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Introduction

Research Program

Microcystin-LR: a potential contaminant of concern for Iowa surface waters

Basic Information

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2. Schultz, Alissa C., Leah C. Osterhaus, Edward J. Brown and Maureen E. Clayton. April 2001. Microcystin-LR: A Potential Contaminant of Concern for Surface Water Quality in Iowa. A poster presentation at the UNI Sigma Xi Student Research Conference, Cedar Falls, IA.

Introduction

Surface water quality is currently one of the most important environmental issues facing the state of Iowa because the ecological, recreational and aesthetic values of these water bodies are threatened by non point source (NPS) pollution. The traditional threat to lake water quality from NPS contaminants is eutrophication resulting from nutrient loading. Preliminary evidence from a study of two Iowa lakes by a multidisciplinary team of researchers at the University of Northern Iowa, however, indicates that algal species composition may also be altered by nutrient loading. The dominance of cyanobacteria in eutrophic lakes suggests that water quality and human and wildlife health may be endangered by exposure to toxin producing strains of cyanobacteria.

Summer blooms of cyanobacterial populations are common in eutrophic lakes and ponds. These blooms represent very high concentrations of cyanobacteria in the water column, and may result in a discoloration of the water and in the formation of what is colloquially known as "pond scum". Blooms are initiated and exacerbated by factors including excessive nitrogen (N) and phosphorus (P) loading, surface water temperatures $> 20\ ^{o}C$, stratification of the water column, long water residence time, organic matter enrichment, low total and dissolved N:P ratios, and high irradiance and long daylength. These conditions are common in Iowa lakes and ponds, especially those which are impacted by nutrient loading from agricultural runoff.

High cyanobacterial concentrations may reduce recreational use of surface waters because of the aesthetic impact of the discolored water. In addition, some species of cyanobacteria produce biotoxins that can result in severe health impacts and death in humans and wildlife. Microcystin-LR is the most toxic of the cyanobacterial biotoxins; it is known to be a potent inhibitor of protein phosphatases 1 and 2A (Toivola et al 1994, Runnegar et al 1995, Sahin et al 1995, Craig et al 1996), as well as a tumor promotor (Lam et al 1995). Neurotoxic illness and liver failure have been observed in exposed humans (Jochimsen et al 1998, Pouria et al 1998). Microcystin-LR may also be transferred through the food chain (Kotak et al 1996, Prepas et al 1997) and can result in sublethal effects, including ionic imbalance, decreased grazing, and reduced growth, in fish and other aquatic organisms (Beveridge et al 1993, Keshavanath et al 1994, Bury et al 1995).

Recent research on microcystin-LR has primarily focused on the following areas: (1) laboratory experiments to elucidate the mechanism of action (e.g. Toivola et al 1994, Lam et al 1995, Runnegar et al 1995, Sahin et al 1995, Craig et al 1996), (2) laboratory studies to examine the effects of nutrients on toxin production (e.g. Parker et al 1997, Rapala et al 1997), (3) improved analytical methods for toxin detection (Lambert et al 1994, Lin and Chu 1994, Liu et al 1996, Ward et al 1997), and (4) mechanisms of toxin degradation (Tsuji et al 1994, Bourne et al 1996). This research suggests that cyanobacterial toxins may be environmentally relevant contaminants if toxin-producing strains are abundant, or if the strains are exceptionally toxic.

Species that are known to produce microcystin-LR (*Microcystis* sp. and *Anabaena* sp.) have been observed as dominant members of the phytoplankton assemblage in two

Iowa lakes, Silver Lake and Casey Lake (Osterhaus 1999). Unfortunately, however, toxic and non-toxic strains are difficult or impossible to distinguish morphologically (Boney 1975). A direct competitive ELISA using antibodies against microcystin-LR (Lin and Chu 1994, Liu et al 1996) is now available to quantitatively determine the microcystin concentration in surface waters. We used this ELISA to determine the degree of the potential threat posed by microcystin-LR to the health of the humans and wildlife (as well as the ecosystem) that utilize two Iowa lakes. The primary objective of the study was to determine the potential threat from cyanobacterial toxins by determining the toxin content in surface waters and correlating these concentrations to cyanobacterial abundance and species composition, and therefore indicating whether or not microcystin-LR should be considered as a contaminant of concern in surface waters. Nutrient concentrations were also determined in the lakes because the nutrients N and P may stimulate cyanobacterial growth, while increased P is correlated with increased microcystin concentration within the cell (Rapala et al 1997); both pathways (increased cyanobacteria populations and increased toxin content per cell) will result in increased toxicity within the water column. The results of this research will provide information to assist resource managers in determining the extent of the problem and in suggesting the need for novel remediation strategies for hypereutrophic Iowa lakes.

Materials and Methods

Two lakes in northeastern Iowa were chosen for this study. Silver Lake, located in the town of Delhi (Delaware County – 42°25'N, 91°19'W), is heavily impacted by land use practices within the watershed, which are primarily agricultural (81% cropland and small hog farms); a small residential area and school ball fields also border the lake. Silver Lake does not currently support its designated use as a recreational water body; winter fish kills are common, stocked fish do not thrive, and cyanobacterial blooms discolor the water. The Iowa Department of Natural Resources has installed an aerator as a remediation effort, but this attempt has been unsuccessful to date. On the other hand, Casey Lake, located in Hickory Hills Park (Tama County – 42°16'N, 92°19'W), is located in a watershed that is primarily parkland (58%), although there is agricultural activity in the form of cropland (31%) within the watershed. The entire shoreline is publicly owned. The lake supports a thriving recreational fishery, including ice fishing tournaments. Fishing, swimming, picnicking, and camping are common recreational activities around the lake.

Both lakes are relatively small and shallow and originated as impoundments. Casey Lake is the larger of the two, with an area of 54 acres and a mean depth of 3.1 m (maximum depth 7.6 m). Silver Lake is approximately 34 acres in area, with a mean and maximum depth of 1.9 and 4.6 m, respectively. The watershed of Silver Lake is smaller, at approximately 5.4 times the area of the lake, while Casey Lake has a watershed area that is 13.6 times the area of the lake (Bachman et al 1994). Casey Lake has three permanent inflows, while Silver Lake is fed entirely by precipitation and runoff. The volume of both lakes is controlled by gated outflows.

Water samples were collected weekly from June 20th to August 10th at stations located along a series of transects within the body of Silver Lake and Casey Lake in conjunction

with the UNI Lake Water Quality Study. During sample collections, *in situ* measurements of dissolved oxygen, temperature, turbidity and secchi disk depth were conducted. Samples for microcystin analyses were collected weekly from the surface in acid rinsed sterile bottles from 14 stations in Casey Lake and 10 sites in Silver Lake. The samples were transported to the laboratory on ice, where they were stored at 4 ^{o}C and processed as soon as possible. In the lab, phytoplankton cells were separated from lake water by centrifugation (Silver Lake) or gravity filtration (Casey Lake). Cell lysate was obtained by centrifugation after sonication in phosphate buffered saline, pH 7.2 (McDermott et al 1995). The lake water (cell free) and cell lysate samples were stored at -20ºC.

Lake water samples were also collected for the analysis of chlorophyll *a* concentrations and determinations of phytoplankton species diversity. Water samples for chlorophyll *a* analyses were collected from the surface at the same stations as for microcystin analysis and transported to the laboratory on ice, where they were stored at 4^{o}C prior to analysis. Chlorophyll *a* was extracted in 90% acetone from water samples filtered through glass fiber filters, and then measured spectrophotometrically by a method that corrects for the presence of phaeophytin, a chlorophyll *a* degradation product (Clesceri et al 1998). Phytoplankton samples were collected from two sites within the body of each lake with an 80 μm mesh plankton net. After being transported to the laboratory on ice, the samples were preserved in Lugol's solution for later analysis (Clesceri et al 1998). The phytoplankton were identified to the lowest possible taxonomic unit and were counted using a hemocytometer. Relative abundances of cyanobacteria and green algae were computed from the count data.

Microcystin-LR concentrations were determined in lake water and cell lysate samples by direct competitive enzyme linked immunosorbent assay (ELISA) (Lin and Chu 1994, Liu et al 1996), using kits purchased from EnviroLogix (Portland, ME). The kit is based on a monoclonal antibody developed against microcystin-LR. Other microcystins may cross react with the antibody to a lesser degree. Therefore, HPLC studies (McDermott et al 1995) are in progress to verify that the ELISA assay results are due to the presence of microcystin-LR in the samples. Until these analyses are completed, the data will be reported as concentration of microcystin.

The results of the chlorophyll *a* analyses were used to select samples for toxin analysis. For each sampling date, three samples were selected from each lake; these samples corresponded to a station at or near the mean chlorophyll *a* concentration in the lake on that date, as well as the stations with the highest and lowest chlorophyll values. This approach was selected in order to adequately characterize the spatial and temporal variation in the lakes without the prohibitive expense of analyzing each sample. Purified microcystin-LR standards were run on each plate along with the lake samples. Each sample (standards, lake water and cell lysate) was analyzed in triplicate; mean microcystin concentrations and standard deviations are reported. Lake water samples are reported as μg microcystin/L, and cell lysate toxin concentrations are reported as μg microcystion/mg cells (wet weight).

Lake water samples were also collected at each station and transported to the laboratory in acid rinsed bottles on ice for nitrate and phosphate analysis. Nitrate concentrations were measured with an ion selective electrode (Vernier, Beaverton, OR). Unfortunately, this method proved to be unreliable, occasionally producing erroneously high nitrate values; this data will, therefore, not be presented. Total phophorus was measured by the ascorbic acid colorimetric method after persulfate digestion (Clesceri et al 1998). Soluble reactive phosphorus was also determined after filtration of Silver Lake samples through 0.45 μm membrane filters.

Toxin concentrations in both cell free lake water and cell lysate samples were evaluated in relation to the other water quality parameters. Linear regressions were used to determine the statistical significance of the observed relationships.

Results and Discussion

Concentrations of microcystin in two Iowa lakes ranged from non-detectable levels (<0.16 μg/L) to more than 1.6 μg/L. Microcystin concentrations in Silver Lake were consistent during the sampling period (0.9 μg/L), and were always higher than concentrations at Casey Lake, where microcystin peaked from 7/20 to 8/2 (0.8 μg/L) before returning to background levels (0.2 μg/L) (Figure 1). These values are consistent with results from previous studies of lakes in Wisconsin (McDermott et al, 1995) and Alberta, Canada (Kotak et al, 1996). The observed concentrations are lower than those found to result in reduced growth of brown trout (41-68 μg/L – Bury et al, 1995). Even though these values would not appear to result in direct effects on fish populations, they are near the 48h LC_{50} for *Daphnia pulex* (1 μg/L – Reinikainen et al, 1994), suggesting that fish and other aquatic organisms could be impacted by effects at lower levels of the lake food web.

Microcystin concentrations in cell lysates ranged from 0.1 to 320 μg/g, and were always higher in Casey Lake (155 μg/g) than in Silver Lake (2 μg/g) (Figures 2 and 3). These values are consistent with the results of studies of cyanobacterial blooms (Jungmann and Benndorf, 1994 ; McDermott et al, 1995) and laboratory strains of *Microcystis* (Jungmann and Benndorf, 1994). The results suggest that a higher percentage of the phytoplankton in Casey Lake produce toxin, or that each toxin producing cell in this system makes more microcystin.

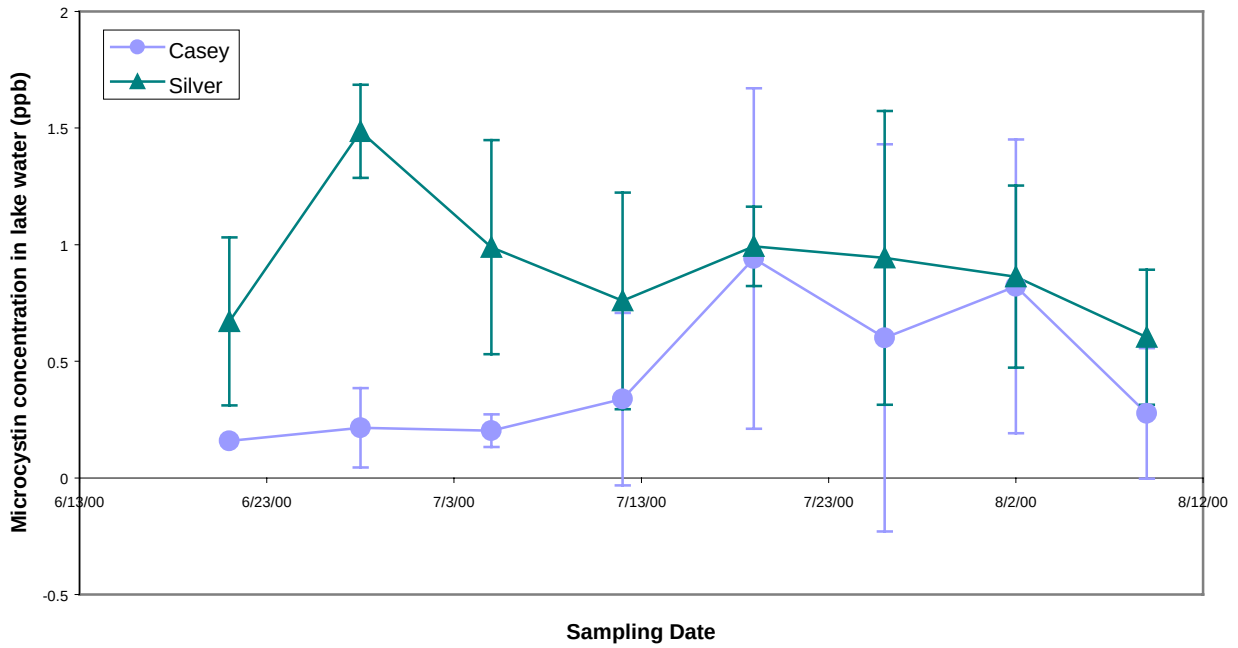

Figure 1. Temporal trends in microcystin concentrations in cell-free lake water samples. Means ± standard deviations are shown for Casey Lake in the circles and for Silver Lake in the triangles.

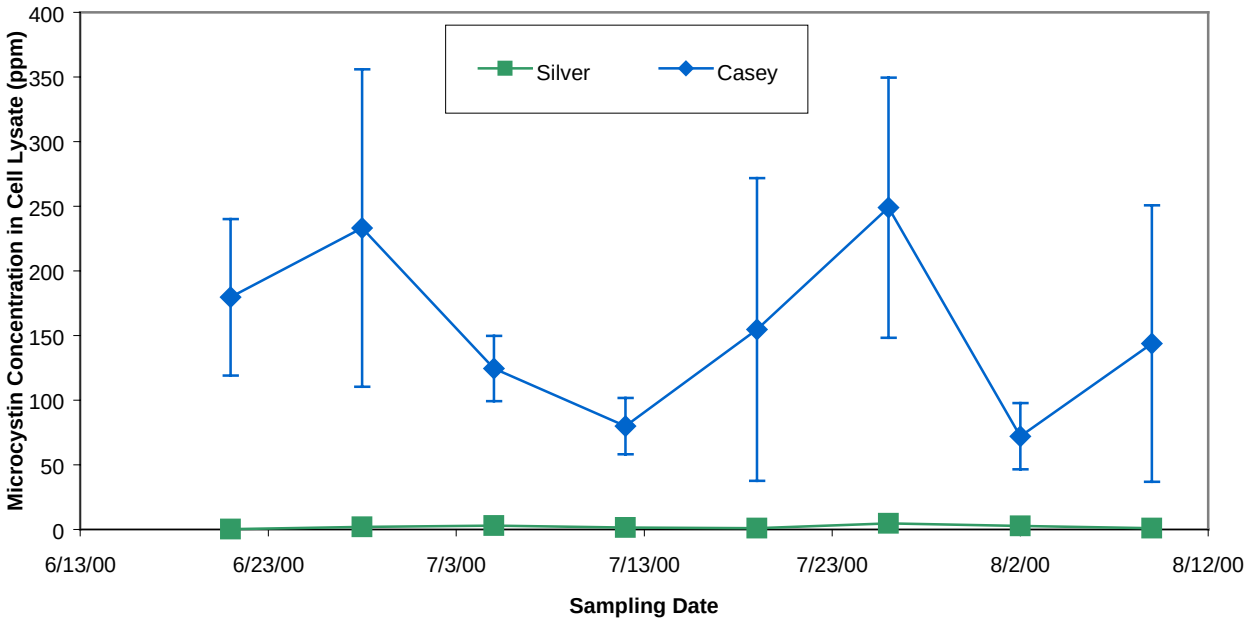

Figure 2. Temporal trends in microcystin concentrations in cell lysate samples, given as µg microcystin per mg cells (wet weight). Concentrations in cells (mean ± standard deviation) from Casey Lake are shown as diamonds; Silver Lake samples are shown as squares.

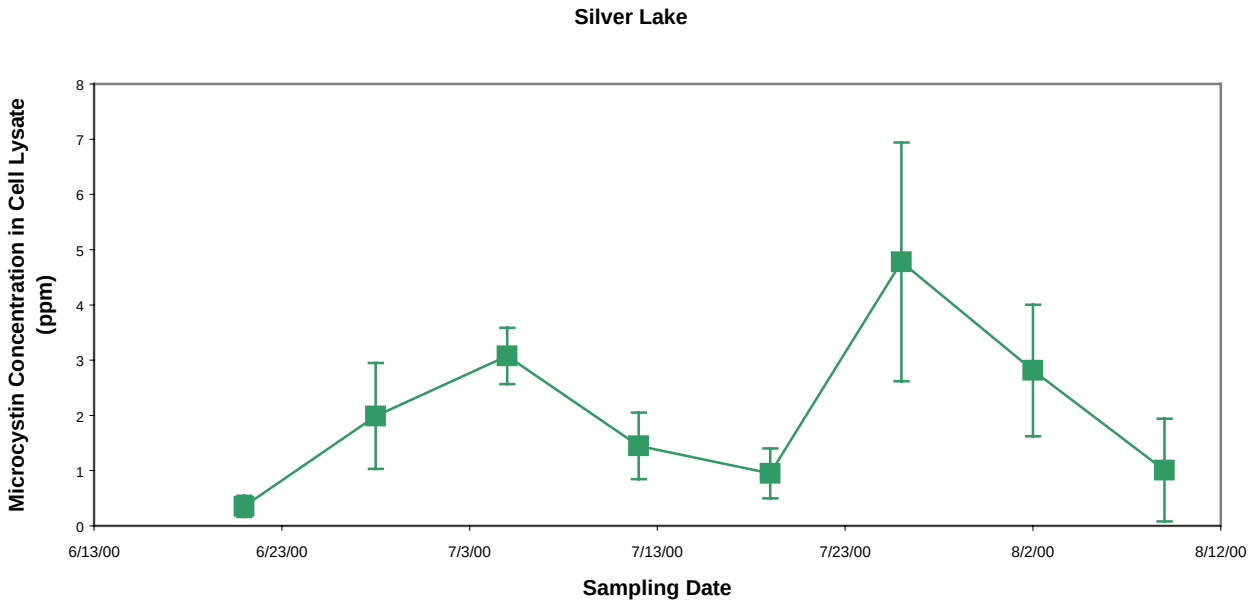

Figure 3. Temporal trends in microcystin concentrations (µg/mg wet weight) in cell lysate samples from Silver Lake phytoplankton. Note the difference in the scale of the y-axis from Figure 2.

