed or eliminated from water.

Table 2. Concentrations of sulfonamides in manure, soils, and water.

Compound	Concentration	Conditions	Reference
General Sulfonamides	> 20 μg L^{-1}	Manure lagoons, measured average of 8 sites in Iowa and Ohio	Campagnolo et al., 2002
	Up to 20 mg kg^{-1} (liquid manure)	Six grab samples taken in Switzerland from manure pits	Haller at al., 2002
Sulfamethazine	0.08 – 0.16 μg L^{-1}	Groundwater	Hirsch et al., 1999; Boxall et al., 2001
	0.22 μg L^{-1}	Surface water	Lindsey et al., 2001; Kolpin et al., 2002
	0.13 – 8.7 mg kg^{-1} (wet sample)	Manure lagoon grab samples	Haller et al., 2002
	11 μg kg^{-1} soil	Soil fertilized with manure	Höper at al., 2002
	Up to 0.05 μg L^{-1}	River	Thiele-Bruhn and Aust, 2004
^{4} N -Acetyl-sulfamethazine	<0.1 – 2.6 mg kg^{-1} (wet sample)	Manure lagoon grab samples	Haller et al., 2002
Sulfamethoxazole	~1 - 2 μg L^{-1}	River water/surface water	Halling-Sørensen et al., 1998; Lindsey et al., 2001; Kolpin et al., 2002
	0.1 - 2 μg L^{-1}	Effluent from sewage treatment plant	Hirsch et el., 1999; Andreozzi et al., 2003
	Up to 0.48 μg L^{-1}	Surface water	Hirsch et el., 1999
	Up to 0.47 μg L^{-1}	Ground water	Hirsch et el., 1999
	0.22 μg L^{-1}	Groundwater	Lindsey et al., 2001

Table 2. Concentrations of sulfonamides in manure, soils, and water (Continued)

Compound	Concentration	Conditions	Reference
Sulfamethoxazole	0.41 µg L^{-1}	Groundwater	Sacher et al. 2001
	230 – 570 ng L^{-1}	Raw influent of the WWTPs	Göbel et al., 2007
^{4}N-acetylsulfamethoxazole	850–1600 ng L^{-1}	Raw influent of the WWTPs	Göbel et al., 2007
Sulfadimethoxine	0.06 - 15 µg L^{-1}	Surface water	Lindsey et al., 2001; Kolpin et al., 2002
sulfamethizole	0.13 µg L^{-1}	Stream	Kolpin et al., 2002
sulfapyridine	60–150 ng L	Raw influent of the WWTPs	Göbel et al., 2007
Sulfathiazole	0.08 µg L^{-1}	Surface water	Lindsey et al., 2001
	<0.1 – 12.4 mg kg^{-1} (wet sample)	Manure lagoon grab samples	Haller et al., 2002

2.5 Fate of sulfonamides in the environment

Up to 30 to 95 percent of the administered dose of sulfonamidesis are excreted as the parent compound, and if metabolites of sulfonamides are included, the excreted amount can be as high as 50 to 100 percent of the administered dose (Kay et al., 2004; Thiele-Bruhn and Aust, 2004). Fate of sulfamethazine and other sulfonamides in the environment is influenced by the physical-chemical and biological reactions between the compounds and soils and soil microorganisms (Boxall et al., 2002; Thiele-Bruhn and Aust, 2004; Kurwadkar et al., 2007). However, sulfonamides are not strongly sorbed to soils and may be relatively mobile in the soil environment (Thiele, 2000; Tolls, 2001; Boxall et al., 2002). This can be seen by the detection of sulfonamides in groundwater and surface water samples collected throughout the U.S. (Lindsey et al., 2001).

2.5.1 Sorption

Sorption of sulfonamides onto soils is dependent on the soil properties such as amount of soil organic matter, composition of organic matter, soil pH, soil surface area, concentration and composition of clay minerals, and cation exchange capacity. Studies on sorption of some important sulfonamides on soils with different characteristics have been done and the sorption coefficients from these studies are presented in Table 3. Since sulfonamides can be ionized, sulfonamides can be sorbed to soil organic matter and soil minerals. However, work done by others has shown that sorption was more dependent on soil organic matter rather than soil minerals (Kaiser and Zech, 1998). There are several possible mechanisms for the sorption of sulfonamides. These include hydrophobic partitioning, cation exchange, cation bridging, surface complexes, hydrogen bonding, and electrostatic interactions (Holten Lützhøft et al., 2000; Tolls, 2001). Thiele (2000) found that the sorption of sulfapyridine on soils was affected by the quantity and quality of soil organic matter. The Freundlich sorption isotherm and the K_{oc} for sulfapyridine to manure-fertilized soils were found to be higher than non-fertilized soil. Thiele (2000) speculated that the reason for increased sulfonamide sorption when the soil organic carbon content increased was that the polar components of soil organic matter interacted with the binding sites of sulfonamides. In contrast to Thiele (2000), Thiele-Bruhn and Aust (2004) investigated the impact of pig manure addition on the sorption of sulfonamides (sulfadimidine, sulfadiazine and sulfapyridine) to soil, and sorption was found to decrease with the addition of acidic manure at a ratio of 50:1. The increase in mobility of sulfonamide (sulfachloropyridazine) due to addition of manure was also found by Boxall et al. (2002). Manure addition resulted in small change of pH and total organic carbon but the dissolved organic carbon increased dramatically. The decrease in sorption of sulfonamides (sulfadimidine, sulfadiazine and sulfapyridine) may be explained as the dissolved organic matter in manure competing with sulfonamides for the soil sorption sites. Another impact of dissolved organic matter in manure on the mobility of sulfonamides in soils is an increase in sulfonamides loss from manure-fertilized soil probably due to colloid-facilitated transport (Tolls, 2001; Burkhardt et al., 2005). Therefore, an increase in dissolved organic carbon in soil may enhance the transport of sulfonamides.

Table 3. Sorption coefficients of sulfonamides at various conditions

Compound	Sorption coefficient (L kg^{-1})			Conc. (solution per kg soil)	Condition	Reference
	$^{++}K_f$	K_d	K_{oc}	(mg kg^{-1})		
Sulfachloropyridazine			41-82		N/A	Kay at al., 2004
		1.8		0.05-20	Clay loam, pH 6.5	Boxall et al., 2002
		0.9			Sandy loam, pH 6.8	
		4	129		Clay loam, 3.1% OC, pH 6.2	Tolls et al., 2002
Sulfanilamide	1.65	0.57	35.4	0.1-40	Unfertilized soil, silt loam, 1.6% OC, pH 7.5	Thiele-Bruhn and Aust, 2004
		0.59	36.6		Soil:slurry 1:50 (w/w), silt loam, 1.6 % OC, pH7.4	
		1.7		0-10	Unfertilized silt loam, 1.6% OC, pH 7.0	Thiele-Bruhn et al., 2004
Sulfamethazine	2.72	0.79	49.1	0.1-40	Unfertilized soil, silt loam, 1.6% OC, pH 7.5	Thiele-Bruhn and Aust, 2004
		0.74	45.9		Soil:slurry 1:50 (w/w), silt loam, 1.6 % OC, pH7.4	
		1.2	174	0.2-25	Sand, 0.9% OC, pH 5.2	Langhammer, 1989
		3.1	125		Loamy sand, 2.3% OC, pH 5.6	
		2.0	208		Sandy loam, 1.2% OC, pH 6.3	
		1.0	82		Clay silt, 1.1% OC, pH 6.9	
		2.4			Soil 1.6 % OC, pH 7	Thiele et al., 2002
		3	97		Clay loam, 3.1% OC, pH 6.2	Tolls et al., 2002
		2.4		0-10	Unfertilized silt loam, 1.6% OC, pH 7.0	Thiele-Bruhn et al., 2004

Table 3. Sorption coefficients of sulfonamides at various conditions (Continued)

Compound	**Sorption coefficient (L kg^{-1})**			**Conc. (solution per kg soil)**	**Condition**	**Reference**
	$^{++}K_f$	K_d	K_{oc}	(mg kg^{-1})		
Sulfadiazine	3.27	2.0	124	0.1-40	Unfertilized soil, silt loam, 1.6% OC, pH 7.5	Thiele-Bruhn and Aust, 2004
		1.18	73.2		Soil:slurry 1:50 (w/w), silt loam, 1.6 % OC, pH7.4	
		2.0		0-10	Unfertilized silt loam, 1.6% OC, pH 7.0	Thiele-Bruhn et al., 2004
		2.5	81		Clay loam, 3.1% OC, pH 6.2	Tolls et al., 2002
Sulfadimethoxine	4.41	0.73	45.3	0.1-40	Unfertilized soil, silt loam, 1.6% OC, pH 7.5	Thiele-Bruhn and Aust, 2004
		0.62	38.4		Soil:slurry 1:50 (w/w), silt loam, 1.6 % OC, pH7.4	
		2.3		0-10	Unfertilized silt loam, 1.6% OC, pH 7.0	Thiele-Bruhn et al., 2004
		10	323		Clay loam, 3.1% OC, pH 6.2	Tolls et al., 2002
Sulfapyridine	4.30	1.02	63.4	0.1-40	Unfertilized soil, silt loam, 1.6% OC, pH 7.5	Thiele-Bruhn and Aust, 2004
		1.22	75.7		Soil:slurry 1:50 (w/w), silt loam, 1.6 % OC, pH7.4	
		3.5		0-10	Unfertilized silt loam, 1.6% OC, pH 7.0	Thiele-Bruhn et al., 2004
	2.2		101	0 -500	Silt loam, 1.6% OC, pH 7.0	Thiele, 2000
	5.5		308		Silt loam, 2.4% OC, pH 6.9	
Sulfathiazole		3	97		Clay loam, 3.1% OC, pH 6.2	Tolls et al., 2002

$^{++}$ unitless for Freundlich adsorption coefficient K_f

2.5.2 Impact of soil pH on sorption of sulfonamides

Sulfonamides, as amphoteric compounds, can be present as positively, neutral, and negatively charged compounds where sorption can be controlled by pH of the matrices (Langhammer, 1989; Thiele, 2000; Boxall et al., 2002; Thiele-Bruhn et al., 2004). Sorption of sulfachloropyridazine to soils was found to decrease as soil pH increased (Boxall et al., 2002; Thiele-Bruhn et al., 2004). Therefore, the mobility of sulfonamides in soils via runoff and preferential flow was impacted by soil pH, i.e., where sulfonamides may be mobile in soil with high pH (Burkhardt et al., 2005). At high soil pH, the fraction of deprotonated species increased resulting in a decrease in sorption. The degree of sorption of each species to soils will be dependent on the sorption mechanism and the soil properties. Cationic sulfathiazole were found to be the most important species for sorption to clay minerals and followed by neutral species (Kahle and Stamm, 2007). Sorption of anionic sulfonamides typically occurs at positively charged surfaces of pedogenic oxides in the clay minerals.

2.5.3 Degradation

Sulfamethazine is partly metabolized in liver by two different isoenzymes to metabolic derivatives (Vree et al., 1980) and removed from body in both parent form and metabolic forms. The majority of SMZ is excreted through urine (Mitchell et al., 1986; Hardman et al., 2001). The major metabolites of SMZ from metabolism in swine are *N*4-acetylsulfamethazine, desaminosulfamethazine, and *N*4-D-glucosyl sulfamethazine (Matusik et al., 1982; Nouws et al., 1985; Paulson et al., 1985; Adams, 2001, Grant et al., 2003).

Among the metabolites, *N*4-Acetylsulfamethazine was found to have the highest percentage in animal excretions. The *N*4-Acetylsulfamethazine, which is less polar than SMZ, is no longer an antibacterial compound but may still maintain the toxic property of the parent compound (Hardman et al., 2001), and can be reconvert to the parent SMZ (Langhammer, 1989).

Bio-degradation

Sulfamethazine and sulfathiazole in manure slurry have been found to decrease by 60% and 40%, respectively, after five weeks of storage (Langhammer, 1989). Blackwell (cited in Kay et al., 2004) also suggested that degradation of sulfachloropyridazine in soils took place rapidly but the rates were not reported. However, Haller et al. (2002) stated that sulfonamides are resistant to degradation, which was also concluded by Ingerslev and Halling-Sørensen (2000). Ingerslev and Halling-Sørensen (2000) found that sulfonamides were not easily degradable using a screening test and due to their hydrophilic character, they can be transported to aquatic system. Degradation was impacted by soil sorption as the half-lives were found to be longer for SMZ sorbed to soil than for the compound alone. The half-lives of various sulfonamides in various conditions were found to range from <1 to 30 days (Ingerslev and Halling-Sørensen, 2000; Kay et al., 2004; Blackwell et al., 2005; Wang et al., 2006b, Accinelli et al., 2007, Blackwell et al., 2007). Degradation of twelve different sulfonamides in activated sludge reactors conducted by Ingerslev and Halling-Sørensen (2000) showed that sulfonamides were degraded after a lag phase of 7 to 10 days, with half-lives (first order kinetics) ranging from <1 to 4 days. The inhibiting effect was found to be at a concentration of 10 mg L^{-1} of sulfadiazine using The International Organization for Standardization (ISO) 15522 protocol. For degradation of sulfonamides in soils, Accinelli et al. (2007) found that concentrations up to 100 mg kg^{-1} of SMZ and sulfachloropyridine had no effect on soil microorganisms (tested by measuring mineralization of glucose and glyphosate) and degradation rates.

The degradation of sulfonamides in manure and in soils was found to be affected by the initial concentration of sulfonamides, moisture, temperature (Wang et al., 2006a), soil type and presence of microbial activity as seen by the half-lives of SMZ and sulfachloropyridine of approximately 19 days in silt loam and 21 days in sandy loam (Accinelli et al., 2007). As reported by Wang et al. (2006a), when the initial concentration of sulfonamides (sulfadimethoxine) in manure increased from 17.8 to 260.5 µmol kg^{-1}, the half-lives decreased from 1.4 to 2.6 days, and this suggested that the microbial activity was inhibited. The effect of manure slurry addition to soils was found to increase the degradation rates of sulfonamides which may be due to an increase in microbial population (Wang et al., 2006b; Accinelli et al., 2007).

Abiotic degradation

Sulfonamides are resistant to chemical or enzymatic hydrolysis (Koeplinger et al., 1999) but not photolysis (Zhou and Moore, 1994). Sulfonamides, like other pharmaceuticals, are not only biodegraded but subjected to photodegradation as well. Five different sulfonamides (sulfamethoxazole, sulfisoxazole, sulfamethizole, sulfathiazole, and sulfamoxole) in buffered water were found to degrade via direct photolysis with the degradation rates ranging from 0.3×10^{-5} s^{-1} to 13×10^{-5} s^{-1} (Boreen et al., 2004). The degradation rate varied with pH but did not follow any trends. Other study on the photocatalytic degradation of sulfacetamide, sulfathiazole, sulfamethoxazole, and sulfadiazine in aqueous solutions with TiO_2 photocatalyst showed the degradation rate constants of these four compounds ranged from 0.01 min^{-1} to 0.03 min^{-1}. The toxicity of intermediate products of sulfonamide degradation was tested in aqueous solution with the green algae and was found to dramatically less toxic than the initial compounds (Baran et al., 2006). Photodegradation of 50 mg L^{-1} SMZ in aqueous solution in the presence of three different photocatalysts, TiO_2 (Degussa, P-25) (anatase/rutile = 3.6/1, surface area 56 m^2 g^{-1}, nonporous), TiO_2 (A) (100% anatase, surface area 10 m^2 g^{-1}, and ZnO, showed that the destruction of SMZ after 60 minutes of illumination were approximately 65 %, 40 % and 90 %, respectively. The kinetics of photodegradation obeyed pseudo-first-order kinetics of the Langmuir–Hinshelwood model (Kaniou et al., 2005).

2.6 Transport of sulfonamides

Detection of sulfonamides in surface water and ground water (Table 2) implied the transport of sulfonamides from sulfonamides-contaminated agricultural fields. Due to the low K_d values of sulfonamides which are lower than 5 L kg^{-1} (Table 3), sulfonamides are characterized as medium to highly mobile in soils and may contaminate waters via surface runoff, drain flow and leaching. The degree of transport is influenced by many factors such as sorption, degradation rate, and solubility of sulfonamides (Boxall, 2008). Transport of strongly adsorbed pharmaceuticals can be enhanced by preferential flow and dissolved organic matter (DOM)-facilitated transport (Williams et al., 2000; Thiele-

Bruhn, 2003; Hoorman et al., 2005), while drain flow is a major route for transport of low sorptive pharmaceuticals. Boxall et al. (2003) found high concentrations of sulfonamide (at the same level as tylosin) in drainflow samples from field applied with spiked pig-slurry and the concentrations of sulfonamide in drainflow declined over time. Although sulfonamides are not strongly sorbed to soils, Tolls (2001) and Thiele-Bruhn and Aust (2004) found that the manure-DOM increased mobility of sulfonamides. The colloid-facilitated transport was proposed by Tolls (2001) to play a major role in the transport of sulfonamides.

2.7 Summary

Fate and transport of veterinary antibiotics in agricultural fields are of interest because some of concerns including the antibiotic resistance produced by bacteria. Sorption and degradation play a major role in transport of these compounds. The soil carbon content and soil pH were found to impact the sorption of antibiotics on soils. Among veterinary antibiotics, SMZ is the fourth largest antibiotics used in livestock and commonly used in swine industries. Iowa produced a huge number of swine compared to other states in the U.S., and the agricultural fields have been applied with swine manure. There are some studies on the sorption of SMZ to different soils and the sorption coefficients have been reported. There are not many studies conducted using soils with a wide range of soil organic carbon content, and soil pH. In addition, a simple model for the prediction of the sorption coefficient of SMZ is not available.

The degradation of sulfonamides has been studied but there are some contradiction on the degradability of sulfonamides. Some studies reported that sulfonamides are readily degraded while some studies reported their resistance to biodegradation. There are some studies investigating the degradation of sulfonamides in manure, in activated sludge, and in soils. However, no study has focus on the degradation of SMZ in soils under aerobic and anaerobic conditions. In addition, data on the concentrations of SMZ that inhibit microbial activities in soils under aerobic and anaerobic conditions have not been determined. The inhibiting concentration is important in order to know if the concentrations of SMZ in manure-applied soil inhibit the microbial activities in the soils.

Vertical transport of SMZ is influenced by the persistence of SMZ in soils, and sorptive affinity of SMZ. The low K_d values of SMZ implies that SMZ is not strongly sorbed to soils and has a tendency to transport via leaching and surface runoff. The time duration between SMZ applied to soil and rainfall may impact the leaching of SMZ because the sorption of SMZ to soil was found to be stronger for longer contact time. Most of studies on the transport of sulfonamides using soil cores were conducted under saturated condition with the steady flow. Information on the transport of SMZ in soil columns with simulated scenario of rainfalls are still lacking.

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CHAPTER 3. EFFECT OF ORGANIC CARBON AND PH ON SOIL SORPTION OF SULFAMETHAZINE

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3.1 Abstract

Batch sorption of sulfamethazine (SMZ) was conducted using five soils with organic carbon (OC) contents ranging from 0.1 % to 3.8 % and solution pHs ranging from 5.5 to 9. Sorption of SMZ was found to be impacted by OC, soil surface area and soil solution pH, with K_d values decreasing as the pH increased. However, OC was found to be the more dominant parameter. Linear sorption coefficients at pH 5.5 were found to be 0.58 ± 0.12 L kg^{-1} for soil with 0.1 % OC and 3.91 ± 0.26 L kg^{-1} for soil with 3.8 % OC. At pH 9, the K_d values were found to decreased by more than 50% to 0.23 ± 0.04 L kg^{-1} (soil with 0.1 % OC) and 1.16 ± 0.03 L kg^{-1} (soil with 3.8 % OC). Hydrophobic sorption was probably involved for pH < 7.4 ($pK_{a,2}$ = 7.4 for SMZ) due to the unionized form of SMZ while surface sorption was probably involved for pH > 7.4 due to the ionized form of SMZ. This was confirmed by regressing the estimated sorption coefficients of cationic, uncharged, and anionic species against the soil properties. A mechanistic model and a linear regression model incorporating the fraction of SMZ ionization and soil properties were developed and were found to estimate the K_d values of other studies using soils of different pH and soil properties.

Keywords: Sorption; Antibiotics; Sulfonamide; Soil pH; Organic carbon content

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3.2. Introduction

The emergence of pharmaceuticals and personal care products in the environment is of concern in many countries. These compounds have been found in soils, ground water, and surface water and their environmental risk are not fully understood. Confined animal feeding operations (CAFOs) are one of the many sources in the release of antibiotics to soils and surface waters (Moorman et al., 2001; Tolls, 2001). Antibiotics fed to animals are not fully absorbed into the animal body and are discharged along with their metabolites through excreta (Thiele, 2000; Tolls, 2001; Thiele-Bruhn and Aust, 2004; Göbel et al., 2005). Swine manure applied to land as fertilizer is one of the many pathways whereby soils and surface waters are contaminated (Thiele-Bruhn and Aust, 2004) as many of the antibiotics are not completely degraded in the manure (Kolz et al., 2005).

One of the major antibiotics used in swine industry is sulfamethazine (SMZ), a commonly used sulfonamide drug (Huang et al., 2001). SMZ is used for therapeutic purposes, for treatment of infections, and as a growth promoter (Bajpai et al., 2000; Tolls, 2001; Grant et al., 2003). Studies conducted in 1988 by The National Center for Toxicological Research indicated that SMZ is carcinogenic and that thyroid tumors developed in rats and mice after receiving 2.4 - 4.8 ppm of sulfamethazine in their diet over 2 years.

