

Water Resources Research Center

Annual Technical Report

FY 2000

Introduction

Fiscal year 2000 was a productive period for the University of Minnesota's Water Resources Center. Significant accomplishments were achieved in research, education, and outreach. The budgets managed by the Center grew to over \$5 million, and the number of staff grew to a total of ~25 individuals, including both on-campus and off-campus extension persons. Approximately 40 University faculty were involved in the Center's research programs, and more have indirect involvement through the interdisciplinary graduate program, Water Resources Science, which the Center administers. Research grants and fellowships administered by the Center supported 26 graduate students, three undergraduate students and three postdoctoral associates in 1999-2000. Staff additions included two extension educators in the area of volunteer stream monitoring.

The WRC issued a Request for Proposals in November 1999 for the FY 2000 grant competition. Using the WRI 104(b) grant and funds provided by the University's Agricultural Experiment Station to the Center for Agricultural Impacts on Water Quality (CAIWQ), a unit of the WRC, we were able to fund a total of seven research projects—four new ones and the second year of three projects beginning in March 2000. In addition, the Center administered one continuing project from the era in which the WRI 104b funds were disbursed by a regional competition.

The FY 2000 projects were: (i) William Arnold (Civil Engineering) Mechanisms of degradation of trifluralin and related herbicides; (ii) Paige Novak and Michael Semmens (Civil Engineering): A novel in situ technology for the treatment of ground water contaminated with agriculturally-derived nitrate; (iii) Neil Hanson (Soil, Water, Climate) and Sagar Goyal (Vet. Diagnost. Med.) Runoff water quality and crop responses to variable manure application rates; (iv) Satish Gupta (Soil, Water and Climate) and Marv Bauer (Forest Resources): Evaluation of bank erosion inputs to the Blue Earth River with airborne laser scanner; (v) Peter Sorensen, Ira Adelman and Heiko Schoenfuss (Fisheries and Wildlife) and Deb Swackhamer (Environmental & Occupational Health): Assessing the effects of endocrine disruptors from a St. Paul sewage treatment plant on sperm viability and testicular development in fish; (vi) Gary Sands (Biosystems and Agricultural Engineering), David Mulla (Soil, Water, and Climate), Lowell Busman (Southern Research and Outreach Center), and Steve Taff (Applied Economics): Feasibility of controlled drainage for mitigating nutrient loss from tile drainage systems in south central Minnesota; (vii) Ray Hozalski (Civil Engineering): Investigation of the factors affecting removal of *Cryptosporidium* and *Giardia* from drinking water supplies by granular media filtration; (viii) Paul Bloom (Soil, Water, and Climate) and Patrick Brezonik (Civil Engineering) Role of photochemical processes and binding by humic substances in mercury cycling in aquatic systems. The first four projects were new in 2000; the next three were in their second (and final) year; and the last project was a hold-over from the regional competition era. Progress or completion reports for each of these projects are provided in the following section.

Another grant was received from the WRI national competition (104(g) program) in FY 2000 to study the role of denitrification as a nitrate sink in agricultural streams. PIs on that project, which began in fall 2000 are Lorin Hatch (Forest Resources) and Patrick Brezonik (Civil Engineering,

WRC).

Work was completed by December 2000 on two major grants: one from the Twin Cities Metropolitan Council on use of satellite imagery to assess lake quality conditions in the seven- county metro area and to develop GIS-based modeling tools to assess non-point source nutrient loadings to lakes and streams in the area, and the other from the U.S. EPA on scale issues in watershed management: Minnesota River Basin as a case study. Completion reports for both projects were submitted to the agencies in spring 2000, and they will be published as WRC reports in 2001.

Other research funded by the CAIWQ during FY 2000 includes hydrologic modeling by John Nieber (Biosystems and Agricultural Engineering), on-site wastewater treatment work of Jim Anderson (Soil, Water, and Climate), MSEA work of Robert Dowdy and John Lamb (SWC), and pesticide work by Bill Koskinen (SWC). Grants totaling over \$830,000 from the Metropolitan Council, MN Pollution Control Agency, and NRCS started in 1999 and continued in 2000. The projects involve a wide range of topics, including research/demonstration projects on alternative residential septic systems, increasing adoption of BMPs to reduce phosphorus in the Blue Earth River, improving whole-farm planning and education on non-point source pollution, developing new educational materials for shoreland management, and increasing citizen participation in watershed management.

Research Program

Basic Information

Title:	Runoff Water Quality and Crop Responses to Variable Manure Application Rates
Project Number:	B-06
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Water Quality
Focus Category:	Agriculture, Nitrate Contamination, Water Quality
Descriptors:	nutrients, phosphorus, coliforms, manure, runoff, agricultural drainage, salmonella
Lead Institute:	University of Minnesota
Principal Investigators:	Neil C Hansen, Sagar M Goyal

Publication

1. Gessel, P.D., J. Webb, N.C. Hansen, and S.M. Goyal. 2001. Persistence of zoonotic pathogens in the mixing zone following land application of liquid hog manure. Currently undergoing internal review.
2. Gessel, P.D., N.C. Hansen, and J.F. Moncrief. 2000. The impact of phosphorus based manure application on water quality and soil properties. Poster at Agronomy Society of America Annual Meeting, November 5-9, 2000, Minneapolis, Minnesota.

3. Gessel, P.D., J. Webb, N.C. Hansen, and S.M. Goyal. 2001. Persistence of zoonotic pathogens in the mixing zone following land application of liquid hog manure. To be presented as a poster at Agronomy Society of America Annual Meeting, October 21-25, 2001, Charlotte, North Carolina.

Project No.: B-06

Project Title: Runoff Water Quality and Crop Responses To Variable Manure Application Rates

Investigators: Dr. Neil C. Hansen, Dept. of Soil, Water, and Climate, College of Agriculture, Food, and Environmental Science; Dr. Sagar Goyal, Department of Veterinary Diagnostic Medicine, College of Veterinary Medicine, University of Minnesota

Period: 3/1/00–2/28/01

Introduction

Livestock producers and society want to protect water in their environment. Applications of manure to cropland can contaminate water if applied improperly. Contamination of water with excess nutrients or pathogens limits the use and function of water and may compromise public health. In Minnesota, some of these water quality problems have been attributed to livestock production. Manure management practices that promote water quality need to be identified and adopted and costs of adoption need to be determined.

One practice that impacts both water quality and crop production is the use of livestock manure in crop production systems. Limiting manure application to rates equal to the crop requirements for phosphorus would prevent the buildup of soil phosphorus that occurs with the currently recommended nitrogen-based rates. However, the assumption that lower phosphorus based application rates would improve water quality may not be correct. When manure is applied, water infiltration into the soil is improved and runoff is reduced. An environmentally-based manure application would be at a rate where a reduction in runoff would combine with the increase in soil phosphorus or pathogen levels to produce the least total loss of contaminants in runoff. There is currently insufficient information to develop an environmentally-based application rate. In this study, the impacts of varying manure application rates on soil hydraulic properties, runoff, and the transport of both phosphorus and pathogens in runoff were studied. The results will be helpful to both livestock producers and regulators in refining current manure management guidelines.

Nature and Objectives

This project involved field research to monitor runoff from snowmelt and rainfall events in cultivated land. Both chemical and biological water quality standards were evaluated. The specific project objectives were to:

1. Determine how manure applied at various rates impact soil hydraulic properties and the loss of nutrients in surface runoff.
2. Determine the survival of the pathogens *Salmonella anatum*, coliphages, and fecal coliform indicator bacteria in surface soil and in runoff water.

Materials and Methods

The study was located at the West Central Research and Outreach Center in Morris, MN on a calcareous soil with uniform slope. Runoff plots sized 22 m by 3 m were bordered with corrugated sheet metal driven vertically into the soil to isolate surface runoff. On the down-slope side of the plot a metal collection flume channeled runoff to a tipping bucket flow meter. The tipping buckets were monitored continuously with a data logger to determine runoff rate and volume. A fraction of the runoff from individual events were composited and collected for quantification of sediment, nutrients, and organisms. Plots were cropped to corn in 1999 and soybeans in 2000. Experimental treatments were:

1. 0 - no manure applied to the plot
2. 0.5 X - liquid swine manure applied at half the phosphorus-based rate
3. 1.0 X - liquid swine manure applied equal to a phosphorus-based rate
4. 2.0 X - liquid swine manure applied at double the phosphorus-based rate

Manure was broadcast applied in October of 1998 and 1999 and immediately incorporated by disking. The manure application rate for the 1.0 X treatment was based on soil test results and University of Minnesota phosphorus fertilizer recommendation (Rehm et al., 1995). The P content of the manure can be found in Table 1. No additional fertilizer was applied to the corn or soybean crop.

Results

Runoff was dominated by 5 events in 1999 and 4 events in 2000. A large event in June of 1999 dominated the total runoff for the season. In 2000, the events were roughly equal in magnitude. All events occurred from June through August in both years. Cumulative runoff totals ranged between 18 mm for the 2.0x treatment and 34 mm for the control (Fig. 1). For both years, runoff was less as manure rate increased. The reduction in runoff led to a reduction in nutrient loading as well (Fig. 2). The reduction in runoff was most likely due to improvement in soil physical properties and water infiltration rates from application of manure. This is consistent with what has been observed at higher manure rates (Ginting et al., 1998). It is important to note that this study evaluates manure application to a soil with a relatively low level of extractable P. It is expected that for soils with very high levels of extractable P the reduced runoff will not offset the high P concentrations possible in runoff.

Total Phosphorus (TP) loads from the plots ranged from 1.7 kg/ha on the control plots to 0.7 kg/ha on the 2.0x plots. The reduction in phosphorus in runoff occurred despite the increase in applied P in the manure. This illustrates the importance of hydrology in contaminant transport. Predictive models currently used to develop manure and nutrient management policy are generally not sensitive to improvements in soil physical properties as a result of applied manure. However, the results from this study are being used in the development of a Phosphorus Index for Minnesota.

The results of the pathogen analysis have been detailed elsewhere (Gessel et al., 2001). Salmonella increased dramatically in the soil after manure application but died off quickly, lasting only 10 days or less on all plots. Fecal coliforms dropped to near-background levels within 4 days. Somatic coliphages persisted for up to 143 days after manure application, but male-specific coliphages survived 24 days or less. Only fecal coliforms were found in the runoff sample collected. However, similar levels were observed in runoff from manure treated and control plots, suggesting that the fecal coliforms did not originate from the manure.. No rate-related persistence in the soil could be detected for any of the pathogens in any of the treatments. Further research is necessary to determine the potential for pathogen loss in runoff immediately following manure application and incorporation.

Conclusion

Two years of runoff data suggest that liquid hog manure applied at P based agronomic rates improves infiltration and reduces runoff. As a result, associated particulate pollutant losses are also reduced. Soluble pollutant losses are not affected by manure application, but total P is reduced as manure increases.

While the manure application rate initially raises pathogen levels in the soil, the rate seems to have a negligible effect on the fatality of the pathogens. No rate-related persistence in the soil could be detected for any of the pathogens in any of the treatments. There appears to be a fairly weak relationship overall between application rate and pathogen populations. Salmonella died off rapidly on the soil surface, suggesting that it is a minimal health risk at best when manure is land applied and incorporated. Due to the unpredictable nature of the weather, further research is necessary to determine the real potential for pathogen loss in runoff, especially right after manure application and incorporation. A series of rainfall simulations would seem to be the logical choice for this type of research.

References

Gessel, P.D., J. Webb, N.C. Hansen, and S.M. Goyal. 2001. Persistence of zoonotic pathogens in the mixing zone following land application of liquid hog manure. Internal review.

Rehm, G., M. Schmitt, and R. Munter. 1995. Fertilizer recommendations for agronomic crops in Minnesota. Minnesota Extension Service BU-6240-E. University of Minnesota. St. Paul, MN.

Table 1: Manure application, 1998 and 1999

<u>Manure Application</u> rate	vol, kL/ha	<u>P, kg/ha</u>	
		98	99
Control	0	0	0
0.5x	17	8	4
1x	35	16	8
2x	75	32	16

Figure 1: Cumulative runoff from research plots

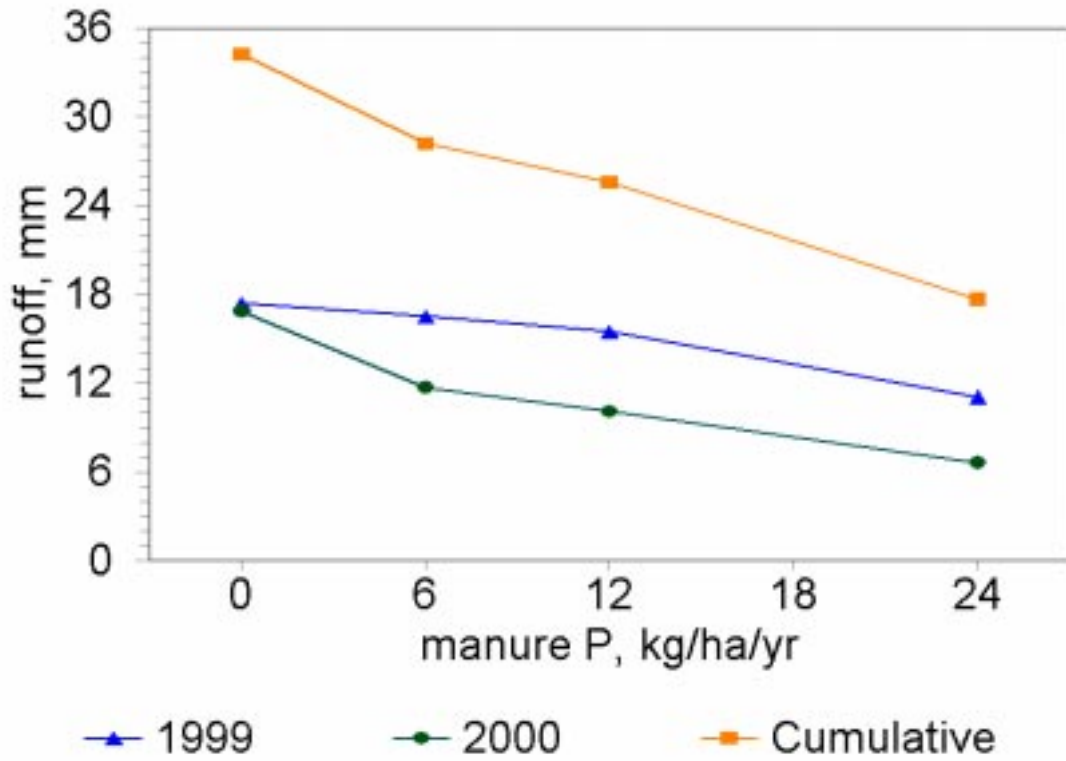
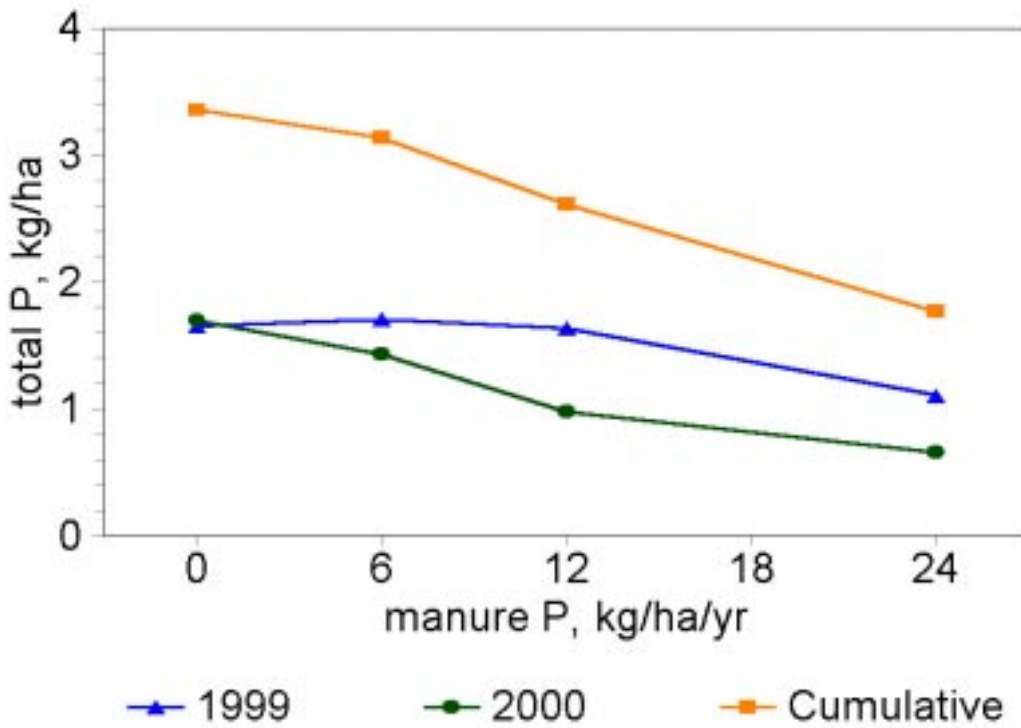


Figure 2: Cumulative total phosphorus loading from research plots



List of Publications

Gessel, P.D., J. Webb, N.C. Hansen, and S.M. Goyal. 2001. Persistence of zoonotic pathogens in the mixing zone following land application of liquid hog manure. Currently undergoing internal review.

Conference Presentations

Gessel, P.D., N.C. Hansen, and J.F. Moncrief. 2000. The impact of phosphorus based manure application on water quality and soil properties. Poster at Agronomy Society of America Annual Meeting, November 5-9, 2000, Minneapolis, Minnesota.

Gessel, P.D., J. Webb, N.C. Hansen, and S.M. Goyal. 2001. Persistence of zoonotic pathogens in the mixing zone following land application of liquid hog manure. To be presented as a poster at Agronomy Society of America Annual Meeting, October 21-25, 2001, Charlotte, North Carolina.

Students Supported by the Grant

Peter Gessel, M.S. Water Resources Science candidate.