There were no significant relationships between microcystin concentrations in lake water or cell lysate and chlorophyll *a* (Figures 4-7), relative cyanobacterial abundance (Figures 8-9), total phosphorus (Figures 10-13), dissolved oxygen (data not shown), turbidity (data not shown), secchi disk depth (data not shown), or water temperature (data not shown). In contrast, cyanobacterial toxin concentrations would be expected to correlate with cyanobacterial concentrations or other similar measures, and with nutrient concentrations in the water column. In fact, Rapala et al (1997) found increased concentrations of microcystin in *Anabaena* cells in culture with increasing phosphorus concentrations, but the examined concentrations (0.5-5 mg PO_4-P/L) were much lower than the values observed in Silver and Casey Lakes. Perhaps the cyanobacteria in these two lakes have sufficient nutrients and therefore these measures are no longer correlated with toxin production.

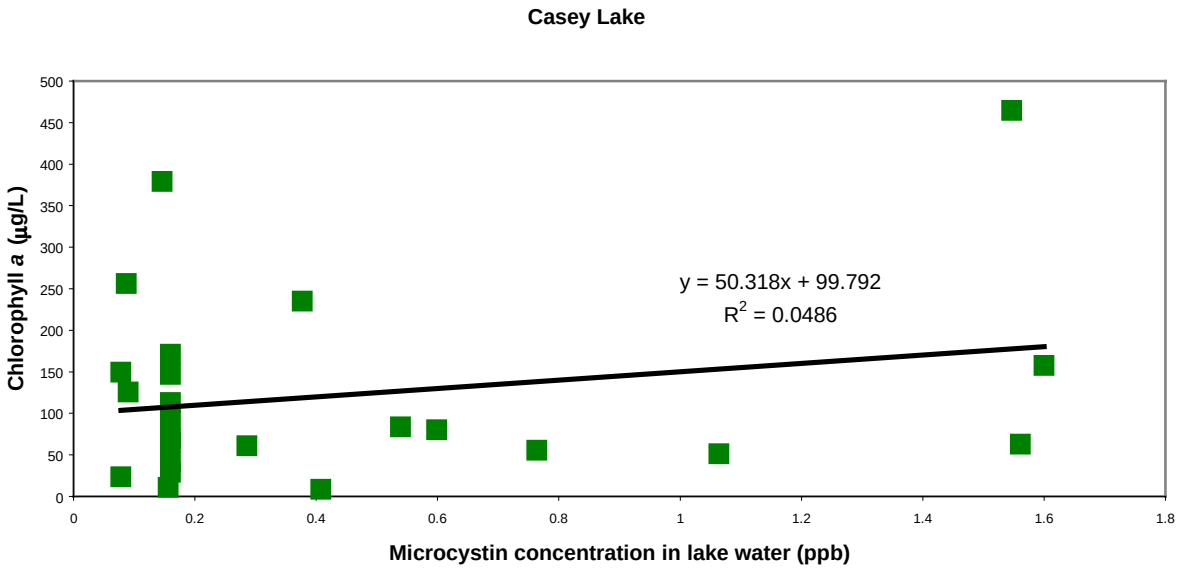

Figure 4. Microcystin concentrations (µg/L) in Casey Lake are not correlated (R^2=0.05) with lake phytoplankton populations measured by chlorophyll *a* concentrations (µg/L) in samples collected at the same time and station.

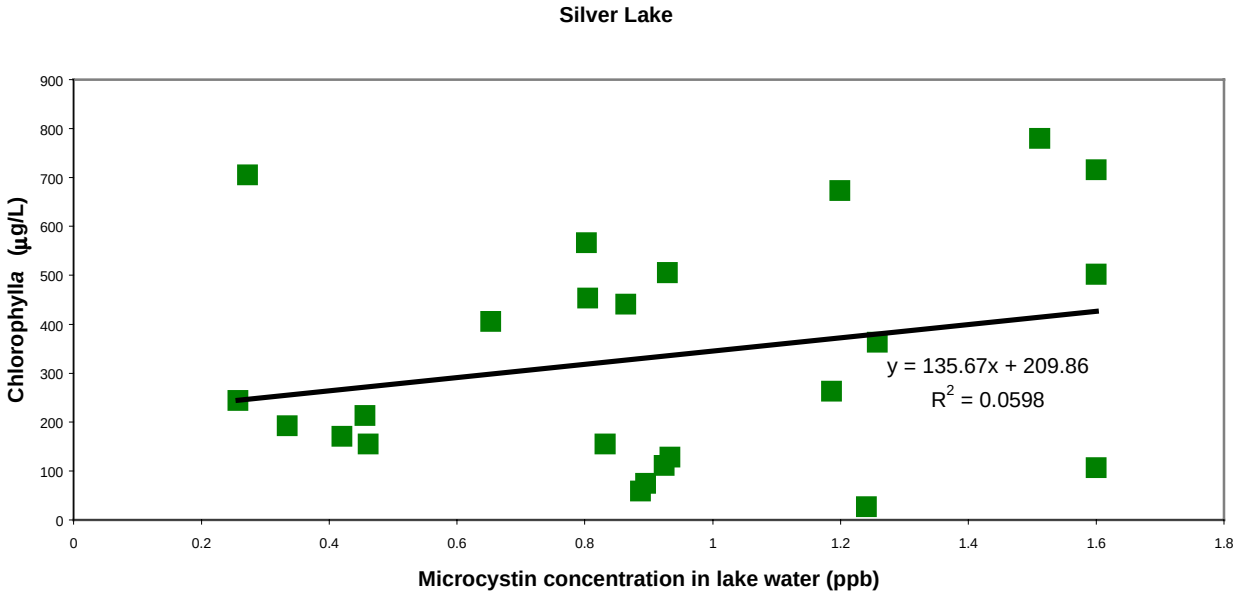

Figure 5. Microcystin concentrations (µg/L) in Silver Lake are not correlated (R^2=0.06) with lake phytoplankton populations measured by chlorophyll *a* concentrations (µg/L) in samples collected at the same time and station.

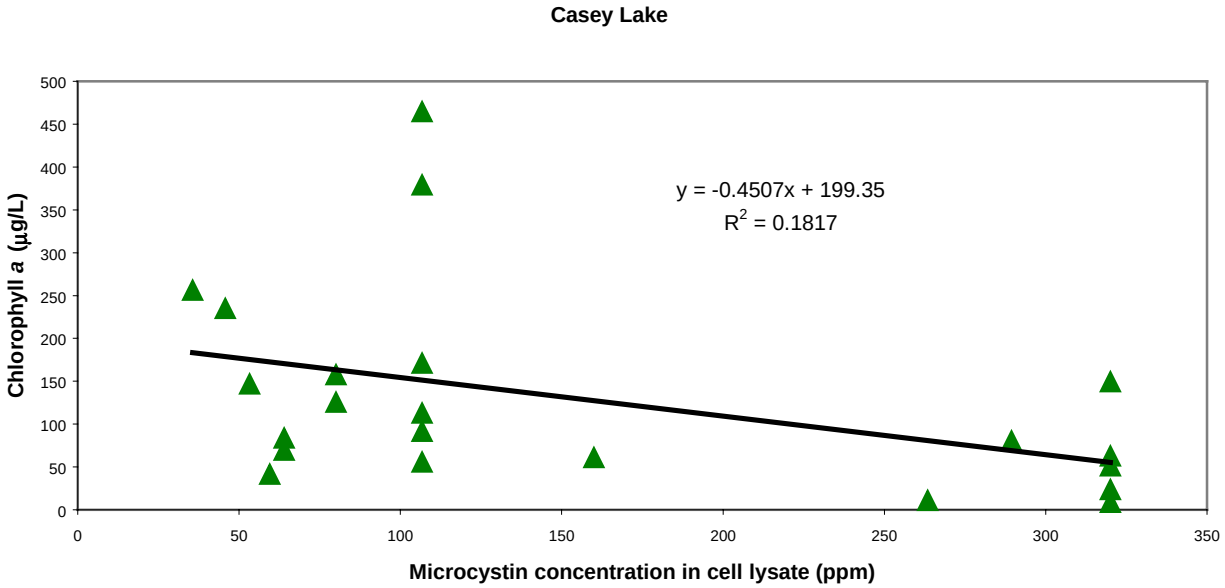

Figure 6. Microcystin concentrations (µg/mg) in cell lysate samples of Casey Lake phytoplankton are not correlated (R^2=0.2) with lake phytoplankton populations measured by chlorophyll *a* concentrations (µg/L) in samples collected at the same time and station.

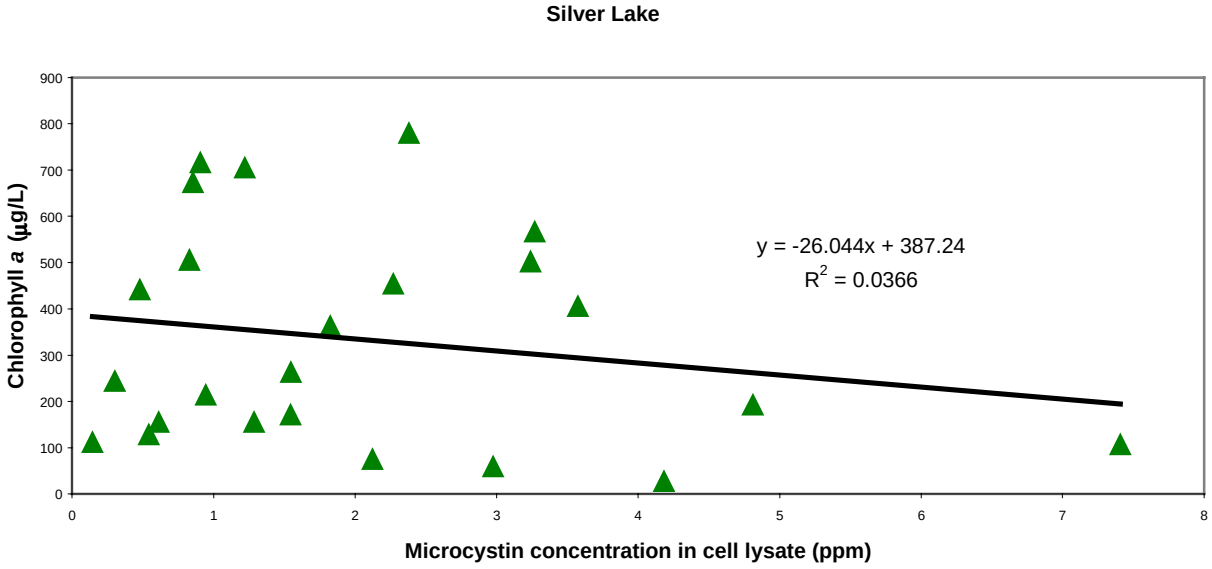

Figure 7. Microcystin concentrations (µg/mg) in cell lysate samples of Silver Lake phytoplankton are not correlated (R^2=0.04) with lake phytoplankton populations measured by chlorophyll *a* concentrations (µg/L) in samples collected at the same time and station.

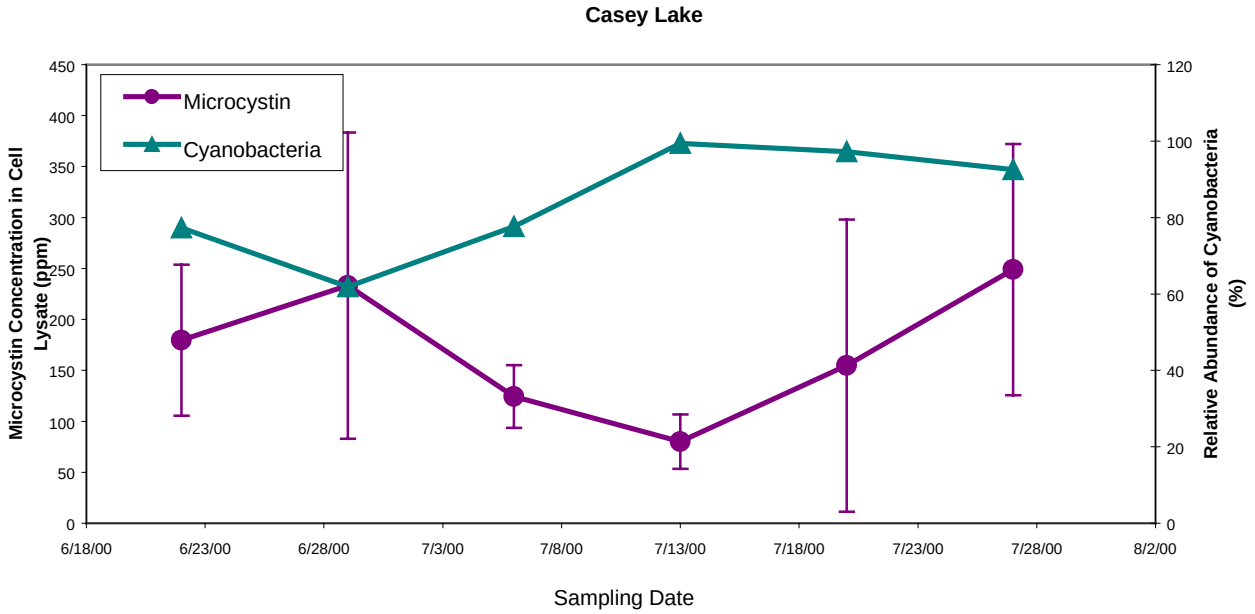

Figure 8. Temporal trends in microcystin concentrations (µg/mg wet weight) in cell lysate (circles; mean ± standard deviation) and relative abundance of cyanobacteria (triangles) in Casey Lake. Based on linear regression analysis (not shown), the relationship between the two parameters is not significant (R^2=0.2).

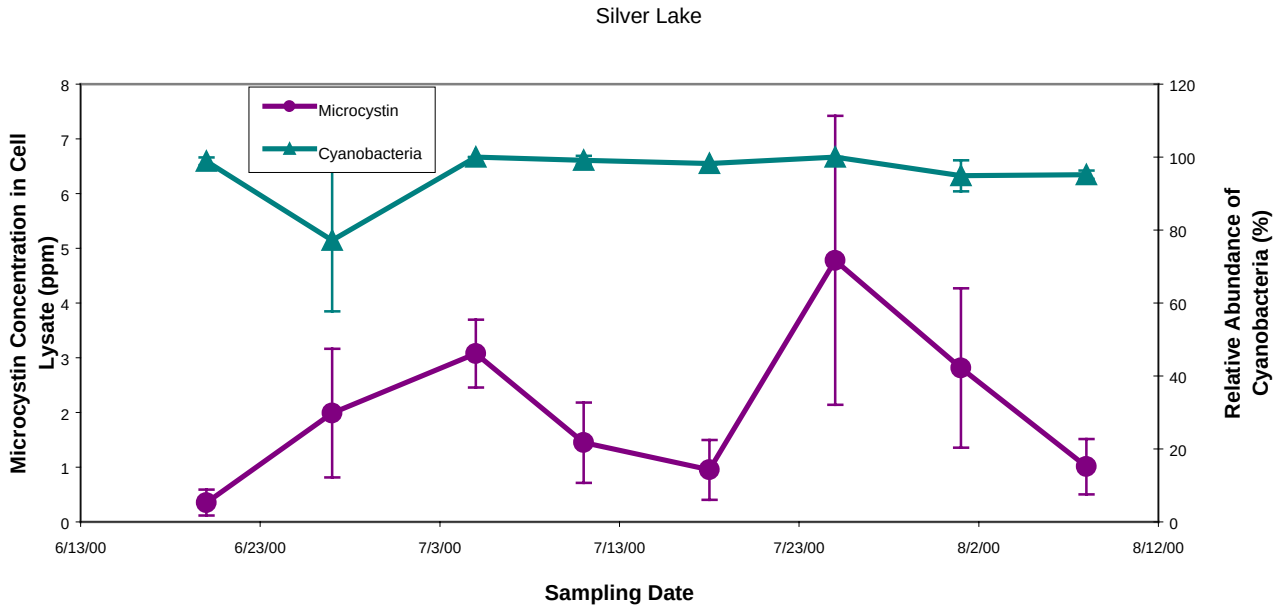

Figure 9. Temporal trends in microcystin concentrations (µg/mg wet weight) in cell lysate (circles; mean ± standard deviation) and relative abundance of cyanobacteria (triangles) in Silver Lake. Based on linear regression analysis (not shown), the relationship between the two parameters is not significant (R^2=0.01).

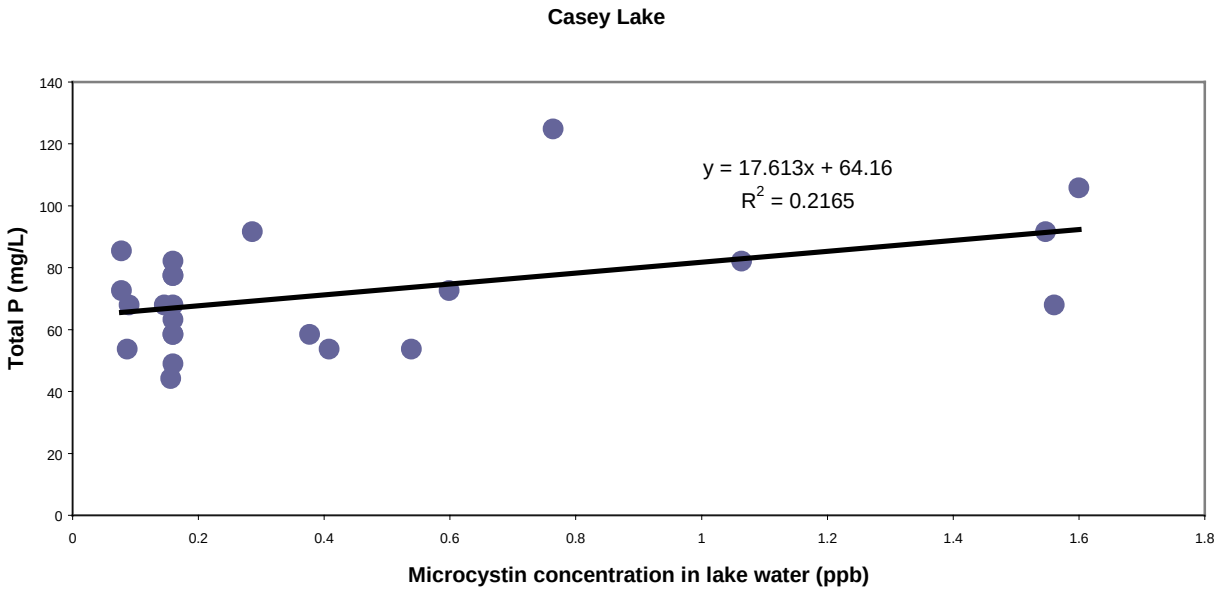

Figure 10. Microcystin concentrations (µg/L) in Casey Lake are not correlated (R^2=0.2) with lake phosphate concentrations measured as total phosphorus (mg/L) in samples collected at the same time and station.

