A Multiscale Investigation of Monensin Sorption, Facilitated Transport, and Abiotic Degradation in Soil

Sanjai J. Parikh* and Thomas Harter¹

Project Objectives

The overarching objective of this research is to improve our understanding of antibiotic transport from the molecular to the field scale in order to determine best land management practices for concentrated animal feeding operations (CAFOs), such as dairies in the Central Valley of California. This study addresses how interactions between common veterinary antibiotics (e.g., monensin, oxytetracycline, sulfamethazine) and soil, along with individual soil components (i.e., soil minerals), affect sorption and transport utilizing: (1) field-scale sampling of monitoring wells affected by dairy operations and (2) micro/macro aggregate-scale batch studies. The specific objectives of this work are:

- To assess the potential for rapid transport of common veterinary antibiotics (oxytetracycline, chlortetracycline, sulfamethazine, sulfamerazine, sulfadiazine, sulfamethoxazole, sulfadimethoxine, and monensin) present in dairy manure to a shallow groundwater aquifer following fertigation of an agricultural field in the Central Valley of California.

- To determine the role of soil minerals (i.e., Mn-oxides, Fe-oxides, kaolinite) on sorption and facilitated transport of common veterinary antibiotics used in dairy operations throughout CA.

In addition to monensin, other common antibiotics used in California Dairies have been added to the project to give a more representative view of antibiotics in soils adjacent to concentrated animal feeding operations (CAFOs) (Table 1).

Approach and Procedures

Field study site. Groundwater samples were taken from an agricultural field adjacent to a dairy farm in Modesto, CA, in the San Joaquin Valley. Samples were collected in September and October of 2010, before and after field fertilization with dairy lagoon water. Figure 1 shows the schematic map of the sampling field and the sampling well locations of the research dairy. A detailed description of the study area is given in Watanabe et al. (2008; 2010). Briefly, the sampling site is located in the Stanislaus and Tuolumne River alluvial fan, east of the northern San Joaquin Valley trough. The groundwater level ranges between 2 to 5 m below the ground surface with a groundwater flow rate of 5 x 10⁻⁷ m s⁻¹ from east to west (Figure 1). The soil at the site is classified as a well-drained sandy loam, characterized as Oakdale (coarse-loamy, mixed, active, thermic Mollie Haploxeralfs) and Chualar (fine-loamy, mixed, superactive, thermic Typic Argixerolls). The sediments comprise alternating layers composed of sands, silts and clays. The sampling field was instrumented with two monitoring wells, designated as monitoring well 5 (MW5) and monitoring well 6 (MW6), from which the groundwater samples were taken. Irrigation water samples were collected from an irrigation outlet at Check 2.

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Figure 1. Schematic map of the sampling site.

Table 1. Chemical structures and physical properties of selected antibiotics used in this study.

| Sulfadiazine | Sulfamerazine |
A Multiscale Investigation of Monensin Sorption, Facilitated Transport, and Abiotic Degradation in Soil – Parikh

<table>
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</tr>
</tbody>
</table>

CAS = CAS Registry Number; MW = molecular weight; pKa = dissociation constant; log K<sub>ow</sub> = octanol-water partition coefficient; Sol<sub>aq</sub> = solubility in water; DMF = dimethylformamide.


**Groundwater and irrigation water samples.** Groundwater and irrigation water were sampled in duplicate from MW5 and MW6 (Figure 1) using a stainless steel submersible pump, after purging at least five well-volumes and field water quality parameters (temperature, electric...
conductivity, and pH) were stable. Water samples were not filtered, but directly collected in 1 L amber glass bottles equipped with Teflon lined caps and stored on ice in the dark until they reached the laboratory where they were stored at -20°C prior to analysis. For general water chemistry data, a single 1 L filtered sample was collected in a clear plastic bottle. Every ten samples a duplicate was taken for quality control. Water samples were analyzed by UC Davis Analytical Laboratory for the following properties: pH, electrical conductivity (EC), dissolved organic carbon (DOC), total Kjeldahl nitrogen (TKN), ammonium (NH$_4$-N), nitrate (NO$_3$-N), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), total phosphorus, bicarbonate (HCO$_3$), chloride (Cl), sulfate (SO$_4$), boron (B), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and total selenium (Se). For antibiotic analysis, the frozen groundwater and irrigation water samples were covered by aluminum foil to prevent possible photodegradation of tetracyclines (Chen et al., 2008; Kim et al., 2005) while thawing either in a fridge at 4°C or at room temperature.