Kory Werk, undergraduate summer intern

Basic Information

Title:	A Novel In-Situ Technology for the Treatment of Groundwater Contaminated with Agriculturally-Derived Nitrate
Project Number:	B-02
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Engineering
Focus Category:	Nitrate Contamination, Water Supply, Treatment
Descriptors:	nitrate, groundwater, denitrification, water treatment, membrane technology
Lead Institute:	University of Minnesota
Principal Investigators:	Paige J Novak, Michael J Semmens

Publication

1. Katrina Haugen a civil engineering graduate student has conducted this research as an MS thesis project. She will complete the research and the thesis in the fall of 2001. In addition, Ms. Haugen will author a paper on her research finding for submission to Water Research this summer (2001), and present her research findings at the Midwest Chemistry Workshop in October 2001.

Project No.: B-02

Project Title: A Novel In-Situ Technology For The Treatment of Groundwater Contaminated with Agriculturally-derived Nitrate

Investigators: Paige Novak Ph.D., P.E.; Michael J. Semmens Ph.D., P. E. and Katrina Haugen, Graduate Research Assistant, Department of Civil Engineering, University of Minnesota

Period: 3/1/00–2/28/01

Introduction

The experiments described within this report were designed to evaluate the use of *in situ* hydrogenotrophic denitrification for remediating nitrate-contaminated groundwaters.

Denitrifying bacteria were cultured from soil from the University of Minnesota St. Paul agricultural station. Denitrifying cultures were started June 23, 2000 by adding 50 grams of soil to 100 ml of anoxic synthetic groundwater media. A series of bottle experiments were performed to develop denitrifying cultures that could use hydrogen gas to reduce nitrate.

To evaluate hydrogenotrophic denitrification *in situ* using hollow fiber membranes, a flow-through box reactor was constructed as shown in Figure 1. The box was designed to simulate groundwater flow through an aquifer. The central rectangular test section measured 2.5 cm in width, 1.83 m in length, and 0.304 m deep and was packed with aquarium rocks (Estes' Ultrastone) and soil from the University of Minnesota to simulate *in situ* conditions. Additional tapered inlet and exit sections measuring 0.3m in length were designed to promote uniform flow in the central test section. The 14.2L reactor was equipped with a removable lid. The lid contained two holes to supply H₂ to the membrane module. The membrane module consisted of six reinforced silicone hollow fiber membranes. Seven gas-tight sampling ports were installed at mid-depth along the length of the reactor for sample collection.

Methylene blue tracer studies were conducted on the box and it was shown that the water moved through the box uniformly in a plug-flow manner.

Prior to the flow through experiments the reactor was seeded with buffered synthetic groundwater containing an active culture of denitrifying bacteria was allowed to stand for a week. A synthetic, slightly oxic groundwater flow was then pumped through the reactor at a rate of 1.6 ml/min. For three weeks medium was pumped through the reactor without the addition of H₂. After three weeks the membrane module was supplied with 100 % H₂ at a gas flowrate of 0.5 ml/min.

Liquid samples were collected from the reactor sample ports P1 – P7 weekly. P1 was located close to and just downgradient from the membrane injection point. P7 was the furthest distance downgradient of the membranes. NO₃⁻ and NO₂⁻ concentrations were measured using ion chromatography. Dissolved H₂ concentrations were determined by gas chromatography. Influent and effluent samples were also analyzed weekly for total organic carbon (TOC), pH, redox potential and protein content.

Results

The study successfully demonstrated that the membrane modules can supply H₂ to groundwater in sufficient concentration to effectively remove nitrate. The dissolved H₂ concentration is shown as a function of operating time in Figure 2. The H₂ concentration dropped to very low level between days 25 and 60, but after the H₂ gas pressure was elevated to 5 psi, high residual dissolved H₂ concentrations were observed.

The reactor required time to develop a microbial population capable of effective NO₃⁻ and NO₂⁻ removal. (shown in Figure 3). Nevertheless, in this study we were able to demonstrate that both NO₃⁻ and NO₂⁻ were effectively removed *in situ* by biological denitrification. The variability in performance and the time required to achieve full removal was influenced by operating conditions, namely H₂ pressure in the membrane module. The system was H₂ and carbon dioxide limited for some time. Once this was recognized and corrected, excellent performance was achieved. This implies that in the field a carbon source (such as carbon dioxide) may need to be added for effective remediation. The removal of nitrate was achieved with little increase in TOC or effluent turbidity, which is critical if this system is to be used in the field for denitrification.

Summary and Future Research

This seed research project was extremely successful. We were able to demonstrate that the process works effectively and has little impact on groundwater quality. Drs. Novak and Semmens used these early results to apply for follow-on funding from the State of Minnesota, Legislative Commission on Minnesota's Resources. They have received approximately \$250,000 in funding for a field trial beginning in the summer of 2001. The funding of this project was due in part to the successful completion of this project.

Publications/ Presentations:

Katrina Haugen a civil engineering graduate student has conducted this research as an MS thesis project. She will complete the research and the thesis in the fall of 2001. In addition, Ms. Haugen will author a paper on her research finding for submission to Water Research this summer (2001), and present her research findings at the Midwest Chemistry Workshop in October 2001.

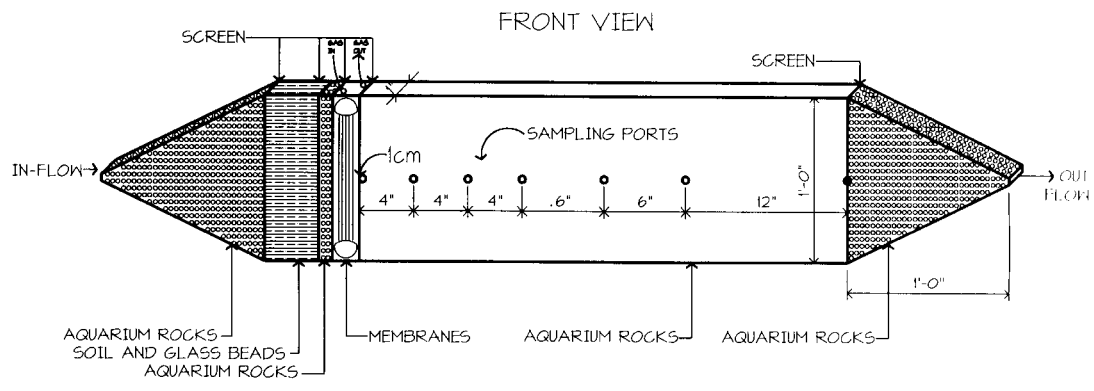


Figure 1. Schematic of flow-through box reactor

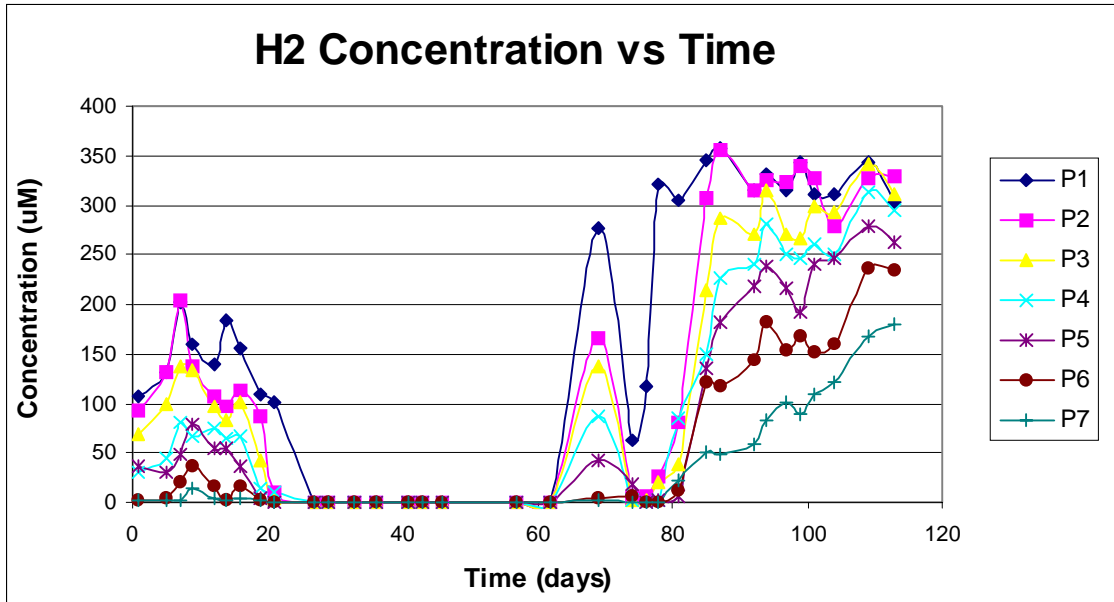


Figure 2 Dissolved hydrogen concentration observed downstream of the membranes as a function of operating time in days. P1 is closest to the membrane and P7 is the greatest distance downgradient of the gas injection point.

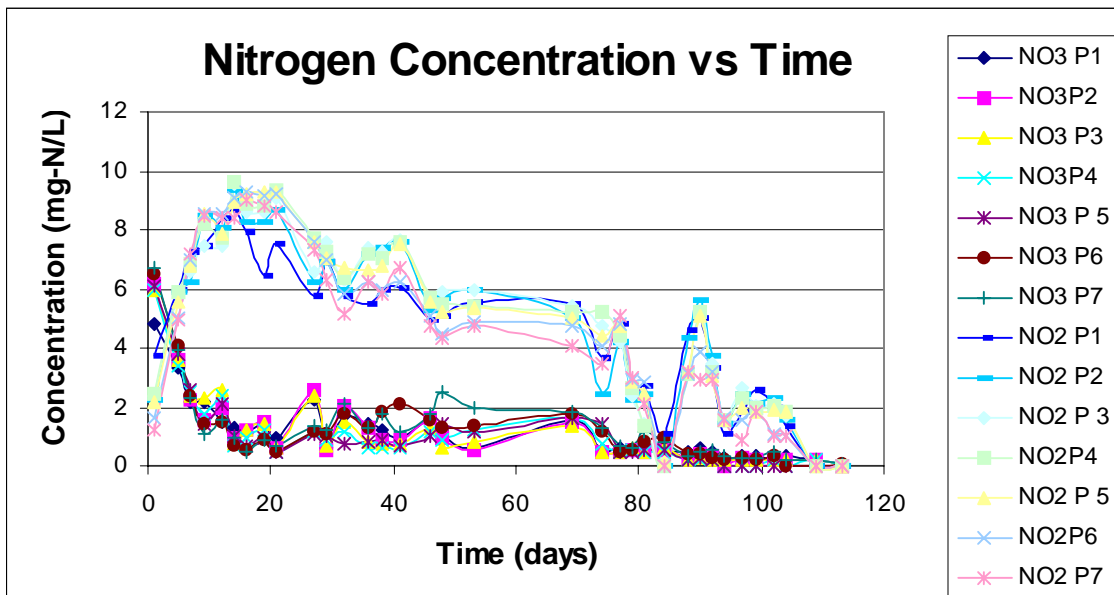


Figure3 Dissolved nitrate and nitrite concentrations observed downstream of the membranes as a function of operating time in days. P1 is closest to the membrane and P7 is the greatest distance downgradient of the gas injection point.

Basic Information

Title:	Evaluation of Bank Erosion Inputs to the Blue Earth River with Airborne Laser Scanner
Project Number:	B-05
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Climate and Hydrologic Processes
Focus Category:	Water Quality, Agriculture, Geomorphological Processes
Descriptors:	streambank erosion, remote sensing, sediment transport, agricultural drainage
Lead Institute:	University of Minnesota
Principal Investigators:	Satish Chander Gupta, Marvin E Bauer

Publication

Project No.: B-05

Title: Evaluation of Bank Erosion Inputs to the Blue Earth River with Airborne Laser Scanner

Investigators: D.P. Thoma, S.C. Gupta, Department of Soil, Water, and Climate, College of Agricultural, Food, and Environmental Sciences; and M.E. Bauer, Department of Forest Resources, College of Natural Resources, University of Minnesota

Period: 3/1/00–2/28/01

Introduction

Nutrients, pesticides and sediment from agricultural activities are the leading surface water pollutants in the U.S. Many of the same issues that link agricultural activities to declines in water quality are common in the eight corn belt states which account for 80% of U.S. agricultural production (Fausey et al. 1995). At the root of the water quality issue are agricultural practices that allow sediment and nutrient transport to surface water bodies. Soil cultivation and drainage are practices necessitated by the climate and soils of the Midwest that make farming possible. Cultivation exposes soil to forces that can dislodge and transport it, and surface and tile drains provide a conduit for transport of sediment and soluble nutrients to surface waters. The interactions between cultivation of 55.7 million hectares and drainage on 20.6 million hectares has already affected the hydrologic regimes of many rivers with unintended consequences on channel erosion.

One such river in the upper mid-west is the Minnesota River, a tributary of the Mississippi River. The Minnesota River flows through a relatively flat agricultural landscape, but is fed by tributaries that are incised with steep and unstable stream banks. According to one estimate, Minnesota River carries from 0.2 to over 2 million tons per year of suspended sediment at Mankato, MN. It is presently not known what proportion of this load is from surface runoff versus stream bank collapse. Gupta and Singh (1996) estimated the bank erosion contributions at 48-55% of the total sediment load in the Minnesota River at Mankato for the 1990-1992 water years. Another report estimated that 25% of the sediment in the Minnesota River were from non-agricultural sources. (<http://www.soils.agri.umn.edu/research/mn-river/doc/watqual.html#nine>). About 55% of the suspended sediment load in the Minnesota River at Mankato is from the Blue Earth River. Bauer (1998) estimated that 36 to 84% of the sediment in the Blue Earth River was from bank erosion. However, these estimates were based on ground measurements of a few banks. Not only are these measurements labor intensive but also the extrapolation procedures are full of assumptions and errors. Procedures are needed that can fully characterize the bank erosion along the complete length of the river.

The Minnesota Pollution Control Agency (MPCA) has stated that a 40% reduction in sediment load at the edge of the Minnesota River Watershed is required to meet federal water quality standards in the lower Minnesota River. To achieve this goal MPCA recommends a 40% reduction in sediment transport to the river to meet this requirement. Thus far, the implicated

sources of these pollutants are agricultural practices on lands in the Minnesota river basin that promote delivery of sediments and nutrients to the river (Randall et al. 1996). Land management activities such as tillage and cropping practice directly affect soil movement, hence agricultural practices that promote keeping soil in place are the focus of current best management practices (BMP) (Randall et al. 1996; Gupta and Singh, 1996). The MPCA assumption that 40% reduction in upland delivery of sediment to the river will reduce the sediment transport to the edge of the watershed by 40% implies most of the sediment in the river has its source in the uplands. This argument has not been verified. In fact, three non-point source pollution models used to estimate the effect of different management practices on Minnesota River water quality have revealed important gaps in our understanding of processes that are acting to control sediment delivery to the river (Gupta and Singh, 1996) .

In order to implement effective management practices to address pollution sources there must be a clear understanding of which mechanisms are the largest contributors. If monitoring showed that for instance that large quantities of sediment were being contributed from stream bank collapse some effort could be re-directed to focus more attention on hydrologic processes both in-stream and via drainage networks that influence channel morphology.

This research will determine the potential of a laser scanning altimeter to provide comprehensive stream reach mass wasting estimates. Operationally, this technology would be used by resource managers at federal, state and local levels to determine allocation of resources to projects with the greatest potential for pollution abatement. Additionally, isolating stream bank inputs and upland contributions by difference with total sediment load will help determine effectiveness of upland BMP soil erosion control efforts. Estimates of stream bank erosion are of special interest to NRCS, MPCA, MDA, BWSR, and Joint Powers Boards which all have roles in improving water quality of the Minnesota River.

The goal of this research is to determine how accurately mass failure rates on the Blue Earth River can be measured with airborne scanning laser altimetry. Airborne laser altimetry measurement for stream bank mass failure rates have not been attempted anywhere, yet it has great potential for government agency and commercial application in erosion and water quality management. Collecting airborne laser altimetry data is an expensive investment, but the initial cost can be justified when considering the cumulative resources already spent addressing sediment pollution, even without a good understanding of pollutant source and mechanisms of delivery.

The objective of this research was to use an helicopter mounted scanning laser to construct a high resolution digital elevation model of the Blue Earth river valley along its length. For calculating mass wasting rates, the procedure will involve making two scans of the river valley at two different times. The first scan will serve as a baseline to which scans taken a year or more later will be compared. Changes in the 3-D topography measured by the laser scans will indicate the location and quantity of material that eroded into the river due to bank erosion.

Theory:

General description of the scanning laser

The scanning laser is flown in a small aircraft at low altitude (300-400m) with a high precision inertial navigation system (INS). The distance between the aircraft and the land surface

is determined as a function of the time it takes a laser pulse to be transmitted to the land surface reflect and return to the sensor. Thousands of pulses per second provide high spatial resolution coverage along a flight line even at air speeds greater than 100 m/s. The lateral positions of laser pulses are georeferenced using GPS and the INS, while the elevation of the measured surface above the ellipsoid is determined as the difference between aircraft elevation above the ellipsoid and distance between the aircraft and the ground (Krabill and Martin, 1987). Laser radiation is considered eye-safe at distances greater than 30 m (Krabill et al., 1984).

The base line elevation accuracy is determined by comparing elevations of a scanned stable surface such as an airport runway to elevations carefully surveyed by conventional or GPS survey methods. Accuracy of elevation measurements during the flights is calculated by a surface elevation differencing technique which determines error in elevation measurements for the same geographic location scanned on intersecting flight lines collected on the same day. This is accomplished by taking the difference in elevation of each point in one file from elevations of points within a fixed buffer in the other file. Both mean difference and root mean square error can then be calculated for the two data sets which serve as the standard for internal validity check (Krabill et al. 1995). This technique is only reasonable for flat homogenous surfaces. The range finding capability, or vertical error of scanning lasers are often as good as 10 cm (Abdalati and Krabill, 1999), but can range up to 20 cm (Krabill et al. 1995). Alignment of annual flight paths for repeat coverage is required for estimates of volume change due to stream bank slumping.

Data georeferencing

In addition to maintaining flight path alignment scanner data is georeferenced by flying over fixed land surface features such as highway bridges that serve as ground control points. These features which are identifiable in the scanner data and have known geographic coordinates serve as match points for co-registering data collected on different dates. A minimum of two fixed features are included in each flight path to ensure appropriate registration accuracy throughout the full length of the flight. The equations for precise georeferencing of laser data using GPS and INS have been described by Vaughn et al. (1996).