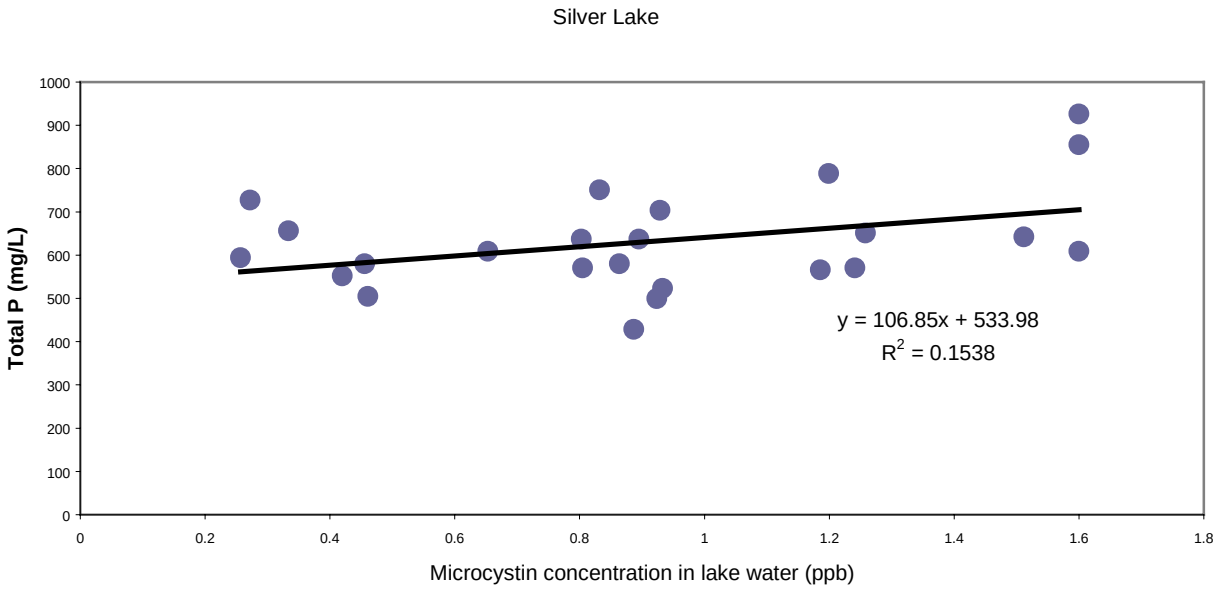

Figure 11. Microcystin concentrations (µg/L) in Silver Lake are not correlated (R^2=0.2) with lake phosphate concentrations measured as total phosphorus (mg/L) in samples collected at the same time and station.

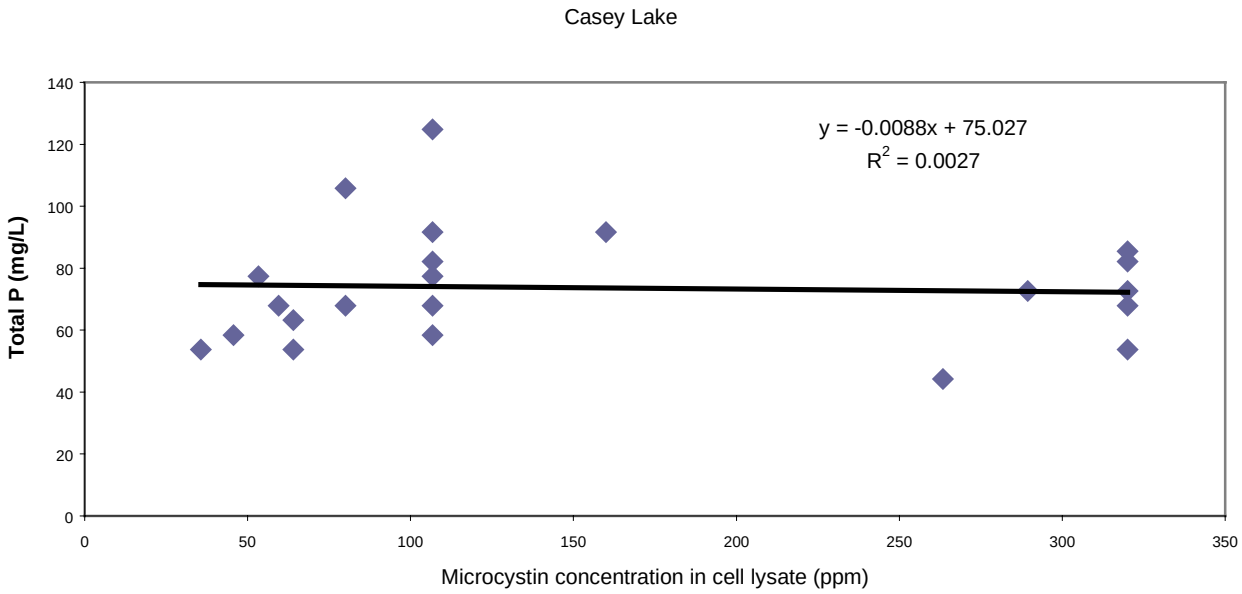

Figure 12. Microcystin concentrations (µg/mg) in cell lysate samples from Casey Lake are not correlated (R^2=0.003) with lake phosphate concentrations measured as total phosphorus (mg/L) in samples collected at the same time and station.

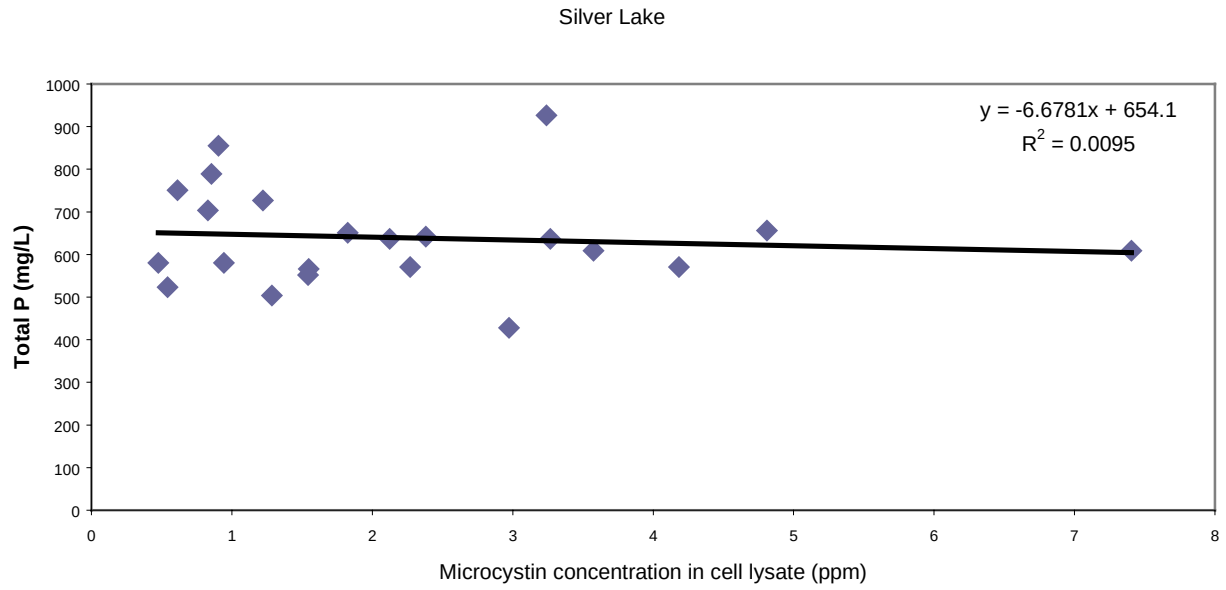

Figure 13. Microcystin concentrations (µg/mg) in cell lysate samples from Silver Lake are not correlated (R^2=0.01) with lake phosphate concentrations measured as total phosphorus (mg/L) in samples collected at the same time and station.

Conclusions

Concentrations of microcystin in Silver and Casey Lakes in the summer of 2000 ranged from non-detectable levels (<0.16 μg/L) to more than 1.6 μg/L, which is consistent with studies of lakes in Wisconsin and Alberta, Canada. Microcystin concentrations in Silver Lake were consistent during the sampling period (0.9 μg/L), and were always higher than concentrations at Casey Lake, where microcystin peaked from 7/20 to 8/2 (0.8 μg/L) before returning to background levels (0.2 μg/L). These levels have not been shown to have effects on fish populations, although there are relatively few studies in this area, especially for chronic exposure. Observed levels in both lakes, however, approach the *Daphnia* 48-hour LC_{50}, suggesting that aquatic organisms in these systems are being impacted. Further study is needed in order to completely define the toxicological risks to indigenous species.

Microcystin concentrations were always higher in the highly eutrophic lake (Silver Lake) than at the control site (Casey Lake). There were, however, no significant relationships between microcystin concentrations in lake water or cell lysate and chlorophyll *a*, total phosphorus or relative cyanobacterial abundance. This result is puzzling, and contradicts at least one study in which microcystin concentrations were correlated with phosphate, although the investigated phosphorus concentrations were much lower than observed in these lakes. Future studies should elucidate the species composition and toxin profiles of cells from the two lakes to provide more information on the potential risks to humans and wildlife, and on the relationship between nutrients and toxin profiles.

Impacts of Research

The implications of this research for potential toxicological risks to aquatic organisms, and ultimately the health of lake ecosystems will be of great benefit in discussions pertaining to the regulation of non point source pollution, not only in Iowa lakes but also in all lakes potentially threatened by harmful algal blooms. Further research is needed to understand the relationship between toxin concentrations and nutrient profiles in eutrophic lakes with seasonal cyanobacterial populations. The results of this study may also indicate the need to consider the need for and to develop novel non-conventional mitigation actions for impacted lakes. Reduction in sources of nutrients to Iowa lakes will surely result in better water quality. Potassium (K) levels should also be considered, however, because potassium has been shown to suppress the growth of *Microcystis* (Parker et al 1997) and may therefore be a novel means of control of toxic cyanobacterial blooms.

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Information Transfer Program

The results of the proposed research will be of interest to resource managers and environmental scientists concerned with water quality. This work was presented at the Agriculture and the Environment: State and Federal Water Initiatives workshop at Iowa State University in March. Additionally, in conjunction with the UNI Lake Water Quality Study, we have developed working relationships with individuals from the Iowa Department of Natural Resources, the Delaware and Black Hawk County Conservation Boards, the Natural Resources Conservation Service, and citizens groups concerned with water quality issues in Silver Lake and Lake Delhi. Representatives of these groups are members of an advisory board associated with the UNI Lake Water Quality Study. The results of this research will be transmitted to resource managers and other interested parties as part of the next advisory board meeting. I have also been in contact with Mr. Bud Cann of the New Jersey Department of Environmental Protection Lakes Program about this research and the potential significance of cyanobacterial blooms resulting from nutrient enrichments, so the outcomes of this research will be also be disseminated to interested resource managers outside of the state of Iowa. The scientific results of and conclusions from this study will also be communicated to environmental scientists through a manuscript which is in preparation for publication in the peer reviewed literature.

Effects of Grazing Management on Sediment and Phosphorus Losses from Pastures

Basic Information

Title:	Effects of Grazing Management on Sediment and Phosphorus Losses from Pastures
Project Number:	2001IA1221B
Start Date:	7/1/2001
End Date:	6/30/2004
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Water Quality
Focus Category:	Nutrients, Management and Planning, Surface Water
Descriptors:	Phosphorus, Water Run-off, Riparian Areas, Pastures,Grazing
Principal Investigators:	James R. Russell, Thomas M Isenhart, John L. Kovar, Steven K. Mickelson, Wendy J. Powers, Richard C Schultz

Publication

Problem and Research Objectives:

Because of the association of phosphorus in runoff with eutrophication of surface water sources, it is likely that the U.S. Environmental Protection Agency will implement regulations to control the Total Maximum Daily Loads (TMDLs) in watersheds within the next five years. To date, phosphorus loads in runoff from agricultural lands in the United States are poorly quantified. This is particularly true for pasturelands in the Midwest. Thus, the database for phosphorus loads that will be used to set TMDLs will likely be developed from models that rely heavily on factors like slope of the terrain. Unfortunately, these models do not consider the potential benefits of management practices like rotational or deferred grazing, regulating sward height, or hay harvest that are likely to reduce sediment losses while increasing forage production and quality. Furthermore, these models do not consider the effects of grazing management on phosphorus balance and excretion of cows. Thus, grazing could be unnecessarily be prohibited on much of the pastureland bordering 8,868 of rivers and streams in Iowa alone. Even if grazing were allowed with fencing of buffer strips, it would cost Iowa farmers nearly $37.5 million in fencing.

In order to develop a database on the relationship of grazing management and sediment and phosphorus runoff from highly erodible soils in Iowa, a project is being conducted with the objectives of:

1) Quantifying the amounts of sediment, P and N in runoff from pastures grazed with different stocking systems during the summer or managed by hay harvest during summer and stockpiled forage grazing during winter.
2) Determining the effectiveness to riparian buffer strips at controlling losses of sediment and P in surface runoff from pastures managed by different systems.
3) Quantifying the impacts of animal nutrition and forage grazing management on P balances and losses from grazing-based production systems.
4) Developing site-specific models for Comprehensive Nutrient Management Plans to control nutrient runoff from pastures through management of forage growth and harvest, animal nutrition, and grazing strategies.

Methodology:

Three blocks of approximately 2.75 hectares along hillsides were identified within in a smooth bromegrass pasture on the Iowa State University Rhodes Research Farm. A 6-m lane was placed along the top of each block for movement of cattle between pastures. To evaluate the efficacy of buffer strips to control nutrient runoff, a 10-m buffer strip (a 10:1 pasture:buffer ratio) was located along the bottom of each block. Each block was divided into five 0.4-hectare paddocks with a 3-m buffer strip separating each paddock. To limit movement of sediment and nutrient between treatments, sandbags were placed in the fencelines between the paddocks and the lane at the above the paddocks and between different paddocks. To attain equal initial phosphorus concentrations, soils were sampled at the 0 to 5 cm and 5 to 15 cm depths, analyzed for P by the Bray 1 method, and fertilized with P to an adequate level. Prior to grazing, each paddock was fertilized with

N as ammonium-nitrate at a rate of 67 kg/ha. Waterers for stock were placed at the top of each paddock.

In each block, 5 forage management treatments were allotted to the five paddocks. One paddock was not grazed. To evaluate the effects of grazing systems on sediment and nutrient losses, one paddock in each block was grazed by 3 non-pregnant cows during summer to a sward height of 5 cm as determined with a rising plate meter (4.8 kg/m^2) which was maintained by a put-and-take stocking system thereafter simulating an intensively grazed continuous stocking system. Two paddocks were grazed with 3 non-pregnant cows to sward heights of 5 or 10 cm as measured with a rising plate meter. When sward heights decreased below the minimal sward height, cows were removed from that paddock and placed on an adjacent smooth bromegrass pasture to provide the paddocks with a 35-day rest period simulating rotational grazing systems with different residual forage heights. To limit the amount of additional phosphorus added to the pasture as feces and urine, cows were not supplemented with phosphorus, either while they were stocked on the experimental paddocks or on the adjacent smooth bromegrass pasture between periods of experimental grazing.

The remaining paddock in each block was used to evaluate nutrient run-off from pastures used for a management system that integrated summer hay harvest with winter stockpiled grazing. Forage from these paddocks was harvested as small bales in June. Although a second hay cutting was planned to occur in August, the amount of forage available for baling was inadequate for baling. Therefore, in August, forage in these paddocks was mowed and left in the field. All pastures were fertilized with N at 45 kg/ha as ammonium nitrate. Residual forage in paddocks assigned the hay/stockpile treatment was allowed to stockpile for late fall grazing. On November 12, each stockpiled paddock was stocked with 3 non-pregnant cows and grazed to a sward height of 5 cm on November 21.

To evaluate the effects of treatments on sediment and nutrient losses, six monitoring sites were located on two different slopes in each paddock. An additional six sites were located in the buffer strip either immediately or 10 m below the treatment paddocks. Water infiltration and sediment and phosphorus at each monitoring site were measured in June, August, and October, 2001 and in April, 2002 using 0.5 x 1.0 m drip rainfall simulators (Bowyer-Bower and Burt, 1989) with a precipitation rate of 6 cm/hr for 90 minutes.

To validate the use of rainfall simulations to predict nutrient losses from pastures, runoff from natural rainfall events was also collected in the paddocks with either no grazing or the three summer grazing treatments. In each of these paddocks, two 3 m x 24.4 m collection plots were constructed to include both the buffer and the upslope pastures using area ratios of 5:1 and 10:1. All plots were hydrologically isolated from their surroundings by sheet metal borders driven in the ground. A collector at the bottom of each plot directed runoff water to a 2.4-m diameter x 0.6 m deep collection tank. Runoff amounts were measured volumetrically after each rainfall event. Sediment concentration in runoff water was determined by oven-drying. Subsamples of runoff water are being analyzed for total phosphorus and dissolved reactive phosphate.

To measure the effects of treatments on soil and plant characteristics, soil samples, soil physical measurements and forage samples were collected adjacent to each monitoring site and within each buffer strip simultaneous to the infiltration experiments. Soil samples were collected from 0 to 5 and 5 to 15 cm depths and analyzed for the contents of moisture, total phosphorus, and soluble phosphorus. Soil penetration resistance was measured to a depth of 35 cm with a pentetrometer. Soil surface roughness was measured by image analysis of digital photographs of a 40-pin meter. Hill slope was measured with a digital level. Proportions of bare soil were measured from image analysis of digital photographs and by the point method. Forage sward height was measured with a rising plate meter (4.8 kg/m^2). Forage samples were hand-clipped, weighed, and analyzed for dry matter and phosphorus.

