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

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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 (i.e., monensin, oxytetracycline, sulfamethazine) and soil, along with individual soil components (i.e., dissolved organic matter [DOM] and soil minerals), affect sorption and transport through: (1) micro/macro aggregate-scale batch/column studies, (2) Fourier transform infrared (FTIR) spectroscopy to probe molecular-scale interactions, (3) field-scale sampling of monitoring wells affected by dairy operations, (4) and hydrologic modeling to link laboratory scale process research to plot, field, and farm scale observations. The specific objectives of this work are:

- To determine the role of soil minerals (i.e., Mn-oxides, Fe-oxides, kaolinite) and DOM on sorption and facilitated transport of common veterinary antibiotics used in dairy operations throughout CA.
- To use ATR-FTIR spectroscopy to determine molecular mechanisms and reaction rates of antibiotic sorption and abiotic degradation by soil minerals.
- To develop hydrologic models using experimental data, incorporating molecular level information, macroscopic transport data, and measured concentrations from soil and groundwater near dairy farms in the Central Valley, CA; providing essential information for developing recommendations regarding storage and land application of antibiotic rich manures.

Approach and Procedures

Laboratory micro/macro aggregate-scale and molecular-scale monensin sorption, degradation, and transport experiments are being employed to simulate natural soil conditions (i.e., temperature, pH ionic strength). Data interpretation will include extrapolation from our laboratory experiments, when appropriate, to representative field conditions. Field-scale data are also being collected and antibiotic concentrations in groundwater under a dairy operation (Central Valley, CA) will be measured. Compilation of the collected data will provide information required for predicting the mobility of monensin in soil and aquatic environments. Investigation of other common antibiotics used in California Dairies have been added to project to give a more representative view of antibiotics in soils adjacent to concentrated animal feeding operations (CAFOs) (Table 1).

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Table 1. Chemical structure and physical properties of selected antibiotics detected in soil and groundwater influenced by dairy farms in the Central Valley, CA.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Chemical Structure</th>
<th>Sol$_{aq}$</th>
<th>MW (g mol$^{-1}$)</th>
<th>pK$_{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td><img src="image" alt="Oxytetracycline" /></td>
<td>313 mg L$^{-1}$</td>
<td>496.5</td>
<td>3.6, 7.5, 9.4</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td><img src="image" alt="Sulfamethazine" /></td>
<td>1500 mg L$^{-1}$</td>
<td>278.33</td>
<td>2.4, 7.4</td>
</tr>
<tr>
<td>Monensin</td>
<td><img src="image" alt="Monensin" /></td>
<td>3x10$^{-3}$ mg L$^{-1}$</td>
<td>669.9</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Results

During this time period we have developed methods for analysis of oxytetracycline, sulfamethazine, and monensin on our newly acquired LC-MS/MS (Agilent 6320 Ion Trap). By using parameters from existing methods found in the literature (e.g., Watanabe et al., 2008) we have developed a method specific to our instrument to analyze antibiotics from laboratory experiments and soil, groundwater, and manure samples. The analysis uses solid-phase extraction (Oasis HLB Cartridges) for sample cleanup and concentration prior to analysis via LC-MS. The LC-MS method uses an Agilent Zorbax Eclipse C18 Column (2.1 x 100 mm; 3.5 μm) at 40° C to measure concentrations of all three antibiotics simultaneously. The current analysis method uses a 3 µL inject volume and a flow rate of 0.3 mL min$^{-1}$ with a mobile phase of 1% formic acid and acetonitrile (ACN). A gradient is used with ACN increasing from 20 to 32% from 0 to 3 min, then increasing to 95% from 3 to 4 min, and remaining constant until 10 min. The mass spectra are acquired in positive ion electrospray mode with drying gas at 12 mL min$^{-1}$ (350° C), nebulizer pressure at 35 psi, and the capillary voltage set at -2500V. Mass spectra are collected using an electron multiplier setting of 1550, and abundant target of 30,000, a maximum accumulation time of 200 ms, with an isolation width of 2.0 m/z, and a fragmentation amplitude of 1.0 V.

Experiments to examine the abiotic degradation of antibiotics on redox active minerals have also been initiated. To date we have reacted sulfamethazine and oxytetracycline with birnessite (δ-MnO$_2$) for 22 and 44 h. The results indicate that in less than 22 h of reaction with δ-MnO$_2$ significant compound oxidation occurs. ATR-FTIR spectra collected after reaction with δ-MnO$_2$ have numerous new peaks, particularly for oxytetracycline, corresponding to phenolic, carboxyl, and aliphatic moieties (Fig. 1).
Discussion

Redox active minerals such as Fe and Mn (hydr)oxides, 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., 2006) there is a lack of studies examining abiotic degradation pathways in soil. In the current study, the increased functionalization of the parent compounds (i.e., oxytetracycline, sulfamethazine) 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-MnO₂ to induce polymerization of phenolic compounds (Shindo and Huang, 1984; Stone and Morgan, 1984; Ukrainczyk and McBride, 1992).

The specific degradation products will be resolved via LC-MS and the reaction pathway determined.

Our research investigating the reaction of monensin, sulfamethazine, and oxytetracycline with soil minerals and DOM is ongoing. We are currently conducting experiments to monitor the oxidation reaction of antibiotics via δ-MnO₂ (in situ) using rapid-scan ATR-FTIR spectroscopy. Additional FTIR experiments will be conducted to evaluate the mechanisms of binding to non-redox active minerals. This data, in conjunction with sorption data will be used to develop hydrologic models to predict macroscopic transport of veterinary antibiotics in the San Joaquin Valley, CA.

References


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