Internal data registration is insured by flying stream reaches in the upstream direction, then downstream to obtain data overlap that can be used for internal validity check. Systematic elevation error for a single flight line can be as high as 7 cm, but this error can be significantly reduced by combining laser pulse footprints from independent flight lines over the stream reach collected on the same day. Systematic error in the sensor position recorded by the INS is an independent variable and can be reduced by combining multiple data from multiple passes. On the same day elevations of a single location will not change, thus allowing accuracy assessment of successive scans. Subtle differences in elevation on rough stream bank surfaces on the order of a few centimeters are expected due to random scatter and system noise.

As the aircraft moves along a predetermined flight line up to 7000 pulses per second are directed by a rotating mirror to the ground in a circular pattern centered on the flight line. Up to five echos from each laser pulse are received by the sensor. Typically the first returned pulse is the top of vegetation canopy while the last is usually the ground. In situations where the last echo return is not the ground, filtering must be employed to remove these elevation data if interest is purely in the bare earth elevation (Ritchie 1994). Resulting data resolution depend on aircraft

elevation and speed as well as laser pulse rate, scan width and scan rate. In a recent study of ice sheets in Greenland a flight altitude of 400m and laser pulse rate of 3000 sec⁻¹ yielded approximately one elevation measurement per 6 m². Data were resampled and interpolated for statistical analysis to evenly spaced 1*1m grid cells (Abdalati and Krabill 1999). Working in deciduous forests can decrease the sample density due to interference from vegetation. However, sampling density can be optimized by collecting data with a slow flying helicopter in the fall or winter during leaf-off conditions.

Methods:

Study area

This study was conducted on the Blue Earth River, a tributary of the Minnesota River. Bauer (1998) identified 136 eroding stream bank sites along 156.9 km the Blue Earth River between Mankato and Blue Earth, MN. The range in area of eroding stream banks was 102 to 18364 m². This stretch of the Blue Earth River has steep (up to 90°) and unstable banks as high as 30m (Fig. 1). Bauer classified banks into minor, moderate and severely eroding. We focussed on river reaches between the confluence of the Blue Earth and Wantonwan rivers and Amboy (~56km river length) which contain 10 minor sites, 30 moderate and 15 severely eroded sites greater than 3m high. Figure 2 shows an example of the bank sloughing along the Blue Earth River. There are 3 county road bridges that will be used as GCP's in this stretch of the river.

Field work

In February 2001, 35 miles of the river between Rapidan Dam and Vernon Center were mapped with a March III GPS to 5m accuracy (Fig. 3a) and stream banks on either side of the river were photographed and categorized qualitatively by size, erosion activity, and vegetation coverage. The start and end points of all large banks were recorded. This data set will be used to aid interpretation of the laser scan and to independently compute bank lengths.

Two stream banks were surveyed with a total station during February 2001 at better than 1cm accuracy (Fig. 3b). These surveys will be used as reference standards to determine absolute accuracy of the airborne scanning laser system. On April 28 and 29, 2001, 8 locations representing dominant vegetation cover types were surveyed for vegetation canopy density using a Geographic Resource Systems densitometer (Fig. 3c).

On April 24 and 25, 2001 Aerotec Inc. completed a 35 mile scan of the river corridor (between the confluence of the Watonwan and Blue Earth rivers and Amboy, MN) using the Saab Topeye laser range finding system (Figs. 4 & 5). The scan specifications were:

flight platform - Eurocopter Astar 350 series helicopter
flight altitude - 375 m
flight speed - < 25 m/s
distance between foot prints - 0.305 m
laser foot print diameter - 0.116 m
laser pulse rate - 7000 Hz
number returned echos - 5

scan width - 273 m
scan angle - 20 degrees
mirror frequency 12.5 Hz
ground reference station - Trimble 4000 SSi dual frequency receiver

Results:

The data has been filtered to remove presence of vegetation resulting in a bare earth model. The filtered data is currently being used to grid the river valley and then draw the contour. Figure 6 shows an example of the two scanned banks along the meandering Blue Earth River. Usually, there will not be any reflection of the laser pulses from the water body because of its tendency to adsorb infrared light. In Figure 6, there was some reflection of the laser pulses because of the presence of sediment in the river water. Plans are underway to compare the elevation measurements from the laser scan against the total station derived elevations. We are also in the process of undertaking a 3-D animation of the river valley using the facilities of the Minnesota Supercomputing Institute.

Depending upon the availability of funds, a second scan will be made in another year or so. The two 3-D images will be compared to calculate the volume change. The volume change will be converted to mass wasting rates using the bulk density values measured on bank sediments along the length of the Blue Earth River.

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Fig 1: A view of the bank sloughing along the Blue Earth River. Hanging fence showing the recent failure in the top portion of the bank. According to owner of this field, the banks have receded about 30 feet in his life time (60 years) giving bank erosion rate of 0.5 ft/year.

GPS survey



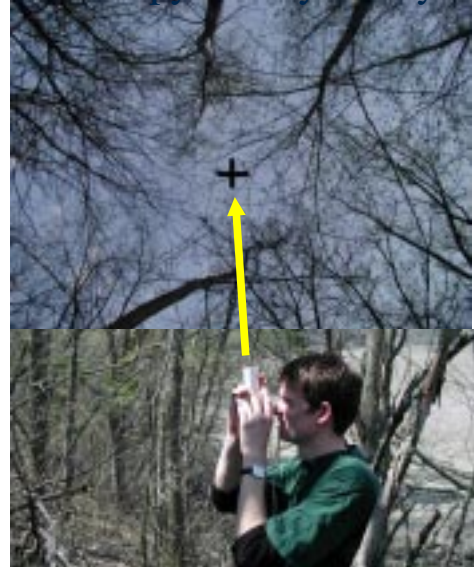
25 river miles

Total station survey



Two sites

Canopy density survey



Eight sites

Fig
. 2:
Tw
o

views of a bank along the Blue Earth River showing the occurrence of seasonal erosion. The light colored area in the lower right of April 2001 image is where some sloughing has occurred since February 2001.

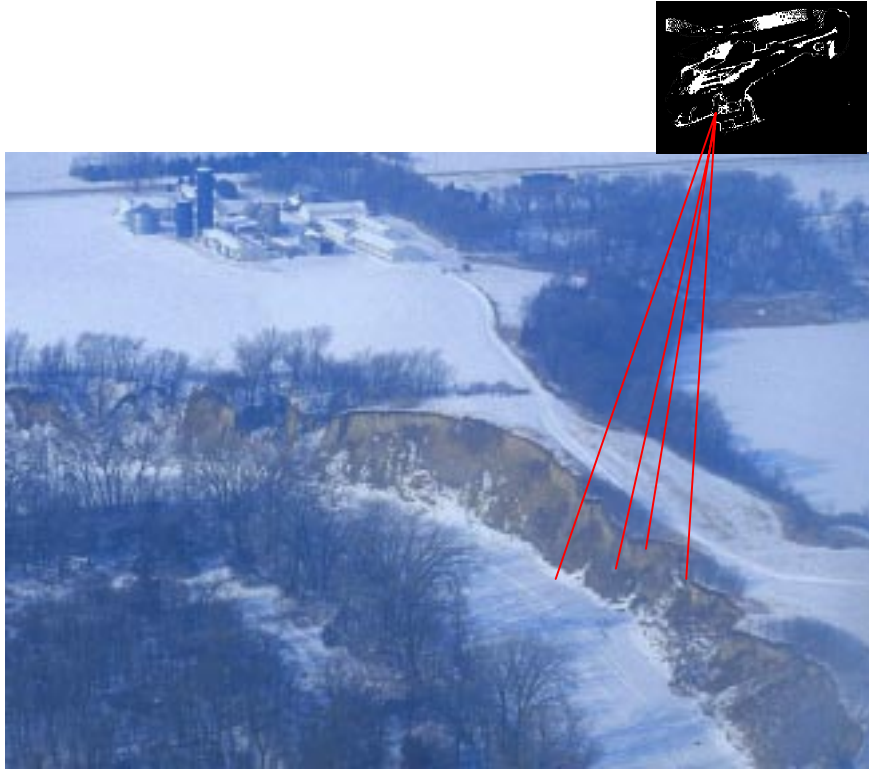
February 2001

April 2001



Arrows indicate the same tree in photographs

Fig 3: GPS, canopy characterization of the Blue Earth River. This was used to verify the laser data.



elevation, and cover of the River Banks. information interpret and accuracy of the

Fig. 4: Components of the helicopter-based laser system used to scan the Blue Earth River.

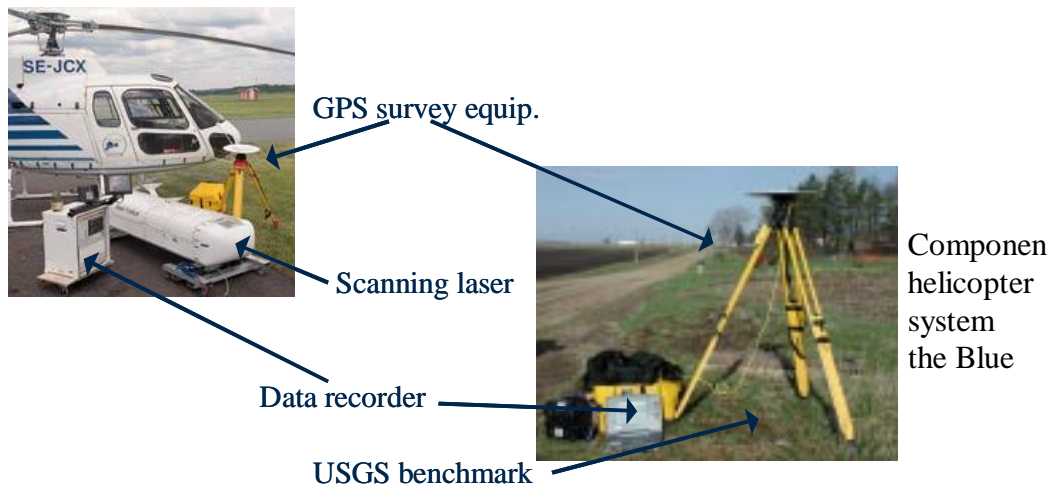


Fig. 5: An aerial view of an eroding bank along the Blue Earth River.

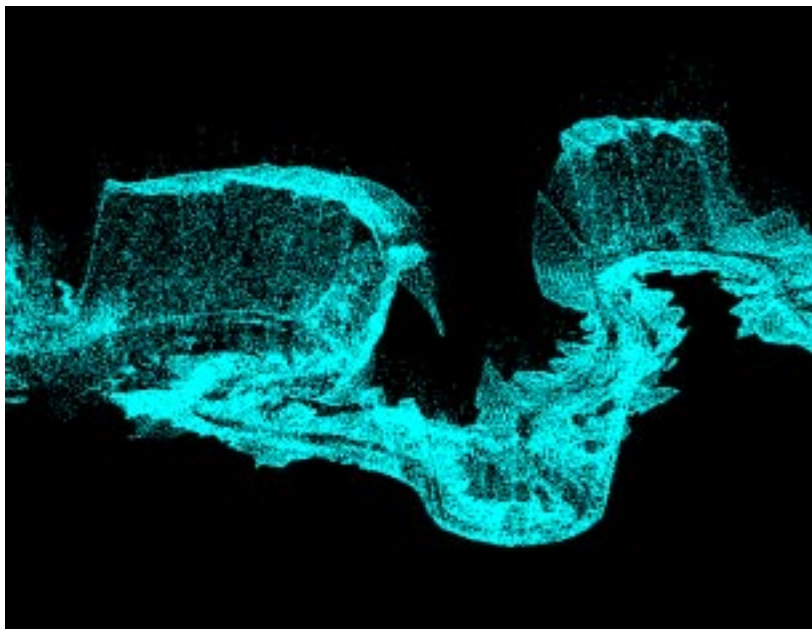


Fig 6:
Scanned view of
two banks along the
Blue Earth River.

Basic Information

Title:	Investigation of the Abiotic Reduction of the Herbicides Trifluralin and Pendimethalin
Project Number:	B-07
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Water Quality
Focus Category:	Toxic Substances, Geochemical Processes, None
Descriptors:	herbicides, pesticides, trifluralin, pendamethalin, abiotic degradation, fate and transport,
Lead Institute:	University of Minnesota
Principal Investigators:	William Alan Arnold

Publication

1. Manuscript to be submitted to either Environmental Science and Technology or Water Research will be prepared during the summer of 2001.
2. Wang, S.; W.A. Arnold, 2000. Reduction of nitroaromatic herbicides. Poster presented at the Midwest Environmental Chemistry Workshop, Kalamazoo, MI, October 7-8, 2000.
3. W.A. Arnold; S. Wang 2001. Abiotic reduction of dinitroaniline herbicides. Paper to be presented at the 33rd Central/Great Lakes Joint Regional American Chemical Society Meeting, Grand Rapids, MI, June 11-13, 2001.

Project No.: B-07

Project Title: Report for “Investigation of the Abiotic Reduction of the Herbicides Trifluralin and Pendimethalin”

Investigators: William A. Arnold, Department of Civil Engineering, Institute of Technology, University of Minnesota

Period: 3/1/00–2/28/01

Introduction

The widespread use of dinitroaniline herbicides, such as trifluralin and pendimethalin, throughout the Midwestern United States has led to their detection as contaminants in groundwater, surface water, air, and precipitation (1-3). According to previous works, reduced inorganic forms of iron and sulfur such as Fe(II) and HS-/H₂S are important reductants under typical groundwater conditions (4-8). Although reduction rates of nitroaromatic compounds by aqueous Fe(II) and HS-/H₂S may be slow, transformation rates of nitroaromatic compounds are dramatically increased when sulfide is in the presence of natural organic matter (NOM) or when Fe(II) is adsorbed onto the surface of goethite (α -FeOOH). The primary objective of this study was to investigate the importance of abiotic reduction as a loss process for a suite of widely used dinitroaniline herbicides (structure shown in Figure 1). It was also desired to develop linear free energy relationships (LFERs) that could be used to predict the transformation rates of untested dinitroaniline herbicides.

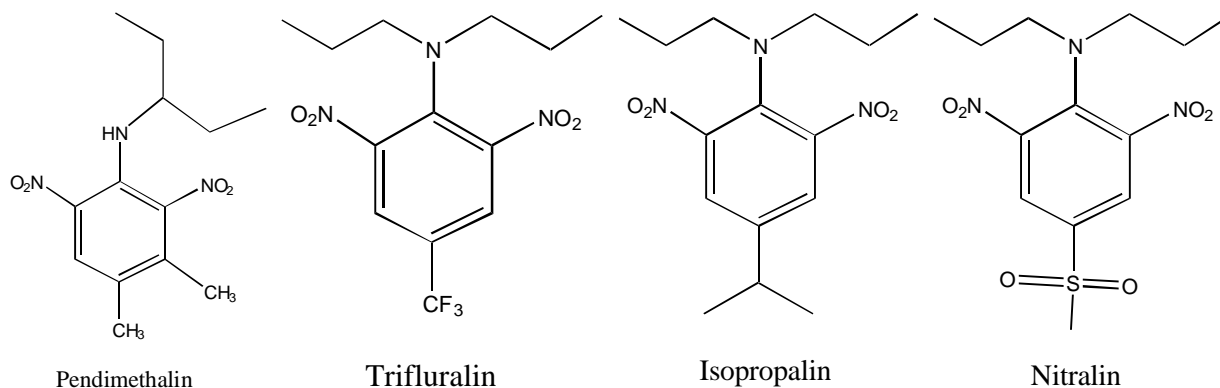


Figure 1. Structures of the dinitroaniline herbicides used in the study.

Methods and Procedures

Experiments were conducted in 164-mL serum flasks sealed with Teflon faced-butyl rubber stoppers. All experiments were prepared in an anaerobic glovebag, and all solutions were purged with argon to remove oxygen prior to use. For the experiments using sulfide, Na₂S and HCl stock solutions were prepared using deoxygenated, deionized water. A stock solution of juglone (an NOM model compound) was prepared in oxygen free methanol. Aliquots of Na₂S, HCl, and juglone stock solutions were added to the serum flasks containing a 50 mM phosphate buffer. For experiments with Fe(II) and goethite, a 3-morpholinopropanesulfonic acid (MOPS) deoxygenated buffer solution was added to the serum flasks. A 1.0 M Fe(II) stock solution was

prepared by filtering 10 mL of a solution of 1.1 M $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ through a 0.02 μm pore diameter GTTP filter directly into 1 mL of 1M HCl. This stock was added to the serum bottle to obtain the desired Fe(II) concentration. All reactors were allowed to equilibrate for at least one day before the initiation of kinetic experiments. The bottles containing goethite were equipped with a Teflon-coated magnetic stir bar and mixed on a magnetic stirrer. The kinetic experiments were initiated by adding 50 μL of 0.6 g/L methanolic stock solution of the desired herbicide to the reactor. Samples were taken at appropriate time intervals by injecting 1 mL of deoxygenated buffer into the serum flasks, and simultaneously withdrawing an equal volume of aqueous sample. Aqueous samples were extracted with hexane (1:1) on a vortex mixer for 1 minute. The extracts were analyzed by GC-ECD.

Results and Discussion

Herbicides reduction by HS/juglone

The rate of disappearance of the dinitroaniline herbicides in a given system could be described by a pseudo-first-order rate law:

$$\text{rate} = -\frac{dC}{dt} = k_{obs} C \quad (1)$$

where k_{obs} is the observed pseudo-first-order rate constant under the system conditions, and C is the aqueous concentration.

Figure 2 reveals that k_{obs} increases linearly with increasing juglone concentration. In the absence of juglone, the reduction rate is quite slow. This result indicates the dinitroaniline herbicides are susceptible to reduction by HS/juglone, and juglone acted as an effective electron carrier in this system.

