Water Resources Research Center

Annual Technical Report

FY 1999

Introduction

The past year was a highly productive period for the University of Minnesota Water Resources Center. Significant accomplishments were achieved in research, education, and outreach. The size of the budgets managed by the Center grew to more than $4 million, and the number of staff grew to about 15 individuals on campus and an additional 10 off-campus extension persons. Approximately 35 Twin Cities faculty were directly involved in the Center's research programs, and more have indirect involvement through the WRC-administered Water Resources Science graduate program. Research grants and fellowships administered by the Center supported 27graduate students and three postdoctoral associates in 1999. Although the entities from which the WRC was formed in 1996 had coalesced into a single operational unit (albeit with distinct parts and programs) by 1998, efforts continued during 1999 to build a greater sense of community and common purpose among the activities and programs that comprise the WRC. Staff additions included a part-time accountant, a second extension educator in the area of water economics and policy, a partnership with the Board on Water and Soil Resources to support partially three educators. In addition, the staff person for the DNR-funded MinnAqua program (a youth fishing education program) joined the WRC and was given office space in the WRC complex. The Center's accomplishments over this review period are described below.

Research Program

After a three-year experiment with a regional grant competition program, the WRRI 104(b) research program was switched back to a program run by the individual state WRRIs in late 1998. This resulted in a Request for Proposals in December 1998 for a grant competition that used a combination of financial resources from the WRRI grant and funds from COAFES to the Center for agricultural Impacts on Water Quality. With combined funds from the two sources, we were able to approve five new research projects for funding beginning in March 1999 (at least two more than we would have been able to support with WRRI funds alone). Progress reports are included below for the five projects plus three projects that were active in 1999 but funded during the previous regional competitions. The Center continued to be successful during 1999 in securing research funding. The Minnesota Department of Natural Resources approached the WRC in spring 1999 for assistance on a study of cumulative impacts of development on lakes and provided $90,000 for a study that involved Pat Brezonik, Jim Perry, and Marv Bauer as co-PIs. Work continued on a grant from the Twin Cities Metropolitan Council to develop a protocol for using 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. The Center's work involving satellite imagery expanded with the funding of a large NASA project (RESAC) to Marv Bauer (ERSAC, Forest Resources); Pat Brezonik and his students have the primary responsibility for the aquatic portions of that project. Work was completed on a task group report funded by the U.S. EPA/NOAA as part of a federal interagency assessment on hypoxia in the Gulf of Mexico. The report concerned effects of nutrient source reductions on water quality in the Mississippi River Basin and on hypoxia in the Gulf of Mexico. It was submitted in November 1998, and after extensive peer review and editing, the final report was completed in April 1999. An

interdisciplinary group of faculty continued to study issues of spatial scale in watershed management work as part of a large grant from NSF-EPA's Water and Watersheds program, which began in fall 1996. The focus of the study is on the Minnesota River Basin; faculty associated with the project are members of the WRS graduate faculty, and most of the students supported by the project are majors or minors in the WRS program. Ongoing research funded by the WRC's Center for Agricultural Impacts on Water Quallity during the reporting period includes hydrologic modeling efforts of John Nieber (Biosystems and Agricultural Engineering), on-site wastewater treatment work of Jim Anderson (Soil, Water, and Climate), MSEA work by Robert Dowdy and John Lamb (SWC), and pesticide work by Bill Koskinen (SWC). Grants totaling over $830,000 were obtained from the Metropolitan Council, MPCA, NRCS by the Extension and CAIWQ components of the WRC in 1999. They involve a wide range of topics, including research and 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 nonpoint source pollution, developing new educational materials for shoreland management, and increasing citizen participation in watershed management. **Academic activities** (Note: this section should be in a separate section on education or in text under student support, but there is no text option under that section. I realize that most WRRIs don't have an education component, but a few of us do.) The Water Resources Science program continued to grow. The number of students active in the WRS program during academic year 1998-99 reached ~75 students, of whom about one-fourth are located in Duluth and the rest in the Twin Cities. Enrollment remained between 70 and 80 students (depending on time of the year) during academic year 1999-2000. About 25 students are in the Ph.D. program, and 50 are M.S. students. The quality of students continues to be very good. All students in the program have faculty advisors, and almost all students receive some kind of financial support. About two-thirds are being supported as 25-50% RAs. Thirteen students completed their M.S. work before August 1999, and six more finished during fall 1999. The WRS water policy class had enrollments of 30 students in winter quarter 1999 and 28 in spring semester 2000. The program continues to receive plaudits from visitors to its web site, and without doubt this has been an important factor in attracting applicants to the program. WRS has an active student organization that promotes social and professional activities for WRS students.

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Remediation of Atrazine Contamination in Municipal Drinking Water
Project Number	C-06
Start Date	09/01/1997
End Date	02/28/2000
Research Category	Biological Sciences
Focus Category #1	Toxic Substances
Focus Category #2	Water Supply
Focus Category #3	Treatment
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Michael J Sadowsky	Professor	University of Minnesota	01
Lawrence P Wackett	Professor	University of Minnesota	02

Problem and Research Objectives

Atrazine is a widely used herbicide for the control of broadleaf weeds. It is the predominant member of a broad class of *s*-triazine herbicides which are used to control weeds in corn, sorghum and other crops. Atrazine is relatively persistent in soils with an average half-life ranging from 4 to 57 weeks. Because of its widespread use over the last thirty years, for both selective and nonselective weed control, atrazine and other *s*-triazine derivatives have been detected in soils and in ground and surface water in most states in the Midwestern U.S. The median concentration of atrazine in 132 streams during post?planting periods was 3.8 ppb, which exceeds the EPA maximum contaminant level goal for this herbicide (3.0 ppb). Consequently, many community water supplies in the Midwestern corn belt may be in violation of the revised Safe Drinking Water Act with respect to atrazine and other triazine herbicides. Many small drinking water treatment plants in the Midwest and other regions of the U.S. are not equipped to eliminate atrazine from drinking water, since removal of atrazine from ground and drinking water requires expensive chemical adsorption procedures, usually using activated charcoal. However, federal mandates, health concerns, and public interest requires that potable drinking not contain more than 3 ppb of atrazine. Consequently, rapid, inexpensive, and effective atrazine removal from drinking water supplies is needed. The research proposed below addresses this major national problem by developing an effective atrazine remediation technology. While results from initial research studies will be targeted towards small municipal drinking water suppliers, the technology to be developed will be equally useful for larger municipal suppliers of drinking water. If successful, this proposed technology will address these concerns by providing an effective and inexpensive method to remove atrazine from drinking water. In 1995, we reported the isolation of a pure bacterial culture, identified as *Pseudomonas* sp. strain ADP, which degraded a high concentration of atrazine (>1,000 ppm) under growth and non-growth conditions. Pseudomonas sp. strain ADP uses atrazine as a sole source of nitrogen for growth and the organism completely mineralizes the *s*-triazine ring of atrazine under aerobic growth conditions. We have cloned and expressed the atrazine-metabolizing genes from *Pseudomonas* sp. ADP in *Escherichia coli* and delineated the degradation genes and their products. This has revealed the metabolic pathway, provided sequence data to elucidate the evolutionary derivation of the genes, and allowed for enzyme overproduction to facilitate purification. The first metabolic step in the *Pseudomonas* sp. ADP atrazine degradation pathway is carried out by atrazine chlorohydrolase (AtzA) and yields hydroxyatrazine. This represents the best possible step from an environmental remediation standpoint, since hydroxyatrazine is non-phytotoxic, non-regulated, and is accepted to be non-toxic and non-carcinogenic. mammals. The research described below is designed to bridge the gap between basic research that has developed promising water resource remediation technology to its application in treating municipal drinking water, principally focused in the Midwestern Cornbelt. The research will provide us with (1) fundamental data on the atrazine hydrolyzing enzyme's performance under actual field water conditions, and (2) the beginnings of technology for scale-up for enzyme production for use a drinking water treatment facilities. In the studies outlined below, we began to explore the remaining issues in enzyme use that are needed to move the technology forward from the laboratory stage to applications at municipal drinking water treatment facilities. To do this, AtzA performance evaluations, done under environmental conditions found in contaminated drinking water supplies, need to be conducted. This will not be done by commercial companies, but we feel that these experiments are

crucial for forwarding the application of enzymes in water treatment most broadly. These studies will allow us to rapidly move from laboratory and pilot-scale experiments to atrazine remediation technologies for use at drinking water reservoirs and treatment facilities.

