THE FATE AND TRANSPORT OF THE ANTIMICROBIALS

SULFADIMETHOXINE AND ORMETOPRIM

IN THE ENVIRONMENT

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THE FATE AND TRANSPORT OF THE ANTIMICROBIALS SULFADIMETHOXINE AND ORMETOPRIM IN THE ENVIRONMENT

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THESIS ABSTRACT

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Over the past decade, there have been increasing concerns regarding antimicrobial contaminants in the environment. To date, there exists limited research regarding the fate of these compounds in the environment once discharged in human and animal waste. The fate and transport of two antimicrobials, sulfadimethoxine (SDM) and ormetoprim (OMP), was investigated in two soils and sand using several series of batch sorption equilibrium experiments as well as miscible displacement column studies. Because OMP and SDM are often administered in combination, their sorption and mobility was investigated in combination as co-solutes as well as individually as single solutes.

Results from multiple experiments indicate relative mobility and subsequent low sorption of these compounds in soils and sand. OMP illustrated a greater tendency to bind to soil than SDM and was quite retarded in column studies. Although OMP illustrated a fairly significant retardation; the compound was readily released from the soil and transported with the mobile water. Overall, sorption of both antimicrobials increased in soils and sand as the organic matter, clay content, and cation exchange capacity increased.

Batch sorption experiments suggested an enhanced sorption of OMP when in combination with SDM; however, this was not observed in the column studies. Additionally, sorption results from batch and column experiments were inconsistent, leading to the notion of rate-limited sorption during antimicrobial transport.

The results from these experiments confirm the potential threat to surface and groundwater by SDM and OMP. Because these compounds will be mobile in the environment, further research investigating their possible environmental impacts and developing management practices to reduce these impacts is necessary.

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Style manual or journal used: Auburn University Graduate School: Guide to Preparation and Submission of Theses and Dissertations.

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CHAPTER 1 - INTRODUCTION

Background

Pharmaceuticals and other personal care products (PPCPs) have recently been classified as "emerging" environmental contaminants. Over the past decade, there has been a surge of research investigating all aspects of these compounds, from occurrences in the environment, to potential toxicities, to their fate and transport in soils, water, and sludge. One of the most comprehensive occurrence studies was performed in 1999-2000 by the United States Geological Survey, where they found at least one of the ninety-five analyzed organic wastewater contaminants (OWC) present in 80% of the 139 sampled streams (Kolpin *et al*., 2002). The OWCs primarily included the emerging PPCPs such as antibiotics, hormones, steroids, and prescription and non-prescription drugs.

The occurrences of antibiotics in the environment have raised particular concerns because their bioactivity often remains after excretion in human and animal wastes (Wollenberger *et al.,* 2000) and because they have been linked to the development of antibiotic resistant genes in nature (Costanzo *et al.,* 2005; Oliveira *et al.,* 2006; Hamscher *et al.,* 2004). Antibiotics belong to a class of bacterial static and bactericidal compounds collectively referred to as antimicrobials. Antimicrobials are used for therapeutic purposes in human and veterinary medicine, and also for nontherapeutic purposes such as growth promotion in farm animals (Mellon *et al.*, 2001; Halling-Sørensen *et al.*, 2002). These compounds have been detected in various environmental compartments in the

United States, Germany, and Switzerland, including soils, swine waste lagoons, surface waters, fish hatcheries, and wastewater effluents (e.g., Meyer *et al.*, 2000; Kolpin *et al.*, 2002). Typical detected antimicrobials include tetracyclines, sulfonamides, β-Lactams, macrolides, diaminopyrimidines, and fluoroquinolones with concentrations commonly in the low $\mu g \cdot L^{-1}$ range (Heberer, 2002; Hirsch *et al.*, 1999; Thiele-Bruhn, 2003).

Sulfadimethoxine (SDM) and ormetoprim (OMP) are two veterinary antimicrobials belonging to the antimicrobial classes of sulfonamides and diaminopyrimidines, respectively. These compounds are generally administered in combination because their combined effects are greater than when administered alone (FDA, 1984). The drug combination of SDM and OMP is approved for use in dogs, chickens, partridges, ducks, catfish, salomids, and turkeys (The Green Book, 2005). Of particular interests are its usages in catfish and chickens because of the potentially high risks of environmental contamination. The SDM and OMP combination administered to poultry is sold under the trade name Rofenaid ® 40 and would primarily enter the environment through land applied poultry waste. In catfish ponds, SDM and OMP are applied as medicated feed (Romet ® 30) and the fate of unused antimicrobial is the sediment (directly, via fish feed or via fish excrements).

The poultry and aquaculture industries are an important food supply and generate a great percentage of the economic gains in many southern states. Alabama, Arkansas, Louisiana and Mississippi alone have 173,010 acres of catfish farms (SRAC, 2005) where they raise ninety-four percent of the nation's catfish. The poultry industry in Alabama generates an economic impact of $8.5 billion, which is ten percent of the state's economy (ALFA, 2007). These important industries must rely on the use of

antimicrobials to maintain healthy animals; however the environmental risks of using these antimicrobials should be fully understood. In order to quantify the ecological risks posed by antimicrobials and to develop management practices to reduce these risks, sound estimates are needed of predicted environmental concentrations (PECs). Calculating accurate PECs requires a thorough understanding of fate and transport processes. The fate of SDM and OMP is often the soil or sediment. Therefore, understanding the SDM and OMP transport in soil is important for the determination of their potential mobility to surface and ground water.

Objectives

The objectives of this research were therefore to study the fate and transport of SDM and OMP in soils and sand representative of poultry and aquaculture in the southeastern United States. More specifically, to

- Utilize batch sorption equilibrium experiments to determine the sorption trends of OMP and SDM and calculate their respective sorption coefficients
- Compare the sorption of OMP and SDM when administered in combination as co-solutes as well as when administered individually as single solutes
- Employ miscible displacement column studies to evaluate the sorption, mobility, and subsequent transport parameters of SDM and OMP individually and in combination
- Compare results of batch sorption experiments to those given by miscible displacement column studies

Organization of Thesis

This thesis is organized according to guidelines outlined in the *Guide to Preparation and Submission of Theses and Dissertations* provided by the Auburn University Graduate School. The thesis contains two manuscripts and utilizes the publication format.

A detailed review of the relevant literature is presented in Chapter 2 in order to explore the breadth of information provided by others in this field.

Chapters 3 and 4 contain the bulk of the research and are presented as two manuscripts. Chapter 3 pertains to the batch sorption experiments of OMP and SDM, while Chapter 4 is with regard to the miscible displacement column studies.

Conclusions and recommendations for future work are included in Chapter 5. And lastly, the appendix includes sample calculations as well as supplemental information.

CHAPTER 2 - LITERATURE REVIEW

Antimicrobials, such as sulfadimethoxine (SDM) and ormetoprim (OMP), belong to a group of "emerging" environmental contaminants generating great concern among environmental scientists and engineers. Understanding the risks of antimicrobials in the environment requires a broad understanding of these compounds. By identifying possible sources of contamination, understanding the fate and transport of these pollutants, and assessing ecosystem effects, researchers can better understand the environmental risks associated with these compounds and develop management strategies to reduce those risks.

Many researchers (Kolpin *et al.,* 2002; Haggard *et al.*, 2006; Hamscher *et al.,* 2006; Campagnolo *et. al.*, 2002) have focused on antimicrobial occurrences in the environment and have verified various sources and sinks for contamination. Additionally, several studies have identified resistant genes from environmental contamination (Costanzo *et al.,* 2005; Oliveira *et al.,* 2006; Hamscher *et al.,* 2004). Only a limited number of studies, however, have considered the fate and transport of antimicrobials in the environment or investigated the ecological effects.

The environmental contamination of SDM and OMP has been studied very infrequently. Only one known study assessed both SDM and OMP in the environment (Dietze *et al*., 2005) and only one known study evaluated the fate, transport, and effects

of both antimicrobials in the environment (FDA, 1984). The sulfonamides, including SDM, are found much more often in the literature than OMP; likely because OMP is only administered in combination with SDM, whereas, SDM can be administered alone (The Green Book, 2005). Because OMP is found seldom in the literature, this paper will often discuss trimethoprim, another diaminopyrimidine having very similar chemical and physical properties to OMP. Some countries, such as Switzerland, use trimethoprim rather than OMP as the veterinary sulfonamide potentiater (Haller *et al*., 2002).

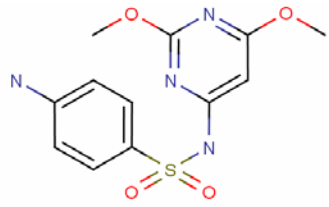
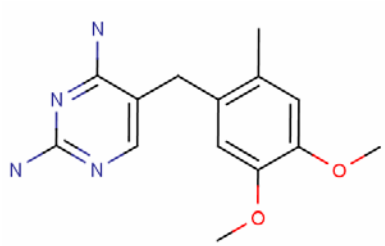
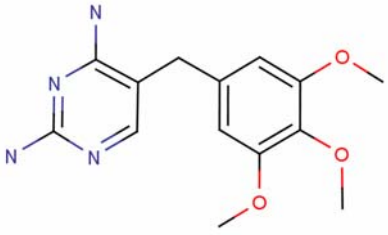
Sulfadimethoxine and Ormetoprim Chemistry

Ormetoprim (OMP, 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl) pyrimidine) and sulfadimethoxine (SDM, N'-(2,6 Dimethoxy-4-pyrimidinyl) sulfanilamide) are two synthetic antimicrobials often used in combination as a potentiated sulfonamide (Manchand *et al*., 1992; FDA, 1984). Each of the antimicrobials possesses antibacterial action alone, yet when combined are more effective and require a lower dosage (FDA, 1984). Three United States Food and Drug Administration (FDA) approved veterinary medicines use the combination of SDM and OMP: Rofenaid ® 40, for chickens, partridges, ducks and turkeys; Primor ®, for dogs; and Romet ® 30, for catfish and salomids (The Green Book, 2005). This study particularly focuses on the applications of Romet ® 30 and Rofenaid ® 40 because of the increased potential for exposure in the environment (described later).

Pharmaceuticals, although often referred to collectively, are a highly diverse group of compounds, generally classified based on molecular structure. Between groups there exist diverse chemical and physical properties (Table 2.1); however within groups their properties are very similar (Thiele-Bruhn, 2003; Cunningham, 2004). For example,

researchers have shown that the sulfonamides act similarly in soil and water (Thiele-Bruhn *et al*., 2004; Díaz-Cruz *et al*., 2003) and that studying a few of them may collectively access their fate. Selected physiochemical properties of SDM and OMP are displayed in Table 2.1. To illustrate the similarity of trimethoprim and OMP, trimethoprim is also shown in Table 2.1.

Table 2.1. Physical and chemical properties of Sulfadimethoxine, Ormetoprim, and Trimethoprim

Antimicrobial	Key Properties
Sulfadimethoxine (SDM)* 	*Structure Class:* Sulfonamide. *Molecular Formula:* $C_{12}H_{14}N_4O_4S$. *Molecular Weight:* 310.33. *Water solubility:* 343 mg/L. *log* K_{ow}*:* 1.63. pK_{a1}/pK_{a2}*:* 2.4/6.0 K_H*:* 1.3E-14 atm-m^3/mol *Vapor Pressure:* 1.59E-9 mm Hg *Atm OH Rate Const:* 2.02E-10 cm^3/molecule-sec
Ormetoprim (OMP)* 	*Structure Class:* Diaminopyrimidine *Molecular Formula:* $C_{14}H_{18}N_4O_2$ *Molecular Weight:* 274.32 *Water Solubility:* 1540 mg/L *log* K_{ow}*:* 1.23 *Weak base* K_H*:* 4.45E-13 atm-m^3/mol *Vapor Pressure:* 2.28E-8 mm Hg *Atm OH Rate Const:* 6.34E-11 cm^3/molecule-sec
Trimethoprim* 	*Structure Class:* Diaminopyrimidine *Molecular Formula:* $C_{14}H_{18}N_4O_3$ *Molecular Weight:* 290.32 *Water Solubility:* 400 mg/L *log* K_{ow} 0.91 pK_a*:* 7.12 K_H*:* 2.39E-14 atm-m^3/mol *Vapor Pressure:* 9.88E-9 mm Hg *Atm OH Rate Const:* 2.03E-10 cm^3/molecule-sec

*Structure and Physiochemical properties retrieved from NLM. 2006. ChemIDplus Lite.

Use of Romet ® 30 and Rofenaid ® 40

The poultry and aquaculture industries are vital to the economy of many southern states. Alabama, Arkansas, Louisiana and Mississippi alone raise ninety-four percent of the nation's catfish on 173,010 acres of catfish farms (SRAC, 2005) and the poultry industry in Alabama alone generates more than 78,000 jobs and accounts for 43% of the state's farm and forestry cash receipts. Although necessary in animal agriculture, it is estimated that these industries use nearly 38,000 pounds of Romet ® 30 (NAA, 2005) and more than 160,000 pounds of Rofenaid ® 40 (FDA, 1984) per year leaving possible residues in the environment. It is very unlikely and unfeasible for these industries to cease the use of antimicrobials. However, understanding the fate and transport of these antimicrobials once exposed to the environment will allow researchers to develop management practices to reduce the potential risks associated with their use.

Routes of Exposure

Antimicrobials are used for therapeutic purposes in human and veterinary medicine, and also for nontherapeutic purposes such as growth promotion in farm animals (Mellon *et al*., 2001; Halling-Sørensen *et al*., 2002). These compounds are designed to be completely excreted from the body after relatively short residence times, mostly as the parent compounds (Thiele-Bruhn, 2003). As a result, a significant fraction of the total amount administered is discharged in human and animal waste and ultimately enters the environment.

In urban areas, antimicrobials enter the environment mainly via treated municipal wastewater (Daughton and Ternes, 1999). Because municipal wastewater treatment plants are not designed to degrade these compounds, much of the mass entering treatment

plants eventually enters the environment by one of two routes: either by passing through the plant in the liquid phase and entering receiving water bodies, or by adsorbing to sludge which is then applied to land (Golet *et al.*, 2003; Wilson *et al.*, 2003).

In agricultural areas (Figure 2.1), antimicrobials enter the environment mainly via land application of stored farm-animal waste or aquaculture ponds from fish production (Jørgensen and Halling-Sørensen, 2000; Halling-Sørensen *et al.*, 2002). The land-applied antimicrobials (e.g. Rofenaid ® 40) from poultry and livestock leach into the soil and are transported to farm ponds and streams via subsurface flow, or are transported directly to these water bodies via surface runoff (Halling-Sørensen *et al.*, 2002; Boxall *et al.*, 2003b). In aquaculture ponds, antimicrobials are applied as medicated feed (e.g., Romet ® 30) and the fate of unused antimicrobials is the sediment (directly, via fish feed or via fish excrements). Here they are either sorbed to the soil, degraded, or slowly leached back into the surrounding water (Hirsch *et al.*, 1999).

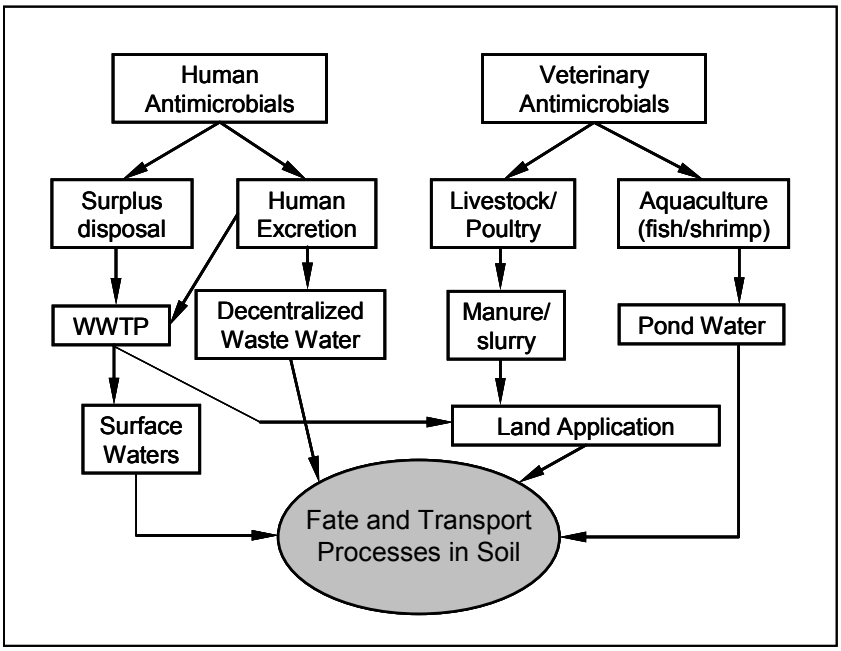


Figure 2.1. Key system components and processes contributing to antimicrobial contamination of surface and groundwater systems.

Occurrence in the Environment

Antimicrobials have been detected in various environmental compartments in the United States, Germany, and Switzerland, including soils, swine waste lagoons, surface waters, fish hatcheries, and wastewater effluents (e.g., Meyer *et al.*, 2000; Kolpin *et al.*, 2002). Typical antimicrobials detected include Tetracyclines, Sulfonamides, β-Lactams, Macrolides, Diaminopyrimidines, and Fluoroquinolones with concentrations commonly in the low $\mu g \cdot L^{-1}$ range (Heberer, 2002; Hirsch *et al.*, 1999; Thiele-Bruhn, 2003).

Thurman *et al.* (2002) of the United States Geological Survey found that out of 189 water samples at 13 state operated fish hatcheries, 27 samples from five fish hatcheries contained trace levels of antimicrobials. Of the antimicrobials detected, SDM was most commonly detected yielding a frequency of 12 percent. The general detection concentration was less than 1 $\mu g \cdot L^{-1}$, with one sample exceeding 15 $\mu g \cdot L^{-1}$. The majority of the water samples (86%), however, did not contain detectable concentrations of antimicrobials.

Dietze *et al.* (2005) continued the previous study on the occurrence of antibiotics in fish hatcheries particularly focusing on the ingredients in Romet® 30, SDM and OMP and also another aquaculture antimicrobial, oxytetracycline. Water samples were collected for two years during and after treatments had occurred in 13 fish hatcheries from across the United States. The authors found that SDM persisted for a period up to 48 days in the pond water, whereas, OMP was less persistent lasting only 28 days (Romet ® 30 only applied in ponds for the first 5 days). SDM was the most frequently detected antibiotic (max 23%) next to OMP (max 17%) then oxytetracycline (max 8%). Samples were collected from intensive (concrete raceways) and extensive (earthen ponds) ponds

and illustrated higher concentrations of each SDM, OMP, and oxytetracycline in intensive ponds (36, 12, 10 $\mu g \cdot L^{-1}$, respectively) versus extensive ponds (1.2, not detected, 0.31 $\mu g \cdot L^{-1}$, respectively). The detection of any antimicrobial in pond effluents was rather uncommon; however, the occurrence of these antimicrobials in the pond water indicated that aquaculture ponds are a potential source of low level antimicrobial environmental contamination.

A study conducted by the United States Geological Survey in 1999 and 2000 found various pharmaceuticals, including antibiotics, in 139 streams from across the United States. Four out of seven tested sulfonamide antimicrobials were detected at frequencies from 1.2 to 19%. The maximum detected concentration of SDM was low at 0.06 $\mu g \cdot L^{-1}$. Trimethoprim was detected at a maximum frequency and concentration of 27.4% and 0.71 $\mu g \cdot L^{-1}$, respectively. Overall, antibiotics were detected in nearly 50% of the sampled streams (Kolpin *et al.,* 2002).

Haggard *et al.* (2006) surveyed several streams in North-Central and Northwestern Arkansas for various pharmaceuticals and other organic chemicals. The authors particularly focused their study upstream and downstream of wastewater effluents because wastewater effluents are generally more susceptible to contamination. Of the selected 18 sites, antibiotics were only detected in water samples at two sites which happened to be downstream from the same wastewater effluent. No antibiotics were detected upstream from wastewater effluents. Out of 45 screened antibiotics, only a maximum of eight were detected. OMP was not detected, but Trimethoprim was detected at a frequency of 24% with a maximum concentration of 0.19 $\mu g \cdot L^{-1}$. SDM was also detected with a frequency of 10% but a maximum concentration estimated to be only

0.004 $\mu g \cdot L^{-1}$ Overall, this study found that wastewater effluents increased the number and concentration of organic pollutants in the receiving water bodies; however, antibiotics were only found in water samples downstream from one of six waste waster treatment discharges.

A study by Miao *et al.* (2004) of stream samples down river from 8 wastewater treatment plants in Canada detected six of the 16 screened sulfonamides. The sulfonamides detected did not include SDM, but it did include the ones that were most frequently prescribed for humans. Overall, none of the detected antimicrobial concentrations exceeded 1 $\mu g \cdot L^{-1}$. Although trimethoprim was not detected in this study, it was detected in most WWTP effluents and some receiving water bodies in a previous study in Canada (Metcalfe *et al.,* 2003).

Sulfonamides were detected in surface and bottled mineral water samples collected by Perret *et al.* (2006), but were not detected in municipal drinking water. Concentrations of all sulfonamides detected were low, from 9 $ng \cdot L^{-1}$ to 402 $ng \cdot L^{-1}$, and the concentrations of SDM ranged from 11 $ng \cdot L^{-1}$ in bottled mineral water to 74 $ng \cdot L^{-1}$ in surface water. The surface waters where sulfonamides were detected were either proximal to a hospital or a cattle-breeding operation. The authors attribute the absence of sulfonamides in municipal water to their removal in the water treatment facility.

Antimicrobial contamination in aquaculture pond water and sediments was investigated by Hamscher *et al.* (2006) in Northern Germany. The authors focused on pond sediment and water contamination by tetracyclines and sulfonamides because of their use as medicated feeds in aquaculture. Only one in 13 sediment samples showed SDM (7.7 $\mu g \cdot L^{-1}$); however, four in 15 water samples contained detectable concentrations

of SDM (0.14 - 0.88 $\mu g \cdot L^{-1}$). The authors concluded that the aquaculture antimicrobial contamination pathway of aquaculture to water to sediment is of minor importance in Germany relative to other environmental contamination pathways.

Campagnolo *et. al.* (2002) detected multiple classes of antimicrobials in swine waste storage lagoons as well as in surface and groundwater samples proximal to poultry and swine farms. SDM was not detected in any monitoring wells, but was detected in two out of 12 surface waters proximal to poultry farms at concentrations less than 0.5 $\mu g \cdot L^{-1}$ as well as at seven swine waste lagoons at concentrations of 2.5 $\mu g \cdot L^{-1}$. trimethoprim was also detected at concentrations of 2.5 $\mu g \cdot L^{-1}$ in all swine waste lagoons in addition to four surface water samples at concentrations not exceeding 0.3 $\mu g \cdot L^{-1}$. The authors found that multiple classes of antimicrobials were present in all swine waste lagoon samples and in water samples proximal to swine and poultry farms at frequencies of 31% and 67%, respectively.

High environmental concentrations of sulfonamides have also been reported in a study by Haller *et al.* (2002) where they found concentrations greater than 12 $mg \cdot kg^{-1}$ in manure slurry from cattle and pigs. These concentrations actually exceeded typical minimum inhibitory concentrations for sulfonamides (e.g. 1 $mg \cdot kg^{-1}$). The authors also detected Trimethoprim in one of the six test sites; however it was below 0.1 $mg \cdot kg^{-1}$.

Sulfonamide and macrolide antimicrobials, along with trimethoprim were investigated in raw and effluent wastewater as well as activated and digested sewage sludge in a study by Göbel *et al.* (2005). Sulfonamide concentrations up to 1900 $ng \cdot L^{-1}$ entered the wastewater treatment facility and 180 to 880 $ng \cdot L^{-1}$ remained in the wastewater after tertiary treatment. Sulfamethoxazole and trimethoprim, both detected in

wastewater samples, were also present in activated sludge with concentrations of 68 and 41 $mg \cdot kg^{-1}$ (dry weight), respectively. These compounds, though, were not detected in the digested sludge. In human pharmaceutical treatment, sulfamethoxazole and trimethoprim (a sulfonamide and diaminopyrimidine) are typically administered in a 5:1 ratio, respectively. This same ratio was evident in influent wastewater (5.4:1), but in the final effluent the ratio had changed to 8.6:1. The higher concentration of the sulfonamide is presumed to be due to the transformation of N^4-acetyl sulfa metabolites back to the original compound (Göbel *et al.,* 2007).