**Groundwater Analysis.** Thawed samples were spiked with 0.1 μg L$^{-1}$ of simeton, which was used as a surrogate to ensure that antibiotics were not lost during sample preparation. All aqueous samples were vacuum filtered with 1 μm precombusted (400°C for four hours) glass microfiber filters (Multigrade GMF 150 graded density; Whatman Inc., Clifton, NJ) to prevent clogging of solid phase extraction (SPE) cartridges. The pH of the filtrates was determined using a Thermo Scientific Orion 4 Star portable pH/conductivity meter (Thermo Fisher Scientific, Waltham, MA) and was adjusted to pH 4 ± 0.05 using a 3 M HCl solution while stirring. EDTA was added to each sample in order to suppress the complexation of tetracyclines with metal ions or divalent cations (Blanchflower et al., 1997; Lindsey et al., 2001; Vartanian et al., 1998). The amount added was 1/4000 of the weight of the aqueous sample. The bottles were capped and shaken periodically for 1 to 2 hours prior to SPE.

Sample clean-up and pre-concentration were performed using a 500-mg, 3 mL strong anion exchange (SAX) Discovery cartridge (Supelco, Bellefonte, PA) and an Oasis™ hydrophilic-lipophilic balance (HLB) cartridge (3cc, 60 mg) (Waters, Milford, MA) in tandem. The cartridges were conditioned with 20 mL of methanol (LC-MS grade), followed by 6 mL BNP water, and 6 mL of 0.05 M citric acid solution which was previously adjusted to pH 3 using a 1 M NaOH solution. Sample was delivered to the cartridges by vacuum through plastic tubing connecting the sample bottle and SAX cartridge. Extraction was performed at an average flow rate of 2 mL min$^{-1}$. After the sample solution passed through, both cartridges were then washed with 6 mL of 0.05 M citric acid solution (pH 3) to remove excess EDTA and dried under vacuum for 15 min to remove excess water. The SAX cartridge was then removed, and the HLB cartridge was eluted by gravity by adding 2 mL of methanol. The eluent was collected in a 2-mL amber glass LC-MS-vial. The sample was divided into two amber glass LC-MS vials and stored at -80°C until LC-MS analysis.

**Irrigation Water Analysis.** The irrigation water samples were spiked with 0.1 μg L$^{-1}$ simeton as a surrogate. Before filtration, samples were distributed to 40-mL Nalgene Oak Ridge PTFE Teflon centrifugation tubes (Nalgene, Rochester, NY) and centrifuged for 20 min at 34,500 rcf. The clear supernatant was transferred back to amber glass bottles and then filtered similarly to the groundwater. The pellets from the centrifugation tubes were carefully removed using a Teflon scoop and placed into another Teflon-lined centrifuge tube. The filter paper was
transferred into the same Teflon-lined centrifuge tube, covered in aluminum foil and stored at -20° C in a freezer for further analysis as described in the following section.

Irrigation water samples were concentrated by SPE in the same manner as the groundwater samples. Elution of the cartridges was performed by gravity by adding 2 mL of methanol (LC-MS grade) to each cartridge into a 25 mL concentration vial. The concentration tubes were then transferred to an N-Evap nitrogen evaporator (Organomation, Associates Inc., South Berlin, MA) and were concentrated to a volume of 0.1 mL under a gentle flow of nitrogen gas in a 37 ± 2° C water bath. The extracts were brought to a final volume of 2 mL by adding methanol. Samples were transferred into LC-MS vials and then vortexed on a Digital Vortex Mixer (Fisher Scientific, Fair Lawn, NJ). Particles in the extracts were visible. Therefore, the extracts were filtered through 0.22 μm polyvinylidene fluoride (PVDF) syringe filters (Fisher Brand, Fisher Scientific, Pittsburgh, PA). Extracts were transferred into LC-MS vials and stored at -80° C until LC-MS analysis.