Methodology

Experiments were performed at the Department of Soil, Water, and Climate; the Department of Biochemistry; and the Biological Processes Technology Institute at the University of Minnesota. Atrazine degradation studies using bench and pilot scale water treatment facilities were done at Montgomery-Watson, Minneapolis, MN. All studies used purified atrazine chlorohydrolase (AtzA). Large scale batches of enzyme were purified from 200 liters cultures of cells grown at the Biological Process Technology Institute. The atrazine chlorohydrolase was purified from cell-free extracts of *E. coli* (pMD4) by precipitation with 28% (w/v) NH_4SO_4. AtzA was further purified using a DEAE-Macroprep column (BioRad, Hercules, CA). Fractions containing peak atrazine chlorohydrolase activity were pooled and stored at -70°C until use. Three batches of enzyme (purified according to the same protocol at different times) were required to complete all tests. The concentration of each batch was between 1 and 2 mg protein/mL. The activity of the first and third lots was comparable. The second lot showed a lower activity; only the process simulation experiment was performed using this enzyme lot. Initial studies were designed to characterize the performance of the enzyme atrazine chlorohydrolase for use in various stages of the drinking water treatment process. Initial testing was designed to determine the amount of enzyme required to dechlorinate atrazine in raw water from 20ppb to 3 ppb in 30 minutes. Three enzyme concentrations (10, 1, and 0.1 mg/L Atz A) were tested in 60 mL raw water spiked with atrazine to about 20 ppb. This test was run for 30 minutes with constant mixing on a magnetic stir plate. 10 mL samples were drawn at t = 0 and t = 30 min. Each sample was immediately treated with heat (2 minutes at 100°C) in order to arrest enzyme activity. Results of this study indicated that 1.0 mg/L Atz A enzyme was sufficient to meet our goal. Next, the test was repeated using 1.0 and 0.5 mg/L Atz A enzyme in 250 ml raw water spiked to 20 ppb atrazine. 10 mL samples for atrazine analysis were taken at intermediate time points (t = 0, 5, 10, 17, 25, and 30 min.) in order to establish the time course of atrazine removal. **Effect of Raw Water and Chemicals on Atz A Activity.** Untreated water from a surface reservoir used as the drinking water supply in Vandalia, MO was shipped to the Montgomery Watson Laboratory in Plymouth, MN. Most tests were performed with 1 L volumes of raw water amended with atrazine to a final concentration of about 20 ppb. Two liter Pyrex beakers were used as test vessels. Rapid (80 rpm) and slow (30 rpm) mixing were provided using a six paddle stirrer (Phipps and Bird, Inc., Richmond, VA). Water treatment chemicals were obtained from Hawkins Chemicals, Inc. (Minneapolis, MN): commercial strength (38%) ferric chloride; commercial alum (equivalent 49% dry alum, $Al_2(SO_4)_3 \cdot 14H2O$); powered activated carbon; and hydrated lime.

Testing began when the appropriate treatment chemical was added to the test vessel and mixed rapidly (80 rpm) for 15 seconds. Atz A enzyme was added and rapidly mixed for an additional 15 seconds. The mixing speed was reduced to 30 rpm for the next 25 minutes and 5 minutes were allowed for settling. Ten ml samples were taken for atrazine analysis and heat treated at t = 0 and t = 30 min. Samples were stored at 4°C until analyzed. Separate 1 mL samples were taken for Atz A enzyme analysis at t = 30 sec (immediately after the mixing speed was reduced) and t = 30 min. These samples were frozen until analyzed. Raw sample water was spiked with atrazine prior to testing. Although the intended initial atrazine concentration was 20 ppb, analysis showed that it was less than 15 ppb. Tests were run for a duration of 80.5 min. During the first 30 sec, samples were mixed rapidly (80 rpm). This was followed by 20 min of flocculation, or slow mixing (30 rpm). Finally, samples were allowed to settle for 1 hour. Samples treated with enzyme received Atz A at a concentration of 0.5 mg/L. Enzyme was added at one of two possible time points during testing: t = 0 (before rapid mix) or t = 20 min (prior to settling). Treatment chemicals were added to test volumes immediately before testing was begun. Ten ml samples

for atrazine analysis were taken before addition of treatment chemicals and at t = 80 min. All samples were heat treated immediately after drawn from the test vessel. One ml samples for Atz A enzyme analysis were taken at 25 and 75 min. These were frozen until analyzed. A range of concentrations was tested for each treatment chemical. The only test run without enzyme was the no chemical, no enzyme control. Chemical combinations were not tested. Ten ml samples for atrazine analysis were drawn before the addition of treatment chemicals and at 80 minutes after addition. All samples were heat treated immediately after drawn from the test vessel. **Filtration Simulation.** Anthracite and sand, both previously seasoned as filter media, were obtained from the municipal water treatment plant in Minneapolis, MN. Average particles size for anthracite and sand were 0.9 mm and 0.35 mm, respectively. Nonionic polyacrylamide (Hawkins Chemcials, Inc., Minneapolis, MN) was used as filteraid. Columns for the filtration study were constructed using 1000 ml, 2 inch I.D. glass burettes. Nylon mesh was placed in the bottom to prevent filtration media from clogging the outlet. Glass beads (1 - 5 mm dia.) occupied the bottom 2 - 3 inches of the column. After inserting a disk of nylon mesh on top of the beads, a total of 18 inches of pre-washed filtration media was packed into the column. The column was packed with constant flow of water and backwashed to eliminate trapped air bubbles. The total mass of sand and anthracite used to pack the column was 1224 g and 650 g, respectively. Sample water was fed into the column through a siphon controlled with a stopcock. The feed rate was adjusted to 250 mL/min prior to each test. A total of 5050 mL of sample water was passed through the column for each test. Columns were flushed with additional sample water between tests. Each 50 mL sample was split: 10 mL for atrazine analysis, and 1 mL for Atz A enzyme analysis. Samples for atrazine analysis were heat treated, and stored at 4°C until analyzed. Atz A samples were frozen until analyzed. **Sample Analysis.** Atrazine was assayed using EnviroGardä Triazine Plate Kit (Strategic Diagnostics, Inc., Newark, DE). Absorbance was read at 450 nm using a Microwell Strip Reader (Bio-Tek, Winooski, VT). The Envirogardä triazine immunoassay was run according to manufacturer's guidelines. All samples were run in duplicate against a set of standards unique to each run. Immunoassays for enzyme concentration were performed in 96-well polystyrene microtiter plates (Nalge Nunc International, Rochester, NY). Anti-Atz A rabbit IgG was produced and purified (18). Alkaline phosphatase conjugated to goat anti-rabbit IgG (Sigma, St. Louis, MO, cat# A3687) served as secondary antibody. p-Nitrophenyl phosphate was used as colorimetric substrate (Sigma, St. Louis, MO), and its absorbance was measured at 405 nm. Sensitivity of the enzyme assay ranged from 0.01 to greater than 0.5 mg/L.

Principal Findings and Significance

Summary: 1) An adequate enzyme concentration to decrease atrazine in the test water from 20 ppb to about 3 ppb in 30 minutes was found to be 0.5 mg/L of Atz A. This enzyme concentration was used throughout the study, except during the filtration study. 2) Atz A enzyme treatment was very effective in the presence of all treatment chemicals, except for lime. Alum, ferric chloride, and PAC all appear to have no deleterious effects on enzyme activity. PAC tests did not show atrazine removal beyond that of the no-chemical control. The effectiveness of the enzyme in the presence of lime was about 25% less than the no chemical control. 3) Atrazine disappearance curves for each treatment showed steady removal of atrazine in the presence of alum, ferric, and PAC. The rate of degradation may be slowed by ferric chloride, although the final atrazine concentration after 30 min is comparable to that of the no-chemical control. 4) The point in the treatment process at which enzyme is added appears to have no effect on final atrazine concentrations. 5) During filtration, there appears to have been minimal adsorption of either atrazine or enzyme to the filtration media. As far as the results are able to show, the enzyme eluted from the column at about 500 mL after loading, possibly earlier for the sand medium. Coal medium held the enzyme longer, but only slightly. By 1000 mL after enzyme loading, nearly all enzyme was removed from the sand column. **Stability of Cross-linked *E. coli* cells over-expressing**