The fate of SMZ in the soils, ground waters or surface waters is dependent on the sorptive affinity and solubility of SMZ in these media. The partition coefficients (K_d) of sulfonamides reported previously were found to vary with respect to the types and properties of the soils (Boxall et al., 2002; Thiele-Bruhn and Aust, 2004; Kurwadkar et al., 2007). For example, the K_d values for sulfachloropyridazine were 1.8 and 0.9 L kg^{-1} for clay loam and sandy loam, respectively, and K_d values were found to decrease with an increase in the soil pH (Boxall et al., 2002). When manure was added to the soils which caused an increase in pH, a decrease of K_d values was observed (Boxall et al., 2002). Thiele-Bruhn and Aust (2004) found similar effects on sorption of sulfonamides due to pH changes when manure was added. In their study, the K_d values of five different sulfonamides, ranging from 0.3 to 2.0 L kg^{-1} for a silt loam soil, increased when acidic manure was added to the soil. In another study, the sorption of sulfamethazine and sulfathiazole to three soils (loamy sand, sandy loam, and loam soil) decreased as pH was increased (Kurwadkar et al., 2007). For example, the K_d of sulfamethazine for loamy soil was 17 L kg^{-1} at pH 3.1 and decreased to 3.1 L kg^{-1} at pH 7.3. SMZ which is the focus of this study has a $pK_{a,2}$ value of 7.4. The objective of this study is to investigate the impact of pH and soil properties on the sorption of SMZ and to construct a mechanistic sorption model based on the various species of SMZ and soil properties for the prediction of the sorption of SMZ onto soils. Experiments were conducted at pH 5.5, 6, 7, 8, and 9 to cover the pH range typically found in soils. The five soils used had organic carbon content ranging from 0.1 % to 3.8 %.

3.3. Materials and methods

3.3.1 Soil sampling and analysis

Five soil samples identified as Clarion-1, Clarion-2, Clarion-3, Nicollet, and Harps series were collected from different agricultural fields in Ames, Iowa. Soils collected were surface soils at depths of 0 - 15 cm, except for Clarion-1, which was a subsoil and collected at a depth of 152 - 212 cm. The soils were thoroughly homogenized, partially-dried at room temperature, sieved using a 2-mm opening sieve, and stored moist in a refrigerator. The organic carbon (OC) content was measured using a NC Soil Analyzer (Flash EA, 1112 series) (CE Elantech Inc, Lakewood, NJ). Ethylene glycol monoethyl ether (EGME) sorption was used to measure the specific surface areas of the soils. Soil moisture contents were determined by weight difference by drying the soil in an oven at 105° C for at least 24 hours. The properties of the five soils are presented in Table 1.

3.3.2 Chemicals

Sulfamethazine (4-amino-N-[4, 6-dimethyl-2-pyrimidinyl]-benzenesulfonamide, $C_{12}H_{14}N_4O_2S$, CAS number 57-68-1) with a purity of 99 % was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and HPLC and chromatography grade water for HPLC analysis were purchased from Burdick & Jackson (Muskegon, MI). A stock solution of 50 mg L^{-1} of SMZ in 0.01 M $CaCl_2$ solution was prepared. For pH adjustment, 0.1 M of KOH and 1 M of HCl solution were used. To maintain the soil-water ratio in the batch sorption experiments, 0.01 M $CaCl_2$ solution was used.

The chemical structures of sulfamethazine and its ionized forms (cationic and anionic with the negative charge exhibited at the nitrogen of sulfonamide group) are presented in the Figure 1. Physical-chemical properties of SMZ include: molecular weight = 278.34, log K_{ow} = 0.89 (Tolls, 2001) solubility = 1.5 g L^{-1}, $pK_{a,1} = 2.65 \pm 0.2$, and $pK_{a,2} = 7.4 \pm 0.2$. The fraction of non-ionized and ionized forms of SMZ as a function of pH may affect the sorption of SMZ to soils. For a given pH, the fraction of anionic SMZ can be estimated by the following (Snoeyink and Jenkins, 1980; Schwarzenbach et al., 1993):

$$\alpha_1 = \frac{[A^-]}{C_{T,A}} = \frac{[A^-]}{[HA] + [A^-]} = \frac{1}{1 + \frac{1}{10^{(pH - pKa)}}} \qquad (1)$$

3.3.3 *HPLC analysis*

SMZ was analyzed using an Agilent HPLC Series 1100 (Eagan, MN) with diode array detection. The injection volume used was 50 μL and the initial eluent flow rate was 0.5 mL min^{-1}. Mobile phase A was water with 1 mM ammonium acetate and 0.1 % (v/v) glacial acetic acid while mobile phase B was acetonitrile and 0.1 % (v/v) glacial acetic acid. The mobile phase B increased from 10 % to 25 % in 12 minutes and to 100 % from 12 to 30 minutes at a flow rate of 0.7 mL min^{-1}. Mobile phase B is then reduced to 10 % in 30 to 40 minutes and the flow rate returned to 0.5 mL min^{-1}. Detection wavelength was set at 254 nm. SMZ calibration curves were prepared for each pH tested.

3.3.4 *Batch sorption experiments*

Nine grams (dry weight) of Clarion-1, Clarion-2, Clarion-3, Nicollet, and Harps soils were weighed and placed into 50-mL fluorinated ethylene propylene (FEP) centrifuge tubes. The soil-water ratio was maintained at 1: 3 (dry mass: volume of water). For each soil, different volumes of 0.1 M of KOH or 1 M of HCl solution and 0.01 M of $CaCl_2$ solution were added to adjust the pH of the solution to the target pHs of 5.5, 6, 7, 8, and 9. Preliminary tests were conducted before the batch sorption experiment to estimate the volumes of acid or base needed for pH adjustment. The tubes were sealed with ethylene-tetrafluoroethylene

(ETFE) caps and shaken for approximately 24 hours to allow the soil pH to stabilize. Due to the pH adjustment processes, the ionic strength of the samples ranged from 0.03 to 0.19. The soil slurries were then spiked with SMZ stock solution to give concentrations of approximately 1.1, 2.8, 5.6, 11.1, and 22.2 mg L^{-1} (or 3.3, 8.4, 16.8, 33.3, and 66.6 μg g^{-1} soil). The total volume of liquid in each tube, i.e., $CaCl_2$ solution, acid or base, and SMZ solution, was maintained at 27 mL. Triplicate samples were prepared. After addition of SMZ, the tubes were vortexed, and left for 10 minutes to allow the soil particles to settle. The pHs of the supernatants were measured (identified as the initial pH). Samples were then shaken for 24 hours at 22 °C. The 24-hour incubation time was selected for batch equilibrium study because previous studies on sorption of some sulfonamides including SMZ showed that near equilibrium conditions can be reached in less than 24 hours (Thiele, 2000; Kurwadkar et al., 2007). The tubes were then centrifuged at 5000 rpm for 20 minutes. The pHs of supernatants were measured (identified as final pH). The differences between initial pH and final pH were found to be less than 0.2 pH unit for all sorption experiments (data not shown). The supernatants were filtered with 0.2 μm nylon membrane filter (13 mm polypropylene encased) (Alltech, Deerfield, IL) and 2 mL of the filtrate were transferred to HPLC vials for analysis.

Sorption of chemical onto tubes and caps were investigated before starting the batch equilibrium experiments and the tests showed 96 % ± 2 % recovery (data not shown). Therefore, the sorption onto tubes and caps were assumed to be negligible.

Linear sorption coefficients, K_d (L kg^{-1}) values, and Freundlich sorption constants, K_f (unitless) values, were estimated for the sorption of SMZ to soil. Sorption coefficients were normalized with respect to organic carbon content. All K_d, K_f, and K_{oc} values were reported on an oven-dried weight basis.

3.4. Results and discussion

3.4.1 Sorption Isotherms

The mass of SMZ sorbed per unit mass of soil (C_s), were plotted against the equilibrium concentrations of SMZ (C_w) as shown in Figure 2. Linear sorption coefficients K_d were obtained using linear regression. Nonlinear Freundlich coefficients (K_f) and n were estimated by the linear form of the Freundlich isotherms. Linear regressions were found to best fit the sorption of SMZ for all soils ($R^2 \geq 0.91$). The K_d, the estimated K_{oc}, and K_f and n values are given in Table 2. Control sorption experiments were conducted with soils without added SMZ. No SMZ were detected in the soil solutions.

The estimated K_d values ranged from 0.23 ± 0.04 to 3.91± 0.26 L kg^{-1} depending on the type of soil, soil organic carbon, and soil pH. The K_d values obtained in this study were in the same range as the values reported by other researchers. For instance, Langhammer (1989) reported K_d values of 1.0 to 3.1 L kg^{-1} for sulfamethazine at concentrations between 0.2-25 mg kg^{-1}, soils with 0.9 - 2.3 % OC and different pHs ranging from 5.2 to 6.9. The K_d values for sulfadimidine (a synonym of SMZ) were reported to be 2.4 L kg^{-1} for a soil with 1.6 % OC and a pH of 7 and (Thiele et al., 2002), and 3 L kg^{-1} for soil with 3.1 % OC and a pH of 6.2 (Tolls et al., 2002).

Except for Clarion-1 soil, the estimated K_{oc} values ranged from 30.4 to 47.8 L kg^{-1} for pH 9 and from 86.9 to 139.7 for pH 5.5. The log K_{ow} of SMZ (0.89) indicates that SMZ is not strongly hydrophobic and the K_d values of 0.2 – 3.9 L kg^{-1} denoted that SMZ may be mobile with a potential to leach and eventually pollute ground water and surface water.

3.4.2 Effect of pH

Since the pK_a's of SMZ are 7.4 ± 0.2 and 2.65 ± 0.2, the deprotonated (anionic) form of SMZ is prevalent at alkaline pH, but for neutral pH and lower, the unionized SMZ and the cationic SMZ would be dominant. The portions of anionic SMZ at pH 5.5, 6, 7, 8, and 9 according to Eq. 1 were 1.2 %, 3.8 %, 28.5 %, 79.9 %, and 97.6 %, respectively.

To assess the impact of pH on the sorption of SMZ to various soils, the K_d's were plotted against pH and the fractions of anionic SMZ as shown in Figure 3 (a) and (b). All five soils showed gradual decrease in sorption as pH increased (for example, at pH 5.5 and pH 9, K_d values for Clarion-2 were 1.22 ± 0.04 and 0.49 ± 0.04, respectively) but except for Clarion-1 soil, all the other soils showed SMZ sorption to be highest at pH 5.5 (see Figure 3 (a)). Essentially, the anionic form of SMZ appeared to sorb less than the unionized form (see Figure 3 (b)). At pH 8 and pH 9 (> $pK_{a,2}$ of SMZ), the anionic SMZ was 80 % of the total SMZ. The trend observed in this study was similar to the study reported by Boxall et al. (2002) but for another sulfonamide compound, sulfachloropyridazine, in clay loam and sandy loam for pHs between 4.6 and 7.8. It is probable that the anionic SMZ resulted in significantly less hydrophobic interactions than the unionized form of SMZ. At pH less than the $pK_{a,2}$, hydrophobic sorption with organic matter may dominate due to the unionized nature of SMZ. Therefore, at high pH, the lower sorption may be attributed to the anionic SMZ where hydrophobic sorption would be impacted by the polar-polar interactions of the anionic SMZ and the negative nature of the soil surface at high pH. It is also probable that some sorption may occur by cation bridging where a polyvalent cation bridges between the anionic SMZ and the negative charge sites on clay or organic materials.

The impact of soil OC on the sorption of SMZ is plotted as shown in Figure 4. For the pH used and soils tested, Clarion-1 with the lowest OC gave the lowest sorption of SMZ and Harps with an OC of 3.8 % gave the highest sorption. To assess the role of organic carbon content, the K_d values were normalized with OC to obtain K_{oc} as shown in Table 2. Ignoring Clarion-1 soil and for pH 9, the K_{oc} values for the remaining four soils were fairly similar ranging from 30.4 to 47.8 L kg^{-1} and at pH 5.5, the K_{oc} values ranged from 86.9 to 139.7 L kg^{-1}. The fairly similar K_{oc} values typically indicate the influence of OC in sorption of organic compounds (Karickhoff et al., 1979). The plot of K_{oc}'s (except for Clarion-1 soil) against pH as in Figure 5 further illustrates and accentuates the impact of OC and pH on sorption of SMZ.

As shown in Table 2, the K_{oc} values of Clarion-1 soil were considerably higher than the other four soils. The low OC content in Clarion-1 was probably one of the reasons for the high values. The clay:OC ratios ranged from 6.7 to 9.1 for the four soils, but was 180 for Clarion-1 soil. Gao and Pedersen (2005) showed that SMZ speciation and clay surface charge density were important factors for the sorption of SMZ to clay surfaces. For soils with high clay content and low OC, sorption of SMZ to clay surfaces may become more important, thereby inflating the K_{oc} values for Clarion-1 soil. Qualitative differences in the OC of subsoil (Clarion-1) compared to the other surface soils may also affect sorption and the K_{oc}.

3.4.3 Model Development

The overall sorption of SMZ at a given pH may be assumed to be equal to the sum of the sorption of each SMZ species meaning that the overall sorption coefficient (K_d) is equal to the sum of the fraction of the SMZ species multiplied by the sorption coefficients of the SMZ species (Schwarzenbach et al., 1993). Gao and Pedersen (2005) found the sorption of zwitterionic did not make a significant difference in the overall sorption of SMZ, therefore, the overall sorption coefficient can be expressed for cationic, unionized, and anionic species as shown below:

$$K_d = \alpha^{+} K_d^{+} + \alpha^{0} K_d^{0} + \alpha^{-} K_d^{-} \qquad (2)$$

where

K_d = overall sorption coefficient (L kg^{-1})

K_d^{+}, K_d^{0}, and K_d^{-} = sorption coefficients of cationic, unionized, and anionic species, respectively

α^{+}, α^{0}, and α^{-} = mass fraction of individual species in solution

Sorption coefficients for each species (K_d^{+}, K_d^{0}, and K_d^{-}) were estimated by fitting the overall sorption coefficients with Eq. 2 for each soil-pH combination using the statistical software, SigmaPlot 10.0 (SyStat Software, Inc., San Jose, CA). The estimated sorption coefficients for each individual species for all five soils ($R^2 = 0.97$) are present in Table 3. The K_d^{+} ‘s for the five soils were found to be highest among the individual SMZ species indicating strong sorption of cationic SMZ to negatively charged clays but this sorption may not play a role in typical soils pH range of 5.5 to 9 as the fractions of cationic SMZ present at this pH range are very small.

Sorption coefficients of each SMZ species (K_d^+, K_d^0, and K_d^-) were regressed against the soil properties of the five soils using stepwise regression (SPSS 14.0, SPSS Inc., Chicago, IL). K_d^+ was found to be significantly correlated with the soil specific surface area (Eq. 3) while other dependent variables were excluded indicating the sorption of cationic SMZ to the negatively charged surface of the soil. For K_d^0 and K_d^-, the stepwise regression showed that the percent OC was the most important dependent variable that controlled the sorption of both unionized and anionic SMZ (Eqs. 4 and 5).

$$K_d^+ = 0.09 \times SA - 2.31 \qquad R^2=0.95 \tag{3}$$

$$K_d^o = 0.81 \times \%OC + 0.38 \qquad R^2=0.92 \tag{4}$$

$$K_d^- = 0.29 \times \%OC + 0.20 \qquad R^2=0.90 \tag{5}$$

where

% OC = organic carbon of soil (%)
SA = soil specific surface area ($m^2\ g^{-1}$)

Figure 6a shows that the above model (Eqs. 2 - 5) predicted fairly well the experimentally determined K_d values.

Another approach is to regress the overall sorption coefficients at different pHs against the physical-chemical properties of the soils (Table 1) and the fraction of the anionic form of SMZ using stepwise regression (SPSS 14.0, SPSS Inc., Chicago, IL). Only the OC and IF were retained as variables in the model ($p < 0.05$) while the other soil properties were excluded (p >0.05). The multiple regression model with a R^2 of 0.831 is as follows:

$$K_d = (0.63 \times \%OC) - (1.50 \times IF) + 0.96 \qquad (6)$$

where

IF = fraction of anionic SMZ at given pH

The Pearson correlations between K_d and %OC, and IF, were 0.728, and -0.549, respectively, showing that OC was more important than IF for the prediction of K_d. When the data of Clarion-1 were excluded since the %OC of Clarion-1 was very low, the R^2 of regression model yielded an R^2 of 0.873, an insignificant improvement. Figure 6b shows that the regression model (Eq. 6) predicted fairly well the experimentally determined K_d values.

To simplify the model (Eq. 6) for quick prediction of SMZ sorption, a regression model using K_{oc} was developed (data for Clarion-1 soil were excluded). The regression model with a R^2 of 0.81 is given below:

$$K_{oc} = -(69\ IF) + 104.78 \qquad (7)$$

The predicted K_{oc} values from Eq. 7 at each pH were compared to the experimental K_{oc} using *one-sample t test* at level of 95% confidence. The sample means (mean of experimental K_{oc} values at a certain pH) were not significantly different from the predicted K_{oc} (considered as a population mean) for all pH in this study with the two-tailed p values ranging from 0.571 to 0.993. Table 4 shows the predicted K_{oc} and mean experimental K_{oc} and two-tailed p value for all pH_s performed.

To assess the suitability of the models developed, the K_d's of SMZ reported by other researchers were compared to the K_d's predicted by the model described by Eqs. 2 – 5 and the model described by Eq. 6 (Table 5). Since some of the previous studies did not include the soil specific surface area (SA), the specific surface area was estimated based on the clay fraction in soil as given by Eq. 8 (Goldberg et al., 2005):

$$\mathrm{SA} = 5.654 + 348.9(\mathrm{Clay\ \ Mass\ \ Fraction}) \qquad (8)$$

Both models predicted the K_d's reported by other researchers except for Kuwadkar et al. (2007) who estimated the K_d using the initial portion of their sorption curve of low concentration ($C_w < 0.5$ mg L^{-1}) instead of the whole range of sorption concentrations of their experiments.

3.5. Conclusion

The sorption behavior of SMZ onto soil is needed to know the fate and transport of this compound in soil and to estimate the risk posed by this compound. Linear sorption isotherms were found to describe the sorption of SMZ for the soils tested and for the concentration range. Linear sorption coefficients (K_d) determined at various pH conditions, were found to be maximum at the lowest pH tested, i.e. pH 5.5 but were lower for higher pH. Based on the experimental results, pH of the soil-solution had an impact on the sorption affinity of SMZ, due to the ionization fraction of SMZ. At pH less than 7.4, hydrophobic sorption was probably involved due to the unionized form of SMZ. At pH greater than 7.4, the lower sorption may be due to the anionic SMZ and the negatively charged surfaces of the soils at high pH. The K_d values were also found to be dependent on the organic carbon of the soils. This behavior may have an impact on the transport of this compound in subsoil due to low organic matter content. Two models incorporating mass fraction of SMZ species and various soil physical-chemical properties were developed to predict the K_d values for various soils for different pHs. The models were found to predict the K_d's of other reported studies. It should be noted that the models were developed based on experimentally determined sorption coefficients for Iowa soils (mollisols) and further verification is needed using experimentally determined sorption coefficients of soils from other areas. However, this model should be highly applicable for soils in Iowa, Southern Minnesota, and Illinois where mollisols are widely distributed.

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Table 1. Physical-chemical properties of soils

Soil	Clarion-1	Clarion-2	Clarion-3	Nicollet	Harps
Soil pH (1: 1)	8.2	7.8	5.4	5.5	8.2
Depth of soil (cm)	152 - 212	0 - 15	0 – 15	0 - 15	0 – 15
Organic Carbon (%)	0.1	1.4	2.2	2.7	3.8
Inorganic Carbon (%)	1.9	0.3	nd[a]	nd	1.4
Cation Exchange Capacity (meq/100g)	12.1	10.7	15.7	20.0	23.3
Specific Surface Area (m^2 g^{-1})	45	37	66	110	129
Sand (%)	56	68	46	44	38
Silt (%)	26	22	34	38	36
Clay (%)	18	10	20	18	26
Texture	Sandy Loam	Sandy Loam	Loam	Loam	Loam

[a] not detected

Table 2. Estimated sorption coefficients, K_d and K_{oc}, with 95 % confidence interval, and Freundlich sorption coefficients (K_f), nonlinearity constant (n)for five soils for pH from 5.5 to 9

Soil	OC (%)	pH	Linear sorption		K_{oc}^{+} (L kg^{-1})	Freundlich sorption		
			K_d (L kg^{-1})	R^2		K_f (L kg^{-1})	R^2	n
Clarion-1	0.1	5.5	0.58 ± 0.12	0.89	579 ± 117	0.87	0.98	0.88
		6	0.48 ± 0.12	0.80	481 ± 120	0.91	0.90	0.82
		7	0.64 ± 0.23	0.72	639 ± 234	1.62	0.87	0.73
		8	0.32 ± 0.06	0.94	320 ± 61	0.14	0.81	1.24
		9	0.23 ± 0.04	0.94	228 ± 44	0.03	0.92	1.67
Clarion-2	1.4	5.5	1.22 ± 0.04	1.00	86.9 ± 3.2	1.06	0.99	1.09
		6	1.18 ± 0.10	0.99	84.1 ± 7.2	1.27	0.97	1.01
		7	0.98 ± 0.08	0.99	70.3 ± 5.8	0.13	0.88	1.94
		8	0.55 ± 0.07	0.98	39.3 ± 5.3	0.05	0.94	2.03
		9	0.49 ± 0.04	0.99	34.9 ± 2.8	0.34	0.99	1.11
Clarion-3	2.2	5.5	2.52 ± 0.40	0.97	114.5 ±18.4	3.81	0.88	0.85
		6	2.42 ± 0.12	1.00	109.9 ± 5.3	2.29	0.99	1.05
		7	1.98 ± 0.17	0.99	90.1 ± 7.9	0.93	0.93	1.40
		8	1.33 ± 0.12	0.99	60.3 ± 5.5	0.16	0.90	1.99
		9	1.05 ± 0.05	1.00	47.8 ± 2.4	1.27	1.00	0.93
Nicollet	2.7	5.5	3.77 ± 0.09	1.00	139.7 ± 3.2	3.73	1.00	1.02
		6	3.06 ± 0.22	0.99	113.2 ± 8.3	3.13	0.98	1.03
		7	2.55 ± 0.16	1.00	94.3 ± 6.0	1.31	0.91	1.38
		8	1.38 ± 0.10	0.99	51.3 ± 3.7	0.29	0.87	1.75
		9	1.07 ± 0.04	1.00	39.8 ± 1.6	1.32	1.00	0.92
Harps	3.8	5.5	3.91 ± 0.26	1.00	102.8 ± 6.8	4.68	1.00	0.93
		6	3.05 ± 0.27	0.99	80.3 ± 7.0	3.23	0.98	1.02
		7	2.84 ± 0.22	0.99	74.8 ± 5.9	1.42	0.94	1.40
		8	1.70 ± 0.09	1.00	44.7 ± 2.5	0.80	1.00	1.39
		9	1.16 ± 0.03	1.00	30.4 ± 0.9	1.39	0.93	0.93

$^{+}K_d$ normalized to organic carbon

Table 3. K_d^+, K_d^0, and Kd^- for five soils[a]

Soil	K_d^+ (L kg^{-1})	K_d^0 (L kg^{-1})	K_d^- (L kg^{-1})	R^2
Clarion-1	1.46 ± 1.27	0.45 ± 0.14	0.25 ± 0.06	0.98
Clarion-2	1.50 ± 0.88	1.19 ± 0.08	0.44 ± 0.06	0.99
Clarion-3	3.61 ± 0.78	2.39 ± 0.08	1.03 ± 0.06	1.00
Nicollet	9.45 ± 2.74	2.96 ± 0.25	1.03 ± 0.18	1.00
Harps	8.83 ± 6.62	3.14 ± 0.61	1.23 ± 0.43	0.97

[a] values are mean ± 95 % confidence interval

Table 4. Comparison of mean estimated K_{oc} for four soils (excluding Clarion-1) at each pH and the K_{oc} predicted by Eq. 7 using one-sample t test

pH	Estimated K_{oc} Mean ± 95 % CI[++] (L kg^{-1})	K_{oc} predicted by Eq. 7 (L kg^{-1})	Two-tailed sig. (*p*)
5.5	110.98 ± 21.80	103.92	0.571
6	96.88 ± 16.72	102.12	0.582
7	82.38 ± 11.39	85.02	0.680
8	48.90 ± 8.86	49.31	0.933
9	38.26 ± 7.29	37.08	0.779

[++] 95 % confidence interval

Table 5. Comparison of K_d reported by other researchers and predicted K_d using Eqs. 2 – 5 and Eq. 6.