Extraction of antibiotics from particulate matter of irrigation waters collected on filter paper was performed to evaluate the potential input of antibiotics during irrigation. The Teflon-lined centrifuge tubes containing the filter papers and the pellets obtained after the centrifugation of the irrigation water were filled up with 15 mL of pH 2 phosphate buffer (0.14 M NaH₂PO₄ · H₂O/85% H₃PO₄) and vortexed for 5 min. The pH of the suspension was adjusted to pH 4 using this phosphate buffer solution, followed by vortexing after each addition. After adding 20 mL acetonitrile to each centrifuge tube, the samples were sonicated (Branson 3200, Branson Ultrasonics Co., Danbury, CT) for 30 min. The samples were centrifuged for 15 min at 15,340 rcf and the extract was decanted into a 250 mL evaporating flask. Acetonitrile (15 mL) was added to the centrifuge tubes again and the same extraction step was repeated once more. The extracts obtained (approximately 150-200 mL) were concentrated to a final volume of approximately 25 mL using rotary evaporators (Büchi Water B-480 and Büchi Rotavapor R-114, Flawil, Switzerland; Laboratora 4000-efficient, Heidolph, Schwabach, Germany) at 50° C. Immediately after the evaporation, 200 mL BNP water and EDTA were added to the evaporating flask. The amount of EDTA added was 1/4000 of the weight of the sample. Samples were swirled to mix. SPE, elution, and concentration steps were carried out as described previously. Due to clogged cartridges, more than one cartridge was used to perform SPE. After the concentration step, particles in the extracts were visible. The extracts were filtered through 0.22 μm PVDF syringe filters (Fisher Brand, Fisher Scientific, Pittsburgh, PA). Extracts were transferred into LC-MS vials and then stored at -80° C until LC-MS analysis.

**Antibiotic sorption to soil minerals.** The sorption of monensin and sulfamethazine to soil minerals was investigated through laboratory batch experiments. Minerals included in the study are kaolinite, a 1:1 clay mineral, gibbsite (γ-Al(OH)₃), birnessite (δ-MnO₂), and goethite (α-FeOOH). Kaolinite was purchased from Fluka Analytical (purum, natural grade) and aluminum hydroxide from Acros Organics (extra pure, NJ, USA). The aluminum hydroxide was determined by X-ray diffraction (XRD) to be gibbsite. Due to observed signal enhancements for monensin on the LC-MS/MS, gibbsite was purified by dialysis against 0.1 mM HCl, changing the solution daily until the electrical conductivity (EC) remained constant over a period of 12 hours. Goethite and birnessite were both synthesized in the lab. Goethite was synthesized by adding enough 2.5 M KOH to 825 mL of 0.146 M Fe(NO₃)₃ x 9H₂O to reach pH 12 (Atkinson et al., 1967). The solution was aged at 60° C for 24 hours in an oven, after which the solid was
washed three times with 1 mM HCl by repeated centrifugation, decantation, and resuspension in fresh 1 mM HCl. After the final rinse, the solid was resuspended in 0.1 mM HCl and dialyzed against 0.1 mM HCl until the EC remained constant over a 12 hour period. The product was freeze dried and ground with a mortar and pestle before use. Birnessite (δ-MnO₂) was synthesized by adding concentrated HCl to a boiling solution of KMnO₄ (McKenzie, 1971). Following synthesis the birnessite was dialyzed against BNP water until the EC remained constant over a 12 hour period.