Atrazine Chlorohydrolase. Another approach to remediate atrazine-contaminated water is to apply killed recombinant *E. coli* cells which over-expressed AtzA to drinking and surface waters. This is essentially the same as adding a "bag of enzyme" to atrazine-contaminated water. The killed *E. coli* represent the "bag," and we have shown that atrazine continually permeates killed cells and is reduced to hydroxyatrazine by intracellular AtzA. This alleviates problems associated with enzyme purification and distribution of enzyme within water systems. Several cell surface cross-linking agents have been used to kill cells and have been evaluated for alteration in enzyme activity. While cross-linking agents can cause some loss in AtzA activity, the residual activity is extremely stable. There was very little loss of activity even after overnight incubation of cells at 55^{o}C and while untreated cells were more stable to temperature than free enzyme, and cross-linked cells were more stable still. Activity of the untreated cells peaked at 45^{o}C and of the cross-linked cells at 65^{o}C. Moreover, the cross-linked cells are stable at room temperature in buffer for many months. We currently are evaluating the maximum time for enzyme stability in this state. There was a remarkable difference in the stability of the AtzA activity in whole cells harvested in stationary phase vs harvested in exponential phase. The activity of the cells per g of cells was the same for the exponential and stationary phase cells, but the activity was much more stable to heat in the stationary phase cells. Cells retained more activity if cross-linked in borate buffer and the presence of 0.1 to 0.4 M NaCl in the cross-linking buffer also helps to maximize the retention of enzyme activity. Initial soil studies have shown that the cross-linked cells are very effective at remediating atrazine contaminated soil (see manuscript by Strong). **Improvement of AtzA Activity.** To make it economical to use recombinant *E. coli* to degrade atrazine in water, we are going to have to increase the specific activity over what we have now with wild type atzA in *E. coli* (pMD4). To do this, we performed mutagenesis by DNA shuffling of the wild type gene and selection for improved atrazine degraders. The gene was amplified from the plasmid by PCR. About 8,000 colonies were plated. Colonies which created clearing zones the fastest were marked, picked, and grown in 1.5 mL each of liquid medium. Cells from each culture were pelleted, washed with buffer, resuspended in buffer, and then assayed for atrazine degradation velocity in a liquid assay. About 60 colonies were tested. Most were faster than the wild type, with the fastest being more than four times as fast as the wild type. The DNA from 24 improved mutants have been pooled together for a second round of mutagenesis. This work is currently in progress. However, a new variant of atzA expressing five times more activity than the wild-type strain has been isolated using this procedure. A manuscript on this work is in progress.

Descriptors

Articles in Refereed Scientific Journals

Strong, L. C., McTavish, H., Sadowsky, M. J., and Wackett, L. P. 2000. Field-scale remediation of atrazine-contaminated soil using recombinant Escherichia coli expressing atrazine chlorohydrolase. Environ. Microbiol. 2:91-98. Mattan, C., DiSteno, F., Wackett, L. P., McTavish, H., and M. J. Sadowsky. 2000. Enzymatic dechlorination of atrazine in a simulated drinking water treatment process. Environ. Sci Tech.: To be Submitted. Sadowsky, M. J., Z. Tong, M. de Souza, and L. P. Wackett. 1998. AtzC is a new member of the amidohydrolase protein superfamily and is homologous to other atrazine-metabolizing enzymes. J. Bacteriol. 180:152-158. de Souza, M. L., D. Newcombe, S. Alvey, D. E. Crowley, A. Hay, M. J. Sadowsky, and L. P. Wackett. 1998. Molecular basis of a bacterial consortium: interspecies catabolism of atrazine. Appl. Environ. Microbiol. 64:178-184. de Souza, M. L., J. Seffernick, B. Martinez, M. J. Sadowsky, and L. P. Wackett. 1998. The atrazine catabolism genes atzABC are widespread and highly conserved. J. Bacteriol. 180:1951-1954. de Souza, M. L., L. P. Wackett and M. J. Sadowsky. 1998. The atzABC genes encoding atrazine catabolism genes are located on a self-transmissible plasmid in Pseudomonas strain ADP. Appl. Environ. Microbiol. 64:2323-2326. Boundy-Mills, K. L., M. L. de Souza, L. P. Wackett, R. Mandelbaum, and M. J. Sadowsky. 1997. The

atzB gene of Pseudomonas sp. strain ADP encodes hydroxyatrazine ethylaminohydrolase, the second step of a novel atrazine degradation pathway. Applied Environ. Microbiol. 63:916-923. de Souza, M. L., M. J. Sadowsky, and L. P. Wackett. 1996. Atrazine chlorohydrolase from Pseudomonas sp. ADP: gene sequence, enzyme purification and protein characterization. J. Bacteriol. 178:4894-4900. de Souza, M. L., L. P. Wackett, K. L. Boundy-Mills, R. T. Mandelbaum, and M. J. Sadowsky. 1995. Cloning, characterization, and expression of a gene region from Pseudomonas sp. strain ADP involved in the dechlorination of atrazine. Appl. Environ. Microbiol. 61: 3373-3378.

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Wackett, L.P., M. J. Sadowsky, M. L. de Souza, and R. T. Mandelbaum. 1998. Atrazine hydrolysis by a bacterial enzyme, pp. 82-87. In: Triazine Herbicides: Risk Assessment, L. Ballantine, J. McFarland, and D. Hackett (eds.), American Chemical Society, Washington, D.C. Sadowsky, M. J., L. P. Wackett, M. L. de Souza, K. L. Boundy-Mills, and R. T. Mandelbaum. 1998. Genetics of atrazine degradation in Pseudomonas sp. strain ADP, pp. 88-94. In: Triazine Herbicides: Risk Assessment, L. Ballantine, J. McFarland, and D. Hackett (eds.), American Chemical Society, Washington, D.C.

Other Publications

Patents. Four patents have been filed by the University of Minnesota concerning this technology, one has issued. The patents cover technology related to the use of cells and enzyme for treating atrazine? contaminated water, including "improved" enzyme variants. A fifth patent disclosure document has been filed with the University of Minnesota concerning technology derived from this project. The technology involves the creation of cross-linked cells containing atzA for remediating atrazine contamination in groundwater and soils. The University is currently evaluating a request by Hugyh McTavish, the inventor, to determine whether a patent will be filed covering this technology.

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Organic Matter Binding and Photoreduction of Mercuric Ion and Methylmercury in Surface Water
Project Number	C-07
Start Date	09/01/1998
End Date	02/28/2001
Research Category	Water Quality
Focus Category #1	Toxic Substances
Focus Category	Hydrogeochemistry

#2	Hydrogeochemistry
Focus Category #3	Surface Water
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Patrick L. Brezonik	Professor	University of Minnesota	01
Paul R. Bloom	Professor	University of Minnesota	02

Problem and Research Objectives

Mercury (Hg) pollution is a global-scale problem with both ecological and human health implications. Thousands of lakes in Europe, Canada and the United States, many in remote and pristine 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, and especially common in the soft-water, acid-sensitive lakes of northeastern Minnesota, northern Wisconsin, and Upper Michigan. For example, the State of Minnesota has issued fish consumption advisories for more than 500 lakes because of elevated Hg levels in one or more species of game fish. Even this number reflects the relatively small fraction of Minnesota lakes from which fish have been collected for Hg analysis rather than an accurate estimate of the magnitude of the problem in the state. Although the primary concern with elevated Hg levels in fish relates to human health effects, wildlife, especially water fowl and top predators, also can be impacted negatively by elevated Hg levels in fish, as has been demonstrated by studies in such disparate areas as northern Minnesota and the Florida Everglades. In spite of significant advances 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 fossil fuel combustion and incinerators are thought to be major emission sources for Hg. Nonetheless, the occurrence of elevated Hg levels in fish of lakes within a small geographic area is patchy, despite the regional homogeneity in atmospheric loading. Among the water chemistry factors thought to be responsible for these differences in lake responses, interactions between Hg forms and natural organic matter (e.g. humic material) are perhaps the most important. Unfortunately, our understanding of Hg-organic matter interactions is based mostly on qualitative observations and statistical correlations, and quantitative, mechanistic information is lacking on this key issue. This study is focused on quantifying the strength of binding (chemical complexation) of Hg^{2+} and CH_3Hg^+ to natural dissolved organic matter (NDOM) and determine the chemical nature of the binding. We also will evaluate the role of complexation by NDOM in mediating the photoreduction of these Hg species to elemental Hg, which is volatile and thus is subject to subsequent efflux from water bodies. A full understanding of the transport and transformations of Hg in natural waters requires quantitative information on these processes. Results of the proposed study will greatly aid in modeling of the behavior of Hg in surface waters and in understanding the bioavailability of Hg bound to NDOM. The specific objectives of this project are to: (1) determine the strength of chemical binding of Hg^{2+} and CH_3Hg^+ by NDOM and its component humic matter as a function of solution conditions and origin of the NDOM and determine the importance of sulfur (thiol) and carboxyl binding sites in binding the Hg forms. (2) determine the

importance of NDOM complexation in photochemical reduction of Hg^{2+} and CH_3Hg^+ to Hg^o.

