Report for 2002IA4B: Predicting sorption, mobility, accumulation, and degradation potential of antibiotics in Iowa's soil/water environment

- Conference Proceedings:
  - Pils, J.R.V. and D.A. Laird. 2004. Adsorption of Tetracycline and Chlorotetracycline on K- and Ca-saturated Soil Clays and Humic Substances in 41st Annual Meeting Program and Abstracts, Clay Minerals Society, 57 pp.
- Other Publications:
  - Pils, J.R.V., D.A. Laird, and T.B. Moorman. 2004. Degradation of Tetracycline Sorbed on K- and Ca-saturated Soils and Soil Components. Agron. Abstr. CD-ROM S11-pils5035.pdf.
  - Pils, J.R.V. and D.A. Laird. 2003. Adsorption of Tetracycline and Chlorotetracycline on K- and Ca-saturated Soil Clays and Humic Substances. Agron. Abstr. S02-pils672004-O.pdf.
  - Pils, J.R.V., V.P. Evangelou, and D.A. Laird. 2002. Smectite tactoid formation induced by confined and double layer monovalent cations. Agron. Abstr. S02-pils152704-O.pdf.
- Dissertations:
  - Pils, Jutta. 2005. The role of soil clays and clay-humic complexes in processes controlling colloidal stability and the sorption and degradation of tetracyclines. Ph.D. Dissertation, Dept. of Agronomy, College of Agriculture, Iowa State University, Ames, IA.

Report Follows

Predicting Sorption, Mobility, Accumulation, and Degradation Potential of Antibiotics in Iowa's Soil/Water Environment

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Problem and Research Objectives

Approximately 31.6 million pounds of antibiotics are used in the production of poultry (10.6 million pounds), hogs (10.3 million pounds), and cattle (3.7 million pounds) in the United States each year (Mellon et al., 2001). Over three fourths of these antibiotics (24.6 million pounds) are given to healthy animals in low doses to promote growth (Levy, 1997). Most of the antibiotics given to farm animals are not metabolized *in vivo*, rather they are excreted in the active form (Lee et al., 2000). The fate of antibiotics introduced into soil and aquatic environments with manure and other animal wastes is largely unknown. However, there is much concern that the presence and persistence of low levels of antibiotics in soil and aquatic environments could encourage the buildup of existing, and the development of new, antibiotic-resistant bacterial populations (Henry, 2000).

In Iowa, Earthen Waste Storage Structures (lagoons) are widely used for temporary storage of liquid animal wastes with the intent of protecting surface and ground water from contamination and allowing farmers to use the wastes in a timely fashion. Liquid animal wastes are generally spread on agricultural soils both as a means of disposal of the wastes and as a nutrient source for crop production. The Iowa Dept. of Public Health (1998) found relatively high concentrations of chlorotetracycline (11 to 540 μg/L) and erythromycin (10 to 275 μg/L) in such liquid animal wastes. The report also indicated that many of the 18 *E. coli* isolates, all three Salmonella species, and an isolate of Enterococcus demonstrated resistance to one or more of the antibiotics.

The antibiotics most commonly added to livestock feed as growth promoters (1 to 100 mg per head per day) are chlorotetracycline (Aureomycin), oxytetracycline (Terramycin) and macrolide (erythromycin) (Sewell, 1993; FAC, 1998; Herman et al., 1995). The fate of these compounds in Iowa soils depends on sorption and desorption of the antibiotics on soils, leaching, and the rates of chemical, photochemical, and microbial decomposition of the antibiotics. The basic hypothesis of the study is that the fate (sorption/desorption, leaching, and decomposition) of antibiotics in soil environments is strongly influenced by the chemical reactions between the antibiotics and soil constituents.

Specific Objectives:

1. Characterize three common Iowa soils and isolate and characterize reactive soil components (clay-humic complexes, clay minerals, and humic materials) from these soils;
2. Quantify sorption of tetracycline and chlorotetracycline on the soils and soil components;
3. Determine the effects of saturating cation (Ca vs. K) and ionic strength (I=0.05 and I=0.005) on sorption of tetracycline and chlorotetracycline on the soils and soil components; and
4. Quantify the influence of sorption on tetracycline and chlorotetracycline degradation rates.

Methodology

Soil samples, surface (0-15 cm) and subsurface (≥ 15 cm), were collected from three sites representing three different soil series and a range of soil physical and chemical properties. Both the studied soils and the general sampling locations had been previously characterized (McBride et al., 1987). Based on interviews with the landowners or operators, specific sampling sites that had never received manure applications were selected. The soils were characterized using standard analytical procedures to determine pH in $CaCl_2$, pH in KCl, pH in water, organic C, organic H, organic N, % sand, % coarse silt, % fine silt, % clay and extractable cations (Ca, Mg, Na, and K).