References	OC (%)	pH	Fraction of anionic SMZ (%)	Surface area (m^2 g^{-1})	K_d measured (L kg^{-1})	K_d predicted using Eqs. 2 - 5 (L kg^{-1})	K_d predicted using Eq. 6 (L kg^{-1})
Langhammer, 1989	0.9	5.2	0.63	23^{++}	1.2	0.81	1.52
	2.3	5.6	1.56	34^{++}	3.1	2.07	2.39
	1.2	6.3	7.36	41^{++}	2.0	1.29	1.61
	1.1	6.9	24.03	180^{++}	1.0	1.16	1.30
Tolls et al., 2002	3.1	6.2	5.94	128^{++}	3.0	2.96	2.83
Thiele et al., 2002	1.6	7	28.48	17.9	2.4	1.38	1.54
Thiele-Bruhn and Aust, 2004	1.6	7.5	55.73	N/A	0.79	N/A	1.14
Kuwadkar et al., 2007	1.0	5.3	0.79	1.8	4.6	0.58	1.58

$^{++}$Surface area estimated using Eq. 8

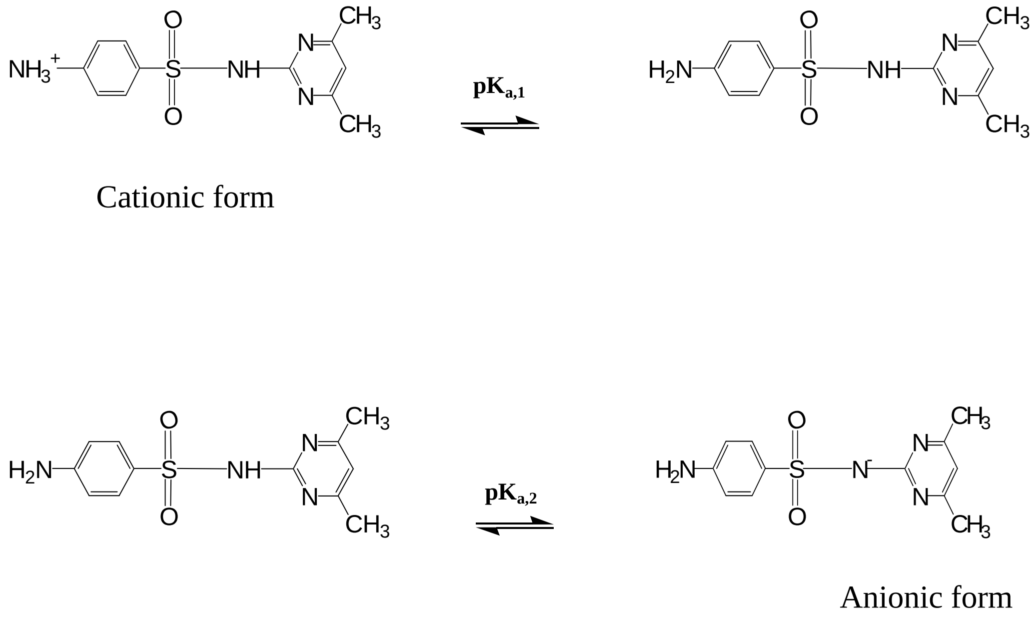


Figure 1. Chemical structure of sulfamethazine and its anionic and cationic forms

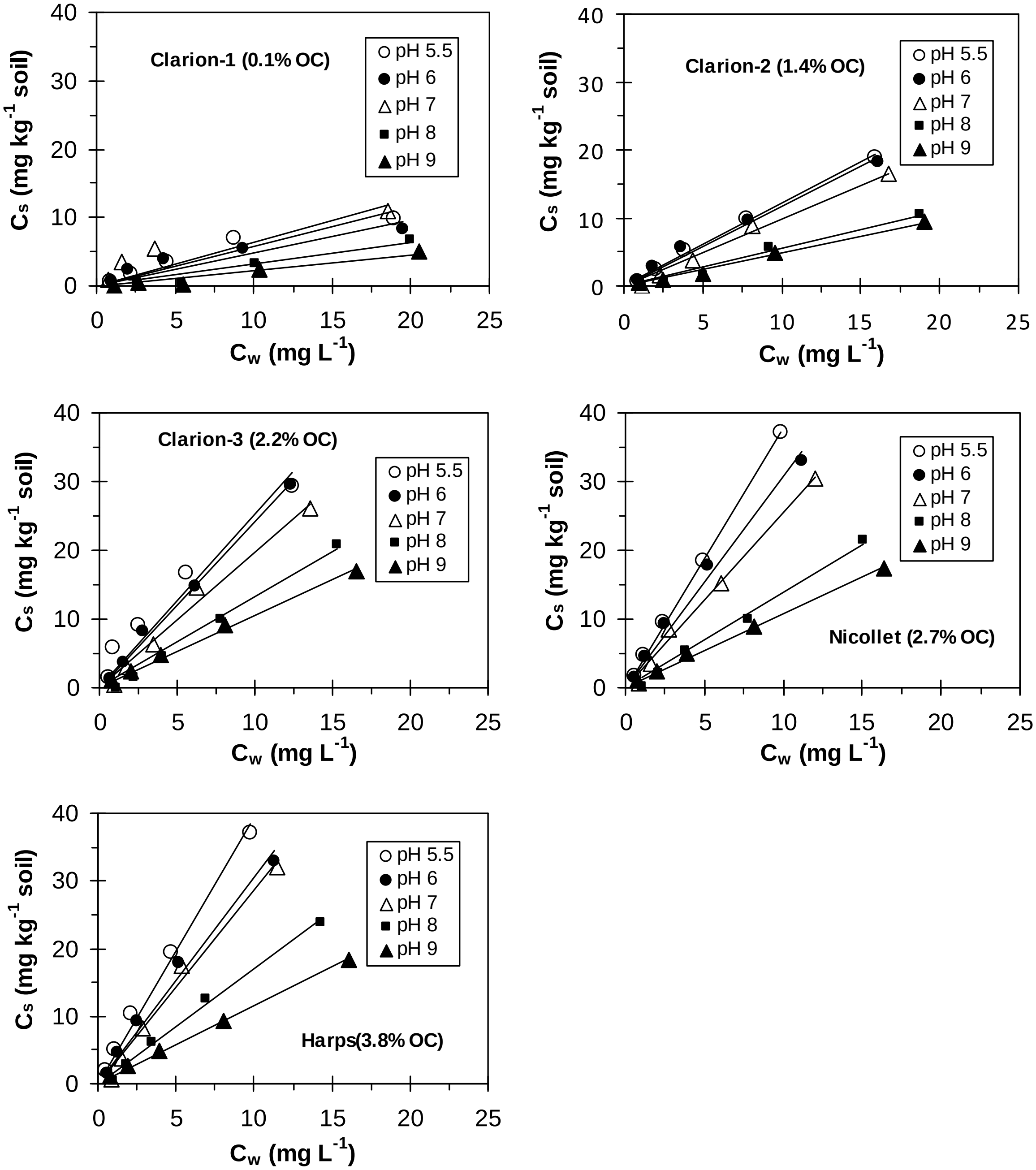
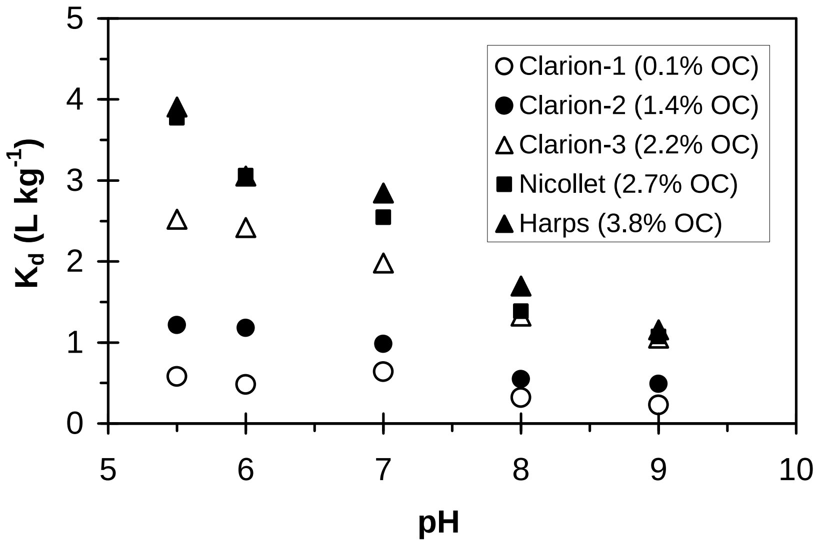
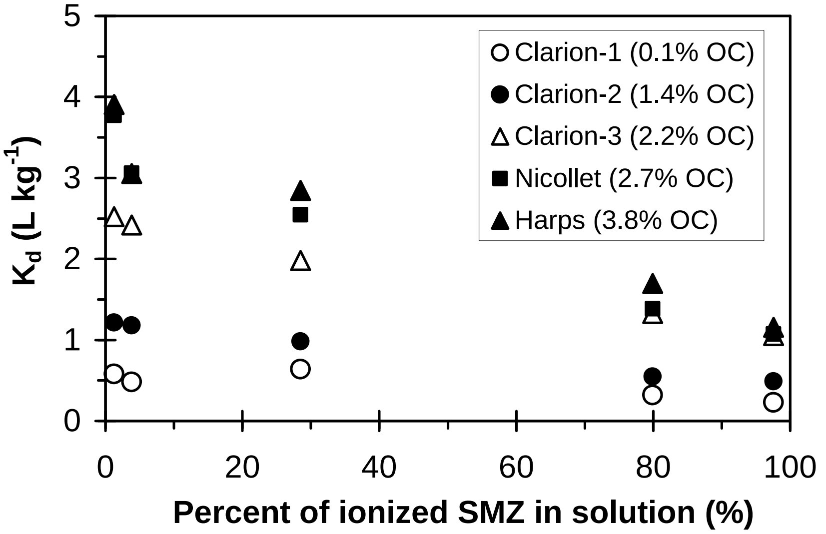


Figure 2. Sorption isotherms of sulfamethazine for five soils at pH 5.5, 6, 7, 8, and 9. Solid lines show the linear isotherms obtained by least squares regression.



(a)



(b)

Figure 3. Sorption (K_d) of sulfamethazine to five soils (Clarion-1, Clarion-2, Clarion-3, Nicollet, and Harps) as a function of (a) pH, and (b) percent of anionic sulfamethazine in solution

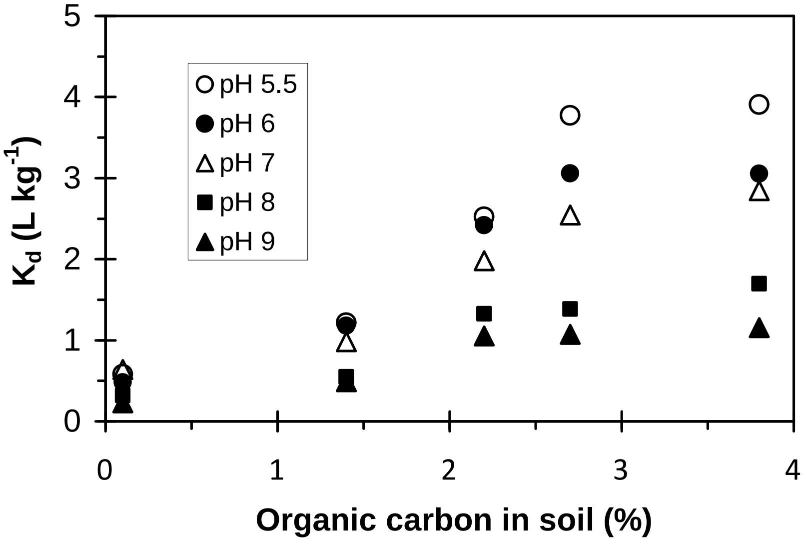


Figure 4. Relationship of sulfamethazine sorption and soil organic carbon

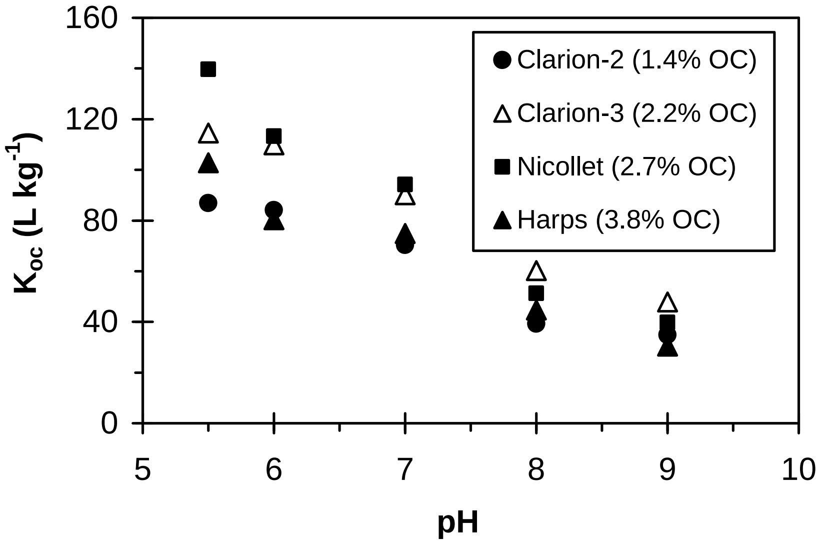


Figure 5. Relationship of K_{oc} (except for Clarion-1) and soil pH

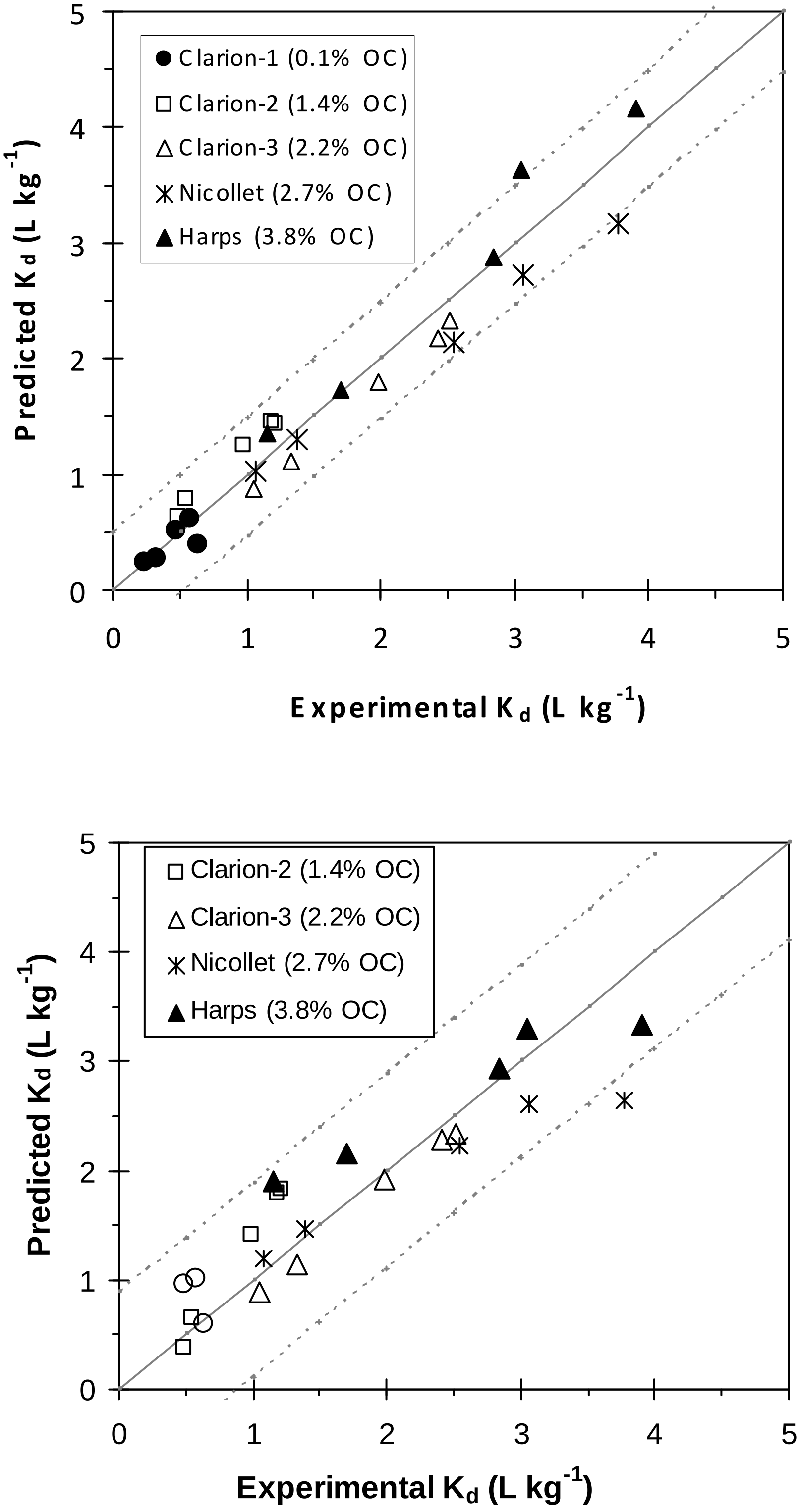


Figure 6. Plot of experimental K_d and predicted K_d predicted using (a) Eqs. 2 – 5 and (b) Eq. 6

CHAPTER 4. DEGRADATION OF SULFAMETHAZINE IN SOIL AND MANURE-AMENDED SOIL

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4.1 Abstract

The impact of initial concentration of SMZ, addition of manure, and aerobic and anaerobic conditions on degradation of SMZ in soils was studied. Inhibitory effects were observed at SMZ concentrations of 50 mg kg^{-1} or higher for anaerobic conditions, and between 50 mg kg^{-1} and 100 mg kg^{-1} for aerobic conditions in soils alone. Disappearance of SMZ was modeled using the availability-adjusted first-order model. Disappearance of SMZ was faster in manure-amended soils than in soils alone, for initial concentrations of 0.5 and 5 mg kg^{-1} but not for concentrations of 50 and 100 mg kg^{-1}. The fate of SMZ in soil determined by using ^{14}C-SMZ showed that 70 to 91 % of ^{14}C-SMZ was bound to soils. Only 0.1 to 1.5 % of ^{14}C-SMZ was completely mineralized to $^{14}CO_2$ with the highest mineralization found in soils without manure under aerobic conditions. Between 5 and 10 % of SMZ were in the form of metabolites. In addition, the results implied that as initial SMZ concentration increased, the fraction bound to soils decreased.

Keywords: Fate; Sulfonamide; Inhibiting; Concentration; Binding

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4.2. Introduction

Veterinary antibiotics from animal feedlots are of concern as they may result in an increase in antimicrobial resistant bacteria (Tolls, 2001; Sengeløv et al., 2003) and they may have an impact on aquatic organisms and humans. Sulfamethazine (SMZ), a sulfonamide compound, is a common antibiotic used in the swine industry (Huang et al., 2001). Sulfonamide antibiotics cannot be totally absorbed into the animal body and are excreted as both the parent compound and various metabolites reaching the environment via application of manure on arable land (Haller et al., 2002). *N*4-acetyl sulfamethazine (*N*4-acetyl-SMZ), desamino sulfamethazine (desamino-SMZ), and N-1-methyl sulfamethazine (*N*-1-methyl –SMZ) are the main metabolic forms of SMZ often found. The acetyl metabolite form of SMZ can be cleaved back to the parent compound (Langhammer, 1989) especially during manure storage. The studies showed that the total sulfonamide concentrations of up to 20 mg kg^{-1} (wet manure) (Haller et al., 2002), SMZ concentrations up to 7 mg kg^{-1} (dry matter) were found in liquid manure (Hamscher et al., 2005) and the concentration of sulfonamides greater than 20 μg L^{-1} were found in the manure lagoons in Iowa and Ohio (Campagnolo et al., 2002). Concentrations of SMZ as high as 11 μg kg^{-1} were found in soils (Höper at al., 2002, Hamscher et al., 2005).