Sorption of monensin at five concentrations ranging 0.2 to 1.0 mg L⁻¹ was examined for each mineral at two environmentally relevant pH levels, approximately 6 and 8, with a constant electrolyte background of 5 mM NaCl. Sulfamethazine sorption was explored similarly for gibbsite (pH 6), goethite (pH 8), and birnessite (pH 6 and 8). In order to see only the abiotic effects of each mineral on the antibiotic sorption and possible transformation, sodium azide was added to each tube (0.077 mM final concentration) to restrict biological activity. Mineral solutions were pH adjusted with either HCl or NaOH, depending on the desired pH. For all initial experiments, a sorbent to solution ratio of 1:100 was maintained. Batch experiments were carried out in 40-mL Nalgene Oak Ridge PTFE Teflon centrifugation tubes (Nalgene, Rochester, NY) to reduce the possibility of antibiotic loss by sorption to the tubes. Solutions of monensin and mineral were rotated end-over-end for 24 hours on a LabQuake rotisserie, centrifuged, and filtered by 0.22 µm PVDF syringe filters. Antibiotic concentration was determined by solution analysis on LC-MS/MS. Based on initial findings, the interaction of monensin with goethite was investigated further by adjusting the solution ionic strength to 5, 50, and 100 mM NaCl.

**LC-MS/MS analysis of antibiotics.** LC-MS/MS analysis was performed using an Agilent series 1200 HPLC coupled to an Agilent 6320 ion trap MS (Agilent Technologies, Palo Alto, CA) equipped with a binary pump, a vacuum degasser, an autosampler, and a diode-array detector coupled to an Agilent 6320 Ion Trap LC/MS (Agilent Technologies, Palo Alto, CA) mass spectrometer. The column temperature was kept at 40° C. Solvent A was 0.1% formic acid in BNP water and solvent B was 0.1% formic acid in methanol. Quantanalysis LC/MSD Trap Software 5.3 was used to process the integration and calculation of peaks for all antibiotics studied.

**Tetracyclines.** Chromatographic separation was carried out on a reverse-phase Agilent Zorbax Eclipse Plus C₁₈ analytical column (150 mm x 4.6 mm x 5 µm), which was protected by a guard column with the same stationary phase (12.5 mm x 2.1 mm x 5 µm) (Agilent Technologies, Palo Alto, CA). Tetracyclines were eluted under gradient conditions beginning with 5% B, and ramping to 80% B over 10 mins, at 0.4 mL min⁻¹. Five microliters were injected onto the LC-MS system using an Agilent series 1200 auto injector. MS data were collected in the negative ESI MS² mode. Nebulizer temperature was 350° C, nebulizer pressure was 50 psi, and drying gas flow rate was 10.0 L min⁻¹. The MS was manually tuned by infusing a 1 µg mL⁻¹ tetracycline mixture in methanol into a 50% B LC effluent at 30 µL min⁻¹.

**Sulfonamides.** Chromatography separations were carried out on a Zorbax Eclipse XDP-C₁₈ column, 4.6 mm x 150 mm x 5 µm (Agilent Technologies, Palo Alto, CA) equipped with a Zorbax Eclipse C₁₈ guard column (Agilent Technologies, Palo Alto, CA). Sulfonamides were eluted under gradient conditions (5% to 100% B) at 0.3 mL min⁻¹. Five microliters were injected onto the LC-MS system using an Agilent series 1200 auto injector. MS data were collected in the positive ESI MS² mode. Nebulizer temperature was 350° C, nebulizer pressure was 50 psi, and
drying gas glow rate was 10.0 L min\(^{-1}\). The MS was manually tuned by infusing a 1 μg mL\(^{-1}\) sulfonamide mixture in methanol into a 50% B LC effluent at 30 μL min\(^{-1}\).

**Monensin.** Chromatography was carried out on a Gemini C18 column, 2.0 mm x 50 mm x 3 μm (Phenomenex, Torrance, CA). Separation of monensin was achieved under gradient conditions beginning with 70% B, and ramping to 90% B over 5 min, at 0.5 mL min\(^{-1}\). Ten microliters were injected onto the LC-MS system using an Agilent series 1200 auto injector. MS data were collected in the positive ESI MS\(^3\) mode. Nebulizer temperature was 350° C, nebulizer pressure was 20 psi, and drying gas glow rate was 10.0 L min\(^{-1}\). The MS was manually tuned by infusing a 1 μg mL\(^{-1}\) monensin sample in methanol into a 50% B LC effluent at 30 μL min\(^{-1}\).