Methodology

Sample collection and characterization. Large volumes (20-40 L) of water samples were obtained from three peatlands (bogs and fens) and three lakes in the Marcell Experimental Forest (~30 miles north of Grand Rapids, MN) by clean procedures. Raw water was filtered through 0.7 µm pre-fired glass fiber filters followed by concentration of the fulvic acid on DEAE cellulose columns (Miles et al. 1983). Fulvic acid was removed from the columns by dilute NaOH, acidified using H^+-saturated cation exchange columns, and freeze dried. Total Hg in extracted NDOM samples was determined after photooxidation/digestion by atomic fluorescence spectroscopy. NDOM was analyzed for total organic carbon (Leco CR-12 Carbon Determinator), total sulfur (Leco CR-132 Sulfur Determinator), total N (persulfate digestion). Total acidity was determined by titration. The relative quantity of reduced S in the fulvic acid and NDOM samples is being determined by XANES and EXAFS x-ray spectroscopy..

Determination of binding constants for NDOM with Hg2+ and CH_3Hg^+. Binding constants are being determined for NDOM using a modification of the Br^- complexation method used to determine K_d values in soil organic matter (Skyllberg et al. 1997). A reference sample of International Humic Substances Society Suwannee River fulvic acid is included for comparison. Solutions containing 200 mg/L of NDOM are prepared in 0.3 M KBr and adjusted to the desired pH using dilute KOH. The desired quantity of Hg^{2+} or CH_3Hg^+ is added, and 50 mL of the solution is transferred to a 500 MWCO cellulose ester dialysis tubing. The quantity of Hg added is adjusted to encompass a range of NDOM-bound Hg from less than typical Hg concentrations in lake waters to several times that concentration (e.g. ~10-200 pg/L for methylmercury; ~0.2-10 ng/L for mercuric ion). After tying the end, the tubing is placed in a 250 mL Teflon bottle containing 150 mL of 0.3 M KBr. The bottles are agitated gently, and the external solution is sampled for Hg after sufficient time for equilibration. Concentrations of Hg^{2+} in the internal and external solutions are determined by CVAFS (Bloom and Fitzgerald, 1988) in a clean room. Methylmercury is determined by the method of Bloom (1989). NDOM in the external solution is determined with a Dorhmann DC-80 analyzer. The quantity of Hg complexed by Br^- and activity of Hg forms will be calculated using MINTEQA2. Apparent binding constants will be determined over a pH range of 3 to 7 using the computer program FITEQL (Herbelin and Westall, 1995). **Photochemical Processes and Hg Transformations.** Experimental work on Hg photochemistry is focusing on photoreduction reactions forming Hg^o (also called dissolved gaseous mercury or DGM) from oxidized Hg forms because this is a potentially important loss process for Hg in aquatic systems. Photooxidation of DGM also is being explored as a potential source of bioavailable Hg in water. Experiments are conducted in the laboratory under controlled conditions of light, temperature and water chemistry using photoreactor facilities that include a merry-go-round reactor, uv light source, and gc/hplc instrumentation to measure concentrations of chemical probes. In order to measure rates in the laboratory in convenient incubation times, it is necessary to use light intensities greater than those in the ambient environment, but experiments are done in ways that allow extrapolation to ambient conditions. For methylmercury photodegradation and reduction of Hg^{2+} to DGM, we are determining the role NDOM in accelerating or inhibiting rates, as well as evaluating the role of photo-intermediates like hydroxyl radicals, singlet oxygen, and hydrogen peroxide, which are produced in the photolysis of aquatic humus. Because of their low concentrations, we use chemical probes (butyl chloride for hydroxyl radicals) and furfuryl alcohol for singlet oxygen) to measure steady-state concentrations of the photo-intermediates.

Principal Findings and Significance

Much of the first year of the project was spent collecting and processing water samples and characterizing the isolated aquatic humic matter (AHM) from the samples. Large volumes (~40 L) of water were collected and processed to obtain sufficient quantities of AHM for characterization and for the Hg binding and photochemical experiments. Water was obtained and processed from three colored lakes in the Marcell Forest and from three peatlands (a low-pH bog, a high-pH fen, and an intermediate-pH fen). The isolated AHM samples have been characterized in terms of elemental analysis (C, N, S), concentration of acidic functional groups, trace metal content, and ^{13}C NMR spectroscopy for structural features. Samples have been prepared for EXAFS and XANES analysis of sulfur speciation at Brookhaven National Laboratory later this summer. This is critical information because we believe that sulfur groups are the binding sites for mercury forms in AHM. Binding studies of mercury with the isolated AHM samples are scheduled to begin in late summer. Substantial efforts were put into upgrading our Hg analytical facilities during the first year; this included purchasing a new Hg AFS analyzer and setting up a distillation system for separating methylmercury from samples prior to analysis. Experiments have been conducted on mechanisms of methylmercury photolysis, and we have obtained evidence that hydroxyl radicals play a significant role in this process in the laboratory. Field experiments are underway to verify that this mechanism can account for observed rates of methylmercury photolysis in lakes. Additional field studies are underway to measure rates of elemental mercury (DGM) production from both mercuric ion and methylmercury. Laboratory experiments using model compounds have been conducted to determine the nature of the functional groups in AHM that may act as reducing agents for oxidized Hg forms. Additional laboratory experiments using model compounds have been conducted to determine the nature of the functional groups in AHM that may act as reducing agents for oxidized Hg forms. **References.** Bloom, N.S. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can. J. Fish. Aquat. Sci. 46: 1131-1140. Bloom, N.S., and W.F. Fitzgerald. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapor atomic fluorescence detection. Analytica Chimica Acta 208: 151-161. Herbelin, A.L. and J.C. Westall. 1996. FITEQL. Version 3.2. A computer program for determination of chemical equilibrium constants from experimental data. Rept. 96-01, Dept. of Chemistry, Oregon State Univ., Corvallis, OR. Miles, C.J., Jr., J.R. Tuschall, Jr., and P.L. Brezonik. 1983. Isolation of aquatic humus with diethylaminoethyl cellulose. Anal. Chem. 55: 410-411. Skyllberg, U., P.R. Bloom, E.A. Nater, K. Xia, W. Bleam. 1997. Binding of Hg(II) by reduced sulfur in soil organic matter. 4th Inter. Conf. Biogeochem. Trace Elements. June 1997, Berkeley CA.

Descriptors

Articles in Refereed Scientific Journals

None

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Initial results are scheduled to be presented at the biennial conference of the International Humic Substances Society in Toulouse, France in July, 2000.

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Controls on Biomass:Nutrient Ratios in Streams and Rivers
Project Number	C-08
Start Date	09/01/1998
End Date	08/31/2000
Research Category	Biological Sciences
Focus Category #1	Ecology
Focus Category #2	Nutrients
Focus Category #3	Surface Water
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Robert Sterner	Professor	University of Minnesota	01

Problem and Research Objectives

Excessive nutrient input to streams and rivers is one of the major sources of their degradation. Point and non-point sources of nitrogen and phosphorus stimulate algal growth, causing unsightly blooms, elevated BOD, and other problems. Scientists and managers are well aware of the general positive correlation between nutrients and algae, and quantitative modeling of nutrients and algae is relatively advanced in lakes. However, considerable uncertainty remains about the precise quantitative relationship between these two variables, especially in running waters. In studies of aquatic eutrophication, correlations typically are expressed on log-transformed variables, which is a statistically sound procedure, but also tends to visually disguise the large remaining unexplained variation. This project will determine the stoichiometry of conversion of inorganic nutrients into biomass in streams and rivers, while also seeking a mechanistic understanding. Fundamental to any management decision regarding nutrients in streams or rivers is the overall stoichiometry of conversion of inorganic nutrients into biomass. Algae are known to exhibit highly variable stoichiometry between measures of biomass such as carbon or chlorophyll compared to the nutrients P or N. Thus, for example, the C:P ratio of individual algae in culture, or of phytoplankton-dominated seston in lakes, ranges from about 100 to about 1000 (all ratios will be given as mol:mol). This variation means that for a single unit of P in biomass, approximately a tenfold range of total biomass (closely related to carbon) may occur. It can easily be

imagined that in some situations, a C:P stoichiometry near the bottom of this range would indicate little problem with eutrophication, while near the top of this range, considerable environmental degradation might be expected. Hence, understanding carbon:nutrient stoichiometry in surface waters is an issue of great practical importance. Predictive models of nutrient releases such as from treatment plants, agricultural activities such as feedlots, or from generally increased atmospheric deposition of nitrogen, will depend on improved understanding of the C:P stoichiometry in streams and rivers. The objectives of this project are: 1) measure the C:N:P stoichiometry in streams and rivers in Minnesota, relating them in particular to stream order and land use practices; 2) describe the degree of nutrient limitation and how that relates to C:N:P stoichiometry; and 3) test a mechanistic model of how light and nutrients relate to the conversion of N or P into aquatic biomass.