The effects of forage treatments on uptake of soil nutrients is being determined using six 1-m^2 grazing exclosures in each paddock. At the initiation of each month, grazing exclosures were moved to a new position within the paddock and a forage sample was clipped from a .25-m^2 area adjacent to the exclosure. At the end of each month, a sample was clipped from a .25-m^2 area within the exclosure. Simultaneously, forage samples was clipped from within the vegetative buffer strip. Forage harvested as hay was also sampled at each harvest. Forage samples were weighed, dried and are being analyzed for nitrogen and phosphorus to determine nutrient uptake and harvest by the forage plants. Root samples were collected in the fall to determine the effects of grazing management on below-ground biomass.

Simultaneous to forage sampling, soil samples were collected and are being analyzed for available phosphorus by the Bray 1 method, exchangeable potassium, calcium, magnesium, sodium, total nitrogen and total carbon so that changes in nutrient status with time can be monitored.

To evaluate the impacts of forage management on phosphorus balance within a system and phosphorus losses from a system, phosphorus balance is being determined in each cow monthly. To estimate phosphorus excretion, cows were bolused with an indigestible marker (chromic oxide) daily for 4-day adjustment and 3-day collection periods. During the collection period, fecal samples were collected and analyzed for chromium and phosphorus to determine total feces and phosphorus excretion. The concentration of in vitro digestible organic matter and phosphorus in forage consumed by grazing cows and the total fecal excretion are being to determine phosphorus intake. Phosphorus balance in the cows is being determined as the difference between the amounts of phosphorus consumed and excreted. These values along with the forage phosphorus uptake and phosphorus runoff data will be used to model phosphorus flow within the system which will be used to develop Comprehensive Nutrient Management Plans for farms with grazing enterprises.

Principal Findings and Significance:

Rainfall Simulations.

Paddocks that were ungrazed, harvested as hay and grazed as stockpiled forage, grazed to 5 cm by continuous stocking, and grazed to 5 or 10 cm by rotational stocking provided 0, 47, 492, 360, and 274 cow-days/ha of grazing.

Over the 4 rainfall simulation periods, the proportions of rainfall running off of simulation plots in pastures in which forages were harvested as hay and grazed as stockpiled forage or grazed were 2.66 times greater than ungrazed pastures. This difference, however, was significant only in June and August (Table 1). Over the 4 periods, the mean proportion of water runoff from pastures rotationally stocked to a residual sward height of 10 cm tended lower than pastures harvested as hay and grazed as stockpiled forage or grazed to 5 cm by continuous or rotational stocking. However, in no month was the difference in runoff significant between any of the forage harvest or grazing treatments.

Table 1. LS means of proportions of rainfall runoff from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

Pasture management	Month of rainfall simulation			
	June	April	October	August
	% of water applied			
Ungrazed	21.1[a]	2.2[a]	3.1[a]	4.5[a]
Hay/Stockpiled grazing	45.5[b]	14.4[b]	10.5[a]	12.2[a]
5 cm continuous stocking	37.8[b]	16.4[b]	24.5[a]	6.2[a]
5 cm rotational stocking	43.1[b]	18.9[b]	22.4[a]	4.3[a]
10 cm rotational stocking	32.3[b]	7.2[b]	16.6[a]	8.1[a]

[ab]Differences between means with different superscripts are significant.

Sediment concentrations of runoff from pastures that were ungrazed, harvested as hay or grazed by any of the summer stocking systems did not differ between treatments in June, October, or April (Table 2). However, sediment concentrations of pastures grazed by continuous or rotational stocking to 5 cm were greater than those pastures that were ungrazed, harvested as hay or grazed by rotational stocking to 10 cm. Annual sediment flows, calculated as the sum of measurements during the four rainfall simulation periods, of ungrazed pastures were 22% of pastures in which forage was harvested as hay and grazed as stockpiled forage in November or grazed by any of the stocking systems during

summer. But there was no difference in sediment flow from pastures harvested by hay or grazed by any of the stocking systems.

Concentrations of total P in runoff in June from pastures grazed by continuous or rotational stocking to 5 or 10 cm were greater than paddocks that were ungrazed or harvested as hay (Table 3). However, there was no difference between treatments in the concentrations of total P in the runoff in August, October, or April. Annual flows of total P, calculated as the sum of measurements during the four rainfall simulation periods, from ungrazed pastures were 18% of pastures in which forage was harvested as hay and grazed as stockpiled forage in November or grazed during summer. Total P flows did not

Table 2. LS means of sediment concentrations and flows from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

	Month of rainfall simulation				
Pasture management	June	August	October	April	Total annual
			Sediment concentration, g/L		
Ungrazed	.05[a]	.01[a]	.01[a]	.02[a]	
Hay/ Stockpiled grazing	.05[a]	.02[a]	.01[a]	.02[a]	
5 cm continuous stocking	.08[a]	.16[b]	.04[a]	.03[a]	
5 cm rotational stocking	.06[a]	.41[b]	.05[a]	.05[a]	
10 cm rotational stocking	.05[a]	.03[a]	.02[a]	.10[a]	
			Sediment flow, kg/ha		
Ungrazed	10.0[a]	.6[a]	.6[a]	.3[a]	11.5[a]
Hay/ Stockpiled grazing	28.1[a]	4.3[a]	.4[a]	1.3[a]	34.1[b]
5 cm continuous stocking	37.9[a]	9.3[a]	13.5[a]	2.0[a]	62.8[b]
5 cm rotational stocking	37.3[a]	19.5[a]	8.9[a]	1.9[a]	67.6[b]
10 cm rotational stocking	21.6[a]	8.5[a]	3.9[a]	9.7[b]	43.7[b]

Table 3. LS means of total P concentrations and flows from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

	Month of rainfall simulation				
Pasture management	June	August	October	April	Total annual
			Total P concentration, mg/L		
Ungrazed	.17[a]	.13[a]	.01[a]	.19[a]	
Hay/ Stockpiled grazing	.23[a]	.24[a]	.04[a]	.21[a]	
5 cm continuous stocking	.35[b]	.52[a]	.37[a]	.12[a]	
5 cm rotational stocking	.40[b]	.48[a]	.40[a]	.19[a]	
10 cm rotational stocking	.39[b]	.10[a]	.56[a]	.27[a]	
			Total P flow, kg/ha		
Ungrazed	.03[a]	.01[a]	0[a]	.01[a]	.05[a]
Hay/ Stockpiled grazing	.12[b]	.04[a]	.01[a]	.03[a]	.21[b]
5 cm continuous stocking	.12[b]	.12[b]	.06[a]	.01[a]	.33[b]
5 cm rotational stocking	.22[b]	.09[b]	.04[a]	.01[a]	.37[b]
10 cm rotational stocking	.16[b]	.03[a]	.03[a]	.03[a]	.26[b]

differ between pastures harvested as hay and grazed as stockpiled forage or grazed by continuous or rotational stocking at 5 or 10 cm.

Because an average of 85.4% of the total P was soluble P, most trends in soluble P followed were similar to those of total P. Concentrations of soluble P in paddocks grazed to 5 cm by either continuous or rotational grazing were greater than ungrazed paddocks in June, August, and October (Table 4). Similarly, concentrations of soluble P were greater in paddocks grazed by rotational stocking to 10 cm than ungrazed paddocks in June and October and greater in paddocks harvested as hay than ungrazed paddocks in August. As

a result, the annual flow of soluble P were greater in paddocks grazed to 5 cm by continuous or rotational stocking than in paddocks under the other forage management systems. In addition, the annual flow of soluble P were greater in paddocks harvested for hay and grazed as stockpiled forage or grazed to 10 cm by rotational stocking than ungrazed paddocks.

Table 4. LS means of soluble P concentrations and flows from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

	Month of rainfall simulation				
Pasture management	June	August	October	April	Total annual
			Soluble P concentration, mg/L		
Ungrazed	.14[a]	.12[a]	.01[a]	.11[a]	
Hay/ Stockpiled grazing	.23[a]	.22[b]	.04[ab]	.09[a]	
5 cm continuous stocking	.36[b]	.35[c]	.27[b]	.04[a]	
5 cm rotational stocking	.44[b]	.34[c]	.16[b]	.03[a]	
10 cm rotational stocking	.39[b]	.05[a]	.21[b]	.07[a]	
			Soluble P flow, kg/ha		
Ungrazed	.02[a]	.01[a]	0[a]	.01[a]	.04[a]
	.12[a]	.04[a]	.01[a]	.01[a]	.19[b]
5 cm continuous stocking	.17[b]	.08[b]	.04[a]	.01[a]	.30[c]
5 cm rotational stocking	.27[b]	.08[b]	.01[a]	0[a]	.36[c]
10 cm rotational stocking	.15[b]	.02[ab]	.02[a]	.02[a]	.21[b]

As designed, the forage masses, estimated by sward height or clipping, were greater in ungrazed paddocks than paddocks in which the forage was harvested as hay or grazing in all months and were greater in paddocks grazed to 5 cm by continuous stocking in August and October than paddocks harvested as hay and grazed as stockpiled forage or grazed to 10 cm by rotational stocking (Table 5). Although both were grazed to a sward height of 5 cm, paddocks by rotational stocking to 5 cm had greater sward heights

Table 5. LS means of sward heights, forage masses and surface covers from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

Pasture management	Month of rainfall simulation			
	June	April	October	August
		Sward height, cm		
Ungrazed	23[a]	16.1[a]	11.2[a]	7.6[a]
Hay/Stockpiled grazing	9.8[b]	9.3[b]	10.9[b]	6.8[ab]
5 cm continuous stocking	12.1[b]	5.3[c]	3.7[c]	4.2[b]
5 cm rotational stocking	10.5[b]	7.6[b]	5.3[cb]	5.3[b]
10 cm rotational stocking	13.0[b]	10.3[b]	7.8[b]	6.4[ab]
		Forage mass, kg DM/ha		
Ungrazed	3663[a]	4659[a]	3870[a]	2856[a]
Hay/Stockpiled grazing	852[b]	1392[b]	2157[b]	1736[b]
5 cm continuous stocking	2111[b]	1099[c]	960[c]	961[b]
5 cm rotational stocking	1840[b]	1521[b]	1439[d]	1170[b]
10 cm rotational stocking	2098[b]	1992[b]	1841[d]	1833[b]
		Surface cover, %		
Ungrazed	-	98.0[a]	-	100.0[a]
Hay/Stockpiled grazing	-	91.5[b]	-	95.5[b]
5 cm continuous stocking	-	77.8[c]	-	87.8[c]
5 cm rotational stocking	-	86.0[b]	-	94.0[b]
10 cm rotational stocking	-	92.1[b]	-	94.0[b]

August and greater clipped forage masses in August and October than paddocks grazed by continuous stocking to 5 cm. There were no differences between paddocks grazed by rotational stocking to 10 cm and those harvested for hay and grazed as stockpiled forage for sward height in any month or clipped forage mass in June, August, or April. Similar

to sward height and forage mass, surface covers in August and April were greater in paddocks that were ungrazed than paddocks in which forage were harvested. Furthermore, surface covers of paddocks harvested as hay and grazed as stockpiled forage or grazed by rotational stocking to 5 and 10 cm sward heights than paddocks grazed by continuous stocking to 5 cm.

Surface roughness measured with a 40-pin meter did not differ between paddocks that were not grazed or in those in which the forage was harvested by hay harvest or grazing in June, August or October (Table 6). In contrast, penetration resistances were lower in ungrazed paddocks than paddocks which were harvested as hay and grazed as stockpiled forage, grazed by continuous stocking to 5 cm or grazed by rotational stocking to 5 or 10 cm in the upper 10.5 cm of soil in October and April and in the 10.5 to 21 cm depth in April. However, there were no differences in penetration resistance in paddocks in which hay was harvested or grazed by continuous or rotational stocking.

Table 6. LS means of surface roughness and penetration resistance of soils from pastures that were ungrazed, harvested as hay and grazed as stockpiled forage in November, grazed by continuous stocking to a sward height of 5 cm or grazed by rotational stocking to a sward height of 5 or 10 cm.

Pasture management	Month of rainfall simulation			
	June	April	October	August
		Standard deviations of 40 pins, cm		
Ungrazed	.61[a]	.67[a]	.79[a]	-
Hay/Stockpiled grazing	.59[a]	.80[a]	.68[a]	-
5 cm continuous stocking	.69[a]	.75[a]	.71[a]	-
5 cm rotational stocking	.63[a]	.75[a]	.75[a]	
10 cm rotational stocking	.68[a]	.83[a]	.78[a]	-
		Penetration resistance, kgf		
		0 – 10.5 cm		
Ungrazed	15.3[a]	31.0[a]	21.8[a]	14.0[a]
Hay/Stockpiled grazing	13.6[a]	34.0[b]	25.8[ab]	20.4[b]
5 cm continuous stocking	14.9[a]	35.5[b]	28.7[b]	20.5[b]
5 cm rotational stocking	14.1[a]	42.1[b]	27.7[b]	20.4[b]
10 cm rotational stocking	16.9[a]	38.8[b]	29.9[b]	19.2[b]
		10.5 – 21.0 cm		
Ungrazed	20.2[a]	34.7[a]	25.7[a]	19.2[a]
Hay/Stockpiled grazing	18.2[a]	36.9[a]	29.9[a]	23.2[b]
5 cm continuous stocking	20.3[a]	35.2[a]	28.1[a]	22.8[b]
5 cm rotational stocking	18.2[a]	43.1[a]	27.6[a]	21.9[b]
10 cm rotational stocking	20.5[a]	20.5[a]	30.7[a]	22.0[b]

Runoff plots. Preliminary results of the amount of surface runoff, and sediment and PO_4-P concentrations in runoff from all treatment plots for the natural rainfall events from July 2001 to October 2001 are presented in Figures 1 and 2. These figures show some mixed results for the amount of runoff and sediment yield in the early rainfall events (Figure 1a-1c). Treatment effects are not very visible in the earlier part of the year. However, Figure 1d shows some grazing/buffer treatments effects more prominently on surface runoff and sediment concentration during the rainfall event on the 22nd of October 2001. Figure 1 also shows that the continuous grazing treatment had highest runoff and sediment concentrations. Figure 2 also depicts some treatment effects on PO_4-P concentrations with surface runoff. Phosphorus concentrations with runoff were found to be highest under continuous grazing system. Although this was the first year of the study, the preliminary data showed the effects of various grazing systems and management practices on the losses of sediment and nutrients with surface runoff.

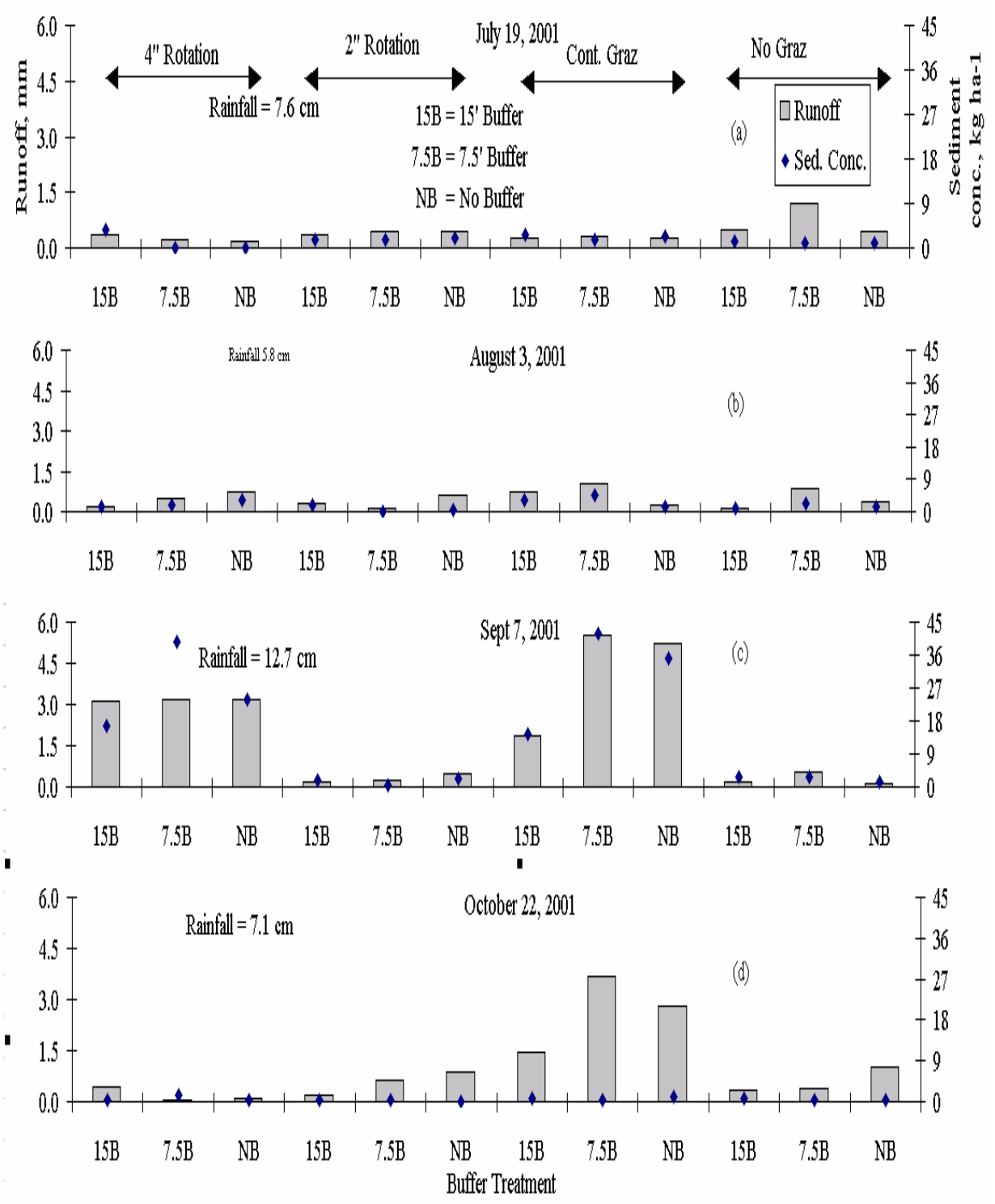

Figure 1. Amount of surface runoff and sediment in runoff water for the natural rainfall events during 2001.

