Report for 2001MN1041G: Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields

- Other Publications:
  - Kumar, Kuldip, A. Thompson, A.K. Singh, and S.C. Gupta. 2002. Adsorption of antibiotics on soils. Agronomy abstract.
  - Chander, Y., K. Kumar, S.C. Gupta, A.K. Singh, and S.M. Goyal. 2003. Antimicrobial activity of soil bound antibiotics. Agronomy Abstract S11-Chander 633887.
- Articles in Refereed Scientific Journals:
  - Kumar, Kuldip, A. Thompson, A.K. Singh, Y. Chander, and S.C. Gupta. 2004. Enzyme-linked immunosorbent assay for ultratrace determination of antibiotics in aqueous samples. J. Environ. Qual. 33: 250-256.
- unclassified:
  - Gupta, S.C., K. Kumar, A. Thompson, A.K. Singh, and Y. Chander. 2003. Antibiotic adsorption of soil in batch and flow through set-ups. Agronomy Abstract S02-Gupta 886285.

Report Follows

Antibiotic losses in runoff and drainage from manure-applied fields

Satish Gupta, Professor and PI, (gupta002@umn.edu), Department of Soil, Water, and Climate; **Ashok Singh**, Veterinary Diagnostic Laboratory; **Kuldip Kumar, Anita Thompson,** and **David Thoma,** Department of Soil, Water, and Climate, University of Minnesota

Project duration: September 4, 2001 to September 3, 2003
Funding source: USGS-WRRI 104G National Grants Competition

The objective of this research is to quantify the effects of liquid swine manure application on antibiotic and nutrient (N and P) losses via surface runoff and subsurface drainage under a conventional (moldboard plowing) and a conservation (chisel plowing) tillage system. The field experiment is set up at the University of Minnesota Southwest Research and Outreach Center, Lamberton, Minnesota. The soil at the experimental site is a Webster clay loam soil (fine-loamy, mixed mesic Typic Haplaquoll), a common soil series in the Minnesota River Basin. The experiment is a randomized split-plot design with four replications (Figure 1). The main plots consist of two tillage treatments: (1) fall moldboard plowing followed by two passes of field cultivation before corn planting; and (2) chisel