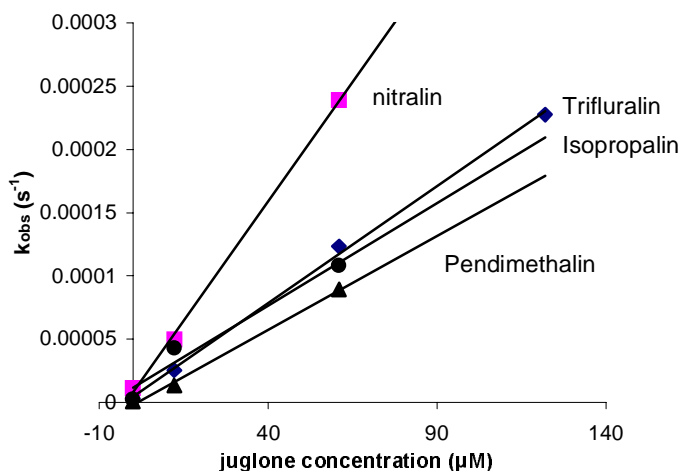


Figure 2. Observed pseudo-first-order rate constants versus juglone concentration the dinitroaniline herbicides. Experiments were performed at pH 6.60 with 4.57 mM total sulfide.

Development of an LFER to predict the rates of dinitroaniline herbicide reduction

The one-electron reduction potential, E_h^1 (shown in eq. 2), of a given (poly)nitroaromatic compound ((P)NAC) is an appropriate parameter for relating and evaluating reduction rates of a series of (P)NACs (7-8). The selected herbicides have a mixture of electron withdrawing ($-\text{SO}_2\text{CH}_3$, $-\text{CF}_3$) and electron donating groups ($-\text{CH}_3$, isopropyl), as a result, their reaction rates are amenable to the LFER analysis.



Because the E_h^1 values of the four dinitroaniline herbicides are not available in the literature, we performed experiments using three NACs (with known E_h^1 values, ref. 8) in the sulfide-cured

juglone system and determined their second-order rate constants with equation (4). The values obtained are given in Table 1. Using these values, an LFER relating $\log k_{HJUG}$ to $E_h^{1'}$ was developed (equation 5).

$$k_{obs}^{juglone} = k_{obs}^{HS^-/H_2S} + k_{HJUG} [HJUG] \quad (4)$$

$$\log k_{HJUG^-} = 0.94 \frac{E_h^{1'}}{0.059V} + 6.85 \quad r^2 = 0.94 \quad (5)$$

Table 1. One-electron reduction potentials ($E_h^{1'}$) and reaction rate constants (k_{HJUG}) of the nitroaromatic herbicides and other NACs.

<i>Compound</i>	$E_h^{1'} (mV)$	$k_{HJUG} (M^{-1}s^{-1})$
Nitrobenzene (NB)	-486 ^a	7.39×10^{-2}
4-chloronitrobenzene (4-Cl-NB)	-450 ^a	9.16×10^{-1}
4-acetylnitrobenzene (4-AC-NB)	-358 ^a	1.11×10^1
Nitralin	-399 ^b	3.44×10^{-0}
Trifluralin	-413 ^b	1.97×10^{-0}
Isopropalin	-417 ^b	1.71×10^{-0}
Pendimethalin	-425 ^b	1.25×10^{-0}

^aValues obtained from refs. 5 and 8.

^bValues calculated using equation (5) and experimentally determined values of k_{HJUG} .

The slope and intercept in equation (5) are consistent with those of other researchers (8, 11). By using equation (5), the $E_h^{1'}$ values of the four dinitroaniline herbicides can be estimated. The resulting LFER is shown in Figure 3. These $E_h^{1'}$ values allow the prediction of the reaction rates of these dinitroaniline herbicides under other sets of conditions.

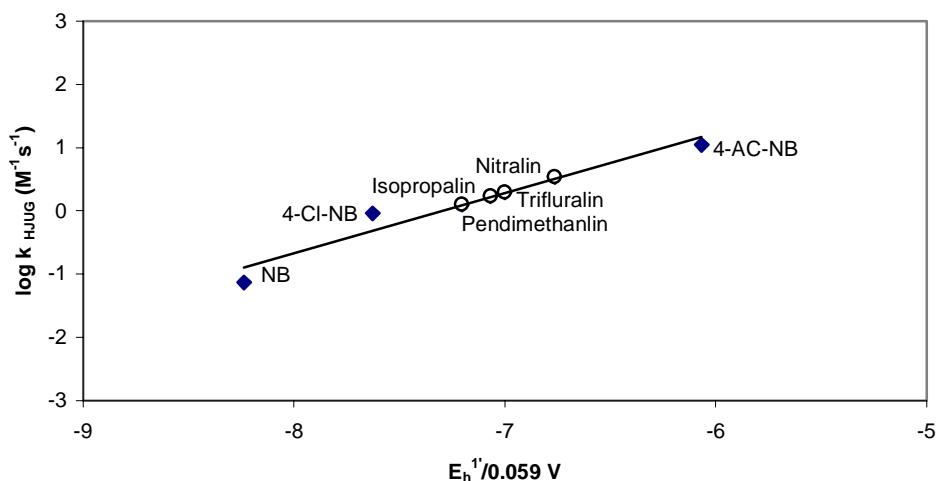


Figure 3. Plot of the second-order rate constants k_{HJUG} of various (P)NACs versus the $E_h^{1'}$ of the compounds. All k_{HJUG} values are measured under the following conditions: pH 6.6 phosphate buffer, 4.57 mM H_2S , and varying juglone concentrations (0-100 μM).

Reduction of dinitroaniline herbicides by Fe(II)/goethite

The kinetics of dinitroaniline herbicide reduction were also studied in the Fe(II)/goethite system. The results indicate that these dinitroaniline herbicides are susceptible to rapid reduction by reduced iron species adsorbed to minerals. Figure 4 shows the observed pseudo-first-order rate constants can also be correlated with the $E_h^{1'}$ values, which allows an LFER for the dinitroaniline herbicides in the Fe(II)/goethite model system to be established.

The dinitroaniline herbicides were not degraded in the presence of goethite alone. At the pH studied (7.2), reduction by aqueous iron (Fe^{2+}) was slow, but the rate markedly increased

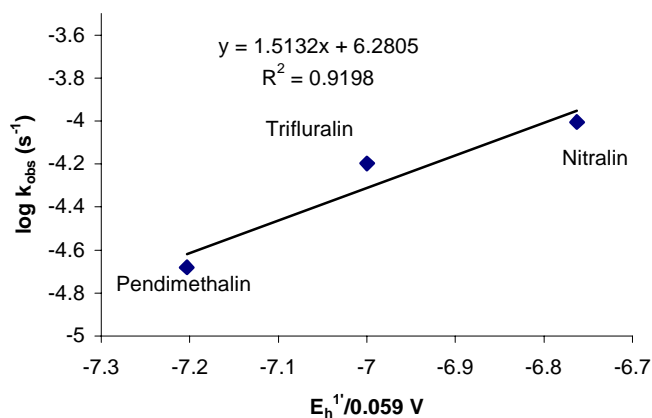


Figure 4. Correlation of k_{obs} of three herbicides (nitralin, pendimethanlin, trifluralin) with the $E_h^{1'}$ values calculated from $\text{HS}^-/\text{juglone}$ experiments

with increasing pH. Further studies are underway to determine the role of pH in reductions mediated by both aqueous and mineral-bound iron.

Conclusions

This study demonstrated that sulfur adducts of NOM and Fe(II) adsorbed onto mineral surfaces will play an important role in the abiotic reductive transformations of dinitroaniline herbicides under the appropriate redox conditions. The rapid kinetics of these reactions are consistent with the infrequent detections and low concentrations of these herbicides in groundwater. Linear free energy relationships were successfully established that could be used to predict the rates of dinitroaniline herbicide reduction by sulfur adducts of juglone and Fe(II) adsorbed onto a mineral surface. Future work focus on product identification. The potential nitroso-, hydroxylamine-, and amino-substituted products of dinitroaniline herbicide reduction are also be of environmental concern.

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Publications

Manuscript to be submitted to either *Environmental Science and Technology* or *Water Research* will be prepared during the summer of 2001.

Presentations

Wang, S.; W.A. Arnold, 2000. Reduction of nitroaromatic herbicides. Poster presented at the Midwest Environmental Chemistry Workshop, Kalamazoo, MI, October 7-8, 2000.

W.A. Arnold; S. Wang 2001. Abiotic reduction of dinitroaniline herbicides. Paper to be presented at the 33rd Central/Great Lakes Joint Regional American Chemical Society Meeting, Grand Rapids, MI, June 11-13, 2001.

Students Supported

Song Wang, M.S. candidate in Civil Engineering.

Basic Information

Title:	Feasibility of Controlled Drainage for Mitigating Nutrient Loss from Tile Drainage Systems in South Central Minnesota
Project Number:	B-03
Start Date:	3/1/1999
End Date:	2/28/2001
Research Category:	Climate and Hydrologic Processes
Focus Category:	Agriculture, Water Quality, Groundwater
Descriptors:	agricultural drainage, tile drainage, best management practices, water table management,
Lead Institute:	University of Minnesota
Principal Investigators:	Gary R Sands, Steven J Taff, David J Mulla, Lowell M Busman

Publication

1. Feyereisen, G, and G. R. Sands. 2000. Water Quality Impacts of Various Watertable Management Scenarios in Minnesota: A Simulation Study. Presented at the 2000 ASAE International Meeting, Paper No. 002118. ASAE, 2950 Niles Road, St. Joseph, MI 49085-9659 USA.
2. Feyereisen, G, and G. R. Sands. 2000. Investigating the Agronomic and Environmental Impacts of Controlled Drainage for Southern Minnesota: Methodology and Preliminary Modeling Results. Presented at the Minnesota Water 2000 Conference.

Project No.: B-03

Project Title: Feasibility of Controlled Drainage for Mitigating Nutrient Loss From Tile Drainage Systems in South Central Minnesota

Investigator: Gary R. Sands, Department of Biosystems and Agricultural Engineering, College of Agricultural, Food, and Environmental Sciences and Institute of Technology, University of Minnesota

Period: 3/1/99–2/28/01

This report describes progress achieved from 7/1/00–6/30/01. The goal of this project is to determine the feasibility of controlled drainage for maintaining or increasing crop yields while reducing nutrient losses from subsurface (tile) drainage systems.

Installation of field instrumentation was completed and data collection began in the fall of 2000 with water table and crop yield measurements. A new flow measurement system was developed for the conventionally drained plots incorporating a flume and sump pump combination. Water quality and flow measurements began in the spring, 2001. The instrumentation systems appear to be working well.

A Biosystems and Agricultural Engineering Masters student is performing a computer simulation study that seeks to devise "optimum" water table management strategies to minimize loss of nitrates from the field, while maintaining, or improving crop yield. The computer drainage model, DRAINMOD, is being used for the simulation study. The strategies recommended by the simulation study will be those implemented in the field.

Publications/Presentations during reporting period

Feyereisen, G, and G. R. Sands. 2000. Water Quality Impacts of Various Watertable Management Scenarios in Minnesota: A Simulation Study. Presented at the 2000 ASAE International Meeting, Paper No. 002118. ASAE, 2950 Niles Road, St. Joseph, MI 49085-9659 USA.

Feyereisen, G, and G. R. Sands. 2000. Investigating the Agronomic and Environmental Impacts of Controlled Drainage for Southern Minnesota: Methodology and Preliminary Modeling Results. Presented at the Minnesota Water 2000 Conference.

Basic Information

Title:	Assessing the Effects of Endocrine Disrupters (EDCs) from a St. Paul Sewage Treatment Plant on Sperm Viability and Testicular Development in Fish: Adding a New Dimension to an Existing Project
Project Number:	B-04
Start Date:	3/1/1999
End Date:	2/28/2001
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, None
Descriptors:	endocrine disrupters, fish, wastewater effluent
Lead Institute:	University of Minnesota
Principal Investigators:	Peter W Sorensen, Heiko Schoenfuss, Ira R Adelman, Deborah L Swackhamer

Publication

1. Assessing the Effects of Endocrine Disrupters Found in Sewage Treatment Plant Effluent on the Fertility of Male Fish. Minnesota Water 2000. Heiko L. Schoenfuss et al. April 25, 2000, Minneapolis.
2. Effects of Environmental Estrogens on Fish in the Vicinity of the Twin City's Waste Water Treatment Plant: A Project Overview. Emerging Issues Conference. Ira R. Adelman et al. June 8, 2000, Minneapolis.
3. Assessing the Effects of Endocrine Disrupters Found in Sewage Treatment Plant Effluent on the Fertility of Male Fish. Emerging Issues Conference. Heiko L. Schoenfuss et al. June 8, 2000, Minneapolis
4. Cause and effect of vitellogenin induction in fish downstream of a sewage treatment plant. Heiko L. Schoenfuss, Deborah L. Swackhamer, Peter W. Sorensen, and Ira R. Adelman. Annual Meeting of the Society of Environmental Toxicology and Chemistry. November 12-16, 2000, Nashville, TN.
5. Variable Effects of Municipal Sewage Plant Discharge on Reproductive Health of Male Goldfish. Heiko L. Schoenfuss, James T. Levitt, Glen Van Der Kraak and Peter W. Sorensen. Manuscript in preparation for publication in Environmental Toxicology and Chemistry.

Project No.: B-04

Project Title: **Assessing the Effects of Endocrine Disrupters (EDCs) from a St. Paul Sewage Treatment Plant on Sperm Viability and Testicular Development in Fish: Adding a New Dimension to an Existing Project**

Investigators: **Peter W. Sorensen, Ph.D., Heiko L. Schoenfuss, Ph.D., Ira R. Adelman, Ph.D., Department of Fisheries and Wildlife, College of Natural Resources; Deborah Swackhamer, Ph.D., Division of Environmental and Occupational Health, School of Public Health, University of Minnesota**

Period: 3/1/99–2/28/01

Research

Introduction: The objective of this study is to determine whether fish exposed to compounds found in the effluent of the St. Paul (Minnesota) Sewage Treatment Plant (STP) experience sex reversal and suffer from reduced sperm viability as a result of exposure to endocrine disrupting compounds (EDCs). This study was part of a larger effort to identify the effects of endocrine disrupters on the reproductive health of fish populations. EDCs are man-made or naturally occurring compounds that are found in the environment and disrupt hormonal pathways causing harm to the exposed organisms or their offspring. Previous studies in the United Kingdom have demonstrated that STP effluent is resulting in feminized male fish with abnormal reproductive organs. One defining characteristic of male fish exposed to STP effluent in the UK, as well as wild carp and walleye captured below the St. Paul STP (our study site), is that they contain high concentrations of vitellogenin (VTG: female egg-yolk protein) in their blood. However, no study has examined whether there might be a correlation between endocrine disruption, as indicated by the presence of VTG in male fish caught in STP, and adverse effects on the fertility of these fish. Our study seeks to examine this by examining the effects of exposing male goldfish to STP effluent under laboratory conditions. We have been assessing fertility by examining milt (sperm and seminal fluids) volume and sperm motility (a function of swimming speed). Sperm motility is an especially important parameter to examine in fish for most are external fertilizers. Goldfish were utilized for this study as they have been studied extensively and constitute the best understood endocrine vertebrate model. An apparatus for goldfish exposure to EDCs and sewage effluent was built and a protocol for assessing sperm quality was established. Three experiments were completed and are described below.

Experiment I: This experiment was designed to determine whether exposure to EDCs has adverse effects on milt quantity and sperm motility. Fish were exposed for eight weeks to estradiol or estrone, two known EDCs at relevant concentrations. Fish exposed to 50ng/L estradiol were found to have a milt volume decrease by 76% while total motile sperm count decreased by 84%. 100ng/L estradiol caused milt volume

decrease by 90% and total motile sperm decrease by 95%. Fifty ng/L estrone exposure resulted in a complete loss of milt after eight weeks.

Experiment II and III: These experiments were designed to determine whether exposure to STP effluent results in decreased milt volume and sperm motility. Male goldfish were exposed under laboratory conditions for ten weeks to a well water control, 25_1/1 ethanol carrier control, 50ng/l estradiol, or effluent collected daily from the St. Paul STP. Besides small adjustments of the experimental design, only the time of the year in which effluent was collected varied between the two experiments. Winter effluent (winter experiment) is not chlorinated and might have different properties than summer effluent (Asummer experiment@), which is chlorinated.

Milt was collected at the end of the treatment period and five sperm measures were analyzed for this study: (i) *spermatocrit*: the number of sperm per milliliter milt; (ii) *motile spermatocrit*: the number of motile sperm per milliliter sample; (iii) *curvilinear velocity*: the average speed of sperm along their path; (iv) *straight line velocity*: the average distance covered by the sperm in a sample per second; (v) *lateral head displacement*: the amount of divergence of the sperm head from the sperm path (a indication of sperm symmetry).

Results: Vitellogenin induction was found to be significant ($P < 0.05$, Kruskal-Wallis non-parametric test) in estradiol (0.50.2 mg/ml) and effluent (0.90.3 mg/ml) exposed fish in the winter experiment and estradiol (0.380.076 mg/ml) exposed fish in the summer experiment. In both experiments, vitellogenin induction was not homogeneous across all fish in an affected treatment group and some fish did not experience vitellogenin induction at all.

Gonadosomatic Index: Only estradiol exposed goldfish in the summer experiment experienced a significant decrease in testis size (GSI; $P < 0.05$, one-way ANOVA). No pathological features were noted during examination of the extirpated testis.

Sperm Quality Parameters: No significant differences in sperm quality parameters were noted between any of the treatment groups in either experiment.

Sperm Quantity Parameters: The percentage of fish with expressible milt declined in estradiol exposed fish in both experiments (see Fig. A, below). Fish exposed to winter effluent experienced a significant reduction in motile sperm (Fig. B; $P < 0.05$, Kruskal-Wallis non parametric test).

Conclusions: This study has documented for the first time in a controlled laboratory experiment a significant decrease in the reproductive health of male fish due to the exposure to ecologically relevant concentrations of estradiol. However, vitellogenin induction does not appear to correlate closely with exposure or reproductive health. STP effluent appears to vary in estrogenicity and the effects of effluent exposure on reproductive health of male goldfish appear to be limited to periods of peak estrogenicity. However, minor impairments in the reproductive health of a fish could be amplified by its interactions with un-compromised fish in the wild. Thus, effluent exposure could have serious effects on fish populations that are not evident from examining a small number of individual fish. This study is likely applicable to other STP in the North America as the St. Paul STP is representative of many municipal sewage treatment plants.