Holm *et al.* (1995) studied a landfill with no leachate collection system that was once used as the disposal for pharmaceutical waste. Groundwater samples up to 260 meters down gradient from this landfill in Grindsted, Denmark showed detectable concentrations of sulfonamides. Groundwater concentrations near the source often exceed 1000 $\mu g \cdot L^{-1}$; however, by a distance of 260 meters and a depth of 10 meters all sulfonamide concentrations were less than 20 $\mu g \cdot L^{-1}$.

To evaluate the effects of nearby a CAFO (Combined Animal Feeding Operation) on local groundwater, Batt *et al.* (2006) took samples from six private wells formerly used as drinking water sources. The well locations were either down gradient from the CAFO or within 1600 feet. All six wells revealed contamination by two sulfonamide antimicrobials, one which was SDM (concentrations from 0.046 to 0.068 $\mu g \cdot L^{-1}$). When the CAFO waste lagoon was analyzed (Tesch and Carlson, 2003) a concentration of SDM was reported at 2.033 $\mu g \cdot L^{-1}$. The CAFO's corresponding well had a SDM concentration of 0.107 $\mu g \cdot L^{-1}$. Because the antimicrobials tested in this study are only approved for veterinary medicine, all of the groundwater contamination is attributed to the CAFO.

This study indicates antimicrobials, such as sulfonamides, used in CAFOs may be a source for groundwater pollution.

Results on detections of sulfonamides and diaminopyrimidines from across the world indicate that these compounds are potentially mobile as they have commonly been detected in surface waters, wastewaters, and ground waters. Few studies have found these compounds bound to sludge, soil, or sediments.

Fate and Transport

Little information is available on the fate, transport, and bioavalability of antimicrobials in soil (Thiele-Bruhn, 2003). Upon entrance into the environment, antimicrobials can undergo several processes including biodegradation, sorption, chemical transformation, and hydrolysis. They can partition into air, water, soil, or sludge depending on the physiochemical characteristics of the antimicrobial and the receiving environment (Boxall *et al.,* 2003b). Recent fate and transport studies on different classes of antimicrobials suggest that while some antimicrobials such as tetracyclines and fluoroquinolones are tightly bound to sewage sludge and soil, others such as the sulfonamides are quickly transported to receiving water bodies (Boxall *et al.,* 2003b; Thiele-Bruhn, 2003). Depending on the environmental conditions such as the presence of organic matter, clay content, microorganisms, or metals and especially pH, each of these antimicrobials would respond differently.

Sorption

Sorption plays a very important role in the fate of antimicrobials in the environment. Soil sorption is particularly interesting because of the many potential pathways of antimicrobials to the soil (Figure 2.1). As an antimicrobial is sorbed to soil

or organic matter from solution, its bioavailability decreases (Hamscher *et al.,* 2004), greatly reducing the toxicity to the surrounding environment; however if the pH or other environmental conditions change, the antimicrobial may quickly be desorbed and become available to microorganisms.

Most antimicrobial fate studies are conducted using batch sorption equilibrium experiments (described later) in soil/slurry systems to identify a sorption coefficient, K_d ($L^3 \cdot M^{-1}$), which relates the amount of antimicrobial at equilibrium remaining in the solid phase, S ($M_{solute} \cdot M_{soil}^{-1}$), to the amount remaining in the liquid phase, C ($M_{solute} \cdot V_{solution}^{-1}$) (Equation 2.1).

$$K_d = \frac{S}{C} \qquad (2.1)$$

Isotherms, both linear and nonlinear such as the Freundlich or Langmuir, are used to model sorption. The linear model, as the name suggests, is a linear representation of Equation 2.1. As the amount remaining in solution increases, the sorbed amount increases at the same rate. The Freundlich and Langmuir are commonly used nonlinear models accounting for the degree of nonlinearity and a maximum number of sorption sites, respectively (Papiernik *et al.,* 2002). The Linear and Freundlich model have shown to adequately fit the antimicrobial sorption data in studies by Thiele-Bruhn *et al.* (2004) and Boxall *et al.* (2002). Additionally, Thiele-Bruhn (2000) showed that the Freundlich equation rather than the Langmuir equation gives a better fit to the sorption data of sulfonamides.

Because the pK_a values for antimicrobials are often in the range of soil pH values, they undergo protonation/deprotonation as the pH changes. The antimicrobial speciation

and sorption strongly depends on the soil solution pH (Tolls, 2001). Boxall *et al.* (2002) found that the sulfonamide, sulfachloropyridazine, had a pH dependent sorption which increased with decreasing pH. Additionally, the authors studied the effects of adding basic manure to the soil resulting in a decreased sorption in which they attributed to the pH effects.

Sorption of sulfonamides to soil has been found to be relatively weak with sorption coefficients ranging from 0.3 to 10 $L \cdot kg^{-1}$ in soils and up to 295 $L \cdot kg^{-1}$ in activated sludge (Boxall *et al.,* 2002; Thiele-Bruhn and Aust 2004; Göbel *et al.,* 2005; Thiele-Bruhn, 2003). Thiele-Bruhn and Aust (2004) found that SDM had a K_d value of 0.73 $L \cdot kg^{-1}$ in a soil with a pH of 7.5 and a soil organic matter (SOM) of 1.61 %. The authors evaluated the effects of adding different ratios of acidic manure to the soil to the adsorption of several sulfonamides. They found that as the pH of the mixture decreased, the sorption actually decreased which is contrary to the pH effects states by Boxell *et al.* (2002). Although the pH effect of the acidic manure would contribute to increased sorption, the competitive nature of the dissolved organic matter (DOM) in manure actually had a greater effect. Because manure components and sulfonamides have similar chemical properties (e.g. amino acids and urea in manure are ammonia-N containing soluble hydrocarbons), the DOM competed for soil sorption sites making the sulfonamides more mobile. The authors also found an increased mobility at lower environmentally relevant concentrations.

Gao and Pedersen (2005) studied three sulfonamide antimicrobials in the presence of clay minerals and also found strong pH dependent sorption. The authors highlighted

the importance of considering clay surface charge density, pH, ionic strength, and antimicrobial speciation in predicting the transport of these antimicrobials.

Sorption of two sulfonamides, sulfapyridine and sulfamethazine, along with trimethoprim were found to sorb to activated sludge with sorption coefficients ranging from 114 to 418 $L \cdot kg^{-1}$ (Göbel *et al.,* 2005). The higher K_d values found in this study than in other studies of soil (Boxall *et al.,* 2002; Thiele-Bruhn and Aust 2004; Thiele-Bruhn, 2003) were attributed to approximately 40% organic matter content in activated sludge.

Trimethoprim sorption to montmorillonite KSF clay was studied by Bekçi *et al.* (2006) as a function of pH, temperature, ionic strength, and time. The results indicated defined pH dependence with a maximum sorption at pH 5.04; below and above this value, the sorption declined. At pH values above 5.04, the pH nears the pKa value of trimethoprim; here the neutral compound exists and no significant ionic attraction was observed. Ionic strength increases caused trimethoprim sorption to decrease because of the competition between the Na^+ ions and the antimicrobial to the clay surface. The results from temperature experiments indicated that more trimethoprim was sorbed at the lower temperature of 303 K over temperatures of 311 and 318 K. At 303 K trimethoprim sorption illustrated a freundlich sorption coefficient (K_f) of 0.824 $mmol \cdot g^{-1}$. Additionally, the rate at which trimethoprim was sorbed drastically decreased at 2.5 hours and by 5 hours of contact time appeared to have reached equilibrium.

Qtaitat (2004) also studied trimethoprim sorption to montmorillonite. This study found that sorption was dominated by cation-exchange mechanisms at low pH and by physical attractions at high pH. At pH 7.5, much more trimethoprim was sorbed at all

concentrations than at pH 2.0. These findings are similar to those of Bekçi *et al.* (2006), where more trimethoprim at pH 7.5 was sorbed to montmorillonite KSF than at pH less than 2.5.

In the environmental impact analysis report for the approval of Romet ® 30 and Rofenaid ® 40, the authors found that when 0.08% Rofenaid ® 40 was applied in duck feed, concentrations of SDM and OMP in feces reached 34 and 30 ppm, respectively (FDA, 1984). Several environmental analyses were performed including leaching studies, stability studies, and environmental sampling studies. The leaching studies indicated that OMP sorbed more strongly than SDM and the three soils could hold a maximum of 345 and 275 $mg \cdot ft^{-3}$ of OMP and SDM, respectively. Additionally, OMP appeared to be more stable in feces, soil-feces, and water-feces than SDM. In all media, SDM was removed after 40 days but OMP remained detectable even at 55 days. The authors concluded that the environmental concentrations of SDM and OMP remaining after degradation and dilution were low ($< 1\ \mu g \cdot L^{-1}$) and did not present a significant environmental threat. However, concentrations in the ppb range have more recently been detected and found to potentially pose ecological threats (Boxall *et al.*, 2003a).

Transport

Once antimicrobials are applied soils, they can be transported to surface waters by surface runoff or can leach through the soil to groundwater. Additionally, when applied to aquaculture ponds or discharged in wastewater effluents, they may reach sediment bottoms and continue to leach into groundwater. The transport of antimicrobials, particularly those having relatively low sorption potential such as the sulfonamides and diaminopyrimidies, is of particular importance in their environmental fate.

Antibiotic transport via runoff and soil loss was studied by Davis *et al.* (2006). Seven veterinary antimicrobials were applied to a bare, tilled, sandy clay loam soil in Fort Collins, Colorado. Two of these antimicrobials were sulfonamides, sulfathiazole and sulfamethazine. After application, three rainfall events were simulated and the concentrations of the antimicrobials in the water runoff and the sediment runoff were measured. The two sulfonamides along with monensin had the lowest relative losses associated with sediment and the highest concentrations found in the runoff water. Other antimicrobials which sorbed more strongly indicated that traditional soil erosion practices would aid in controlling loss of those antimicrobials from fields. The sulfonamides and monensin, however, would not benefit from these practices as they are not tightly bound by the soil.

Kreuzig *et al.* (2005) showed similar findings of sulfonamide runoff from manured, irrigated soils. The sulfonamide runoff reached up to 28% of the amount initially applied, but the emissions by means of sediment were much lower at only 0.02%. The authors found that soil cultivation would reduce both the runoff volume and sulfonamide concentrations.

Kay *et al.* (2005b) also found sulfonamides in runoff. However, contrary to Kreuzig *et al.* (2005), these authors found higher runoff concentrations in disturbed soil (703 $\mu g \cdot L^{-1}$) than in undisturbed soil (15.5 $\mu g \cdot L^{-1}$) when 25.58 $mg \cdot L^{-1}$ was initially applied via manure. Previously, Kay *et al.* (2004) found that when the sulfonamide, sulfachloropyridazine, was applied to soil in pig slurry for two years, concentrations up to 365 $\mu g \cdot kg^{-1}$ were detected in soil. Additionally, a maximum concentration 613 $\mu g \cdot L^{-1}$ was detected in drain flow, but mass losses to receiving water bodies was less than 0.5%.

Kay *et al.* (2005a) continued their leaching study by investigating the transport of sulfachloropyridazine, oxytetracycline, and tylosin in laboratory soil columns. Again, 25.58 $mg \cdot L^{-1}$ of sulfachloropyridazine in slurry was applied to the top of both undisturbed and disturbed soil columns. The leachate was measured for a period of 64 days after application during which the undisturbed columns contained high effluent concentrations of sulfonamide (mean 1264 $\mu g \cdot L^{-1}$). The disturbed column illustrated no mass loss in the column effluent while the undisturbed column had a 56%. Contrary to what one might think, the concentration of sulfonamide remaining in the disturbed column was much less than that in the undisturbed column. The authors attributed this to increased exposure to degrading microorganisms in the disturbed soil versus the undisturbed soil.

The study by Boxall *et al.* (2002) not only found that sorption coefficients for sulfonamides were low, but also that concentrations up to 590 $\mu g \cdot L^{-1}$ were observed in drain flow. On the other hand, the authors performed a leaching study in a sandy soil where they concluded that the sulfonamides had a low potential to leach into ground water.

To date, little information is available on the transport of antimicrobials in soils, particularly for SDM and OMP. Additionally, more realistic solute transport information can be gained from miscible displacement soil column experiments than the leaching studies presented thus far or from traditional batch sorption experiments. Miscible displacement is a well-established technique for studying solute transport in the subsurface environment, but has not frequently been employed for antimicrobial transport. These soil column leaching studies allow estimation of important solute transport parameters (e.g., diffusion-dispersion coefficient, retardation factor, distribution

coefficient; described later). Miscible displacement column studies have frequently been used to assess possible contamination of soil and groundwater (Porro and Wierenga, 1993; Singh and Kanwar, 1991; Tipton *et al.,* 2003). Casey *et al.* (2003) successfully used miscible displacement transport studies to evaluate 17β-Estadiol in soil-water systems, and Rabølle and Spliid (2000) used it to evaluate the transport of four antibiotics.

Theory of Solute Transport

The one-dimensional convection-dispersion transport equation (CDE) for reactive solutes under steady state flow in a homogenous soil is given by

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \mu_l C - \mu_s \frac{\rho_b}{\theta} S \qquad (2.2)$$

where, R, is a dimensionless retardation factor, C is the solute concentration in the liquid phase ($M \cdot L^{-3}$), t is time (T), D is the solute dispersion coefficient ($L^2 \cdot T^{-1}$), x is the distance in the direction of flow (L), v is the average pore velocity ($L \cdot T^{-1}$), μ_l and μ_s are the liquid and solid first order decay coefficients (T^{-1}), ρ_b is the dry bulk density of the soil ($M \cdot L^{-3}$) θ is the volumetric water content ($L^3 \cdot L^{-3}$), and S is the mass of solute in the sorbed phase per mass of solid ($M^3 \cdot M^{-3}$) The retardation factor is written as

$$R = 1 + \frac{\rho_b}{\theta}\frac{\partial S}{\partial C} \qquad (2.3)$$

where $\delta S/\delta C$ is the slope of the sorption isotherm. For a linear sorption isotherm the slope is represented by K_d , the sorption coefficient ($L^3 \cdot M^{-1}$).

Non-reactive solutes such as bromide are not retarded in the soil, R=1, and the decay coefficients are zero (Casey *et al.,* 2003). Reactive solutes (ie. antimicrobials) are often modeled by chemical nonequilibrium transport where sorption is described by two

sites ("type-1" and "type-2" sorption sites) (Selim *et al.,* 1976, 1977). In a two-site model, type-1 sorption sites, S^e, are subject to instantaneous sorption, while type-2 sorption sites, S^k, obey a kinetic rate law (Skaggs and Leij, 2002).

$$S = S^e + S^k \tag{2.4}$$

Using a linear equilibrium sorption isotherm, S^e, is represented by

$$S^e = K_d C \tag{2.5}$$

and the type-2 sorption sites are given by

$$\frac{\partial S^k}{\partial t} = \alpha[(1-f)K_d C - S_k] - \mu_{s,k} S_k \tag{2.6}$$

where α is a first-order kinetic sorption coefficient (T^{-1}), f is the fraction of exchange sites always in equilibrium with the solution phase, and $\mu_{s,k}$ is the solid phase first order kinetic decay coefficient (T^{-1}). The concept of two-site sorption is implemented in the CXTFIT (Toride *et al.,* 1999) model, which can analyze experimental data and estimate transport parameters. For nonequilibrium transport of reactive solutes it useful to simultaneously observe the mobility of the reactive solute as well as a nonreactive solute. Parameters, such as the dispersion coefficient, D, should presumptively be equal in a given media for the nonreactive and reactive solute (Toride *et al.,* 1999). Therefore, the dispersion coefficient, D, that is estimated for the nonreactive solute can be used with the reactive solute to provide a more reliable estimation of additional transport parameters.

Degradation/ Transformation

Antimicrobials may undergo degradation and/or transformation processes once released into the environment. These may include photodegration, biodegradation, chemical transformation, and hydrolysis. Conflicting information on the degradation of

sulfonamides exists in the literature. Halling-Sørensen *et al.* (2003) found that the sulfonamide, sulfadiazine remained unaffected under aerobic conditions in pure water in the dark as well as pure water exposed to light. A 10-hour exposure to activated sludge, however, reduced the concentration by nearly 19%. Thiele-Bruhn *et al.* (2003) found that SDM underwent a slight photodegradation during 28 days of exposure to light; however, in soils under typical field conditions, photodegradation for sulfonamides was found to be negligible as compared to other environmental processes. The environmental impact analysis report of SDM and OMP indicates that both compounds are stable in the presence of light as well as for long storage times (FDA, 1984).

Guerard and Chin (2006), on the other hand, found SDM to degrade in the presence of light in pure water and was enhanced by the presence of fulvic acid and iron. Boreen *et al.* (2005) investigated the photodegradation rates of five sulfonamide antimicrobials. All of the sulfonamides, except SDM, illustrated increased degradation in the presence of dissolved organic matter. The authors estimated a maximum half life for SDM to be only about 420 hours.

Wang *et al.* (2006b) found that no significant degradation of SDM was observed in sterilized soil over a period of 60 days. A remarkably faster degradation rate was experienced in the non-sterile soil, indicating that microorganisms are responsible for the majority of SDM degradation in soil. Wang *et al.* (2006a) also found that SDM did not illustrate significant degradation in sterilized acidified manure. However, the sterile non-acidified manure did undergo a chemical degradation which obeyed the first order kinetic model. Overall, the degradation in non-sterile manure was much greater than that in

sterile manure, indicating that microbes may play an important role in eliminating antimicrobials in manure.

Bakal and Stoskopf (2001) concluded that the half-lives of SDM and OMP in the aquatic environment must exceed one year even in the presence of salinities up to 30 ppt and in a pH range of 2 to 12. On the contrary, Capone *et al.* (1996) found that SDM and OMP appeared to be very short-lived in marine sediments and were only detected 2 days after the cessation of their use. The limits of analytical detection, however, were high (125-250 $\mu g \cdot L^{-1}$) relative to current practices. Additionally, Capone *et al.* (1996) did not test the surrounding water for antimicrobial contamination, so even though the antimicrobial may be short-lived in sediments, it cannot be concluded that it degraded.

SDM is possibly demethylated in sediments (Thiele-Bruhn, 2003) or even biodegraded to an N_4-acetyl metabolite (Kishida and Furusawa, 2004). One potential concern for degradation products is that they may contain antimicrobial activity or microbial toxicity (Thiele-Bruhn, 2003). Additionally, some antimicrobials are excreted as metabolites, but under environmental conditions are actually transformed back to their original compound (Göbel *et al.,* 2007). It is clear from the literature that degradation and degradation products play an important role in the fate of antimicrobials in the environment.

Effects

The effects of unwanted antimicrobials in the environment are not fully understood or studied. A few studies comment on sulfonamide potencies and effects to microorganisms. For example, Halling-Sørensen *et al.* (2003) found that although the concentration of the sulfonamide, sulfadiazine, was not affected in aerobic conditions

exposed to light and dark conditions, the potency was affected. In fact, these authors found that the potency was reduced by 56% under dark conditions and by nearly 40% in the light. When exposed to activated sludge, sulfadiazine was reduced in concentration by 18.6% and potency by 5.3%. Similarly, Halling-Sørensen and Ingerslev (2000) found that microbial growth was not inhibited when in the presence of multiple sulfonamides.

Information on the toxicity of antimicrobials to a variety of test organisms is generally available from the risk assessment data when the antimicrobial is approved (Boxall *et al.,* 2003a). However, data about soil-dwelling organisms is very limited. Only a few studies have assessed effects of antimicrobials on the structure or function of soil microbial communities. They show that antimicrobials can decrease soil respiration and dimethyl sulfoxide reduction, inhibit the growth of soil microorganisms, inhibit dung decomposition within soil, and cause persistent changes in microbial diversity and community structure (Westergaard *et al.,* 2001). Although several studies have found antimicrobial resistant bacteria in the environment when antimicrobials are present (Costanzo *et al.,* 2005; Oliveira *et al.,* 2006; Hamscher *et al.,* 2004), no general conclusions about environmental exposure of antimicrobials and resistant bacteria can be drawn at this time (Hamscher *et al.,* 2004). However, because antimicrobials are highly bioactive and potentially toxic to microorganisms at low concentrations (Wollenberger *et al.,* 2000), important microbial mediated ecosystem functions may be affected by their presence. Further studies are needed to assess the potentially chronic effects from low-level exposures over a long period of time as well as ecosystem effects.

CHAPTER 3 - SORPTION OF THE ANTIMICROBIALS SULFADIMETHOXINE AND ORMETOPRIM IN SOIL

Introduction

Antimicrobials belong to a class of pharmaceuticals used for therapeutic purposes in human and veterinary medicine, and also for nontherapeutic purposes such as growth promotion in farm animals (Mellon *et al.*, 2001; Halling-Sørensen *et al.*, 2002). In animal agriculture, antimicrobials are often administered in medicated feeds such as Romet® 30 for aquaculture and Rofenaid® 40 for poultry. Because antimicrobials are designed to be completely excreted from the body after relatively short residence times, a significant fraction of the total amount administered is discharged in urine and feces and ultimately enters the environment (Thiele-Bruhn, 2003).

Romet® 30 and Rofenaid® 40 consist of two veterinary antimicrobials, sulfadimethoxine (SDM) and ormetoprim (OMP). These antimicrobials typically enter the environment via land application of stored poultry waste or aquaculture ponds from fish production (Jørgensen and Halling-Sørensen, 2000; Halling-Sørensen *et al.*, 2002). Once in the environment, they may leach into the soil, sorb to the soil and sediment, be transported to ground or surface waters, or be degraded (Halling-Sørensen *et al.*, 2002; Boxall *et al.*, 2003b; Hirsch *et al.*, 1999). Concentrations of SDM and OMP up to 36 and 12 $\mu g \cdot L^{-1}$, respectively, have been detected in pond water (Dietze *et al.*, 2005), while

sulfonamide concentrations in animal slurry have been detected up to 12 mg/kg (Haller *et al.*, 2002).

The poultry and aquaculture industries are very important to the southeastern United States economy and, more importantly, to the nation's food supply. These industries must rely on modern medicinal practices to maintain healthy animals as well as to prevent widespread illness. It is estimated that nearly 38,000 pounds of Romet® 30 (NAA, 2005) and more than 160,000 pounds of Rofenaid® 40 (FDA, 1984) are used per year, leaving possible residues in the environment. It is impractical to think that the use of antimicrobials in animal agriculture would cease; however, in order to develop management practices to reduce their potential ecological risks, sound information is needed on their fate in the environment. This information can partially be provided by understanding antimicrobial sorption in soils. By quantifying the sorption capacity of antimicrobials in various soils, adequate predictions can be made about their transport potential in the aquatic environment (Hamscher *et al.* 2004) as well as their bioavailability to microorganisms. The objective of this study was, therefore, to understand the sorption characteristics of SDM and OMP, two antimicrobials commonly used in aquaculture and poultry industries, in sand and two soils from the southeastern United States.