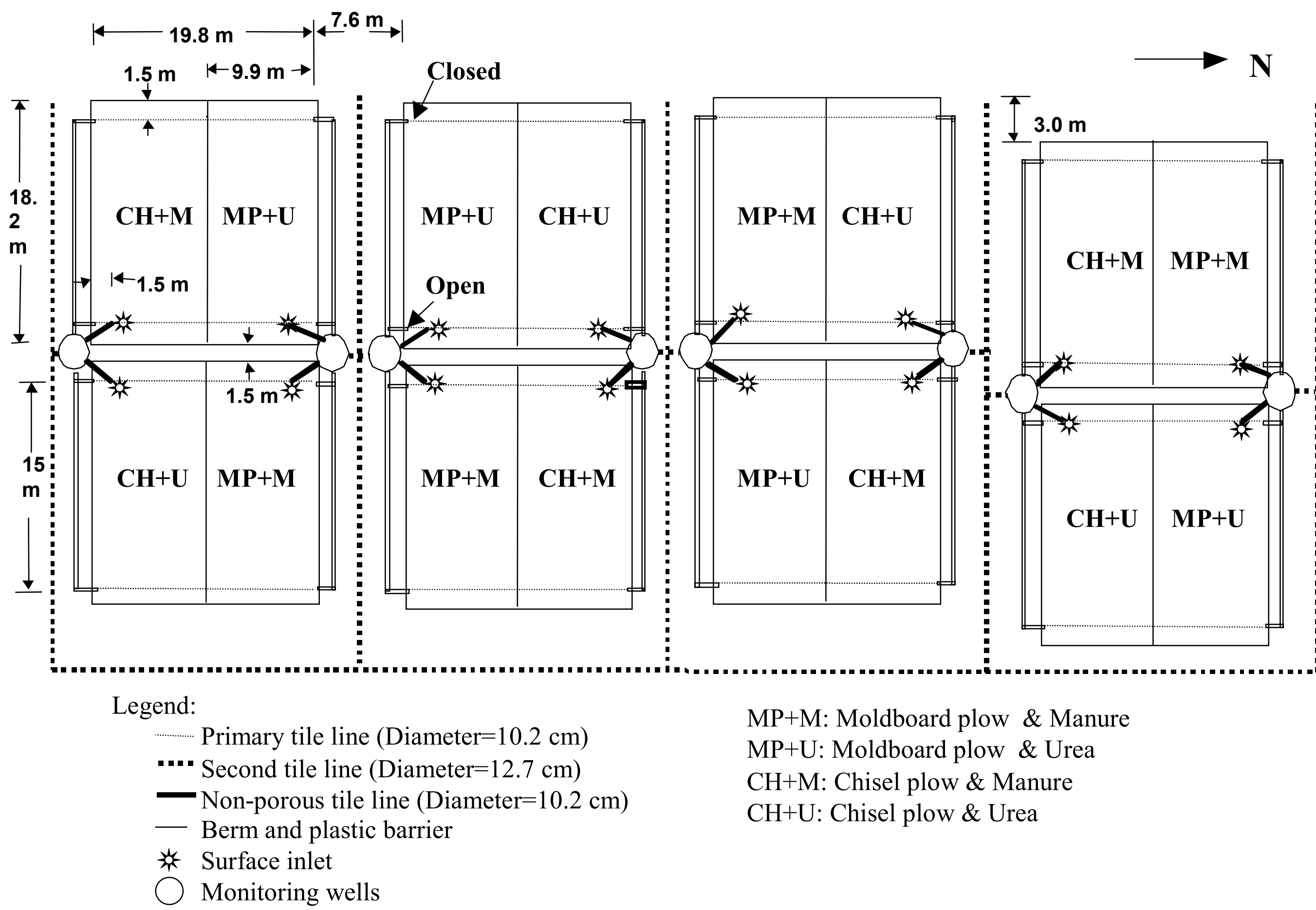

Figure 1. Surf-n-sub plot lay out at the Southwest Research and Outreach Center in Lamberton, MN.

plowing followed by two passes of field cultivation before corn planting. The subplots are two annually applied nutrient management treatments: fall injected (10 cm depth) liquid swine manure *versus* spring-applied and incorporated (5 cm depth) urea.

The drainage plots are 18.2 m long and 9.9 m wide. Each plot is isolated to a depth of 1.8 m by trenching around plot borders and installing a 0.3 mm plastic sheet (Zhao et al. 2001). A perforated plastic tile drain, 10 cm in diameter, is installed at 1 m depth and 1.5 m away from the plot boundary along its width. This arrangement drains 16.7 m (18.2 m minus 1.5 m) length of the plot, one-half side of tile drains that may be 33.4 m apart. Tile drains empty into a monitoring well. Surface inlets are located at the lowest point in the plots and also drain surface runoff into the monitoring well.

Manure and urea application rates are based on the University of Minnesota recommendations corresponding to a yield goal of 150 bu/acre. Both surface runoff and subsurface tile drainage are measured by tipping bucket devices that are connected to CR-10 data loggers. Volume- distributed (composite water sample over a certain number of tips) runoff samples from surface inlets are taken by automated ISCO® samplers. Time-distributed (composite water sample over a certain time interval) subsurface drainage samples are collected manually once a day. The other details of sampling set-up and protocol are given in Zhao et al. (2001), and Thoma and Gupta (2001).

For the 2001-2002 crop year, primary tillage was done October 4, 2001, and subsequently liquid hog manure was injected on November 5, 2001, in half of the plots @ 4944 gallons/acre. This corresponds to N application of 239 lbs/acre. We had planned to apply manure corresponding to a yield goal of 150 bu/acre. However, our manure analysis from the manure pit before application showed lower N concentration than what was actually applied. The low concentration might have been due to lack of mixing of the manure in the pit prior to collection of the sample used to determine application rate. When we analyzed the actual manure sample injected in the soil, N concentrations were higher, resulting in higher N application rates than needed for the yield goal.

Two passes of secondary tillage were made on May 1, 2002. In the remaining half of the plots, urea was applied at an equivalent of 150 lbs-N/acre just before the secondary tillage. Corn was planted on May 1, 2002, right after secondary tillage. Both runoff and tile line samples were collected as per event. The samples are being analyzed for sediment, nutrients, and antibiotics losses.

