

FINAL PROJECT REPORT

for

**Fate of Representative Fluoroquinolone, Macrolide, Sulfonamide and Tetracycline
Antibiotics In Subsurface Environments**

Submitted to

University of Wisconsin Water Resources Institute

by

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PROJECT SUMMARY

Title: Fate of Representative Fluoroquinolone, Macrolide, Sulfonamide and Tetracycline Antibiotics in Subsurface Environments.

Project I.D.: WR03R008

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Background/Need:

The problem of antibiotics entering the environment from municipal and agricultural sources is well recognized and is a matter of growing concern. Several reports documenting the presence of antibiotics in groundwater, both in the U.S. and Europe, have been published within the last decade. Since soils are the recipients of various types of wastes and have the capability to serve as potential “sinks,” a better understanding of processes controlling the interaction of antibiotics with soils is required. Quantification of the extent of sorption and elucidation of underlying mechanisms of interaction with soil constituents is especially important, because sorption determines the fraction of antibiotics that is available for transport to groundwater or is biologically active. Antibiotics possess molecular properties expected to favor their sorption to soil components. There is a paucity of information related to the fate and transformation of antibiotics in soil/water environments.

In a recent WI statewide survey of wastewater treatment plants funded by the GCC (Karthikeyan and Meyer, 2006), members of five antibiotic compound classes were detected in the following order of frequency: tetracyclines and trimethoprim > sulfonamides > macrolides > fluoroquinolones. These compounds were also detected in the first nationwide survey conducted by the USGS (Kolpin et al., 2002). In the WI survey, sulfamethoxazole (a sulfonamide) and tetracycline were also detected in groundwater monitoring wells adjacent to the treatment systems. Sorption of these antibiotics to soils can be expected to be controlled by the nature of their interactions with important soil components, such as clay minerals, hydrous metal oxides, and organic matter (both in dissolved and particle-bound forms).

Objectives:

The study objectives were to: (1) quantify the extent of sorption of selected antibiotics (one compound from each family of antibiotics detected in the WI survey: tetracyclines, sulfonamides, macrolides, fluoroquinolones) to humic substances (HS) associated with hydrous iron and aluminum oxides and smectitic clays; and (2) investigate antibiotic association with dissolved organic matter and how such association facilitates antibiotic transport under unsaturated flow conditions.

Methods:

A combination of macroscopic batch experiments and spectroscopic (using FTIR) analyses was employed to accurately quantify the extent of sorption as well as to elucidate underlying mechanisms. Radiolabeled versions of antibiotic compounds were used to facilitate monitoring the extent of parent compound sorption and transformation. Antibiotic-sorbent systems studied include: (a) tetracycline interaction with hydrous Al oxide (HAO), hydrous Fe oxide (HFO), humic acid (HA), and HAO-HA complexes; (b) ciprofloxacin (a fluoroquinolone) interaction with HAO and HFO; (c) sulfamethazine (a sulfonamide) interaction with montmorillonite, kaolinite, and humic acid-clay complexes; and (d) clarithromycin (a macrolide) complexation with HA. All experiments were conducted in the Departments of Biological Systems Engineering and Soil Science at the University of Wisconsin – Madison.

Results and Discussion:

Both tetracycline and ciprofloxacin formed strong inner-sphere complexes with HAO and HFO and the zwitterionic species appeared to be primarily involved in sorption interactions. The surface interaction of

antibiotics with hydrous oxides occurred primarily on the edge Al/Fe atoms of HAO/HFO minerals, since these sites are not fully coordinated with the hydroxyl groups and are highly active. Ligand-promoted dissolution of hydrous oxides was observed during sorption and this process could conceivably increase the mobility of antibiotics. The tetracycline-promoted dissolution was more pronounced than ciprofloxacin, attributable to differences in the formation constants for the corresponding metal-antibiotic complexes.

Sulfonamide antimicrobial interaction with clay minerals was strongly pH-dependent, consistent with sorbate speciation and clay properties. Surface charge density appeared to influence sorption by determining adsorption domain size. Adsorption of uncharged sulfamethazine to montmorillonite was relatively insensitive to pH, ionic strength and type of exchangeable cation, while that to kaolinite was highly sensitive to ionic strength. Adsorption of cationic sulfamethazine to montmorillonite exceeded that of the neutral species by one to two orders of magnitude, but was unimportant for kaolinite at the pH values examined. Cation exchange appeared to contribute to sorption of cationic sulfonamide species to montmorillonite. Anionic sulfamethazine adsorption was negligible. The nature of the variable sulfonamide R group influenced the degree of adsorption of cationic and neutral species.

The interaction of tetracycline and clarithromycin with humic substances (HS) was influenced by both pH and ionic strength. Complexation of cationic/zwitterionic tetracycline species with deprotonated sites in HA (mainly carboxylic functional groups) appeared to be the major underlying sorption mechanism. Humic acid possessed a moderate sorption capacity for tetracycline with maximum sorption being less than 10% of tetracycline added ($\log K_{\text{doc}}$ values up to ~ 3.4). In contrast, clarithromycin sorption was strong ($\log K_{\text{doc}}$ values up to ~ 4.1). Clarithromycin was cationic over most of the pH range examined. Sorption of clarithromycin was strongly influenced by HA speciation. Aggregation of HA was observed to occur in the presence of tetracycline, which could help explain the hysteretic behavior observed in the sorption-desorption isotherms. This hysteretic behavior, indicative of irreversible sorption, could impact the bioavailability and degradation of humic-complexed tetracycline as well as enhance its mobility. Clarithromycin interaction with HA did not display sorption-desorption hysteresis.

Significant suppression of tetracycline sorption to HAO was observed in the presence of HA. Therefore, the presence of HS, both in dissolved and particle forms, could increase the subsurface mobility of tetracycline. It is expected that in organic-rich soil environments, tetracycline would have a high potential for off-site migration via both surface and subsurface pathways. In contrast, the sorption of sulfamethazine increased when clay minerals were coated with HA, and sorption-desorption hysteresis was observed.

Conclusions/Implications/Recommendations:

The study results indicate that propensity for antibiotics to migrate from the soil surface to groundwater depends on number of factors including their pH-dependent speciation, the ionic strength of the infiltrating water and the composition of the soil. Sulfonamide antimicrobials interact rather weakly with clay minerals and HS, suggesting that these compounds will be relatively mobile. In contrast, tetracycline and fluoroquinolone antibiotics interact strongly with HAO and HFO. Soils rich in these components (and relatively poor in HS) are expected to hinder the movement of these antibiotics through soil to groundwater. On the other hand, dissolved HS could increase the mobility of antibiotics by facilitating transport or by hindering their sorption to mineral surfaces (in the case of tetracyclines), thereby enabling inter-compartmental transfer of these compounds.