Data on the sorption and degradation of sulfonamide are important in understanding the fate and impact of sulfonamide in the environment. Studies have shown that SMZ and other sulfonamides are not strongly sorbed to soils (Langhammer, 1989; Tolls, 2001, Sarmah et al., 2006; ter Laak et al., 2006) and are potentially mobile. Degradation experiments, conducted for various sulfonamides at various conditions, showed that the half-lives of sulfonamide ranged from <1 to 30 days (Ingerslev and

Halling-Sørensen, 2000; Kay et al., 2004; Blackwell et al., 2005; Wang et al., 2006b, Accinelli et al., 2007, Blackwell et al., 2007). The longer the half-lives of the compounds, the more persistent is the compound in the environment. Although sulfonamides are subjected to photodegradation (abiotic degradation) (Boreen et al., 2004), sulfonamide-contaminated soils under the soil surface are not exposed to sunlight and therefore may be subjected to only biodegradation by microorganisms. Aerobic biodegradation is the main process of veterinary pharmaceutical compounds degradation in soils (Aga, 2008). When sulfonamides migrate deeper into the soil, the sulfonamides may be subjected to anaerobic degradation. Generally, the degradation of veterinary pharmaceuticals in soils is impacted by the environmental conditions such as temperature, soil type, soil pH, organic carbon content, soil nutrients and density of bacteria (Kümmerer, 2004). The degradation of sulfonamides in manure and in soils was found to be affected by the initial concentration of sulfonamides, moisture, temperature (Wang et al., 2006a), soil type and presence of microbial activity (Accinelli et al., 2007). The effect of manure slurry addition to soils was found to increase the degradation rates of sulfonamides which may be due to an increase in microbial population (Wang et al., 2006b; Accinelli et al., 2007).

Sulfonamides are antibiotics designed to kill bacteria and, at a certain concentration, they may have an adverse effect on soil bacteria and consequently biodegradation of sulfonamide. Colinas et al. (1994) reported that the bacteria population in oxytetracyclins-applied soils were found to reduce to a fifth of the original population. Doses of sulfapyridine that inhibited microbial activity by 10 % and 50 % (ED_{10} and ED_{50}) in a Fe(III) reduction test were found to range from 0.003 to 1.14 mg kg^{-1}, and from

6.45 to 86.5 mg kg^{-1}, respectively (Thiele-Bruhn and Beck, 2005). In the same study, the ED_{10} and ED_{50} of oxytetracycline hydrochloride in the Fe (III) reduction test were found to range from 5.50 to 7.35 mg kg^{-1} and from 9.68 to 156 mg kg^{-1}, respectively. There appeared to be no studies on the inhibiting effect of SMZ on soil microbial processes under aerobic or anaerobic conditions.

This study investigated the degradation of SMZ at different initial concentrations under aerobic and anaerobic conditions in soils and in manure-amended soils, the extent of SMZ mineralization using ^{14}C-SMZ and the distribution of SMZ and its metabolites in soils. In addition, this study investigated the inhibiting effect of SMZ on soil microbial processes.

4.3. Materials and methods

4.3.1 Soil sampling and swine manure

A sample of Clarion soil was collected from a corn field in Ames, Iowa. Soil collected was surface soil at depths of 0 - 15 cm. The soil was thoroughly homogenized, partially dried at room temperature, sieved using a 2-mm opening sieve, and stored moist in a refrigerator. Soil moisture content was determined by weight difference between the moist soil and dry soil by drying the soil in an oven at 105º C for at least 24 hours. The soil is a loam with: pH (1:1) of 6.4, 2.6 % organic carbon content, 44 % sand, 36 % silt, 20 % clay, and a cation exchange capacity of 13.9 meq 100 g^{-1} (analyzed by Midwest Laboratories, Omaha, NE). Swine manure slurry was collected from a deep pit near Boone, Iowa, and stored in a refrigerator until use. The pH of liquid manure was 8.9. The liquid manure was sent to Swine Odor and Manure Management Research (USDA, Ames, IA) to analyze the carbon content in manure using CNS analyzer (Elementar Vario MAX CNS Analyzer, Germany). Manure has dry matter of 8.1 %, dried manure carbon of 36.8 %, ashed manure carbon of 0.1 %, dried manure nitrogen of 3.9 %, and ashed manure nitrogen of 0.01 %.

4.3.2 Chemicals

Sulfamethazine (4-amino-N-[4, 6-dimethyl-2-pyrimidinyl]-benzenesulfonamide, $C_{12}H_{14}N_4O_2S$), CAS number 57-68-1, with a purity of 99 % was purchased from Sigma-Aldrich (St. Louis, MO). The structure of sulfamethazine is presented in the Figure 1. Other properties of SMZ include a molecular weight of 278.34, log K_{ow} of 0.89, solubility of 1.5 g L^{-1}, pK_1 of 2.65 ± 0.2, and pK_2 of 7.4 ± 0.2. A stock solution of 375 mg L^{-1} of SMZ in 10 % methanol and 90 % deionized water was prepared. Concentrations of 37.5 mg L^{-1} and 3.75 mg L^{-1} of SMZ solutions, were prepared by diluting the 375 mg L^{-1} solution with deionized water.

^{14}C-SMZ was purchased from Sigma-Aldrich Corp. (St. Louis, MO). Acetonitrile, HPLC and chromatography grade water for HPLC analysis, and methanol were purchased from Burdick & Jackson (Muskegon, MI).

4.3.3 Sulfamethazine effects on microbial respiration

Aerobic and anaerobic respiration experiments in soils and manure-amended soils treated with SMZ at concentrations of 0, 0.5, 5, 10, 50, 100, and 150 mg kg^{-1} soil were prepared by placing 15 g (dry weight) of moist soil in 40-mL screw-top amber-glass tubes. For manure-amended soils, 1 mL of liquid manure was added into each tube.

SMZ stock solutions were added to the soil to obtain the initial concentration listed above. Deionized water was added to achieve a soil moisture of 25 %. Anaerobic conditions were obtained by capping the tubes tightly with screw caps with rubber septum and evacuating and purging the tubes five times with helium gas to flush out all air in the tubes. As for the aerobic experiments, tubes were capped loosely and weighed. Tubes were uncapped once every three days to allow fresh air into tubes and weighed to check if water was needed to maintain the 25 % moisture in soil. The tubes were incubated at 22±1 °C. Soil without addition of SMZ was used as a control. Gas produced from samples were analyzed for carbon dioxide and methane at day 2, 4, 6, 10, 14, 18, 24, 32, and 40 for aerobic incubations, and day 6, 14, 24, 32, 40, 52, 66, and 80 for the anaerobic incubations.

For each sampling event in the aerobic experiment, tubes were uncapped to release all gas accumulated previously, and then capped tightly and incubated for exactly one hour. After the one-hour incubation, 10 mL of nitrogen gas was injected into the capped tube using a needle and syringe to mix the headspace gas. Ten mL of the headspace was withdrawn and injected back into tube. Mixing was repeated for three times, then 10 mL of headspace was collected and injected into an evacuated 8 mL glass vial with a grey butyl rubber septum and aluminum seal (Alltech, Deerfield, IL). The gas sample vials were prepared before use by evacuating and purging the vials five times with helium gas. For anaerobic incubations, tubes were evacuated and purged with helium gas three times to flush out all gas produced earlier, and incubated exactly for one hour. The procedures for collecting headspace gas were the same as in aerobic incubations. Gas samples were analyzed for methane and carbon dioxide using SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA) with a flame ionization detector (FID) and HaySep D column (Alltech, Deerfield, IL) using an autosampler as described by Arnold et al. (2001). The oven temperature was set at 50 ºC. The amounts of carbon dioxide, and methane (for anaerobic incubations) produced were calculated to represent the hourly rate of gas generation at the particular time of sampling.

After sampling the headspace for aerobic respiration, tubes were uncapped, weighed, and water was added (if needed) to replenish moisture loss. All tubes were incubated further as described for aerobic respiration until the next sampling time. For anaerobic respiration, tubes were evacuated and purged with helium gas three times and incubated further until the next sampling time. Standard curves were established using SCOTTY®II standard gases (Scott Specialty Gas, Plumsteadville, PA). Ten mL of standard carbon dioxide and methane gas at concentrations ranging from 503 to 100,400 ppmv, and from 2.01 to 107 ppmv, respectively, were used.

4.3.4 Aerobic degradation of SMZ

Aerobic degradation of SMZ was conducted in a similar manner as the microbial respiration experiments. Moist soil was weighed (15 g dry weight) and placed in 40-mL screw-top amber-glass tubes. SMZ stock solutions were added to the soil to give initial SMZ concentrations of 0.5, 5, 50, and 100 mg kg^{-1} soil. Deionized water was added to achieve a soil moisture content of 25 %. For manure-amended soil, 1 g of liquid swine manure was added to each tube with the SMZ and deionized water added as for the soil samples without manure. The tubes were capped, weighed, and incubated at 22±1 °C. Each tube was weighed every three days interval to determine soil moisture, and water was added if needed. Triplicate samples for all treatments were prepared. To determine the disappearance of SMZ, tubes were sacrificed, and samples extracted and analyzed with HPLC for SMZ at various times: 4, 7, 14, 21, and 28 days after the start of the experiment.

To prepare sterilized samples, soils and manure-amended soils were weighed and placed in tubes in the same way as mentioned in the non-sterilized samples. Tubes containing soils or manure-amended soils were autoclaved for 30 minutes. To further ensure inhibition of microbial activities in soils and manure-amended soils, sodium azide solution was added into autoclaved soils or manure-amended soils with a total amount of 700 mg of sodium azide per tube. Sterile soils were used as controls for degradation experiments. Samples in the tubes were extracted at each sampling time and analyzed for the SMZ using HPLC.

4.3.5 Anaerobic degradation of SMZ

Soil and manure-amended soils for anaerobic degradation of SMZ were prepared in a similar manner as the aerobic treatment but after the tubes were capped tightly with plastic screw caps with rubber septa, the tubes were evacuated and purged five times, with helium gas. The anaerobic experiments were conducted over a 63-day period. Sampling times were at 7, 14, 21, 35, and 63 days.

4.3.6 Extraction of SMZ

The extractant used was a mixture of 80 % methanol and 20 % 0.1 M of KOH. Potassium hydroxide was added in the extractant to adjust the pH of the soil-solution system to be higher than the pK_2 of SMZ to increase extractability. To determine the mass of SMZ in soil, 10 mL of methanol/KOH mixture was added to each tube. Samples were shaken for 3 hours at 22±1 °C, and centrifuged at 5,000 rpm for 15 minutes. The supernatants were then transferred to 15 mL glass volumetric tubes. Extraction was conducted for a total three times for a given soil sample. The combined supernatants were evaporated using nitrogen gas in a N-EVAP analytical evaporator (Organomation Associates, Berlin, MA) at 41 °C, and the remaining residuals re-dissolved with 80 % Phase A and 20 % Phase B of HPLC mobile phase (details presented later). The liquid was filtered with 0.2 μm nylon membrane filter (13 mm polypropylene-encased) (Alltech, Deerfield, IL) and 2 mL of the filtrate were transferred to HPLC vials for analysis.

Preliminary tests were conducted before the experiments to investigate the recoveries of SMZ. The extraction recoveries for a spiked concentration of 5 mg kg^{-1} in soils alone, manure-amended soil, and sterilized manure-amended soil were 90 %, 88 %, and 92 %, respectively.

4.3.7 HPLC analysis

SMZ was analyzed using an Agilent HPLC Series 1100 (Eagan, MN) with a diode array detector. The detection wavelength was 254 nm. The mobile phase was made up of Phase A consisting of water with 1 mM ammonium acetate and 0.1 % (v/v) glacial acetic acid and phase B consisting of acetonitrile and 0.1 % (v/v) glacial acetic acid. Triplicate injections were made and each injection volume used was 50 μL. The initial eluent flow rate was 0.5 mL min^{-1} and was changed accordingly with different percentages of phase A and B as follows:

Time (min)	% B	Flow (mL min^{-1})
0	10	0.5
6	15	0.5
10	15	0.5
12	25	0.55
16	65	0.6
30	100	0.7
35	100	0.7
40	10	0.7
50	10	0.5

4.3.8 Fate of ^{14}C- SMZ

Soils were prepared and treated in the same manner as aerobic and anaerobic degradation experiments with total SMZ (unlabeled and radioactive labeled SMZ) concentrations of 0.5, 5, and 50 mg kg^{-1} soil with 1.04×10^5 disintegrations per minute (dpm) of [^{14}C-phenyl]-SMZ per tube. A 2 mL glass vial containing 1 mL of 1 M NaOH was placed in each tube to trap $^{14}CO_2$ evolved. Tubes were then capped and incubated. At each sampling time, NaOH solution was transferred into a scintillation vial and 6 mL of Ultima Gold^{TM} XR cocktail (Perkin Elmer, Waltham, MA) was added. The radioactivities in the NaOH solutions were counted for 5 minutes using a Packard 1900TR liquid scintillation analyzer (Perkin Elmer, Waltham, MA). New vials filled with fresh NaOH were then placed back into the tubes.

At the last sampling event (28 days for aerobic treatment and 77 days for anaerobic treatment), soils were extracted in the same manner as the degradation experiments except that extracts were evaporated to 5 mL and were not re-dissolved. One mL of the extracted liquid was subsampled and transferred into scintillation vials, and 6 mL of Ultima Gold^{TM} XR cocktail was added, and then counted for 5 minutes in a liquid scintillation counter (identified as the total extractable ^{14}C). The remaining portions of extracted liquids were analyzed and counted for extractable ^{14}C-SMZ using a HPLC (Hewlett-Packard series 1100, Palo Alto, CA) with a mobile phase of 30 % methanol. The detection wavelength was 254 nm. The flow rate was 1 mL min^{-1} and 200 µL injection volume. The HPLC was connected with a Beta-RAM radioactive detector (IN/US Systems, Tampa, FL) with a 30-second residence time and IN-FLOW® cocktail of 1:1 ratio. After extraction, the soils were air-dried and ground, and 0.5 g of the soils were sub-sampled and combusted at 900 °C using a OX500 Biological Oxidizer (R.J. Harvey Instrument Corporation, Tappan, NY). ^{14}C-SMZ bound to soil was determined by the amount of $^{14}CO_2$ generated from the oxidation and liquid scintillation counting. Mass balances were conducted by using the extractable ^{14}C, $^{14}CO_2$ evolved and ^{14}C in bound residue soils.

4.3.9 Degradation kinetics

The kinetics of degradation for SMZ were evaluated using the availability-adjusted first-order model as shown below which was used for pesticide and organic contaminant degradation in soil by Wang et al. (2006b) and Wang and Yates (2008)

$$\frac{dC}{dt} = -k''Ce^{-(at)} \tag{1}$$

$$C_t = C_0 e^{-\frac{k''(1-e^{-(at)})}{a}} \tag{2}$$

where

C_t = concentration of the target compound at time t (mg kg^{-1})

C_0 = initial concentration of the target compound (mg kg^{-1})

t = time (d)

k'' = adjusted rate constant ($k'' = k\xi$) (d^{-1})

k = first-order rate constant (d^{-1})

ξ = fraction of non-adsorbed amount in the total amount of the target compound at t = 0

a = coefficient describing change in availability (d^{-1})

Half-lives ($t_{1/2}$) for SMZ were estimated as follow:

$$t_{1/2} = -\frac{1}{a}\ln(1 - \frac{0.693a}{k''}) \tag{3}$$

4.4. Results and discussion

4.4.1 SMZ effects on microbial respiration

Using the CO_2 production rates for each sampling time, the cumulative CO_2 evolved over time for various concentrations of SMZ and controls were estimated. The net cumulative CO_2 evolved for all samples (cumulative CO_2 evolved minus the cumulative CO_2 evolved for the control (0 mg kg^{-1}) were plotted against time for aerobic and anaerobic conditions as shown in Figure 2 and Figure 3, respectively. For aerobic degradation of SMZ in soils alone (Figure 2a), CO_2 was found to be evolved almost immediately for SMZ treatments with 0.5, 5, 10 and 50 mg kg^{-1} (based on Day 2 measurements) with a decrease in CO_2 evolved relative to the control after days 4 to 6 while there was a lag phase for SMZ treatments of 100 and 150 mg kg^{-1} with a decrease in CO_2 evolved after days 14 to 18. The net cumulative CO_2 evolved was the highest for 50 mg kg^{-1} treatment at a level of 42 mg but higher SMZ treatment (100 and 150 mg kg^{-1}) resulted in lower net cumulative CO_2 evolved ($\approx$ 35 mg).

For the manure-amended soils (Figure 2b), the cumulative CO_2 evolved for 5 and 10 mg kg^{-1} treatments were initially higher (day 2 and 4) but were then lower than the cumulative CO_2 for the control (0 mg kg^{-1} treatment). The amount of CO_2 evolved for these two treatments may be due to the slight differences in the amount of manure added. For 50 mg kg^{-1} treatment, CO_2 evolution exceeded the control almost immediately (based on Day 2) while for 100 and 150 mg kg^{-1} treatments, the net cumulative CO_2 evolved for 100 and 150 mg kg^{-1} treatments were lower than for 0.5, 5 and 10 mg kg^{-1} treatments for the first six days of incubation. The subsequent increase in respiration in soil treated with the 100 and 150 mg kg^{-1} of SMZ may be due to activity of SMZ-resistant microorganism. Eventually, the net cumulative CO_2 evolved for 150 mg kg^{-1} (after 40 days) exceeded that for 50 and 100 mg kg^{-1} treatment. Based on the above results, it appeared that microbial respiration was inhibited when SMZ concentration was higher than 50 mg kg^{-1} for the soils alone treatment while in the manure-amended soils, inhibition was initial for SMZ concentration higher than 50 mg kg^{-1} but the microbes eventually become acclimatized to the SMZ. Another possible reason for the low net cumulative CO_2 in 100 and 150 mg kg^{-}

^{1} treatments during the first ten days incubation and was higher after day 10 was that the SMZ with time became bound to the soils and manure and therefore was rendered ineffective with time.

Under anaerobic conditions, soil treated with SMZ concentrations of 50, 100, and 150 mg kg^{-1} had cumulative CO_2 evolved that were less than the control CO_2 evolved for the whole incubation period (see Figure 3). For manure amended-soils, similar trends were observed as in soils alone, except that the cumulative CO_2 evolved from soil treated with 50 mg kg^{-1} at day 6 was higher than the control and the net cumulative CO_2 evolved were less negative than the SMZ treatments of 100 and 150 mg kg^{-1}. In soils alone, the net cumulative CH_4 produced in 5 mg kg^{-1} treatment was found to be greater than CH_4 produced in 0.5 mg kg^{-1} treatment by 1.6 times, and was about sixty-fold greater than the CH_4 produced in 50 mg kg^{-1} treatment (data not shown). Similar trends were observed for the manure-amended soils, where the CH_4 produced in 5 mg kg^{-1} treatment was 1.4 times larger than 0.5 mg kg^{-1} treatment but the CH_4 produced in 50 mg kg^{-1} treatment was less than the control. The results implied that for anaerobic conditions, SMZ appeared to inhibit microbial respiration at concentrations as low as 50 mg kg^{-1}.

The net maximum cumulative CO_2 evolved for all samples and their initial SMZ concentrations are plotted in Figure 4 (a and b). The Figure clearly shows that under anaerobic conditions, the SMZ concentration of 50 mg kg^{-1} or higher resulted in inhibit of anaerobic microbial respiration in both soils alone and manure-amended soils while under aerobic conditions, the inhibiting effect was observed to be at concentrations between 50 mg kg^{-1} and 100 mg kg^{-1} in soils alone. For manure-amended soils under aerobic conditions, the inhibiting concentration could not be conclusively determined, based on the CO_2 evolved data.

4.4.2 Aerobic and Anaerobic Degradation of SMZ

The persistence of SMZ in soils and manure-amended soils under aerobic conditions for different initial concentrations are presented in Figure 5 (a and b). The fraction of non-adsorbed amount in the total amount of SMZ at day zero (ξ) were set at 90 % in soil and 88 % in manure-amended soils. These fractions were obtained from the extraction of soils and manure-amended soils immediately after spiked with SMZ

In both soils and manure-amended soils experiments, the concentrations of SMZ were found to decrease to close to zero $\mu g\ g^{-1}$ for 0.5, 5 and 50 mg kg^{-1} initial concentration. For 100 mg kg^{-1} initial concentration, the SMZ concentration was reduced and reached an asymptotic concentration at about 40 % of the initial concentration.

The SMZ concentration in the sterilized soil was found to decrease to about 50 % of the initial concentration indicating that the degradation of SMZ was caused by both chemical and biological processes. The loss of SMZ in sterilized control was evidence of chemical dissipation processes which may be result in SMZ being strongly sorbed to the soils over time making it unavailable.