Methodology

The project was divided into two 12-month phases. The first phase examined stream order and the second phase is examining land use. Stream reaches were selected in the Mississippi River drainage north of the Twin Cities. For the stream order phase, reaches of stream orders from headwater streams (order one) to order five were selected, with several replicates of lower order streams as practical. Low order streams minimally impacted by human activities were chosen in this phase. This phase was designed to explore how the light:nutrient balance shifts with stream order, and how that affects the stoichiometry of conversion of nutrients into algal biomass. For the land use phase, several reaches each of forested, agricultural, and urban low order streams are being examined. This phase is designed effects of human practices (lumbering, agriculture, urbanization and drainage, etc.) on algal stoichiometry. Procedures are divided into three components: **A. Determination of C, N and P stoichiometry related to light and nutrient availability.** This component relies on observation and field measurement to infer how nutrients and biomass are related. Total suspended solids (TSS), ash-free dry weights (AFDW), chlorophyll, and particulate CHN & P are measured in suspended matter, periphyton, and sediments, at several sites in each reach, as appropriate. Particulate matter is collected on pre-ashed, acid-rinsed glass fiber filters (Whatman GF/C). Some samples are dried and weighed on a microbalance, and some are ashed, and re-weighed. Others are oxidized with persulfate, and phosphorus is measured. Still other filters are analyzed by a CHN analyzer (Perking Elmer CHN 2400). Samples for chlorophyll are frozen after initial filtration, and chlorophyll is subsequently extracted with 90% acetone, and Chl a fluorescence is measured using a Turner AU fluorometer equipped with Chl a filters. Chemical fractions in the dissolved pool, including SRP, TDP, NO_3^-, TDN, DOC, NH_4^+ and SRSi are measured using standard methods. Light at the substrate surface is measured as a function of distance from shore and water depth using a Biospherical Instruments micro sensor. Multiple measurements are made at each study site. Combined estimates of light available for photosynthesis in the water and at the substrate surface are calculated for each reach. Background information (temperature, pH, dissolved oxygen, current speed) are collected, as well. To derive an independent assessment of P availability, a subset of the periphyton and suspended particles are assayed for alkaline phosphatase activity. **B. Nutrient limitation experiments.** The identity of the limiting nutrient and degree of nutrient limitation is estimated at each reach of lower order streams by use of nutrient-diffusing, agar-filled, clay pot experiments. A control (agar only) and three sets of nutrient amendments are to agar are run: N (0.5 M $NaNO_3$), P (0.05 M Na_2HPO_4), N and P (0.5 M $NaNO_3$ and 0.05 M Na_2HPO_4). Five replicates of each treatment are run (total of 20 pots per experiment). Pots (8.8 cm diameter, 8.0 cm height) are covered with mesh to exclude large grazers. Experiments are run for approximately three weeks. At the end of each experiment, periphyton on the pots is collected by scraping, and Chl a is measured. A subsample of algae is preserved in Lugol's solution for qualitative assessment of algal taxonomic composition. A 2 X 2 ANOVA analysis on chlorophyll growth rates was designed to identify whether the separate nutrients (N or P) or their interaction influenced the algae to different degrees. A comparison of growth in the

controls (algae obtaining nutrients only from the natural stream water) to the nutrient amendments (nutrients also available from the agar) provides a dynamic response variable for the bioavailability of nutrients. **C. Laboratory cultures.** During summer 1999, single species algal isolates of dominant phytoplankton taxa were obtained by use of sterile micropipets. A small culture bank of Minnesota stream periphyton thus was created. Incubations were designed to expose periphyton to known supplies of light and nutrients. Experiments are run on plexiglass "sandwiches" with two parallel plates enclosing a narrow (several mm) space. Sandwiches are incubated at an inclined slope, and medium is delivered at the top of the slope and spent medium is collected at the bottom. By altering the nutrient composition of the inflowing medium and the light climate under which the algae are incubated, we achieve high control over these two variables. Samples for Chl a, CHN, and particulate P is collected and analyzed at several growth stages (log phase, early stationary phase and late stationary phase).

Principal Findings and Significance

The yield of algal biomass per unit nutrient input is a stoichiometrically-variable parameter in lakes and streams. Periphyton C:N:P ratios may depend on abiotic factors such as available light and nutrients. In this project, we are investigating the relationship between the C:P ratio (yield of algae per unit nutrient) of stream periphyton and the relative amount of light and phosphorus available. A higher periphyton C:P indicates a greater yield of algal biomass per unit of available nutrient. We predict that as the light:nutrient ratio increases, the C:P of periphyton will also increase. In summer 1999, we sampled five stream reaches in Clearwater and Hubbard Counties, Minnesota. We measured nutrient concentrations and relative available light. We collected and analyzed rock periphyton and suspended particulate matter for carbon, nitrogen and phosphorus. In addition, we performed experiments with nutrient diffusing artificial substrates in which we manipulated levels of nitrogen, phosphorus and light. The periphyton growth on the substrates is being analyzed for C:N:P and pigment composition. The pigment analysis will provide information on general taxonomic algal composition. Periphyton C:P in an open canopy reach was greater than in a closed canopy adjacent reach, suggesting that light intensity can influence periphyton C:P. Additionally, when looking at all five sites, as light:TP (total phosphorus) increased, both periphyton C:P and suspended matter C:P increased significantly as well. The light:TP had a similar effect on both the periphyton and suspended matter C:P. Disturbed stream ecosystems may produce more algae per unit of nutrient input. When light conditions are altered in a stream ecosystem, the amount of algal biomass produced per unit of available nutrient (periphyton C:P) may increase. We are further investigating controls on biomass:nutrient ratios in streams through a survey in which we are sampling approximately 40 streams in Minnesota. We hope to further elucidate the relationships between light, nutrients and periphyton nutrient composition.

Descriptors

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Balance of Available Light and Nutrients and the Elemental Composition of Stream Periphyton. Andrea

B. Plevan and Robert W. Sterner. Presented at Minnesota Water 2000, Minneapolis, MN, April 25-26, 2000.

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Investigation of a novel biomolecule active in the degradation of common groundwater contaminants
Project Number	B-02
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Engineering
Focus Category #1	Groundwater
Focus Category #2	Toxic Substances
Focus Category #3	Treatment
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Paige Novak	Assistant Professor	University of Minnesota	01

Problem and Research Objectives

Chlorinated solvents are classified as priority pollutants by the United States Environmental Protection Agency and represent a human and ecological health risk. Many remediation technologies exist for the degradation or removal of chlorinated solvents from contaminated groundwater supplies; however, they are not always effective and can be expensive. In addition, if biological treatment is used to remediate a site, the degradation kinetics may be prohibitively slow at groundwater temperatures of 15ºC or below. Additional technologies to degrade chlorinated solvents, particularly ones that are effective at low temperatures, are needed.

Methodology

Principal Findings and Significance

We have found that the methanogen Methanosarcina thermophila secretes a biological catalyst that is capable of rapidly transforming carbon tetrachloride (CCl4) (Novak et al., 1998). It may be possible to develop a new remediation technology using this secreted catalyst to degrade chlorinated solvents in groundwater under conditions that pose problems for other remediation technologies. The research sponsored by the USGS has focused on further characterizing this biological catalyst in order to determine if such a use would be feasible.

The catalyst has been found to rapidly degrade not only CCl4, but also CHCl3 and C2H3Cl3. It also slowly degrades perchloroethene (C2Cl4) and trichloroethene (C2HCl3). The secreted catalyst has an extremely high transformation capacity for CCl4 (Figure 1). Examination of the affect of pH on the activity of the catalyst indicated that activity was greater under alkaline conditions. Degradation of CCl4 and CHCl3 was quite slow at a pH of 5.5 but very rapid at a pH of 8.5. Temperature also impacted the activity of the biological catalyst. Rapid CCl4 degradation occurred from 35-65¨¬C, with activity increasing as temperature increased. The secreted catalyst was also extremely active at low temperatures (Figure 2), which is particularly promising for its use in extreme and inhospitable environments where traditional biological systems do not function well. When oxidized, the biological catalyst did not function; however, reduction of the catalyst after oxidation returned CCl4 degradation activity.