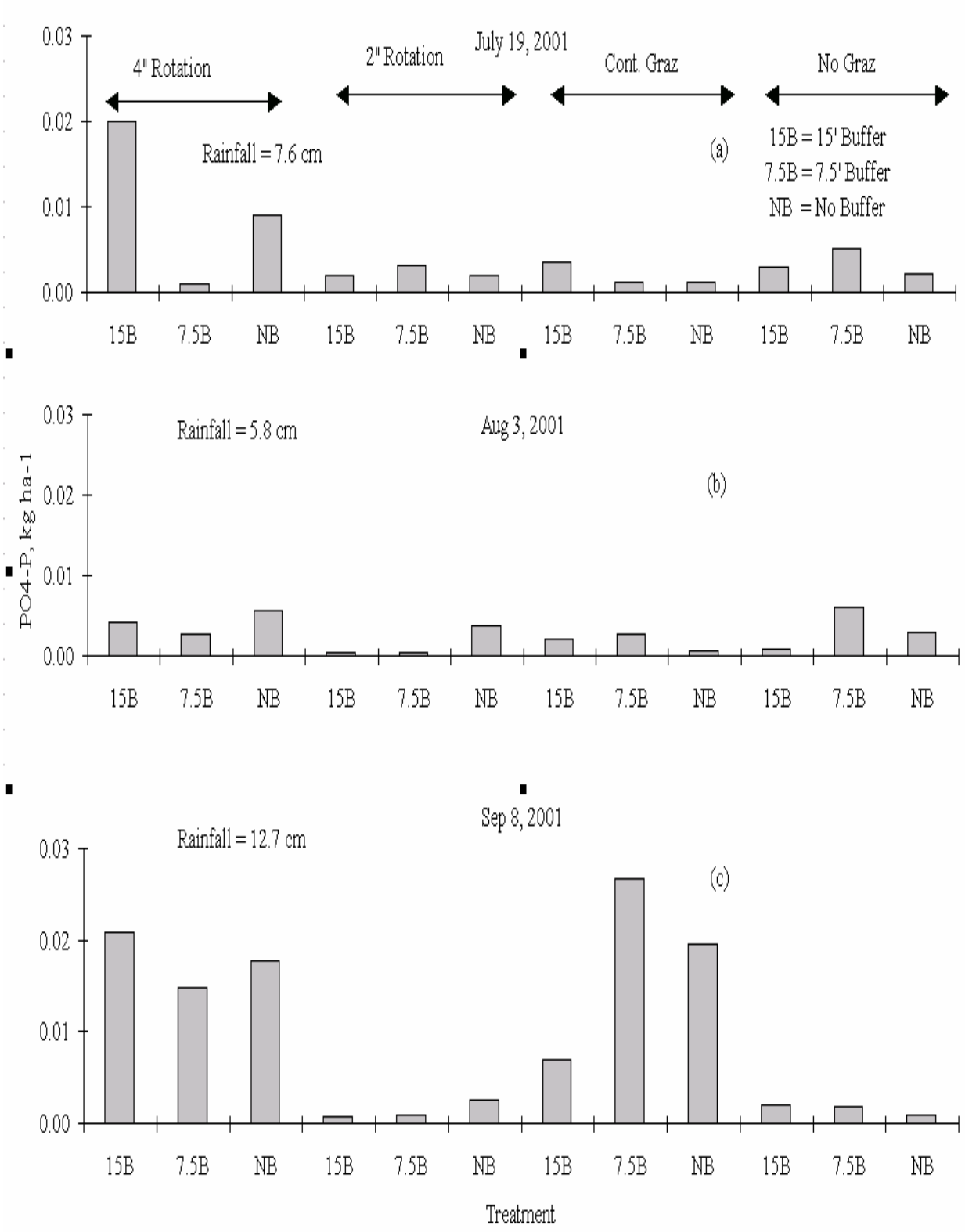

Figure 2. Amount of PO4-P in surface runoff for the natural rainfall events during 2001.

Soil and root sampling. Total P content of the soil samples collected May 7, 2001 was analyzed following digestion using the Aqua Regia (H_2SO_4 + HNO_3) method. Total P in the surface (0-5 cm) layer ranged from 337 to 526 ppm in the 15 paddocks. The buffer area totals ranged from 175 to 436, slightly lower than the paddocks (Table 7).

Table 7. Mean total P content of surface soils (0-5 cm layer) within paddocks and buffer areas of each grazing treatment.

Treatment	Total P, ppm	
	Paddock	Buffer
Control	475	332
Hay/Stockpile	374	255
4" Rotational	451	323
2" Continuous	429	293
2" Rotational	449	309

Soil core samples collected June 21, 2001 for initial root length density were processed using a hydropneumatic root separation system. The samples were processed further to remove plant residues and debris. Root length will be determined by scanned images of each sample. Mean root length density will then be calculated for each of the treatment areas.

Whole-farm phosphorus flow estimates.

Monthly throughout the summer of 2001, cows were dosed with chromic oxide and forage and fecal samples were collected for the determination of dry matter and phosphorus intake. Samples were currently being analyzed.

Significance of preliminary results.

Preliminary results imply that harvest of forage either by hay harvest or grazing increases rainfall run-off and losses of sediment, total P and soluble P. These losses tend to be greater when forage is grazed to a residual height of 5 cm by either continuous or rotational stocking than when forage is grazed to a residual sward height of 10 cm or forage is harvested as hay. The greater losses with greater intensity of grazing seem related to the shorter sward heights, lower forage masses, lower canopy cover and greater soil penetration resistance in the upper 10.5 cm of soil which occur compared to no grazing, hay harvest, or grazing to a residual sward heights of 10 cm.

Effect of Swine Waste Effluent Field Application on Tn916 Content of Surface Waters

Basic Information

Title:	Effect of Swine Waste Effluent Field Application on Tn916 Content of Surface Waters
Project Number:	2001IA1241B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Water Quality
Focus Category:	Waste Water, Water Quality, None
Descriptors:	Sphaerotilus, antibiotic resistance, environmental genetic exchange, Tn916,Enterococcus faecalis
Principal Investigators:	Robert E. Andrews

Publication

1. Haack, Bradley and Robert Andrews, 2000 Isolation of Tn916-like conjugal elements from swine lot effluent. Can J Microbio 46:542-549.

Problem and Research Objectives:

Our previous work has shown that (i) Tn*916* may be introduced in to the soil by application of manure containing wastes, (ii) that Tn*916* mediated genetic exchange occurs in the soil, and (iii) that members of the normal soil microflora may receive Tn*916*. The experiments contained herein are designed to provide evidence that Tn*916*-containing enterococci appear in the surface waters after application of manure-containing wastes. The results will substantially "close the loop" by showing that fecal enterococci the water supply, which would then provide a mechanism by which these may be taken up by animals and humans. The strength of the current approach is that the analysis will depend on detection of specific DNA sequence responsible for gene mobilization as well as the presence of the specific antibiotic resistance gene.

To test the hypothesis, three lines of experimentation will be conducted

Objective 1

Examine effect of swine manure on the Tn*916*-containing fecal enterococci in surface water after waste application to nearby fields. Water samples from streams, ditches and drainage tiles will be examined. If the hypothesis is correct, one should observe that application substantially increases the Tn*916* content in the enterococci found therein.

Objective 2

Determine if manure application in the field results in increased numbers of Tn*916*-encoded tetracycline resistant microbes in nearby surface waters. The content in these microbes will be determined in surface waters in the same sites as described in the previous objective. If the hypothesis is correct, application should increase the Tn*916* content of these microbes.

Objective 3

Evaluate the *Sphaerotilus-Leptothrix* group of bacteria as an indicator of "genetic pollution" by antibiotic resistance genes. One of the requirements for monitoring "genetic pollution" will be the development of indicators that show that such events have occurred. Although the enterococci are important indicators in the short term, their survival in aquatic systems will be limited (2). Thus it is proposed to examine organisms that are more persistent in aquatic ecosystems for the presence of Tn*916*. Accordingly the members of the *Sphaerotilus-Leptothrix* group, particularly *Sphaerotilus* spp., will be examined for Tn*916* content. If Tn*916* were being passed through the water one would expect these organisms to acquire the genetic element after manure application.

Methodology:

Objective 1. The site selected for study was a field approximately 6 km north of Jewell Iowa. Water in drain tiles prior to manure application were sampled for the presence of enterococci, then after application, repeated samples were taken. Enterococcal isolation was done according the using selection of KF agar (5). Antibiotic resistance of these isolates was done according to the methods previously described (4).

Objective 2. The extraction of DNA from soils that is suitable for PCR amplification has been somewhat problematic. Currently we are using a method involving breakage of the cells in a Bead Beater followed by purification using HPT and gel filtration (3). DNA from pit wastes was extracted by using a modification of the method of Yeates et al (14).

Objective 3. The methods for isolation and growth of *Sphaerotilus* species are as described elsewhere in the literature (1, 6, 11). DNA was isolated from *Sphaerotilus* isolates by using the method of Mandel (7).

PCR methods. The PCR methods used herein were those described by Haack and Andrews (5) except that, in some experiments, the following primers were used:

*tet*M primers
Upper primer
GCGTACAAGCACAAACTCGT
Lower primer
CTCTAACCGAATCTCGTAAAT

ORF13 primers
Upper primer
GGGTACTTTTAGGGCTTAGT
Lower primer
GGCTGTCGCTGTAGGATAGAG

Principal Findings and Significance:

Objective 1. To begin this objective, it was of interest to determine the nature of the tetracycline resistance in fecal enterococci. Isolates of *Enterococcus faecalis* obtained from a swine farrowing house outflow were examined for genetic elements similar to Tn*916*. Of the enterococci isolated, 71% were resistant to tetracycline. Among the tetracycline-resistant enterococci isolated from the outflow samples, approximately 34% were able to transfer the tetracycline resistance phenotype to *Bacillus thuringiensis* in cross-genus matings. The frequencies of transfer for 10 random isolates were comparable to those for transfer of Tn*916* from *E. faecalis* to *B. thuringiensis*. In addition, these elements were shown to mobilize plasmid pC194 between *Bacillus* species, as did Tn*916*. Southern blot and polymerase chain reaction (PCR) analysis showed these elements share extensive structural homology with Tn*916*. The selected conjugal elements were capable of transfer to a *Bacillus* recipient in a soil environment. When the swine waste was introduced into the soil, the tetracycline resistant fecal enterococci levels rose from essentially undetectable levels to approximately 4×10^4 and remained at this level for 4 weeks. After six months, including one winter, levels had decreased to 5×10^3.

In samples from the field tiles, the patterns of tetracycline resistance in fecal enterococci was quite different. In the tiles, and in soil from the field, approximately 50% were resistant to tetracycline and of about 80% of these isolated appeared to contain Tn*916*, based on the ability to transfer the element to *Bacillus* recipients. Thus, it appears that Tn*916* is more stable in the field than are other tetracycline resistance elements.

Based on these results, it was of interest to compare the fecal enterococci in the pit samples with those from field samples for their antibiotic resistances. For antibiotics are chosen for these studies are shown in Table 1.

Table 1. Percent of *Enterococcus faecalis* isolates resistant to various antibiotics.

Antibiotic	Percent resistant in pit samples[1]	Percent resistant in the field1
ampicillin	60	22
kanamycin	52	48
neomycin	46	60
tetracycline	72	50
vancomycin	66	62
streptomycin	32	38

[1]Based on a sample size of 50 isolates obtained without antibiotic selection. These isolates were then examined for their resistance to the various antibiotics.

These data show that some antibiotic markers are more stable than others when the enterococci are in the soil. The basis for the variable instability under field conditions remains unclear.

Objective 2. To isolate DNA from swine pit samples a modification of the method of Picard was used (8). The method resulted in the isolation of DNA that was readily amplifiable using the ORF13 primers described in the methods section above. Figure 1 shows the amplified product from one such experiment.

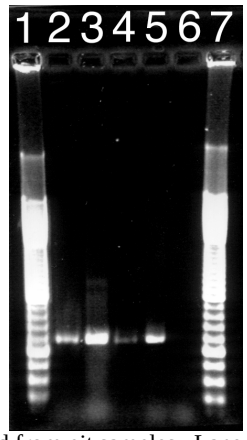

Figure 1. PCR products from DNA extracted from pit samples. Lane assignments, Lane 1, 100 bp ladder; Lane 2, positive control, Lane 3, undiluted extract, Lane 4; extract diluted 1:5; Lane 5, extract diluted 1:25; Lane 6, Negative Control, Lane 7, 100 bp ladder.

These data indicated that a non-antibiotic resistance-encoding region of Tn*916* abounds in the pit samples.

It was of further interest to examine this material for the presence of antibiotic resistance genes. In initial experiments the *tet*M primers described in the methods section of this report were used to amplify a fragment of DNA encoding a portion of the *tet*M gene, which exists in several forms in nature, including that found on Tn*916*. In these experiments, (data not shown) a fragment of DNA was readily amplified from the pit waste extract.

To further analyze these fragments, denaturing gel gradient electrophoresis (DGGE) was used. The methods for DGGE are described elsewhere (9, 10, 12, 13). The DGGE procedure allows one to examine the contents of a PCR reaction for the presence of different forms of very similar genes; the forms of the gene may differ in as few as 1 bp. Figure 2 shows the results from one such experiment.

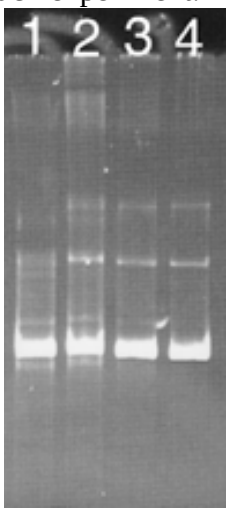

Figure 2. DGGE analysis of PCR product from pit waste. Lane assignments, ; Lane 1, positive control, Lane 2, undiluted extract, Lane 3; extract diluted 1:5; Lane 4, extract diluted 1:25.

In these experiments at least two unique bands are observed in PCR reactions from pit sample extracts suggesting that there are at least 3 forms of Tn*916* conjugation gene in the pit sample wastes. Further characterization of these products is presently underway.

Objective 3. With regard to the third objective in the current proposal, we needed to perfect our techniques for *Sphaerotilus* isolation. Mat samples from the trickling filter were obtained from the Nevada, IA municipal sewage treatment plant. These isolates were brought to the laboratory and washed five times in sterile tap water. The mat was then broken up by using a Dounce homogenizer and diluted in sterile water. Samples from each dilution were plated on a medium modified from that of Stokes (11). The Stokes medium, which was designed to select for *S. natans* based on the low nutrient content of the medium, used 0.1% glucose and 0.1% tryptone in basal salts. In our hands, however, problems were encountered with the original Stokes medium. Many non-*S. natans* colonies appeared on the medium and we found that molds tended to overgrow the *S. natans* colonies that did come up. After several attempts we settled on a basal salts medium that contained no tryptone, glucose at 0.1%, 5 µg/L vitamin B_{12}, and 40 mg/L cyclohexamide to control mold growth.

When tetracycline (40 µg/ml) was included in the isolation medium, approximately 10% of the isolated appeared to be tetracycline resistant. DNA was extracted from 30 of these purified and used in PCR reactions using the primers of Haack and Andrews (5). Two of the samples exhibited a positive reaction, indicating the presence of a Tn*916*-like element. Thus the initial data suggests that Tn*916* may exist in *S. natans* in a domestic sewage plant. With this information we have established that Tn*916*-like elements do appear in *S. natans.*

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

Basic Information

Title:	Predicting Sorption, Mobility, Accumulation, and Degradation Potential of Antibiotics in Iowa's Soil/Water Environment
Project Number:	2001IA1261B
Start Date:	3/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Not Applicable
Focus Category:	Agriculture, Non Point Pollution, Toxic Substances
Descriptors:	Adsorption and sorption, Leaching, Organic compounds, Manure lagoons, Solute transport, Biodegradation
Principal Investigators:	Steven Fales

Publication

Research problem:

Approximately fifty million pounds of antibiotics per year are released into the environment. About half of the total antibiotics produced are given to farm animals, fish, and trees for disease prevention and growth promotion at dosages below those recommended for fighting bacterial infections (Levy, 1997). These lower dosages are believed to be more conducive to building antibiotic resistance by pathogenic bacteria. Most of the antibiotics given to farm animals are not metabolized in the body, 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 antibiotics in soil and aquatic environments could encourage the buildup of existing and potentially 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. The 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. Recent data for lagoon liquid wastes in Iowa (Unknown author, Iowa Dept. of Public Health, 1998) showed relatively high concentrations of antibiotics. For example, chlortetracycline concentrations ranged from 11 to 540 µg/L and erythromycin concentrations ranged from 10 to 275 µg/L. The report also shows that many of the 18 *E. coli* isolates, all three Salmonella species and isolate of Enterococcus demonstrated resistance to a particular antibiotic or combination of antibiotics.

The antibiotics most commonly added to livestock feed as growth promoters (1 to 100 mg per head per day) are chlortetracycline (Aureomycin), oxytetracycline (Terramycin) and macrolide (erythromycin) (Sewell, 1993; FAC, 1998; Herman et al., 1995). The fate of these compounds in Iowa soils will depend 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 behavior of antibiotics in soil environments with respect to sorption/desorption, leaching, and decomposition is predictable from a knowledge of the chemical structure of the antibiotics.

Specific objectives:

1) Evaluate the potential of the antibiotics tetracycline, chlortetracycline, oxytetracycline and erythromycin to sorb onto soil surfaces involving organo-surfaces (humic substances) and clay mineral surfaces.
2) Evaluate the potential of the selected antibiotics to mobilize through the soil profile under various compositions of the soil solution and soil exchange phase.

3) Evaluate the potential of the selected antibiotics to persist in the soil environment: quantify the half-life for these antibiotics in soil and elucidate for modeling purposes abiotic and biotic cooperative/anti-cooperative effects of sorption on half-life.

Methodology:

Surface (0-15 cm) and subsurface (≥ 15 cm) soil samples were collected from three sites representing three different soil series. The soils were selected to represent a range of physical properties and because these soils and general locations had been previously characterized (McBride et al., 1987). Based on interviews with the landowner 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).

Preliminary chemical characterization of tetracycline, chlortetracycline, and oxytetracycline were performed. UV-vis absorbance spectra of the antibiotics dissolved in water, and various concentrations of KCl, $CaCl_2$, $MgCl_2$, and $AlCl_3$ were obtained using a UV-vis spectrophotometer (Varian Instruments, Cary 50 Bio model, Walnut Creek, CA, USA). Calibration curves for quantifying concentrations of the various antibiotics dissolved in water were developed for two wave lengths, near 270 nm (W1) and 370 nm (W2). Solubility of the oxytetracycline in water was measured by determining the concentration where the absorbance-concentration relationship deviates from Beer's-law. Work on developing an HPLC method for quantifying antibiotics was initiated.

Principal Findings and Significance:

Work on the project to date has focused on collecting and characterizing soil samples, characterizing chemical properties of the antibiotics, and the development of analytical methods for antibiotic quanitation.

The soils sampled for this study are listed in Table 1. Clay content of the sampled soils ranged from 19.2 % in the Nicollet surface sample to 34.6% in the Clarinda subsoil sample. Organic C content ranged from 0.44% for the Fayette subsoil sample to 1.65 for the Fayette surface soil sample. Total exchangeable cation ranged from 13.6 $cmol_c\ kg^{-1}$ for the Nicollet surface soil to 19.7 $cmol_c\ kg^{-1}$ for the Fayette subsoil. The pH values in KCl ranged from 4.5 in the Clarinda subsoil sample to 6.5 in the Fayette surface soil sample. In general, the properties of the sampled soils are sufficiently diverse to allow a reasonable assessment of the influence of soil properties on the fate of antibiotics.