The results of the anaerobic degradation experiments are presented in Figure 6 (a and b). The changes in SMZ concentrations were similar to the aerobic experiments with a fast initial decrease in the concentration and followed by a slow decrease. However, degradation under aerobic conditions, the residual SMZ became fairly constant at about 20 % of the initial concentration. Furthermore, the asymptotic concentrations for the 100 mg kg^{-1} of initial SMZ concentration were similar to that of the sterilized manure-amended soil. The impact of concentration was similar to that of aerobic conditions with slower disappearance of SMZ applied at higher concentrations and faster disappearance of SMZ in manure-amended soil than in soils alone. The degradation of SMZ in manure-amended soil was found to be generally faster than in soils alone except for 100 mg kg^{-1} under anaerobic conditions which was similar to other reports (Wang et al., 2006; Accinelli et al., 2007). This might be attributed to the higher microbial population in manure-amended soils as compared to the soils alone experiments. Wang et al. (2006b) reported there were 0.7 – 2.6 times higher bacterial populations in soils amended with 1 and 10 % of manure in soil than that in soil alone. Surprisingly, at a high-level of SMZ (100 mg kg^{-1}), the degradation in manure-amended soil was found to be slower than in soils alone. This suggests that the stimulatory effects of manure were overcome by the inhibitory effect of the SMZ.

Extracts of soils alone and manure-amended soils from anaerobic degradation experiments at day 63 were analyzed for potential SMZ metabolites, using LC-MS and MRM MS/MS (analyzed by National Soil Tilth Laboratory, Ames, IA). The analysis confirmed *N*-4-acetyl-SMZ and desamino SMZ were found in all degradation experiments except for the sterilized samples, but there was no evidence of *N*-1-methyl-SMZ in all degradation experiments (data not shown).

The degradations of SMZ for aerobic and anaerobic treatments were modeled using availability-adjusted first-order model. The availability-adjusted first order model is a pseudo first-order where the availability of the target compound for degradation is incorporated as discussed in Wang et al. (2006b). Adjusted degradation rate constants of degradation, k´´, and *a* values, in soils alone and manure-amended soils are presented in Table 1. R^2 values for all the regressions were found to be > 0.88 for aerobic treatment and > 0.90 for anaerobic treatment. Similar modeling efforts using 1st order model for both aerobic and anaerobic treatments did not fit the data well.

Table 1 shows that for soils alone under aerobic conditions, the k´´ value was found to be the highest for 0.5 mg kg^{-1} treatment and decreased for an increase in concentration except for 50 and 100 mg kg^{-1} treatments where the rates were not significantly different. For manure-amended soils under aerobic conditions, k´´ values were not significantly different between 0.5 and 5 mg kg^{-1} treatments (≈ 0.6 d^{-1}), and between 50 and 100 mg kg^{-1} treatments (≈ 0.2 d^{-1}). The impact of manure addition on the k´´ values could be observed at 5 and 50 mg kg^{-1} treatments for aerobic degradation experiments, but only for 0.5 mg kg^{-1} treatment under anaerobic treatment. Similar trends were observed for anaerobic experiments.

The relationships of adjusted rate constant, k´´ and initial concentration of SMZ for aerobic and anaerobic conditions and for soils alone and manure-amended soils are plotted in Figure 7. Figure 7 shows that concentration of SMZ greater than 5 mg kg^{-1} had an inhibitory impact on the aerobic and anaerobic biodegradation of SMZ which is in line with the earlier experiments showing inhibition of SMZ on microbial respirations (Figure 2 and 3).

Half-lives ($t_{1/2}$) of SMZ in soils and manure-amended soils ranged from 1 to 7 days, and 2 to 15 days, under aerobic and anaerobic conditions, respectively. Other studies reported the half-lives of some sulfonamides ranging from 10 to 30 days (Kay et al., 2004; Blackwell et al., 2005; Wang et al., 2006b, Accinelli et al., 2007). For a concentration of 100 mg kg^{-1} under anaerobic conditions, the half-lives could not be estimated, but they were roughly known to be more than 63 days. At a given concentration, half-lives of SMZ in soils and manure-amended soils under aerobic conditions were shorter than under anaerobic conditions. A plot of the half-lives for aerobic and anaerobic conditions against the initial concentrations of SMZ is presented in Figure 8.

4.4.3 Fate of ^{14}C-SMZ

Mass balances of ^{14}C-SMZ are presented in Figure 9 and Figure 10. For soils alone under aerobic conditions experiments, about 0.5 to 1.5 % of the SMZ was mineralized to CO_2 while a lower percentage of 0.1 to 0.2 %.were found for under anaerobic conditions. For manure-amended soils, the percent of mineralization ranged from 0.2 to 0.7 % under aerobic conditions which was significantly lower than for soils alone, and from 0.1 to 0.3 % under anaerobic conditions. The lower mineralization in manure-amended soils may be attributed to lower availability of SMZ to microorganisms. A similar scenario was found in Henderson (2008) where mineralization of ^{14}C-SMZ to $^{14}CO_2$ in fresh water sediment was about 1% and was found to be higher than in fresh water sediment with manure addition (0.2%).

Most of the ^{14}C-SMZ was bound to soil and was immobile. Bound residue of ^{14}C ranged from 80 to 90 % and 70 to 90 %, in soils alone and manure-amended soils, respectively. The form of ^{14}C-compound bound to soil was not investigated and may be ^{14}C-SMZ and/or SMZ metabolites. Unbound residue of ^{14}C-SMZ or extractable ^{14}C-SMZ ranged from 5 % to 25 %. The higher the initial SMZ concentration, the lower was the portion of SMZ bound. This may be due to the limited reaction sites/bonds of the soils. There was no difference in ^{14}C bound residues between aerobic and anaerobic treatments. Manure also decreased the amount of bound residue at the 5 and 50 mg kg^{-1} concentrations. Even though the sorption of sulfoamides increased with an increase in the organic matter content of soils, Thiele-Bruhn and Aust (2004) found that when low concentration (2 %) of pig manure slurry was added to soils, the sorption of sulfonamides decreased when compared to soils alone. They suggested that this was due to the competitive adsorption of dissolved organic matter in manure onto the soils. Competition between commonly found compounds in manure such as amino-N-containing soluble compounds (Liang et al., 1996) and N-heterocyclic hydrocarbons, and sulfonamides for specific soil exchange site may be another possibility. Organic matter in manure may be associated with the soil minerals resulting in adsorption of ionic organic chemicals to soil minerals (Kaiser and Zech, 1998). A probable reason for the high percentage of nonextractable SMZ in soil may be due to cross-coupling of SMZ to soil organic matter by covalent bonds as suggested by Bialk et al. (2005). The extent of cross-coupled SMZ product in soils is dependent on the existence of phenoloxidase, manganese oxide surfaces, and natural organic matter.

Table 2 presents the percent of the total ^{14}C recovered from the extracts of the soils and the percent of ^{14}C-SMZ found in the extracts. The percent of transformation products of ^{14}C-SMZ varied from 5 to 10 % which is given by the differences between the percent of total ^{14}C recovered and the percent of ^{14}C-SMZ. The data had a good agreement with other studies which reported that the non-extractable ^{14}C-sulfonamides remained in soils were approximately 90 % and higher for ^{14}C-sulfadiazine (Heise et al., 2006; Schmidt et al. 2008) and ^{14}C-sulfamethoxazole (Heise et al., 2006), and only 2 % of ^{14}C-sulfadiazine was mineralized (Schmidt et al., 2008).

4.5. Conclusion

In soils alone under aerobic conditions, the net cumulative CO_2 evolved for 100 and 150 mg kg^{-1} treatments were found to be lower than for 50 mg kg^{-1} indicating inhibitory effects for SMZ concentrations between 50 and 100 mg kg^{-1}. Concentrations of SMZ greater than 50 mg kg^{-1} in soils alone and in manure-amended soils were found to have an inhibitory effect on the respiration of soil microorganisms under anaerobic conditions. Degradation of SMZ under aerobic conditions was found to be faster than under anaerobic conditions. Addition of manure resulted in faster disappearance of SMZ, except for concentrations of 50 and 100 mg kg^{-1}. In addition, the degradation of SMZ in soils was found to be dependent on the initial concentration. As the initial concentrations increase, degradation became slower presumably due to the availability of SMZ and SMZ inhibition on the microbes present. The kinetics of SMZ degradation fitted well with the availability-adjusted first-order model but not first-order kinetics. The *a* values had no strong relationship with initial concentration.

Less than 2% of ^{14}C-SMZ was mineralized for all concentrations and conditions, with the highest mineralization percentage for soils alone under aerobic conditions. In soils alone, bound residue of ^{14}C was about 80% or greater depending on initial concentration but the percent of ^{14}C bound to soil decreased in manure-amended soils. It is possible that SMZ was cross-coupled to soil organic matter by covalent bonds resulting in a high portion of ^{14}C-bound residues. *N*-4-acetyl-SMZ and des-amino SMZ were found in the extracts indicating that a fraction of the SMZ was at least biotransformed to SMZ metabolites.

4.6. References

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Table 1. Degradation rate constants and half-lives of aerobic degradation, and anaerobic degradation of SMZ in soils alone and manure-amended soils (with 95 % confidence interval)

Treatment (mg kg^{-1})	Rate constant k'' (d^{-1})	Availability coefficient *a* (d^{-1})	R^2	Half life $t_{1/2}$ (days)
Aerobic-soils alone				
5 (sterilized)	0.06 ± 0.08	0.09 ± 0.14	0.88	36.2
0.5	0.58 ± 0.14	0.16 ± 0.08	1.00	1.3
5	0.40 ± 0.04	0.11 ± 0.02	1.00	1.9
50	0.23 ± 0.02	0.05 ± 0.02	1.00	3.2
100	0.20 ± 0.14	0.19 ± 0.16	0.95	5.9
Aerobic-manure-amended soils				
5 (sterilized)	0.11 ± 0.16	0.15 ± 0.14	0.92	17.1
0.5	0.62 ± 0.25	0.17 ± 0.14	1.00	1.2
5	0.63 ± 0.18	0.17 ± 0.10	1.00	1.2
50	0.17 ± 0.00	0.02 ± 0.00	1.00	4.1
100	0.17 ± 0.04	0.17 ± 0.04	1.00	6.6
Anaerobic-soils alone				
5 (sterilized)	0.06 ± 0.04	0.16 ± 0.10	0.97	NA[++]
0.5	0.20 ± 0.06	0.12 ± 0.04	1.00	4.3
5	0.21 ± 0.08	0.15 ± 0.04	0.99	4.4
50	0.10 ± 0.04	0.15 ± 0.08	0.98	15.1
100	0.12 ± 0.08	0.24 ± 0.16	0.98	NA
Anaerobic-manure-amended				
soils	0.05 ± 0.04	0.09 ± 0.08	0.93	NA
5 (sterilized)	0.37 ± 0.18	0.19 ± 0.10	0.99	2.3
0.5	0.19 ± 0.06	0.13 ± 0.06	0.99	4.9
5	0.12 ± 0.06	0.11 ± 0.06	0.97	9.1
50	0.11 ± 0.14	0.21 ± 0.27	0.90	NA
100				

[++] NA = not applicable

Table 2. Fraction of total ^{14}C, and ^{14}C-SMZ in extracts after 28 and 77 days incubation under aerobic and anaerobic conditions, respectively (average with 95 % confidence interval)

Treatment	Initial Conc. (mg kg^{-1} soil)	Extractable ^{14}C (%)	
		Total ^{14}C	^{14}C-SMZ
Aerobic-soils alone	0.5	5.4 ± 0.6	0.4 ± 0.2
	5	10.5 ± 0.4	4.5 ± 2.4
	50	15.8 ± 1.6	7.6 ± 1.0
Aerobic-manure-amended soils	0.5	6.0 ± 0.4	0.00
	5	12.1 ± 6.9	8.7 ± 1.4
	50	23.9 ± 0.8	18.3 ± 2.4
Anaerobic-soils alone	0.5	11.3 ± 0.4	5.5 ± 3.7
	5	11.1 ± 1.8	4.2 ± 1.4
	50	13.8 ± 3.9	8.5 ± 2.5
Anaerobic-manure-amended soils	0.5	10.0 ± 2.0	1.3 ± 1.2
	5	14.2 ± 2.2	5.3 ± 3.3
	50	22.4 ± 8.4	15.9 ± 4.7

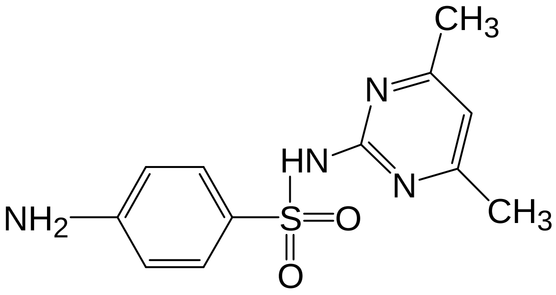


Figure 1. Chemical structure of sulfamethazine

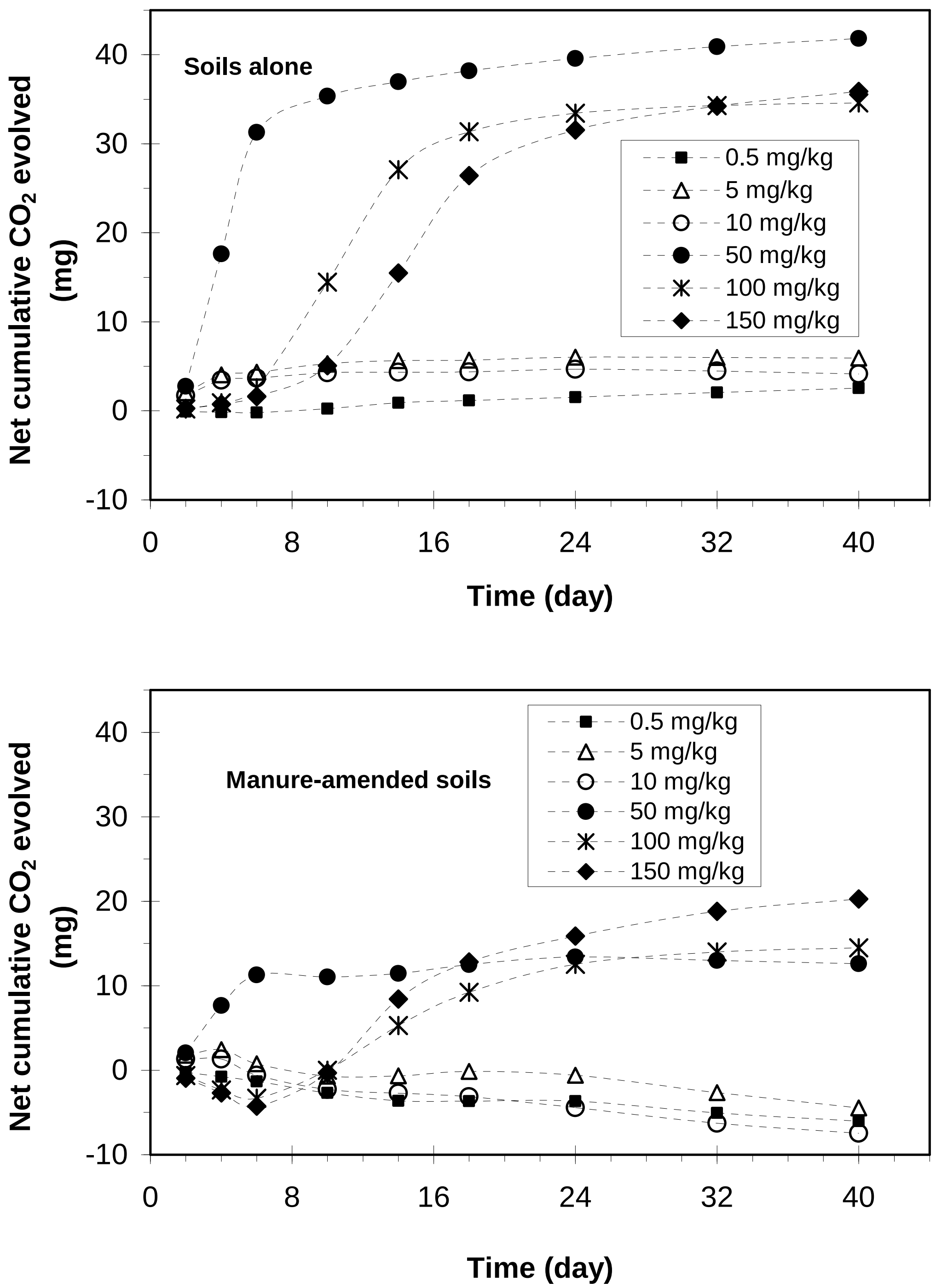


Figure 2. Net cumulative amounts of CO_2 evolved at initial SMZ concentrations of 0.5, 5, 10, 50, 100, and 150 mg kg^{-1} soil in soils alone and manure-amended soils under aerobic conditions over a 40-day incubation.

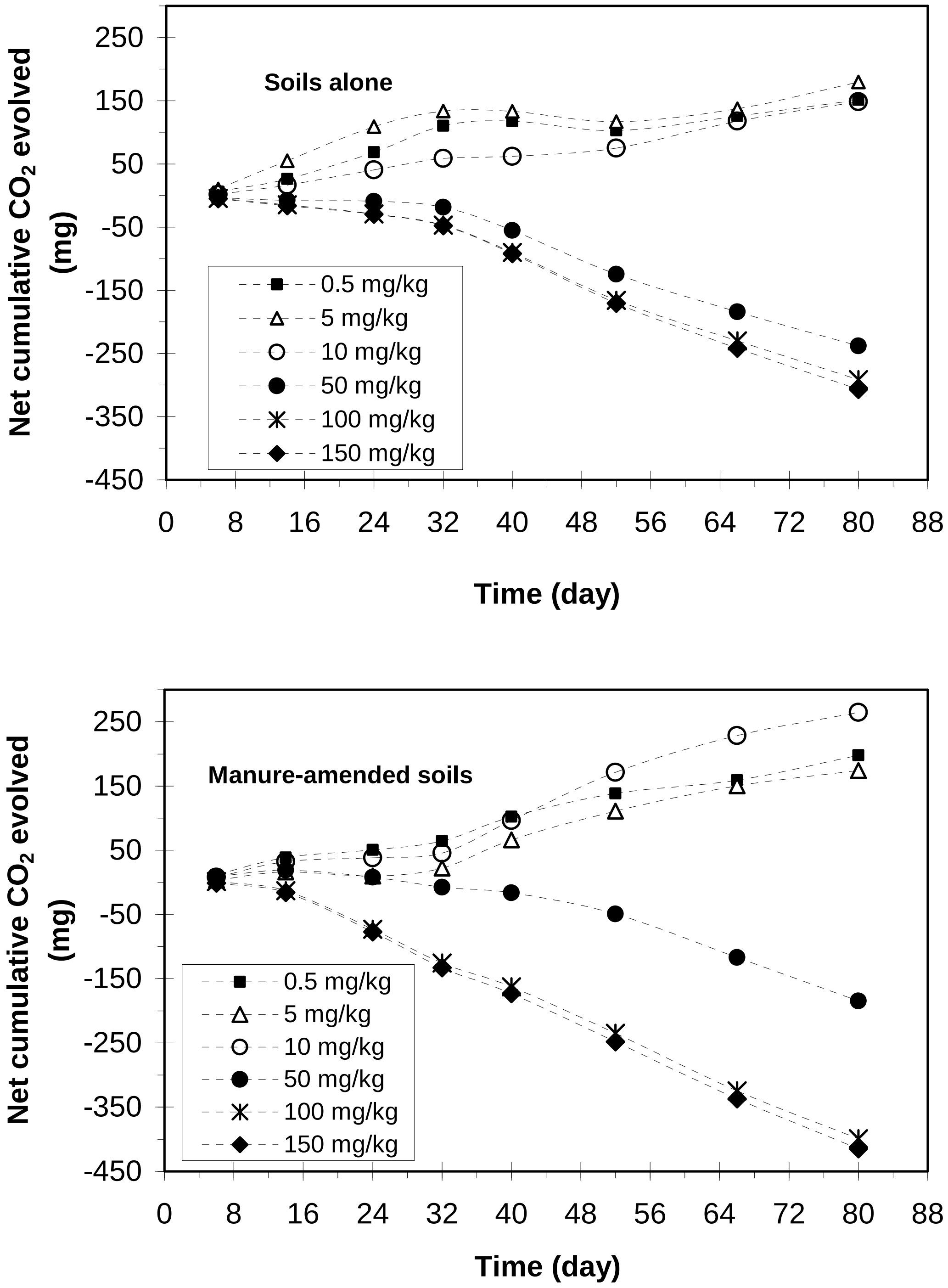


Figure 3. Net cumulative amounts of CO$_2$ evolved at initial SMZ concentrations of 0.5, 5, 10, 50, 100, and 150 mg kg^{-1} soil, in soils alone and manure-amended soil under anaerobic conditions over a 80-day incubation.