Materials and Methods

Selected Soils

Two soils representative of agriculture and aquaculture in the southeastern United States were chosen for this experiment. One, a Coastal Plain soil (Soil 1), was collected from Geneva County, AL and the other, a Tennessee Valley soil (Soil 2), was collected

from Sevier County, TN. Because southeastern U.S. soils are generally sandy, pure sand, Ottawa 4.0 was also used to understand the sorption by sand alone (i.e., no clay minerals or organic matter). Antimicrobial sorption will at a minimum be affected by clay and organic matter content, cation exchange capacity and pH; therefore, the selected soils represent several of the physical and chemical characteristics commonly found in the southeastern U.S. Major soil physical and chemical properties (Table 3.1) were determined at the Soil Testing Laboratory at Auburn University. Both soils were air-dried, ground, and sieved (≤ 2mm diameter). The sand was muffled at 550°C for four hours to eliminate any possible organic matter. Following this, the sand and soils were irradiated using a ^{60}Co source at 5MRads to eliminate microorganisms that could potentially biodegrade the antimicrobials. A microbial plate count with ½ strength nutrient agar (4 g nutrient broth/ L) of the two soils and sand confirmed that radiation had eliminated all of the bacteria (0 colonies on plate after 14 days). ^{60}Co irradiation is highly effective as a soil sterilization method, causing minimal changes to soil physical and chemical properties especially in oven dried soil (McNamara *et al.*, 2003).

Table 3.1. Physical and chemical properties of the selected soils and sand

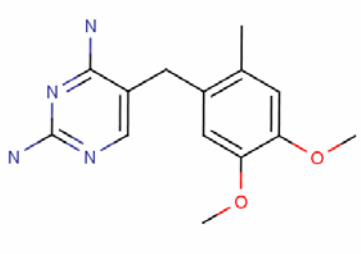
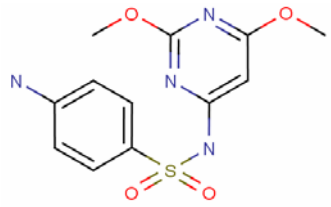
Soil Acronym	Description	Order	pH	%Organic Matter	%Sand	%Silt	%Clay	eCEC* ($cmol_c/kg$)
Soil 1	Plintic Kandiudults	Ultisol	5.03	1.5	81.5	13.5	5	3.19
Soil 2	Typic Eutorchrepts	Inceptisol	4.66	2.07	52	38	10	6.64
Sand	Ottawa 4.0	---	4.92	0	100	0	0	0.33

* eCEC: effective cation exchange capacity

Antimicrobials

The antimicrobials, ormetoprim (OMP; 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl) pyrimidine) and sulfadimethoxine (SDM; N'-(2,6 Dimethoxy-4-pyrimidinyl) sulfanilamide), were obtained from Chem Service, Inc., West Chester, PA and Sigma Aldrich, St. Louis, MO, respectively. Molecular structures and selected physiochemical properties of SDM and OMP are shown in Table 3.2. For the development of the stock solutions, both antimicrobials were initially dissolved in methanol (HPLC grade, obtained from Fisher Scientific International, Inc., Hampton, NH) such that the final working solutions contained less than 0.2% methanol.

Table 3.2. Physiochemical characteristics of Ormetoprim and Sulfadimethoxine

Antimicrobial	Key Properties
Ormetoprim (OMP)* 	*Structure Class:* Diaminopyrimidine *Molecular Formula:* $C_{14}H_{18}N_4O_2$ *Molecular Weight:* 274.32 *Water Solubility:* 1540 mg·L^{-1} *log K_{ow}* 1.23 *Weak base* *K_H:* 4.45E-13 atm-m^3·mol^{-1} *Vapor Pressure:* 2.28E-8 mm Hg *Atm OH Rate Const:* 6.34E-11 cm^3·molecule-sec^{-1}
Sulfadimethoxine (SDM)* 	*Structure Class:* Sulfonamide *Molecular Formula:* $C_{12}H_{14}N_4O_4S$ *Molecular Weight:* 310.33 *Water solubility:* 343 mg·L^{-1} *log K_{ow}:* 1.63 *pK_{a1}/pK_{a2}:* 2.4/6.0 *K_H:* 1.3E-14 atm-m^3·mol^{-1} *Vapor Pressure:* 1.59E-9 mm Hg *Atm OH Rate Const:* 2.02E-10 cm^3·molecule-sec^{-1}

*Structures and Physiochemical properties retrieved from NLM. 2006. ChemIDplus Lite.

Batch Kinetic Experiments

In order to determine the time for SDM and OMP to reach sorption equilibrium (dC/dt = 0) in Sand, Soil 1, and Soil 2, batch kinetic experiments were conducted at the natural, unaltered pH of the media. Preliminary soil/solution ratio experiments were performed and the optimum ratio for all combinations was determined to be 1:20 (soil:solution). Antimicrobials were administered to a working solution of 0.01 M $CaCl_2$ in deionized water. A 100 $\mu g \cdot L^{-1}$ concentration was chosen for these experiments because it is approximately the midpoint concentration for batch equilibrium experiments. One gram of each soil or sand was weighed and placed into a 50 mL polypropylene centrifuge tube, the sorbate (OMP or SDM, 20 mL) was added, and the suspension was placed on a reciprocating shaker for a specified time at 25°C. Duplicate samples were removed from the shaker after 1, 2, 5, 8, 16, 24, 39, 48, and 68 hours, respectivley. Additionally, duplicate controls were run for each time slot (no sand or soil; solution only). Although photodegradation has been found to be negligible for sulfonamides (Thiele- Bruhn *et al.*, 2003; FDA, 1984) and is likely not important for OMP (FDA, 1984), to ensure that photodegradation was not a factor, the experiments were performed in dark by covering the tubes with aluminum foil. After the specified times, samples were centrifuged at 1200 *g* for 45 minutes. The supernatant was determined to be clear, immediately removed from the soil (sorbent), filtered with a 0.45 µm PTFE membrane filter, placed in 750 µL polypropylene autosampler vials, and acidified with formic acid for sample preservation so that the final amount of acid was less than 1%.

Batch Sorption Equilibrium Experiments

Batch sorption equilibrium experiments were used to determine the sorption of SDM and OMP individually as single solutes and in combination as co-solutes in the selected soils and sand. In other words, three separate experiments were performed in each soil and sand, one with OMP only, one with SDM only, and one with OMP and SDM administered in combination. Concentrations of 5, 10, 50, 100, 250, and 500 $\mu g \cdot L^{-1}$ were employed for sorption isotherms because they equated to the range of antimicrobial concentrations found in the environment (Kolpin *et al.*, 2002; Thurman *et al.*, 2002; Campagnolo *et al.*, 2002; Meyer *et al.*, 2000). Each soil or sand (1 g) was placed into a 50 mL polypropylene centrifuge tube, solution was added (20 mL), and the suspension was equilibrated on a reciprocating shaker in the dark at 25°C. The above procedure was performed in triplicate for each of the six concentration levels. Additionally, duplicate controls for each concentration (no sand or soil; solution only) were run to verify the initial concentration, while a blank (sand or soil, $CaCl_2$ solution only) was analyzed to verify the lack of laboratory contamination. After equilibration, the samples were centrifuged and prepared for analysis using the procedure described in the previous section (Batch Kinetic Experiments).

Chemical Analysis

Antimicrobial samples were analyzed in cooperation with the Food and Drug Laboratory at the Alabama Department of Agriculture and Industries, Montgomery, AL. All samples were stored at 4°C until they were transported on ice (2–4°C) to the Food and Drug Laboratory. The analysis of the extracts utilized a liquid chromatograph triple quadrupole mass spectrometer (LC/MS/MS). A sample run consisted of seven standard

curve solutions followed by an initial calibration verification (ICV) and an initial calibration blank (ICB). This was followed by a repeating sequence of 10 samples and subsequently by a continuing calibration verification (CCV) and continuing calibration blank (CCB).

The analytical method uses a gradient separation with the following solvents: acetonitrile and 0.1% formic acid in water. Analytes were separated chromatographically using a Phenomenex® Gemini C18 column (5 μm, 150mm x 2.00 mm) and samples were analyzed using a Thermo Finnigan TSQ Quantum LC/MS/MS system. Compounds of interest were ionized using atmospheric pressure chemical ionization (APCI) in positive mode generating an M+H ion of the parent compound or electrospray ionization (ESI). A minimum of three daughter ions were generated from the parent compound using selective ion monitoring mode (SIM). Compounds were identified by the presence and ratio of all daughter ions and the retention time of the compound as compared to the calibration standards. Quantification was based on the area of the peak of the most abundant daughter ion of the analyte and regressed against the seven point standard curve. A typical chromatogram for OMP illustrating the parent compound and three most abundant daughter ions is shown in Figure 3.1.

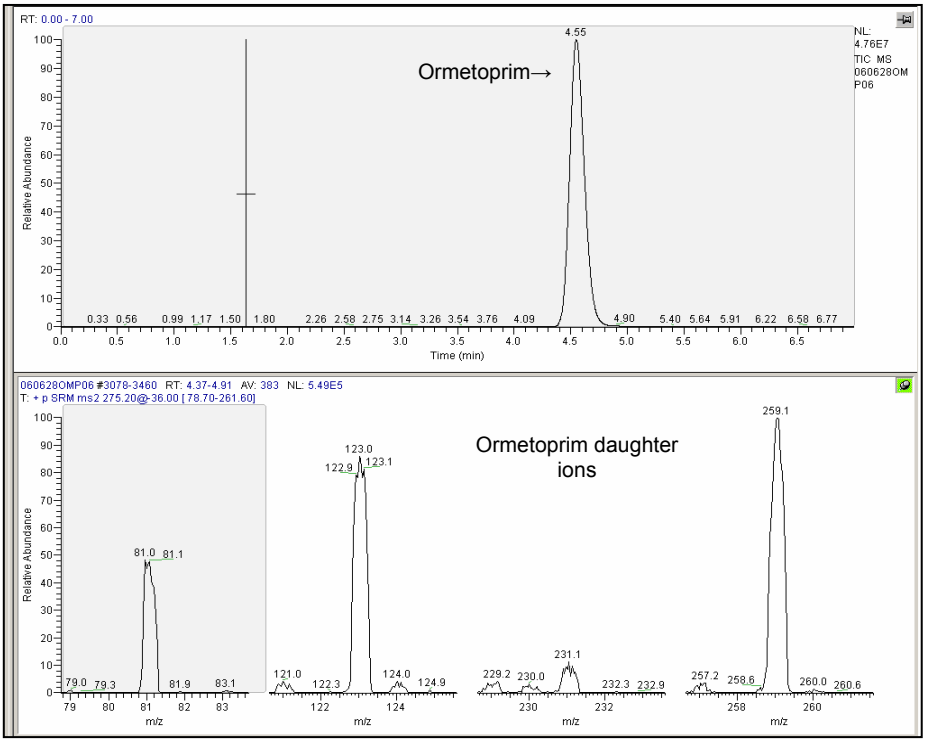


Figure 3.1. Sample OMP Chromatogram with three most abundant daughter ions.

Results and Discussion

Sorption Modeling

Once the amount of antimicrobial remaining in solution after equilibrium was determined, a mass balance was performed to determine the amount of antimicrobial retained by the soil. The sorbed antimicrobial concentration was calculated from the difference between the initial and final antimicrobial concentration. The following equation illustrates the calculation of the concentration of antimicrobial sorbed to the soil, S ($\mu g \cdot g^{-1}$):

$$S = \frac{V_{aq}}{M_s} \cdot (C_i - C) \tag{3.6}$$

where V_{aq} is the initial solution volume (L), M_s is the soil mass (g), C_i is the initial concentration ($\mu g \cdot L^{-1}$), and C is the concentration remaining in solution after equilibrium is reached ($\mu g \cdot L^{-1}$). Several studies and test methods (e.g., Figueroa *et al.*, 2005, Gao *et al.*, 2005, and EPA, 1998) actually use the control concentration to subtract from rather than the initial concentration**.** However, we chose not to do this because it eliminates other possible reactions that could be occurring. This would also bias correct the data.

All results were modeled by both linear and nonlinear Freundlich isotherms. This allowed us to not only fit the data with the best model, but also report a sorption (distribution) coefficient, K_d, from the linear isotherm that could be compared with data from other studies. The linear isotherm is denoted by:

$$S = K_d \cdot C \tag{3.7}$$

where there is a direct linear relationship between the amount sorbed and the amount remaining in the solution. The Freundlich isotherm is represented by:

$$S = K_{Fr} \cdot C^{n} \tag{3.8}$$

where K_{Fr} is the Freundlich sorption coefficient and n is an empirical constant denoting the degree of nonlinearity. It should be noted that if $n = 1$ then $K_{Fr} = K_d$ (Equation 3.7) (Papiernik *et al.*, 2002). Based on other sorption studies of sulfonamides, the linear and Freundlich isotherms adequately fit the sorption data (Thiele-Bruhn *et al.*, 2004; Boxall *et al.*, 2002). Additionally, Thiele-Bruhn (2000) showed that the Freundlich equation rather than the Langmuir equation gave a better fit to the sorption data of sulfonamides.

Since soil organic matter is highly variable among soils, an approach for sorption coefficients accounting for the organic carbon (OC) was used. This carbon-normalized sorption coefficient (K_{oc}, ($L \cdot kg^{-1}$)) reduces the variability in sorption data among soils for a particular compound (Tolls, 2001). The carbon-normalized sorption coefficient is given by

$$K_{oc} = \frac{K_d}{f_{oc}} \tag{3.9}$$

where f_{oc} is the organic carbon fraction ($g \cdot g^{-1}$) of the soil. The f_{oc} was estimated by assuming soil organic matter (SOM) to have an OC content of about 58% (i.e., SOM*0.58) (Sylvia *et al.*, 2005; Cunningham, 2004).

Karickhoff (1981) suggested an equation to predict the carbon normalized sorption coefficient of an organic compound in instances where laboratory sorption studies have not been performed. The predicted carbon normalized sorption coefficient (K'_{oc}, ($L \cdot kg^{-1}$)) is estimated based on the octanol water partition coefficient (K_{ow}) of each antimicrobial. The K'_{oc}, is given by

$$\log K'_{oc} = 0.989 \cdot \log K_{ow} - 0.346 \tag{3.10}$$

which is an empirical equation based on a study of multiple organic pollutants in sediments and soils (Karickhoff, 1981). The correlation between K_{oc} and K_{ow} is attributed to the solute solubility being the main determinant in both K_{oc} and K_{ow} values (Chiou, 2002). Using K_{ow}, an easily obtainable physiochemical property, to estimate K_{oc} has successfully been used in many pesticides and industrial chemicals, although it has been shown to underestimate K_{oc} values for antimicrobials (Tolls, 2001; Boxall *et al.*, 2004). Possible explanations of this underestimation include differences in binding mechanisms between antimicrobials and other organic chemicals or the dissociation of antimicrobials in pH dependent environmental conditions.

Kinetics

The results for the kinetic experiments to determine the time when sorption equilibrium was approached are shown as a fraction of the initial concentration remaining in solution versus time for OMP (Figure 3.2) and SDM (Figure 3.3). Although apparent equilibrium was reached for both SDM and OMP in all three media after 24 hours, 48 hours was chosen as the equilibrium time for both antimicrobials. For OMP less than a 5% decrease in relative concentration was observed from 16 hours to 68 hours. Similarly, less than a 5% decrease in SDM relative concentration was observed from 39 hours to 68 hours. All kinetic experiments were performed at the natural, unaltered pH of the media.

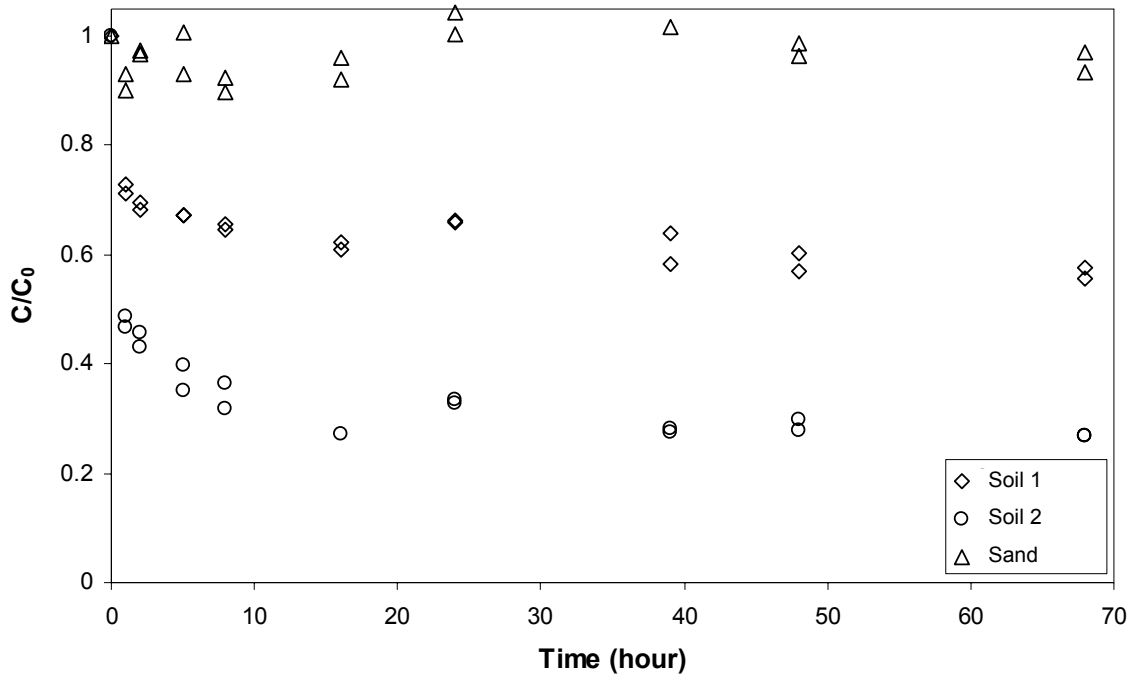


Figure 3.2. Results of OMP kinetic experiments: aqueous fraction as a function of time. Soil:Solution ratio = 1:20. C_0 = 100 μg·L^{-1}. Performed at the natural soil pH.

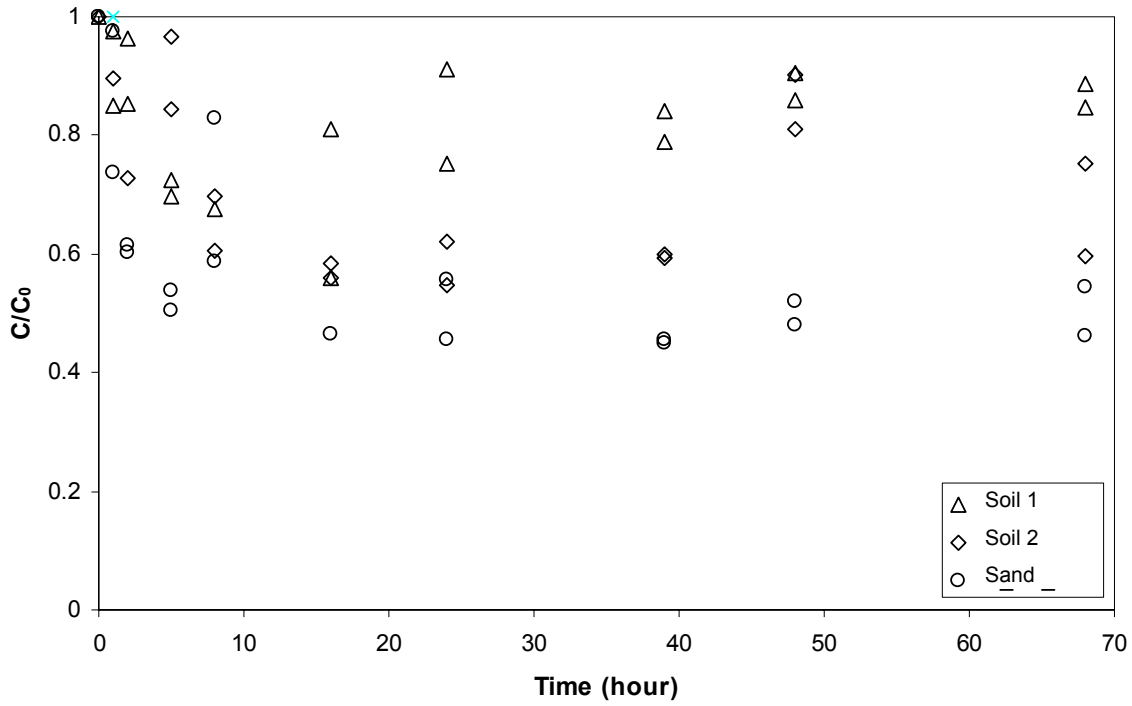


Figure 3.3. Results of SDM kinetic experiments: aqueous fraction as a function of time. Soil:Solution ratio = 1:20. C_0 = 100 μg·L^{-1}. Performed at natural soil pH.

Sorption

OMP and SDM sorbed differently in Sand, Soil 1, and Soil 2 (Table 3.3). All distribution coefficients and Freundlich sorption coefficients were similar to those found for other mobile antimicrobials (Tolls, 2001; Sassman *et al.*, 2005; Thiele-Bruhn, 2003). OMP linear sorption coefficients, K_d, for the single solute and co-solute experiments ranged from 1.3 to 89.7 $L \cdot kg^{-1}$. Similar sorption data in soils have been found for other antimicrobials, such as Tylosin, with sorption coefficients ranging from 8.3 to 128 $L \cdot kg^{-1}$ (Tolls, 2001). Additionally, Halling-Sørensen (2000) found that trimethoprim, another diaminopyrimidine with similar structure and properties to OMP, had a distribution coefficient of 76 $L \cdot kg^{-1}$ in sludge. The trimethoprim distribution coefficient is within the range of the OMP distribution coefficients determined in this study. Research performed on other sulfonamides have determined low sorption coefficients, K_d, in the range of 0.62-10 $L \cdot kg^{-1}$, indicating a relatively high mobility of these compounds (Thiele-Bruhn *et al.*, 2004; Tolls, 2001; Boxall *et al.*, 2002; Díaz-Cruz *et al.*, 2003). The SDM K_d value in Soil 1 is 25.8 $L \cdot kg^{-1}$ which is higher than the K_d values for other sulfonamides in soils reported by Tolls (2001), but more similar to that of Gao and Pedersen (2005) where they found K_d values for sulfonamides in clay minerals up to 22.2 $L \cdot kg^{-1}$. Thiele-Bruhn *et al.* (2003) reported a distribution coefficient of 0.73 $L \cdot kg^{-1}$ for SDM in soil, which is generally lower than the findings presented in this research; however, their goodness of fit (R^2) for the linear isotherm was only 0.34. The distribution coefficients and Freundlich sorption coefficients presented here (Table 3.3) indicate more sorption than was found by Thiele-Bruhn *et al.* (2003) and Thiele-Bruhn (2003). This perhaps can be

attributed to the differences in soil properties such as organic matter content, mineral fraction, cation exchange capacity, and pH.

Because the pK_a values for antimicrobials are often in the range of soil pH values, the antimicrobials may protonate or deprotonate as the pH changes. The aqueous speciation of antimicrobials in various pH environments strongly affects their sorption (Tolls, 2001). SDM, like other sulfonamides, has two ionizable functional groups relevant to the soil pH range, the anilinic amine and the amide moieties (Gao and Pedersen, 2005). The neutral species (SDM^0) dominates between the pK_a and pK_b values (Table 3.2), the cationic species (SDM^+) dominates at pH values below the pK_a, and the anionic species (SDM^-) dominates at pH values above the pK_b. Because soils generally carry a net negative charge, SDM sorption would tend to be greater at low pH values where the cationic species dominates. The cationic species (SDM^+) would have a higher attraction to the negatively charged surface and would therefore exhibit more sorption than either the neutral or the anionic species. The OMP pH dependent sorption would be similar to those found by Bekçi *et al*. (2006) for trimethoprim. These authors found that at low pH, all of trimethoprim was in the protonated form (cationic species), and that at pH values near neutral, the weak base was near its pK_a and was subsequently in is neutral species. More trimethoprim sorption was found between pH 4-6 than above and below these values. The authors found that above pH 6, the neutral species dominated and had little attraction for the negatively charged surface. At low pH the protonated trimethoprim was in competition with the decreasing hydrogen ions in solution and little sorption occurred.