Table 1: Soil sampled for the study.

Sample Site	Soil Series	Classification
Tama Co.	Fayette	Fine-silty mixed superactive mesic, Typic Hapludalfs
Boone Co.	Nicollet	Fine-loamy mixed superactive mesic, Aquic Hapludolls
Clarke Co.	Clarinda	Fine smectitic mesic, Vertic Argiaquolls

Potentiometric titrations indicate two and possibly three pKa's for the tetracyclines. The solubility of oxytetracycline was found to be approximately 300 mg L^{-1}. UV-VIS spectroscopy revealed two prominent absorption maxima near 280 and 360 nm for oxytetracycline and tetracycline and two prominent sorption maxima near 280 and 370 nm for chlortetracycline. Absorption spectra for all three tetracyclines were only slightly affected by background $CaCl_2$ (0 to 50 meq L^{-1}) and $MgCl_2$ (0 to 40 meq L^{-1}) concentrations. By contrast, the presence of as little as 2 meq L^{-1} $AlCl_3$ substantially altered the absorbance spectra for all three tetracyclines. The cause of change in the absorbance spectra in the presence of $AlCl_3$ is not clear, but may indicate either a pH effect or the formation Al-tetracycline complexes. More work is needed to resolve the cause of this effect. The results demonstrate that tetracycline, chlortetracycline, and oxytetracycline concentrations in water and both $CaCl_2$ and $MgCl_2$ solutions can be quantified by UV-VIS spectroscopy with linear response for the 0 to 20 mg L^{-1} concentration range. The presence of Al in aqueous solution, however, may cause problems with spectrometric analysis. Preliminary work on developing an HPLC technique for analysis of the oxytetracycline resulted in an r^2 of 0.98. This is not satisfactory for most analyses, and indicates that more work is needed to develop HPLC methods.

It is too early in the study to draw conclusions about the fate of antibiotics in soils. However, the work performed to-date has provided critical background information necessary for successful completion of the proposed research. Future work will focus on building on this foundation and focus on quantifying interactions between the antibiotics and soils.

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Estimation of the Nutrient Load to Clear Lake from Groundwater Using Analytic Element and Parameter Estimation Models

Basic Information

Title:	Estimation of the Nutrient Load to Clear Lake from Groundwater Using Analytic Element and Parameter Estimation Models
Project Number:	2001IA1521B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Water Quality
Focus Category:	Nutrients, None, None
Descriptors:	parameter estimation, analytic elements, groundwater modeling,Nutrients
Principal Investigators:	William W. Simpkins

Publication

1. Simpkins, William; Keri Drenner; and Tamara Ewoldt. 2001. Characterizing groundwater-lake interaction: a tool for understanding long term phosphorus input for lake restoration. Abstracts of the 63rd Midwest Fish and Wildlife Conference, Dec. 12, 2001, Des Moines, IA. p. 31.

Problem and Research Objectives:

Although the inputs of nitrogen (N) and silica (Si) are important for aquatic life, phosphorus (P) is the principal limiting nutrient in freshwaters. Assessment and control of P losses in agricultural areas is now of broad interest, because agricultural landscapes lose more P than undisturbed lands. Although lakes have been impacted by excess nutrients, the contribution from groundwater to lakes is rarely estimated. It has been assumed that P is sorbed to sediment and that transport of P in groundwater is limited. However, evidence is accumulating that P occurs in groundwater in agricultural areas (Rodvang and Simpkins, 2001).

The groundwater flow system in contact with a lake must be known in order to estimate the load of nutrients into a lake. Traditionally, groundwater discharge into lakes has been estimated using seepage meters, along with hydraulic head and hydraulic conductivity (K) measurements that are used in Darcy's Law calculations. These data are essentially point measurements that must then be extrapolated to large areas of shoreline where measurements are lacking. Discharge measurements from seepage meters are notoriously variable and generally work best in lakes with clean sandy bottom sediments. Hydraulic head and K measurements are also often difficult to measure in lake sediments. Groundwater modeling has proven to be a valuable tool in understanding groundwater-lake interaction. Recent research has shown that the Analytic Element (AE) method is applicable to groundwater-lake systems (Haitjema, 1995; Hunt and Krohelski, 1996; Hunt et al., 1998; Hunt et al., 2000). The AE model can be calibrated and the model solution constrained by coupling the results of the AE model with a parameter estimation model, such as UCODE (Poeter and Hill, 1998).

The purpose of this study is to apply the AE modeling method to Clear Lake, a 1400-ha recreational lake in north central Iowa, for the purpose of estimating groundwater discharge and recharge from the lake. The proposed research continued the groundwater part of the *Clear Lake Diagnostic and Feasibility Study (CLDFS)*, a large interdisciplinary study that is funded by the Iowa Department of Natural Resources (DNR) to Dr. John Downing of Iowa State University (ISU). The objectives of the proposed study are to:

1) simulate shallow groundwater flow near Clear Lake using an Analytic Element model;
2) calibrate the model using a parameter estimation model;
3) estimate groundwater discharge to the lake with the calibrated model and compare with estimates from seepage meters and Darcy's Law to find representative values of discharge;
4) calculate nutrient load from groundwater to the lake using representative discharge (from the model and field data) and nutrient concentration (from monitoring wells) data.

Methodology:

Piezometer (monitoring well) nests installed at 11 sites on the perimeter of the lake in summer 2000 were used in this study. They were installed at depths between 3 and 31 ft (0.9 and 9.5 m) using hollow-stem augers. Coordinates (X-Y) and absolute elevation of the standpipes (and the USGS lake stage gage) to within 1 cm were obtained from a professional surveyor using GPS and total station equipment. Hydraulic conductivity (K) was estimated in the shallow piezometers using falling and rising-head slug tests during summer 2001. Hydraulic head was measured monthly and lake-stage data was retrieved in digital form from the U.S. Geological Survey. Additional temporary piezometers were installed in the eastern part of the watershed in November 2001 using a Geoprobe. Locations and elevations of those piezometers were surveyed in using GPS technology. A Windows-based, AE model, GFLOW 2000 (version 1.2), was used to simulate the groundwater flow system and to calculate groundwater discharge into and out of the lake. Input data included elevations of stream and drainage tiles, K values, and estimates of groundwater recharge. Because the model does not explicitly incorporate lakes, Clear Lake was given a very high K value to simulate that effect, as suggested by Hunt et al. (2000) and Anderson et al. (2002). An unpublished glacial geologic map (based on digital soil survey data) from the Iowa Geological Survey Bureau was used to identify geological units that could affect flow in the vicinity of the lake. Creek discharges were also measured at approximately 25 points within the model domain to provide additional calibration points for the model.

Dedicated polyethylene tubing was installed in each piezometer to facilitate groundwater sampling. Groundwater was sampled and analyzed for total P, dissolved P, total N, Si, pH, electrical conductivity, NO_3-N, and Cl on a monthly basis. Standard methods of analysis were employed by the Water Research Laboratory, Department of Animal Ecology, at Iowa State University, for determination of total P (persulfate digestion and spectrophometric method), dissolved P (spectrophometric method), total N (second derivative spectroscopy method), and Si (molybdate reactive method) concentrations, pH, and electrical conductivity. Samples were analyzed in triplicate. Determination of NO_3-N, Cl, and SO_4 concentrations were made by ion chromatography in the Department of Geological and Atmospheric Sciences at Iowa State University. Nutrient and contaminant loads from groundwater to Clear Lake were calculated from estimates of groundwater inflow and outflow and estimates of the concentrations of nutrients (primarily P, N, and Si) and Cl in groundwater. Nutrient load per time was calculated by multiplying discharge (L^3/T) by concentration (M/L^3). Because of Clear Lake's nature as a flow-through lake, nutrients will be added to the lake in areas of inflow and lost from the lake in areas of outflow.

Principal Findings and Significance:

The GFLOW model was run until reasonable agreement was reached with the hydraulic heads measured in the piezometers around the lake and lake stage. Values of K used for this model were 2×10^{-4} m/s and recharge was 10 percent of mean annual precipitation (3.2 in/yr). The K values for this model were judged too high for the till areas around the

lake. Clear Lake is set within the Algona-Altamont Moraine Complex containing till; however, a large outwash deposit occurs on the eastern edge of the lake (bounded by orange or lighter gray border in Figure 1). It is approximately aligned with the Clear Creek outlet and leads into what appears to be an outwash fan east of the City of Clear Lake. Subsequent runs included a more conductive zone on the east end (Figure 1). Assigning a K value of 3.5×10^{-3} m/s to the outwash allowed K values in the till in the rest of the watershed to be lowered to 5.3×10^{-5} m/s. Although this is still a high value for till, it is in keeping with K values estimated from slug tests at the lake. Using a recharge value of 81.3 mm/yr (3.2 in/yr or 10 percent of mean annual precipitation), the final model produced heads similar to those observed in the field (Figure 1) and produced inflow and outflow in the areas indicated by field data. The maximum departure from the field data was 0.55 m and the mean absolute difference was 0.27 m. Flux inspection lines were drawn along areas that the model shows to be inflow and outflow zones. The results suggest that groundwater inflow (discharge) to Clear Lake is approximately 7.9×10^3 m^3/d and groundwater outflow is approximately 8.9×10^3 m^3/d. Although these values are higher than those suggested by the Darcy's Law analysis, they are within the same order of magnitude (Table 1). They are significantly higher than the seepage meter estimates. Parameter estimation using UCODE has met with only moderate success in improving these parameter estimates and that work continues. A new version of GFLOW2000 (version 1.3) was released in April 2002 that allows the lake to be modeled as a lake with a specified stage. Preliminary simulations indicate that parameter values will change slightly in this new model. The newer version using the additional calibration points will be discussed in the final report.

Table 1. Comparison of groundwater discharge values estimated by the three methods.

Method	**GW Inflow (m^3/d)**	**GW outflow (m^3/d)**	**Net (m^3/d)**
Seepage meters	5.4E+05	None	5.4E+05
Darcy's Law	4.7E+03	-6.2E+03	-1.5E+03
AE GW Model	7.9E+03	-8.9E+03	-1.0E+03

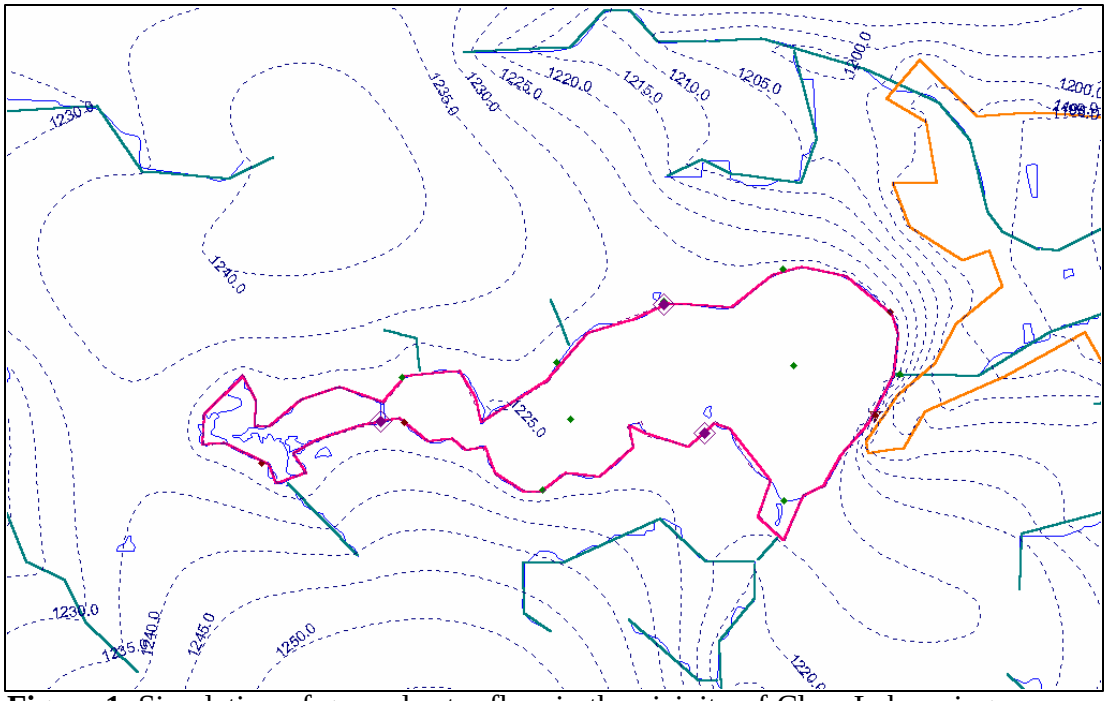

Figure 1. Simulation of groundwater flow in the vicinity of Clear Lake using GFLOW2000 (version 1.2). Contour interval is 5 ft. Outwash deposit is outlined in orange (lighter gray). Original calibration points (piezometers) shown as diamonds.

Total P concentrations were similar to that reported by Simpkins et al. (2001) with a mean value of 237.7 µg/L and a standard deviation of 232.9 µg/L (Table 2). Because some very high concentrations skew the distribution, the median value of 172.9 µg/L may be a better measure of the central tendency. Most of the total P is apparently in the form of dissolved P. In contrast to P, concentrations of Total N were considerably less (Table 2). The mean, standard deviation and median values are 1.1, 1.57 and 0.8 mg/L, respectively. The lack of NO_3-N in these samples is consistent with the lack of dissolved O_2 and use of alternate electron acceptors in the system. Concentrations of SiO_2 were within the range common for groundwater systems at near-neutral pH in these materials (Table 2). Mean, standard deviation, and median concentrations were 40.4, 14.3, and 37.3 mg/L, respectively. Chloride concentrations in groundwater ranged from 1.0 to 73.1 mg/L, with a mean of 17.1 mg/L. The mean Cl concentration in Clear Lake is about 16 mg/L. Because there is no known natural source of Cl in the glacial sediment, its origin must be anthropogenic. Potential sources include agricultural fertilizers, septic systems and road de-icing salts and solutions. Preliminary nutrient loads to Clear Lake for the major nutrients and Cl (calculated using groundwater discharges from the model) are given in Table 3. Work continues to refine water quality statistics and nutrient load estimates to the lake.

Table 2. Statistics for Total P, Total N, and SiO_2 concentrations in groundwater from 32 piezometers. Total P concentrations in µg/L. Total N and SiO_2 concentrations in mg/L.

Analyte	N	Mean	Median	Std. Dev.	SE Mean	Min.	Max.
Total P	219	237.7	172.9	232.9	15.7	< 0.01	1783.1
Total N	219	1.1	0.8	1.57	0.11	0.001	11.54
SiO_2	219	40.4	37.3	14.3	0.96	17.6	131.3

Table 3. Summary calculations of nutrient and contaminant load to Clear Lake.

Flow direction	Q (m^3/d) from Part I	Nutrient or contaminant	Median conc. (mg/L)	Load (kg/d)
In	7.9E+03	Total P	0.173	1.37
	"	Total N	0.8	6.32
	"	Silica (SiO_2)	37.3	294.70
	"	Cl	14.3	112.98
Out	-8.9E+3	Total P	0.174	-1.54
	"	Total N	1.31	-11.66
	"	Silica (SiO_2)	38.7	-344.47
	"	Cl	15.4	-137.08

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Modeling, GIS, and Technology Transfer in Support of TMDL Development and Implementation in Iowa

Basic Information

Title:	Modeling, GIS, and Technology Transfer in Support of TMDL Development and Implementation in Iowa
Project Number:	2001IA1542B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Water Quality
Focus Category:	Water Quality, Models, None
Descriptors:	environmental policy, modeling, GIS, water quality,TMDL
Principal Investigators:	Udoyara Sunday Tim

Publication

Project Rationale and Significance:

Under the provisions of the federal Clean Water Act (CWA) Section 303(d), states (herein referred to the collection of states, territories, and authorized tribes) are required to identify water bodies and stream segments that are impaired from point and nonpoint pollution sources. To improve the water quality problems in these water bodies, total maximum daily loads (TMDLs), defined as "the sum of the individual waste load allocations for point sources and load allocations from nonpoint sources and natural backgrounds" (40 CFR 130.2) are to be developed. TMDLs form the basis for developing best management practices for stream water quality restoration and play a key role in stakeholder involvement in watershed management and watershed restoration strategies. All stages in the TMDL development process requires sound science and the ability to translate complex data and information into a coherent and concise package so that agencies and stakeholders can understand the issues and be able to evaluate alternative remedial options.

The basis for TMDL development rests on a wide range of factors, including source water assessments, expected ability to meet the TMDL limits, terrestrial and aquatic ecosystem modeling and monitoring, and resource economics. Although the Environmental Protection Agency (EPA) has provided a compendium of tools and models to aid in the TMDL development and implementation plan, the reliability of these models for general applications remain questionable. There remain considerable gaps between models developed for the prediction of point and nonpoint source pollution and those that can be used to support TMDL analysis and development. Indeed, many in the agriculture and water resource communities have expressed concerns over the lack of science behind the TMDL modeling and planning process. For example, former Secretary of Agriculture, Dan Glickman and many others have expressed concern over the TMDL program. Specifically, Mr. Glickman stated, *"the USDA is concerned about the science being used in assessing and attributing the effects of nonpoint source pollution. These models have a high degree of uncertainty and there are gaps in the data regarding what is natural background pollution versus what is caused by human activity.*" Given these uncertainties, there is critical need for an objective evaluation and refinement of existing models and tools used in TMDL development as well as a concise demonstration of how these models can be used to establish quantitative measures of the relationship between pollutant sources and water quality impacts. Equally critical is the need for effective information dissemination, training, and stakeholder engagement programs that build capacity for effective and technically defensible implementation of TMDLs.

The problems faced in developing TMDLs vary widely across water quality issues and problems. Iowa, like many states, currently needs to develop TMDLs for nearly 49 watersheds and 157 affected streams; efficient and equitable development of TMDL requires a sound scientific and technical base and appropriate tools not currently available. Successful water quality management in the U.S. has always depended on applying good science and on the efficacy of modeling techniques. This project will undertake a comprehensive review of models, recommend modifications to models as appropriate, assess existing data sources for Iowa and develop a database for model application, and refine and apply the SWAT model to assessing sediment, nutrients, pesticides, and microbial pathogen impairment related to TMDLs.