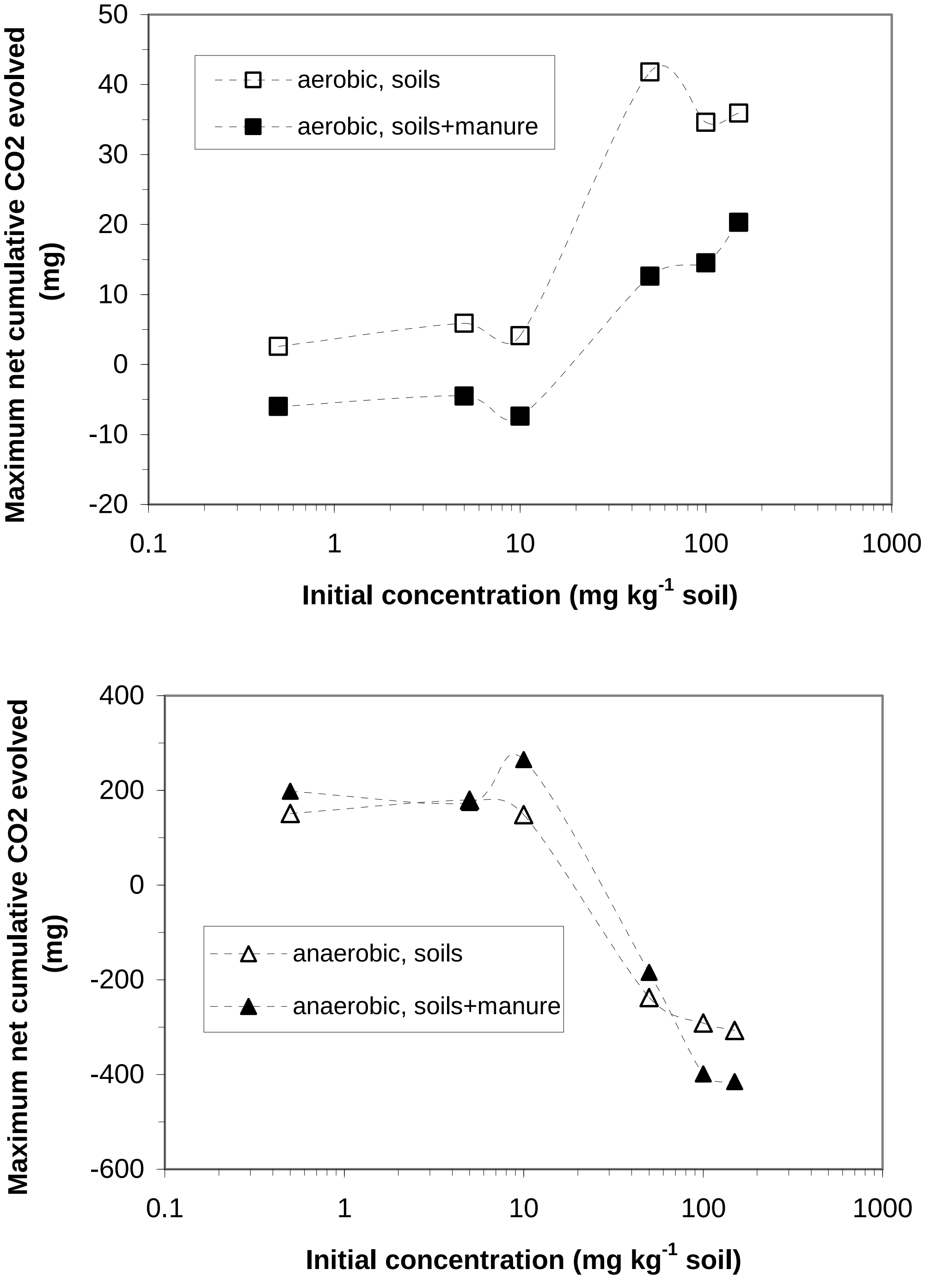
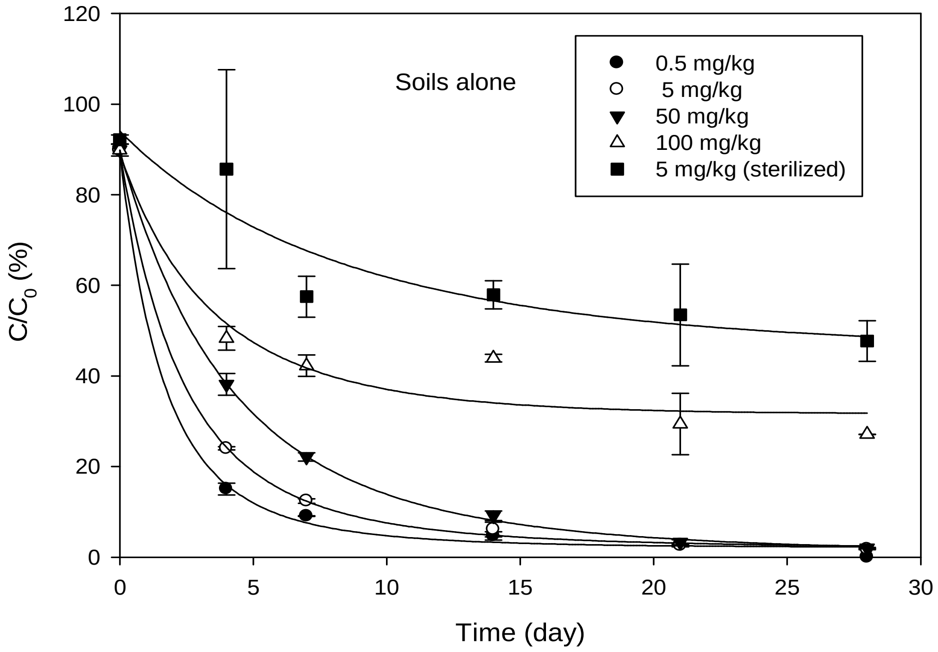


Figure 4. Maximum net cumulative CO_2 evolved from soils alone and manure-amended soils under aerobic (40-day incubation) and anaerobic (80-day incubation) for various SMZ initial concentrations.



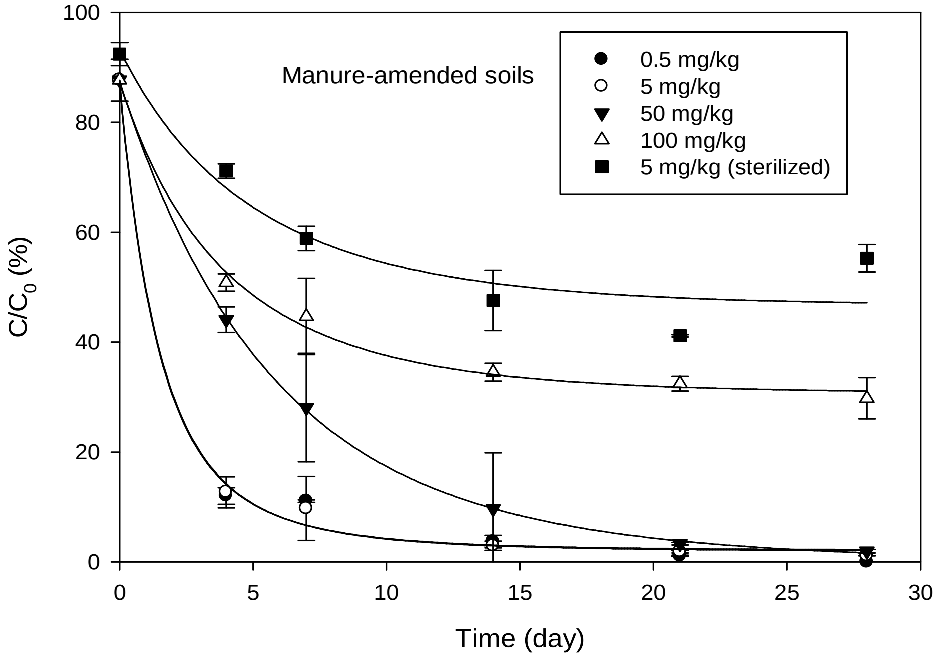


Figure 5. Percent of extractable sulfamethazine (SMZ) remaining in (a) soils alone and (b) manure-amended soils, under aerobic conditions, for various initial SMZ concentrations (points are experimental, and solid lines are fitted results of availability adjusted first-order kinetic model).

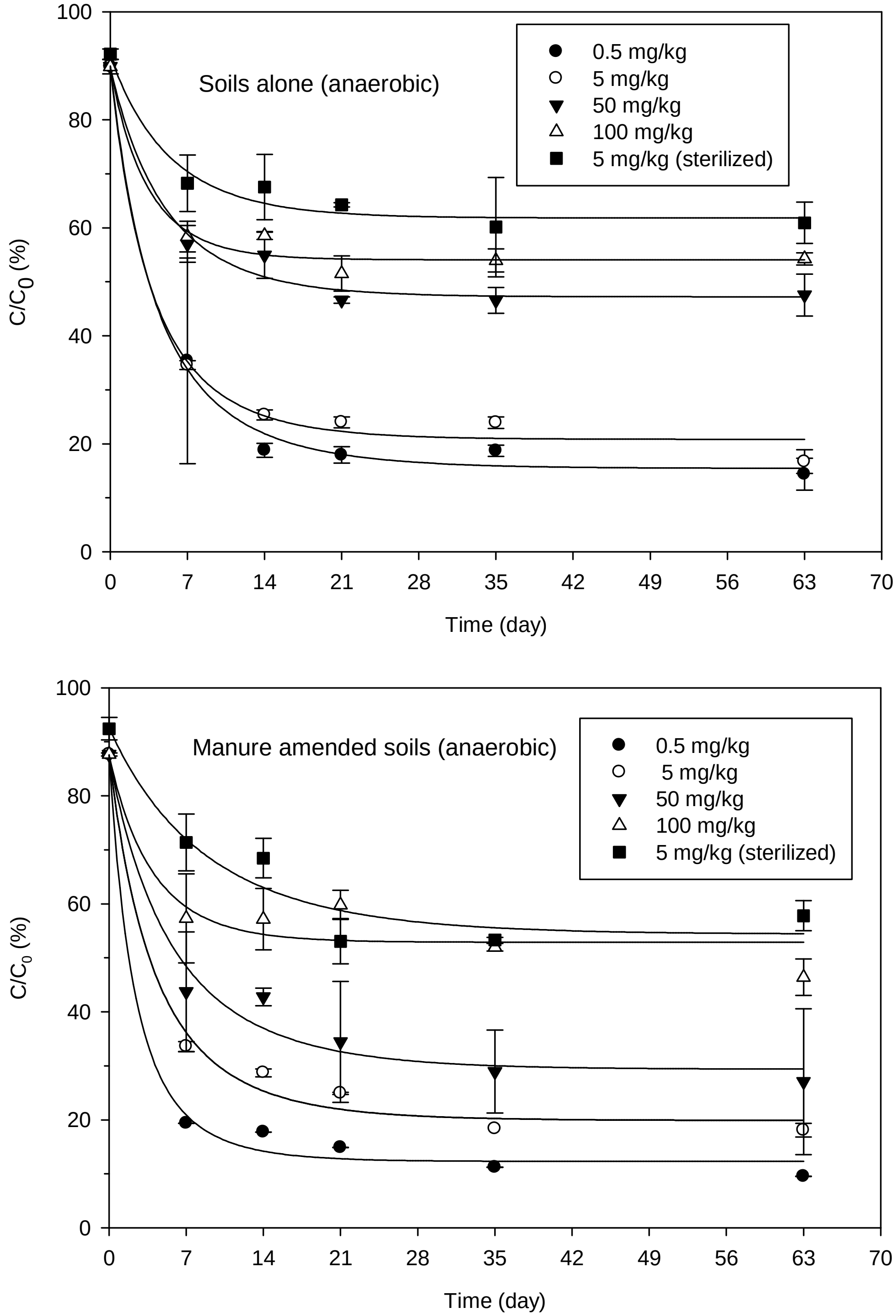


Figure 6. Percent of extractable sulfamethazine (SMZ) remaining in (a) soils alone (b) manure-amended soils, under anaerobic conditions for various initial concentrations (points are experimental, and solid lines are fitting results of availability adjusted first-order kinetic model).

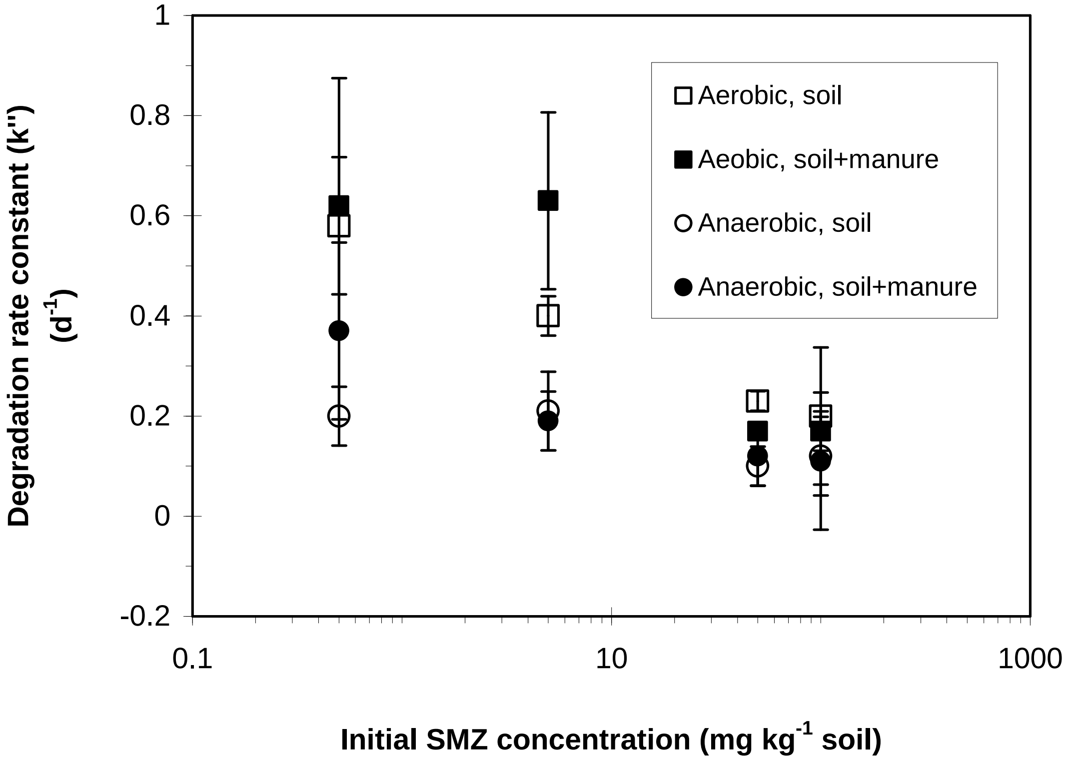


Figure 7. Adjusted rate constant (k´´) (± 95 % confidence interval) and initial SMZ concentration in aerobic (28-day incubation) and anaerobic degradation (63-day incubation) experiments for various initial SMZ concentrations

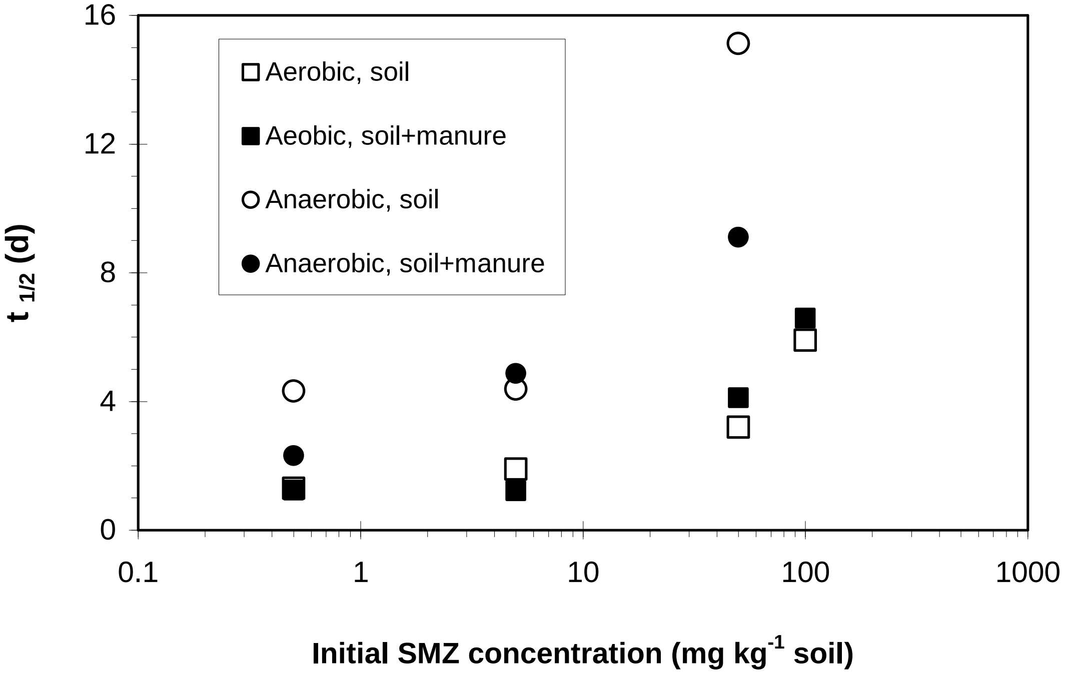


Figure 8. Half-lives ($t_{1/2}$) and initial SMZ concentration in aerobic (28-day incubation) and anaerobic degradation (63-day incubation) experiments for various initial SMZ concentrations.

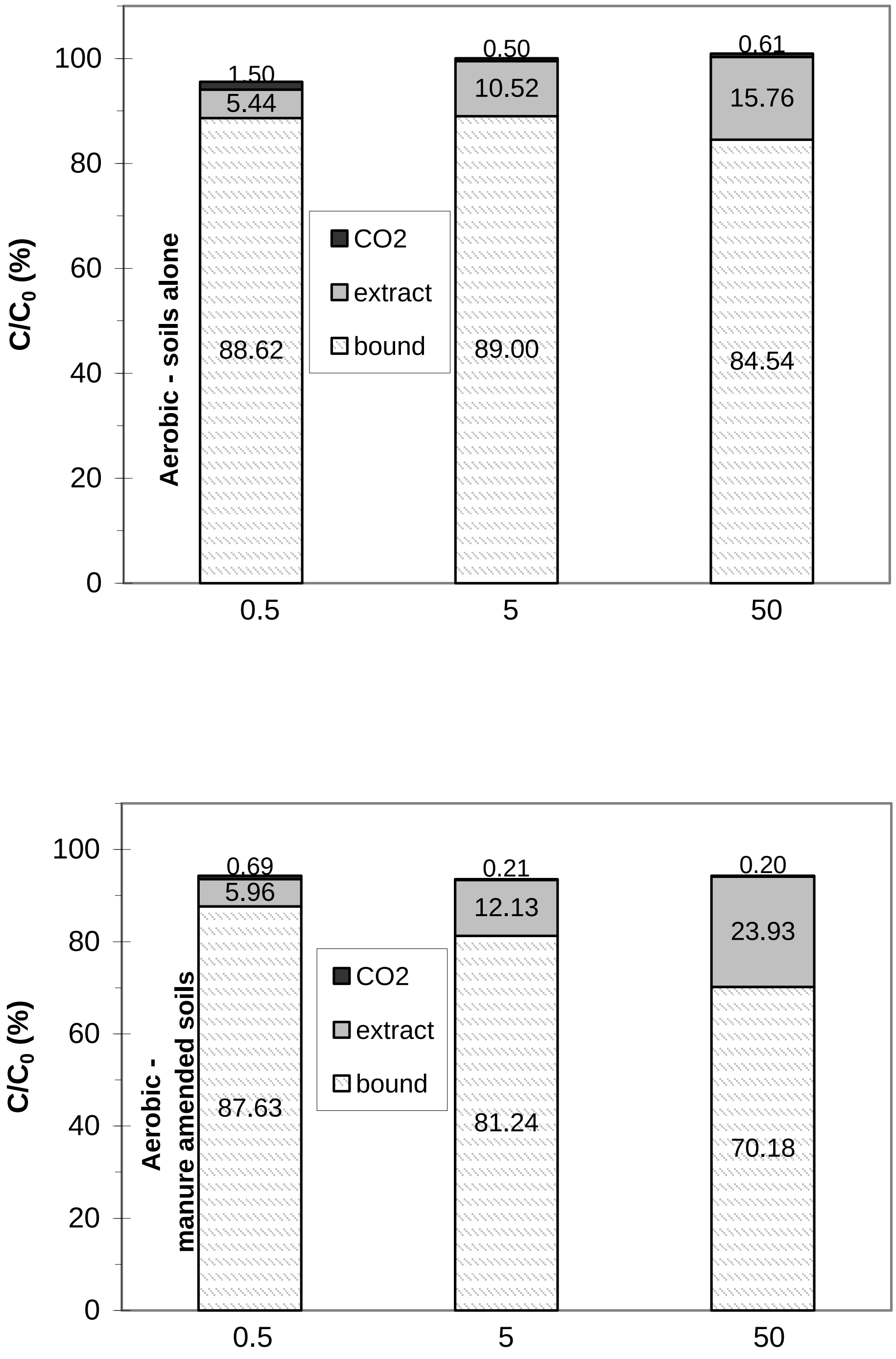


Figure 9. ^{14}C mass balances for $^{14}CO_2$ evolved, ^{14}C extracted, and ^{14}C bound residues in (a) soils alone and (b) manure-amended soils over 28-day period of aerobic degradation