Currently, there are no standard methods for analysis of antibiotics in soil and water samples. Therefore, most of our effort this year has gone into the development of analytical methods for antibiotic in manure, water, and soil samples. The farmer supplying manure for our experiment mentioned that he is mixing aueromycin (chlortetracycline) and tylosin in swine feed. Therefore, our methods development was geared towards quantification of chlortetracycline and tylosin. Analysis of the hog manure from the supplier lagoon showed presence of chlortetracycline (5.0 mg/L of manure slurry) and tylosin (5.6 mg/L of manure slurry). At 4,944 gallons/acre, this is equivalent to 92.7gm/acre of chlortetracycline and 103.8 gm/acre of tylosin. At 150 lbs-N/acre, the manure application rate would have been 3103 gallons/acre and the addition of antibiotics would have been 58.2 g of chlortetracycline and 65.2 g of tylosin. Antibiotic analysis in manure sample was done on HPLC (High Performance Liquid Chromatography).

Subsequent analysis of runoff and tile line samples showed that concentrations of both chlortetracycline and tylosin were too low to detect with HPLC. Therefore, a new method based on immuno assay (ELISA-Enzyme-Linked Immunosorbent Assay) was used to analyze runoff samples. Because the ELISA plates are expensive and our laboratory showed that chlortetracycline is readily and highly adsorbed on soil particles, most of our effort so far has gone into analysis of tylosin. We have detected the presence of tylosin in only a few runoff samples. In most of these runoff samples, tylosin concentrations were generally <1 parts per billion (ppb). There were other samples where tylosin concentration was between 1–3 ppb. In one sample, we detected tylosin concentrations as high as 5.4 ppb (Table 1). These concentrations are relatively low compared to the concentration of tylosin in the manure sample (5.6 ppm). We are in the process of buying the ELISA plates for chlortetracycline. In the next report, we should have results on the concentration of chlortetracycline in runoff and tile line samples and also the concentration of tylosin in the tile line samples.

Other efforts in this project have gone into characterizing the adsorption characteristics of tetracycline, chlortetracycline and tylosin on two different soil types (Webster clay loam and Hubbard sandy loam). Adsorption studies were done both in batch (Figure 2) and in flow-through (Figure 3) set-up. The surface samples of Webster clay loam soil were taken from urea plots of our field experiment at Lamberton. Hubbard sandy loam is a glacial outwash soil and represents a major soil group in Central Sands of Minnesota. Batch experiments showed that tetracycline and chlortetracycline are strongly adsorbed on both soils than tylosin. Among the soils, Webster clay loam has higher adsorption capacity than the Hubbard sandy loam. The differences in soil types are due to differences in clay and

Table 1. Samples Tested Positive for Tylosin.

Sample (Plot-Number)[a]	Sample Date	Manure or Urea[b]	Filtered or Unfiltered[c]	ELISA Test[d]	Tylosin concentration (ppb)
2-1	08/04/2002	M	F	Aug 14 #1	1.77
9-bkt	08/04/2002	M	F	Aug 14 #1	1.14
9-bkt	08/04/2002	M	U	Aug 14 #1	1.46
1-5to8	08/09/2002	U	U	Aug 14 #1	0.17
2-1	08/09/2002	M	F	Aug 14 #1	0.13
2-bkt	08/09/2002	M	F	Aug 14 #1	1.40
2-bkt	08/09/2002	M	U	Aug 14 #1	1.85
8-1	08/09/2002	M	F	Aug 14 #2	1.02
8-1	08/09/2002	M	U	Aug 14 #2	1.68
8-bkt	08/09/2002	M	F	Aug 14 #2	1.75
9-bkt	08/09/2002	M	F	Aug 14 #2	0.05
9-bkt	08/09/2002	M	U	Aug 14 #2	0.81
11-1	08/09/2002	M	F	Aug 14 #2	3.27
11-1	08/09/2002	M	U	Aug 14 #2	0.92
13-bkt	08/09/2002	M	F	Aug 14 #2	0.88
16-1	08/09/2002	M	F	Aug 14 #2	1.92
16-1	08/09/2002	M	U	Aug 14 #2	0.90
16-2	08/09/2002	M	F	Aug 14 #2	0.78
16-2	08/09/2002	M	U	Aug 14 #2	0.49
16-3	08/09/2002	M	F	Aug 14 #2	1.03
16-3	08/09/2002	M	U	Aug 14 #2	0.32
8-1	07/30/2002	M	U	Aug 19	0.44
9-1	07/30/2002	M	F	Aug 19	5.38
1-1	08/04/2002	U	F	Sept 17	0.64
4-1	08/04/2002	M	F	Sept 17	0.50
1-4	8/9/02	U	F	Sept 17	0.52
10-1	8/9/02	U	F	Sept 17	0.20
12-1	8/9/02	U	F	Sept 17	0.34
12-2	8/9/02	U	F	Sept 17	0.41
14-1	8/9/02	U	F	Sept 17	0.12
15-1	8/9/02	U	F	Sept 17	0.06
1-1	8/22/02	U	F	Sept 17	0.04
2-1	8/22/02	M	F	Sept 17	1.21
8-1	8/22/02	M	F	Sept 17	1.36
14-1	8/22/02	U	F	Sept 17	0.25
15-1	8/22/02	U	F	Sept 17	2.91
15-2	8/22/02	U	F	Sept 17	0.03

[a]bkt refers to samples collected from the tipping bucket
[b]M=manure plots, U=urea plots
[c]F=filtered sample, U=unfiltered sample
[d] Dates refers to dates ELISA test was done

organic matter content of soils. Webster clay loam is higher in both clay and organic matter contents (34% & 4.4%) than the Hubbard sandy loam (10.4% & 2.2%). Flow-through experiment with Hubbard sandy loam showed results consistent with the batch experiment; i.e., chlortetracycline and tetracycline are more strongly adsorbed on the soil than tylosin.

Linear sorption coefficients (K_d) of chlortetracycline, tetracycline and tylosin on Webster clay loam were 2386, 2370, and 92 L/kg as compared to 1280, 1147, and 66 L/kg for Hubbard sandy loam. Thus, at saturation, the retardation coefficients of chlortetracycline, tetracycline and tylosin in Webster clay loam will be 6083, 6042, 236, as compared to 4466, 4002, 231

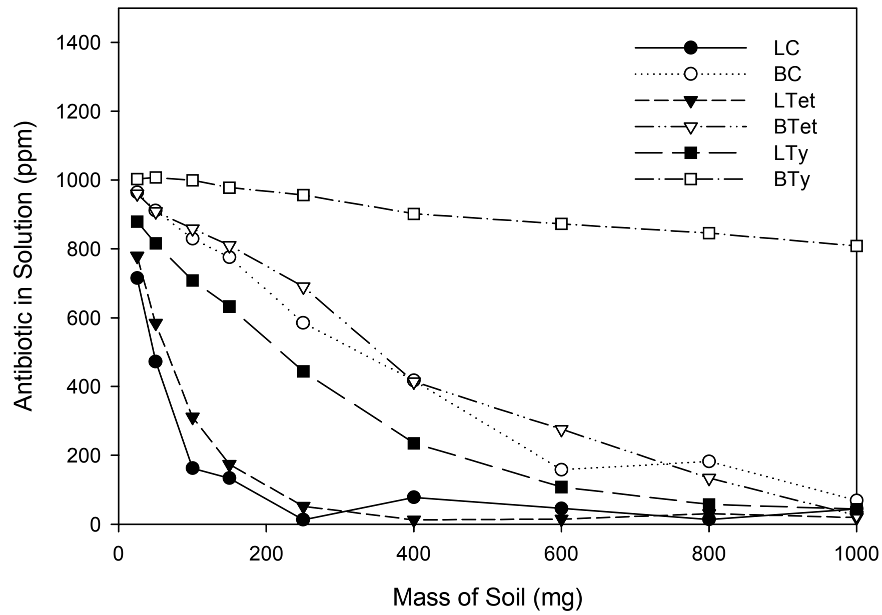

Figure 2. Antibiotic remaining in solution after shaking 1000 ppm of antibiotic solution with various amounts of soil. L= Webster clay loam, B=Hubbard sandy loam, C=chlortetracycline, Tet=tetracycline, Ty=Tylosin. Top three curves are for Hubbard sandy loam soil whereas bottom three curves are for Webster clay loam soils. Tetracycline and chlortetracycline are more strongly adsorbed on both soils than tylosin. Among the soils, Webster clay loam soil has higher adsorption capacity than the Hubbard sandy loam soil.

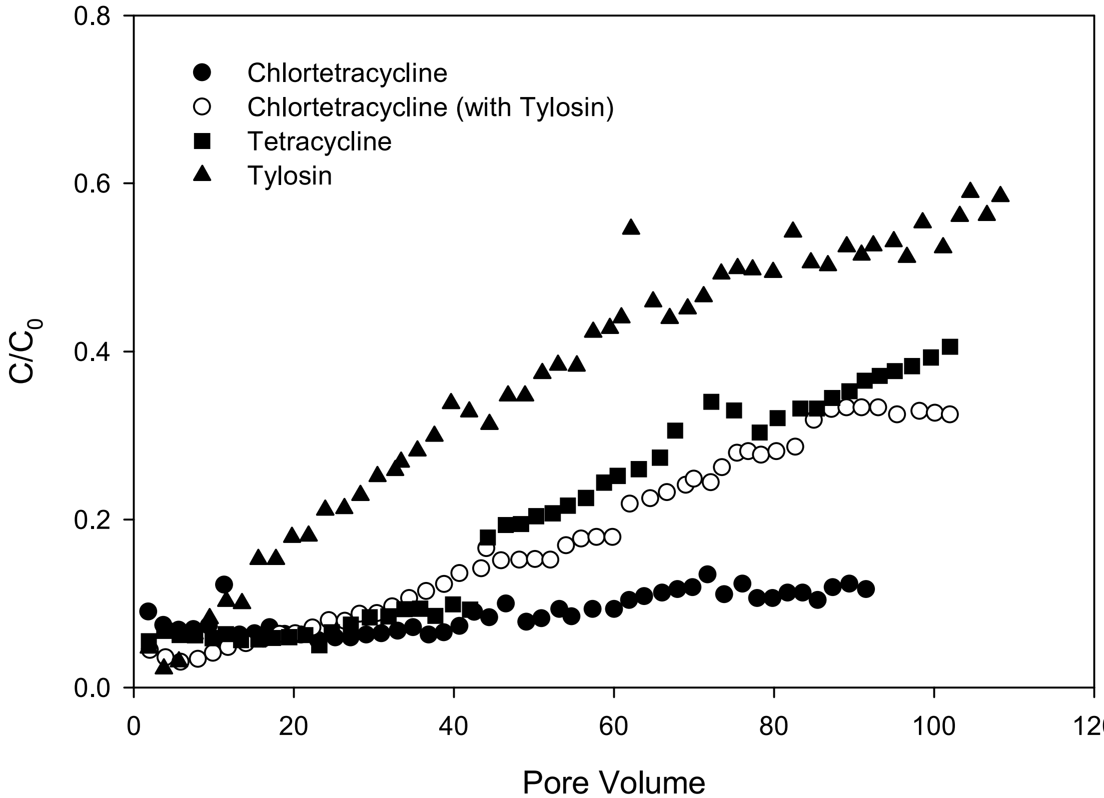

Figure 3. Breakthrough Curves for the Hubbard sandy loam soil for three antibiotics. Each data point is the average of three replicates. As shown by batch adsorption studies, chlortetracycline and tetracycline are more strongly adsorbed on the Hubbard sandy loam soil than tylosin.

for Hubbard sandy loam. The higher the retardation value, the greater is the adsorption potential of that chemical for a given soil. This number also reflects the quantity of water needed to displace a chemical through soil to the same distance as the non-adsorbing chemical. In other words, chlortetracycline will need 6083 times more water to displace than chloride in a Webster clay loam at saturation. The variation in K_d values reduced when it was normalized with clay or organic carbon contents, thus suggesting that clay and organic carbon may be the primary adsorption sites for these antibiotics.

Breakthrough experiments with Hubbard sandy loam also showed similar differences in the mobility of these three antibiotics. At C/C_o=0.2, the pore volume needed to displace tylosin was 24 compared to 52 for tetracycline and >100 for chlortetracycline.

We have finished the nitrate and ammonium concentration of tile line samples. We are still processing the runoff samples for sediment and phosphorus. We will report those results along with other antibiotic analyses in our next report.

References

Thoma, D.P. and S.C. Gupta. 2001. Tillage and Nutrient Source Effects on Water Quality From Flat Lands in the Minnesota River Basin. A report submitted to Minnesota Dept. of Agriculture.

Zhao, S. L., S.C. Gupta, D.R. Huggins, and J.F. Moncrief. 2001. Tillage and nutrient source effects on surface and subsurface water quality at corn planting. *J. Eniron. Qual.* 30: 998-1008.