2) PUBLICATIONS AND CONFERENCE PROCEEDINGS

Assessing the Effects of Endocrine Disrupters Found in Sewage Treatment Plant Effluent on the Fertility of Male Fish. *Minnesota Water 2000*. Heiko L. Schoenfuss et al. April 25, 2000, Minneapolis.

Effects of Environmental Estrogens on Fish in the Vicinity of the Twin City's Waste Water Treatment Plant: A Project Overview. *Emerging Issues Conference*. Ira R. Adelman et al. June 8, 2000, Minneapolis.

Assessing the Effects of Endocrine Disrupters Found in Sewage Treatment Plant Effluent on the Fertility of Male Fish@. *Emerging Issues Conference*. Heiko L. Schoenfuss et al. June 8, 2000, Minneapolis.

Cause and effect of vitellogenin induction in fish downstream of a sewage treatment plant. Heiko L. Schoenfuss, Deborah L. Swackhamer, Peter W. Sorensen, and Ira R. Adelman. Annual Meeting of the *Society of Environmental Toxicology and Chemistry*. November 12-16, 2000, Nashville, TN.

Variable Effects of Municipal Sewage Plant Discharge on Reproductive Health of Male Goldfish. Heiko L. Schoenfuss, James T. Levitt, Glen Van Der Kraak and Peter W. Sorensen. Manuscript in preparation for publication in *Environmental Toxicology and Chemistry*.

Student Support

Dr. Heiko L. Schoenfuss, a postdoctoral research associate in the Department of Fisheries & Wildlife, University of Minnesota, was supported 3/1/99 through 2/29/00 with 33% salary and 7% fringe benefits through this grant.

Khuong Vuong, an undergraduate student in Biochemistry, University of Minnesota, was given a research opportunity through funding provided by this grant. He was able to purchase laboratory supplies to study the breakdown of estradiol in exposure aquaria used in this study.

Mira Rivera, an exchange student from Puerto Rico in the Department of Fisheries & Wildlife, U of MN, was given the opportunity to develop a reproductive assay to assess the reproductive success of endocrine disrupted fish.

Achievements and Awards

Khuong Vuong was awarded third place for his oral presentation at the Minority Scholars Development Program. His presentation was titled: AA Monitor on Estradiol Concentrations in Experimental Tanks@. August 1999, Minneapolis.

Basic Information

Title:	Investigation of Factors Affecting the Removal of Cryptosporidium parvum Oocysts in Porous Media Filters
Project Number:	B-01
Start Date:	3/1/1999
End Date:	2/28/2001
Research Category:	Engineering
Focus Category:	Treatment, Water Quality, None
Descriptors:	water treatment, Giardia, cryptosporidium, filtration
Lead Institute:	University of Minnesota
Principal Investigators:	Raymond M Hozalski

Publication

Project No.: B-01

Project Title: Investigation of Factors Affecting the Removal of *Cryptosporidium parvum* Oocysts in Porous Media Filters

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Period: 3/1/99–2/28/01

Executive Summary

Cryptosporidium parvum is a protozoan pathogen that can cause gastroenteritis in humans and possibly lead to the death of immunocompromised individuals.

Cryptosporidium enters water supplies as the hardy oocyst form by input of feces from infected animals or humans. Because chlorine is ineffective at inactivating *Cryptosporidium* oocysts, properly designed and operated filters are essential for physically removing the oocysts from the water supply. The condition of the source water can influence the surface characteristics of the filter media, which, consequently, can influence the deposition of the oocysts during the filtration process. The purpose of this research project was to evaluate the impact of typical filter media surface changes on oocyst removal, including biofilm coating and natural organic matter (NOM) coating.

Bench-scale filtration experiments were performed with clean glass beads (0.55 mm), biofilm coated glass beads and NOM coated glass beads. The filters were operated at a loading rate of 5 m/h and 0.01 M CaCl₂ was added as “coagulant” in a direct filtration mode. Samples were collected from the filter effluent and the oocysts were enumerated by staining with the immunofluorescent antibody (IFA) assay, collection onto 0.2 µm pore size polycarbonate filters, and direct counting by epifluorescence microscopy at 400X magnification. In addition, the zeta potential (a measure of surface charge) of oocysts with and without NOM coating over a range of ionic strength was measured at pH 6.7 using a Laser Zee Meter (Model 501). The hydrophobicity of the oocysts was also evaluated by measuring oocyst partitioning between an aqueous phase and an organic hexadecane phase.

Development of a biofilm or NOM coating on the filter media resulted in a significant decrease in oocyst removal efficiency. The removal efficiency decreased from 51% for the clean bed to 23% for the biofilm coated bed. The impact of NOM coating was also evaluated by pre-equilibrating the filter media and oocysts with a 5 ppm (as C) NOM solution prior to filtration of oocysts. The removal efficiency in the presence of NOM was 15%. The oocyst removal for an experiment with a combination of biofilm coating and NOM coating was similar to that for the experiment with NOM coating alone. The zeta potential values of the oocysts pre-equilibrated with NOM were significantly more negative than the values for the “clean” oocysts. This suggests that NOM enhanced electrostatic repulsion between the oocysts and the negatively charged glass filter media in addition to possible steric effects. The NOM coating on the oocysts

also increased oocyst hydrophobicity, which may also have had some effect on deposition.

The results of these experiments suggest that source waters with high organic matter concentrations have a greater potential for oocyst breakthrough. Thus, to ensure that *Cryptosporidium* oocysts are effectively removed from high NOM source waters during filtration, treatment options include enhanced coagulation to remove NOM and destabilize the oocysts, frequent backwashing with disinfectant to limit biofilm buildup, or the use of a two stage filtration system (biofilter followed by a standard filter).

Introduction

Cryptosporidium parvum is a protozoan pathogen that is responsible for the waterborne disease called cryptosporidiosis, which can be life threatening for immunocompromised individuals.^[3] The form of *Cryptosporidium* found in water supplies is the hardy oocyst, and widespread contamination of surface waters with this organism has been reported in the literature.^[2,5,9] The oocyst is one stage in the life cycle of *Cryptosporidium* and is the form that is excreted by the host and enters the environment. Water treatment plants that serve more than 10,000 people and use surface water as their source water will be required to remove 99% of oocysts according to the Interim Enhanced Surface Water Treatment Rule (SWTR).^[15]

Unlike bacteria or viruses, *Cryptosporidium* oocysts are highly resistant to chlorine disinfection.^[8] Because chlorine is the most common disinfectant used in the United States, removal of oocysts is predominantly done by physical processes. In a typical water treatment plant, oocysts, like other particles, are susceptible to removal by the processes of sedimentation and filtration.^[1] Filtration serves as the main treatment barrier to oocysts. For treatment plants that use chlorine as the primary disinfectant, failure of filtration could lead to an outbreak of waterborne cryptosporidiosis. The Milwaukee outbreak in 1993, during which over 400,000 people were infected, was likely caused by insufficient treatment.^[13] The factors causing the treatment breakdown have not been definitively elucidated.

Recently, a great deal of research has been conducted to evaluate biofiltration for drinking water treatment.^[4,7] In biofiltration, biological treatment is coupled with the filtration process, by allowing biofilm to accumulate on the filter media. Biofiltration has received a lot of attention recently, because biofilters can significantly decrease the total organic carbon (TOC) concentration in water^[7], resulting in reduced disinfection by-product (DBP) formation and reduced potential for bacterial regrowth in distribution systems. However, the impact of biofilm growth in filters on particle removal is not well known. Rittmann and Wirtel^[12] reported that biofilm can increase cohesion efficiency of particles. Goldgrabe et al.^[4] reported lower particle removal in biological filters than in non-biological filters. More work is needed to clarify the impact of biofilm on particle removal.

Natural organic matter (NOM) is ubiquitous in water supplies and can adsorb onto solid surfaces immersed in water. Adsorbed NOM can alter the surface characteristics of filter media and suspended particles. For example, NOM can increase the mobility of bacteria in soil^[10]. The impact of NOM on the removal of oocysts in a porous media filter is unknown.

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The objective of this research was to evaluate the effect of NOM and biofilm on the removal of *Cryptosporidium parvum* oocysts in granular media filters. The ultimate goal is to develop recommendations or guidelines for achieving effective removal of protozoa from drinking water supplies.

Material and Methods

2.1 Simulated Bench Scale Filtration System

A schematic diagram of the filtration system used in this research is shown in Figure 1. A photograph of the setup is included as Figure 2. The filter consists of a 2.54 cm (1 inch) ID \times 30.5 cm (12 inch) polycarbonate plastic column, packed with 0.55 mm spherical glass beads (MO-SCI Corporation) to a depth of approximately 25.4 cm (10 inches). The porosity of the bed was 0.40. Peristaltic pumps (Cole-Parmer Instrument Company) were used to pump the oocyst suspension and coagulants into the system. Calcium chloride (CaCl_2 , 0.01 M) served as the coagulant. The hydraulic characteristics of the system, computed from a bromide tracer experiment, are given in Table 1. Polystyrene latex microspheres (Interfacial Dynamics Corp.) were used as model particles in preliminary experiments to evaluate the performance of the experimental setup and accuracy of our methods. All experiments were performed at room temperature (20 – 25°C).

2.2 Establishment of the Biofilm

A pure culture of *Pseudomonas aeruginosa* (American Type Culture Collection, ATCC strain 27853) was used to establish a biofilm on the filter media. The *P. aeruginosa* were maintained on tryptone yeast extract (TYE) agar slants. To prepare a liquid culture, a loop of bacteria from a slant was inoculated into approximately 200 mL of TYE broth (10 g/L tryptone, 8 g/L NaCl, and 1 g/L yeast extract) one day prior to inoculating the column. After incubating at 35 °C for 24 hours, the resulting suspension (approximately 10^9 cells/mL) was then used to inoculate the column.

In order to establish a uniform biofilm coating throughout the filter bed depth, mixing is necessary to limit the substrate concentration gradient throughout the column. Therefore, the column was operated as an upflow fluidized bed with effluent recycle during establishment of the biofilm to achieve good mixing in the column. To fluidize the bed, a feed flow rate of 3.6 mL/min with a high recycle ratio of 20 was used. The feed solution consisted of sodium acetate (23 mg/L) as substrate for bacterial growth dissolved in phosphate buffered mineral media (21.8 mg/L K_2HPO_4 ; 8.5 mg/L KH_2PO_4 ; 17.7 mg/L Na_2HPO_4 ; 2.7 mg/L NaNO_3 ; 22.5 mg/L MgSO_4 ; 0.25 mg/L FeCl_3 ; 36.4 mg/L CaCl_2). After inoculating the column with 20 mL of fresh *P. aeruginosa* suspension ($\sim 10^9$ cells/mL), the feeding and recycle flow was continued for 5 to 6 days before the column was used for a filtration experiment.

The biofilm growth was monitored by measuring the effluent suspended bacteria concentration using the heterotrophic plate count (HPC) method. In addition, after steady state was achieved, the column was shut down and filter media samples were taken to measure the biomass density. Samples of glass beads were removed from both the top and bottom of the filter bed to quantify the biofilm density. Approximately 10 grams of beads were transferred to a 125 mL bottle and 100 mL of polyphosphate solution (0.1%

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Na₄P₂O₇) was added. The bottles were placed on a Burrell Wrist Action™ shaker and shaken for 15 minutes to transfer the bacteria from the glass beads to the solution. The desorbed biomass was quantified using three different methods, HPC, total direct count and total protein and the results were normalized by the surface area of the beads in the bottle.

2.3 Enumeration of oocysts

Cryptosporidium oocysts were enumerated using EPA Method 1622.^[14] Briefly, water samples were vacuum filtered onto a 0.22 µm pore size membrane filter (Fisher Scientific), and then approximately 330 µL of immunofluorescence antibody solution (Waterborne Inc.) was applied to the filter. The stained oocysts were counted by epifluorescence microscopy at 400× magnification with a Nikon microscope (model Eclipse E600) equipped with mercury vapor lamp, video camera, and image analysis system. Figure 3 is a photograph of fluorescently-stained oocysts on a black polycarbonate 0.22 µm filter.

2.4 NOM Source and NOM Coating Procedure

Suwannee River whole NOM was obtained from the International Humic Substances Society in powder form (48.8% carbon, 7.0% ash). The NOM powder was dissolved to 5 ppm in buffered water (5×10^{-5} M NaHCO₃, pH = 6.7-7.0) for the experiments. The filter bed was flushed with the NOM solution for 30 minutes prior to a filtration experiment to allow NOM to coat the glass beads. In addition, *Cryptosporidium* oocysts were coated with NOM by introducing a suspension of oocysts into a 5 ppm NOM solution and mixing for 30 minutes.

2.5 *Cryptosporidium* oocysts

Cryptosporidium oocysts were purchased from the Sterling Parasitology Laboratory, Department of Veterinary Science and Microbiology, University of Arizona. The oocysts were shipped in antibiotic solution and stored at 4 °C.

2.6 Determination of Filtration Performance

Cryptosporidium removal was evaluated by performing oocyst filtration experiments. In these experiments, the effluent oocyst concentration was monitored following step input of a suspension of oocysts. After flushing the filter with a particle free solution for 30 minutes to pre-equilibrate the system, a 3-way valve was switched to allow flow from the oocysts suspension to begin the experiment.

From the study with latex microspheres (Figure 4), the effluent concentration stabilized after approximately 10 minutes (approximately 1.6 pore volumes). In subsequent experiments, 84.5 mL effluent samples were collected for 2 minutes each during the period of 10 to 16 minutes after oocyst feed was initiated. The average concentration of the three effluent samples was then computed and compared to the influent concentration.

The oocyst feed suspension was prepared by diluting the oocyst stock solution ($\sim 7.1 \times 10^7$ /mL) in pH buffer (5×10^{-5} M NaHCO₃), to obtain a final concentration of approximately 1.5×10^3 /mL. Three samples were taken from the feed suspension to determine the influent concentration by the IFA method.

2.7 Evaluation of Oocyst Surface Characteristics

The surface charge and hydrophobicity of clean oocysts and NOM pre-coated oocysts were evaluated. Zeta potential served as an indicator of surface charge. The zeta potential of oocysts was measured using a Laser Zee Meter (Model 501). Hydrophobicity was evaluated by measuring K_{hw} , which is the ratio of the oocyst concentration in hexadecane ($C_{16}H_{34}$) to that in water at equilibrium. For the K_{hw} measurement, a known amount of oocysts were spiked into a flask containing 30 mL of buffered water and 30 mL of hexadecane and the flask was mixed by handshaking for approximately 5 minutes. The hexadecane and water phase were allowed to separate under quiescent conditions for 4 hours, then samples of the water phase were withdrawn to measure the oocyst concentration. The oocyst concentration in hexadecane phase was calculated by mass balance. The K_{hw} value was obtained by simply dividing the concentration in hexadecane by that in water.

Results and Discussion

3.1 Preliminary Evaluation of Experimental System Performance

The results from the filtration experiments with model particles are plotted in Figure 4. The normalized effluent latex microsphere concentration was 0.62, which is slightly below that predicted by the filtration model of Yao et al.^[16] (0.68), using the Rajagopalan and Tien^[11] expression for collection efficiency and assuming a collision efficiency (α) value of 1.0. This experiment suggested that the experimental system was working well and that our methodology was sound. We then proceeded to use this system to evaluate oocyst removal over a range of conditions.

3.2 Establishment of a biofilm on the filter media

A biofilm was established on the filter media by inoculating the column with a suspension of *P. aeruginosa* and operating the filter as a fluidized bed system while continuously feeding a solution of sodium acetate in phosphate buffered mineral media. The effluent bacteria concentration was monitored every 12 hours by heterotrophic plate count (HPC) and the results are plotted in Figure 5. The effluent concentration did not vary significantly over the five-day period suggesting that the bacterial growth quickly reached steady state. Samples of glass beads were also removed from the top and bottom of the filter bed to quantify the biofilm density. Surface area normalized HPC, total direct count and total protein values are shown in Table 2. The similarity of the values for the top and bottom of the filter bed suggested that the biofilm growth on the filter media throughout the column was uniform.

Environmental Scanning Electron Microscopy (ESEM) was used to evaluate the uniformity of the coating on the beads to determine if coverage was complete or patchy. Figure 6 includes two ESEM photographs, one of the surface of a clean glass bead (Figure 6A) and another of a biofilm coated glass bead. After a base-acid wash procedure, the glass bead surface was very clean and smooth except for a rare surface blemish used as a point of focus in the photograph. After inoculation and feeding for five days in a fluidized bed mode, the glass bead surface was completely covered with biofilm, which consists of bacteria and their extracellular polymeric substances (EPS)

(Figure 6B). Examination of several beads from the filter bed suggested that the biofilm coverage was complete and relatively uniform.

3.3 Effect of Biofilm Coating on Oocysts Removal

Oocyst removal in clean and biofilm-coated filters was compared. The results of these experiments are shown in Table 3. A biofilm coating on the filter media significantly reduced oocyst removal. Two factors might contribute to this effect, a change in flow pattern through the filter (i.e. short circuiting) or a change in the surface character of the filter media.

Compared with the clean bed, the biofilm-coated bed is likely to have more short circuiting and reduced porosity which could impair particle removal. Figure 7 shows the observed difference in the breakthrough curves for the system with and without biofilm. The residence time of the system decreased from 6.60 minutes with a clean bed to 6.26 minutes with a biofilm coated bed. The residence time for the system is dominated by tubing and the small reduction for the system does not indicate the significance of the change in residence time for the bed alone. When the residence time in the tubing and other volumes are subtracted away, the residence time for the 10 inch packed bed actually decreased by 28% from 1.19 minutes to 0.86 minutes. Thus, the reduction in bed porosity was significant; however, the effect of this porosity reduction on particle removal is unclear. Higher flow velocity through the bed as a result of reduced porosity would reduce particle removal; conversely, accumulation of biofilm would provide more surface area for particle deposition, which would enhance particle removal. It was not possible to separate these effects in the experimental system used in this research; however, based on the observed decrease in oocyst removal efficiency, it appears that short circuiting and higher flow velocity were the dominant effects in this system.