Since pH plays such an important role in sorption it is important to compare sorption data from other studies with caution and to pay particular attention to the soil pH values. In fact, the soil pH in the sorption studies of sulfonamides by Thiele-Bruhn *et al.* (2003) and Thiele-Bruhn (2003) was greater than 7, which is significantly higher than the pH values of the soils presented in this paper. These authors found lower sorption coefficients than observed in this research, which would be expected based on the higher pH.

The carbon normalized sorption coefficient was determined for each antimicrobial in the single solute and co-solute systems for Soil 1 and Soil 2 (Table 3.3). The Tennessee soil sorbed more antimicrobial than the Alabama soil, even when normalized to the organic carbon fraction. The carbon normalized sorption coefficients were nearly twice as much in Soil 2 than in Soil 1 for OMP single solute, OMP co-solute, and SDM single solute. The SDM co-solute K_{oc}, however, only exhibited a 28% increase in Soil 2 compared to Soil 1. The large increase in K_{oc} values between soils either indicates that factors other than soil organic carbon are contributing to the antimicrobial sorption or that the carbon of one soil was more reactive than that of the other soil. Soil 2 has a higher cation exchange capacity, higher clay content, and lower pH than Soil 1. At the lower pH, more of SDM and OMP would be in the cationic species and would likely have a stronger attraction to the negatively charged soil surface.

Each, an increased cation exchange capacity and clay content, and a reduced pH could enhance sorption and appear to be significant based on the dramatic differences in the K_{oc} values of the two soils. Additionally, Sand had a relatively low sorption capacity for either antimicrobial, which indicates that the sand in the soils likely does not play a

large role in sorption. However, to further investigate the sorption of SDM and OMP onto sand, an iron, iron oxide, manganese, and aluminum extraction was performed on the sand. This procedure followed the concerns that after muffling, the sand turned a slightly red color, indicating the presence of iron or manganese. Additionally, Zhang and Huang (2007) and Figueroa and Mackay (2005) found that iron oxides contributed to sorption of other antimicrobials. Results from the extraction procedure, however, revealed that the sand possessed less than 0.01% iron oxide or iron, less than 0.0001% manganese, and no detectable aluminum, leading to the conclusion that these fractions were not likely contributors to the antimicrobial sorption by sand. Sorption of SDM to sand was also noted by Thiele-Bruhn et al. (2004) where SDM and other sulfonamides actually sorbed more to sand than to three other soils investigated.

The predicted carbon normalized sorption coefficients were calculated (Equation 3.10) based on antimicrobial octanol/water partition coefficients (Table 3.2). Tolls (2001) and Boxall *et al.* (2004) have shown that the predicted carbon normalized sorption coefficients based on K_{ow} actually underestimate K_{oc} for antimicrobials, as is the case in this research for OMP and SDM. The predicted carbon normalized sorption coefficient, K'_{oc}, for OMP was calculated to be 7.42 $L \cdot kg^{-1}$, which is much less than the resulting K_{oc} values for OMP (Table 3.3). The SDM predicted carbon normalized sorption coefficient (K'_{oc} = 18.45 $L \cdot kg^{-1}$) also underestimated the actual carbon normalized sorption coefficient. Tolls (2001) suggested that because the normalized-carbon sorption coefficient reduces the variability between soils based on hydrophobic interactions it may not explain the variability for hydrophilic interactions such as those with some antimicrobials. It is also worth noting that the sorption pattern presented by SDM and

OMP is different from traditional hydrophobic compounds in that the more hydrophobic compounds actually have higher sorption coefficients (Weber *et al.,* 2002). Our results indicate that SDM, the more hydrophobic compound, sorbs less than OMP, the more hydrophilic compound.

Table 3.3. Sorption model coefficients for OMP and SDM administered as single solutes and co-solutes in two soils and sand. OMP and SDM administered concentration range, 5-500 $\mu g \cdot L^{-1}$. Performed at the natural, unaltered soil pH.

		Linear parameters			Freundlich parameters		
Sorbent	**Solute**	**K_d [a]**	**R^2**	**K_{oc} [a]**	**K_{Fr} [b]**	**n**	**R^2**
Sand	OMP single solute	1.3 (1.0-1.6)[c]	0.60	--	16.0 (11.3-22.8)	0.58 (0.50-0.67)	0.94
	OMP co-solute	4.96 (4.2-5.7)	0.85	--	12.2 (9.5-15.7)	0.86 (0.79-0.92)	0.98
	SDM single solute	0.4 (0.4-0.5)	0.95	--	3.1 (2.1-4.6)	0.64 (0.52-0.75)	0.98
	SDM co-solute	2.5 (1.2-3.8)	0.72	--	79.8 (31.7-201.0)	0.44 (0.24-0.64)	0.65
Soil 1	OMP single solute	21.9 (19.7-24.1)	0.93	2517.24	47.1 (34.0-65.4)	0.90 (0.80-0.99)	0.96
	OMP co-solute	30.9 (28.4-33.4)	0.95	3551.72	50 (38.4-65.1)	0.93 (0.85-1.01)	0.98
	SDM single solute	10.4 (9.2-11.6)	0.94	1195.40	2.1 (0.9-5.1)	1.32 (1.09-1.55)	0.92
	SDM co-solute	12.5 (10.0-15.0)	0.80	1436.78	134.9 (82.9-219.8)	0.54 (0.41-0.67)	0.89
Soil 2	OMP single solute	58.3 (53.9-62.8)	0.96	4855.91	84.9 (57.8-124.6)	0.97 (0.84-1.10)	0.94
	OMP co-solute	89.7 (84.6-94.9)	0.98	7471.26	115.1 (93.8-141.2)	0.98 (0.90-1.05)	0.98
	SDM single solute	25.8 (23.1-28.5)	0.94	2148.93	14.5 (10.3-20.4)	1.10 (1.01-1.20)	0.98
	SDM co-solute	22.1 (19.7-24.6)	0.94	1840.75	106.8 (80.1-142.5)	0.71 (0.63-0.79)	0.97

[a] K_d , K_{oc} in units $L \cdot kg^{-1}$

[b] K_{Fr} in units $L \cdot kg^{-1}$ if n=1, else in units $\mu g^{1-n} * L^n \cdot kg^{-1}$

[c] Confidence Intervals (95%)

Single Solute Sorption

OMP overall was best fit by the Freundlich equation with all correlation coefficients (R^2) ≥ 0.94 (Figure 3.4); however, the linear isotherm also yielded R^2 ≥ 0.93 for both Soil 1 and Soil 2 but not for Sand, which did not yield a good linear fit for OMP. This was contributed to the low sorption potential of sand with OMP, in which the sand approached a limited number of sorption sites; however, it was not fit well with the Langmuir isotherm (data not shown). SDM also fit reasonably well with the Freundlich equation (R^2 > 0.92) (Figure 3.5), but was perhaps best fit with a linear isotherm for Soil 1 and Soil 2. The Freundlich models for Soil 1 and Soil 2 with SDM yielded slightly unfavorable sorption (n > 1) indicating that these two soils have very little sorption capacity for SDM at low concentrations, but have an increased capacity as the SDM concentration increases. An isotherm of this nature may be classified as an *S-curve* isotherm, as noted by Sposito (1989), where there is an initially small slope followed by a period of increasing sorption. For organic compounds, the *S-curve* is often the result of cooperative interactions among the sorbed molecules where they become more stable on the surface as the concentration increases.

Both SDM and OMP were found to fit better with the Freundlich or linear relationship than by the Langmuir equation. The latter resulted in Langmuir correlation coefficients for SDM (R^2) ≤ 0.33 and OMP (R^2) ≤ 0.42 (data not shown). This is likely because the concentrations used in these experiments were limited to the relatively low concentrations found in the environment. Perhaps at higher concentrations, the soils or sand would reach a maximum number of sorption sites for the OMP and SDM.

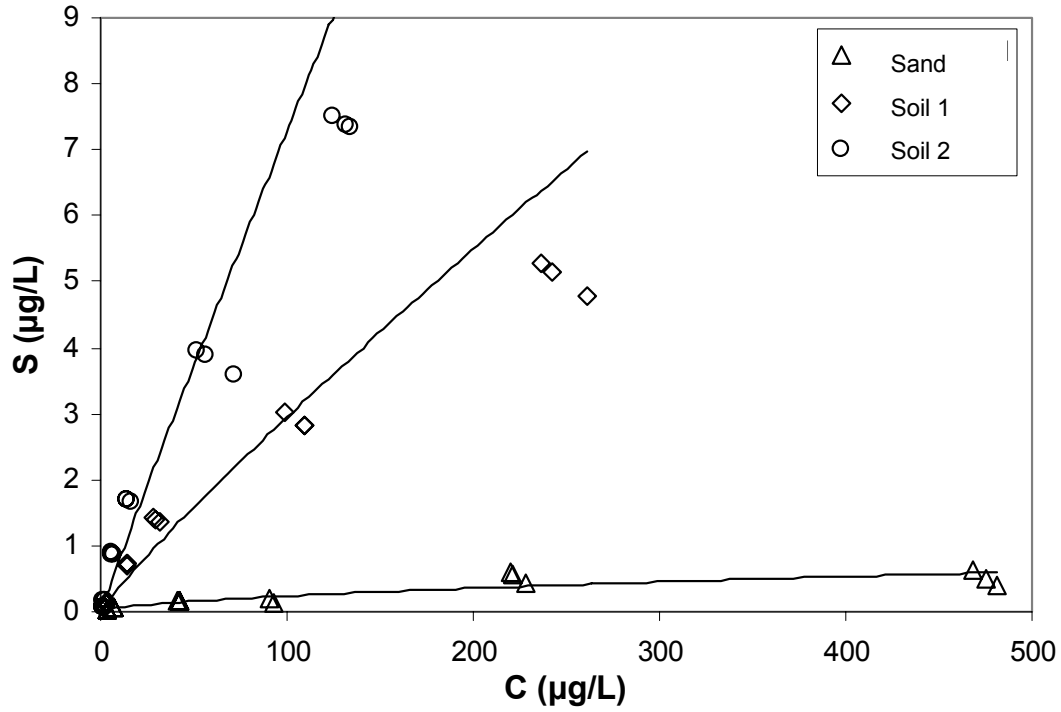


Figure 3.4. Freundlich Sorption Isotherm for OMP administered individually in sand and two soils. Lines denote fitted isotherm and points are the observed data. Performed at the natural, unaltered soil pH.

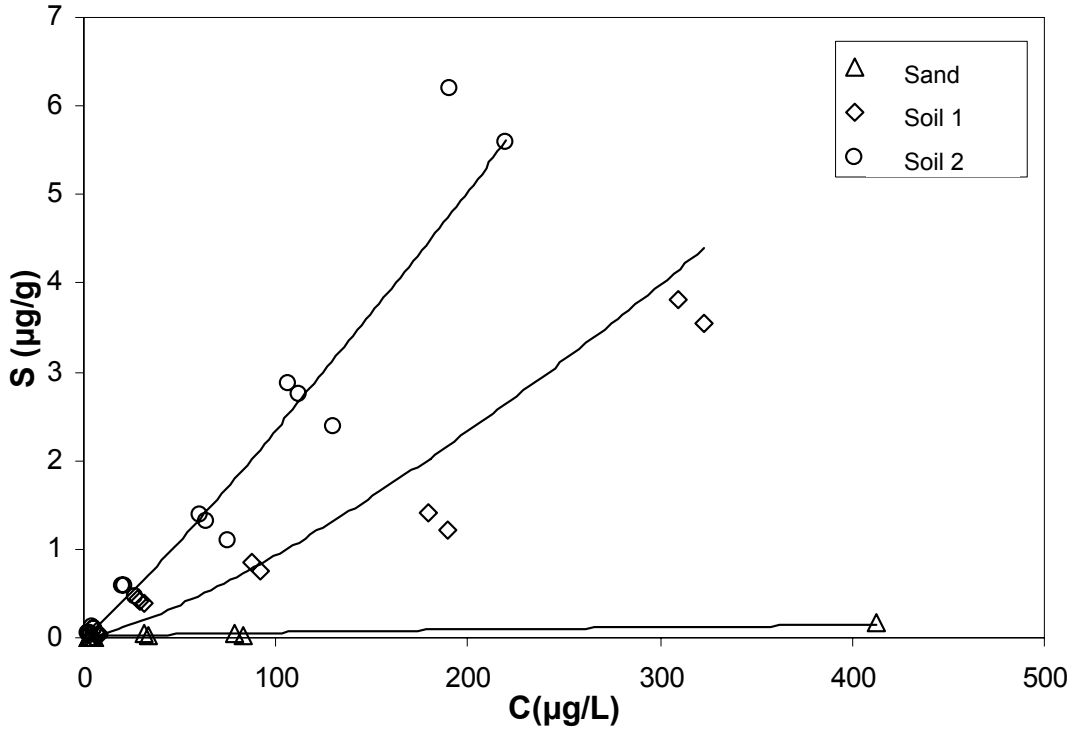


Figure 3.5. Freundlich Sorption Isotherm for SDM administered individually in sand and two soils. Lines denote fitted isotherm and points are the observed data. Experiments were performed at the natural, unaltered soil pH.

Co-solute Sorption

The co-solute batch sorption equilibrium experiments involved the same approach as the single solute systems except in co-solute batch experiments both antimicrobials were administered together. Model results from these experiments are also presented in Table 3.3. SDM and OMP followed the same sequence of sorption for co-solute (Figures 3.6 and 3.7) as for single solute systems, viz., Sand < Soil 1 < Soil 2. This was necessarily expected because it follows the sequence of increasing soil organic matter (SOM), cation exchange capacity, clay content (more surface area for sorption), and decreasing pH (Table 3.2). As stated earlier, the decreasing pH would cause cationic speciation of SDM and OMP, where there would be more affinity for the negatively charged soil surface.

The co-solute sorption of OMP was fit well by both the linear and Freundlich equation (Figure 3.6). However, in the co-solute sorption, the Freundlich nonlinearity coefficient, *n*, is closer to 1 than in the single solute sorption, indicating that sorption for OMP is more linear in the co-solute system. Additionally, by examining Figures 3.8a and 3.8b as well as Table 3.3, it can be noted that more OMP sorption occurs when in combination with SDM than when administered as a single solute. Specifically, as the OMP concentration increases, there is a statistically significant difference between the means of the single solute and co-solute systems for Soil 1 (Figure 3.8a) at concentrations of the initial solution of 250 and 500 $\mu g \cdot L^{-1}$ and for Soil 2 (Figure 3.8b) at initial concentrations greater than 100 $\mu g \cdot L^{-1}$ (one-way ANOVA, $p < 0.05$). The enhanced sorption capacity at the high OMP co-solute concentration level has also been observed in soils for other organics and pharmaceuticals such as 17α-Ethinyl Estadiol and

Naphthalene as well as other polycyclic aromatic hydrocarbons (PAHs) (Bucheli *et al.*, 2000; Yu and Huang, 2005; Weber *et al.*, 2002) where the enhanced sorption is attributed to the swelling of soil organic matter (described later)..

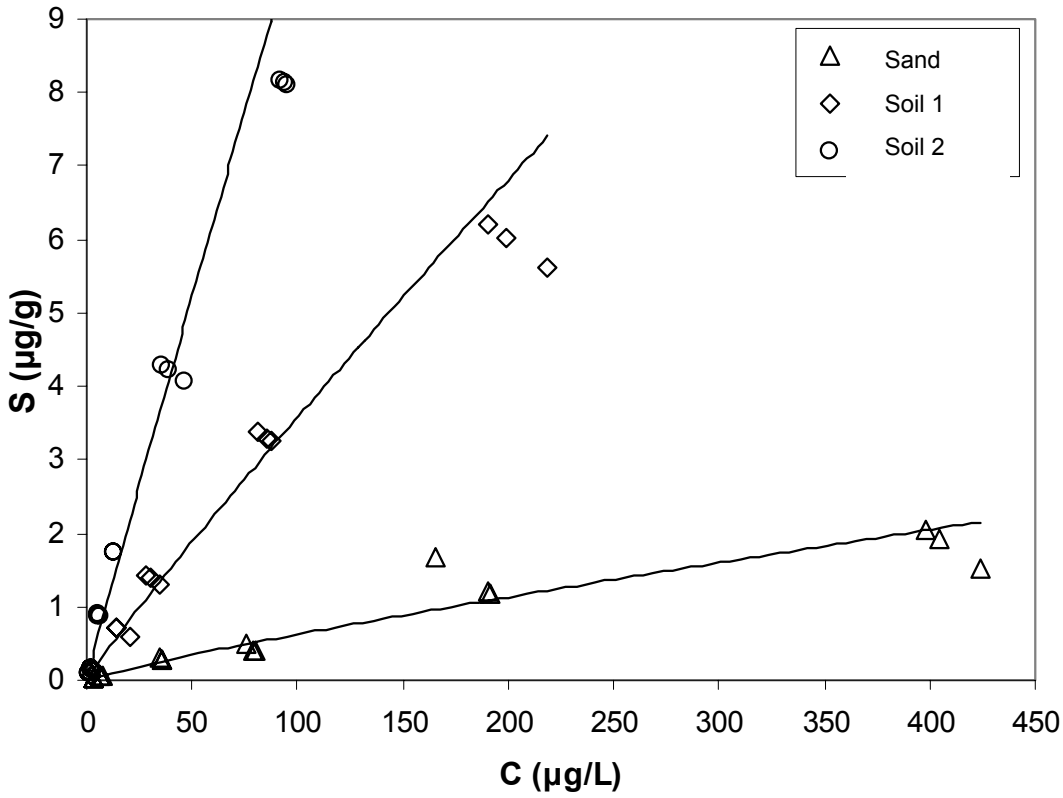


Figure 3.6. Freundlich Sorption Isotherm for OMP administered in combination with SDM in sand and two soils. Lines denote fitted isotherm and points are the observed data. Experiments were performed at the natural, unaltered soil pH.

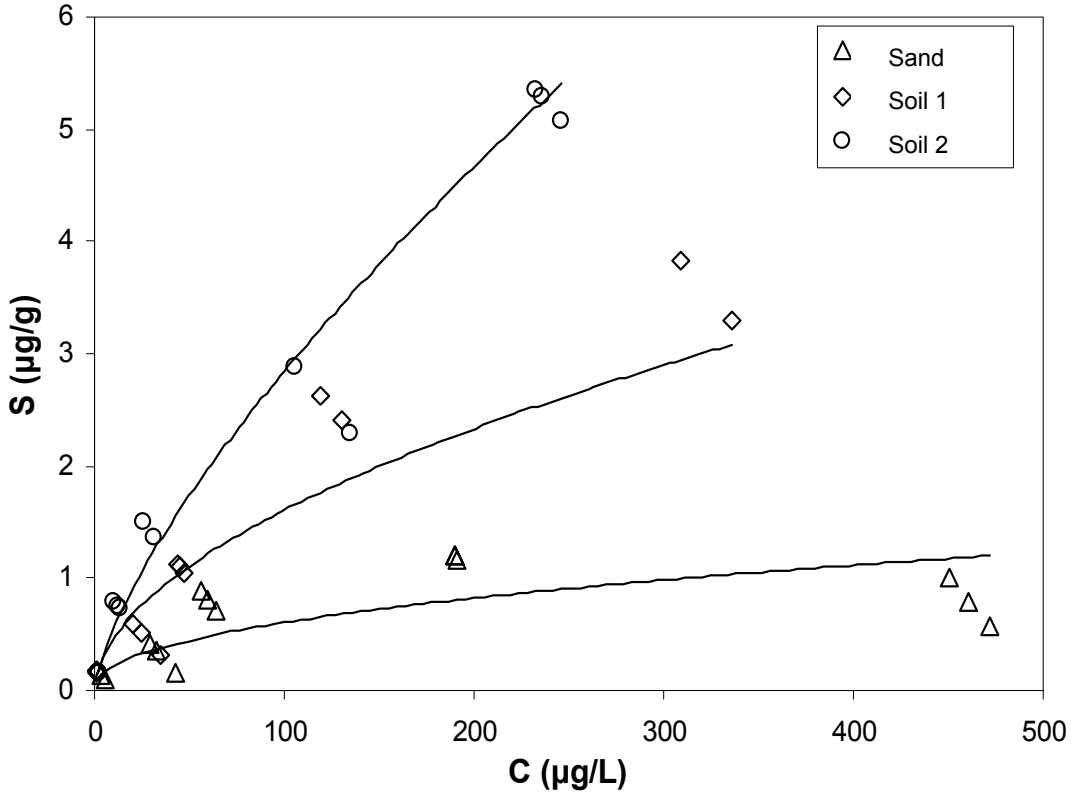


Figure 3.7. Freundlich Sorption Isotherm for SDM administered in combination with OMP in sand and two soils. Lines denote fitted isotherm and points are the observed data. Experiments were performed at the natural, unaltered soil pH.

SDM co-solute sorption is fit best by the Freundlich isotherm (Figure 3.7) and is more nonlinear than the single solute isotherm. At first glance, the SDM sorption in Soil 1 (Figure 3.8c and Table 3.3) appears greater in the co-solute system. However, the linear sorption coefficients, K_d, in the single and co-solute experiments differ only 16.8%. When examining Figure 3.8c it can also be noted that the sorption diverges at the concentration of the initial solutions of 100 and 250 $\mu g \cdot L^{-1}$, but actually converges at the higher concentrations. A similar phenomenon is observed with Soil 2 (Figure 3.8d). There is an initial divergence between the single solute and co-solute SDM at an initial concentration of 50 $\mu g \cdot L^{-1}$ but then a convergence again at higher concentrations. The SDM co-solute sorption in the two soils exhibited favorable sorption ($n < 1$) whereas the SDM single solute experiment exhibited unfavorable sorption ($n > 1$).

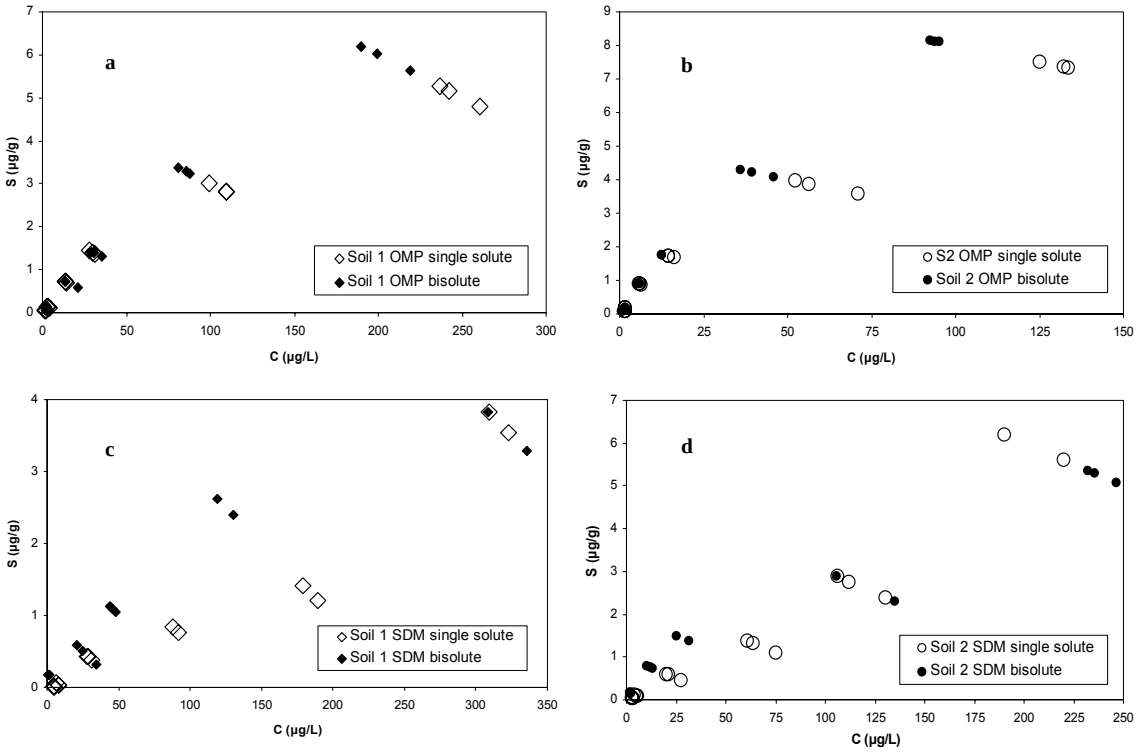


Figure 3.8. Single solute versus co-solute sorption for a) Soil 1 with OMP, b) Soil 2 with OMP, c) Soil 1 with SDM, d) Soil 2 with SDM.