Related Publications:

- Gao, J.; Pedersen, J.A. 2005. Adsorption of sulfonamide antimicrobial agents to clay minerals. *Environ. Sci. Technol.* 39, 9509 - 9516.
- Gu, C.; Karthikeyan, K.G. 2005. Sorption of the antimicrobial ciprofloxacin to aluminum and iron hydrous oxides. *Environ. Sci. Technol.* 39, 9166-9173.
- Gu, C.; Karthikeyan, K.G. 2005. Interaction of tetracycline with aluminum and iron hydrous oxides. *Environ. Sci. Technol.* 39, 2660-2667.
- Gu, C.; K.G. Karthikeyan, J. A. Pedersen, Sibley, S.D. Complexation of the antibiotic tetracycline with humic acid. *Chemosphere (in review)*.

Key Words: Ciprofloxacin, clarithromycin, sulfamethazine, tetracycline, hydrous aluminum oxide, hydrous iron oxide, montmorillonite, kaolinite, humic acid, humic-mineral complexes, FTIR spectroscopy

Funding: UW-Water Resources Institute

INTRODUCTION:

Occurrence of Antibiotics in Groundwater. The problem of antibiotics entering the environment from municipal sources and confined animal feeding operations is well recognized and is a matter of growing concern. The last decade has seen a growing number of published reports documenting the occurrence of antibiotics in groundwater. Some of the earliest reports of antibiotic contamination of groundwater stem from Europe. Holm et al. (1995) reported the presence of sulfonamide antibiotics in a leachate plume emanating from a Danish landfill. Significant attenuation of these seemed to occur in a strongly anaerobic zone of the aquifer. Hartig and Jekel (2001) reported the occurrence of several sulfonamide bacteriostatics in Berlin drinking water wells for which 80% of the groundwater was bank-filtered surface water. Sacher et al. (2001) detected anhydroerythromycin and sulfamethoxazole in groundwater in Germany. Several U.S. studies have identified antibiotics in groundwater downgradient from landfills, and in wells at bank filtration sites. Eckel et al. (1993) tentatively identified four sulfonamide compounds in a leachate plume downgradient from a landfill containing hospital waste. Heberer et al. (2001) reported that a water supply well in a Nebraska bank filtration site was contaminated with sulfamethoxazole and trimethoprim. Lindsey et al. (2001) also detected sulfamethoxazole in groundwater from Washington. In the first national reconnaissance survey on *emerging organic contaminants* conducted by the US Geological Survey (USGS), antibiotics were detected in half of the sites from a network of 139 streams sampled across 30 states (Kolpin et al., 2002). A total of six antibiotic compounds were detected including two sulfonamides, one tetracycline, fluoroquinolone, macrolide, and trimethoprim in our Wisconsin (WI) statewide survey of wastewater treatment plants (Karthikeyan and Meyer, 2006).

Mobility of Pharmaceuticals in Subsurface Environments. Soil serves as a major repository for contaminants in the environment; however, it can act as a source of antibiotic compounds when surface runoff or leaching occurs (Thiele-Bruhn, 2003; Pedersen et al., 2005). Hence, it is important to understand the factors controlling the surface and subsurface mobility of antibiotics. Sorption processes are particularly important, since association of antibiotics with mineral particles and organic matter (OM) will determine their extraction into surface runoff, leaching through soils, and transport in aquifers. Compared to more well-studied xenobiotics (e.g., pesticides, PAHs, PCBs), very little information is available on the fate and transformation of antibiotics in soil/water environments. A striking difference between antibiotics and the xenobiotics mentioned above is in their hydrophobicity and aqueous solubility. The high polarity (i.e., lower K_{ow} values) and aqueous solubility of antibiotics can enhance their environmental mobility. However, antibiotics possess molecular properties expected to favor sorption to soil components (Daughton and Ternes, 1999), which would control the potential for antibiotics to enter groundwater systems and their attenuation in subsurface environments. An adequate understanding of relevant sorption processes is necessary to assess the vulnerability of groundwater resources to antibiotic contamination and to predict their transport and attenuation in subsurface environments.

The uptake of hydrophobic nonionic organic compounds by soils and sediments is usually assumed to be controlled by partitioning to OM-phases and is described by the organic carbon-normalized solid-water distribution coefficient, K_{oc} . Unlike hydrophobic nonionic organic chemicals, the high water solubility and polar nature of many human and veterinary pharmaceuticals indicate that sorption mechanisms in addition to partitioning to OM-phases may be relevant. These additional mechanisms include surface ligand exchange, complexation, hydrolysis, cation bridging, hydrogen bonding, electrostatic and polarization interactions (Stumm, 1992). Determining the relative importance of surface-related sorption mechanisms is essential for developing the ability to predict pharmaceutical movement in subsurface environments. Tolls (2001) reviewed the literature on the sorption of antibiotics used in animal husbandry and concluded the assumption that sorption of these compounds is controlled solely by hydrophobic partitioning to soil OM is conceptually inappropriate. Many of veterinary antibiotics displayed solid-water distribution coefficients (K_d) significantly higher than would be expected from partitioning to OM alone (Tolls, 2001). Interaction with clay mineral surfaces appears to be especially

important for some compounds (Sithole and Guy, 1987; Nowara et al. 1997), although specific interactions with OM are also possible. The relative importance of these processes needs to be elucidated to assess the potential mobility of antibiotics in the subsurface environment.

Interactions between whole soils and antibiotics are expected to be fairly complex with numerous competing reactions and controlling factors. Although sorption to whole soils can be directly quantified, the approach renders the elucidation of underlying interaction mechanisms difficult. Therefore, we chose to systematically evaluate the role to various model soil components on the sorption of selected antibiotics (fluoroquinolones, macrolides, sulfonamides and tetracyclines) as a function of solution chemistry. The model soil components employed included the hydrous oxides of Fe (HFO) and Al (HAO), the clay minerals (montmorillonite, Mte; kaolinite, Kte), soil humic acids (Elliott soil humic acid, ESHA; Summit Hill soil humic acid, SHHA), and organo-mineral complexes (ESHA-HAO, ESHA-Mte). These components were chosen to represent inorganic minerals, soil humic substances (HS), and humic-mineral complexes, respectively. HAO, HFO, Mte and Kte are important mineral components of environmental particles. Montmorillonite is a wide-spread, reactive soil mineral (Borchardt, 1989). Kaolinite is also a common soil mineral, found most abundantly in older soils (Dixon, 1989). In highly weathered soils, HAO/HFO can account for as much as 50% of the total soil mass (Summer, 2000). Although they may not be found in large quantities in soils, the hydrous oxides are considered major “sinks” for many contaminants because of their high surface area and reactivity (Goldberg and Johnston, 2001). Due to their high affinity for natural OM, humic-coated mineral surfaces are prevalent in soils. These sorbents assume significance, since the charge properties and reactivity of mineral surfaces towards organic species are altered by the presence of these secondary coatings (Kretzschmar et al., 1997).