Soil components were physically and chemically separated from the soils and prepared for the sorption and degradation studies. Clay-humic complexes were isolated from the soils by sedimentation (<2 µm e.s.d.). Portions of the clay-humic complexes were K- or Ca-saturated by washing in 1M KCl or 0.5 M $CaCl_2$ and then dialyzed against distilled water and freeze-dried. Other portions of the clay-humic complexes were treated with 30% H_2O_2 for removal of the humic materials before being K- and Ca- saturated, dialyzed against distilled water and freeze-dried. Humic materials were separated from the three soils by hydrolyzing Na-saturated samples in 0.1 M NaOH under and N_2 purge. After the hydrolysis the humic materials were separated by centrifugation, neutralized to pH 7, K- or Ca-saturated, dialyzed and freeze-dried.

A batch equilibration technique was used to measure sorption of tetracycline and chlorotetracycline on the various soils and prepared soil components. HPLC was used to quantify tetracycline and chlorotetracycline in the supernatant solutions and sorption was determined by difference. Variables tested include soil components (clay-humic complexes, clay minerals, and humic substances), saturating cation (K vs Ca), and ionic strength (I=0.05 and I=0.005).

The degradation of tritium-labeled tetracycline in both aqueous and colloidal systems was quantified under both biotic and abiotic conditions. The colloidal systems included soil clays and soil clay-humic complexes that were both K- and Ca-saturated. During the incubations (149 days), we quantified both the disappearance of tetracycline from the aqueous phase by HPLC and total tritium activity in the aqueous phase using a liquid

scintillation counter. Bioavailability of adsorbed tetracycline was assessed using an agar plate technique.

Principal Findings and Significance

Tetracycline (TC) and chlorotetracycline (CTC) are very strongly sorbed on soil clays, soil humic substances, and soil clay-humic complexes. In agreement with previous studies, sorption of TC and CTC was found to increase with decreasing pH and to be greater in Ca-systems compared to K-systems. Also in agreement with previous studies, we found higher sorption of CTC compared to TC, most likely due to the lower solubility of CTC. An important new finding is that TC and CTC are more strongly sorbed on soil clays than on soil humic substances, and that interactions between clays and humic substances significantly diminish sorption of TC and CTC on soil clay-humic complexes. Although TC and CTC are strongly sorbed by soil clays, humic substances, and clay-humic complexes, a small portion of sorbed TC and CTC is released back into an aqueous solution through desorption. Desorption increased with increasing pH and for K-systems relative to Ca-systems. Desorption of TC was greater than desorption of CTC. Desorption of TC and CTC from the clay-humic complexes was substantially greater than desorption from either the humic substances or the soil clays. The results demonstrate that interactions between soil clays and humic substances significantly influence both the sorption and desorption of TC and CTC from soil components. One possible explanation is that humic substances compete with TC and CTC for sorption sites on soil clays.

Despite significant effort, we were not able to find an extraction scheme that would remove more than a small fraction of the adsorbed TC from soil clays and clay-humic complexes. This precluded our ability to directly quantify degradation of adsorbed tetracycline. Also, because TC was so strongly adsorbed on soil clays and clay-humic complexes, it became apparent that there was little likelihood of our being able to successfully quantify the mobility of TC in intact soil columns. Therefore, we switched strategies and used tritium-labeled TC to quantify release of TC degradation products from clays and clay-humic complexes under both biotic and abiotic conditions during long-term (149 days) incubations. The results for abiotic aqueous systems indicated that about 30% of the added TC was transformed into 4-epitetracycline, but, otherwise, there was no degradation of TC during a 21-day incubation. In contrast, during the same period all of the TC in aqueous biotic systems was degraded. In the colloidal systems, all of the TC and 4-epitetracycline disappeared from the aqueous phase after 40 days for both the biotic and abiotic systems. Tritium concentrations in the aqueous phase increased during the first 30 days of the incubation but stabilized thereafter. Both the rate of tetracycline disappearance from the aqueous phase and the rate of tritium appearance in the aqueous phase were only slightly larger for the biotic systems relative to the abiotic systems. Tritium release increased for whole soils < clay-humic complexes < clays.

The results of these studies indicate that TC is very strongly adsorbed on soils and soil components. And, once adsorbed, only small amounts of TC are desorption from the solid phase to the aqueous phase. Although soil clays adsorb more TC than whole soils or soil clay-humic complexes, the TC that is adsorbed to soil clays is more labile than the TC that is adsorbed on whole soils or clay humic complexes. Up to 16% of the

tetracycline that was added to the soil clay systems was degraded during the 149-day incubation. In contrast, only about 4% of the tetracycline that was added to the whole soil systems was degraded during the incubations. The results indicate that TC in soil systems will be subject to both biodegradation and surface catalyzed abiotic degradation. TC interacts so strongly with soil components that there is little likelihood for significant leaching of TC in soils.

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