Weber *et al.* (2002) attributed the enhanced sorption in co-solute experiments to the swelling of SOM matrices in the presence of increased solute concentrations of the co-solute. The authors use the analogy of organic matrix swelling at high temperatures and note that in both high temperature and high concentration scenarios there is an energy input increase. This leads to a disordering of the SOM matrix, which in turn can sorb more of one or both solutes. Perhaps this is the case for OMP in combination with SDM. As the combined solute concentration increases, the SOM matrices swells, allowing enhanced sorption of OMP. This phenomenon could also be occurring for the SDM in the presence of OMP.

It is worth noting that many environmentally relevant antimicrobial concentrations are less than 10 $\mu g \cdot L^{-1}$ and in this range for SDM and OMP there is very little difference between the single solute and co-solute sorption. Nonlinearity has been observed at low concentrations.

Summary and Conclusions

Sorption of OMP and SDM in this study followed the sorption trend of Sand < Soil 1 < Soil 2. This was expected due to the increasing SOM. The relatively low sorption coefficients for SDM and OMP found in this study suggests that both of these antimicrobials will be relatively mobile in soils and have potential to reach surface and groundwaters. In fact, sulfonamides and diaminopyrimidines have been detected in pond water, streams, and/or groundwater by several researchers (Dietze *et al.*, 2005; Koplin *et al.,* 2002; Haggard *et al.*, 2006; Metcalfe *et al.,* 2003; Holm *et al.*, 1995). Because antimicrobials are highly bioactive and potentially toxic to microorganisms at low concentrations (Wollenberger *et al.,* 2000), important microbial mediated ecosystem

functions may be affected by their presence in soil water and/or surface and ground water.

The presence of both SDM and OMP in combination illustrated an enhanced sorption of OMP and a more favorable sorption of SDM ($n < 1$). However, in the low environmentally relevant concentrations, there does not appear to be much difference in the single solute and co-solute experiments. These compounds are often administered in combination (e.g. Romet® 30 and Rofenaid® 40) where the co-solute fate, transport, and effects would dominate. FDA (1984) found that when OMP and SDM are combined they are more effective than when alone. Therefore, fate, transport, and ecotoxity studies should therefore consider competitive or enhanced effects of SDM and OMP in combination.

The results of this study further suggest that more attention be given to predicting antimicrobial sorption than simply relying on the organic carbon sorption predictions alone. For OMP these predictions were shown to underestimate the carbon normalized sorption coefficient. This study reveals that OMP sorbed more strongly in the soils and sand than SDM, but both antimicrobials are likely to be relatively mobile and may be found in nearby water sources. Further research is needed to investigate the potential ecological implications of their presence in soil and water.

CHAPTER 4 - MISCIBLE DISPLACEMENT COLUMN STUDIES TO EVALUATE THE SORPTION AND MOBILITY OF SULFADIMETHOXINE AND ORMETOPRIM IN SOIL

Introduction

Antimicrobials are introduced into the environment primarily through discharges in human and animal waste. These compounds are designed to be toxic at low concentrations; however, their bioactivity may remain after excretion, causing potentially harmful ecosystem effects (Wollenberger *et al.,* 2000). Several studies have found bacterial resistant genes in the environment from overexposure to antimicrobials (e.g., Costanzo *et al.,* 2005; Oliveira *et al.,* 2006; and Hamscher *et al.,* 2004). In addition to the development of antimicrobial resistance, these bacterial static or bactericidal compounds may alter microbial community structure and inhibit the growth of important microorganisms (Westergaard *et al.,* 2001), thereby affecting essential microbial mediated ecosystem functions.

One target route of antimicrobial contamination is animal husbandry. Current agriculture practices require the use of confined animal feeding operations (CAFOs) where it is often necessary to provide antimicrobials for treatment and prevention of disease. In animal agriculture, antimicrobials may make their way into the environment through land applied animal waste used as organic fertilizers or aquaculture ponds where medicated feed is applied in the water. The poultry and aquaculture industires both rely

on antimicrobials, such as sulfadimethoxine (SDM) and ormetoprim (OMP) to maintain healthy animals. SDM and OMP are sold in combination as the U.S. Food and Drug Administration (FDA) approved drugs Romet® 30 and Rofenaid® 40 for aquaculture and poultry, respectively. Recent antimicrobial environmental occurrence studies have detected SDM and OMP in pond water and several others have detected SDM in receiving waters from agricultural sources. Dietze *et al.* (2005) detected both SDM (maximum 36 $\mu g \cdot L^{-1}$) and OMP (maximum 12 $\mu g \cdot L^{-1}$) in pond water where Romet® 30 had been applied. Another pond analysis found SDM in more than 25% of the water samples at concentrations up to 0.88 $\mu g \cdot L^{-1}$, but only found sediment contamination in less than 8% of the samples (7.7 $\mu g \cdot L^{-1}$) (Hamscher *et al.*, 2006). The United States Geological Survey (USGS) found multiple antimicrobial contaminants in streams across the U.S., including low concentrations of SDM (0.06 $\mu g \cdot L^{-1}$) (Kolpin *et al.,* 2002). Poultry farms were also shown to contribute to stream surface contamination in a study by Campagnolo *et. al.* (2002) who found SDM in receiving waters proximal to poultry farms. Other studies (e.g. Kreuzig *et al.*, 2005) have suggested high sulfonamide concentrations, up to 703 $\mu g \cdot L^{-1}$, in runoff from agriculture fields, indicating that land applied antimicrobials are often at risk for surface transport. Limited studies have assessed the occurrences of SDM and OMP in groundwater, but a few have found SDM and other sulfonamides in groundwater at environmentally relevant concentrations (Batt *et al.*, 2006; Holm *et al.*, 1995).

A thorough understanding of antimicrobial fate and transport in the environment is necessary for adequate environmental risk assessments of these compounds. Inadequate knowledge, however, is available on SDM and OMP fate and transport in

soils. The Environmental Impact Analysis Report for the approval of Romet ® 30 is, to date, the only known study to evaluate the sorption and mobility of both, SDM and OMP (FDA, 1984). The authors, however, did not evaluate necessary fate and transport parameters, nor did they possess the analytical capabilities to analyze environmentally relevant concentrations less than 0.05 $mg \cdot L^{-1}$. More specific fate and transport information than given by FDA (1984) is necessary to determine adequate predicted environmental concentrations (PEC).

The objectives of this paper were therefore to (i) use miscible displacement column experiments to study the fate and transport of SDM and OMP individually and in combination in two soils and pure sand from the southeastern U.S and (ii) compare the results from previous batch sorption studies to those of the column experiments.

Materials and Methods

Selected Soils

Two soils representative of agriculture and aquaculture in the southeastern United States were chosen for this experiment. One, a Coastal Plain soil (Soil 1), was collected from Geneva County, AL and the other, a Tennessee Valley soil (Soil 2), was collected from Sevier County, TN. Because southeastern U.S. soils are generally sandy, pure sand, Ottawa 4.0 was also used to understand the sorption by sand alone (i.e., no clay minerals or organic matter). Antimicrobial sorption, at minimum, will be affected by clay and organic matter content, cation exchange capacity and pH. Therefore, the selected soils represent several of the physical and chemical characteristics commonly found in the southeastern U.S. Major soil physical and chemical properties (Table 4.1) were determined at the Soil Testing Laboratory at Auburn University. Both soils were air-

dried, ground, and sieved (≤ 2mm diameter). The sand was muffled at 550°C for four hours to eliminate any possible organic matter. Following this, the sand and soils were irradiated using a ^{60}Co source at 5MRads to eliminate microorganisms that could potentially biodegrade the antimicrobials. Cobalt-60 irradiation is highly effective as a soil sterilization method, causing minimal changes to soil physical and chemical properties, especially in oven dried soil (McNamara *et al.*, 2003). A microbial plate count with ½ strength nutrient agar (4 g nutrient broth/ L) of the two soils and sand confirmed that irradiation had eliminated all of the bacteria (0 colonies on plate after 14 days).

Table 4.1. Physical and chemical properties of the selected soils and sand.

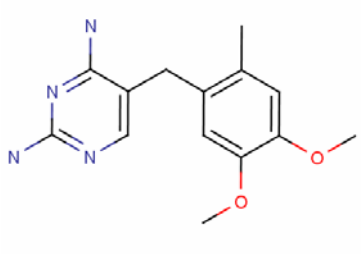
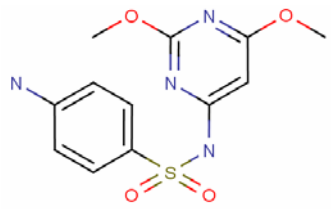
Soil Acronym	Description	Order	pH	%OM	%Sand	%Silt	%Clay	eCEC* ($cmol_c/kg$)
Soil 1	Plintic Kandiudults	Ultisol	5.03	1.5	81.5	13.5	5	3.19
Soil 2	Typic Eutorchrepts	Inceptisol	4.66	2.07	52	38	10	6.64
Sand	Ottawa 4.0	---	4.92	0	100	0	0	0.33

***eCEC: effective cation exchange capacity**

Antimicrobials

The antimicrobials, ormetoprim (OMP, 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl) pyrimidine) and sulfadimethoxine (SDM, N'-(2,6 Dimethoxy-4-pyrimidinyl) sulfanilamide), were obtained from Chem Service, Inc., West Chester, PA and Sigma Aldrich, St. Louis, MO, respectively. Molecular structures and selected physiochemical properties of SDM and OMP are shown in Table 4.2. For the development of the stock solutions, both antimicrobials were initially dissolved in methanol (HPLC grade, obtained from Fisher Scientific International, Inc., Hampton, NH) such that the final working solutions contained less than 0.2% methanol.

Table 4.2. Physiochemical characteristics Ormetoprim and Sulfadimethoxine

Antimicrobial	Key Properties
Ormetoprim (OMP)* 	*Structure Class:* Diaminopyrimidine *Molecular Formula:* $C_{14}H_{18}N_4O_2$ *Molecular Weight:* 274.32 *Water Solubility:* 1540 mg·L^{-1} *log K_{ow}:* 1.23 *Weak base* *K_H:* 4.45E-13 atm-m^3·mol^{-1} *Vapor Pressure:* 2.28E-8 mm Hg *Atm OH Rate Const:* 6.34E-11 cm^3·molecule-sec^{-1}
Sulfadimethoxine (SDM)* 	*Structure Class:* Sulfonamide *Molecular Formula:* $C_{12}H_{14}N_4O_4S$ *Molecular Weight:* 310.33 *Water solubility:* 343 mg·L^{-1} *log K_{ow}:* 1.63 *pK_{a1}/pK_{a2}:* 2.4/6.0 *K_H:* 1.3E-14 atm-m^3·mol^{-1} *Vapor Pressure:* 1.59E-9 mm Hg *Atm OH Rate Const:* 2.02E-10 cm^3·molecule-sec^{-1}

*Structures and Physiochemical properties retrieved from NLM. 2006. ChemIDplus Lite.

Miscible Displacement Experiments

Both soils and the sand (Table 4.1) were each uniformly packed in individual glass columns. Two different columns were used, one with an internal diameter 5 cm and length 10 cm, and the other with an internal diameter 5 cm and length 4 cm. For each experiment soil columns were packed with new soil to the same bulk density. Both ends of the soil columns were covered with several layers of cheesecloth, a paper filter, and Teflon end caps to retain the soil during the experiments. The schematic in Figure 4.1 illustrates the laboratory column apparatus used for these experiments.

The apparatus included a precision constant-volume pump (Masterflex® Quick Load), a fraction collector (Spectra/Chrom* CF-1 or ISCO Retriever II) with 15 mL polypropylene test tubes, glass column with end caps, Masterflex® BioPharm Plus platinum silicone tubing, and polypropylene volumetric flasks for resident, tracer, and antimicrobial solutions. Polypropylene was determined to be chemically inert to SDM and OMP for the duration of these experiments and yielded preferred recoveries over glassware. However, because polypropylene columns were not readily available, glass columns were acid washed and used for these experiments. All tubing, test tubes, pipettes, filters, and flasks were checked for SDM and OMP compatibility. Additionally, column controls (no soil or sand; solution only) with SDM and OMP were performed for the duration of the column experiments. The influent concentrations were regular checked and remained within 5% of the initial concentration.

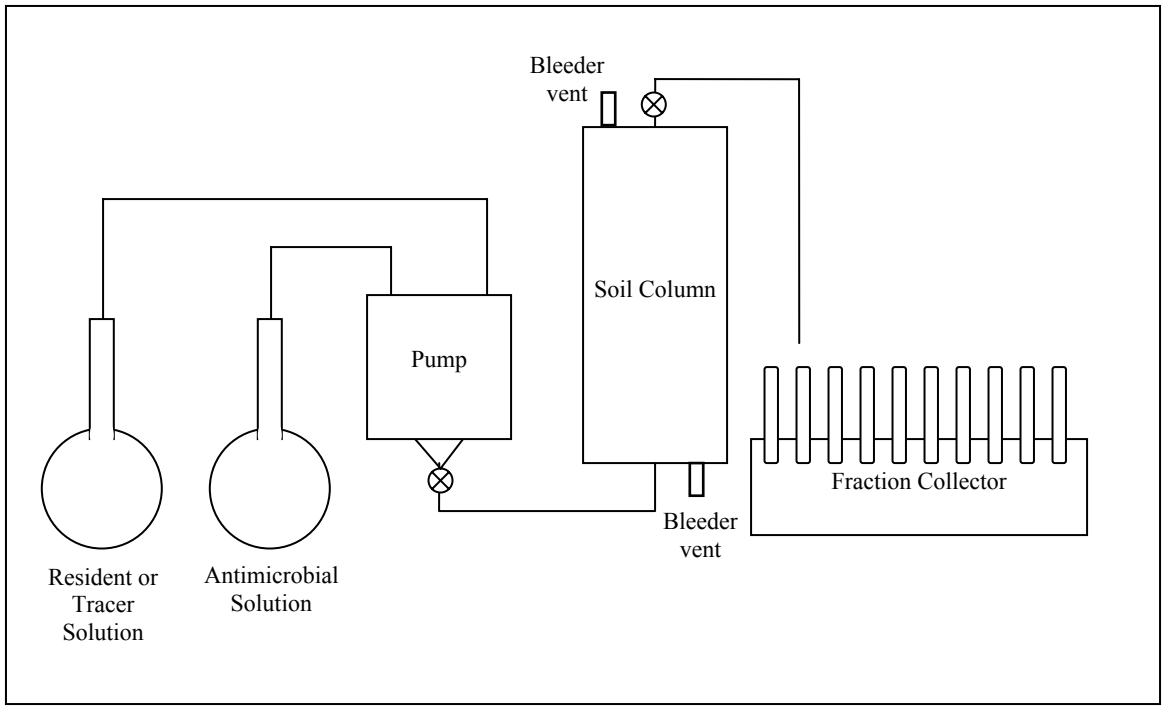


Figure 4.1. Schematic of Laboratory Soil Column Apparatus

The experiments were conducted under saturated flow conditions, because it provided a well-defined and repeatable starting point, and the desired initial chemical conditions in the soil could be established quickly (Skaggs *et al.* 2002). Each soil was wetted, from the bottom up, with a calcium chloride resident solution (0.01 M), and the flow (3 $mL \cdot min^{-1}$) was maintained for a minimum of 24 hours to remove entrapped air and create steady-state saturated flow conditions. The Ca^{2+} in the resident solution minimized clay dispersion and served as an exchangeable cation of known charge and sorption affinity (Skaggs *et al.* 2002). Although photodegradation has been found to be negligible for sulfonamides (Thiele- Bruhn *et al.*, 2003; FDA, 1984) and was likely not important for OMP (FDA, 1984), to ensure it was not a significant factor, the experiments were performed in the dark by wrapping the columns and influent solutions with aluminum foil.

Once steady-state pore water velocity was achieved, the resident solution was replaced with a step feed (for 10 cm long columns) or pulse feed (for 4 cm long columns) of bromide ion tracer solution (0.01 M $CaBr_2$), and fractions of the effluent were collected. The bromide concentration of each fraction was indirectly measured using an Ion Chromatogram (Dionex DX-120), and a breakthrough curve was determined. Calcium chloride (0.01 M) was then flushed through the column for several pore volumes. Following this, each antimicrobial or antimicrobial combination (100 $\mu g \cdot L^{-1}$) was applied as a step feed (for the 10 cm long columns) or pulse feed (for 4 cm long columns) with the resident solution (0.01 M $CaCl_2$) until the estimated time for retardation (described later) had been reached. For the 4 cm columns the antimicrobial pulse was then replaced with the resident solution for the remainder of the experiment.

The column effluent was collected in polypropylene test tubes with increments of 15 mL or 9 mL for the 10 cm and 4 cm columns, respectively. Following collection, samples were immediately prepared for analysis by filtration with 0.45 µm PTFE membrane filters. The filtrates were placed into 750 µL polypropylene autosampler vials and were acidified with formic acid for sample preservation so that the final amount of acid was less than 1% of the total volume.

Chemical Analysis

Antimicrobial samples were analyzed in cooperation with the Food and Drug Laboratory at the Alabama Department of Agriculture and Industries, Montgomery, AL. All samples were stored at 4°C until they were transported on ice (2–4°C) to the Food and Drug Laboratory. The analysis of the extracts utilized a liquid chromatograph triple quadrupole mass spectrometer (LC/MS/MS). A sample run consisted of seven standard curve solutions followed by an initial calibration verification (ICV) and an initial calibration blank (ICB). This was followed by a repeating sequence of 10 samples and subsequently by a continuing calibration verification (CCV) and continuing calibration blank (CCB).

The analytical method uses a gradient separation with the following solvents: acetonitrile and 0.1% formic acid in water. Analytes were separated chromatographically using a Phenomenex® Gemini C18 column (5 µm, 150mm x 2.00 mm) and samples were analyzed using a Thermo Finnigan TSQ Quantum LC/MS/MS system. Compounds of interest were ionized using atmospheric pressure chemical ionization (APCI) in positive mode generating an M+H ion of the parent compound or electrospray ionization (ESI). A minimum of three daughter ions were generated from the parent compound using

selective ion monitoring mode (SIM). Compounds were identified by the presence and ratio of all daughter ions and the retention time of the compound as compared to the calibration standards. Quantification was based on the area of the peak of the most abundant daughter ion of the analyte and regressed against the seven point standard curve.

Fate and Transport Model

Miscible displacement solute transport experiments have frequently been used to assess possible contamination of soil and groundwater (Porro and Wierenga 1993; Singh and Kanwar 1991; Tipton *et al.* 2003) as they yield more realistic solute transport parameters than traditional batch sorption equilibrium experiments. Traditional batch sorption experiments allow estimation of only a sorption or distribution coefficient whereas, miscible displacement column studies account for additional solute transport parameters (e.g., diffusion-dispersion coefficient, retardation factor, distribution coefficient, kinetic rate coefficients, and degradation/transformation rate constants). Models, such as CXTFIT (version 2.1) developed by Toride *et al.* (1999), allow analysis of column breakthrough curves and subsequent determination of these transport parameters. CXTFIT uses an inverse modeling technique to fit a mathematical solution of theoretical transport models to the experimental data (Toride *et al.*, 1999). Several transport models based on the convection-dispersion equation (CDE) are included in CXTFIT. These consist of local sorption equilibrium, chemical non-equilibrium, and physical non-equilibrium.

The one-dimensional convection-dispersion transport equation (CDE) for reactive solutes under steady state flow in a homogenous soil is given by

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \mu_l C - \mu_s \frac{\rho_b}{\theta} S \tag{4.1}$$

where, R, is a dimensionless retardation factor, C is the solute concentration in the liquid phase ($M \cdot L^{-3}$), t is time (T), D is the solute dispersion coefficient ($L^2 \cdot T^{-1}$), x is the distance in the direction of flow (L), v is the average pore velocity ($L \cdot T^{-1}$), μ_l and μ_s are the liquid and solid first order decay coefficients (T^{-1}), ρ_b is the bulk density of the soil ($M \cdot L^{-3}$), θ is the volumetric water content ($L^3 \cdot L^{-3}$), and S is the mass of solute in the sorbed phase per mass of solid ($M^3 \cdot M^{-3}$) The retardation factor is written as

$$R = 1 + \frac{\rho_b}{\theta}\frac{\partial S}{\partial C} \tag{4.2}$$

where $\delta S/\delta C$ is the slope of the sorption isotherm. For a linear sorption isotherm the slope is represented by K_d , the sorption coefficient ($L^3 \cdot M^{-1}$).

Chemical nonequilibrium transport is often used for modeling reactive solutes which exhibit kinetic sorption (Toride *et al.*, 1999). A number of other factors can cause chemical nonequilibrium transport, including nonlinear sorption, desorption hysteresis, and the presence of physical and chemical heterogeneity in soil (Skaggs & Leij 2002). Non-reactive solutes such as bromide are not retarded in the soil and the decay coefficients are zero (Casey *et al.,* 2003).

Chemical nonequilibrium transport is often described by two sorption sites ("type-1" and "type-2" sorption sites) (Selim *et al.,* 1976, 1977). In a two-site model, type-1 sorption sites, S^e, are subject to instantaneous sorption, while type-2 sorption sites, S^k, obey a kinetic rate law (Skaggs and Leij, 2002).

$$S = S^e + S^k \tag{4.3}$$

Using a linear equilibrium sorption isotherm, S^e is represented by

$$S^e = K_d C \tag{4.4}$$

and the type-2 sorption sites are given by

$$\frac{\partial S^k}{\partial t} = \alpha[(1-f)K_d C - S_k] - \mu_{s,k} S_k \tag{4.5}$$

where α is a first-order kinetic sorption coefficient (T^{-1}), f is the fraction of exchange sites always in equilibrium with the solution phase, and $\mu_{s,k}$ is the solid phase first order kinetic decay coefficient (T^{-1}).

Results and Discussion

Upon packing and saturating the columns, major physical properties of the soil in each column were determined (Table 4.3). Columns were packed to the same bulk density (≤ 9% difference). The hydraulic residence time (RT) for the antimicrobial solution in the column was also recorded.

Bromide breakthrough curves were symmetrical and fit well with the equilibrium transport model of the CDE, indicating that bromide transport was nearly ideal and physical nonequilibrium was likely not occurring in the soil columns. The dispersion coefficient, *D*, was determined and is shown for the 10 cm long columns in Table 4.4 and for the 4 cm columns in Table 4.5. The dispersion coefficients determined from the bromide (nonreactive) breakthrough curves were assumed to be equal in a given soil for the OMP or SDM (reactive) breakthrough curves. *D* was therefore used as a fixed parameter when modeling the antimicrobial breakthrough curves because it allowed for more reliable estimation of additional transport parameters (Toride *et al.,* 1999).

Table 4.3. Soil/Sand Column Physical Properties

Sorbent	Column[a]	bulk density P ($g \cdot cm^{-3}$)	volumetric water content θ ($cm^3 \cdot cm^{-3}$)	pore water velocity v ($cm \cdot min^{-1}$)	pore volume PV (cm^3)	pulse input (relative PV)[b]	RT (hr)
Sand	4cm co-solute[c]	1.783	0.327	0.467	25.71	84.01	0.14
	10cm co-solute	1.803	0.320	0.478	62.77	n/a	0.35
	10cm OMP single solute	1.779	0.329	0.465	64.55	n/a	0.36
	10cm SDM single solute	1.800	0.321	0.476	62.99	n/a	0.35
Soil 1	4cm co-solute	1.488	0.438	0.349	34.43	121.30	0.19
	10cm co-solute	1.447	0.454	0.337	89.19	n/a	0.50
	10cm OMP single solute	1.365	0.485	0.315	95.22	n/a	0.53
	10cm SDM single solute	1.360	0.487	0.308	97.48	n/a	0.53
Soil 2	4cm co-solute	1.261	0.524	0.291	41.18	132.87	0.23
	10cm co-solute	1.227	0.537	0.285	105.41	n/a	0.59
	10cm OMP single solute	1.164	0.561	0.273	110.09	n/a	0.61
	10cm SDM single solute	1.161	0.562	0.272	110.31	n/a	0.61

[a] 4cm columns performed only with SDM and OMP co-solute, 10cm columns performed with SDM and OMP co-solute as well as OMP single solute and SDM single solute

[b] Relative pore volume of antimicrobial pulse input for 4 cm columns. Desorption begins after pulse.