**Results**

**Irrigation water and groundwater: general chemical analysis.** The groundwater elevation at both MW5 and MW6 was determined throughout the irrigation season of the agricultural field before groundwater samples for analysis were collected. The data were provided by Unc et al. (paper in preparation). Figure 2 shows the changes in the groundwater elevation above sea level (m) as a function of the sampling events.

The groundwater elevation at both monitoring wells increased after the first irrigation events and reached its maximum after two days of irrigation (9/17/2010). Subsequently, the groundwater elevation slowly decreased. At the end of the irrigation season, a slight increase could be observed again at MW5. Figure 2 also demonstrates that the groundwater elevation at MW6 is higher (12.9 to 13 m above sea level) than at MW5 (12.7 to 12.9 m above sea level). Therefore, MW5 and MW6 are termed as downgradient and upgradient, respectively (Watanabe et al., 2008). Table 2 displays an overview of selected water chemistry data (relevant for the following discussion) for both irrigation water and groundwater at the operation dairy.
The chemical composition and characteristics of the lagoon and groundwater vary. Ammonia (NH$_4$-N), total nitrogen (TKN), potassium (K), sodium (Na), total phosphorus, bicarbonate (HCO$_3$), and chloride (Cl) were the predominant constituents in the lagoon water samples. Metals such as boron (B), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) were detected at lower concentrations in the irrigation water. Other important differences between the groundwater and the lagoon water can be found in the electrical conductivity (EC) and dissolved organic carbon (DOC) content. Due to the high salt content of animal manures, an increase of EC is indicative of the lagoon water reaching the groundwater (Miller et al., 2008). The EC in the irrigation water increased considerably from 4.77 dS m$^{-1}$ at the beginning of the sampling event (9/15/2010, 11:40 am) to approximately 6.7 to 6.8 dS m$^{-1}$ at the end of the sampling event (9/16/2010). With respect to the groundwater samples, EC measurements of MW5 indicate a negligibly small increase from 2.39 to 2.48 dS m$^{-1}$, whereas a slight decrease in MW6 (from 1.22 to 1.1 dS m$^{-1}$) was observed. The same trend can be seen in the dissolved organic matter content (DOC). The lagoon water had a high DOC content with an average concentration of around 250 mg L$^{-1}$. The DOC at MW5 slightly increased during the irrigation event (from 15.9 to 17.0 mg L$^{-1}$). At MW6, however, the DOC content slightly increased at the beginning of the irrigation event from 6.7 mg L$^{-1}$ to a maximum value of 6.9 mg L$^{-1}$ and then decreased to 6.1 mg L$^{-1}$. NH$_4$-N is an indicator for anaerobic conditions in the lagoon water (Watanabe et al., 2008; Watanabe et al., 2010). Mean concentrations of NH$_4$-N and nitrate (NO$_3$-N) in the irrigation water were around 310 and 0.45 mg L$^{-1}$. In the groundwater samples, in turn, NH$_4$-N concentrations were less than the method detection limit (<0.05 mg L$^{-1}$) suggesting that NH$_4$-N did not reach the
Table 2. Overview of selected water chemistry data from the irrigation water and groundwater of MW5 and MW6.

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<th>NO₃-N</th>
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<td>[mg/L]</td>
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groundwater but was converted to NO₃-N by nitrifying bacteria in soils. Consequently, the NO₃-
N content at MW5 increased from around 85 to 91 mg L⁻¹ over time because MW5 is near the
irrigation outlets (Nolan and Stoner, 2000). The opposite is observed at MW6, again due to the
fact that NH₄-N rapidly transformed into NO₃-N in soils before it was transported to MW6.
Based on these results, it can be shown that an aerobic zone exists below the agricultural field
which is in general agreement with statements by Watanabe et al. (2008; 2010). With respect to
the pH, constant values were obtained ranging between pH 7 and 8 for both the irrigation and
groundwater samples throughout the sampling season.