In addition, it is possible that a change in the surface characteristics of the media caused the observed reduction in oocyst removal. From the ESEM photographs (Figure 6), the biofilm-coated surface was physically quite different from the clean glass bead surface. In the biofilm experiments, the oocysts were deposited onto a surface dominated by EPS. Although the exact structure of the EPS in this study was not elucidated, an organic EPS coating (consisting primarily of polysaccharides and proteins) differs significantly from the inorganic surface of clean glass beads (composed primarily of SiO₂). We speculate that the biofilm surface was more hydrophobic and less electronegative than the clean glass surface. The increase in hydrophobicity would be expected to decrease attachment of the relatively hydrophilic oocysts (see section 3.4 below) while the decrease in surface charge would be expected to increase attachment. Thus, it appears that the effect of biofilm on fluid flow through the bed and possibly an increase in the hydrophobicity of the glass beads were responsible for the observed decrease in filtration performance.

3.4 Effect of NOM coating

When NOM was applied to the system, oocyst removal decreased from 51% to 15% (Table 3). There are three possible mechanisms that could be used to explain the impact of NOM coating on oocyst removal: 1) a change in the surface charge of the oocysts, glass beads, or both; 2) a change in the surface hydrophobicity of the oocysts, glass beads, or both; and 3) steric interactions (i.e. steric repulsion). As shown in this

research (Figure 8) and in the literature^[6], *Cryptosporidium* oocysts have a neutral or slightly negative surface charge at circumneutral pH over a range of ionic strengths. Pre-adsorption of NOM onto the oocysts significantly increased the magnitude of the negative charge on the oocysts (Figure 8), and likely enhanced the electrostatic repulsive force between the glass beads and the oocysts. In addition, NOM also increased the hydrophobicity of the oocysts, as the K_{hw} value increased from 0.14 to 1.1 (Figure 9). An NOM coating likely increased the hydrophobicity of the glass beads as well, although this effect was not confirmed experimentally. Because increased hydrophobicity of both the oocysts and glass beads would tend to improve oocyst removal, it appears that the effect of hydrophobicity was insignificant relative to other effects. Extensive attachment of NOM could also result in steric repulsion; however, this is unlikely in these experiments because the moderate pH (6.7 – 7.0) and relatively low ionic strength of the water during NOM coating of the oocysts and glass beads would prohibit a dense accumulation of anionic macromolecules. Therefore, the dominant mechanism in these experiments is likely the increased electrostatic repulsion due to NOM adsorption onto the oocysts.

Conclusions

Based on the experimental results from this research, we offer the following conclusions. Pre-equilibration of *Cryptosporidium* oocysts and filter media with NOM and accumulation of biofilm on the filter media significantly impaired the removal of oocysts relative to a clean filter bed. Thus, when the source water contains high concentrations of NOM, the potential for oocysts to pass through the filter increases. Oocysts have a circumneutral surface charge; however, oocysts pre-exposed to a solution of NOM obtained a significant negative charge. This increased potential for electrostatic repulsion suggests that effective coagulation is imperative to neutralize the surface charge and ensure good oocyst removal. The NOM can also change the hydrophobicity of the oocysts or cause steric repulsion; however, these effects were deemed to be minor in comparison to the enhanced electrostatic repulsion. The results of this research suggest that source waters containing a high concentration of NOM may benefit from enhanced coagulation and/or preoxidation to remove NOM prior to filtration.

When a biofilm was permitted to accumulate in the filter bed, oocyst removal performance was significantly impaired. Thus, when biofiltration is used in a water treatment plant to remove NOM, a two-stage filter system (i.e., biofilter followed by standard filter) may be needed to ensure good oocyst removal. More work is needed to further elucidate the effects of NOM and biofilm on filtration of *Cryptosporidium* oocysts in order to provide guidelines for the operation of filters to ensure pathogen free drinking water.

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Table 1. Hydraulic residence time and dispersion number for the filtration system

Loading rate, m/h	Bed depth, m	Residence time, min	Dispersion number
5.0	0.25	6.63	0.02

Table 2. Comparison of biomass density on the filter media from the top and bottom of the column

	HPC / cm ²	Direct count / cm ²	Total Protein, μg / cm ²
Bottom	2.38 × 10 ⁵	4.83 × 10 ⁶	2.02 × 10 ³
Top	1.53 × 10 ⁵	5.08 × 10 ⁶	4.01 × 10 ³

Table 3. Impact of NOM and biofilm coating on oocyst removal

NOM	No	Yes	No	Yes
Biofilm	No	No	Yes	Yes
Effluent conc., C _{eff} /C _{inf}	0.49±0.10	0.85	0.77±0.03	0.85±0.01
Removal, %	51	15	23	15
Collision efficiency (α)	0.87	0.2	0.32	0.2

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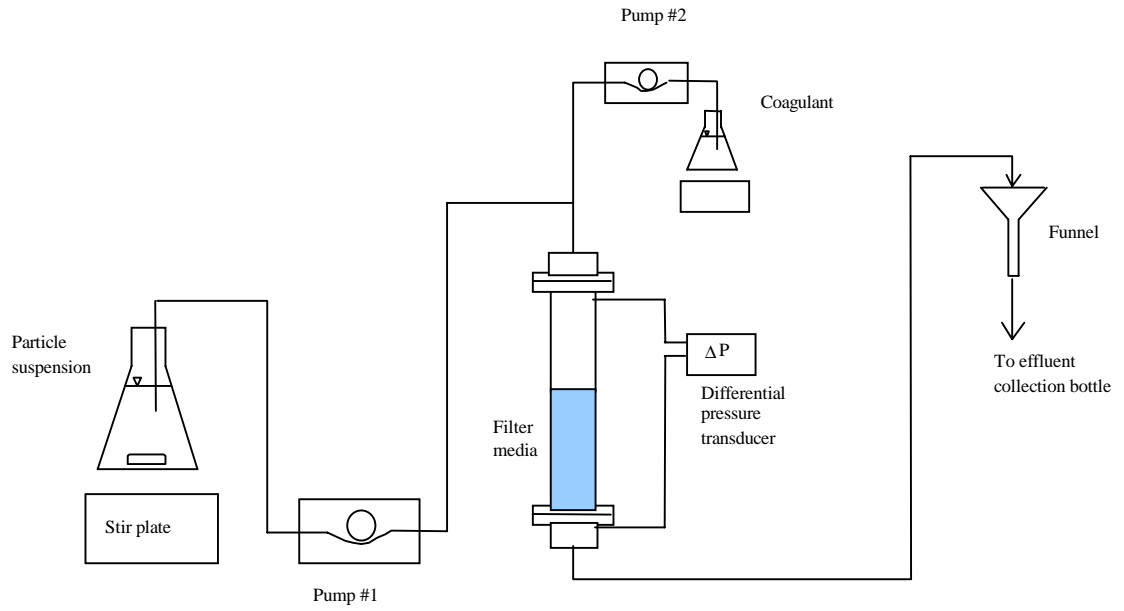


Figure 1. Schematic diagram of the laboratory-scale filtration system

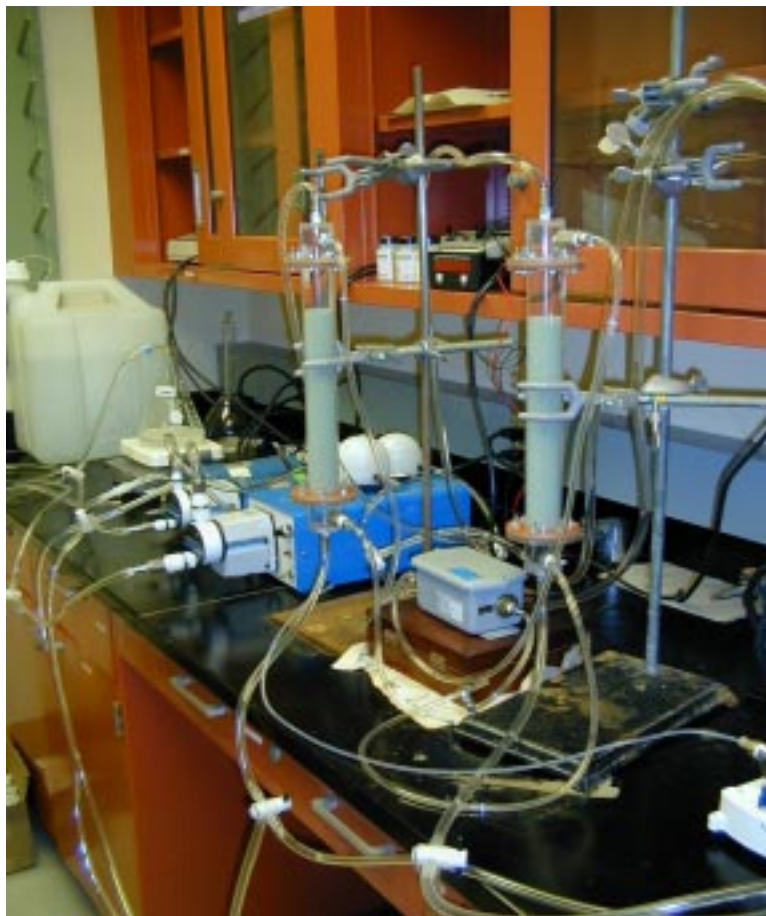


Figure 2. Photograph of the laboratory-scale filtration system
(From left to right: Feed reservoirs, Pumps, Columns, Pressure transducer)

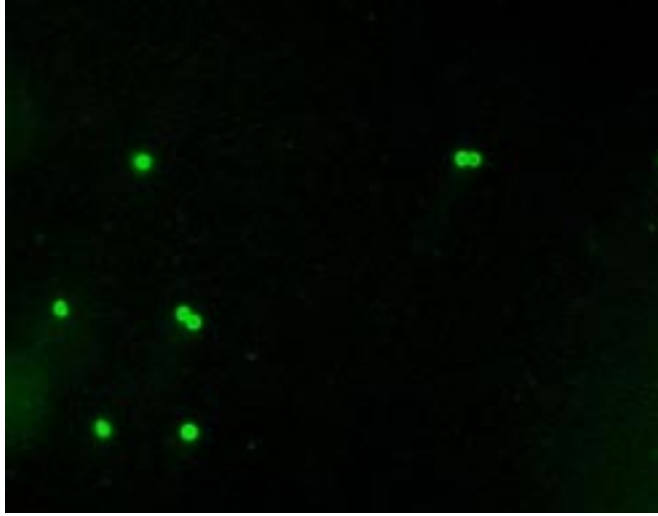


Figure 3. Photograph of *Cryptosporidium parvum* oocysts stained with immunofluorescent antibody and viewed at 400X magnification

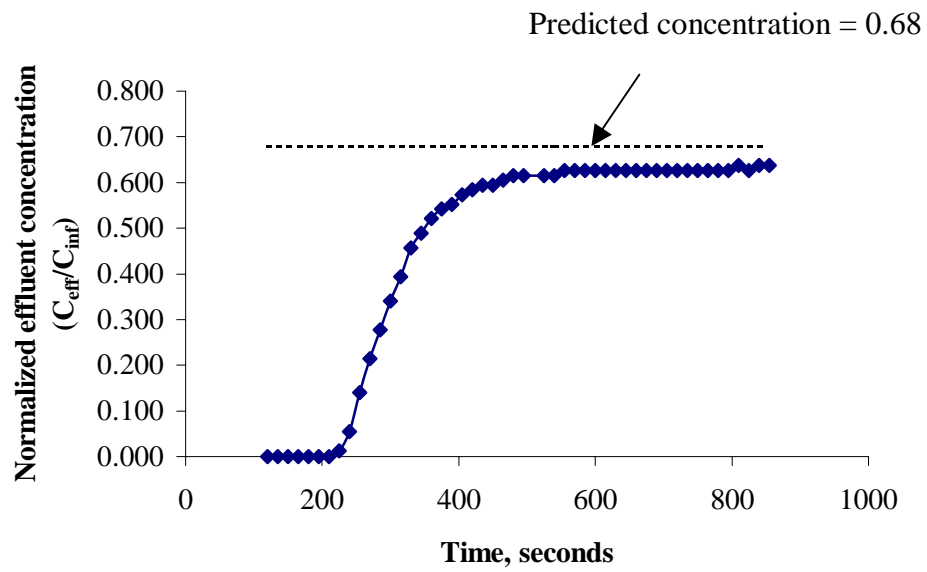


Figure 4. Breakthrough curve for 1 μ m latex microspheres

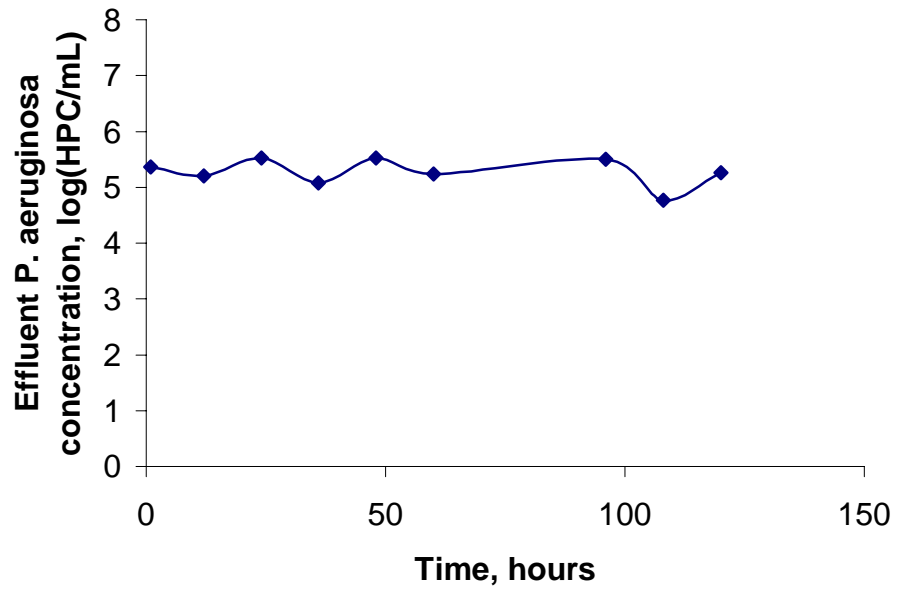


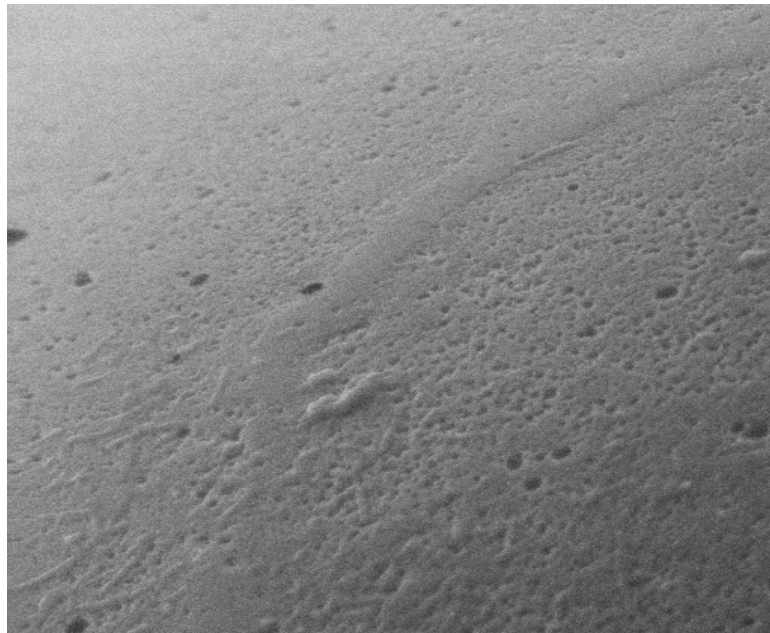
Figure 5. Effluent HPC over time during establishment of the biofilm on the filter media

A



10µm 2000X

B



10µm 2000X

Figure 6. ESEM photographs of clean and biofilm coated glass beads
(A = clean glass bead; B = biofilm-coated bead)

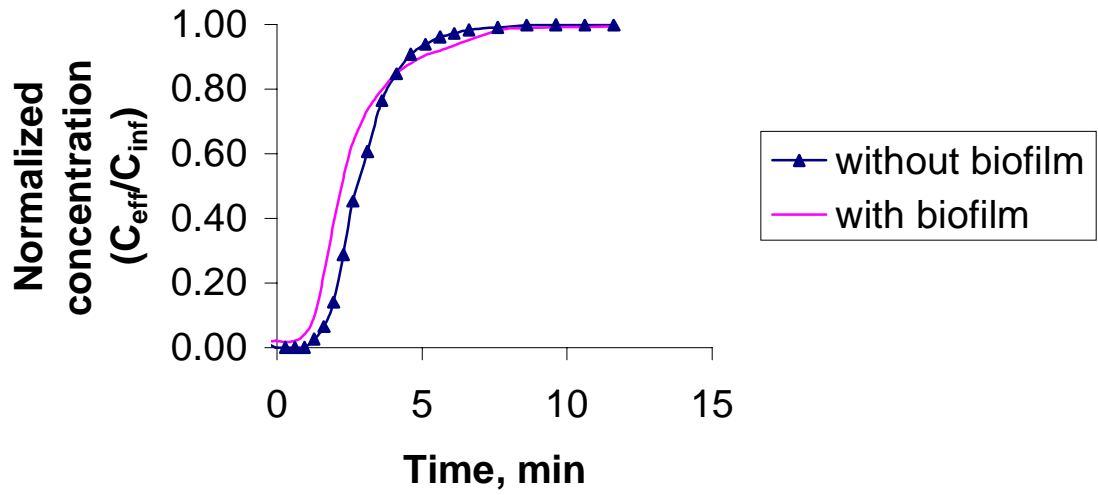


Figure 7. Effect of biofilm accumulation on the tracer breakthrough curve

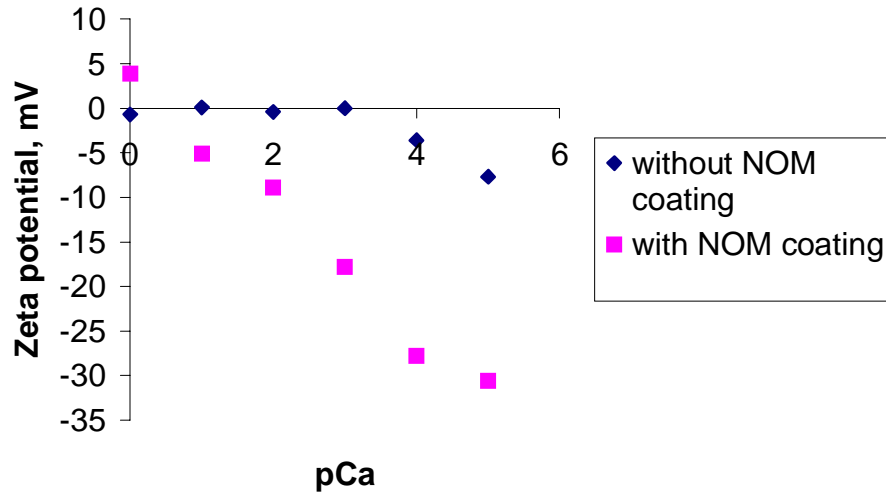


Figure 8. Comparison of the zeta potential of oocysts with and without NOM coating as a function of calcium concentration at pH=6.7
($pCa = -\log[Ca^{2+}]$ where $[Ca^{2+}]$ = calcium concentration in moles/L)