The overall goal of this project was to determine the extent that association with soil minerals, particle-bound OM and dissolved natural OM influences the mobility of antibiotics in soils and subsurface environments. We focused on representative antibiotics from four major classes, namely, fluoroquinolones, macrolides, sulfonamides, and tetracyclines, which have been detected in wastewater influent and effluent in WI (Karthikeyan and Meyer, 2006) and in streams throughout the U.S. (Kolpin et al., 2002). Specific objectives were to: (i) quantify the extent of sorption of tetracycline, fluoroquinolone and sulfonamide antibiotics to iron and aluminum hydrous oxides and smectitic clays; (ii) quantify the extent of sorption of tetracycline and sulfonamide antibiotics to humic substances associated with these minerals; and (iii) investigate the complexation of representative tetracycline and macrolide antibiotics with natural OM.

The results from this study are expected to improve our understanding of the sorption processes and should help in assessing the ability of soil to act as a potential “reservoir” for the antibiotics. In addition, emphasis was placed to determine the ability of dissolved OM to facilitate the sub-surface migration of antibiotics.

PROCEDURES AND METHODS

Antibiotic Compounds. Sorption experiments were conducted with radiolabeled antibiotics ([7-³H]-tetracycline, [3,5-³H]-sulfamethazine, [2-¹⁴C]-ciprofloxacin, [methyl-³H]-clarithromycin). Stock solutions were prepared as mixtures of unlabeled and radiolabeled antibiotic, such that the radiolabeled form contributed <1% the total amount of antibiotic. Fresh stock solutions were prepared for each experiment.

Sorbent Preparation. Clay minerals were obtained from the Clay Minerals Society Source Clays Repository (West Lafayette, IN) and included two Mte clays (SWy-2, Crook County, Wyoming, USA; SAz-1, Apache County, Arizona, USA) and a well-crystallized Kte clay (KGa-1b, Washington County, Georgia, USA). Clays were saturated with Na⁺, and particles with effective hydrodynamic diameters of 0.5–2 μm were obtained by wet sedimentation. Samples of SWy-2 were subjected to homoionization with

Li^+ , K^+ , Ca^{2+} and Mg^{2+} . Permanently charge-reduced SAZ-1 was prepared by the Hoffman-Klemen method following the protocol of Brindley and Ertem (1971).

HAO was synthesized by gradual neutralization of a 0.5 M AlCl_3 solution to pH 7 using 0.5 M NaOH (Huang et al., 1977). HFO was precipitated by dissolving $\text{Fe}(\text{NO}_3)_3$ salt using 0.01 M HCl and then rapidly increasing the solution pH to 7.0 using 0.1 M NaOH (Schwertmann and Cornell, 2000). The hydrous oxides were characterized with BET surface area measurement, zeta potential for determining zero point of charge (ZPC), and X-ray diffraction analysis. The BET surface area and pH_{ZPC} for the hydrous oxides were, respectively: HAO: $386 \pm 2 \text{ m}^2 \text{ g}^{-1}$ and 9.5; HFO: $322 \pm 1 \text{ m}^2 \text{ g}^{-1}$ and 8.7. ESHA (1S102H) was obtained from the International Humic Substance Society (University of Minnesota, St. Paul, MN) and used as received.

ESHA-HAO complexes were prepared following the method of Chorover et al. (1999). Three g of HAO were reacted with ESHA in 1.5 L of 0.01 M NaCl for 72 h at room temperature. Two different initial concentrations of ESHA (18.4 and 106.7 mg C L^{-1}) were chosen, as determined from the ESHA-HAO sorption isotherm, to achieve significantly different humic surface coverages on HAO.

Humic-clay complexes were prepared following the protocol of Wang and Xing (2005). To obtain different HA loadings on the smectite clay minerals, HA stock solution was added at HA-to-clay ratios of 1:5, 1:50 or 1:100 (w/w). After coating, HA-clay complexes were freeze-dried and stored in room temperature.

Batch Sorption Experiments. Experiments were conducted to obtain both pH-dependent relationships (pH 4 to 10) and sorption isotherms at varying sorbate-to-sorbent ratios. For each batch system, 0.03 g (oven dried mass) of hydrous oxides or humic-mineral complexes were added to tared 15 mL glass centrifuge tubes (solid-to-solution ratio of 1:500). Varying proportions of 0.01 M HCl and NaOH were used for pH adjustment. Stock antibiotic solutions were added to obtain a constant initial concentration of 0.1 mM (to examine pH-dependence of sorption) and varying concentrations (5×10^{-4} to 0.5 mM) to generate the sorption isotherms. Suspensions were equilibrated at 25 °C by end-over-end rotation at 7 rpm for 24 h. pH was measured immediately after equilibration by an Accumet AR-50 pH/conductivity meter. Suspensions were centrifuged at 5083 RCF for 20 min and supernatant was analyzed for ^{14}C or ^3H radioactivity levels by liquid scintillation counting and Al/Fe concentrations on an atomic absorption spectrometer. For tetracycline, additional quantification was performed using HPLC. The amount of antibiotics sorbed to the hydrous oxides or humic-mineral complexes was determined based on the difference between initial and equilibrium ^3H or ^{14}C radioactivity levels.

DOM-Complexation Experiments. Equilibrium dialysis was used to investigate antibiotics sorption to HA. Spectra/Pro 6 dialysis membrane with a nominal molecular weight cutoff (MWCO) of 2000 Da was pre-equilibrated in appropriate background electrolyte for 24 h and then washed thoroughly with MilliQ-grade deionized (DI) water prior to placement in a 60-mL amber glass bottle filled with background solution of desired pH and ionic strength (I). Proton activity was adjusted with NaOH or HCl to achieve pH values between 2.5 and 10; I was set at 0.01 or 0.1 M using NaCl. The dialysis cell was filled with the external solution, and a volume of concentrated stock ESHA solution ($480 \text{ mg}_{\text{DOC}} \text{ L}^{-1}$; $\text{pH} \approx 7.0$) was added to provide a dissolved organic carbon (DOC) concentration of $24 \text{ mg}_{\text{DOC}} \text{ L}^{-1}$ and sealed. The total suspension mass was 50 g with 10 g inside the dialysis cell. Stock radiolabeled tetracycline solution was added to the external compartment to obtain a total system tetracycline concentration of 0.1 mM. ESHA-tetracycline suspensions were agitated in the dark at 25 °C in a platform orbital shaker at 60 rpm for 3 d. At the end of the equilibration period, pH and ^3H activity of the internal and external solutions were determined. The quantity of ^3H label removed from solution after interaction with ESHA was determined from the difference between internal (free and ESHA-bound) and external (free) ^3H activities (Carter and Suffet, 1982; Karthikeyan and Chorover, 2000). Sorption isotherms were generated by equilibrating different amounts of tetracycline (5×10^{-4} to 1.5 mM) with ESHA ($24 \text{ mg}_{\text{DOC}} \text{ L}^{-1}$).