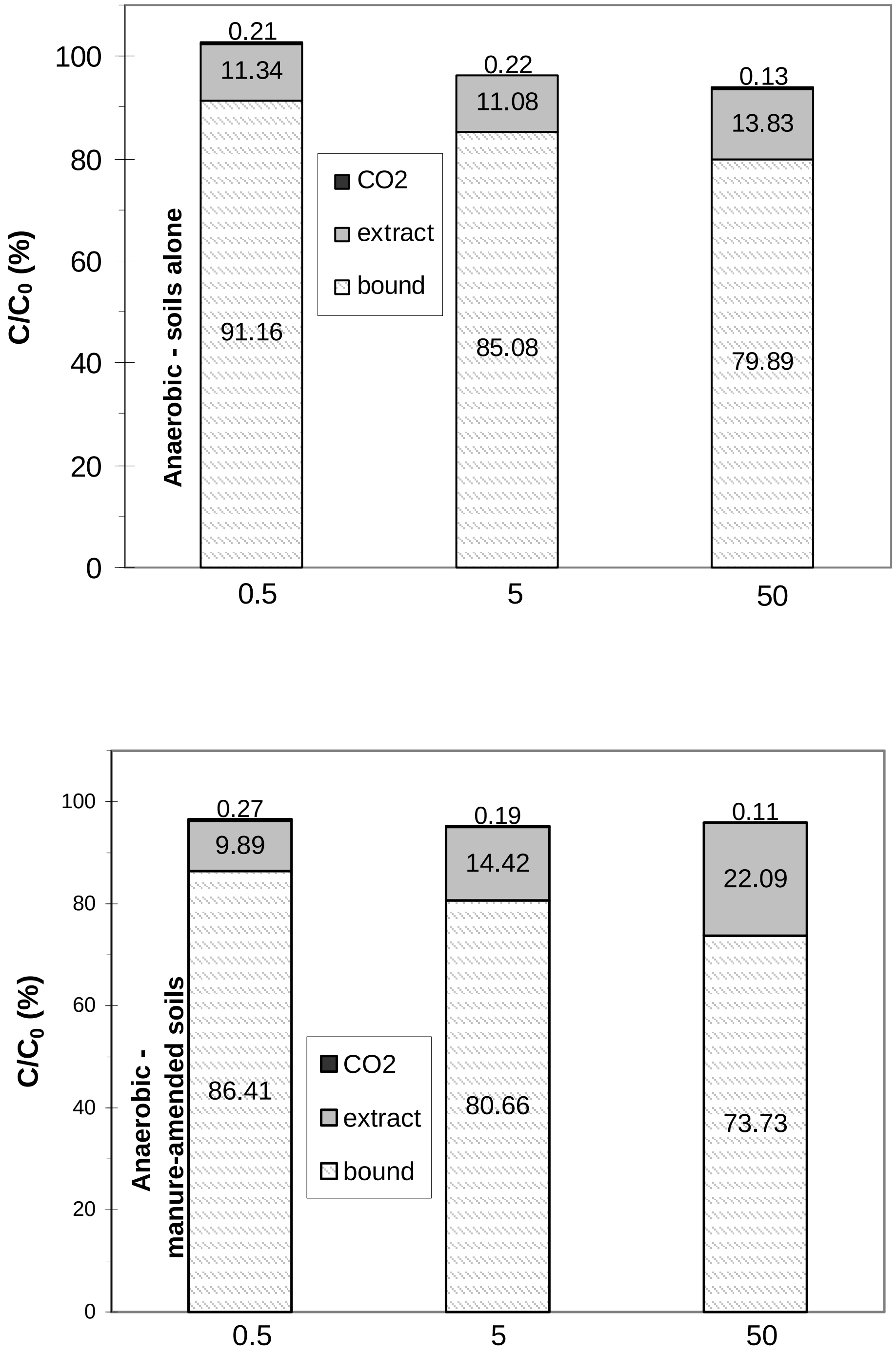


Figure 10. ^{14}C **Mass balances for** $^{14}CO_2$ **evolved,** ^{14}C **extracted, and** ^{14} **C bound residues in (a) soils alone (b) manure-amended soils over 77-day period of anaerobic degradation**

CHAPTER 5. LEACHING OF SULFAMETHAZINE IN SOIL WITH AND WITHOUT MANURE: EFFECT OF DURATION BETWEEN APPLICATION AND RAIN

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5.1 Abstract

Leaching of SMZ from manure-amended soils and soils without liquid manure were investigated using column studies. Topsoil was mixed with SMZ or with SMZ-contaminated manure to a concentration of 7.25 mg kg^{-1} soil. Simulated rainfall was applied at 1, 4 and 7 days after the application of SMZ which was then followed by a second rain event, three days after the first rain event. Concentrations of SMZ in leachate were highest for first day after rainfall with average concentrations of 432 μg L^{-1} and 393 μg L^{-1} in the leachate from soils alone and manure-amended soils, respectively. Concentrations of SMZ in the leachate decreased with longer time duration between application of SMZ and the first rain event. SMZ was also detected after the second rain event indicating that SMZ was mobile and leached from the soils alone and manure-amended soils. The results showed that manure in the soils did not impact the leaching. Measurement of SMZ in filtered and non-filtered samples implied that colloid-facilitated transport may not be a likely process in the transport of SMZ. Depth distribution of SMZ in soils column showed that SMZ were generally retained in the topmost layer, 0 – 10 cm depth of soils.

Keywords: Antibiotics; Sulfonamide; Depth distribution; Leaching; Manure; Rainfall

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5.2. Introduction

The 2002 Market Sales Report of the Animal Health Institute state that approximately 4.7 million kg of antibiotics were used in hogs every year for nontherapeutic purposes (Mellon et al., 2001). Depending on the antibiotics, as much as 90 % of the administered antibiotics are not metabolized in vivo and are released in the manure of the animals (Boxall et al., 2001, Boxall et al., 2002). Veterinary antibiotics have been detected in agricultural fields, surface waters, and ground waters which most probably came from the leaching of antibiotics into surface runoffs from animal feedlots and from land application of digested manure as a fertilizer (Campagnolo et al., 2002; Haller at al., 2002; Hamsher et al., 2002; Schlusener et al., 2003; Yang and Carlson, 2003). The presence of antibiotics in the environment has been of concern as they may affect human and animals (Wollenberger et al., 2000) and may develop antibiotic resistant microorganisms (Chee-Sanford et al., 2001).

Sulfonamides are one of the antibiotic classes widely used in the livestock industries (Bajpai et al., 2000; Lindsey et al., 2001; Tolls, 2001; Grant et al., 2003). Sulfonamide concentrations as high as 20 mg kg^{-1} in liquid manure samples were reported by Haller at al. (2002) while concentrations up to 0.47 μg L^{-1} in ground water were reported by Hirsch et el. (1999). For agricultural soils, sulfamethazine (SMZ) concentrations of 11 μg kg^{-1} in soils was reported by Höper at al. (2002). Sulfonamides have low affinity to soils (Langhammer, 1989; Tolls, 2001; Sarmah et al., 2006; ter Laak et al., 2006) and, with K_d values lower than 5 L kg^{-1}(Langhammer, 1989; Thiele, 2000; Boxall et al., 2002; Tolls et al., 2002; Thiele-Bruhn and Aust, 2004; Thiele-Bruhn et al., 2004), and are expected to have between medium to high mobility and to leach from the soil matrix.

For strongly adsorbed organic pollutants, transport can be enhanced by preferential flow through soil macropores and by dissolved organic matter (DOM)-facilitated transport (Williams et al., 2000; Thiele-Bruhn, 2003; Hoorman et al., 2005). Although sulfonamides have low to medium sorptive affinity in soils, DOM in manure may increase the mobility of sulfonamides by reducing their sorption to soil due to competition by organic matter for sorption sites (Thiele-Bruhn and Aust, 2004) or by colloid-facilitated transport (Tolls, 2001). As such, it is possible that transport of sulfonamides may be enhanced when sulfonamide-contaminated manures are applied to land. In addition, the impact of the frequency of manure application to the fields and the impact of the amount and time periods of irrigation and rain after the manure is land applied on the leaching of sulfonamide are not well understood. The fate of sulfonamides in soils is also impacted by microbial degradation where half-lives of sulfonamides have been found to range from 4 to 30 days (Kay et al., 2004; Boxall et al., 2006; Wang et al., 2006, Accinelli et al., 2007, Blackwell et al., 2007).

The main objective of this study was to investigate the movement of SMZ in SMZ-contaminated-manure-amended soils and the impact of colloidal manure on the transport of SMZ through the subsoil. The impact of time between SMZ-contaminated manure application to land and the commencement of rain or irrigation on the leaching of SMZ was investigated. Soil column experiments were used to simulate and study the movement of SMZ in soils with added SMZ-contaminated manure. The data from this study would provide information that may be used by policy makers to evaluate manure management and irrigation strategies to reduce the transport of sulfonamide antibiotics.

5.3. Materials and methods

5.3.1 Soil and swine manure sampling and analysis

Undisturbed soil cores of Clarion soil were taken from a corn field at the Agronomy and Agricultural Engineering Research Farm, Boone, Iowa using hydraulic soil probes (Giddings Machinery Co., Fort Collins, CO) consisting of a sharp edge steel cylinder with a 10.16 cm inner diameter containing a 55 cm long polyvinyl chloride (PVC) tube insert. The soil probe was pulled out from ground and the PVC tube containing the soil core was removed. The bottom of the PVC tube was capped with a PVC cap while the upper end of tube was wrapped with a plastic wrap to minimize moisture loss.

To measure the soil properties, a soil core was randomly picked, cut into 5 pieces with 10 cm increments. Each 10 cm section was air dried, homogenized thoroughly, sieved using a 2-mm opening sieve, and sent to Midwest Laboratories, Inc. (Omaha, NE) for their soil properties analysis. The properties of soil for each depth increment are presented in Table 1.

Swine manure slurry was collected from a deep pit near Boone, Iowa, and stored in a refrigerator until use. The pH of liquid manure was 8.9. The liquid manure was sent to Swine Odor and Manure Management Research (USDA, Ames, IA) to analyze the carbon content in manure using CNS analyzer (Elementar Vario MAX CNS Analyzer, Germany). Manure has dry matter of 8.1 %, dried manure carbon of 36.8 %, ashed manure carbon of 0.1 %, dried manure nitrogen of 3.9 %, and ashed manure nitrogen of 0.01 %.

5.3.2 Chemicals

Sulfamethazine (4-amino-N-[4, 6-dimethyl-2-pyrimidinyl]-benzenesulfonamide, $C_{12}H_{14}N_4O_2S$, CAS number 57-68-1) with a purity of 99 % was purchased from Sigma-Aldrich (St. Louis, MO). The chemical structure of sulfamethazine is presented in the Figure 1. Physical-chemical properties of SMZ include: molecular weight = 278.34, log K_{ow} = 0.89 (Tolls, 2001) solubility = 1.5 g L^{-1}, $pK_{a,1} = 2.65 \pm 0.2$, and $pK_{a,2} = 7.4 \pm 0.2$. A stock solution of 250 mg L^{-1} of SMZ in deionized water was prepared. Acetonitrile and HPLC and chromatography grade water for HPLC analysis were purchased from Burdick & Jackson (Muskegon, MI). Calcium sulfate solution was prepared at a concentration of 0.01 M for irrigating the soil columns.

5.3.3 Soil column preparation

The PVC cap for the soil cores were replaced with new PVC caps for conducting column studies. The new PVC caps were prepared by drilling a 0.64 cm hole at the center of the cap. Inside the cap, a circular piece of polyamide (PA) monofilament fabric mesh with a 100 µm opening (Sefar Filtration Inc., Depew, NY) was placed in the inner side of cap to cover the hole. Silica sand (Granusil 4030, UNIMIN Corporation, Portage, WI) was then placed inside the PVC cap to serve as a support for the soil core and to prevent clogging of the drainage hole. For drainage a 0.32 cm inner diameter HDPE plastic tubing was inserted through the hole and was glued to the cap using silicone. The new PVC cap was then attached to the PVC tube containing the soil core and the joints were sealed with silicone.

5.3.4 Leaching study

To reduce trapped air in the soil columns, the tubes were immersed in 0.01 M $CaSO_4$ solution at a depth of about 30 cm from the bottom for approximately 24 hours. The tubes were then removed from solution and excess water allowed to drip for about 12 hours.

The leaching of sulfamethazine in soil alone and in manure-amended soil was compared. About 800 g of soil (7 – 8 cm depth) were removed from the top of the soil core, and treated with 20 mL of 250 mg L^{-1} of SMZ stock solution to give a concentration of 6.25 mg kg^{-1} (wet) soil or equivalent to 7.25 mg kg^{-1} oven dried soil. The SMZ-contaminated soil was placed in a fume hood for 6 hours to allow excess moisture to evaporate. When the SMZ-contaminated soil was placed back into tube, bentonite clay was applied to the side of the tube to fill any gap between tube and the soil to prevent any flow of water between PVC column and the soil core.

To determine the effect of manure on leaching of SMZ through the soil columns, manure-amended soils contaminated with SMZ were prepared in the same way as the SMZ-contaminated soils described above except that SMZ solution was added to 140 mL of liquid swine manure and the manure was thoroughly mixed with the soil. It was assumed that application of manure in the field was well mixed with the top 5 to 8 cm of soil. The rate of manure added to the 800 g of soil was equivalent to swine manure application rate of 18,000 gal $acre^{-1}$ for corn grain crop (College of Agricultural Sciences, The Pennsylvania State University, 1997). The total time from adding the SMZ to the soils or manure and placing them in the soil column was approximately 7 hours and this was included in the incubation time.

The soil columns were treated with simulated rainfall separately on day 1, 4 and 7 after the application of SMZ-contaminated media to study the impact of time duration between the application of the SMZ-contaminated media and the commencement of rain on the leaching of SMZ. Three days after the first simulated rain, a second simulated rain was applied at same rate as the first simulated rain. Each time duration experiment had four replicates. To simulate rainfall for the soil columns, 0.01 M $CaSO_4$ solution was applied for one hour at the rate of 9.5 cm^3 min^{-1} using a peristaltic pump and plastic manifold with 60 needles that dripped liquid on the soil surface. The amount of simulated rainfall was equivalent to a total rainfall of 2.76 inches for a storm period of 1 hour (Center for Transportation Research and Education, Iowa State University, 2007).

Leachates from the soil columns were collected with 480 mL mason jars. Each mason jar was weighed and placed under the soil column to collect the leachate on an hourly basis. Since most of the applied water leached out in the first hour, the first sample collected was for 1 hour and the second sample collected was from 1 to 3.5 hours. Leachates from the second rain were collected in a similar manner. The jars containing the leachate were weighed and the mass of the leachate was estimated. The leachate was immediately stored in the refrigerator until it was analyzed.

5.3.5 Samples clean up using SPE

Leachates were cleaned using Oasis® HLB 6-mL solid phase extraction (SPE) cartridges (Waters Corporation, Milford, MA) which were initially equilibrated with 3 mL of methanol, 3 mL of 0.5 N HCl, and 3 mL of distilled water. The cartridges were then loaded with 60 mL sample, washed with 3 mL distilled water, and eluted with 3 mL methanol. The eluate was collected in a 15 mL volumetric conical tube, then evaporated using nitrogen gas until about 0.5 mL at 40 ± 2 °C in an N-EVAP analytical evaporator (Organomation Associates, Berlin, MA). The remaining solution was then, re-dissolved with HPLC mobile phase solution. The liquid was filtered with 0.2 μm nylon membrane filter (Alltech, Deerfield, IL) (13 mm polypropylene encased) and 2 mL of the filtrate were transferred to HPLC vials for analysis.

To determine colloidal-facilitated effect, 60 mL of leachates from all experiments were filtered using 25-mm encased cellulose acetate syringe filters (filter opening - 0.8 μm) (Sterlitech Corporation, Kent, WA). Filtered samples were then loaded through SPE cartridges as mentioned above. The recoveries of SMZ using this SPE method from samples spiked with 0.03 μg SMZ mL^{-1} were 95 ± 4 %.

5.3.6 Soil extraction

After collecting the leachate from the second rain event, soil columns were immediately cut into 5 sections of 10 cm each and weighed. Each 10 cm section was partial air-dried, homogenized thoroughly, weighed, and subsampled to analyze for moisture content and SMZ. Soil moisture content was determined by weight difference by drying the soil in an oven at 105 ºC for at least 24 hours.

The concentration of SMZ in each section was measured by placing 150 g of partially air-dried soil for each section in a 250-mL fluorinated ethylene propylene (FEP) centrifuge bottle and 100 mL of a mixture of 80 % methanol and 20 % of 0.1 M of KOH added as an extractant. Potassium hydroxide was added to adjust the pH of the soil-solution to be higher than the pK_2 of SMZ to increase the extraction of SMZ in its anionic form. The bottles were sealed with ethylene-tetrafluoroethylene (ETFE) caps. Duplicates were prepared. Samples were shaken for 3 hours at 22 ± 1 °C, and centrifuged at 1680 × g for 15 minutes. Extractions of the soils were repeated for a total of three times. Twenty five mL of each extraction were transferred to a 100 mL graduated cylinder. The combined supernatant was gradually transferred to a 15 mL conical volumetric tube and then evaporated to about 0.5 mL using nitrogen gas at 40 ± 2 °C in an N-EVAP analytical evaporator. The remaining liquid was then re-dissolved with HPLC mobile phase solution. The liquid was filtered with 0.2 μm nylon membrane filter (Alltech, Deerfield, IL) (13 mm polypropylene encased) and 2 mL of the filtrate were transferred to HPLC vials for analysis. The extraction recoveries for a spiked concentration of 5 mg kg^{-1} were 90 % and 88 % for soils alone and manure-amended soil, respectively.

5.3.7 HPLC analysis

SMZ was analyzed using an Agilent HPLC Series 1100 (Eagan, MN) with diode array detection. The injection volume used was 25 μL and the initial eluent flow rate was 0.5 mL min^{-1}. Mobile phase A was water with 1 mM ammonium acetate and 0.1 % (v/v) glacial acetic acid while mobile phase B was acetonitrile and 0.1 % (v/v) glacial acetic acid. The mobile phase B increased from 10 % to 25 % of the total flow over the first 12 minutes and to

100 % from 12 to 30 minutes at a flow rate of 0.7 mL min^{-1}. Mobile phase B was then reduced to 10 % from 30 to 40 minutes at a total flow rate of 0.5 mL min^{-1}. The detection wavelength was set at 254 nm. Calibration curves for SMZ were developed using external standards with concentrations ranging from 0.05 to 5 mg L^{-1}.

5.4. Results and discussion

5.4.1 Effect of time duration and manure on SMZ leaching

SMZ concentrations measured in the leachate during the first hour of leaching and during the 1 – 3.5 hours of leaching after the first simulated rain event were not significantly different (p>0.05, data not shown). Therefore, the weighted mean SMZ concentrations for 0 – 1 hour and for 1 – 3.5 hour were used as the SMZ concentrations in leachate from a single rain event (Figure 2).

The average SMZ concentrations (± standard deviation) in the leachate after the first simulated rain event were 432 ± 167, 156 ± 29, and 121 ± 64 µg L^{-1} for experiments with SMZ applied alone and with time durations of 1, 4, and 7 days between application of SMZ and the first rain event (the number of days between the application of SMZ and the first rain event will be referred later as DBFR), respectively. For the second rain event which occurred three days after the first rain event, the SMZ concentrations in the leachate for soils alone were found to be lower at 91 ± 20, 60 ± 15, and 82 ± 39 µg L^{-1} for experiments with time durations of 1, 4, and 7 DBFR.

For manure-amended soils with SMZ, the average concentrations of SMZ in the leachate after the first rain event were 393 ± 209, 92 ± 33, and 79 ± 32 µg L^{-1} for experiments with time duration of 1, 4, and 7 DBFR, respectively. The SMZ concentrations decreased in the second rain event to 224 ± 61, 64 ± 23, and 35 ± 11 μg L^{-1} for experiments with time durations of 1, 4, and 7 DBFR.

For both SMZ-amended and for manure-amended soils with SMZ, the highest concentrations, as expected, were for the shortest DBFR. With 4 and 7 DBFR, the concentrations of SMZ in leachate decreased significantly and were about only one third of the concentrations in the leachate for 1 DBFR. However, the SMZ concentrations for the 4 and 7 DBFR were of same magnitude and in the hundreds μg L^{-1}. Similar patterns in reduction of SMZ concentrations were observed for the manure-amended soils with SMZ. The data showed the same trend as for the transport of herbicides (Isensee and Sadeghi, 1995; Neurath et al., 2004) in soils where lower concentrations in leachate were found with longer contact time and time between rainfall events. These results also showed that SMZ is mobile and can rapidly move through soils to groundwater. Similar findings reported in the studies on sulfachloropyridazine leaching (Boxall et al., 2002; Blackwell et al., 2007) and the detection of sulfonamides in ground water (Hirsch et el., 1999; Lindsey at al., 2001).

However, the volume of simulated rain applied to each column was approximately 25 % to 30 % of pore volume of soil column (data not shown). SMZ found in leachate with this amount of water applied indicated that the leaching was due to macropores in soils which were also observed when soil cores were collected.

The effects of manure addition and DBFR on the concentrations of SMZ in leachate from soil alone and manure-amended soils with SMZ were tested using Two-way ANOVA (SPSS 14.0, SPSS Inc., Chicago, IL) at 95 % confidence intervals. The statistical analysis showed that manure addition to soils had no effect ($p>0.05$) on the SMZ concentrations in the leachate, while DBFR had an effect ($p<0.05$) on the concentrations in the leachate for first rain fall, and second rain fall events. As such, the results implied that the presence of manure in the soils did not impact the leaching of SMZ from soil columns. This was similar to the column studies conducted by Kay et al. (2005) where pig slurry added to soils had no impact on the leaching of oxytetracycline antibiotics.

To investigate the effect of colloid-facilitated transport, leachate from soils alone and manure-amended soils were filtered through 0.8 μm cellulose acetate syringe filters to remove colloidal particles and analyzed for SMZ. Concentrations of SMZ in non-filtered leachate and filtered leachate were not significantly different (p>0.05) at 95 % confidence. For example, concentrations of SMZ in non-filtered leachate and filtered leachate from first rain fall event and 1-DBFR treatment were 432 ± 167 and 419 ± 53 $\mu g\ L^{-1}$, respectively. The results demonstrated that colloid-facilitated transport of SMZ in this study was unlikely to contribute towards the mobility of SMZ.

Total masses of SMZ leached from each soil column were estimated by summing the SMZ mass leached from the first rain and second rain event. For soils with SMZ, the mass of SMZ leached were 4.7 ± 1.9, 1.9 ± 0.6, and 1.8 ± 1.0 % of SMZ added for 1, 4, and 7 DBFR, respectively. In the case of SNZ-manure-amended soils, the masses leached were 5.3 ± 2.4, 1.6 ± 0.7, and 1.2 ± 0.5 % of SMZ added for 1, 4, and 7 DBFR, respectively.

5.4.2 Depth distribution of SMZ

The depth distribution of SMZ in soils alone and manure-amended soils are presented in Figure 3. For soils alone and 1 DBFR, the SMZ concentration were 293 $\mu g\ kg^{-1}$ soil (oven-dried basis) in the 0 – 10 cm depth and decreased sharply to 112, 39, 37 and 18 $\mu g\ kg^{-1}$ for 10 – 20, 20 – 30, 30 – 40 and 40 – 50 cm depths, respectively. The soil concentration of SMZ in the 40 – 50 cm section was about 0.2 % of the initial concentration (7.25 mg kg^{-1}) of the SMZ-contaminated soils added. Assuming that the mass sorbed to the 40 - 50 cm soil was in equilibrium with the leachate concentration, the sorption of SMZ to soil was estimated (using equation 2 – 5 and 8 of Chapter 3) resulting in an estimated leachate SMZ concentration of 472 ± 37 µg L^{-1} in the leachate. This estimated SMZ concentration is close to the measured SMZ concentration of 432 ± 170 µg L^{-1} in the leachate from soils treated with SMZ alone in the 1 DBFR treatment.