Objectives of the Research Project:

This research is structured to provide enhanced decision support for TMDL analysis by: (a) critically evaluating existing terrestrial and aquatic models and TMDL planning tools to insure that they are based on sound science and are used in a sound manner; (b) providing tools for estimating waste loads associated with biological pollutants under different watershed conditions and management practices; (c) developing objective criteria for choosing among models, data sources, and implementation plans based on the priorities of all stakeholders; and (d) demonstrating the use of integrated models for assessing the potential ecological benefits of TMDL implementation at the watershed scale. As outlined in the original proposal, the specific project objectives are:

- To undertake a science-based evaluation of existing models for their use in TMDL development and implementation and suggest areas for future refinements.

- To integrate algorithms developed for waterborne pathogens (specifically bacteria) into the SWAT biophysical model to facilitate use in development of nutrients and microbial TMDLs in tiled drained watersheds in Iowa.

- To assess the potential ecological and water quality benefits of TMDL implementation in an agriculturally dominated watershed in Iowa to serve as a case study.

The overarching goal of the project is to enhance the effectiveness of watershed water quality management efforts and improve the scientific basis and computer models for TMDL development and implementation. The products from this research should increase the likelihood of acceptance of the TMDL process by regulators and stakeholders, and help assure that the entire TMDL development process meets the desired water quality goals.

Progress Made Thus Far:

The project is proceeding well, with considerable progress made in the development of the primary project deliverables. Specifically, the research is progressing in three interrelated phases. In Phase I, we conducted a rigorous, science-based evaluation of existing terrestrial and aquatic ecosystem models that are available or recommended for use in developing TMDL loads and waste-loads. Under this phase, two activities were accomplished. First, a detailed review of the procedures and methodologies used by the states, tribes and territories within the U.S. to develop TMDLs was conducted. The objective of this review was to compare or contrast differences in TMDL methodology, modeling techniques, and implementation strategies. The second activity involved a critical, process-level, science-based evaluation of the terrestrial/in-stream response models used for TMDL development. In Phase II of the project, constitutive equations (algorithms) that govern the movement of microbial pathogens—including the fecal coliform bacteria group—were developed for inclusion into the SWAT biophysical model. Currently we are under Phase III of the project, which involves a rigorous demonstration of the utility of the enhanced SWAT modeling system for TMDL development and using the modeling system to explore the ecological benefits of TMDL implementation within an agriculturally dominated watershed.

Under the Phase III project plan, we have assembled relevant input parameters to support SWAT modeling or water quality conditions in the Upper Maquoketa River watershed. Observed flow and transport parameters have also been assembled for critical evaluation of model reliability. The Upper Maquoketa River watershed is located within the Counties of Buchanan, Clayton, Delaware, and Fayette in northeast Iowa. With a total drainage area of 15,890 ha or 39,248 acres, the watershed land use consists of 78.7% agricultural (i.e., 40.3% corn, 27.2% soybean, and 11.2% oats/hay/alfalfa), 10.5% pasture, and 8.8% forest. Monitoring activities within the watershed consist of flow measurements at four sites and water quality sampling at same sites. Among other constituents, these water quality samples are analyzed for nitrate-nitrogen (N), kjedahl-N, ammonium-N, total phosphorus (P), ortho-P, chemical oxygen demand, and total suspended solids. In this project, data on nitrate and bacterial concentrations and flow will be used to establish the TMDL waste-load numeric criteria. The various tasks under this phase of the project are being conducted with assistance from Dr. Baker and Dr. Kanwar. A research associate, Marius Aqua, was also hired to help in data assemble and data quality checks.

In addition to the tasks described above, our Year 2 activities also involve process-level evaluation of the microbial fate and transport component in the SWAT model. From this evaluation, a more robust modeling component for developing numeric standards for microbial pathogens will emerge, providing much needed analytical tool for predicting movement of not only total and fecal coliform bacteria, but also other pathogens that may be present in land-applied animal manure and sewage.

Future Project Activities:

The project elements that remain to be accomplished will involve application of the modified SWAT model to the Upper Maquoketa River watershed. Here we will conduct model simulation runs, assess the reliability of the model, and suggest techniques for evaluating model uncertainty and error propagation. In addition to model verification and reliability assessment, the Year 2 activities will also include the application of modified SWAT modeling environment in assessing the ecological and water quality benefits of the TMDL program in the watershed. This activity will concentrate on the Upper Maquoketa watershed where continuous water quality (including bacterial) monitoring has been underway for quite a number of years. The lessons learned from this modeling process will form the basis for transferring methods to other watersheds. Technology transfer activities will involve demonstration of the modeling system to personnel at state or federal resource agencies. Upon completion of the Year 2 activities, we will submit a final report that details the project elements, modeling system, deliverables, and accomplishments to ISWRRI and prepare manuscripts for journal publication.

Occurrence and Formation of Nitrosamines in Drinking Water Distribution Systems

Basic Information

Title:	Occurrence and Formation of Nitrosamines in Drinking Water Distribution Systems
Project Number:	2001IA1601B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	
Congressional District:	Iowa 1st
Research Category:	
Focus Category:	Water Quality, Toxic Substances, Water Supply
Descriptors:	Chlorination, Disinfection, Water treatment, Water quality,Water chemistry
Principal Investigators:	Richard L. Valentine

Publication

1. Choi, Junghoon and Richard Valentine. 2001. "Formation of N-nitrosodimethylamine (NDMA) in Chloraminated Water: New disinfection by-product", Proceedings the 221st National Meeting (Environmental Division), San Diego, CA, April 1-5, 2001, Vol 41, No. 1, pg 8-11.
2. Choi, Junghoon and Richard Valentine. 2001. "Studies on the Formation of Nitrosodimethylamine (NDMA) in Drinking Water: A New Chloramination Disinfection By-product", Proceedings of the 2001 National American Water Works Association (AWWA) Annual Conference, Universities Forum-Water Science and Research Division, Chapter TUE22, paper no. 4, 9 pp., June 17-22, 2001-Washington D.C.
3. Choi, Junghoon and Richard Valentine, 2001 "Mechanistic studies of N-nitrosodimethylamine (NDMA) formation in chloraminated drinking water", Proceedings of the 222nd American Chemical Society (Environmental Division), Vol. 41(2), Paper no. 112, pp. 804-809 August 26-30, 2001, Chicago, Illinois.
4. Valentine, Richard and Junghoon Choi. A kinetic model of N-Nitrosodimethylamine (NDMA) formation during water chlorination/chloramination", IAW 2nd World Water Congress, Preprints, Health Standards-Track 8, Paper no. 4, 6 pp., October 15-19, Berlin, Germany, 2001.
5. Choi, Junghoon and Richard Valentine. "Formation of N-nitrosodimethylamine (NDMA) from reaction of monochloramine: a new disinfection by-product", Water Research, Vol. 36, No. 4, pp.

817-824, 2002.
6. Choi, Junghoon, Stephen Duirk and Richard Valentine. "Mechanistic Studies of N-nitrosodimethylamine formation in Drinking Water", Journal of Environmental Monitoring, (2002), 4(2), 249-252.

Research Background:

Many nitrosamines, especially N-dimethylnitrosamine (NDMA), are potent carcinogens. A number of past studies and current observations support the hypothesis that nitrosamine (NA) occurrence and formation could be an especially important problem in some Midwestern drinking water distribution systems. While the exact conditions and mechanisms leading to nitrosamine formation in the environment are not well understood, it is known that they are formed by reaction of nitrite with certain organic nitrogen containing (amine) compounds. These precursors to NA formation are ubiquitous in many Midwest drinking water sources or can actually be formed in distribution systems thus possibly making these supplies susceptible to nitrosamine formation. Additionally, recent observation in California suggests that NDMA formation may be related to disinfection practices, suggesting that at least this nitrosamine should also be considered a "new" disinfection by-product.

Little is known, however, about the occurrence and formation potential of nitrosamines in Midwestern drinking water distribution systems. To date, no systematic studies have considered the potential for nitrosamine formation in distribution systems and possible spatial and temporal variability. Research is needed to characterize the extent of this potential problem, and to determine how water quality, treatment, and distribution system characteristics influence it. The relationship of nitrosamine formation and occurrence to disinfection and in-system processes, especially those influenced by the pipe-water interface, needs to be ascertained. A fundamental understanding of the reaction kinetics and mechanisms would also be of great benefit in developing strategies to minimize exposure to these contaminants.

Objective(s) of the Research Project:

Based on the ascertained research needs, the following specific objectives with rationale were formulated for this research study:

1. Assess the spatial and temporal occurrence of selected nitrosamines, especially NDMA, in several midwestern water distribution systems.
2. Conduct laboratory based studies to examine mechanisms and fundamental factors influencing nitrosamine formation especially disinfection, and the presence of pipe and attached deposit material.
3. Relate field and laboratory findings to provide an assessment of the significance of nitrosamine occurrence and formation as a consequent of source water quality, water treatment and distribution, and to propose strategies to minimize occurrence and formation.

Progress Summary/Accomplishments for Second Year:

Work during the first year focused on the development of analytical methods for NDMA and acquiring data conclusively showing that NDMA is a water treatment disinfection by-product. During the second year reported on here, we focused on developing a comprehensive reaction model describing NDMA formation by reaction of monochloramine and dimethylamine (the hypothesized organic nitrogen precursor). Additional work implicates the possible role of bromide as a catalyst in NDMA formation in chlorinated and chloraminated water. Several water distribution systems have also been sampled for NDMA. NDMA formation appears to be associated with the practice of chloramination in support of the hypothesis that it is a disinfection by-product.

The kinetic reaction model (Table 1 and Figure1) was developed primarily to provide kinetic evidence in support of a proposed mechanism describing NDMA formation in chlorinated water containing ammonia or in drinking waters which are chloraminated. Rate constants were obtained from the literature or fit to the data obtained only at pH 7. Therefore the model is pH specific and not of general use at other pH values without further calibration at these specific pH values. In addition, the model does not include reactions of other potentially reactive constituents such as bromide, nitrite, and NOM.

The proposed mechanism is based largely on studies of hydrazine formation (a rocket fuel) and its oxidation (Lunn et al, 1991; Castegnaro et al, 1986; Cahn and Powell, 1954). The key reactions include the formation of monochloramine from the initially added HOCl (Reaction 1), the reaction of chlorine with DMA to form dimethylchloramine, DMCA (Reaction 2), and the slow transfer of active chlorine from monochloramine to DMA to form more DMCA (Reaction 3). Formation of NDMA is initiated by the formation of 1,1-dimethylhydrazine (UDMH) intermediate from the reaction of DMA with monochloramine (Reaction 4), followed by the oxidation of UDMH by monochloramine or oxygen to NDMA (Reaction 5). It should be pointed out that NDMA is believed to be only a minor product of UDMH oxidation. Aside from NDMA formation, the reaction of UDMH with monochloramine may form other products such as tetramethyltetrazene, methylenedimethylhydrazine, and formaldehyde dimethylhydrazone (Sisler et al., 1969; Mitch and Sedlak, 2002). The presence of other natural organic matter will likely influence the effeciency of NDMA formation from competitive reactions involving intermediates.

Model results, based upon parameters either obtained from literature or determined by fitting selected data sets, generally conform quite well to experimental results at the same pH. Figures 2 and 3 show a close correspondence between measured NDMA concentrations and that predicted by the calibrated model in chloraminated water containing DMA. As can be seen, the formation of NDMA by reaction of monochloramine is relatively slow at this pH. Mitch and Sedlak (2002) reported that NDMA formation by this mechanism is a maximum near pH 8. It should be noted that addition of nitrite to DMA containing solutions yielded very little NDMA. This, as well as comparisons based on literature kinetic constants, suggests that classical nitrosation is not likely an important formation mechanism at drinking water pH conditions unless some unrecognized catalytic process is occurring.

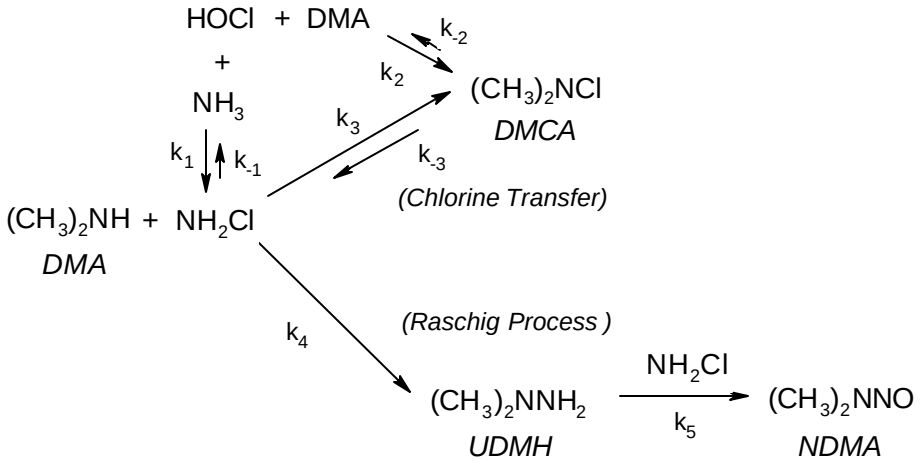

Figure 1. Mechanism of NDMA formation in chlorinated waters containing DMA and ammonia.

Table 1. Proposed reactions and rate constants for NDMA formation from DMA and monochloramine.

Reaction	Rate constant at pH 7 (25°C)	Reference
(1) $HOCl + NH_3 \underset{k-1}{\overset{k1}{\rightleftharpoons}} NH_2Cl + H_2O$	$k_1 = 4.17\times10^{6}\ M^{-1}s^{-1}$ $k_{-1} = 2.11\times10^{-5}\ M^{-1}s^{-1}$	Morris J. C. and Isaac R. A. (1981)
(2) $HOCl + (CH_3)_2NH \underset{k-2}{\overset{k2}{\rightleftharpoons}} (CH_3)_2NCl + H_2O$	$k_2 = 4.23\times10^{4}\ M^{-1}s^{-1}$ [a] $k_{-2} = 1.60\times10^{-6}\ M^{-1}s^{-1}$ [a]	Yoon J. and Jensen J. N. (1993)
(3) $NH_2Cl + (CH_3)_2NH \underset{k-3}{\overset{k3}{\rightleftharpoons}} (CH_3)_2NCl + NH_3$	$k_3 = 1.40\times10^{-1}\ M^{-1}s^{-1}$ $k_{-3} = 5.83\times10^{-3}\ M^{-1}s^{-1}$ [a]	Yoon J. and Jensen J. N. (1993); Isaac R. A. and Morris J. (1983)
(4) $NH_2Cl + (CH_3)_2NH \xrightarrow{k4} (CH_3)_2NNH_2 + H^+ + Cl^-$	$k_4 = 1.28\times10^{-3}\ M^{-1}s^{-1}$	This model
(5) $(CH_3)_2NNH_2 + 2NH_2Cl + H_2O \xrightarrow{k5} (CH_3)_2NNO + 2NH_3 + 2H^+ + 2Cl^-$	$k_5 = 1.11\times10^{-1}\ M^{-1}s^{-1}$	This model

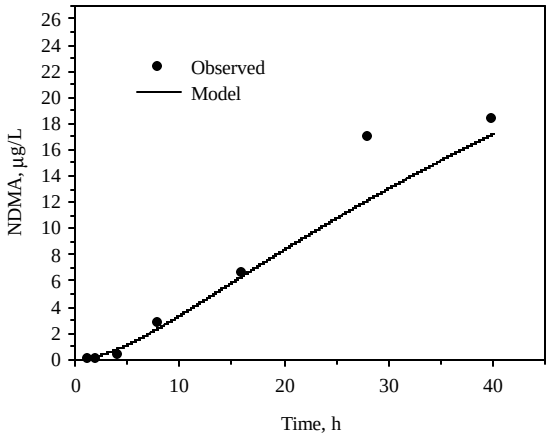

Figure 2. NDMA formation as a function of time. 0.1 mM of DMA was reacted with 0.1 mM of monochloramine. pH 7.0 ± 0.1,1 mM bicarbonate buffer. Model results are calculated based upon reactions and rate constants shown in Table 1.

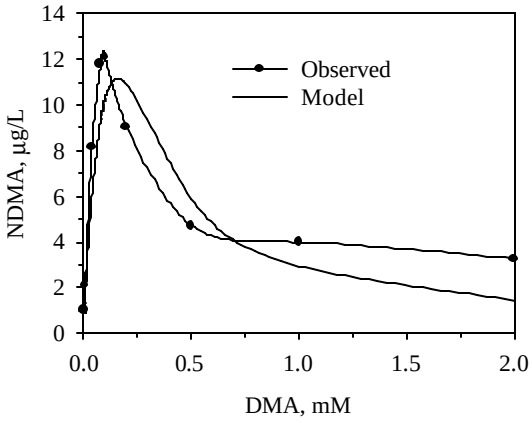

Figure 3. NDMA formation from preformed monochloramine after 24 hours as a function of DMA concentration. Monochloramine 0.1 mM, pH 7.0, 1 mM bicarbonate, 25 ^{o}C.

Additional work shows that the presence of bromide can also increase the formation of NDMA. This is presumed to be through the formation of monobromamine, an analog of monochloramine, formed by the oxidation of bromide by either free chlorine or by monochloramine. In general, its effect is also manifested much more quickly than the reaction of monochloramine. This is believed due to the general increased reactivity of monobromamine compared to monochloramine.

Consistent with this general formation mechanism, significant NDMA formation in some chloraminated distribution systems was observed sometimes in the range of 10-20 ng/L. The highest formation was observed in distribution systems obtaining their source water from supplies impacted municipal and agricultural waste disposal.

Effect of Liquid Swine Manure Application on Water Quality from Soil Infiltration Areas and Wetlands

Basic Information

Title:	Effect of Liquid Swine Manure Application on Water Quality from Soil Infiltration Areas and Wetlands
Project Number:	2001IA1641B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	
Congressional District:	Iowa 3rd
Research Category:	
Focus Category:	Water Quality, Waste Water, Wetlands
Descriptors:	Manure, Animal waste, Subsurface drainage, Bacteria
Principal Investigators:	Jeffery Lorimor, Larry J. Halverson

Publication

State:	Iowa
Project Number:	IA1641
Title:	Effect of Liquid Swine Manure Application on Water Quality from Soil Infiltration Areas and Wetlands
Project Type:	Research Project
Focus Category:	Water Quality, Waste Water, Wetlands
Keywords:	Manure, Animal waste, Subsurface drainage, Bacteria
Start Date:	03/01/2001
End Date:	02/28/2002
Congressional District:	Iowa 3rd
PI:	Jeffery Lorimor Assistant Professor, Iowa State University email:jclorimo@iastate.edu phone:515-294-9806 Larry J. Halverson Assistant Professor, Iowa State University email:larryh@iastate.edu phone:515-294-0495

Abstract

Recent research has shown that soil filtration of liquid swine manure through infiltration areas and subsequent wetland treatment of filtrate effluent from infiltration areas is very effective at removing waterborne nutrients. However bacterial movement to surface and groundwater from areas receiving manure applications is also of concern. This project will examine bacterial movement through the soil in manure application areas to determine whether the bacteria in effluent from application areas and wetlands used for treatment are from the applied manure, or from the soil itself.