Experiments performed to determine the nature of the biological catalyst showed that it was not a protein. The biomolecule was <10 kDa in size, with degradation activity present in the 1-10 kDa and <1 kDa fractions. Purification of the catalyst(s) by column chromatography through a C18 matrix resulted in the collection of three fractions that possessed dechlorination activity and contained elevated levels of cobalt, zinc, and iron, compared to a medium control and an inactive fraction. One of the molecules in these fractions appears to have a molecular weight of approximately 1400 (based on electrospray ionization mass spectroscopy) and also appears to contain an iron porphyrin (heme) group (based on UV-visible spectrophotometry). Research to further purify all of the active catalysts and elucidate their identity, particularly the catalyst containing zinc, is continuing.

Novak, P.J., Daniels, L., and Parkin, G. F. 1998. Rapid Dechlorination of Carbon Tetrachloride and Chloroform by Extracellular Agents in Cultures of Methanosarcina thermophila. Environ. Sci. Technol., 32:3132-3136.

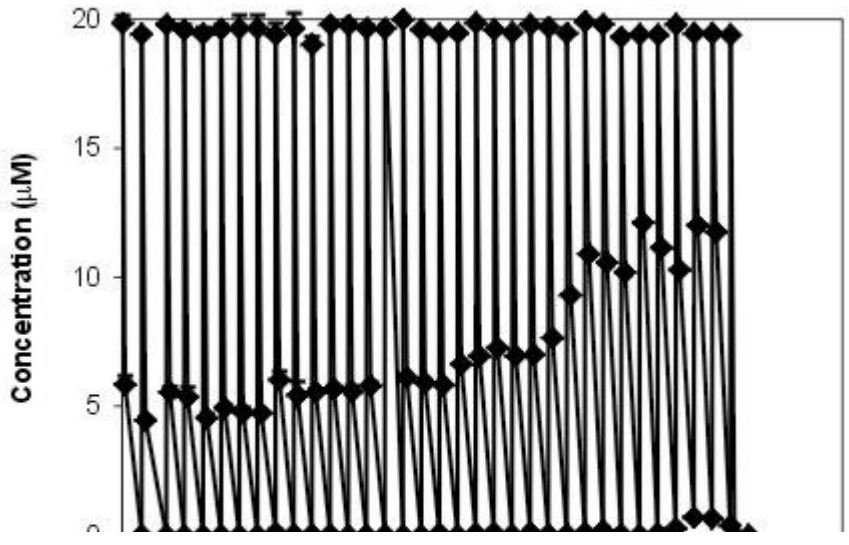

Figure 1.Transformation of CCl, in systems containing filtered (0.22 im) supernatant from *M. thermophila* containing the secreted catalyst; CCl, was added repeatedly (every day from day 0-34).

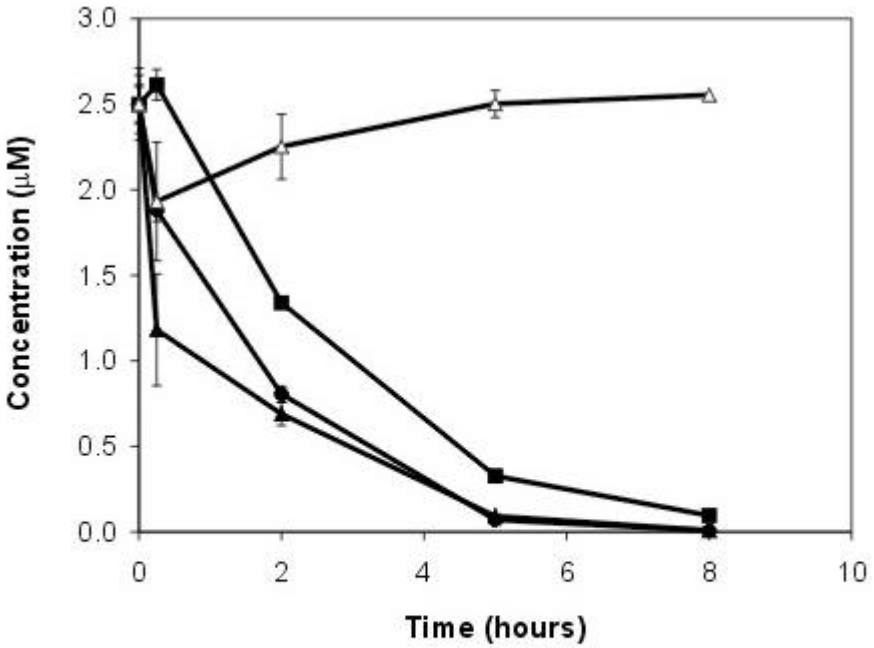

Figure 2.Transformation of CCl, in systems containing filtered (0.22 im) supernatant from *M. thermophila* containing the secreted catalyst incubated at various temperatures; Symbols are filled triange for filtrate at 23°C, filled circle for filtrate at 10°C, filled square for filtrate at 4°C, and hollow triangle for medium control at 23°C.

Descriptors

Articles in Refereed Scientific Journals

Koons, B. W., Baeseman, J. L., and Novak, P. J. 2000. Investigation of Extracellular Biomolecules Active in Carbon Tetrachloride and Chloroform Degradation. Biotechnology and Bioengineering, submitted 4/5/2000.

Book Chapters

Dissertations

Koons, B. W. 1999. “Chlorinated Methane Transformation by a Methanogen-Derived Biomolecule.” M.S. thesis, University of Minnesota.

Water Resources Research Institute Reports

Conference Proceedings

Koons, B. W., and P. J. Novak. 1999. Chlorinated Methane Transformation by a Methanogen-Derived Biomolecule. In Situ and On-Site Bioremediation: The Fifth International Symposium, San Diego, CA, 5(2):41-46. Baeseman, J. L., and Novak, P. J. 1999. "A Novel Biomolecule Active in the Degradation of Common Groundwater Contaminants." Presentation at the Minnesota 2000 Conference, Minneapolis, MN, April, 2000.

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	An investigation of the factors affecting removal of Cryptosporidium and Giardia from drinking water supplies by granular filtration media
Project Number	B-01
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Water Quality
Focus Category #1	Water Supply
Focus Category #2	Surface Water
Focus Category #3	Treatment
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Raymond M Hozalski	Associate Professor	University of Minnesota	01

Problem and Research Objectives

The goal of this project is to evaluate the effects of operational and water quality parameters on the removal of the protozoan pathogens Cryptosporidium and Giardia in granular media filters. Specifically,

the impact of four parameters will be evaluated with a 24 factorial experimental design. These parameters include: 1) the presence or absence of dissolved natural organic matter (NOM) in the water, 2) presence or absence of biofilm on the filter media, 3) filter hydraulic loading rate (5 to 25 m/h), and 4) filter media type (sand or GAC). This work will allow us to develop guidelines for achieving effective removal of pathogenic protozoa from drinking water supplies.

Methodology

The experimental filtration system was setup in summer of 1999 and conservative tracer studies were performed to evaluate the hydraulic characteristics of the filtration system (i.e. hydraulic residence time and dispersion number). A spreadsheet model for predicting filtration performance was developed from the particle mass balance equation of Yao et al. (1971) and the collection efficiency model of Rajagopalan and Tien (1976). The spreadsheet model will be used for evaluation and interpretation of the experimental particle removal data. Preliminary filtration studies were performed with latex microspheres as model particles. The basic purpose of these experiments was to check our experimental methodology by comparing results from a well defined “model” filtration system (spherical particles and spherical filter media) with spreadsheet model predictions. The results from the latex microsphere filtration experiments were in good agreement with the model predictions suggesting that the experimental apparatus and our experimental methods are sound. Finally, other preliminary work completed to date includes the testing and implementation of a method for enumeration of Cryptosporidium parvum oocysts using the immunofluorecence assay. The oocysts are labelled with a fluorescent-antibody, filtered onto a 0.2 mm pore size membrane filter, and then counted by epifluorescence microscopy (Nikon model Eclipse E600 microscope equipped with motorized stage, color video camera and computer with image analysis software).