For SMZ-manure-amended soils and 1 DBFR, the concentrations of SMZ were found for 0 –10 cm depth was 391 µg kg^{-1} soil and decreased to 40, 31, 23 and 16 µg kg^{-1} for the 10 – 20, 20 – 30, 30 – 40 and 40 – 50 cm sections, respectively. The concentrations of SMZ in each depth from columns receiving SMZ alone were not significantly different than the SMZ concentrations of manure-amended soil columns, except for the 0 – 10 cm depth of 1-DBFR and for the 10 – 20 cm depth. The results of this study showed a similar pattern as other studies (Kay at al., 2005; Blackwell et al., 2007) where the highest concentration was at the topmost layer and declined with depth. The results also indicate that SMZ was mobile, moving to the lowest depths of the soil column (50 cm) within the time period for the simulated rain to move through the column.

The mass balances for SMZ in soil columns after two rainfall events are presented in Figure 4. For soil columns treated with SMZ alone, the total SMZ mass recovered from 1, 4 and 7 DBFR treatments were 14.1 ± 2.5, 6.4 ± 1.1 and 6.2 ± 2.3 %, respectively. The masses recovered from manure-amended-soil columns were not significantly different from columns without manure for all treatments. SMZ recovered from 1 DBFR treatment was the highest while SMZ recovered from 4 and 7 DBFR treatments were not significantly different. Assuming that the remaining SMZ in application -soil layer (0 – 8 cm) was the same as in the topmost section (0 – 10 cm), the SMZ remaining in application layer was 4.0 ± 0.8, 2.7 ± 0.2 and 2.5 ± 0.6 % for 1, 4 and 7 DBFR, respectively.

The degradation of SMZ in soils treated with SMZ alone and manure-amended soils under aerobic condition in Chapter 4 showed that the extractable SMZ remaining in soil matrix at the initial concentration of 5 mg kg^{-1} soil was about 10 % at day 7. This is comparable with the percentage of recoverable SMZ in this study from the 7 DBFR treatment, which was about 6 %. The data showed that the disappearance of SMZ in soil columns was not only due to sorption. Degradation in soil columns is expected: our results in Chapter 4 show that the half-lives of SMZ in sterilized soils and manure-amended soils at initial concentration of 5 mg kg^{-1} were 36 and 17 days, respectively.

5.5. Conclusion

The experiments showed that SMZ leached from the soil columns and the SMZ concentrations in the leachate for the first hour were similar to the SMZ concentrations in the leachate flow for 1 to 3.5 hours. With longer time between the application of SMZ alone or SMZ with swine manure and the first rain event, the amounts of SMZ leached were significantly reduced. Also, the presence of SMZ in the leachate after the second rain event 3 days after the first rain event further confirmed the mobility of SMZ. Based on data from this study, it suggested that application of pig manure to soil should be done not less than 7 days before rain fall to avoid movement of SMZ from contaminated-manure-amended soils down to deeper soil layers and eventual movement to tile drains. There was no evidence to show that the presence of manure in the soils impacted the leaching of SMZ from the soils. Similarly, SMZ concentrations in non-filtered leachate and filtered leachate were not significantly different implying that colloid-facilitated transport may not be an important factor in transport of SMZ. Measurements of SMZ concentrations in the soil columns indicate that most of the SMZ remained in the 0 – 10 cm depth of soil. Mass balances showing only 4 to 14 % of SMZ were recovered. The SMZ remaining in the soil layer where it was initially applied ranged from 2 to 5 %. When disappearance of SMZ from soil columns was compared to the degradation of SMZ in Chapter 4, it appears that degradation accounts for the unrecoverable SMZ.

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Table 1. Physical-chemical characteristics of soil for each depth increment

Depth (cm)	Soil pH (1: 1)	Organic Carbon[a] (%)	Cation Exchange Capacity (meq/100 g)	Sand (%)	Silt (%)	Clay (%)	Texture
0-10	6.0	2.6	13.3	56	28	16	Sandy loam
10-20	5.4	2.1	15.1	56	26	18	Sandy loam
20-30	6.0	1.8	14.4	52	28	20	Sandy clay loam
30-40	6.3	1.5	14.6	50	32	18	loam
40-50	6.1	1.4	13.0	54	26	20	Sandy clay loam

[a] Walkley-Black method

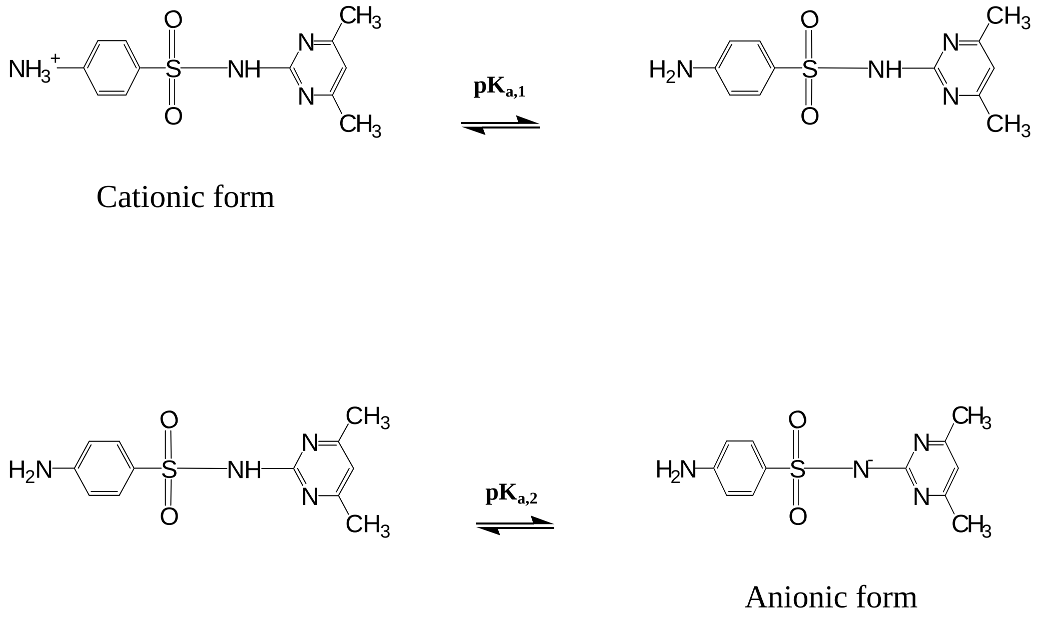

Figure 1. Chemical structure of sulfamethazine and its anionic and cationic forms

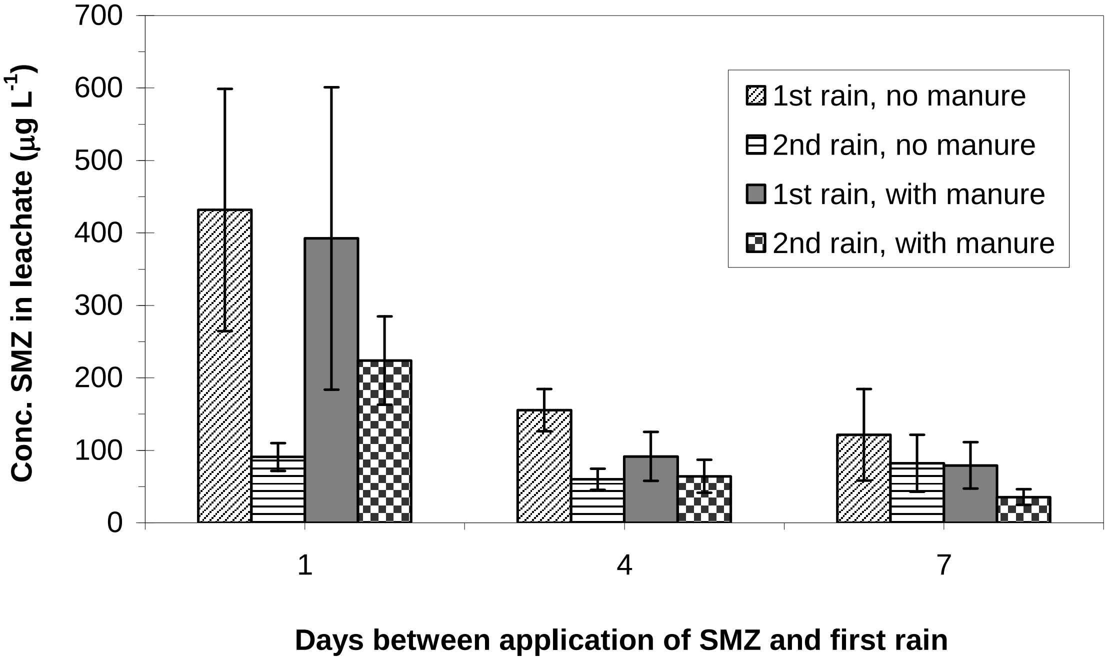

Figure 2. Concentration (± 95% confidence interval) of SMZ in leachate, of soil with and without manure applied, from first and second rain. SMZ was applied 1 day, 4 days, and 7 days before first rain. The second rain occurred 3 days after the first rain.

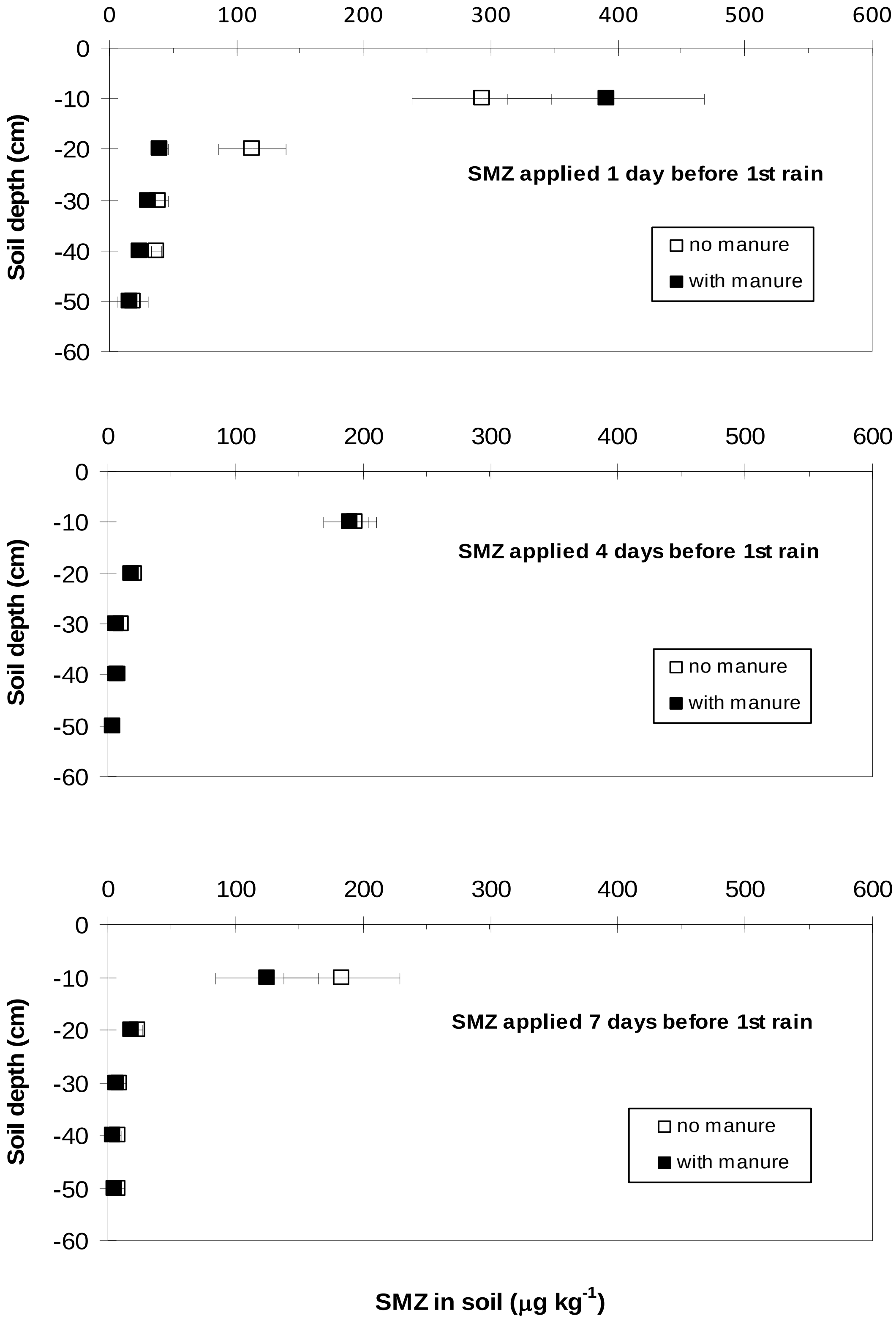

Figure 3. Depth concentration (± 95 % confidence interval) distributions of SMZ in soil, with and without manure, after two rain events

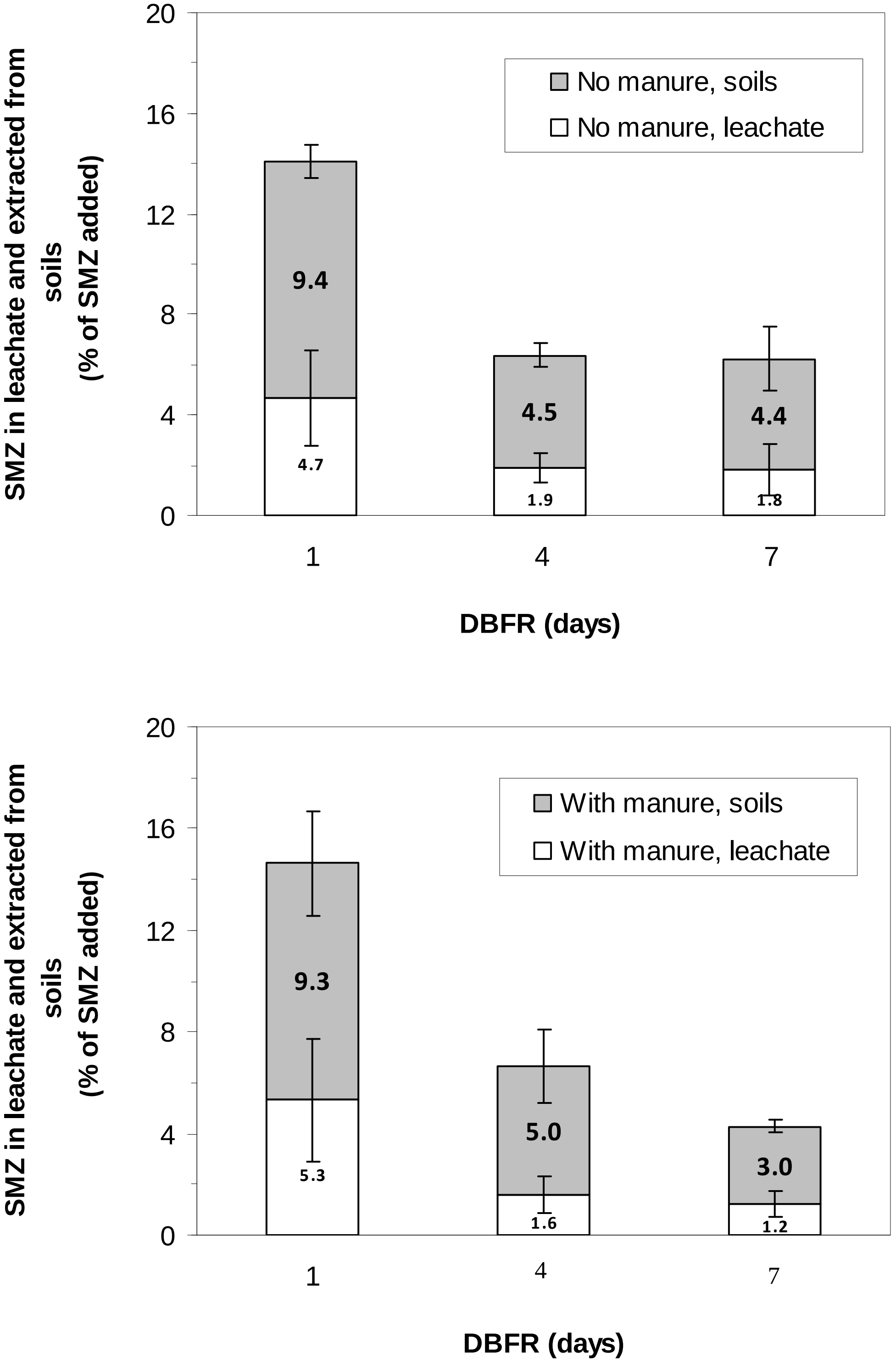

Figure 4. Mass balances (± 95% confidence interval) for SMZ after two rain events showing SMZ leached and in soils for (a) soils treated with SMZ or (b) SMZ and manure-amended soils (100 % SMZ initially applied)

CHAPTER 6. CONCLUSION

Release of antibiotics from animal manure to the environment can take place by application of animal manure to agricultural land as organic fertilizer. The impact of antibiotics on the environment especially the development of antibiotic resistant bacteria has been of concern. SMZ is one of the common antibiotics administered to swine in intensive swine production. The persistence of SMZ and its sorption to soils are the two most important factors controlling the movement of SMZ from soils to surface water or groundwater. This research provides important information on these two processes for the estimation of risk posed by SMZ.

The effect of soil organic carbon content (OC) and soil pH on the sorption of SMZ to soils were investigated and the data are presented in Chapter 3. Linear sorption isotherms described the sorption of SMZ to soils for the initial concentrations ranging from 3.3 to 66.6 mg kg^{-1} soil. Linear sorption coefficients (K_d) determined at various pH conditions, were greatest at the lowest pH tested (pH 5.5) but were lower for higher pH. For example, K_d values for soil with 3.8 % OC were 3.91 ± 0.26 and 1.16 ± 0.03 L kg^{-1} at pH 5.5 and 9, respectively. The pH of the soil-solution had an impact on the sorption of SMZ, due to the ionization of SMZ. At pH less than 7.4, hydrophobic sorption was probably involved due to the unionized form of SMZ. At pH greater than 7.4, the lower sorption may be due to the anionic SMZ and the negatively charged surfaces of the soils at high pH. The K_d values were also found to be dependent on the organic carbon of the soils. For example, K_d values at pH 5.5 were found to be 0.58 ± 0.12 L kg^{-1} for soil with 0.1 % OC and 3.91 ± 0.26 L kg^{-1} for soil with 3.8 % OC. Two models incorporating mass fraction of SMZ species and various soil physical-chemical properties were developed to predict the K_d values for various soils. To evaluate the models, reported K_d values of other studies were compared to K_d values predicted using these two models and the comparison showed that percent difference ranged from 1 to 54 %. It should be noted that the models were developed based on experimentally determined sorption coefficients for Iowa soils (mollisols) and further verification is needed using experimentally determined sorption coefficients of soils from other areas. However, this model should be highly applicable for soils in Iowa, Southern Minnesota, and Illinois where mollisols are widely distributed.

The results obtained from Chapter 4 provide information on the concentration of SMZ that inhibits soil-microbial activities, degradation kinetics, half-lives and fate of SMZ in soils. Concentrations of SMZ greater than 50 mg kg^{-1} in soils with and without manure were found to have an inhibitory effect on the respiration of soil microorganisms under anaerobic conditions. Degradation of SMZ under aerobic conditions was found to be faster than under anaerobic conditions. The half-lives under aerobic condition were approximately 2 to 5 times shorter than anaerobic conditions. The degradation of SMZ in soils was found to be dependent on the initial concentration. As the initial concentrations increase, degradation rate constant decreased due to the availability of SMZ and SMZ inhibition on the microbes present. The kinetics of SMZ degradation fitted well with the availability-adjusted first-order model but not first-order kinetics.

The fate of SMZ under aerobic and anaerobic conditions was determined using ^{14}C-SMZ and the results showed that less than 2 % of ^{14}C-SMZ was mineralized for all treatments with the highest percentage mineralization in soils without manure addition under aerobic conditions. In soils without manure, bound ^{14}C residue was about 80 % or greater, depending on initial concentration but the percent of ^{14}C bound to soil decreased in manure-amended soils compared to soils treated with SMZ alone. It is possible that SMZ was cross-coupled to soil organic matter by covalent bonds resulting in a high portion of ^{14}C-bound residues. *N*-4-acetyl-SMZ and des-amino SMZ were found in the extracts indicating that a small fraction of the SMZ was biotransformed to these SMZ metabolites.

The effect of duration between application of SMZ to soils and rainfall on the leaching of SMZ from soil was the goal of the study described in Chapter 5. The SMZ concentrations in the leachate obtained in the first hour of leaching were similar to the SMZ concentrations in the leachate flow for 1 to 3.5 hours. With longer time between the application of SMZ and the first rain event, the amounts of SMZ leached were significantly reduced. The presence of SMZ in the leachate after the second rain event occurring 3 days after the first rain event further confirmed the mobility of SMZ. The impact of manure addition and the colloid-facilitated transport on the leaching of SMZ in soils were not confirmed in this study. Mass balances showed that only 4 to 14 % of SMZ were recovered. The SMZ remaining in the soil layer where it was initially applied ranged from 2 to 5 %. Data from this study suggested that application of pig manure to soil should be done not less than 7 days before rain fall to avoid movement of SMZ from contaminated-manure-amended soils down to deeper soil layers and eventual movement to tile drains.

The results of this study can be applied to the environmental risk assessment of sulfamethazine including estimation of the persistence of sulfamethazine in the environment.

The results from Chapter 5 also can suggest the time for manure application on agricultural fields to avoid transport of sulfamethazine from manure.