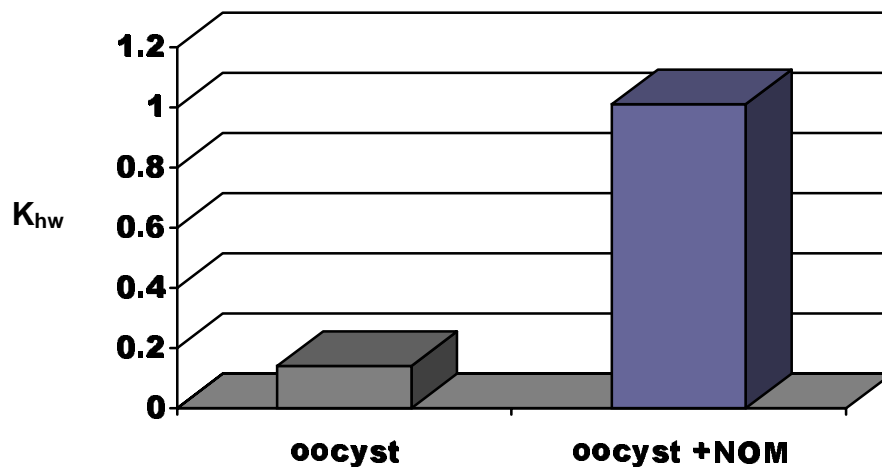


Figure 9. Effect of NOM on the hydrophobicity of *Cryptosporidium* oocysts

Basic Information

Title:	Organic Matter Binding and Photoreduction of Mercuric Ion and Methylmercury Ion Surface Water
Project Number:	C-07
Start Date:	9/1/1998
End Date:	2/28/2001
Research Category:	Water Quality
Focus Category:	Toxic Substances, Geochemical Processes, Non Point Pollution
Descriptors:	mercury, methylmercury, humic substances, photochemistry, metal binding, complexation, lakes, wetlands, peatlands
Lead Institute:	University of Minnesota
Principal Investigators:	Paul R Bloom, Patrick L Brezonik, Abdul R Khwaja

Publication

Project No.: C-07

Title: Organic Matter Binding and Photoreduction of Mercuric Ion and Methylmercury Ion Surface Water

Investigators: Paul R. Bloom, Department of Soil, Water, and Climate, College of Agricultural, Food, and Environmental Sciences; and Patrick L. Brezonik, Department of Department of Civil Engineering, Institute of Technology; and Abdul R. Khwaja, Water Resources Science Program, Research Assistant, University of Minnesota

Period: 9/1/98–2/28/01

Introduction

Mercury (Hg) pollution is a global-scale problem with both ecological and human health implications. Thousands of lakes in the northern hemisphere, many in remote areas, contain fish with methylmercury (CH_3Hg^+) concentrations above public health guidelines for human consumption. The problem is prevalent in the lake regions of the Upper Midwest, especially in soft-water lakes of northeastern Minnesota, northern Wisconsin, and Upper Michigan. Although the primary concern with elevated Hg levels in fish relates to human health, waterfowl and other wildlife can be impacted negatively by elevated Hg levels in fish, as demonstrated by studies in such disparate areas as northern Minnesota and the Florida Everglades.

Despite many studies on Hg cycling over the past decade, critical gaps remain in our understanding of Hg sources, transport, and cycling processes in aquatic and terrestrial systems. For example, it is widely acknowledged that the most important source of Hg in lakes is atmospheric deposition and that most Hg entering the atmosphere (primarily from fossil fuel combustion and incinerators) is transported long distances (hemispheric-scale transport) before being redeposited to earth. Nonetheless, Hg levels in fish are highly variable even in lakes within small geographic areas, despite the regional homogeneity in loading implied by an atmospheric source. Among the water chemistry factors thought to be responsible for the differences in lake responses, interactions between Hg forms and natural organic matter (NOM) are perhaps the most important. Our understanding of Hg-NOM interactions has been based mostly on qualitative observations and statistical correlations; quantitative and mechanistic information is lacking on this issue.

The photochemistry of Hg also is poorly understood, but studies conducted during the past decade indicate that such processes are important for Hg cycling in aquatic systems. For example, the photodecomposition of CH_3Hg^+ in lake water was demonstrated by Sellers et al. (1996). Photoreduction of Hg^{2+} to Hg^0 (often referred to as dissolved gaseous mercury, DGM) has been shown by several research groups, and diurnal patterns in DGM in surface waters (with maximum concentrations in daylight hours) is evidence for the photochemical origin of this mercury species. The role of NOM in these reactions, the mechanisms by which they occur, factors affecting the rates, and their environmental significance all remain to be determined. Because NOM absorbs light in the photochemically important uv and low visible part of the spectrum, it may be an important sensitizing agent in aquatic photochemical reactions.

Objectives

The transport and reactivity of Hg^{2+} and methylmercury (CH_3Hg^+) in surface waters are affected by light and natural dissolved organic matter (NOM), but quantitative information on the extent and nature of Hg-NOM interactions and Hg photochemistry is lacking. We obtained such information related to several important interactions of mercury forms, NOM, and light. In particular, we evaluated the importance, mechanisms and role of NOM in photochemical transformations of Hg^{2+} and CH_3Hg^+ , especially those related to the production of elemental mercury (Hg^0). In addition, we determined binding constants of Hg^{2+} and CH_3Hg^+ with NOM isolated from surface waters in the Marcell Experimental Forest in northern Minnesota. The nature of the binding ligands in NOM was determined by synchrotron-based x-ray spectroscopy.

Specific objectives of the project were to:

- (1) evaluate the mechanisms whereby aquatic NOM affects photochemical reduction of Hg^{2+} to Hg^0 ;
- (2) determine the rates, mechanisms and products of CH_3Hg^+ photolysis and the influence of NOM on this process;
- (3) determine the binding strength of Hg^{2+} and CH_3Hg^+ to aquatic NOM as a function of solution conditions and origin of the NOM; and
- (4) determine the importance of sulfur and carboxyl binding sites in Hg-NOM binding.

Methods

Sample collection and characterization. Natural organic matter was obtained from several surface waters in the Marcell Experimental Forest, ~50 km north of Grand Rapids, MN. Sampling sites included an ombrotrophic bog (S-2), a fen (S-3), and three small lakes (Blandin, Spring, Scrapper) with varying types of wetland influences. Aquatic sites complemented the soil sampling sites used in our separately-funded studies of Hg^{2+} binding by soil organic matter. Two common methods for extracting aquatic NOM were used so that we could compare the chemical and structural characteristics of the isolated NOM. For both methods, surface waters were filtered through 0.7 μm pre-fired glass fiber filters into PVC bottles followed by acidification to pH 2. The NOM (primarily fulvic acid) then was concentrated onto either an XAD-8 column (Thurman and Malcolm, 1981) after acidification at pH2, or a DEAE cellulose column (Miles et al. 1983; Eaton et al. 1995) after pH adjustment to ~7. NOM was removed from the columns by dilute NaOH, acidified using H^+ -saturated cation exchange columns, and freeze dried.

Total Hg and CH_3Hg^+ in extracted NOM and raw water samples were determined by ultra-trace procedures using cold-vapor atomic fluorescence spectroscopy (CVAFS) (Bloom and Fitzgerald 1988; Bloom 1989; Horvat et al. 1993). Extracted NOM was characterized by elemental analysis (C, N, S), uv-visible absorbance spectra, SUVA (specific uv absorbance-absorptivity at 254 nm per mg C), total acidity, and solid-state ^{13}C NMR spectra. Elemental analyses were as follows: carbon (mass spectrometer), sulfur (Leco CR-132 Sulfur Determinator), and nitrogen (mass spectrometer). Total acidity was determined by titration (Ritchie and Perdue 2000). The ^{13}C NMR spectra were run on a 300 MHz solid-state NMR spectrometer with magic angle spinning. The quantity of reduced S in the NOM samples was determined by XANES x-ray spectroscopy.

Photochemical Processes and Hg Transformations. Experimental work on Hg

photochemistry focused on photolysis of methylmercury, because this is the most toxic and bioaccumulated form of Hg, and on photoreduction of oxidized Hg forms to Hg^0 , because this is a potentially important loss process for Hg in aquatic systems. Photooxidation of Hg^0 was explored briefly as a source for bioavailable Hg in water. Experiments were conducted in the laboratory under controlled conditions of light, temperature and water chemistry using a merry-go-round reactor, uv light source (medium-pressure Hg vapor lamp), and gas chromatograph to measure concentrations of chemical probes. Additional measurements were conducted under field conditions at the Marcell Forest to evaluate ambient rates under natural light conditions. Rates of reaction were measured in terms of loss of Hg reactant or formation of specific Hg products (e.g. Hg^0), as appropriate.

For CH_3Hg^+ photodegradation and reduction of Hg^{2+} to Hg^0 , we determined the role NOM in accelerating or inhibiting rates, as well as the role of photo-intermediates like hydroxyl radicals ($\cdot\text{OH}$) produced by photolysis of aquatic humus. Production of Hg^0 from Hg^{2+} and CH_3Hg^+ by a series of model organic compounds was studied to gain an understanding of the functional groups in NOM that are involved in the Hg photochemical transformations. Because of the concentration of $\cdot\text{OH}$ remains low ($\sim 10^{-14}$ - 10^{-16} M) during photochemical reactions, we used a chemical probe, butyl chloride, to measure its steady-state concentration by methods described in Brezonik and Fulkerson-Brekken (1998).

Determination of binding constants for NOM with Hg^{2+} and CH_3Hg^+ . Binding constants were determined by a modification of the Br^- complexation method used to determine K_d values of soil organic matter (Skylberg et al., 2000). A sample of Suwannee River fulvic acid from the International Humic Substances Society was included for comparison. Solutions containing ~ 8000 mg/L of NOM were prepared in 0.5 M KBr and adjusted to the desired pH using dilute KOH. A quantity of Hg^{2+} or CH_3Hg^+ was added prior to making the solutions in KBr, and 3.0 mL of solution was transferred into 100 MW cellulose ester dialysis tubing. The quantity of Hg added was adjusted to encompass a range of NOM-bound Hg from less than typical Hg concentrations in lake waters to several times that concentration (e.g. ~ 50 - 1000 pg/L for CH_3Hg^+ ; ~ 0.2 - 3000 ng/L for Hg^{2+}). The sealed tubes were placed in a 100 mL glass bottles with Teflon caps containing 100 mL of 0.5 M KBr and agitated gently. The external and internal solution was sampled for Hg after 72 h equilibration time. Concentrations of Hg^{2+} and CH_3Hg^+ in the internal and external solutions were determined by CVAFS (Bloom and Fitzgerald, 1988). NDOM in the external solution was determined with a Dorhmann Phoenix 8000 analyzer. The quantity of Hg complexed by Br^- and activity of Hg^{2+} or CH_3Hg^+ was calculated by MINTEQA2.

Results

NOM characteristics. Important chemical characteristics of the waters from which the NOM samples were isolated are summarized in Table 1. The difference in water chemistry between the bog and fen in pH

Table 1. Some chemical characteristics of Marcell surface waters that were the source of extracted NOM

Sample	DOC mg/L	SUVA @254nm	pH	MeHg ng/L	Conductivity microS/cm	Color at 440 nm (Pt, mg/l)	total filtered Hg, ng/L
Bog S2	80	0.0394	4.13	0.318	40	825	53.3
Fen S3	29	0.0264	7.94	0.206	110	177	6.4
Scrapper L.	12	0.0142	8.11	0.126	150	28	25.3
L.Blandin	31	0.0213	6.46	0.76	25	242	11.8
Spring L.	11	0.0207	6.68	0.75	20	67	11.4

and color, and to a lesser extent in DOC and conductivity, is dramatic. The bog receives no water except from direct precipitation, but the fen receives some ground-water inflow. Thus the bog is acidic, low in dissolved ions, and highly colored, but the fen is circumneutral and has a higher ionic content but lower color (and DOC). Scrapper Lake, which drains a large fen, has a high pH, moderately high ionic content, and low color and DOC. The other two lakes have pH values just below 7.0, are low in dissolved ions and have moderate to high color and DOC levels.

The extracted NOM exhibited a fairly wide range of properties (Table 2). SUVA ranged from ~0.023 (Scrapper and Blandin) to 0.048 (S2). This property is a simple indicator of the aromatic content of NOM; thus NOM from S2 likely has a high aromatic content, while NOM from the two lakes is low in aromatic structures. In contrast, Spring Lake NOM has a SUVA similar to that of the fen. The bog and fen extracts are low in acidic functional groups and high in background Hg compared with the lake extracts.

Hg photochemistry. Methylmercury undergoes photolysis with a characteristic time on the order of a few days when exposed to ambient light conditions in surface waters (Figure 1). These results agree generally with those of Sellers et al. (1996) for lakes at the ELA in Ontario. Left unknown by their work and that shown in Figure 1 is information on the mechanism of photolysis, environmental factors affecting the rate, and the nature of the product(s) of photolysis. We undertook experiments to answer these questions.

Table 2. Some chemical characteristics of extracted NOM from Marcell surface waters

	SUVA @ 254 nm	Color @ 440 nm*	E4:E6	Acidity, meq/g C	% C	%N	% S	HgT ng/g
Bog S2	0.0480	458	8.9	17.9	40.0	1.67	0.48	0.50
Fen S3	0.0389	154	10.2	17.2	52.2	3.68	0.82	0.23
Scrapper L.	0.0230	68	10.2	56.8	32.2	2.40	1.10	0.08
L. Blandin	0.0232	87	9.3	61.3	23.5	0.58	0.32	0.04
Spring L.	0.0394	271	9.4	NA**	NA	NA	NA	0.16

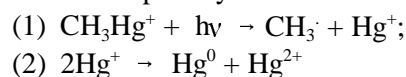
*Color in mg/L as Pt for a 100 mg/L solution of the extract

**Samples still being processed

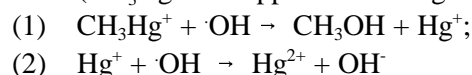
Photolysis of CH_3Hg^+ occurs by both direct and indirect mechanisms (Figure 2). In the former case, the only factors affecting the rate are the concentration of CH_3Hg^+ and light intensity.

CH_3Hg^+ does not absorb light in the visible range, but spectral analysis shows that it does absorb in the uv (~290-330 nm). Indirect photolysis of CH_3Hg^+ is mediated by the highly reactive photo-intermediate, hydroxyl radical ($\cdot\text{OH}$), which are formed photochemically in natural waters by reactions of various sensitizer molecules, including nitrate, ferrous iron, and humic substances. When water was enriched in nitrate to provide a source of $\cdot\text{OH}$, the loss rate of CH_3Hg^+ in the light increased substantially. The loss rate of CH_3Hg^+ in the presence of 20 mg/L of NOM extract from L. Blandin was about the same as that in de-ionized water, indicating that the humic matter from this source was an insignificant source of OH radicals and that direct photolysis was the primary photolysis mechanism in this sample. Further experiments with the three lake extracts of NOM showed that two of the extracts (Blandin and Spring) had a generally inhibitory effect on the rate of CH_3Hg^+ photolysis, with rates decreasing as the concentration of NOM increased, but increasing concentrations of NOM from Scrapper L. caused increasing rates of CH_3Hg^+ photolysis. Further experiments are underway to explain these trends in terms of the structural differences in Scrapper L. NOM and to determine whether NOM from other sources (e.g., S2, S3, Suwannee River fulvic acid) causes a stimulation of CH_3Hg^+ photolysis. The structural characteristics of Scrapper L. NOM differ somewhat from those of the other two lakes (Table 2; also, NMR spectra, not shown) and is generally less colored than that of the other two lakes.

The products of CH_3Hg^+ photolysis were investigated in an effort to elucidate the chemical mechanisms involved in direct and indirect photolysis. In the former case, both Hg^{2+} and Hg^0 are formed; about half the CH_3Hg^+ lost through photolysis was recovered as Hg^{2+} , but a good mass balance was not achieved (<< half of the lost CH_3Hg^+ was recovered as Hg^0); efforts are underway to redo this experiment to achieve closure on the mass balance. Nonetheless, we are confident in the CH_3Hg^+ and Hg^{2+} data (less so for Hg^0 results), and thus it appears that direct photolysis of CH_3Hg^+ does not simply produce Hg^{2+} . A likely mechanism for direct photolysis is:



If true, we should see equal quantities of elemental and divalent Hg formed; we expect to have definitive information confirming (or rejecting) this hypothesized mechanism in the next few months. In contrast, indirect photolysis of CH_3Hg^+ mediated by $\cdot\text{OH}$ produced Hg^{2+} almost exclusively, and a good mass balance was achieved (CH_3Hg^+ loss approximated Hg^{2+} gain). A mechanism that fits these results is:



Photoreduction of Hg^{2+} occurs readily in surface waters and results in the production of Hg^0 (Figure 3). Pseudo first-order rate constants for Hg^0 production from Spring L. samples at ambient levels of Hg^{2+} (about an order of magnitude lower than the spiked samples in Figure 3) were much lower than those calculated from the data in Figure 3. This suggests that under ambient conditions Hg^{2+} is somewhat protected against photolysis, probably as a result of forming strong complexes with NOM. The formation of unreactive or less reactive complexes of various metals and organic contaminants NOM has been demonstrated by several investigators; studies are underway to verify this in relation to Hg-NOM photochemistry.

In an effort to evaluate the functional groups in NOM that provide the reducing power (electron source), we selected a set of model organic compounds with functional groups known to occur in NOM and humic substances. In each case, 10 mg/L of the compound was incubated with 20 ng/L of Hg^{2+} in the laboratory under controlled light conditions, and the production of Hg^0 was measured after 20 minutes of irradiation (Table 3). It is apparent that a wide range of organic compounds can reduce Hg^{2+} to Hg^0 in the presence of uv light, but in general, sulfhydryl groups and typically strong chelating agents (e.g., EDTA) that react by ligand-to-metal charge-transfer (LMCT) mechanisms appear much less important than

aromatic carboxylate, phenolic, and quinone groups.

Hg binding by NOM. A XANES S-K edge spectrum was taken of the S2 aquatic NOM extracted with DEAE. Analysis of the spectrum (Figure 4) revealed that 33% of the total 'S' was present as reduced S (formal oxidation states -I and 0); 25% of the total S was present as most oxidized S (formal oxidation state VI). The rest was present in intermediate oxidation states (formal oxidation states II, IV and V). Based on the XANES study, a sample of the same S2 extract was prepared for EXAFS study. The sample was loaded with 0.7:1 (moles of Hg(II) per mole of reduced S). Analysis of the Hg L_{III}-edge EXAFS spectrum showed that most of the Hg^{II} was bound to S atoms, and very little binding was found to O atoms. It is uncertain whether the small O peak is real or an artifact, and further analysis using *ab initio* calculations with FEFF-6 is underway. The results thus far suggest that Hg^{II} strongly prefers to bind with reduced S functional groups.

Table 3. Photo-reduction of Hg²⁺ to produce Hg⁰ is mediated by a variety of model organic compounds in addition to NOM extracts from the three Marcell lakes.

Model Compounds	Concentration mg/L	Hg(II), ng/L	pH	Hg(0) in control (ng/L)	Hg(0) formed in 20 min of irradiation, ng/L
Salicylic acid	10	20	6.95	2.03	6.99
Citric Acid	10	20	6.45	2.17	7.38
Tartaric Acid	10	20	6.27	3.6	7.6
Catechol	10	20	5.68	0	2.8
Phthalic Acid	10	20	5.01	0	9.42
Hydroquinone	10	20	5.68	0	10.87
Mercaptoacetic Acid	10	20	5.26	0	1.43
EDTA	10	20	5.38	0	0.25
Cysteine	10	20	5.99	0	0.83
Oxalic Acid	10	20	5.63	0	0.03
Acetic Acid	10	20	6.1	0	0.12
NTA	10	20	5.37	0.78	0.93
Aquatic Humic Matter					
Lake Spring	20	20	4.86	1.84	4.04
Lake Blandin	20	20	6.65	2.5	6.09
Lake Scrapper	20	20	4.54	2.72	3.84

Experiments to determine the binding constants of Hg²⁺ and CH₃Hg⁺ with humic substances are under progress. These experiments are being done using the dialysis approach with a competitive ligand like Br⁻. A preliminary experiment with a 100 MW membrane and IHSS Suwannee River fulvic acid loaded with Hg at molar ratio of 0.01:1 for Hg:reduced S gave a K_{NOM} of 1.7 X 10²². Experiments with NOM extracts from the Marcell wetlands and lakes are underway, and results will be reported at the a symposium on chemical processes of mercury in aquatic and soil systems at the summer meeting of the American Chemical Society in Chicago this August. The symposium was organized by the PIs of this project.

Figures

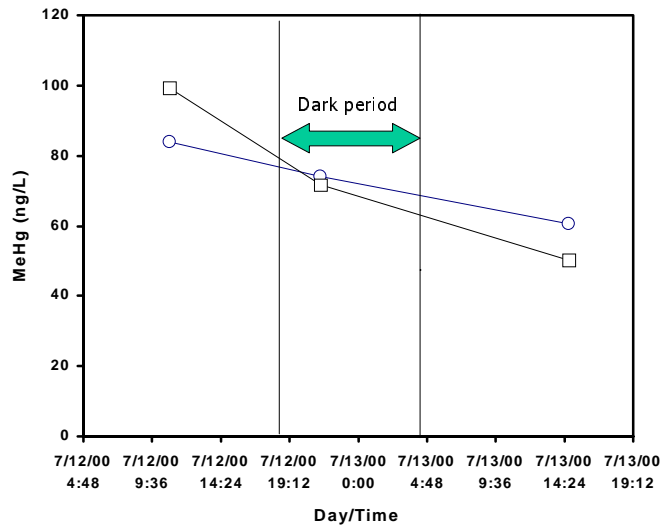


Figure 1. Photochemical loss of methylmercury in filtered water from Spring L. (squares) and bog S2 (circles) incubated in situ.

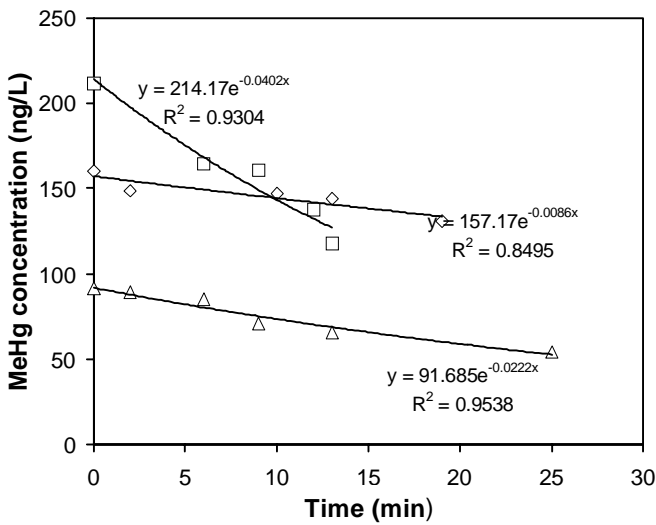


Figure 2 (left). CH_3Hg^+ photolysis occurs by a direct mechanism (diamonds; only CH_3Hg^+ in deionized water) but is faster (squares) in presence of 10 mg/L of nitrate-N, a source of $\cdot\text{OH}$; photolysis rate in presence of 20 mg/L of L. Blandin NOM (triangles) was same as in deionized water.

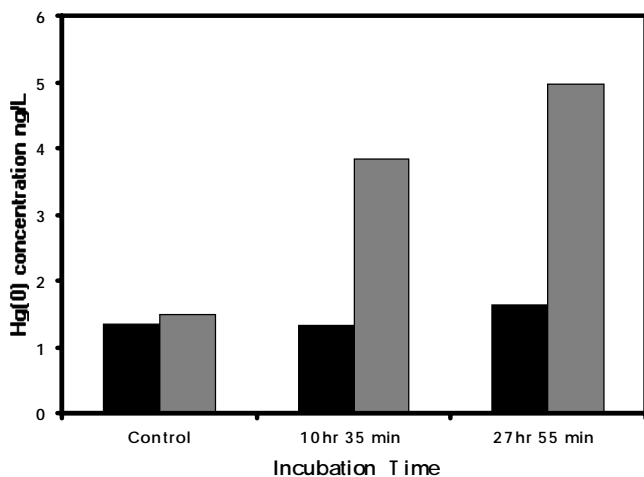


Figure 3. Production of Hg^0 in S2 (black bars) and Spring L. (stippled bars) under ambient light conditions at Marcell in responsible July 2000; 20 ng/L of Hg^{2+} was added to each sample initially.

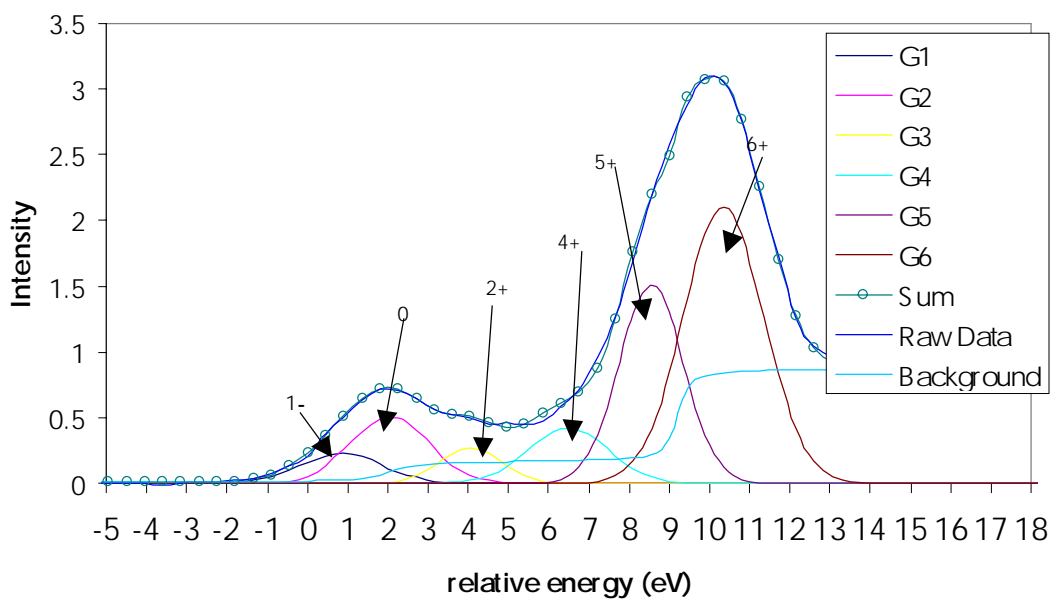


Figure 4. Sulfur K-edge XANES spectrum of NOM extract from bog S2 and nonlinear least square fit of the XANES spectrum showing the different oxidation states.

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Key words: mercury, methylmercury, photochemistry, humic substances, fulvic acid, complexation

Basic Information

Title:	In situ measurement of denitrification in agricultural streams
Project Number:	C-01
Start Date:	9/1/2000
End Date:	8/31/2001
Research Category:	Water Quality
Focus Category:	Nitrate Contamination, Non Point Pollution, Methods
Descriptors:	denitrification, agriculture, surface drainage, isotopes
Lead Institute:	University of Minnesota
Principal Investigators:	Patrick L Brezonik, Lorin Kent Hatch

Publication

Project No.: C-01

Title: *In situ* measurement of denitrification in agricultural streams

Investigators: Patrick Brezonik, Water Resources Center; Lorin Hatch, Department of Fisheries and Wildlife, University of Minnesota

Period: 9/1/00–8/31/01

Introduction

Nitrate contamination of ground water and streams is common in landscapes dominated by agricultural activities. Associated impacts of this pollution range from local violations of drinking water standards designed to prevent methemoglobinemia to national concerns (e.g., Gulf of Mexico hypoxia). Significant quantities of nitrate are exported from agricultural lands through drainage ditches and low-order natural streams, but our understanding of nitrogen transport and transformation in these agricultural streams is far from complete. Denitrification may be an important mechanism for nitrate removal in these streams, and this would mitigate water quality and health hazards downstream. Several methods can be used to measure denitrification, but the most common ones involve laboratory experiments with sediment cores, where conditions are not conducive to obtaining *in situ* rates.

Our main goal is to assess *in situ* denitrification rates in agricultural streams and determine how these rates vary with stream stage, flow, and temperature. We will compare *in situ* results obtained by acetylene blockage, ^{15}N tracer, ^{15}N natural abundance ratios, and mass balance methods. Part of our field work will use in-stream mesocosms for assessments. We will make measurements over a range of discharge and stage and make a preliminary assessment of the usefulness of artificial stream level manipulations to enhance denitrification. Field work is being conducted in the eastern Minnesota River Basin, an agricultural area with high nitrate levels in its streams and rivers.

Our project has two principal objectives, both of which will provide important information to scientists concerned with evaluating the transport and fate of agricultural nitrogen losses from fields to downstream ecosystems:

- (1) evaluate stream denitrification measurement methods;
- (2) determine how denitrification rates vary with stream stage, discharge, and temperature.

Activities

We initially selected the Sand Creek system in the Lower Minnesota River major watershed for part of our work, but for logistic and technical reasons, we have since selected an alternative site (Beauford Ditch) in Blue Earth County, MN. This site provides better opportunities for mass balance measurements than the Sand Creek system, and is closer (~ 20 miles west) to the Waseca site (which we are still including in our study). Figure 1 shows for site locations. Beauford Ditch discharges are several times larger than those in the Waseca Ditch, allowing us to assess denitrification under higher discharge situations. Beauford Ditch also has an older, downstream section which has vegetation both on the banks and in-stream, as well as a new ditch section upstream of the vegetated section that was constructed in 1999. This upstream section is devoid of any vegetation. Comparison of the two sections with respect to mass balance and stable isotope analysis will allow us to assess the impact of in-stream vegetation on nitrogen dynamics. We since installed three piezometers adjacent to the ditch at each of the three locations.

Although our project starting date was September 2000, we were unable to find and hire a suitable research assistant until spring 2001. Fortunately, we found such an individual before the summer 2001 field season began. This individual is involved in the field and laboratory studies underway this summer, and he will continue to be associated with the project in fall 2001. However, he will be paid from a fellowship starting in fall, and thus we have hired an additional research assistant to work on the project beginning in fall 2001.

Our focus for summer/fall 2001 consists of mass balance and stable isotope studies at both the Waseca and Beauford locations. We are conducting 24-hour studies under low and medium flow conditions using autosamplers to collect a series of upstream and downstream samples during four time periods: afternoon, evening, nighttime, and morning. Based on velocity and discharge measurements, we estimate transit time for a given slug of water between upstream and downstream locations. Our goal is to sample the same slug of water downstream that was sampled upstream minutes (or hours) earlier. For example, mid-July transit times between the two Waseca sampling stations (400 m apart) was 160 minutes. We also are collecting and processing water samples along both streams for stable N isotope analysis under low and medium flow conditions this summer and will analyze the samples for $\delta^{15}\text{N}$ values this fall.



Figure 1. The Minnesota River Basin and research sites.

Future Research

We currently are designing mesocosms for in-stream estimation of denitrification rates by use of ^{15}N tracer techniques. We have consulted with our project USGS representative, Frank Triska (Menlo Park, CA) via e-mail and conversations at the February 2000 ASLO conference (Albuquerque, NM) and the June 2000 NABS conference (La Crosse, WI). Light and dark mesocosms will be fitted with battery-powered stirrers for field use. We are also building probes to monitor sediment redox changes over 24-h periods according to depth into the stream bottom sediments. Additional consideration is being given to conducting a solute dynamics study at the Waseca site using a combined chloride/nitrate drip approach. We anticipate that the new research assistant initially will focus on collecting sediment cores at both the Waseca and Beauford locations for use in laboratory experiments on factors affecting denitrification rates in the stream sediments. These experiments will use ^{15}N tracer additions and the acetylene-blockage method to measure rates of denitrification in the intact cores.

Information Transfer Program

The Centers biennial water conference, Minnesota Water 2000, was held at the Minneapolis Convention Center in May 2000. The theme of the conference related to the dawn of a new millennium: A Watershed Year: Looking Back; Planning Ahead. Over 350 people participated in the two-day affair, which featured Bob Hirsch, Chief Hydrologist of the USGS as a keynote speaker. A wide variety of breakout sessions and posters complemented the plenary talks by national and state authorities on water resources.

The Center published four issues of Minnogram, its quarterly newsletter, during FY 2000. Positive comments continue to be received about its appearance and the relevance and timeliness of the information it contains; continuing efforts are made to include articles with significant depth as well as news of interest to water-related professionals in Minnesota. A major overhaul of the WRC and WRS web pages was begun in 2000, and efforts to provide more useful features are continuing in 2001. Fact sheets and videos describing shoreland best management practices were developed and combined with an expanded volunteer training program for shoreland owners. A biennial report covering the activities of the Center for academic years 1998-99 and 1999-2000 was prepared in FY 2000 and published early in FY 2001.

Numerous other outreach activities were conducted through the University of Minnesota Extension Water Quality program (another component of the WRC). Highlights are provided below. Significant efforts have been made in the areas of Environmental Quality Incentives Program (EQIP) education, alternative sewage treatment systems, and manure management. The manure management education effort focused on land application of manure. Considerable efforts were expended to inform producers about changes to Minnesota Rules 7020 governing feedlots, so they can assess whether they are in compliance. Producer-oriented education packets were prepared and distributed explaining rule provisions and how to develop nutrient management plans. Over 60 county meetings were held to bring this information to producers.

USGS Summer Intern Program

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	0	0	0	0	0
Masters	4	1	0	0	5
Ph.D.	1	1	0	0	1
Post-Doc.	1	0	0	0	1
Total	6	2	0	0	0

Notable Awards and Achievements

Ken Brooks and Heinz Stefan, WRS faculty members, received the University of Minnesota Distinguished Teaching Award.

Jim Perry, WRS Director of Graduate Studies, received an Award of Merit from the Minnesota chapter of Gamma Sigma Delta, the Honor Society of Agriculture.

Thomas Jabusch, WRS Ph.D. student, was awarded a 2001 Graduate Student Award in Environmental Chemistry from the American Chemical Society.

Stefanie Miklovic, WRS M.S. student, was awarded the Alexander P. and Lydia Anderson fellowship and the Carolyn M Crosby Fellowship from the University of Minnesota.

Edith Mussukuya-Kerre, WRS M.S. student, received a fellowship from GIWA for study in southern Africa.

Brian Huser, WRS Ph.D. student, received a Fulbright-Hays grant for study in Sweden.

Jay Peterson, WRS Ph.D. student, was awarded a Torske Klubben Fellowship for the 2001-2002 academic year.

Barbara Liukkonen, WRC Water Resources Education Coordinator, won a Gold Award from the Association of Natural Resource Extension Professionals for the "Minnesota Shoreland Management Resources Guide" website.

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