**Irrigation water samples.** In order to examine the occurrence of antibiotics in lagoon
water, samples were collected during fertigation from outlets located in Check 2 at the
agricultural field from one sampling day, 9/15/2011 (Figure 1). Prior to extraction and analysis,
samples were centrifuged and filtered, in order to separate the solution from the manure particles.
The solid residues remaining on the filter papers were also analyzed for antibiotics. Sample
clean-up and pre-concentration steps of both the irrigation water and the filter papers were
performed using the developed tandem SPE method. Figure 3 displays the concentration profiles
obtained for the lagoon water samples and filtrate material. Of the analyzed antibiotics, only
sulfadimethoxine and monensin were detected in the dairy lagoon water. For monensin, the
concentrations in the irrigation water ranged from 1593 to 4772 ng L⁻¹ (LOD: 0.165 ng L⁻¹; LOQ:
0.524 ng L⁻¹). Sulfadimethoxine signals were observed, with concentrations ranging from 16.43
to 116.1 ng L⁻¹ (LOD: 0.217 ng L⁻¹; LOQ: 0.693 ng L⁻¹). In the filter paper residue, relatively
constant antibiotic concentrations of sulfadimethoxine were measured between 0.108 to 1.428 ng
L⁻¹. Monensin concentrations varied from 0.462 to 47.71 ng L⁻¹.

**Groundwater samples.** The detected concentrations of the target antibiotics as a
function of the sampling dates are shown in Figure 4. Sulfamethazine was the only compound
detected in both monitoring wells throughout the sampling season with concentrations above the
LOQ (0.713 ng mL⁻¹), with the exception of three samples (MW5A13, MW5A14, and MW6A1).
Concentrations ranged from 1.435 to 5.103 ng L⁻¹ in MW5 and from 1.624 to 8.310 ng L⁻¹ in
MW6 (one sample having 20.63 ng L⁻¹).

At MW5, sulfadimethoxine was frequently detected with concentrations always measured
above the LOQ (0.693 ng mL⁻¹). Concentrations detected ranged between 1.475 to 2.507 ng L⁻¹
(one sample at 8.702 ng L⁻¹). Lower concentrations (<LOQ) of sulfadimethoxine were found at
MW6 throughout the sampling events with one exception. Monensin was also detected in the
groundwater samples from both monitoring wells. Most concentrations were below the LOD
(0.165 ng mL⁻¹), and a few were detectable (>LOD) but were too low for confident quantitative
determinations. Three concentrations above the LOQ (0.524 ng mL⁻¹) were determined with high
degree of confidence ranging from 1,329 to 2,157 ng L⁻¹.
Figure 3. Concentration profiles of sulfadimethoxine and monensin detected in lagoon water and in the corresponding filter paper extract.
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![Graphs showing concentration of sulfamethoxazole, sulfadimethoxine, and sulfamerazine over time for samples MW5 and MW6.](image-url)
Figure 4. Concentration profiles of sulfadiazine, sulfamethoxazole, sulfadimethoxine, sulfamerazine, sulfamethazine, and monensin detected in groundwater. (*) = samples (MW6A1 to MW6A4) prepared after EPA method 1694; MW5 = monitoring well 5; MW6 = monitoring well 6; LOD = limit of detection; LOQ = limit of quantification.

Frequent signals for sulfamethoxazole were observed throughout sampling at MW5 but always below the LOD (0.539 ng mL\(^{-1}\)), therefore analysis was not feasible. False signals may arise from interferences or background noise during LC-MS analysis (Alfassi, 1998; Armbruster et al., 1994; Willets and Wood, 2000). At MW6 sulfamethoxazole, was detected in all samples but one. Sulfadiazine and sulfamerazine were observed infrequently, mostly below the LOD (0.257 ng mL\(^{-1}\) and 0.607 ng mL\(^{-1}\) for sulfadiazine and sulfamerazine, respectively), although signals above the LOD were detected in a few samples.