Principal Findings and Significance

Experiments were performed to evaluate the impact of biofilm coated filter media on the removal of Cryptosporidium oocysts in the laboratory-scale filters. The removal of Cryptosporidium oocysts in a 24.4 cm deep filter bed of 0.5 mm clean glass beads was 60%. However, addition of a biofilm coating on the filter media resulted in a significant decrease in oocyst removal to 23%. This reduction in removal efficiency in the presence of biofilm may have been due to either short circuiting in the filter bed leading to reduced collisions between oocysts and filter media, or a reduction in the probability of attachment (i.e. the collision efficiency), or both. Experiments will be performed to test these hypotheses including another conservative tracer study to evaluate the possibility of short circuiting in the filter bed. The reduction in oocyst removal performance due to biofilm presents some concerns for the ability of biologically-active filters to prevent the breakthrough of pathogenic particles. However, more experiments are needed to evaluate the significance of these early findings. References 1. Rajagopolan, R. and Tien, C. 1976. Trajectory Analysis of Deep-Bed Filtration with the Sphere-in-cell Porous Media Model. AIChE Journal 22(3): 523. 2. Yao, K.M., Habibian, M.T., and O’Melia, C.R. 1971. Water and Waste Water Filtration: Concepts and Applications. Environ. Sci. Technol. 5(11): 1105.

Descriptors

Articles in Refereed Scientific Journals

None

Book Chapters

Dissertations

None

Water Resources Research Institute Reports

Conference Proceedings

None

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Feasibility of controlled drainage to mitigate nutrient loss from tile drainage systems in soutch central Minnesota
Project Number	B-03
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Water Quality
Focus Category #1	Hydrology
Focus Category #2	Water Quality
Focus Category #3	Agriculture
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Gary R Sands	Assistant Professor	University of Minnesota	01
David Mulla	Professor	University of Minnesota	02
Lowell M Busman	Professional Staff	University of Minnesota	03

Problem and Research Objectives

The goal of the controlled drainage research project is to determine the technical feasibility of maintaining or increasing crop yields while reducing nutrient losses from subsurface (tile) drainage systems.

Methodology

The previous 12 months have been spent planning and installing the drainage system and instrumentation for four controlled drainage plots at the Agro-Ecological Research Farm (AERF) at the Southern Research and Outreach Center in Waseca, Minnesota. Figure 1 illustrates the features of the controlled drainage plot design. Two plots are equipped with outlet control structures and two are conventionally drained. Several innovations have resulted during project design and installation. Perhaps the most important of these is the development of large tipping buckets for measuring drainage flow.

Principal Findings and Significance

Currently, a Biosystems and Agricultural Engineering Masters student (Figure 2) 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. This model was just recently upgraded by its developers to include frozen soils and snowmelt computations, and has been released to the project investigators for further testing. Preliminary modeling results indicate that for a 30-year historical weather record (southern Minnesota), watertable management can produce effects on crop yield and drainage water loss from the system (Table 1). It remains to be seen if these modeling results can be substantiated with field data. Preliminary modeling results and project plans will be presented at the 2000 Annual International ASAE Meeting, in early July (Milwaukee, WI).

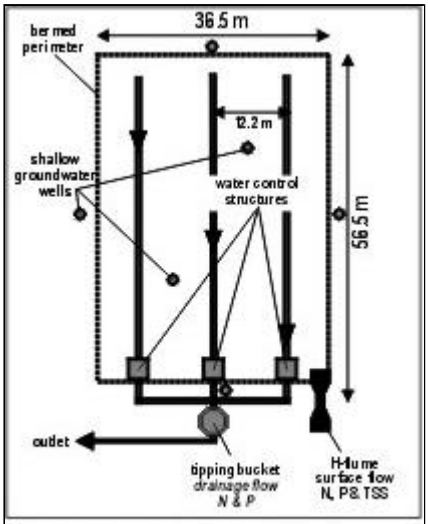

Fig 1. Schematic of controlled drainage plot design.

Table 1. Controlled Drainage Compared to Conventional Drainage (preliminary modeling results)

Predicted Change in Average Annual Values

Infiltration Volume	6.3%	decrease
Evapotranspiration Volume	12.6%	increase
Drained Volume	31.8%	decrease

Fig 2. One of four newly installed H-flumes for measuring surface runoff. Berms can be seen extending away from the wing-walls of the flume. Above, BAE Masters student, Gary Feyereisen, works on installation of the flume.

As of February 2000, drainage pipe, access stations, watertable control structures, buried cable, monitoring wells, and surface runoff flumes had been installed in the field. Figures 2 through 4 illustrate some of these activities and equipment. Data collection is expected to commence during the summer, 2000, to include water quantity and water quality data, along with watertable height, crop yield, and soil temperature and moisture data. Publication of the first-year results is planned for late 2000 or early 2001. An additional year of data collection and more extensive modeling are planned within the current project scope.

One of the milestones during 1999 was a two-day field event held in early August in Waseca. In attendance were farmers, contractors and researchers from in and out of state, including the leading drainage researchers from around the U.S. A workshop was held to discuss drainage issues and communicate the drainage research plans at AERF. On the second day of the event, members of the Minnesota Land Improvement Contractors Association donated their services to install the drainage systems on the research farm.

Fig 4. Installation of a water control structure. At left, inflow and outflow pipes are visible at the bottom of the structure. At right, one of the baffles that are used to set water height, is removed.

Descriptors

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

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

Other Publications

Sands, G.R. Controlled Drainage.1999 Agricultural Drainage Workshop & Field Day, Southern Research and Outreach Center, Waseca, MN.

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Characterizing the fate of Nitrogen fertilizer to improve Nitrogen use
Project Number	S-05
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Water Quality
Focus Category #1	Agriculture
Focus Category #2	Nutrients
Focus Category #3	Non Point Pollution
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Carl Rosen	Professor	University of Minnesota	01
Michael Ruselle	Professor	University of Minnesota	02
Satish D Gupta	Professor	University of Minnesota	03

Problem and Research Objectives

Potatoes are usually grown on coarse-textured soils that are not capable of sustaining high tuber yields and quality without fertilizer and water application. The high N and water amounts applied to meet crop demand often result in leaching of nitrate and consequent contamination of groundwater. The overall objectives of this study were to (1) evaluate the efficacy of a urea-based polyolefin-coated fertilizer (POCU) to reduce NO_3^- leaching while improving tuber yield and quality in a glacial outwash soil under irrigated potato production and (2) determine the fate and recovery of N from POCU compared to conventional urea using the ^{15}N-enrichment method. This was a two-part study encompassing a small plot experiment at Becker, MN, and an on-farm demonstration trial at Clitherall, MN in eastern Otter Tail County.

Methodology

Small Plot Study. This study was conducted at Sand Plain Research Farm in Becker, Minnesota, on an excessively drained Hubbard loamy sand (sandy, mixed, frigid Entic Hapludoll). Depth to the water table is typically 2 m or more. The objective was to evaluate the efficacy of polyolefin-coated urea (POCU) application as a strategy in minimizing nitrate leaching and improving nitrogen use efficiency (NUE) in irrigated Russet Burbank potato. POCU and urea fertilizers were applied at 140 and 280 kg N ha^{-1}. All POCU N was applied at planting, whereas urea was added in three split applications at planting, emergence, and hilling. An additional urea treatment at 190 kg N ha-1 received two extra 45 kg N ha^{-1} applications of a 1:1 mixture of urea and ammonium nitrate after hilling. A control receiving no N was included in each replication. Two irrigation regimes were applied in order to ensure that leaching occurred. Under the standard irrigation regime, a total of 271 mm of water was applied, whereas the corresponding amount for the excessive irrigation experiment was 430 mm. Rainfall during the growing season was 694 mm, which is above the long-term average (550 mm) for the area. Total drainage volumes below the 120-cm depth from the standard and excessive irrigation plots were 275 mm and 382 mm, respectively, during the cropping season. Nitrate concentration was determined in soil water collected at the 120-cm depth using ceramic cup samplers. The amount of N leached between two consecutive sampling dates was calculated as the product of NO_3^--N concentration and drainage volume for the sampling interval. The N loss was summed over the entire growing season to obtain total leaching from each treatment. **On-Farm Demonstration.** Potatoes in the Otter Tail County are grown on sandy soils that are generally infertile with low water holding capacity and rapid drainage. Nitrogen (N) rates and irrigation amounts applied to meet crop demand have been linked to elevated levels of nitrate in groundwater. Use of polyolefin-coated slow-release urea (POCU) on these soils may reduce leaching and improve potato yield and quality by closely matching N release to crop N demand. The objectives of this on-farm demonstration trial were (a) to evaluate the effect of POCU on nitrogen leaching and potato yield and quality on a field scale and (b) to facilitate communication between potato growers and citizens who are concerned about the impact of N fertilizers on groundwater pollution. The study was conducted in 1999 on a center pivot at Clitherall, eastern Otter Tail County, MN. The test cultivar was Russet Burbank, a popular long-season variety grown in the area for processing. One half of the pivot was managed according to conventional farmer practice and received 305 kg N/ha as soluble fertilizer applied at planting (45 kg N ha^{-1}) and in 8 additional split applications with the irrigation water. The other half received 264 kg N ha^{-1}, 72% of which was POCU applied at planting, and the remainder soluble N fertilizer added in two splits with the irrigation water. Nitrate concentration was measured in solutions extracted at 120 cm depth using ceramic suction samplers. Tissue N status was monitored biweekly by measuring nitrate concentration in petioles from the youngest mature leaves. Prior to harvesting in September, vines were chopped by hand from 6 m sections of two harvest rows. The vines were weighed and samples dried for biomass and N determination. Tubers harvested by hand from the 6 m sections were sized and weighed for fresh yield. Samples were taken for determination of quality, dry mass, and N content.