[c] co-solute, SDM and OMP administered together; single solute, OMP or SDM administered separately

Step Input Columns

A total of nine columns used the step input procedure for tracer and antimicrobial solutions. All step input columns had a 10 cm length. Both single solute experiments (containing SDM and OMP individually) and co-solute (containing both SDM and OMP together) were performed for each soil and the sand.

The antimicrobial breakthrough curves (Figures 4.2, 4.3, 4.4) illustrate nonequilibrium transport, which was suggested by the delayed arrival of the antimicrobial or the antimicrobial tailing in the pulse input columns (Figure 4.4). Nonequilibrium transport was determined to be the result of chemical processes (e.g. sorption, chemical transformation, and kinetics) because strong indications of physical nonequilibrium were not present in the bromide tracer breakthrough curves. SDM and OMP breakthrough curves were modeled well by assuming a two-site chemical nonequilibrium model and that degradation was equal in the solid and liquid phase. Antimicrobial breakthrough curves for the step input single solute and co-solute columns are illustrated in Figures 4.2 and 4.3, respectively. The general shape of the breakthough curves for OMP were similar in the two soils but much different in sand, which illustrated a relatively low retardation. SDM was also only slightly retarded in sand, while yielding a higher retardation in the two soils. To further investigate the sorption of SDM and OMP in sand, an extraction for iron, iron oxide, manganese, and aluminum was performed on the sand. This procedure was chosen because the sand had indications (slight red color) of the presence of iron or manganese after muffling. Additionally, other antimicrobials have been shown to sorb to iron oxides (Zhang and Huang, 2007; Figueroa and Mackay, 2005). Results from the extraction procedure, however, revealed that the sand contained

less than 0.01% iron oxide or iron, less than 0.0001% manganese, and no detectable aluminum, leading to the conclusion that these sand fractions were not likely contributors to the antimicrobial sorption. Sorption of SDM in sand was also noted by Thiele-Bruhn et al. (2004) where SDM and other sulfonamides actually sorbed more to sand than to three other soils investigated. A similar phenomenon to the sorption in silica sand was illustrated in the incompatibilities of silica glassware with these antimicrobials, where there was an affinity to the silica active sites. Overall, sorption of OMP and SDM in Sand was relatively low compared to sorption in Soil 1 and Soil 2.

The OMP breakthrough curves in the two soils exemplify strong sorption of OMP where the relative concentrations (C/C_0) never reached 1. Upon first glance, it may seem that the low C/C_0 for OMP in the two soils should be attributed to degradation alone; however significant degradation was not occurring in the sand column which may illustrate irreversible sorption in the soil.

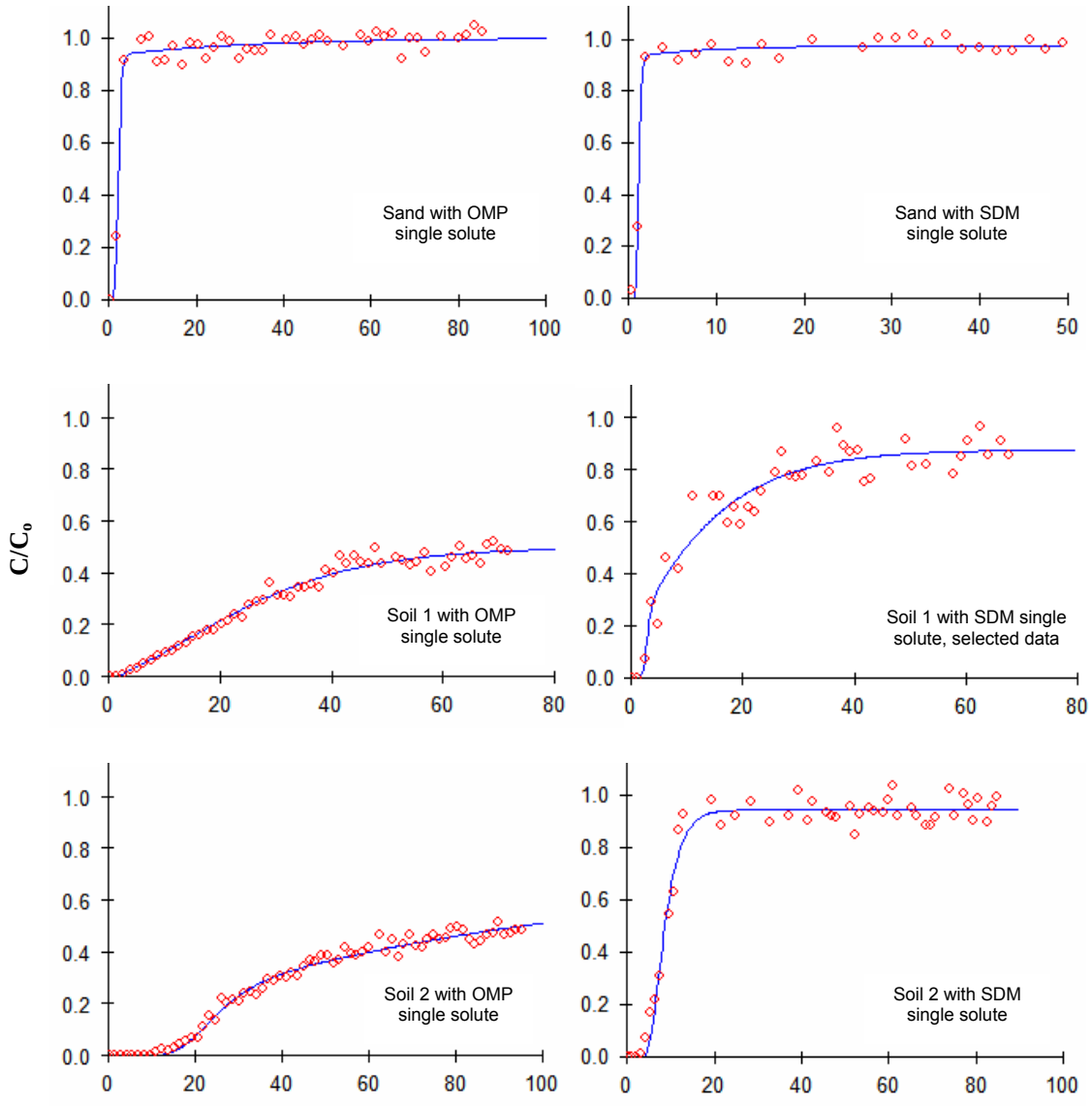


Figure 4.2. Step Input Breakthrough Curves for OMP and SDM single solute column experiments (10 cm long columns). Relative concentration (C/C_0) as a function of relative pore volume.

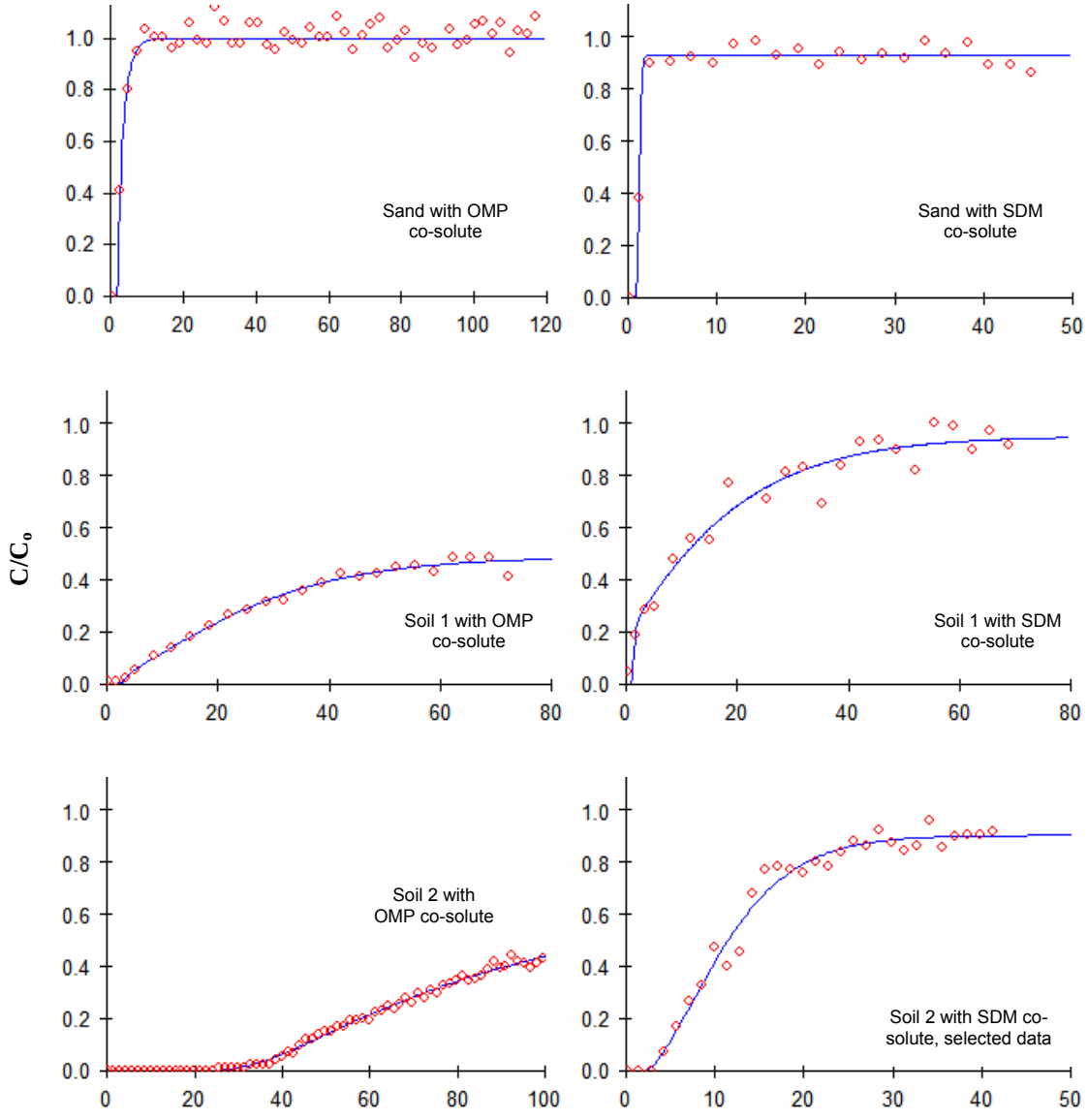


Figure 4.3. Step Input Breakthrough Curves for OMP and SDM combination column experiments (10 cm long columns). Relative concentration (C/C_0) as a function of relative pore volume

Results from the single solute and co-solute sorption experiments indicate that there is little difference in SDM and OMP sorption or mobility when administered alone as single solutes or in combination as co-solutes. This is evident not only in the breakthrough curves of the combination columns and the individual columns (Figures 4.2 and 4.3), but also in the relevant transport parameters (Table 4.4). Retardation factors between single solute and co-solute systems are very similar, indicating similar mobility of these compounds when administered alone and in combination. Additionally, there is less than a 5% difference in sorption coefficients in single solute and co-solute systems for SDM in Sand, SDM in Soil 1, and OMP in Soil 2. Although the single and co-solute sorption coefficients for OMP in Sand, OMP in Soil 1, and SDM in Soil 2 represent a 20-36% difference, in relation to other mobile compounds this difference is rather small. The sorption coefficients for OMP and SDM are much less than sorption coefficients found for highly sorbing classes antimicrobials such as tetracyclines. Sassman and Lee (2005) found linear sorption coefficients for tetracycline, oxytetracycline, and chlortetracycline ranging from 1,229 to 352,911 $L \cdot kg^{-1}$. Sorption of OMP falls in a similar range as sorption of macrolide antimicrobials, such as Tylosin. Rabølle and Spliid (2000) and Tolls (2001) reported tylosin linear sorption coefficients in sandy loam and clay loam between 8.3 and 128 $L \cdot kg^{-1}$.

Table 4.4. Transport parameters for 10 cm long columns with step input

				Column[c]	Batch[c]					
Sorbent	**Solute[a]**	**D ($cm^2 \cdot min^{-1}$)**	**R**	**K_d ($L \cdot kg^{-1}$)**	**K_d ($L \cdot kg^{-1}$)**	**R_{batch}**	**f**	**α (min^{-1})**	**μ (min^{-1})**	**R^2**
Sand	OMP single solute	0.093 (0.062-0.123)[b]	4.26 (3.17-5.34)	0.60	1.3	8.03	0.40	0.0016	4.6E-09	0.97
	OMP co-solute	0.075 (0.065-0.086)	3.36 (2.95-3.77)	0.42	4.96	28.95	0.53	0.0394	4.8E-09	0.93
	SDM single solute	0.136 (0.084-0.188)	1.51 (1.44-1.57)	0.09	0.4	3.24	0.30	0.0055	0.0012	0.98
	SDM co-solute	0.075 (0.065-0.086)	1.52 (1.42-1.62)	0.09	2.5	15.09	0.51	5.5E-07	0.0037	0.98
Soil 1	OMP single solute	0.140 (0.093-0.186)	28.26 (25.56-30.96)	9.69	21.9	62.64	0.09	0.0051	0.0229	0.98
	OMP co-solute	0.143 (0.093-0.193)	26.34 (23.03-29.66)	7.95	30.9	99.49	0.13	0.0044	0.0252	0.99
	[d]SDM single $solute_m$	0.088 (0.061-0.115)	12.31 (9.73-14.88)	4.21	10.4	28.97	0.19	0.0040	0.0042	0.93
	[d]SDM single $solute_a$	0.088 (0.061-0.115)	9.92 (5.93-13.92)	3.32	10.4	28.97	0.19	0.0070	0.0293	0.56
	SDM co-solute	0.143 (0.093-0.193)	14.97 (10.53-19.41)	4.38	12.5	40.84	0.04	0.0037	0.0018	0.96
Soil 2	OMP single solute	0.049 (0.019-0.078)	84.91 (83.07-86.75)	40.44	58.3	121.96	0.33	0.0005	0.0094	0.99
	OMP co-solute	0.146 (0.083-0.209)	91.83 (50.19-133.5)	39.75	89.7	205.96	0.60	0.0013	0.0133	0.99
	SDM single solute	0.097 (0.064-0.131)	9.15 (8.45-9.86)	3.95	25.8	54.30	0.80	0.0170	0.0016	0.98
	[d]SDM co-$solute_m$	0.146 (0.083-0.209)	12.02 (10.98-13.06)	4.82	22.1	51.50	0.33	0.0131	0.0030	0.98
	[d]SDM co-$solute_a$	0.146 (0.0829-0.2087)	11.21 (9.19-13.22)	4.47	22.1	51.50	0.35	0.0171	0.0047	0.74

[a] Single solute represents the antimicrobial was administered individually and co-solute represents the antimicrobial was administered in combination with the other antimicrobial

[b] 95% Confidence Intervals

[c] Linear sorption coefficients were calculated here from column experiments but are also shown from previous batch sorption equilibrium experiments.

[d] SDM single solute for SOIL 1 and SDM co-solute for SOIL 2 were modeled using a visual fit data set, represented by m, and using the actual data set represented by a.

It is important to note that SDM in Soil 1 for the single solute and SDM in Soil 2 for the co-solute have two models (Table 4.4 and Figure 4.4); one represents a fit to the actual raw data and another represents a fit to the selected data. Although all SDM C/C_0 ratios reach at least 0.9 at some point, the effluent concentrations often showed substantial variations throughout the experiment. These high variations did not occur in all SDM experiments which raised many questions as to the cause of this phenomenon. Because these variations were often large, the data was modeled using all of the raw data as well as using selected data points which illustrated the most likely SDM sorption trend based on the other SDM transport experiments. This was considered the best modeling method for this situation (Selim, 2007). Model differences are shown in Figure 4.4. The model variations for Soil 2 with SDM co-solute were very insignificant, and the only difference in the selected data and actual data was that the selected data stopped at 41 relative pore volumes and the actual data continued for 100 relative pore volumes. After 41 pore volumes, the actual data began to have large fluctuations that did not occur early in the experiment. This was contrary to SDM single solute in Soil 1 where the fluctuations began early in the experiment. Although modeled two ways, both SDM single solute in Soil 1 and SDM co-solute in Soil 2 illustrated mobility as noted by the low retardation coefficients. The deviation between models was slightly greater in SDM single solute in S1 than in SDM co-solute in Soil 2. The actual data model for SDM with Soil 1 gave slightly less retardation than the selected data model, but yielded a higher degradation coefficient and a much lower correlation coefficient. Selecting data for modeling did not significantly change the retardation factor. It did, however, illustrate slightly lower degradation coefficients, which were more consistent with those of SDM

co-solute in Soil 1 and SDM single solute in Soil 2.

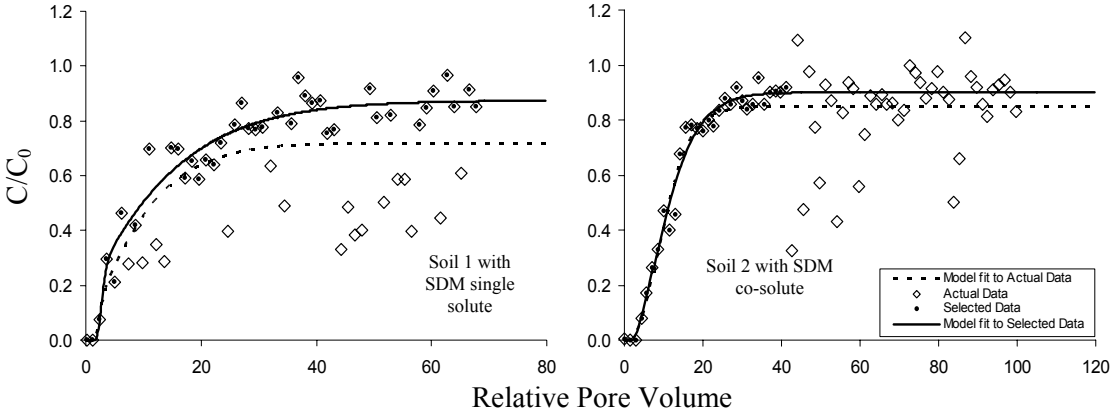


Figure 4.4. Breakthrough curves and model fits for the actual data and selected data SDM single solute in Soil 1 and SDM co-solute in Soil 2 for the step input. Relative concentration (C/C_0) as a function of relative pore volume

One proposed cause for the fluctuations in SDM was a degradation cycle by microorganisms, however the soils were cobalt irradiated and initially sterile. To check the sterility of the soils after the completed SDM and OMP column experiments, microbial plate counts were performed on each of the soils utilizing ½ strength nutrient agar. After incubation at room temperature for 7 days, the plates were examined and found to contain too many colonies to count. This was necessarily expected because of natural air-borne and water-borne microbes that may have been introduced into the columns. From this plate count, however, no conclusions could be made whether these bacteria would degrade SDM or OMP. To further check the possibility of antimicrobial degradation, ½ strength nutrient agar plates were prepared again, but with the additions of either 100 or 500 $\mu g \cdot L^{-1}$ of SDM and OMP. The same soils from the column experiments were plated again on the new nutrient agar plates containing OMP and SDM and incubated at room temperature for 7 days. The hypothesis for the addition of OMP and SDM in the agar was that if the soil bacteria were utilizing OMP and SDM as a nutrient source, then with the addition of OMP and SDM, bacterial growth would be observed. No effect, however, was observed with either the addition of 100 or 500 $\mu g \cdot L^{-1}$ SDM and OMP in the agar. This was a simple indication that neither degradation nor inhibition was occurring during the length of the column experiment (about 7 days). This is consistent with findings by Halling-Sørensen and Ingerslev (2000), who showed that microbial growth was not inhibited in the presence of multiple sulfonamides and that the sulfonamides were not readily biodegradable. Additionally, studies have shown that sulfonamides are stable in the presence of light (FDA, 1984; Halling-Sørensen *et al*., 2003) and others have shown that the half life of OMP and SDM exceeds 1 year in an

aquatic environment (Bakal and Stoskopf, 2001).

Fluctuations in column effluent concentrations have been reported by others working with pharmaceuticals (Scheytt *et al.*, 2004; Scheytt *et al.*, 2006). Propyphenazone concentration fluctuations by Scheytt *et al*. (2004) were partially attributed to degradation and partially to analytical uncertainty. Analytical uncertainty could explain some of the SDM fluctuations, but it is not likely the predominant factor.

The pK_a values for antimicrobials are often in the range of soil pH values, causing antimicrobials to protonate or deprotonate as the pH changes. Antimicrobial sorption is often affected as the aqueous speciation changes in various pH environments (Tolls, 2001). Because pH can strongly influence antimicrobial sorption, the effluent pH of SDM and OMP co-solute in Soil 1 and Soil 2 was evaluated. The effluent pH of Soil 1 varied between 4.75 to 5.01 and Soil 2 between 4.65 to 5.15. No covariance between pH and SDM effluent concentration was found (Soil 1 covariance, -0.0001; Soil 2 covariance, -0.006) indicating that the change in pH did not correspond to the fluctuations in SDM effluent concentration. Although no covariance was found between the SDM effluent concentration and pH, the pH fluctuations are likely important. Even a slight change in pH can affect the speciation of antimicrobials, which in turn affects the affinity for the surface. Gao and Pedersen (2005) found that as the pH dropped below 5, marked increases in sulfonamide sorption were often observed. For SDM, the neutral species (SDM^0) dominates between the pK_{a1} and pK_{a2} values (Table 4.2), the cationic species (SDM^+) dominates at pH values below the pK_{a1}, and the anionic species (SDM^-) dominates at pH above the pK_{a2}. Because soils generally carry a net negative charge, SDM sorption would tend to be greater at low pH values where the cationic species

dominates. The cationic species (SDM^+) would have a higher attraction to the negatively charged surface, and would therefore exhibit more sorption, than either the neutral or the anionic species. The OMP pH dependent sorption would be similar to that found for another diaminopyrimidine, trimethoprim (e.g. Bekçi *et al*., 2006). These authors found that at low pH, all trimethoprim was in the protonated form (cationic species), and that at pH values near neutral, the weak base was near its pK_a and was consequently in is neutral species. More trimethoprim sorption was found between pH 4-6 than above and below these values. The authors found that above pH 6, the neutral species dominated and had little attraction for the negatively charged surface. At low pH, the protonated trimethoprim was in competition with the decreasing hydrogen ions in solution and little sorption occurred. It should be noted that even a slight change in pH could affect the speciation of SDM and OMP and could therefore cause more sorption or desorption to occur. Although soil has a natural buffering capacity, it may not buffer it enough to prevent slight sorption and desorption changes in antimicrobials, particularly for the SDM observed here. The pH changes in the natural environment would play an important role in the sorption and desorption of these compounds.

Batch sorption experiments

Sorption coefficients from previous batch sorption equilibrium experiments utilizing the same soils and antimicrobials are also reported for comparison with those found for the column studies (Table 4.4). The batch studies allowed for initial estimates of retardation factors (R) and were useful in predicting column durations and achieving the minimum for the objective function when modeling using CXTFIT. Sorption coefficients from batch studies considerably overestimated column retardation

coefficients. In fact, batch sorption coefficients and subsequent retardation factors were 31 to 96% higher in the batch sorption studies than in the corresponding column studies. A number of factors can cause this discrepancy including low soil/solution ratios in batch sorption experiments relative to column studies and rate-limited mass transfer in column studies because of advective transport (Barnett *et al.,* 2000; Casey *et al*., 2003). The fraction of exchange sites, *f*, at equilibrium in the column studies was always less than 1, indicating that equilibrium exchange was never reached. Rate limited sorption in soil column studies is the likely cause of the discrepancies between batch and column sorption coefficients and retardation factors.

Results from batch sorption experiments indicated that OMP sorption was enhanced when in combination with SDM. This trend was not observed in the miscible displacement column studies. The high flow rate in column studies did not allow sorption equilibrium to be reached ($f < 1$). Therefore the effects of enhanced sorption of OMP were not shown under flow conditions.

Pulse Input Columns

After modeling the step input for the 10 cm long columns, it was deemed necessary to perform pulse input columns (e.g., square wave) with a shorter column length. Performing the shorter columns would reveal whether the SDM variations were only occurring because of the extended experiment time or whether the variations would also occur when the hydraulic residence time (shorter column) was reduced. Additionally, the shorter columns would be subjected to a pulse input which would evaluate desorption and mass recovery. Because the differences between single solute and co-solute were determined negligible, the pulse input columns were only run with

SDM and OMP in combination.

Breakthrough curves for the pulse input columns are illustrated in Figure 4.5. The same sequence of sorption for OMP and SDM was observed in the pulse input columns, step input columns, and batch sorption tests, viz., Sand < Soil 1 < Soil 2. The increasing sorption in the two soils and sand follows the pattern of increasing organic matter, cation exchange capacity, clay content (more surface area for sorption), and decreasing soil pH. As described earlier, the decreasing pH would likely change the speciation of OMP and SDM to more cationic species, thereby enhancing sorption.

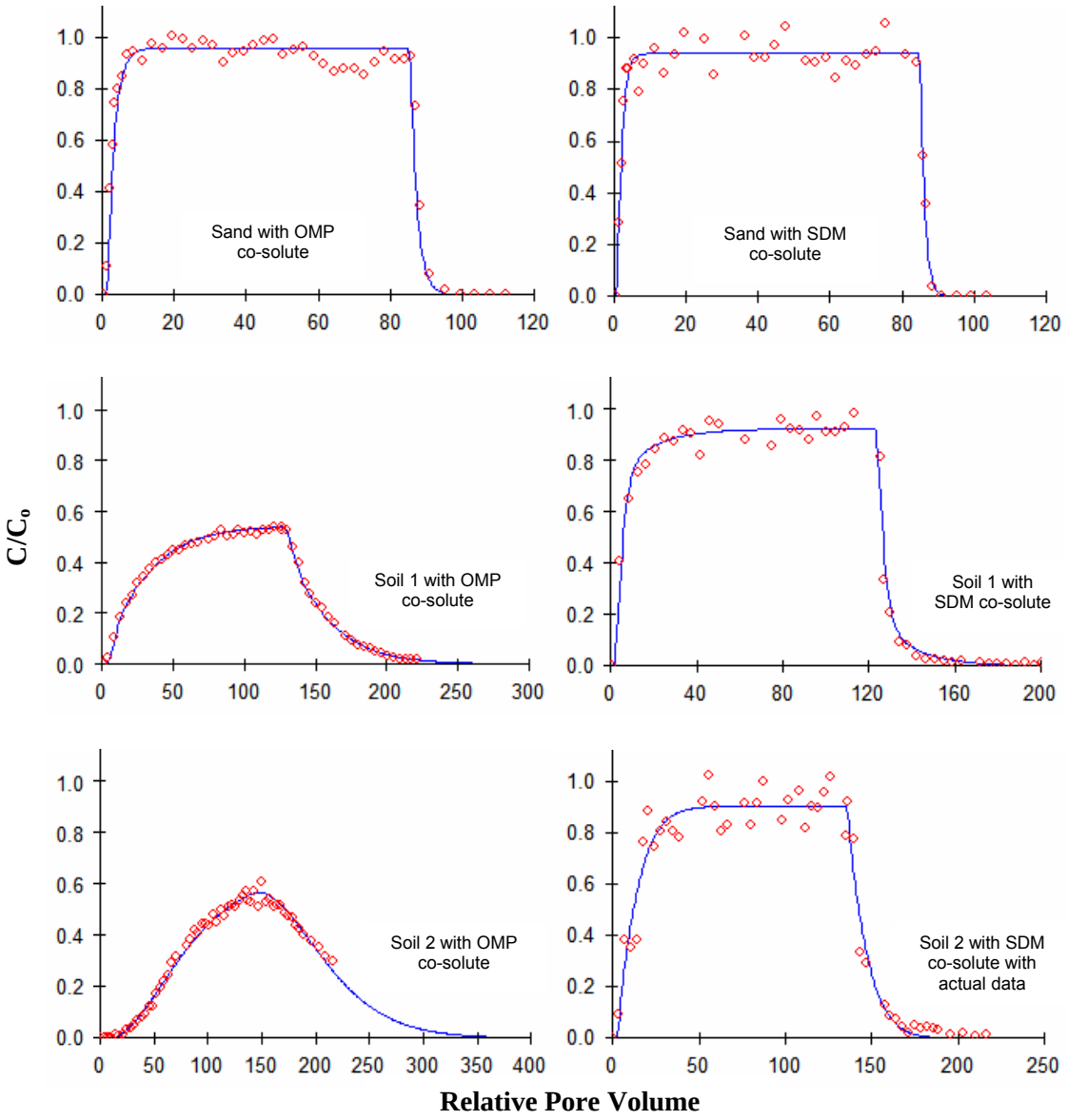


Figure 4.5. Pulse Input Breakthrough Curves for OMP and SDM co-solute column experiments (column length = 4 cm)

SDM was more readily desorbed than OMP as indicated in the model comparisons (Figure 4.6). OMP was not fully desorbed in soil 2, but based on the results of the model with complete desorption, the recovery would not have reached 1. Mass recoveries were greater than 0.90 with the exception of the two soil columns with OMP. Here the mass recoveries in the column effluent were 0.56 and 0.55 for the OMP co-solute in Soil 1 and OMP co-solute in Soil 2, respectively. Low recoveries indicate irreversible sorption, chemical transformations, or degradation. Degradation rate coefficients (μ) which account for degradation, chemical transformations, and irreversible sorption are given in Table 4.4. OMP in the two soils has the highest μ of all of the other combinations.

The influent antimicrobial solutions were regularly checked to ensure no loss of antimicrobial at the inlet (relative percent difference < 10%). Further, to check the effluent variations for possible metabolites, several of the low effluent SDM samples were evaluated in a full ion scan on the LC/MS/MS in MS mode. Sulfonamide metabolites of particular interest were those from hydroxylation or acetylation because they have been detected in chickens, pigs, manure, or milk (Furusawa, 2006; Furusawa and Mukai, 1994; Haller et al., 2002; Kishida and Furusawa, 2004). None of the molecular weights of the expected metabolites were identified for SDM; unknown metabolites may be present, but based on the peaks in the total ion scan, they would represent less than 1 % of the total analyte.

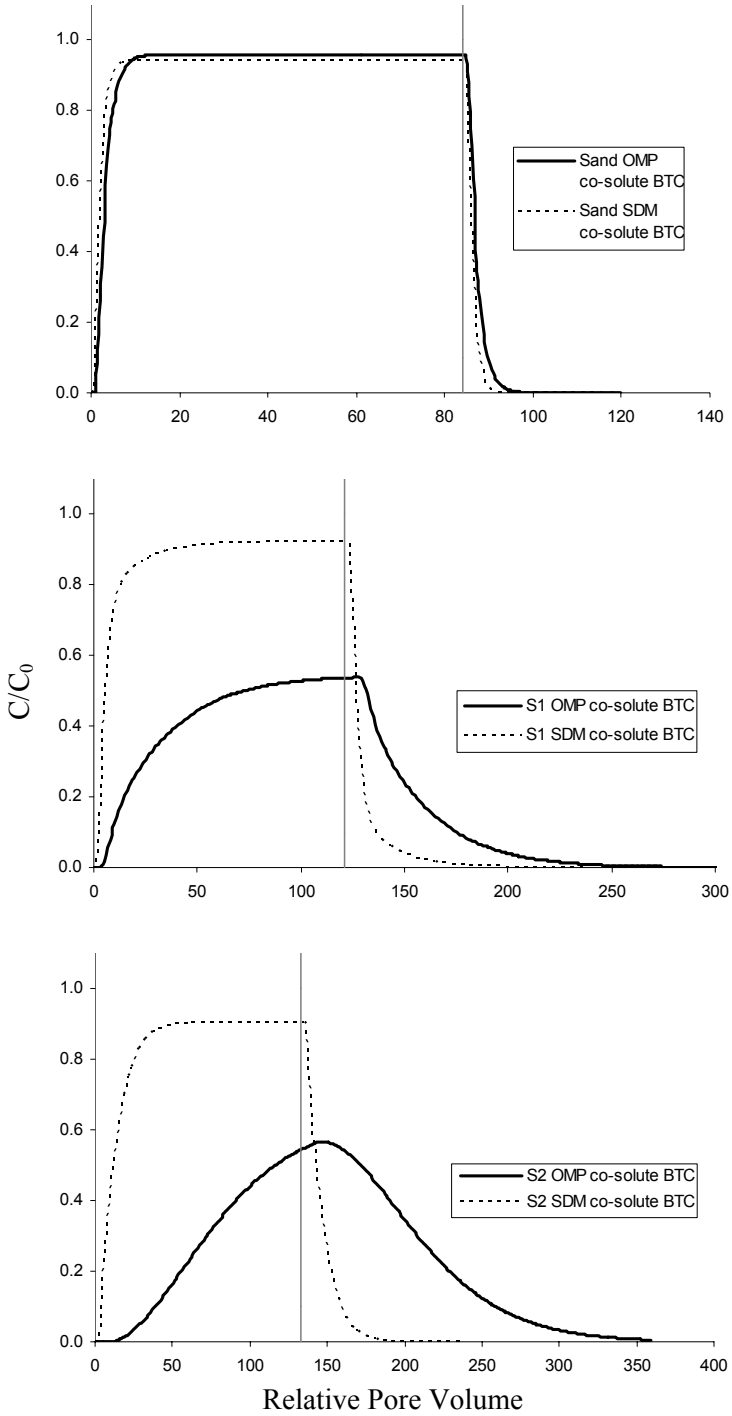


Figure 4.6. Modeled breakthrough curve comparisons for SDM and OMP co-solute in Sand, Soil 1, and Soil 2. Relative concentration (C/C_0) as a function of relative pore volume; vertical line denotes end of pulse.

The Soil 1 SDM single solute and Soil2 SDM co-solute pulse input 4 cm long columns illustrated a similar pattern of SDM fluctuations as the 10 cm long columns. This indicated that the shorter hydraulic residence time did not eliminate the SDM fluctuations. The SDM mass recovery for all 3 SDM columns illustrating fluctuations was greater than 0.90. For the pulse input columns, S1 SDM co-solute illustrated the largest concentration variances, but was modeled well using the actual data (Figure 4.5). A comparison of the models for the actual data and selected data for this column is illustrated in Figure 4.7.

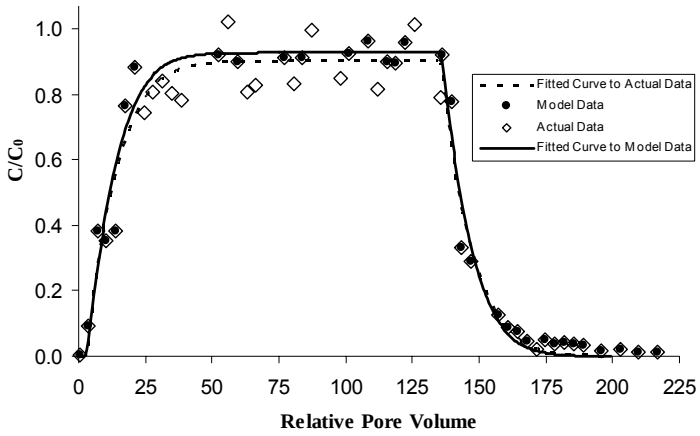


Figure 4.7. Breakthrough curve and model fit for the actual data and selected data for SDM co-solute in Soil 2 pulse input.

Table 4.5. Transport parameters for 4 cm long columns with pulse input

Sorbent	Solute[a]	D ($cm^2 \cdot min^{-1}$)	R	Column[c] K_d ($L \cdot kg^{-1}$)	Batch[c] K_d ($L \cdot kg^{-1}$)	R_{batch}	f	α (min^{-1})	μ (min^{-1})	R^2	Mass Recovery
Sand	OMP co-solute	0.158	3.38	0.44	5.0	28.04	0.70	0.0895	0.0051	0.96	0.95
		(0.052-0.263)[b]	(2.89-3.86)								(0.96)[e]
	SDM co-solute	0.158	2.26	0.23	2.5	14.63	0.52	0.1951	0.0074	0.98	0.94
		(0.052-0.263)	(1.99-2.53)								(0.94)
Soil 1	OMP co-solute	0.202	35.00	10.01	30.9	105.98	0.38	0.0065	0.0586	0.99	0.56
		(0.021-0.384)	(34.12-35.87)								(0.56)
	SDM co-solute	0.202	8.58	2.23	12.5	43.47	0.66	0.0060	0.0073	0.99	0.91
		(0.021-0.384)	(6.93-10.23)								(0.92)
Soil 2	OMP co-solute	0.047	85.86	35.26	89.7	216.86	0.27	0.0057	0.0348	0.99	0.55
		(-0.004-0.097)	(82.03-89.69)								(0.63)
	[d]SDM co-solute$_a$	0.047	13.72	5.29	22.1	54.18	0.32	0.0153	0.0075	0.97	0.90
		(-0.004-0.097)	(12.09-15.34)								(0.90)
	[d]SDM co-solute$_m$	0.047	13.27	5.10	22.1	54.18	0.31	0.0183	0.0055	0.97	0.94
		(-0.004-0.097)	(11.70-14.85)								(0.93)

[a] Co-solute represents the antimicrobial was administered in combination with the other antimicrobial

[b] 95% Confidence Intervals

[c] Linear sorption coefficients were calculated here from column experiments but are also shown from previous batch sorption equilibrium experiments.

[d] SDM co-solute for SOIL 2 was modeled using a visual fit data set, represented by m, and using the actual data set represented by a.

[e] Mass Recovery calculated from model results

Model parameters for the pulse input columns yielded excellent results with $R^2 > 0.96$. OMP linear sorption coefficients ranged from 0.44 to 35.26 $L \cdot kg^{-1}$ and followed the same sequence of sorption in sand and two soils as in the step input columns. Halling-Sørensen and Ingerslev (2000) found that trimethoprim, another diaminopyrimidine with similar structure and properties to OMP, had a distribution coefficient of 76 $L \cdot kg^{-1}$ in sludge. The trimethoprim distribution coefficient is higher than the largest distribution coefficient of OMP in Soil 2; however, the sewage sludge would contain a greater percentage of organic matter for sorption than that of Soil 2 (2.07%). SDM sorption also followed the same sequence of sorption and yielded sorption coefficients from 0.23 to 5.29 $L \cdot kg^{-1}$. The weak sorption of SDM in column studies was similar to the sulfonamide sorption in soils found by others. Boxall *et al.* (2002), Thiele-Bruhn and Aust (2004), and Thiele-Bruhn (2003) report sulfonamide sorption coefficients from 0.3 to 10 $L \cdot kg^{-1}$ in a variety of soils from sand to clay. Gao and Pedersen (2005) found higher K_d values for sulfonamides in clay minerals, ranging from 2.3 to 22.2 $L \cdot kg^{-1}$. The higher K_d values are more similar to the sorption coefficients identified in the previous batch sorption experiments, rather than the column studies. Linear sorption coefficients for SDM were higher in the batch tests than in the column experiments. The sorption differences in batch and column experiments are likely due to rate limited sorption in the column tests.

Conclusions

This study reveals new information on the fate and transport of SDM and OMP in the environment. SDM and OMP are both relatively mobile in sand, but illustrate more retardation in the two southeastern U.S. soils. Comparisons of single solute and co-solute column studies of OMP and SDM indicate that sorption of these compounds in mixture is not considerably different from their individual sorption. Previous batch sorption studies indicate much higher sorption than found in the miscible displacement column experiments. These differences are likely due to rate limited sorption during advective transport of the column studies.

The relative mobility of OMP is much greater than that of tetracycline antimicrobials observed by Sassman and Lee (2005), yet less than sulfonamide antimicrobials, such as SDM. Sorption of SDM from column transport studies in soil and sand was found to be very weak, as noted by other authors studying sulfonamides (Boxall *et al.,* 2002; Thiele-Bruhn and Aust 2004; Göbel *et al.,* 2005; Thiele-Bruhn, 2003).

SDM and OMP transport was modeled well by the chemical nonequilibrium model of the convection-dispersion equation. Neither SDM nor OMP reached sorption equilibrium in the soil columns ($f < 1$) and were therefore both modeled by rate limited sorption and first order kinetics. Results for the pulse input columns yielded mass recoveries > 0.90 for sand and the two soils with SDM and for sand with OMP. The mass recoveries for OMP in the two soils were lower, at values of 0.56 and 0.55 in Soil 1 and Soil 2, respectively, indicating irreversible sorption or chemical transformation.

The antimicrobials SDM and OMP used for animal husbandry exhibit a potential pathway for environmental contamination when released in feces and urine or medicated

feed. Results from this study indicate that both compounds have potential to move through soils, contaminating nearby surface and ground water. SDM demonstrates some sorption capacity, but is readily desorbed, whereas OMP is sorbed more strongly and requires more time for desorption. pH may be an important factor affecting sorption in the natural environment. This study revealed that SDM and OMP sorption increased and relative mobility decreased in soils with increasing cation exchange and SOM, and decreasing pH.

CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS

Summary of Conclusions

New information regarding the fate and transport of sulfadimethoxine (SDM) and ormetoprim (OMP) was revealed through this research. Through a series of laboratory experiments, this research has shown that SDM exhibits mobility in soils and will likely be transported to nearby waters in the natural environment. OMP sorbed more strongly to the soil than SDM, but nonetheless exhibited some mobility. Sorption of SDM and OMP in sand and two soils from the southeastern U.S. followed the sequence: Sand < Soil 1 <Soil 2. This is indicative that the higher the soil organic matter, cation exchange capacity, and clay content, the higher the sorption of these two antimicrobials.

Results from batch sorption kinetic experiments showed that SDM and OMP sorption was rapid over the first 16 hours of experimentation followed by a period of much slower sorption. The batch sorption equilibrium experiments implied an enhanced sorption of OMP when in combination with SDM. This, however, did not hold true in miscible displacement column studies. No considerable differences were determined between co-solute and single solute systems in the column studies indicating that SDM and OMP did not positively or negatively interact for sorption sites during transport.

Differences in miscible displacement column studies and batch sorption equilibrium experiments were also observed in the sorption coefficients. The K_d values from column studies were considerably lower than those found in batch experiments. This is likely because of the high flow conditions and subsequent rate limited sorption occurring in the column experiments.

The column experiments were performed under step input and pulse input conditions. Model results from the pulse input columns were preferred over those from the step input columns because the pulse input columns included desorption and allowed for mass recovery calculations. Mass recoveries were greater than 0.90 for SDM in both soils and sand as well as for OMP in sand. OMP mass recoveries in the two soils were lower, at values of 0.56 and 0.55 for Soil 1 and Soil 2, respectively. Low recoveries were indicative of possible irreversible sorption or chemical transformations.

SDM often illustrated unexplained activity, especially in the column experiments where the effluent concentrations fluctuated throughout the duration of the experiment. Evaluation of this problem in column studies suggested that neither pH nor microorganisms were causing these concentration inconsistencies. Analytical uncertainties could be a contributing factor; however, further investigation into the SDM concentration fluctuations is necessary before making specific conclusions about this problem.

Based on the results of these experiments, SDM and OMP will likely be mobile in natural environments. If present in soil water, these antimicrobials may be bioavailable to microorganisms and hence affect microbial mediated ecosystem functions. Future

research should focus on the ecological effects of these antimicrobials to determine if they are a threat to surface and ground water.

Recommendations

These experiments were intentionally performed in soils from the southeastern U.S. in an attempt to apply the fate and transport results to agriculture and aquaculture areas within this region. These soils, however, represent only a few of the many possible soil types. It is recommended that additional fate and transport experiments of SDM and OMP be performed in other soils, as this will generate a variety of transport data for accurate predicted environmental concentrations of these compounds. The experiments presented in this research were carried out at the natural, unaltered soil pH in an attempt to mimic actual environmental conditions. These compounds, however, may protonate or deprotonate depending on the surrounding pH, thereby affecting their sorption. To further understand the pH effects, it is suggested that pH sorption envelope experiments (% sorbed versus pH at a constant antimicrobial concentration) be performed in future studies.

Because limited data on the ecological effects of SDM and OMP is found in the literature, it is highly recommended that laboratory studies are initiated to investigate these effects. Particularly to this area of research, it is suggested that column studies are performed examining the soil microbial effects as well as the antimicrobial transport when microorganisms are present in the soil. Additional research is suggested to investigate removal strategies of SDM and OMP in natural and engineered environments.

REFERENCES

ALFA. 2007. Alabama Poultry Producers. Alabama Farmers Federation. Montgomery, AL. Available at: www. alfafarmers.org/commodities/poultry.phtml. Accessed 13 March 2007.

Amacher, M.C., Selim, H.M, Iskandar, I.K. 1988. Kinetics of chromium (Vi) and cadmium retention in soils: A nonlinear multireaction model. *Soil Science Society of America Journal* 52:398-408.

Bakal, R.S., Stoskopf, M.K. 2001. In vitro studies of the fate of sulfadimethoxine and ormetoprim in the aquatic environment. *Aquaculture* 195:95-102.

Barnett, M.O., Jardine, P.M., Brooks, S.C., Selim, H.M. 2000. Adsorption and Transport of Uranium (VI) in Subsurface Media. *Soil Science Society of America Journal* 64:908-917.

Batt, A.L., Snow, D.D., Aga, D.S. 2006. Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. *Chemosphere* 64:1963-1971.

Bekçi, Z., Yoldaş, S., Yurdakoç, M.K. 2006. Equilibrium studies for trimethoprim adsorption on monmorillonite KSF. *Journal of Hazardous Materials* B133:233-242.

Boreen, A.L., Arnold, W.A., McNeill, K. 2005. Triplet-Sensitized Photodegradation of Sulfa Drugs Containing Six-Membered Heterocyclic Groups: Identification of an SO2 Extrusion Photoproduct. *Environmental Science and Technology* 39:3630-3638.

Boxall, A.B.A., Blackwell, P., Cavallo, R., Kay, P., Tolls, J. 2002. The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters*. 131:19-28.

Boxall, A.B.A., Fogg, L.A., Blackwell, P.A., Kay, P., Pemberton, E.J. 2003b. Veterinary medicines in the environment. *Reviews in Environmental Contamination and Toxicology* 180:1-92.

Boxall, A.B.A., Kay, P., Blackwell, P.A., Fogg, L.A. 2004. Fate of Veterinary Medicines Applied to Soils. *In* Kümmerer (ed.) *Pharmaceuticals in the Environment, Sources, Fate, Effects, and Risks 2nd Edition*. Springer-Verlag Berlin Heidelberg New York. pp. 165-180.

Boxall, A.B.A., Kolpin, D.W., Halling-Sorensen, B. & Tolls, J. 2003a. Are veterinary medicines causing environmental risks? *Environmental Science and Technology* 37: 286A-294A.

Bucheli, T.D., Gustafsson, O. 2000. Quantification of the soot-water distribution coefficient of PAHs provides mechanistics basis for enhanced sorption observations. *Environmental Science and Technology* 34:5144-5151.

Campagnolo, E.R., Johnson, K.R., Karpati, A., Rubin, C.S., Kolpin, D.W., Meyer, M.T., Esteban, J.E., Currier, R.W., Smith, K., Thu, K.M., McGeehin, M. 2002.

Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. *Science of the Total Environment* 299:89-95.

Capone D.G., Weston, D.P., Miller, V., Shoemaker, C. 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture* 145:55-75.

Casey, F.X.M., Larsen, G.L., Hakk, H., Šimůnek, J. 2003. Fate and Transport of 17B-Estradiol in Soil-Water Systems. *Environmental Science and Technology* 37:2400-2409.

Chiou, C.T. 2002 *Partition and Adsorption of Organic Contaminants in Environmental Systems*. John Wiley & Sons, Inc. Hoboken, NJ, USA. pp. 106-213.

Costanzo, S.D., Murby, J., Bates, J. 2005. Ecosystem Response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin* 51:218-223.

Cunningham, V.L. 2004. Special Characteristics of Pharmaceuticals Related to Environmental Fate. *In* Kümmerer (ed.) *Pharmaceuticals in the Environment, Sources, Fate, Effects, and Risks 2nd Edition*. Springer-Verlag Berlin Heidelberg New York. pp. 13-24.

Daughton, C. G. and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspective*. 107:907-938.

Davis, J.G., Truman, C.C., Kim, S.C., Ascough II, J.C., Carlson, K. 2006. Antibiotic Transport via Runoff and Soil Loss. *Journal of Environmental Quality* 35:2250-2260.

Díaz-Cruz, M. S., López de Alda, M. J., Barceló, D. 2003. Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *Trends in Analytical Chemistry*. 22:340-351.

Dietze, J.E., Scribner, E.A., Meyer, M.T., Koplin, D.W. 2005. Occurrence of antibiotics in water from 13 fish hatcheries, 2001-2003. *International Jorunal of Environmental Analytical Chemistry* 85:1141-1152.

EPA. 1998. Fate, Transport and Transformation Test Guidelines. OPPTS 835.1220 Sediment and Soil Adsorption/Desorption Isotherm. EPA 712-C-98-048. Washington, DC.: United States Environmental Protection Agency.

FDA. 1984. Environmental Impact Analysis Report, Romet-30 (Sulfadimethoxine + Ormetoprim) Medicated Premix for Control of Certain Salmonid Diseases. AMD4-72, 01AMD4. Rockville, MD.: United States Food and Drug Administration.

Figueroa, R. A., Mackay, A.A. 2005. Sorption of Oxytetracycline to Iron Oxides and Iron Oxide-Rich Soils. *Environmental Science and Technology* 39:6664-6671.

Furusawa, N. 2006. Determining sulphamonomethoxine and its acetyl/hydroxyl metabolites in chicken plasma under organic solvent-free conditions. *Analytical and Bioanalytical Chemistry* 385: 1570–1574.

Furusawa, N., Mukai, T. 1994. Simultaneous high-performance liquid chromatographic determination of residual sulphamonomethoxine, sulphadimethoxine and their N4-acetyl metabolites in foods of animal origin. *Journal of Chromatography A* 677:81-85.

Gao, J., Pedersen, J.A. 2005. Adsorption of Sulfonamide Antimicrobial Agents to Clay Materials. *Environmental Science and Technology* 39:9509-9516.

Göbel, A., Mcardell, C.S., Joss, A., Hansruedi, S., Giger, W. 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment* 372:361-371.

Göbel, A., Thomsen, A., Mcardell, C.S., Joss, A., Giger, W. 2005. Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment. *Environmental Science and Technology* 39:3981-3989.

Golet, E. M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W. 2003 Environmental exposure assessment of flouroquinolone antibacterial agests from sewage to soil. *Envronmental Science and Technology.* 37:3243-3249.

Guerard J., Chin Y.P. 2006. Photodegradation of Antibiotics Used in Aquaculture. Poster presented at the 2006 EPA Graduate Fellowship Conference. Washington, D.C. September 24-26.

Haggard, B.E., Galloway, J.M, Green, W.R., Meyer, M.T. 2006. Pharmaceuticals and other organic chemicals in selected North-Central and Northwestern Arkansas streams. *Journal of Environmental Quality* 35:1078-1087.

Haller, M.Y., Muller, S.R., McArdell, C.S., Alder, A.C., Suter, M.J.-F. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. *Journal of Chromotography A* 952:111-120.

Halling-Sørensen, B., Ingerslev, F. 2000. Biodegradability properties of sulfonamides in activated sludge. *Environmental Toxicology and Chemistry* 19:2467-2473.

Halling-Sørensen, B., Lützhøft, H.C.H., Anderson, H.R., Ingerslev, F. 2000. Environmental risk assessment of antibiotics: comparison of meillinam, trimethoprim and ciprofloxacin. *Journal of Antimicrobial Chemotherapy*. 46 (Suppl. S1): 53-58.

Halling-Sørensen, B., Nielsen, S. N., Jensen, J. 2002. *Environmental Assessment of Veterinary Medicines in Denmark*. Copenhagen: Danish Environmental Protection Agency.

Halling-Sørensen, B., Sengelov, G., Ingerslev, F., Jensen, L.B. 2003. Reduced Antimicrobial Potencies of Oxytetracycline, Tylosin, Sulfadiazin, Streptomycin, Ciprofloxacin, and Olaquindox Due to Environmental Processes. *Archives of Environmental Contamination and Toxicology* 44:7-16.

Hamscher, C., Pawelzick, H.T., Hoper, H., & Nau, H. 2004. Antibiotics in Soil: Routes of Entry, Environmental Concentrations, Fate and Possible Effects. *In* Kummer, K. (ed.) *Pharmaceuticals in the Environment, Sources Fate and Risk, 2nd Edition*. Springer-Verlag Berlin Heidelberg New York. pp. 139-147.

Hamscher, G., Priess, B., Nau, H. 2006. A survey of the occurrence of various sulfonamides and tetracyclines in water and sediment samples originating from

aquaculture systems in Northern Germany in Summer 2005. *Archiv fur Lebensmittelhygiene* 57:97-101.

Heberer, T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters* 131:5-7.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L. 1999. Occurrence of antibiotics in the aquatic environment. *Science of the Total Environment* 225: 109-118.

Holm, J.V., Rügge, K., Bjerg, P.L., Christensen, T.H. 1995. Occurrence and Distribution of Pharmaceutical Organic Compounds in the Groundwater Downgradient of a Landfill (Grindsted, Denmark). *Environmental Science and Technology* 29:1415-1420.

Jørgensen, S.E., Halling-Sørensen, B. 2000. Drugs in the environment (editorial). *Chemosphere* 40: 691-699.

Karickhoff, S.W. 1981. Semi-Empirical Estimation of Sorption of Hydrophobic Pollutants on Natural Sediments and Soils. *Chemosphere* 10:833-846.

Kay, P., Blackwell, P.A., Boxall, A.B.A. 2004. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environmental Toxicology and Chemistry* 23: 1136-1144.

Kay, P., Blackwell, P.A., Boxall, A.B.A. 2005a. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. *Chemosphere* 60:497-507.

Kay, P., Blackwell, P.A., Boxall, A.B.A. 2005b. Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere 2*59:951-959.

Kishida, K., Furusawa, N., 2004. Application of shielded column liquid chromatography for determination of sulfamonomethoxine, sulfadimethoxine, and their N_4-acetyl metabolites in milk. *Journal of Chromatography A* 1028:175-177.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zuagg, S.D., Barber, L.B., & Buxton, H.T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environmental Science and Technology* 36: 1202-1211.

Kreuzig, R., Holtge, S., Brunotte, J., Berenzen, N., Wogram, J., Schulz, R. 2005. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. *Environmental Toxicology and Chemistry* 24:777-781.

Ma, L., Selim, H.M. 1997. Evaluation of Nonequilibrium Models for Predicting Atrazine Transport in Soils. *Soil Science Society Journal of America* 61:1299-1307.

Manchand, P.S., Rosen, P., Belica, P.S., Oliva, G.V., Perrotta, A.V., Wong, H.S. 1992. Syntheses of Antibacterial 2,4-Diamino-5-benzylpyrimidines. Ormetoprim and Trimethoprim. *Journal of Organic Chemistry* 57:3531-3535.

McNamara N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R. 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Applied Soil Ecology* 24:117-132.

Mellon, M., Benbrook, C., Benbrook, K.L. 2001. *Hogging It: Estimates of Antimicrobial Abuse in Livestock*. Cambridge, MA: Union of Concerned Scientists.

Metcalfe, C.D., Miao, X.S., Koenig, B.G., Struger, J. 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. *Environmental Toxicology and Chemistry* 22:2881-2889.

Meyer, M. T., Bumgarner, J.E., Varns, J.L., Daughtridge, J.V., Thurman, E.M., Hostetler, K.A. 2000. Use of radioimmunoassay as a screen for antibiotics in confined animal feeding operations and confirmation by liquid chromatography/mass spectrometry. *Science of the Total Environment* 248(2-3): 181-188.

Miao, X.S., Bishay, F., Chen, M., Metcalfe, C.D. 2004. Occurrence of Antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environmental Science and Technology* 38:3533-3541.

NAA. 2005. Drugs Used in the US Aquaculture Industry. Auburn University National Aquaculture Association. Auburn, AL. Available at: www. nationalaquaculture.org. Accessed 26 September 2005.

NLM. 2006. ChemIDplus Lite. National Library of Medicine Specialized Information Services. Bethesda, MD.: Available at: http://chem2.sis.nlm.nih.gov/chemidplus/. Accessed 17 March 2006.

Oliveira, K.A.D.M., Mendonça, R.C.S., Oliveira, G.V.D., Sodré, A.F. 20052006. Antibiotic resistance of *Campylolobacter* from automated broiler farms. *Journal of Food Safety* 26:82-91.

Papiernik, S.K., Yates, S.R., Gan, J. 2002. Processes Governing Transport of Organic Solutes. In Dane and Topp (eds.) *Methods of Soil Analysis, Part 4, Physical Methods*. Soil Science Society of America, Inc. Madison, WI, USA. pp. 1451-1479.

Perret, D., Gentili, A., Marchese, S., Greco, A., Curini, R. 2006. Sulfonamide Residues in Italian Surface and Drinking Waters: A Small Scale Reconnaissance. *Chromatographia* 63:225-232.

Porro, I., Wierenga, P.J. 1993. Transient and steady-state solute transport through a large un-saturated soil column. *Groundwater* 31: 193-200.

Qtaitat, M.A. 2004. Study of the interaction of trimethoprim—montmorillonite by infrared spectroscopy. *Spectrochimica Acta Part A* 60:673-678.

Rabølle, M., Spliid, N.H. 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* 40:715-722.

Sassman, S. A., Lee, L.S. 2005. Sorption of Three Tetracyclines by Several Soils: Assessing the Role of pH and Cation Exchange. *Environmental Science and Technology*. 39:7452-7459.

Scheytt, T.J., Mersmann, P., Heberer, T. 2006. Mobility of pharmaceuticals carbamazepine, diclofenac, ibuprofen, and propyphenazone in miscible-displacement experiments. *Journal of Contaminant Hydrology* 83:53-69.

Scheytt, T.J., Mersmann, P., Leidig, M., Pekdeger, A., Heberer, T. 2004. Transport of Pharmaceutically Active Compounds in Saturated Laboratory Columns. *Journal of Contaminant Hydrology* 83:53-69.

Selim, H.M. 2007. Personal communication. February 14, 2007.

Selim, H.M., Davidson, J.M., Mansell, R.S. 1976. Evaluation of a two-site adsorption-desorption model for describing solute transport in soils. P. 444-448. Proc Summer Comp. Stimu. Conf., Washington, DC, July 12-14, 1976. Simulations Counsels, La Jolla, CA.

Selim, H.M., Davidson, J.M., Rao, P.S.C. 1977. Transport of reactive solutes through multilayered soils. *Soil Science Society of America Journal* 41:3-10.

Singh, P., Kanwar, R.S. 1991. Preferential solute transport through macropores in large undisturbed saturated soil columns. *Journal of Environmental Quality* 20:295-300.

Skaggs, T.H., Leij, F.J. 2002. Solute Transport: Theoretical Background. In Dane and Topp (eds.) *Methods of Soil Analysis, Part 4, Physical Methods.* Soil Science Society of America, Inc. Madison, WI, USA. pp. 1353-1380.

Skaggs, T.H., Wilson, G.V. Shouse, P.J., Leij, F.J. 2002. Solute Transport: Experimental Methods. In Dane and Topp (eds.) *Methods of Soil Analysis, Part 4, Physical Methods.* Soil Science Society of America, Inc. Madison, WI, USA. pp. 1381-1402.

Sposito, G. 1989. *The Chemistry of Soils*. Oxford University Press, Inc. New York, NY, USA. pp. 151-152.

SRAC. 2005. Catfish Facts. Mississippi State University Southern Regional Aquaculture Center. Starkville, MS. Available at: www.msstate.edu/dept/srac/catfish2.htm. Accessed 29 September 2005.

Sylvia, D.M., Hartel, P.G., Fuhrmann, J.J., Zuberer, D.A. 2005. *Principles and Applications of Soil Microbiology. 2nd Edition.* Pearson Education Inc., Upper Saddle River, NJ, USA. p.322

Tesch, C., Carlson, R. 2003. Assessment of water quality in the Sunnyside area, Washington County, Idaho: 2003 Update. ISDA Technical Results Summary No. 19. 14p.

The Green Book. 2005. U.S. Food and Drug Administration. 22 February 2005 <http://www.fda.gov/cvm/Green_Book/Chapter2.htm>.

Thiele-Bruhn, S. 2000. Adsorption of the antibiotic pharmaceutical compound sulfpyridine by a long-term differently fertilized loess Chernozem. *Journal Plant Nutrition and Soil Science* 163: 589-594.

Thiele-Bruhn, S. 2003. Pharmaceutical antibiotic compounds in soils – a review. *Journal Plant Nutrition Soil Science* 166: 145-167.

Thiele-Bruhn, S., Aust, M.O. 2004. Effects of Pig Slurry on the Sorption of Sulfonamide Antibiotics in Soil. *Archives of Environmental Contamination and Toxicology.* 47:31-39.

Thiele-Bruhn, S., Leinweber, P., Halling-Sørensen, B. 2003. Photodegradation and ageing of antibiotics pharmaceuticals on soil surfaces. Poster presented at ENVIRPHARMA, European Conference on Human and Veterinary Pharmaceuticals in the Environment, Lyon, France. April 14-16.

Thiele-Bruhn, S., Seibicke, T., Schulten, H.-R., Leinweber, P. 2004. Sorption of Sulfanamide Pharmaceutical Antibiotics on Whole Soils and Particle-Size Fractions. *Journal of Environmental Quality* 33:1331-1342.

Thurman, E.M., Dietze, J.E., Scribner, E.A. 2002. Occurrence of antibiotics in water from fish hatcheries. *USGS Fact Sheet 120-02* (November 2002; revised May 2003).

Tipton, D.K., Rolston, D.E., Scow, K.M. 2003. Transport and biodegradation of perchlorate in soils. *Journal of Environmental Quality* 32:40-46.

Tolls, J. 2001. Sorption of Veterinary Pharmaceuticals in Soils: A Review. *Environ. Sci. Technol*. 35:3397-3406.

Toride, N., Leij, F.J., van Genuchten, M. Th. 1999. CXTFIT. Code for Estimating Tranport Parameters from Laboratory or Field Tracer Experiment.

Wang, Q., Guo, M., Yates, S.R. 2006b. Degradation kinetics of manure-derived sulfadimthoxine in amended soil. *Journal of Agriculture and Food Chemistry* 54:157-163.

Wang, Q.-Q., Bradford, S.A., Zheng, W., Yates, S.R. 2006a. Sulfadimethoxine Degradation Kinetics in Manure as Affected by Initial Concentration, Moisture, and Temperature. *Journal of Environmental Quality* 35:2162-2169.

Weber, W.J., Kim, S.H., Johnson, M.D. 2002. Distributed reactivity model for sorption by soils and sediments. 15. High concentration co-contaminant effects on phenanthrene sorption and desorption. *Environmental Science and Technology* 36:3625-3634.

Westergaard, K., Müller, A.K., Christensen, S., Bloem, J., Sørensen, S.J. 2001. Effects of tylosin as a disturbance on the soil microbial community. *Soil Biology & Biochemistry* 33: 2061-2071.

Wilson B., Smith, V., Denotelles, F., Larive, C. 2003. Effects of three pharmaceutical and personal care products on natural fresh algal assemblages. *Environmental Science and Technology* 37:1713-1719.

Wollenberger, L., Halling-Sørensen, B., Kusk, K.O. 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. *Chemosphere* 40: 723-730.

Yu, Z., Huang, W. 2005. Competetive sorption between 17a-Ethinyl Estradiol and Naphthalene/Phenanthrene by sediments. *Environmental Science and Technology* 39:4878-4885.

Zhang, H., Huang, C.H. 2007. Adsorption and oxidation of fluoroquinolone antibacterial agents and structurally related amines with goethite. *Chemosphere* 66:1502-1512.

APPENDICES

Appendix A. Sample Calculations

Equation 3.6. Calculation of antimicrobial sorbed to the soil, S (μg·g-1), for Sand with OMP single solute

$$S = \frac{V_{aq}}{M_s} \cdot (C_i - C)$$

Given: Initial solution volume, V_{aq} = 0.02 L; Mass of the soil, M_s = 1 g; Input antimicrobial concentration, C_i = 500 μg·L^{-1}; and Equilibrium antimicrobial concentration, 480.6 μg·L^{-1}

Solution:

$$S = \frac{0.02}{1} \cdot (500 - 480.6) = 0.388 \text{ μg·g}^{-1}$$

Equation 3.9. Calculation of the carbon normalized sorption coefficient, K_{oc}, for Soil 1 with OMP single solute

$$K_{oc} = \frac{K_d}{f_{oc}}$$

where, f_{oc} is given by

$$f_{oc} = SOM \cdot 0.58$$

Given: Soil 1, soil organic matter, SOM = 1.5%; Soil 1 with OMP single solute sorption coefficient, K_d = 21.9 L·kg^{-1}

Solution:

$$f_{oc} = 1.5\% \cdot 0.58 = 0.87\%$$

$$K_{oc} = \frac{21.9}{0.87} = 25.17 \text{ L·kg}^{-1}$$

Equation 3.10. Calculation of the predicted carbon normalized sorption coefficient, K'_{oc}, for OMP

$$\log K'_{oc} = 0.989 \cdot \log K_{ow} - 0.346$$

Given: Octanol water partition coefficient for OMP, $\log K_{ow} = 1.23$

Solution:

$$\log K'_{oc} = 0.989 \cdot 1.23 - 0.346 = 0.8705$$

$$K'_{oc} = 10^{0.8705} = 7.42 \text{ L·kg}^{-1}$$

Sample Calculations for values in Table 4.3. (ex. Sand co-solute 4 cm column)

Bulk density, ρ_b

$$\rho_b = \frac{M_{soil}}{V_{total}}$$

$$V_{total} = \frac{\Pi d^2}{4} \cdot L$$

Given: Mass of the soil, $M_{soil} = 140$ g; Column diameter, $d = 5$ cm; Column length, $L = 4$ cm

Solution:

$$V_{total} = \frac{\Pi 5^2}{4} \cdot 4 = 78.54 \text{ cm}^3$$

$$\rho_b = \frac{140}{78.54} = 1.783 \text{ g·cm}^{-3}$$

Volumetric water content, θ, for saturate soil

$$\theta = 1 - \frac{\rho_b}{2.65}$$

Solution;

$$\theta = 1 - \frac{1.783}{2.65} = 0.327 \text{ cm}^3\text{·cm}^{-3}$$

Pore water velocity, v

$$v = \frac{q}{\theta}$$

$$q = \frac{Q}{A}$$

Given: Flow rate, Q = 3 $cm^3 \cdot min^{-1}$; cross-sectional area, A = 19.63 cm^2

Solution:

$$q = \frac{3}{19.63} = 0.153 \text{ cm} \cdot \text{min}^{-1}$$

$$v = \frac{0.153}{0.327} = 0.467 \text{ cm} \cdot \text{min}^{-1}$$

Pore volume, PV, for saturated conditions

$$PV = V_{total} \cdot \theta$$

Solution:

$$PV = 78.54 \cdot 0.327 = 25.710 \text{ cm}^3$$

Column Residence Time, RT

$$RT = \frac{PV}{Q}$$

Solution:

$$RT = \frac{25.710}{3} = 8.6 \text{ min} = 0.14 \text{ hr}$$

Mass Recovery calculations, Table 4.4 for Sand OMP co-solute

$$\text{Mass Recovery} = \frac{\sum \frac{C}{C_0} \cdot \Delta PV}{PulseRPV}$$

Given: The pulse relative pore volume, *Pulse RPV* = 84.01; The sum of the relative concentration times the change in pore volumes, $\Sigma C/C_0 \cdot \Delta PV = 79.782$

Solution:

$$\text{Mass Recovery} = \frac{79.782}{84.01} = 0.95$$

Appendix B. Illustration of OMP and SDM compatibility with polypropylene

Objective: Determine whether polypropylene or glass tubes were more compatible with OMP and SDM.

Procedure for polypropylene: OMP solutions (C_0= 60 $\mu g \cdot L^{-1}$) were developed in polypropylene volumetric flasks and administered to acid washed and non-acid washed polypropylene tests tubes with polypropylene screw on caps. SDM solutions (C_0= 150 $\mu g \cdot L^{-1}$) were developed in polypropylene volumetric flasks and administered to acid washed and non-acid washed polypropylene tests tubes with polypropylene screw on caps.

Procedure for glassware: OMP solutions (C_0= 60 $\mu g \cdot L^{-1}$) were developed in glass volumetric flasks and administered to acid washed and non-acid washed glass tests tubes with PTFE screw on caps. SDM solutions (C_0= 150 $\mu g \cdot L^{-1}$) were developed in glass volumetric flasks and administered to acid washed and non-acid washed glass tests tubes with PTFE screw on caps.

Analysis: Antimicrobial solutions were left in the test tubes for 3 days. Hereafter, samples were prepared for analysis using the procedure described in Chapter 4, Miscible Displacement Experiments. Samples were analyzed on the LCMSMS in cooperation with the AL Department of Agriculture and Industries, Food and Drug Lab.

Results and Discussion: OMP was compatible with both acid washed and non-acid washed glass and polypropylene tubes. SDM, however, was only compatible with acid washed and non-acid washed polypropylene tubes. It was observed from this experiment that acid washing the polypropylene yielded the same results when polypropylene was not acid washed.

Antimicrobial	Tube Type	Prep Method	C_0 ($\mu g \cdot L^{-1}$)	C ($\mu g \cdot L^{-1}$)	C/C_0	% Diff
OMP	Glass	Acid Wash	60	59.744	1.00	0.4
OMP	Glass	Acid Wash	60	56.157	0.94	6.4
OMP	Glass	No Acid Wash	60	59.859	1.00	0.2
OMP	Glass	No Acid Wash	60	57.464	0.96	4.2
OMP	Polypropylene	Acid Wash	60	55.729	0.93	7.1
OMP	Polypropylene	Acid Wash	60	58.519	0.98	2.5
OMP	Polypropylene	No Acid Wash	60	59.447	0.99	0.9
OMP	Polypropylene	No Acid Wash	60	58.402	0.97	2.7
SDM	Glass	Acid Wash	150	57.054	0.38	62.0
SDM	Glass	Acid Wash	150	62.299	0.42	58.5
SDM	Glass	No Acid Wash	150	25.898	0.17	82.7
SDM	Glass	No Acid Wash	150	11.497	0.08	92.3
SDM	Polypropylene	Acid Wash	150	148.585	0.99	0.9
SDM	Polypropylene	Acid Wash	150	169.062	1.13	12.7
SDM	Polypropylene	No Acid Wash	150	146.469	0.98	2.4
SDM	Polypropylene	No Acid Wash	150	159.560	1.06	6.4

Conclusions: This experiment determined that the use of polypropylene was preferred to that of glassware, especially for SDM. All laboratory containers were therefore changed to polypropylene.