Simeton was also added to the groundwater samples in order to assess analyte loss during sample preparation and analysis. Consistent and satisfactory recoveries of simeton were obtained in groundwater samples, usually ranging between 91.0 and 106.3%, with few exceptions ranging from 59.4 to 88.6%. These recoveries indicate that no significant analyte losses occurred.
Antibiotic interaction with pure minerals. Batch experiments showed negligible sorption of monensin to kaolinite, gibbsite, or birnessite at pH 6 or 8 with a 1:100 sorbent to solution ratio (Figure 5). Increasing the ionic strength of the background solution with NaCl caused a decrease in monensin sorption to goethite (Figure 6). Preliminary data for ongoing research suggest that sulfamethazine was completely removed from solution within 5 hours upon reaction with birnessite, and several sulfamethazine transformation products were observed via LC-MS/MS and FTIR. However, initial results did not show significant, if any, sorption of sulfamethazine to gibbsite at pH 6 or goethite at pH 8 (data not shown).

Figure 5. Sorption isotherms for monensin sorption to kaolinite, birnessite, and goethite (error bars represent standard error for triplicates).

Figure 6. Sorption of monensin to goethite with varying ionic strength (error bars represent standard error for triplicates).
Discussion

Field water samples. The occurrence and fate of antibiotics in dairy lagoon water applied to agricultural fields depends on the properties of the specific antibiotics and soil conditions. Oxytetracycline and chlortetraacyclines are strongly sorbed to soil particles as characterized by their high $K_d$ values, which limits their mobility in the environment (Kumar et al., 2005; Sassman and Lee, 2005; Tolls, 2001). In the current study, the absence of tetracyclines in the groundwater may be due to strong sorption to soil and photodegradation in field, which is consistent with these previous studies. Monensin and sulfonamides have lower $K_d$ values indicating greater potential transport in soils than tetracyclines. Surface runoff, infiltration or leaching into deeper soil layers allow sulfonamides and monensin to reach different environmental waters (Boxall, 2008; Carlson and Mabury, 2006). Sulfonamides are frequently detected in shallow groundwater and groundwater impacted by agricultural activities from farmlands (Bartelt-Hunt et al., 2011; Watanabe et al., 2010). Monensin is neither as mobile as sulfonamides nor able to sorb as strongly as tetracyclines (Carlson and Mabury, 2006; Sassman and Lee, 2007), and has the potential to reach shallow groundwater from manure-applied agricultural fields (Bartelt-Hunt et al., 2011; Song et al., 2010; Watanabe et al., 2008). However, during percolation monensin exhibits some sorption to soil particles (Davis et al., 2006).

Antibiotics in manure which is applied to agricultural land are also exposed to a range of environmental influences such as sunlight, temperature, water evaporation, and rainfall (Davis et al., 2006), hence some degree of degradation is expected. For instance, sulfonamides have been observed to readily undergo hydrolysis (Sukul et al., 2008). In comparison to sulfadimethoxine and sulfamethoxazole, sulfamethazine is less biodegradable under anaerobic conditions in manure and has a very high potential to be transported into the groundwater where it can persist (Watanabe et al., 2010). Sulfadimethoxine is generally more persistent in soils (Wang et al., 2006a).

There are noticeable differences in the concentration profiles of antibiotics in the two monitoring wells. This could be due to the fact that MW5 was placed down-gradient while MW6 was up-gradient at the agricultural field (Watanabe et al., 2008). MW6 is termed an up-gradient well due to its distance from irrigation outlets and is, therefore, less impacted by manure-application on the field (Watanabe et al., 2010). Sulfamethazine was detected at both MW5 and MW6, but higher concentrations were observed at MW6, suggesting that the antibiotic was already present in the groundwater before irrigation. This assumption is supported by the fact that the groundwater elevation increased at both monitoring wells after irrigation. However, the EC and DOC at both monitoring wells did not change considerably as would be expected if the antibiotic contaminated lagoon water reaches the groundwater. Also, although the antibiotic concentration levels seem to strongly vary at each well; these measurements are taken at the ng L$^{-1}$ range. The magnitude of variations is relatively low so that the concentrations detected proved to be relatively constant throughout the sampling events. These constant concentrations suggest that no contaminated lagoon water reached the groundwater.