Spectroscopic Analysis. The interaction between ciprofloxacin and HFO/HAO was investigated using ATR-FTIR spectroscopy in aqueous environments. ATR-FTIR spectra were collected using a FTS7000 spectrometer (256 scans with a spectral resolution of 4 cm^{-1}). For tetracycline, FTIR spectra were generated on freeze-dried samples using a FTS7000 spectrometer with a photoacoustic detector.

RESULTS AND DISCUSSION

Tetracycline Sorption to HAO and HFO. Sorption of tetracycline to HAO increased with increasing pH up to pH 7 (no such trend for HFO) above which it decreased at higher pH values for both the hydrous oxides. Ligand-promoted dissolution is occurring during tetracycline sorption to these hydrous oxides (Fig. 1), which was more significant for HAO than HFO attributable to the difference in labile surface sites between these two sorbents. The ability of tetracycline to form strong complexes with Fe and Al will increase the solubility of these minerals. Sorption of tetracycline was quite rapid and equilibrium was achieved after 8 h. However, soluble metal (Me: Al or Fe) concentrations attained equilibrium only after 24 h. Ligand-promoted dissolution appears to be a two-step process; initially 1:1 Me-tetracycline soluble complexes are formed and as the reaction progresses 2:1 complexes existed. Increasing I (0.01 to 0.5 M) decreased the sorption extent only at higher sorbate-to-sorbent ratios suggesting the dominance of inner-sphere type complexes at low equilibrium tetracycline concentrations. Spectroscopic evidence indicates that tricarbonylamide and carbonyl functional groups of tetracycline could be responsible for sorption to mineral surfaces. These results are further discussed in Gu and Karthikeyan (2005a).

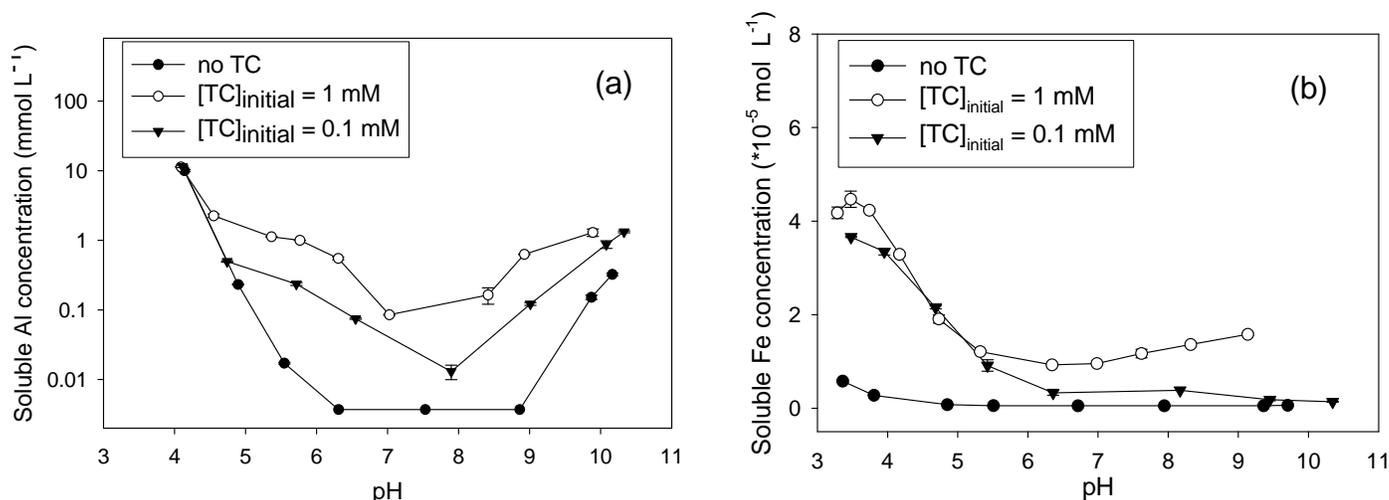


Figure 1. Solubility of (a) HAO and (b) HFO as a function of pH in the presence and absence of tetracycline ($[\text{TC}]_{\text{initial}} = 0.1\text{ mM}; 1\text{ mM}$; ionic strength (I) = 0.01 M NaCl; equilibration time = 24h).

Ciprofloxacin Sorption to HAO and HFO. Sorption of ciprofloxacin to both the hydrous oxides showed a strong pH-dependent behavior, following the fraction of zwitterionic species over the entire pH range studied. Increase in I from 0.01 to 0.5 M had an insignificant effect on the extent of ciprofloxacin sorption, and isotherms ($\text{pH} = 7.1$) were well-described by the Langmuir model. HFO possessed a higher sorption capacity ($0.066\text{ mmol kg}^{-1}$) than HAO ($0.041\text{ mmol kg}^{-1}$). Ligand-promoted dissolution of hydrous oxides, more pronounced for HAO, was observed in the presence of ciprofloxacin, but at a fairly high initial concentration (0.5 mM). Attenuated total reflectance Fourier-transform infrared spectroscopy analysis indicated that different type of ciprofloxacin surface complexes are formed with HAO and HFO (Fig. 2); while a *monodentate mononuclear* complex (with $-\text{COO}^-$) appears likely between ciprofloxacin and HAO, keto-O and one O from COO^- seem to be involved in the formation of a *six-member ring* with Fe on HFO surface. These results are further discussed in Gu and Karthikeyan (2005b).

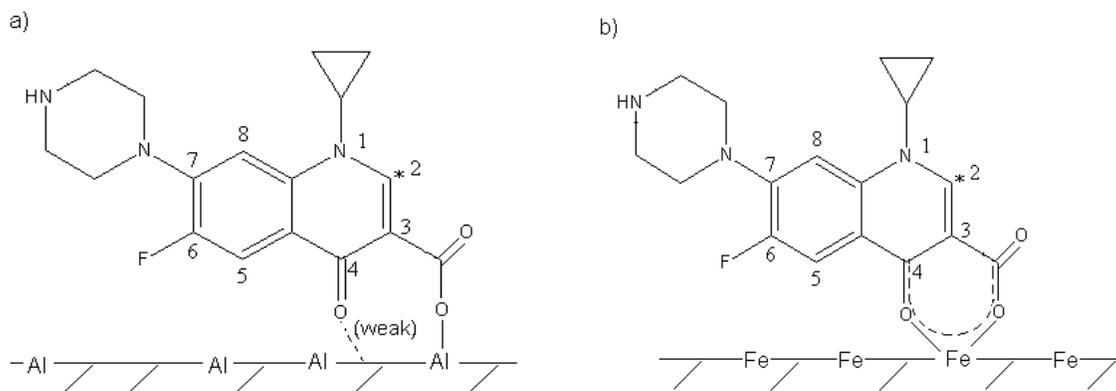


Figure 2. Proposed surface structures for ciprofloxacin complexation on the surface of a) HAO and b) HFO.