12. Effect of Liquid Swine Manure Application on Water Quality from Soil Infiltration Areas and Wetlands

13. State of Critical Water Problems:
Concentration of animals into larger production facilities is a cause for water quality concerns. When manure is misapplied (especially over applied) the quality of nearby waters can be compromised. Recent research at Iowa State University (Prantner et al., 1999; and Yang, 2000) has shown that soil filtration of liquid swine and beef manure through soil, and subsequent wetland treatment of effluent from the infiltration area removes a high percentage of nutrients (70-90% of N and P).

Bacterial movement to surface and groundwater from manure applications is also of concern. More information is needed on the movement of bacteria associated with manure through soils and wetlands, and on the processes involved as the manure liquid moves through the soil water system on different landscapes. We need to increase our knowledge about bacteria movement through infiltration areas, bacterial origin, and bacterial life expectancy. Little research has been conducted in this area. The proposed project will provide very useful information for the state of Iowa about the contribution of bacteria from liquid swine manure to surface and groundwater supplies. Funding is requested for the second year of research for two related ongoing studies.

14. Statement of Results or Benefits:
Determining the bacteriological effects of applying manure to croplands and wetlands, will help determine whether soil filtration and wetland treatment are viable treatment options. If manure-born bacteria are moving through the soil profile, manure application rates and methods may need further adjustments to minimize water quality deterioration, making the treatment system less beneficial. Understanding the processes that are occurring in the soil-water system after the application of manure will help engineers and scientists design better, more efficient, and less polluting land/wetland treatment systems for managing excessive animal manure from large livestock production facilities.

15. Nature, Scope, and Objectives of the Research:
This research is a laboratory scale project conducted on small field plots and lysimeters, both in the laboratory and in the field. The overall objective of this project is to understand the soil microbial and chemical processes occurring within infiltration areas and wetlands used to treat liquid swine manure to enable effluents to be of sufficient quality to be discharged to Iowa's water bodies safely. (Discharges from animal facilities are not allowed now, but may become an option in the future if effective treatment systems can be developed). Rather than answering the question "what's happening?" the proposed project will also answer the question "why is it happening in the proposed treatment system?" Instead of determining whether bacteria are present in the soil filtrate and wetland effluent, we will determine whether the source of that bacteria is from swine manure or from naturally occurring activities in the soil-water system. *This is a request for second year funding to continue the project.*

16. Methods, Procedures, and Facilities
A set of 18 (9 pairs) of 210L plastic containers located in the field and are being used for the study. In each set of two containers, the first is designed for soil infiltration, the second just

below first set serve as wetlands. They are designed so that flows in and out of each container can be monitored and sampled for nitrogen, phosphorus, and bacteria. This set of containers is subject to climatic and hydrologic events occurring throughout the year.

A second set of undisturbed soil cores have been obtained by driving 20 cm diameter steel cylinders into the soil. This set of cores is inside Dr. Lorimor's laboratory at ISU where they are isolated from outside daily climatic and hydrologic events

To determine whether the bacteria from the manure are actually transported through the soil columns, genetically marked bacteria have been isolated to use as tracers. A genetically marked psuedomonad or a bacillus species from the manure system were isolated. The genetic marker used is a green fluorescent protein which provides a readily visible marker since it makes the bacteria turn bright green color. The genetically marked bacteria were introduced into the manure and maintained for some period of time (2 weeks) to verify that the marker has not decreased their ability to survive in the manure. The manure with the marked bacteria were applied to the soil.

The primary independent variables being investigated in the outdoor system are:

Manure –applied every two weeks at full rate based on nitrogen utilization
Manure – applied every two weeks at ¾ N rate
Water only – applied every two weeks based on soil moisture holding capacity

Treatments in the indoor columns are designed to determine if time and desiccation due to drying soils affect bacteria survival and transport:

Manure – one application only to all columns except the checks at 5000 gallons/ac,
Treatment 1 – one application of 2 inches of water at 4 days after manure application again at 8 days, and again at 16 days
Treatment 2 one application of 2 inches of water at 8 days, and again at 16 days
Treatment 3 one application of 2 inches of water at 16 days

Research identifies macropores as one of the primary pathways for bacterial movement. Three tillage treatments designed to destroy macropores superimposed on the above water treatments in the laboratory experiment will help delineate the contribution macropores play in bacterial movement. The treatments are 1) notill manure broadcast, 2) tillage ahead of broadcasting manure, and 3) manure injection. The effluent will be monitored for the marked bacteria. At the end of the experiment, the soil columns will be sampled at the top, middle, and bottom to determine the number of genetically marked and naturally occurring bacteria in the soil.

17. Related Research:

This project is a nice follow-up to other projects in process at ISU utilizing nearby plots and similar types of columns. Warnemunde (PhD dissertation not yet published) has investigated movement under corn and soybean field plots and found large variability in microbial detects in tile water.

18. Training Potential:

One graduate student is receiving training through this project to complete her MS degree in Water Resources at Iowa State University.

19. Investigator's Qualifications:

Jeffery Lorimor
Assistant Professor, Agriculture and Biosystems Engineering

BSAE	Iowa State University	1967
MSAE	Univ. of Nebraska	1970
PhD	Iowa State University	1996

Selected List of Publications:

Lorimor, J.C. and S.W. Melvin. 1996. Nutrient loss from properly manured land. ASAE paper MC96-120. Presented at Midcentral Section of ASAE. St. Joseph, Mo.

Lorimor, J.C., K. Kohl, and G. Wells. 1997. Swine liquid manure nutrient concentrations field study results. ASAE paper 972043.

Lorimor, J.C., and S.W. Melvin. 1998. Nitrates in drainage tile flow from properly manured land. In Proc. of Animal Production Systems and the Environment Conference, July 20-22, 1998. Des Moines, IA.

Lorimor, J.C., and H. Xin. 1998. Manure volume and nutrient concentrations from high rise layer houses. In Proc. of Animal Production Systems and the Environment Conference, July 20-22, 1998. Des Moines, IA.

Lorimor, J.C., A. Millmier, C. Hurburgh, C. Fulhage, H. Person, J. Hattey, H. Zhang. 1998. Near infrared technology to determine manure nutrients. In Proc. of Animal Production Systems and the Environment Conference, July 20-22, 1998. Des Moines, IA.

Lorimor, J.C., and H. Xin. 1999 Manure production and nutrient concentrations in high rise layer houses. ASAE Applied Engineering in Agriculture 15(4): pp 337-340.

Millmier, A., J. Lorimor, C. Hurburgh, Jr., C. Fulhage, J. Hattey, H. Zhang. 1999. Near-infrared sensing of manure nutrients. Submitted to ASAE Transactions.

Prantner, S.R., R.S. Kanwar, J.C. Lorimor, and C.H. Pederson. 1999. Management of swine manure through the use of soil infiltration and wetland systems. ASAE Paper MC99-116. Presented at Midcentral Section of ASAE. St. Joseph, Mo.

Warnemuende, E.A., J.L. Baker, R.S. Kanwar, J.C. Lorimor, S. Mickelson, and S.W. Melvin. 1999. The effects of swine manure application systems on surface and groundwater quality. ASAE Paper 992197. Presented at 1999 ASAE International Meeting, Toronto, Canada.

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Assistant Professor, Agronomy
Ph.D University of Wisconsin-Madison, 1991
M.S. University of Tennessee-Knoxville 1983
B.A. Luther College, Decorah, IA 1981

Research:
My research interests are focused on how environmental factors influence the growth, survival, and diversity of bacteria in natural habitats, particularly those bacteria residing in soil or in association with plant roots. Various environmental factors such as water content, salinity, temperature, nutrient availability, and the presence of organic and inorganic pollutants can strongly influence bacterial ecology. Our approach is to understand the relationship between the physiological, biochemical, and genetic responses bacteria employ to counter the detrimental effect those stresses can impose on them so as to better understand their behavior in natural environments. This information is necessary if we are to utilize soil bacteria for beneficial purposes such as for the remediation of polluted soils, control of plant diseases, or promotion of plant growth.

Teaching:
Micro 302 Biology of Microorganisms; Micro 477/577 Bacterial-plant interactions
A) Identification of genes that confer water deprivation tolerance in *Pseudomonas putida*
B) Evaluation of the biological availability of organic pollutants in soil by using green fluorescent protein (GFP) based bacterial biosensors.
C) Exploration of bacterial strategies for environmental stress adaptation and evolution in soils.
D) Characterization of the relationship between microbial community ecology and malodor production in anaerobic swine manure storage systems.

Selected List of Publications:
Holden, P. A, L. J., Halverson, and M. K. Firestone. 1997. Water stress effects on toluene biodegradation by Pseudomonas putida. Biodegradation 8:143-151.
Halverson, L. J., M. K. Clayton, and J. Handelsman. 1993. Variable stability of antibiotic- resistance markers in Bacillus cereus UW85 in the soybean rhizosphere in the field. Molecular Ecology. 2:65-78.
Halverson, L. J., M. K. Clayton, and J. Handelsman. 1993. Population biology of Bacillus cereus UW85 in the soybean rhizosphere. Soil Biology and Biochemistry. 25:485-493.
Halverson, L. J. and J. Handelsman. 1991. Enhancement of soybean nodulation by Bacillus cereus UW85 in the field and in a growth chamber. Applied and Environmental Microbiology. 57:2767-2770.
Halverson, L. J. and G. Stacey. 1986. Signal exchange in plant-microbe interactions. Microbiological Reviews. 50:193-225.

Evaluating the Effectivensss of Restored Wetlands for Reducing Nutrient Losses from Agricultural Watersheds

Basic Information

Title:	Evaluating the Effectivensss of Restored Wetlands for Reducing Nutrient Losses from Agricultural Watersheds
Project Number:	2001IA1681B
Start Date:	3/1/2001
End Date:	2/28/2002
Funding Source:	104B
Congressional District:	Iowa 3rd
Research Category:	Water Quality
Focus Category:	Wetlands, Nutrients, None
Descriptors:	Wetlands, Watersheds, Nutrient loss
Principal Investigators:	Arnold G Van der Valk, William G. Crumpton

Publication

Problem and Research Objectives:

This study examines at two levels, the subwatershed and wetland, the effectiveness of wetland restorations for reducing nutrients in agricultural runoff in the Iowa Great Lakes watershed. It has two major research objectives: (1) to monitor nutrient concentrations in the outflow of subwatersheds with different percentages of restored wetlands to determine if restored wetlands have significantly reduced the levels of nutrients in outflows, and (2) to monitor nutrient concentrations in the inputs and outputs of restored wetlands to see how effective they are as nutrient sinks.

Methodology:

(a) Subwatersheds

Outflows from a series of subwatersheds with and without restored wetlands are sampled on a weekly basis. These water samples are analyzed for nitrate, total nitrogen and total phosphorus using standard techniques.

(b) Restored Wetlands

Inflows and outflows from a series of restored wetlands are also sampled on a weekly basis. These water samples are analyzed for nitrate, total nitrogen and total phosphorus.

In order to characterize the developmental status of each restored wetlands, their vegetation and litter compartments are sampled using standard techniques. Each restored wetland is divided into ten zones along its longest axis. Randomly placed quadrats (1 m x 1 m) in each zone are sampled in either late July or early August. The abundance of each plant species in each quadrat is estimated using a cover-abundance scale. The entire quadrat aboveground is then harvested. Harvested plant material is separated into live plants, standing litter, and fallen litter. All biomass samples are dried at 80 C before being weighed.

Principal Findings and Significance:

(a) Subwatersheds

In 2001, sampling of outflows from 10 selected subwatersheds with and without restored wetlands were sampled. Weekly samples were collected from 12 sites in these 10 subwatersheds. All of the water samples have been analyzed for nitrate-nitrogen, total nitrogen, and total phosphorous through the end of 2001. There are four subwatersheds that are mostly cropland (E3, E4, W13, and W14), 2 intermediate subwatersheds with some restored wetlands (G3, W2), and four subwatersheds (G5, W3, W9, and W10) that have restored wetlands and land in set-aside programs.

Since they have been monitored, the outflows from the mostly cropland subwatersheds have had mean nitrate concentrations ranging from 6.25 to 9.60 mg/L while the four subwatersheds with restored wetlands have had mean concentrations of nitrate that ranged from 0.12 to 4.38 mg/L. The two intermediate subwatersheds had mean nitrate concentrations of 3.77 and 5.38 mg/L. Subwatershed W9, which has the highest percentage of its area in wetlands, has had the lowest nitrate concentration (0.12 mg/L) while subwatershed G5 that has a largest percent in CRP had the second lowest nitrate concentration (0.41 mg/L). Watershed E4, which has the second largest percent of its area in cropland, had the highest mean nitrate concentration (9.60 mg/L). Concentrations of nitrates in subwatersheds with restored wetlands are much lower than in those without restored wetlands.

Because water samples were collected weekly during this study, our estimates of total phosphorus concentrations are not as reliable as are those for nitrates. Phosphorus concentrations in runoff are a function of volume of flow with the highest concentrations typically in initial flows after a storm event. By contrast, nitrate concentrations are less dependent on volume of flow. Phosphorus concentrations in runoff are also highly affected by the topography and total area of the subwatershed sampled. This makes comparisons among subwatersheds more difficult. Mean total phosphorous concentrations in the mostly cropland subwatersheds ranged from 0.081 to 0.172 mg/L while in the four subwatersheds with wetlands it ranged from 0.117 to 0.273 mg/L. The two intermediate subwatersheds had total phosphorus concentrations of 0.129 and 0.175 mg/L. Subwatershed W9 with the largest percentage of its area in wetlands had the highest phosphorous concentration (0.273 mg/L). Subwatershed W10 with the highest percentage of its area in CRP had a mean total phosphorus concentration of 0.205 mg/L. Watershed E3 that has the largest percent of its area in cropland had among the lowest mean total phosphorous concentration (0.137 mg/L). Total phosphorus concentrations in the outflows of subwatersheds with restored wetlands have not been lower than in those without restored wetlands. The phosphorus data, however, have not been adjusted for differences in topography and area among subwatersheds. Consequently, these results should be considered tentative.

Although there is significant variation in their effectiveness for removing nitrates from subwatershed to subwatershed, as expected, restored wetlands and land set-aside programs are effective in reducing nitrate losses from subwatersheds. For total phosphorus, thought to be the major nutrient responsible for algal blooms in most lakes, restored wetlands may not to be reducing total phosphorus in the outflows from subwatersheds with the highest percent of their area covered by wetlands.

To determine if inadequacies in sampling might be responsible for the lack of congruence between phosphorus and nitrate reductions in the outflow from subwatersheds, two flow-weighted automatic samplers were purchased in the fall of 2001 and will be placed in the field in 2002. One will be placed on a subwatershed primarily in row crops and the other on a subwatershed with restored wetlands. Negotiations with landowners have been initiated to obtain permission to locate these samplers on private land away from roads

and places of public access to reduce the probability that these automatic samplers will be vandalized.

(b) Restored Wetlands

Five wetlands in the Iowa Great Lakes watershed with distinct, easy to sample inputs and outputs were selected in the summer of 2001. Sampling of nutrients in their inflows and outflows began in July 2001 and ended in October because their inflows dried up. Three of the wetlands (7, 8 and 12) are in subwatersheds of West Lake Okoboji while the other two (1 and 16) are in subwatersheds of East Lake Okoboji.

Input concentrations of nitrates ranged from 6.33 to 21.79 mg/L while the output concentrations ranged from 0.05 to 5.84 mg/L. Wetland 7 the largest reduction in nitrate concentrations with mean input concentrations of 21.79 mg/L and output of 0.15 mg/L. Wetland 1 was the least effective with mean input concentrations of 19.65 mg/L and output of 5.84 mg/L. All of the restored wetlands are effective at removing nitrates. Our data are too limited yet to draw any conclusions about phosphorus.

In the summer of 2001, sampling of the vegetation and standing crop of the five selected restored wetlands was initiated. Each wetland was divided into a series of parallel zones and each zone was sampled using a randomly located transect in the zone. Samples were collected in quadrats placed at random intervals along these transects. The cover of each species in each 1m x 1 m quadrat was recorded and then all aboveground vegetation clipped and bagged. All standing crop samples were oven dried and weighed. Altogether over 200 standing crop samples were collected. The mean standing crop in restored wetlands was about 430 g/m^2. This is considerably lower than standing crops found in natural wetlands in northern Iowa, ca. 600 to 1,000 g/m^2 The cover data and vegetation maps derived from recent aerial photography also indicate that the vegetation in restored wetlands is not as well developed as in natural wetlands in the region.

In summary, restored wetlands are not yet as well developed from an ecosystem perspective as natural wetlands. Consequently, their effectiveness as nutrient sinks may still be less than that of natural wetlands. Nitrate losses have been reduced significantly from subwatersheds that contain restored wetlands and set-aside land. Phosphorus losses from these subwatersheds have not shown a similar trend. The reason for this is unclear. It may be due to inadequate sampling of phosphorus in runoff or it may be because restored wetlands are not good sinks for phosphorus. Improved sampling of runoff from subwatersheds with flow-weighted automatic samplers will be initiated to determine of the poor removal of P is a sampling artifact or not.

Complementary Investigations for Implementation of Remote, Non-Contact Measurements of Streamflow in Riverine Environment

Basic Information

Title:	Complementary Investigations for Implementation of Remote, Non-Contact Measurements of Streamflow in Riverine Environment
Project Number:	2001IA1021G
Start Date:	9/1/2001
End Date:	8/31/2004
Funding Source:	
Congressional District:	1st congressional district of Iowa
Research Category:	
Focus Category:	Water Quantity, Methods, Non Point Pollution
Descriptors:	
Principal Investigators:	

Publication

Information Transfer Program

Student Support

None

Notable Awards and Achievements

Publications from Prior Projects

1.
2. Haan, Mathew; James Russell; Wendy Powers; Steven Mickelson; Syed Ahmed; John Kovar; Richard Schultz. 2002. Effects of Grazing Management on Sediment and Phosphorus Runoff. 5 p. IN: 2002 Beef Research Report-Iowa State University, Ames, IA.
3. Powers, Wendy; James Russell; Mathew Haan; Richard Schultz; Emily Stauffer, George Zaimes; Jennifer Hunkins; John Kovar; Jamie Boehm; Steven Mickelson; and Syed Ahmed. 2002. Grazing Systems to Maximize Forage Use and Minimize P, N, and Sediment Pollution of Streams. pp. 23-33. IN: Proceedings of Agriculture and the Environment: The Challenge of Change. Iowa State University, Ames, IA.