After application of lagoon water to the soil, a series of interactions between organic matter, antibiotics, microorganisms, and soil will occur. During the time that the lagoon water migrates through the soil profile the composition of the water will change. Hence, antibiotics will bind to soil particles and be degraded via light, microorganisms, and redox active minerals; reducing concentrations from lagoon water. It is also possible that a large portion of the lagoon
water did not reach the groundwater during the sampling events. The lagoon water in the subsurface soil might have moved existing soil water present in the vadose zone (the unsaturated zone which is between the ground surface and water table (Harter, 2003)) that, in turn, might have moved downward to groundwater. This would result in a higher water elevation and no significant changes of the EC and DOC of the groundwater over time.

If most of the lagoon water reached the groundwater during the sampling interval, it would be diluted through mixing with groundwater already present below the field. The dynamics and mixing behavior in the groundwater needs to be considered, as instantaneous mixing and dilution will not occur. The depth of the monitoring well and rate of diffusion and mixing all will influence concentrations of antibiotics in groundwater samples. Therefore, measured concentrations may not reflect the concentrations of antibiotics in groundwater stemming from this irrigation event. Rather, sampling of groundwater after mixing and diffusion are complete may give more reliable values.

Another factor to consider is that antibiotic may reach the monitoring wells via lateral transport. Water movement from off-site or from on-site groundwater influenced by the lagoon waste water system at the dairy farm may be responsible for some of the antibiotics analyzed in this study. Although the lagoon system is located further away from the monitoring wells (from MW5 and MW6 approximately 300 and 500 m, respectively) and that the groundwater flow is from the east to west (Figure 1), it could be considered as a potential source of antibiotic contamination.

Based on the results obtained, it has been demonstrated that antibiotics other than the administered antibiotics (monensin, oxytetracycline and sulfamethazine) are present in the shallow groundwater below the manure-applied agricultural field. Furthermore, the results suggest that sulfadiazine and sulfamerazine were present in the samples but too low for a confident quantification.

**Antibiotic sorption to pure mineral phases.** Sorption experiments for the two antibiotics most commonly found (i.e., monensin, sulfamethazine) in groundwater samples provide additional insight into the field scale transport mechanisms of veterinary antibiotics. Our preliminary sorption data reveal no sorption of sulfamethazine to mineral phases studied besides birnessite and therefore suggest transport through soil. For monensin, of the minerals evaluated, only goethite was shown to bind monensin. This is likely due to limited binding of monensin to the birnessite and kaolinite surface within this pH range where both sorbate and sorbent are both negatively charged. Goethite, however, is positively charged within this range, and monensin is negatively charged. The decreased sorption of monensin to goethite with increasing ionic strength suggests competition for surface sites and an outer-sphere sorption mechanism. This is theorized as sodium ions have large hydrated radius and commonly bind via outer-sphere mechanisms.

Redox active minerals such as Fe and Mn (hydr)oxides (e.g., goethite, birnessite), which are commonly found in soils, can both sorb and abiotically degrade organic compounds (Huang, 1990; Lehmann et al., 1987; Parikh et al., 2004). Therefore, in addition to serving as a sorption sink for antibiotics, these mineral surfaces could lead to degradation of parent compounds. While many studies have investigated the degradation of antibiotics in the presence of bacteria (Dolliver et al., 2008; Kumar et al., 2005; Lee et al., 2007; Wang et al., 2006b) there is a lack of studies examining abiotic degradation pathways in soil. In the current study, increased functionalization of sulfamethazine observed upon reaction with δ-MnO₂ indicates cleaving of
aromatic rings, and that abiotic degradation is occurring. Although not previously shown for antibiotics, this result is in agreement with previous studies documenting the ability of δ-MnO$_2$ to induce polymerization of phenolic compounds (Shindo and Huang, 1984; Stone and Morgan, 1984; Ukrainezyk and McBride, 1992). Ongoing research aims to identify the specific degradation products via LC-MS/MS and reaction pathways.

In addition, experiments with soil from the field sites and pure mineral phases continue via both batch and spectroscopic methods. It is anticipated that current research efforts will help to determine the specific mechanisms for sorption and transport of the veterinary antibiotics studied in this project. This information will be highly relevant for developing recommendations for the application of antibiotic-rich manures to agricultural fields based on the specific mineralogy of soils present.

References


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