Sulfamethazine Sorption to Clay Minerals. Adsorption of three sulfonamide antimicrobials to clay minerals was investigated as a function of pH, I and type of exchangeable cation. Sulfonamide antimicrobial adsorption exhibited pronounced pH dependence consistent with sorbate speciation and clay properties (Fig. 3). Sulfonamide antimicrobials did not intercalate into montmorillonite (SWy-2, SAz-1), and surface charge density influenced sorption by determining adsorption domain size. Adsorption edge data were best fit to a model including terms for the cationic and uncharged species. Adsorption of uncharged sulfamethazine to montmorillonite was relatively insensitive to pH, I (Fig. 3) and type of exchangeable cation, while that to kaolinite (KGa-1b) was highly sensitive to ionic strength. Adsorption of cationic sulfamethazine to montmorillonite exceeded that of the neutral species by one to two orders of magnitude, but was unimportant for kaolinite at the pH values examined. Cation exchange appeared to contribute to sorption of cationic sulfonamide species to montmorillonite. Anionic sulfamethazine adsorption was negligible. The nature of the sulfonamide R group influenced the degree of adsorption of cationic and neutral species. Our results highlight the importance of considering sulfonamide speciation and clay surface charge density in predicting the transport of these antimicrobials. These results are further described in Gao and Pedersen (2005).

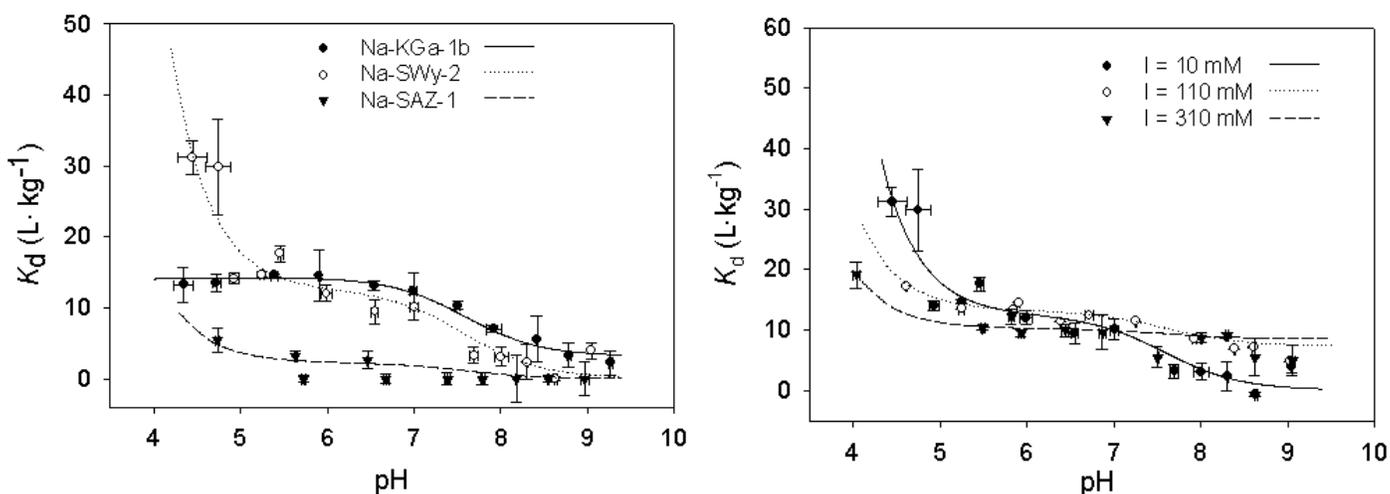


Figure 3. Effects of (a) pH and (b) I on sulfamethazine adsorption to near homoionic clays. In (a) $I = 10$ mM. In (b) solutions were 10 mM NaHCO_3 ; the desired ionic strength was achieved by addition of NaCl . Initial SMZ concentration, $C_0 = 3.5 \mu\text{M}$. Symbols represent means of triplicate measurements; error bars indicate one standard deviation. Trendlines represent best fits to a linear model accounting for the cationic and uncharged (and for $I = 110$ and 310 M, anionic) species (Gao and Pedersen, 2005).

Tetracycline Complexation with Humic Acid. Tetracycline speciation strongly influenced its sorption to ESHA over the entire pH range studied. Sorption was strongly pH-dependent with a maximum near pH 4.3, and competition with H^+ and electrolyte cation (Na^+) was evident. The pH-dependent trend is consistent with complexation between the cationic/zwitterionic species of tetracycline and deprotonated sites in ESHA (mainly carboxylic functional groups) as the primary underlying sorption mechanism. Modification of ESHA by Ca^{2+} addition increased tetracycline sorption suggesting that ternary complex formation (ESHA-metal-tetracycline) could be important at higher multivalent metal concentrations. The macroscopic data (pH-envelope and sorption isotherms) were successfully modeled using a discrete log K function ($pK_a = 3, 5, 7$ and 9) with the FITEQL 4.0 chemical equilibrium program indicating that ESHA-tetracycline interaction could be reasonably represented as complex formation of a monoacid with discrete sites in humic acid. Sorption-desorption isotherms revealed the hysteretic behavior of tetracycline (Fig. 4), and both isotherms were well described by the Freundlich equation. Aggregation of ESHA in the presence of tetracycline was observed by flow field-flow fractionation measurement, which could have resulted in different microscopic pathways during sorption and desorption processes causing the observed hysteretic behavior. These results are further discussed in Gu et al. (*in review*).

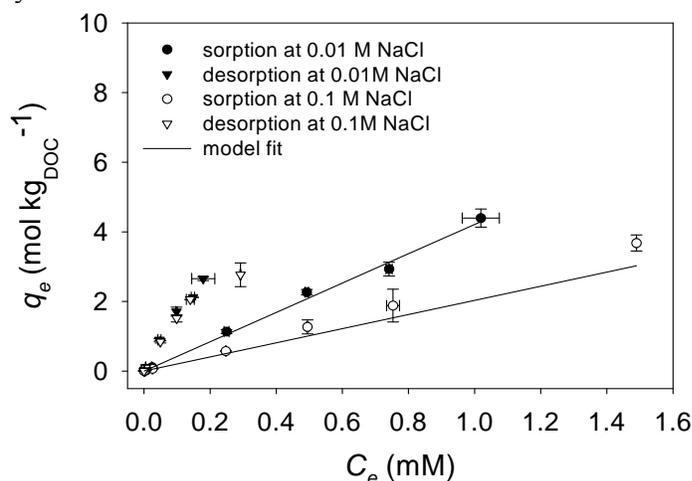


Figure 4. Sorption-desorption isotherms for tetracycline interaction with ESHA at two ionic strength (I) values ($pH = 4.3 \pm 0.1$). C_e denotes the equilibrium tetracycline concentration, and q_e represents the amount of tetracycline sorbed to ESHA. Mean values and ranges for q_e and C_e are reported (range values if not shown are within the symbols).

Clarithromycin (CLAR) Complexation with Humic Acid. We investigated the interaction of radiolabeled H^3 -clarithromycin, a semi-synthetic derivative of erythromycin, with dissolved ESHA. We determined K_{doc} values using equilibrium dialysis over a range of environmentally relevant proton activities and solution chemistries. Clarithromycin association with ESHA: (1) reached a maximum at near-neutral pH values (Fig. 4); (2) was well described by the Freundlich model and was reversible (i.e., no sorption-desorption hysteresis); (3) decreased with increasing concentrations of competing molecules (e.g., erythromycin); and (4) was larger than predicted by hydrophobic partitioning alone. Overall, these trends suggest the importance of electrostatic interactions and hydrogen-bonding for CLAR association with colloidal organic matter (COM). Furthermore, our data demonstrate the critical need to consider a variety of solution conditions when assessing the environmental fate of ionic organic compounds such as CLAR. This is especially true when COM (the chemistry of which is also strongly dependent on solution conditions) is considered an important phase in partitioning and transport. For example, CLAR association with ESHA increased from pH 4–6.3, while CLAR remained predominately cationic and deprotonation of ESHA carboxylic acids resulted in electrostatic attraction. K_{doc} decreased above pH 6.3, as the neutral form of CLAR becomes more abundant and the charge of ESHA carboxylic acid and phenolic moieties becomes increasingly negative (Fig. 5). Furthermore, deprotonation of ESHA diminishes its H-bond-donating capacity and decreases its interaction with CLAR, which possesses an abundance of H-bond accepting oxygen functional groups. Sorption and desorption isotherms displayed nonlinearity ($n = 0.85 - 0.90$) suggesting that in addition to hydrophobic partitioning, specific interactions with ESHA were important in the association of CLAR with dissolved humic substances.

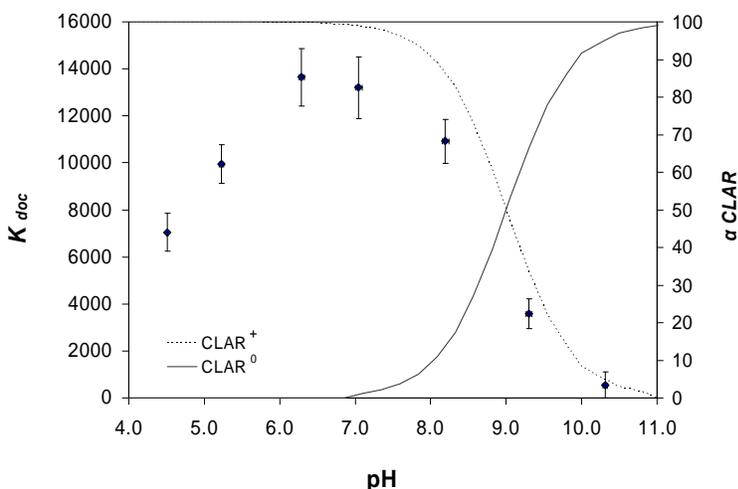


Figure 5. K_{doc} vs. pH (1.3×10^{-6} M total clarithromycin, CLAR). The mass percent of CLAR species (α_{CLAR}) is shown on the secondary y-axis. Error bars for pH and K_{doc} represent ± 1 standard deviation for triplicate incubations.

Tetracycline Sorption to Humic-Mineral Complexes. Strong interaction between ESHA and HAO led to ESHA-promoted dissolution of HAO and surface charge reversal. The ESHA-HAO sorption-desorption isotherms were successfully described using a modified Langmuir model. A surface coverage affinity parameter and a hysteresis coefficient were required to account for the heterogeneity of HAO surface and ESHA, and sorption-desorption reversibility, respectively. Ligand exchange was proposed as the major interaction mechanism, and the edge Al atoms on HAO surface were considered as the sorption sites for ESHA macromolecules. Sorption results were compared for the binary ESHA-tetracycline and HAO-tetracycline systems, and the ternary HAO-ESHA-tetracycline system. The coating of ESHA on HAO significantly suppressed tetracycline sorption levels, attributable to altered HAO surface charge characteristics and competition (between ESHA and tetracycline) for potential sorption sites.

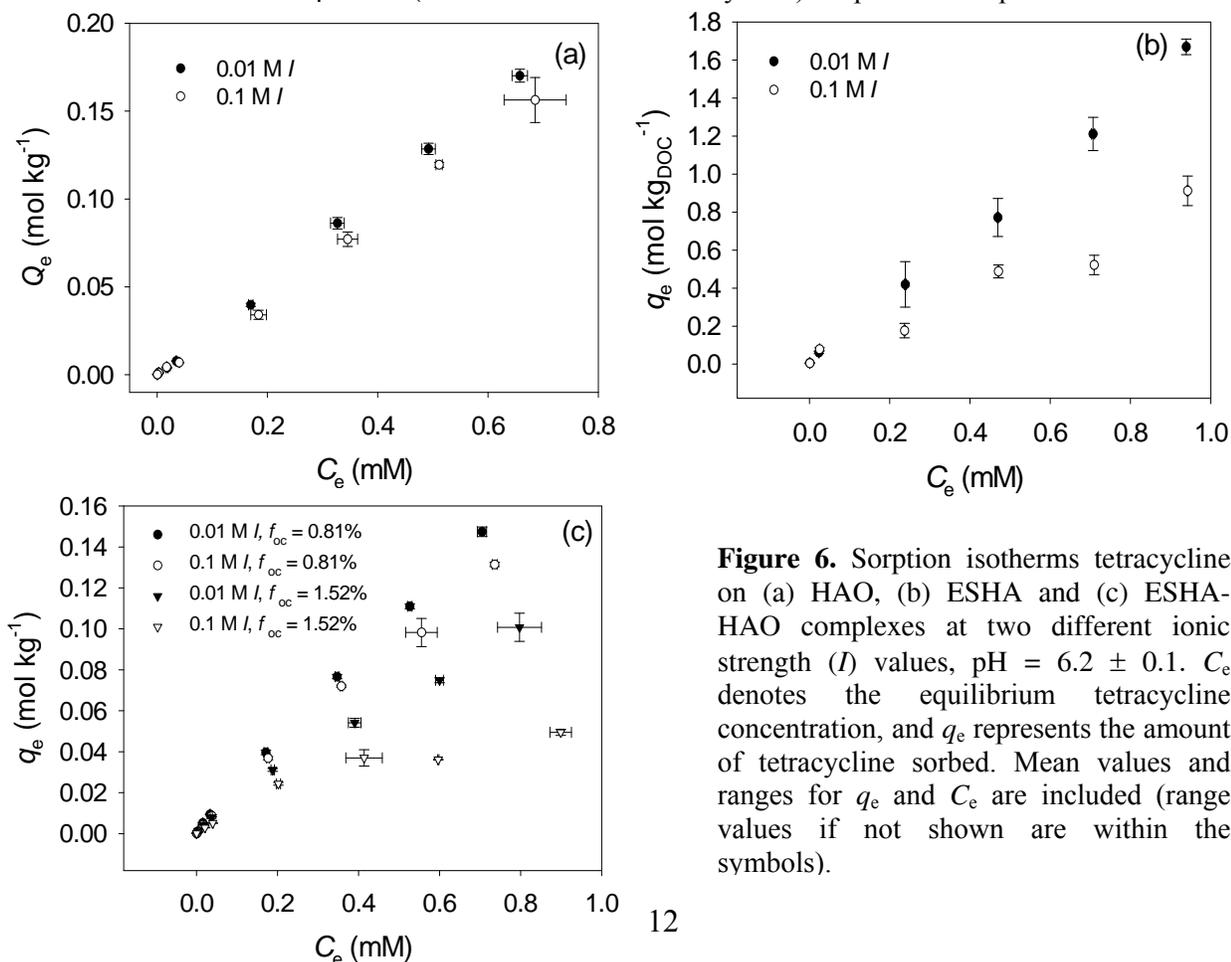


Figure 6. Sorption isotherms tetracycline on (a) HAO, (b) ESHA and (c) ESHA-HAO complexes at two different ionic strength (I) values, pH = 6.2 ± 0.1 . C_e denotes the equilibrium tetracycline concentration, and q_e represents the amount of tetracycline sorbed. Mean values and ranges for q_e and C_e are included (range values if not shown are within the symbols).

The two initial ESHA levels (i.e., 18.4 and 106.7 mg C L⁻¹) used to coat the HAO surface yielded significantly different organic carbon contents (f_{oc}) of 0.81 and 1.52%, respectively, on HAO. Figure 6c shows the sorption isotherms for tetracycline on ESHA-HAO complexes at 0.01 and 0.1 M NaCl *I*. The presence of ESHA significantly suppressed tetracycline sorption onto HAO (compare Fig. 6a and 6c), with a greater reduction noticed at the higher f_{oc} level (Fig. 6c). In previous experiments, we observed that at pH 6.2 tetracycline had a higher affinity for HAO (35% of initial tetracycline sorbed) than for ESHA (<10% sorption level). Weaker ESHA-tetracycline interaction could explain suppression of tetracycline sorption to ESHA-HAO complexes (compared to HAO). Surface charge of HAO was altered by the presence of HAO. Sorption of ESHA reversed the surface charge on HAO (from positive to negative), which would result in repulsive interactions between the hydrous oxide surface and deprotonated tricarbonylamide and carbonyl groups of tetracycline. Blockage of potential tetracycline sorption sites in HAO by ESHA could have occurred as well. Since the edge Al atoms of HAO are proposed as the sorption sites for both ESHA and tetracycline, the associated humic macromolecules would inevitably suppress the sorption of tetracycline. With increasing ESHA surface loading, the interaction of tetracycline with ESHA-HAO complexes became more *I* dependent (Fig. 6c) along with a decrease in isotherm linearity, indicating the greater influence of humic substances when present at higher concentrations.

Sulfamethazine Sorption to Humic-Mineral Complexes. The sorption and desorption of the sulfonamide antimicrobial sulfamethazine to three reference smectites coated with soil HA (HA-to-clay ratios of 1:5, 1:50 and 1:100) were examined. Infrared and UV-Vis spectroscopy indicated that aliphatic components of HA exhibited higher affinity to smectite surfaces than aromatic components at lower humic-to-clay ratios (1:50 and 1:100). Humic acid coating on clay minerals enhanced sulfamethazine sorption, especially at the humic to clay ratio 1:5, at which sorption linearity was decreased significantly (Fig. 7). Both sorption and desorption isotherms of sulfamethazine were well fit with the Freundlich Model. The linearities of sorption and desorption isotherms did not differ significantly. Desorption hysteresis was observed and thermodynamic irreversibility index (TII) was calculated, which was relatively concentration independent. Apparent competitive sorption with another sulfonamide (sulfapyridine) was found for humic-clay complexes at the highest HA loading.

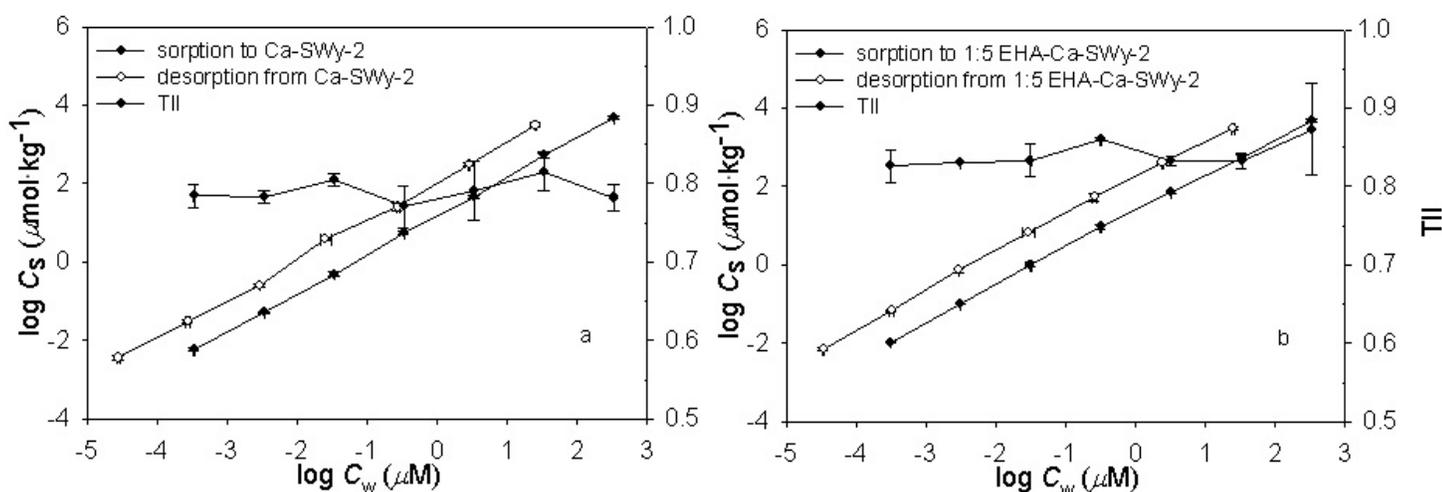


Figure 7. Representative isotherms for the sorption and desorption of SMZ to (a) SWy-2 and (b) 1:5 EHA-SWy-2 complexes. Data points represent means of triplicate measurements; error bars indicate one standard deviation. TII, Thermodynamic Index of Irreversibility (Sander et al., 2005).

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicate that propensity for antibiotics to migrate from the soil surface to groundwater depends on number of factors including their pH-dependent speciation, the ionic strength of the infiltrating water and the composition of the soil (e.g., presence of specific mineral phases, organic carbon content). Sulfonamide antimicrobials interact rather weakly with clay minerals and HS, suggesting that these compounds will be relatively mobile. However, work conducted in the Pedersen laboratory under other funding indicates that these antimicrobials may covalently bind with soil OM, a process that would render them immobile and reduce their bioactivity (Bialk et al., 2005; Bialk et al., 2006). Tetracycline and fluoroquinolone antibiotics interact very strongly with HAO and HFO to the extent of causing antibiotic-promoted dissolution of these hydrous oxides at sufficiently high concentrations. The zwitterionic species of tetracycline and ciprofloxacin appears the most reactive. In contrast, sorption to HS is weak with maximum sorption extent being <10% of the initial tetracycline concentration. Soils rich in HFO and HAO are expected to hinder the movement of these antibiotics through soil to groundwater; however, HA coating appears to suppress sorption to HAO particles. These results suggest that soil can act as a “buffer” to strongly affect the environmental fate of tetracycline and fluoroquinolone antibiotics. Inorganic minerals (i.e., hydrous oxides) have a strong affinity for these antibiotic compounds, which could promote their retention and sequester them in subsurface environments. Dissolved HS appear able to increase the mobility of antibiotics by facilitating transport (e.g., macrolides like clarithromycin) or by hindering their sorption to mineral surfaces (e.g., tetracyclines), thereby enabling inter-compartmental transfer of these compounds.

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APPENDIX A:

Peer-reviewed Research Articles

1. Gao, J.; Pedersen, J.A. 2005. Adsorption of sulfonamide antimicrobial agents to clay minerals. *Environ. Sci. Technol.* 39, 9509 - 9516. doi: [10.1021/es050644c](https://doi.org/10.1021/es050644c).
2. Gu, C.; Karthikeyan, K.G. 2005. Sorption of the Antimicrobial Ciprofloxacin to Aluminum and Iron Hydrated Oxides. *Environ. Sci. Technol.* 39, 9166-9173.
3. Gu, C.; Karthikeyan, K.G. 2005. Interaction of Tetracycline with Aluminum and Iron Hydrated Oxides. *Environ. Sci. Technol.* 39, 2660-2667.
4. Gu, C.; K.G. Karthikeyan, J. A. Pedersen, Sibley, S. Complexation of the Antibiotic Tetracycline with Humic Acid. *Chemosphere (in review)*.

Published Conference Proceedings

1. Gao, J.; Bialk, H.M.; Pedersen, J.A. 2003. Sorption of sulfonamide antimicrobials to soil constituents. 226th American Chemical Society National Meeting, September 7-11, New York, NY (extended abstract).
2. Gao, J.; Pedersen, J.A. 2004. Potential for sulfonamide antibiotics to leach from septic system drainfields. *Proc. 10th International Drainage Symposium*, American Society of Agricultural Engineers, Sacramento, CA, March 21-24, 2004.
3. Gu, C.; Karthikeyan, K.G. 2004. Sorption of Tetracycline to Iron and Aluminum Hydrated Oxides. Presented at the 228th American Chemical Society National Meeting, Aug 22-26, Philadelphia, PA (extended abstract).
4. Gao, J.; Pedersen, J.A. Adsorption of sulfonamide antimicrobial agents to clay minerals. Presented at the 228th American Chemical Society National Meeting, Aug 22-26, Philadelphia, PA (extended abstract).
5. Sibley, S.D.; Pedersen, J.A. Interaction of clarithromycin with dissolved humic acid. Presented at the 230th American Chemical Society National Meeting, Aug. 29 – Sept. 1, 2005, Washington, D.C. (extended abstract).

Papers Presented

1. Gao, J.; Bialk, H.M.; Pedersen, J.A. 2003. Sorption of sulfonamide antimicrobials to soil constituents. *Abstracts of Papers of the American Chemical Society*, **226**, 159-ENVR Part 2, September 7-11, 2003.
2. Gu, C.; Karthikeyan, K.G. 2003. Sorption of Tetracycline and Fluoroquinolone Antibiotics to Inorganic Mineral Surfaces. Presented at the 2003 Society of Environmental Toxicology and Chemistry Meeting, Nov 9-13, Austin, TX (Abstract).
3. Pedersen, J.A.; Gao, J.; Bialk, H.M. Sorption of sulfonamide antimicrobial agents. In *Annual Meetings Abstracts* [CD-ROM]. ASA, CSSA and SSSA: Madison, WI; 2003 (invited).
4. Gao, J.; Bialk, H.M.; Pedersen, J.A. Sorption of sulfonamide antimicrobial agents to smectitic clays and natural organic matter. Abstract no. 66179. In *Geological Society of America Abstracts with Programs*, **35**(6), 2003.
5. Pedersen, J.A.; Gao, J.; Bialk, H.M. Sorption of sulfonamide antimicrobial agents to soil organic matter and clay minerals. Society of Environmental Toxicology and Chemistry Annual Meeting, PED-1054-309491, Austin, TX, November 9-13, 2003 (invited).
6. Gao, J.; Pedersen, J.A. Potential for sulfonamide antibiotics to leach from septic system drainfields. 10th International Drainage Symposium, American Society of Agricultural Engineers, Sacramento, CA, March 21-24, 2004.
7. Gao, J.; Pedersen, J.A. Adsorption of sulfonamide antimicrobial agents to clay minerals. *Abstracts Papers Amer. Chem. Soc.*, **228**, 310-ENVR, Part 2, August 22-26, 2004.

8. Gao, J.; Pedersen, J.A. Adsorption of sulfonamide antimicrobials to clay minerals. 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, MN, October 13-15, 2004.
9. Gu, C.; Karthikeyan, K.G. 2004. Interaction of Tetracycline with Aluminum and Iron Hydrous Oxides. Poster presentation at the 27th Annual Midwest Environmental Chemistry Workshop, Oct 15-17, Madison, WI (Abstract).
10. Gu, C.; Karthikeyan, K.G. 2004. Interaction of Ciprofloxacin with Aluminum and Iron Hydrous Oxides. Presented at the 4th International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Oct 13-15, Minneapolis, MN (Abstract).
11. Gu, C.; Karthikeyan, K.G. 2004. Interaction of Tetracycline and Fluoroquinolone Antibiotics with Inorganic Minerals. Presented at the American Society of Agricultural Engineers Annual International Meeting, Aug 1-4, Ottawa, Canada (Abstract).
12. Sibley, S.D.; Pedersen, J.A. Interaction of clarithromycin with dissolved humic acid. *Abstracts Papers Amer. Chem. Soc.*, **230**, 130-ENVR, Part 2, 29 August – 1 September 2005.
13. Gao, J.; Pedersen, J.A. Sorption of sulfonamide antimicrobials to humic-clay complexes. In *Annual Meetings Abstracts* [CD-ROM]. ASA, CSSA and SSSA: Madison, WI; 2005.
14. Gao, J.; Pedersen, J.A. Sorption of sulfonamide antimicrobials to humic-clay complexes. 18th World Congress of Soil Science, International Union of Soil Scientists, Philadelphia, PA, 9-15 July 2006.
15. Gu, C.; Karthikeyan, K.G. 2005. Interaction of Tetracycline with Dissolved Humic Substances. Presented at the 2005 Soil Science Society of America International Annual Meeting, Nov 6-10, Salt Lake City, UT (Abstract).