Principal Findings and Significance

Small Plot Study. Total tuber yields were higher with POCU (82 Mg ha^{-1}) than urea applied in three (71 Mg ha^{-1}) or five (70 Mg ha^{-1}) split applications under excessive irrigation. However, yields were similar (mean 77 Mg ha^{-1}) under standard irrigation, probably due to low leaching early in the season. All N treatments increased total nitrate leaching compared to the control. At 280 kg N ha^{-1}, cumulative

nitrate leaching was higher with urea than POCU application. For the standard irrigation experiment, the leaching amounts were 84.2 kg NO_3^--N ha^{-1} for urea versus 55.8 kg NO_3^--N ha^{-1} for POCU, whereas the respective amounts under excessive irrigation were 228 and 142.5 kg NO_3^--N ha^{-1}. At the lower N rate, however, the difference in leaching between the two N sources was not significant, with both sources, taken together, averaging 64 and 96 kg NO_3^--N ha^{-1} under standard and excessive irrigation, respectively. Leaching from five split applications of soluble N was comparable to POCU under excessive irrigation (mean 139 kg nitrate-N ha^{-1}), but lower under normal irrigation (89.8 versus 55.8 kg nitrate-N ha^{-1}). Based on the drainage volume for standard irrigation stated above and the N rate of 280 kg ha-1 commonly applied to the potato crop in the study area, the N losses translate to average nitrate-N concentrations in water draining past the 120 cm depth of about 31 mg L^{-1} and 20 mg L^{-1} for urea and POCU, respectively. With excessive leaching, the corresponding concentrations would be 60 and 37 mg nitrate-N, respectively, for urea and POCU. Fertilizer N recovery efficiency (RE) estimated by the difference and ^{15}N isotope methods was higher with POCU than urea at equivalent N rate regardless of irrigation regime. The RE was similar for both sources under low leaching conditions, averaging 68% for POCU and 67% for three splits of urea. Under severe leaching (excessive irrigation), however, recovery of fertilizer N was higher with POCU (68%) than urea (48%). Leaching from five applications of soluble N was higher compared to POCU regardless of irrigation amount. Higher recoveries were obtained at lower N rate, with POCU outperforming urea regardless of irrigation amount. Results from this study suggest that use of POCU in potato production may reduce nitrate leaching and improve N use efficiency compared to split applications of soluble N fertilizer. **On-Farm Demonstration.** Nitrate concentrations in leachate samples at 120-cm depth were higher ($p < 0.001$) with urea (mean 24 mg N L^{-1}) than POCU (mean 19 mg N L^{-1}). Total potato tuber yields were 7% higher ($p < 0.1$) with POCU (60.9 Mg ha^{-1}) than urea (56.5 Mg ha^{-1}). Tissue nitrate concentration was at least within the sufficiency range for both sources, but tended towards excessive during the midseason for urea. Slow-release urea may reduce nitrate leaching and improve yield of irrigated potato. Adoption of slow-release fertilizer will depend on cost of the fertilizer relative to price of potatoes.

Descriptors

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Basic Project Information

Basic Project Information	
Category	**Data**
Title	Assessing the effects of endocrine disrupters on sperm viability and testicular development in fish
Project Number	B-04
Start Date	03/01/1999
End Date	02/28/2001
Research Category	Biological Sciences
Focus Category #1	Methods
Focus Category #2	None
Focus Category #3	None
Lead Institution	University of Minnesota

Principal Investigators

Principal Investigators			
Name	**Title During Project Period**	**Affiliated Organization**	**Order**
Peter W Sorensen	Professor	University of Minnesota	01
Ira Aldeman	Professor	University of Minnesota	02
Deborah Swackhamer	Professor	University of Minnesota	03

Problem and Research Objectives

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). 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. Among the many potential sources of EDCs, STPs have received much attention due to their omnipresence and possible implications for human health. 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.

Methodology

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. This study is conducted in conjunction with a larger investigation into effects of EDC exposure on the reproductive behavior and success of male goldfish. 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. Two experiments have been completed and are described below.

Principal Findings and Significance

The first experiment was designed to determine whether exposure to EDCs has adverse effects on milt quantity and sperm motility. Fish were exposed for eight weeks with 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. A second experiment was designed to determine whether exposure to STP effluent results in decreased milt volume and sperm motility. Male goldfish were exposed under laboratory conditions to a well water control, 50ng/l estradiol, or effluent collected daily from the St. Paul STP. Total number of motile sperm was then calculated for each fish ('Motility') as shown below. 5 Week Exposure 10 Week Exposure Milt Motility VTG Milt Motility VTG (g) [10^6] [mg/mL] (g) [10^6] [mg/ml] Well Water 0.145 1505 0.186 0.115 2688 0.159 50ng/l Estradiol 0.098 474 *0.817 0.079 *2578 *0.596 STP Effluent 0.088 1682 0.043 0.091 *2041 *0.892 Median values are presented. * indicates significant differences from well water control at $p<0.05$ (Kruskal-Wallis Analysis.) Only eight of the eighteen effluent-exposed fish exhibited high VTG concentrations after a five week exposure. After ten weeks effluent-exposed fish had significantly decreased sperm motility and eleven fish had exhibited increased VTG concentrations. Fish reproductive behavior, an additional parameter to assess fertility, was also quantified and did not vary between treatment groups. In conclusion, this study has documented for the first time in a controlled laboratory experiment a significant decrease in male fish fertility due to exposure to sewage effluent. It is also clear that milt volume does not to correlate with sperm motility in unstimulated fish and should not be used to gauge fertility in fish. VTG concentrations in STP exposed male goldfish did not rise uniformly, suggesting intra-specific differences in sensitivity to effluent exposure, even among male fish from the same stock and age class. This possibility is being investigated further in year 2. Reproductive behavior appears not to be affected by effluent exposure for ten weeks, raising the possibility that male fish, whose sperm has compromised fertility, can successfully compete with unexposed male fish for spawning opportunities. Thus effluent exposure could have serious effects on fish populations that are not evident from examining a few 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. A histological analysis of reproductive tissues of exposed male goldfish will be conducted in year 2 to address whether effluent exposed male fish suffered sex reversal.

Descriptors

Articles in Refereed Scientific Journals

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

Book Chapters

Dissertations

Water Resources Research Institute Reports

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.

Other Publications

Information Transfer Program

WRC outreach activities expanded greatly by inclusion of the Extension Water Quality Program in the Center. A satellite conference was hosted on October 28, 1999 on the need for proper on-site wastewater treatment. It reached 45 down-linked sites in Minnesota counties and 35 other sites across the country, reaching nearly 1,000 people. The Center published four issues of Minnegram, its quarterly newsletter, during the 1999-2000 reporting period. Positive comments continue to be received about its appearance and the relevance and timeliness of the information it contains–both articles with significant depth and news of interest to water-related professionals in Minnesota. The WRC and WRS web pages continued to be updated during 1998-99 (a never-ending task) to include more useful features. Fact sheets and videos describing shoreland best management practices were developed and combined with an expanded volunteer training program for shoreland owners. The Center published a report called *Minnesota Rivers: A Primer* in 1999 as a companion to its primer on limnology, which focuses on lakes. The 70-page rivers report has a similar purpose--to serve as a resource guide for students and the interested public on how rivers work, stream water uses, laws and agencies regulating rivers in Minnesota, and agencies involved in river and stream monitoring in the state. A biennial report covering the activities of the Center for academic years 1996-98 was distributed in December 1998. Work is underway (as of June 2000) to prepare a similar report covering the period July 1998 to June 2000. This report will be distributed in early fall 2000.

USGS Internship Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	3	N/A	N/A	N/A	N/A
Masters	3	N/A	N/A	N/A	N/A
Ph.D.	4	N/A	N/A	N/A	N/A
Post-Doc.	2	N/A	N/A	N/A	N/A
Total	12	N/A	N/A	N/A	N/A

Awards & Achievements

Publications from Prior Projects

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications