Water Resources Research Center
Annual Technical Report
FY 2003

Introduction

The Minnesota WRRI program is a component of the University of Minnesotas Water Resources Center (WRC). The WRC is a collaborative enterprise involving several colleges across the University, including the College of Natural Resources (CNR), the College of Agriculture, Food, and Environmental Sciences, the Minnesota Extension Service, and the University of Minnesota Graduate School. The WRC reports to the Dean of CNR. In addition to its research and outreach programs, the WRC is also home to the Water Resources Sciences graduate major. The WRC has two co-directors that divide the activities of administering its programs. Until September 2003, Professor Patrick Brezonik served as Co-Director, and was then replaced by Professor Deborah Swackhamer. The other Co-Director is Professor James Anderson. Dr. Swackhamers principle duties include administering the WRRI program and the graduate program. Dr. Anderson primarily oversees the activities related to agriculture and extension. Together they share responsibility for much of the research and outreach.

Research Program

Characterization of Nitrifying Bacterial Populations in Wastewater Treatment Bioreactors

Basic Information

Title:	Characterization of Nitrifying Bacterial Populations in Wastewater Treatment Bioreactors
Project Number:	2002MN1B
Start Date:	3/1/2002
End Date:	2/28/2004
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Congressional District:	Fifth
Research Category:	None
Focus Category:	Treatment, Waste Water, Surface Water
Descriptors:	None
Principal Investigators:	Timothy Michael LaPara

Publication

Characterization of nitrifying bacterial populations in wastewater treatment bioreactors

Principal investigator
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Funding Source: USGS-WRRI 104B National Grants Competition
Project Duration: 3/01/2002-2/29/2004

Summary
Excessive nitrogen loading to the Mississippi River basin has been recently linked to the development of a large hypoxic zone in the northern Gulf of Mexico. As a result, there is renewed interest in achieving complete nitrogen removal during the treatment of municipal and industrial wastewater. One of the most critical challenges that must be addressed before complete nitrogen removal can be consistently achieved is to reduce and eliminate upsets in the nitrification process. Nitrifying bacteria are well known to be susceptible to numerous factors such as temperature, pH, and toxic compounds. One of the problems with eliminating nitrification upsets is that there is a virtually complete lack of knowledge regarding the community dynamics of nitrifying bacteria. The research described herein tracked both nitrifier community structure and total nitrifier biomass at a municipal wastewater treatment facility for a period of one year. Samples were collected from the aeration tanks of the Metropolitan Wastewater Treatment Facility twice per month from June 2002 through May 2003. From these samples, total nitrifying bacterial biomass (as *amoA* gene copy number) was quantitified. The physiologically relevant nitrifying bacteria were also identified using a nested PCR-DGGE approach targeting 16S rRNA gene fragments as well as a PCR-cloning approach targeting *amoA* gene fragments. There was a weak negative correlation between the quantity of *amoA* genes and the effluent ammonia concentrations from the treatment facility. This research also identified specific nitrifying bacteria that were associated with excellent nitrification efficiency.

Introduction
Nitrogenous pollutant removal from wastewater has received renewed interested recently due to a large hypoxic zone that has developed over the last several decades in the northern Gulf of Mexico. This large area (> 8,000 km^2) has exhibited severely depleted dissolved oxygen concentrations (DO < 2 mg L^{-1}) near the Mississippi River delta since the 1950s and 1960s (Rabalais et al., 2001). Nitrogenous compounds, particularly nitrate, have long been suspected to be the cause of this hypoxic zone, which has an adverse affect on aquatic life and commercial fisheries. River basins in southern Minnesota, Iowa, Illinois, Indiana, and Ohio have been identified as the primary N-sources (Goolsby et al., 2001). Preliminary efforts are currently underway to reduce nutrient loading to the Mississippi River basin, including the construction of wetlands, riparian forests, and flood plains (Boesch and Brinsfield, 2000). These efforts will undoubtedly be extended to the regulation of municipal and industrial wastewater discharges.

Numerous process designs exist to adequately remove nitrogenous pollutants from wastewater. Operational control of these processes is hindered by inconsistent nitrification performance. Ammonia-oxidizing bacteria (AOB) are sensitive to pH, temperature, dissolved oxygen, and toxic compounds. AOB are also slow-growing, so their recovery from perturbation is slow. One problem with controlling nitrification during wastewater treatment is an inadequate knowledge

of the AOB population dynamics. Numerous cultivation-based techniques have been developed to quantify the biomass densities of nitrifying and denitrifying communities (e.g., the most-probable-number assay) (APHA, 1992), however these methods are infamous for underestimating the actual population density by an order of magnitude or more (Amann *et al*., 1995). Furthermore, cultivation-based assays are time-consuming because nitrifying bacteria grow slowly and fail to provide relevant data on microbial community structure.

The goal of this research is to examine the AOB community at a full-scale municipal wastewater treatment plant over a period of one year. This objective was achieved through the application of novel molecular-genetic techniques for the analysis of bacterial communities without cultivation. The total number of AOB was quantified using quantitative competitive polymerase chain reaction (PCR). The types of AOB were determined using denaturing gradient gel electrophoresis.

Methods

Site description and sample collection

Biomass samples were collected from the aeration tanks of the Metropolitan Wastewater Treatment Plant (St. Paul, Minn.) between June 2002 and May 2003. This facility treats an average of 225 million gallons of municipal wastewater each day and discharges to the Mississippi River. The majority of this wastewater is generated by residential and commercial activity (> 90%). The activated sludge process at this facility has a hydraulic residence time of 5 hours and a mean cell retention time of 7-10 days. The target dissolved oxygen concentration in the bioreactors is 2.5 mg l^{-1}. Biomass samples were collected directly from the aeration tanks and were transported on ice to the University of Minnesota (< 30 min.) and processed immediately.

PCR-DGGE

Biomass samples (1 ml each) were collected from the reactor, centrifuged, and resuspended in 1 ml lysis buffer (120 mM sodium phosphate, 5% sodium dodecyl sulfate, pH 8.0). Cells were lysed by performing three consecutive freeze-thaw cycles and a 90 minute incubation at 70°C. Genomic DNA was then extracted using a Fast DNA Spin Kit (Qbiogene; Vista, Calif.) per manufacturer's instructions.

Partial 16S rRNA genes were amplified by PCR using a PTC 100 thermal cycler (MJ Research; Watertown, Mass.). An initial PCR amplified a 465 bp fragment of the 16S rRNA gene biased towards the clade of known AOB from the *Betaproteobacteria* using primers CTO189f (5′-GRA AAG YAG GGG ATC G-3′) and CTO654r (5′-CTA GCY TTG TAC TTT CAA ACG C -3′) (Kowalchuk et al., 1997). The PCR protocol included a 5 min initial denaturation at 94°C, 35 cycles of 92°C for 1 min, 57°C for 1 min, and 72°C for 2 min, followed by a final extension at 72°C for 5 min. PCR products were then diluted 10^4- to 10^6-fold and used as template for PCR of the V3 region of these 16S rRNA genes using the PRBA338F (5′-ACT CCT ACG GGA GGC AGC AG-3′) (Lane, 1991) and PRUN518R (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993) primers with a GC-clamp (Muyzer et al., 1993) attached to the forward primer. The PCR protocol included a 5 min initial denaturation at 94°C, 30 cycles of 92°C for 45 sec, 55°C for 45 sec, and 72°C for 45 sec, followed by a final extension at 72°C for 10 min. The first and second reaction mixtures (volume = 50 μl) contained 1× PCR buffer with $MgCl_2$ (Promega;

Madison, Wis.), 4 nmol deoxynucleoside triphosphates, 25 pmol of forward and reverse primers, and 1.25 units of *Taq* DNA polymerase (Promega).

Denaturing gradient gel electrophoresis (DGGE) was performed using a D-Code apparatus (BioRad; Hercules, Calif.). Approximately equal amounts of PCR products were loaded onto 8% w/v polyacrylamide gels (37.5:1, acrylamide: bisacrylamide) in 0.5× TAE buffer (Sambrook et al., 1989) using a denaturing gradient ranging from 25 to 50% (100% denaturant contains 7 M urea, 40% v/v formamide in 0.5× TAE buffer). Electrophoresis was performed at 60°C, initially at 20 V (15 min) and then at 200 V (180 min). The gel was stained with SYBR Green I (Molecular Probes; Eugene, Oreg.; diluted 1:5000 in 0.5× TAE buffer), viewed on a UV transilluminator, and photographed with a CCD camera (BioChemi System; UVP; Upland, Calif.). The contrast and brightness of the photographs were adjusted using Adobe PhotoShop v 6.0.

Specific PCR-DGGE bands were manually excised from the gel, suspended in 20 µl of sterile water, and incubated overnight at room temperature. PCR-DGGE was repeated using these samples as template until a single band remained in each lane. A final PCR step was performed without the GC clamp attached to the forward primer. PCR products were then purified using the Geneclean II Kit (QBiogene) and nucleotide sequences were determined fully in both directions for each PCR-DGGE band using PRBA338F and PRUN518R as sequencing primers. Sequencing was performed at the Advanced Genetic Analysis Center at the University of Minnesota using an ABI 3100 Genetic Analyzer (Applied Biosystems; Foster City, Calif.). Reported nucleotide sequences do not include the original PCR primer sequence. Reference nucleotide sequences were obtained from the GenBank database.

PCR-Cloning

Fragments of *amoA* genes were amplified by PCR using primers amoA-1F (5′-GGG GTT TCT ACT GGT GGT-3′) and amoA-2R (5′-CCC CTC KGS AAA GCC TTC TTC-3′) (Rotthauwe et al., 1997). PCR products were purified, ligated into the pGEM-T Easy cloning vector (Promega), and transformed into competent *E. coli* DH5α cells (Sambrook et al., 1989). Transformants were plated onto LB agar plates and clones with putative *amoA* gene inserts were selected by the blue-white screening method. Plasmids were purified by the alkaline lysis procedure (Sambrook et al., 1989) and used as template for DNA sequencing using primers amoA-1F and amoA-2R.

Competitive Quantitative PCR

Ammonia oxidizing bacterial populations were quantified in each biofilm slice by competitive PCR (cPCR) of the ammonia monoxygenase (amoA) gene fragment (Dionisi et al., 2002). A competitor of 100 nucleotides in length was synthesized by AlphaDNA (Montreal, Canada) and diluted to generate a range of copy numbers. Reaction mixtures (50 µl) contained 1× PCR buffer (Roche Diagnostics, Indianapolis, IN), 175 µmol of $MgCl_2$, 4 nmol of deoxynucleoside triphosphates, 2% bovine serum albumin, 25 pmol (each) of forward and reverse primers (Table 1), and 1.25 units of AmpliTaq polymerase (Roche Diagnostics). PCR was performed with the following protocol: initial denaturation at 94°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, ending with a final 7 min at 72°C. Products were run on a 3% wt/vol NuSieve 3:1 agarose gel (BioWhittaker, Rockland, ME) in 1× Tris-borate-EDTA

(TBE) buffer (Sambrook et al, 1989) stained with ethidium bromide. Band intensities of target DNA and competitor DNA were quantified using LabWorks Image Acquisition software (UVP). For each biofilm sample slice (target), competitive PCR amplifications were performed as a series of four reactions of constant target DNA concentration and decreasing competitor copy number. Target copy number was determined as the value of the y-intercept of a linear plot of log competitor copy number versus the log of the ratio of target to corrected competitor band intensity. Competitor band intensity was corrected for the difference in amplicon length by multiplication by a factor of 121/100.

Chemical Analysis

Wastewater samples were collected over a 24 hour period and composited. All analytical assays (BOD_5 and TKN) were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1992). All assays were performed by personnel from the Metropolitan Council Environmental Services.

Statistical Analysis

Tukey's studentized *t* test was used for pairwise comparison of *amoA* gene copy numbers to determined statistically significant ($P < 0.05$) differences. Linear regression of *amoA* gene copy numbers versus TKN concentrations was performed using Microsoft Excel 2002.

Results

Treatment performance

The effluent quality of the municipal wastewater treatment facility was consistently high with respect to the presence of biodegradable organic compounds (Fig. 1a). During the period of study, the removal efficiency for biochemical oxygen demand was at least 95% for all but 18 days. The treatment efficiency for the removal of ammonia and organic nitrogen (the sum were measured as total kjeldahl nitrogen [TKN]) was also excellent throughout the majority of the study period (Fig. 1b). As the wastewater temperature began to decline in the winter months (Fig. 1c), however, the quantity of TKN in the effluent substantially increased, exceeding a concentration of 5 mg/l for substantial portions of the period between December 2002 and March 2003.

PCR-DGGE of the Entire Bacterial Community

Fingerprints of the total bacterial communities growing in the aeration basin were studied by PCR-DGGE (Fig. 2). The fingerprints were complex (mean = 25.9 bands per lane; s.d. = 3.7) and varied gradually throughout the duration of the study. The patterns gradually changed as a function of time, although a substantial number of the bands were detectable throughout the study (11 out of 40 bands were detected throughout). Cluster analysis by the UPGMA algorithm confirmed this gradual adaptation in community structure, but further suggested that the bacterial community did not return to any previously observed structure (Fig. 3).

Nested PCR-DGGE of AOB

Fingerprints of the AOB communities growing in the aeration basin were studied by PCR-DGGE (Fig. 4). One population was especially dominant throughout the study (Band G), whereas numerous other populations appeared sporadically. The majority of these prominent bands were excised so that their nucleotide sequences could be determined (Table 1). The majority of the

less prominent bands were phylogenetically related to *Nitrosomonas* spp. (Bands A-C, E) or *Nitrosospira* spp. (Band I). Several other bands, however, were not phylogenetically related to any known AOB, including the two most prominent bands (Bands G-H).

The number of AOB detected by nested PCR-DGGE negatively correlated to the effluent TKN concentrations from this treatment facility. During the time periods in which excellent TKN removal efficiency was observed (25 June 2002 – 14 November 2002 and 17 March 2002 – 16 May 2003), the number of putative AOB populations detected per sample (mean = 1.6; s.d. = 0.9; n = 16) was substantially higher than the number AOB populations detected when poor TKN removal efficiency was observed (mean = 0.8; s.d. = 1.0; n = 8) (27 November 2002 – 7 March 2003).

PCR-Cloning of amoA Gene Fragments

The community of AOB was also analyzed in two samples (19 August 2002 and 24 January 2003) by PCR-cloning of *amoA* gene fragments (Table 2). From the 19 August 2002 sample, 33 different clones were analyzed to give 3 different gene sequences. Two of these *amoA* sequences were closely related to *Nitrosomonas oligotropha* (97% of the clones screened), while the third sequence was closely related to another *Nitrosomonas* spp. From the sample collected on 24 January 2003, 28 different clones were analyzed to provide the same 3 *amoA* gene sequences. The distribution of these clones, however, was substantially different in that *Nitrosomonas oligotropha*-like populations comprised less than 90% of the clone library.

Quantification of AOB populations

Nitrosomonas oligotropha-like AOB populations were quantified in the aeration tanks by competitive quantitative PCR of *amoA* gene fragments (Fig. 5a). The quantity of *amoA* genes detected throughout the year varied substantially, from a minimum of 3.9×10^5 ml^{-1} (24 January 2003) to a maximum of 3.5×10^6 ml^{-1} (30 June 2002). During the winter months (December 2002 – March 2003), a statistical decrease in the quantity *amoA* gene copy number occurred compared to samples collected during the warmer months. Because the lowest concentration of *amoA* gene copy numbers roughly coincided with the highest effluent concentrations of total kjeldahl nitrogen, these two parameters were correlated using data generated on the same date (Fig. 5b). A negative correlation was observed, however this correlation was not statistically significant ($P > 0.3$). Visual inspection of a plot containing both *amoA* copy numbers and effluent total kjeldahl nitrogen (plot not shown) suggested that these two parameters were out of phase, such that total kjeldahl nitrogen concentrations lagged by approximately one week (Fig. 5c). A stronger negative correlation was observed, however, it too was not statistically significant ($P > 0.1$).

Summary of findings

This project has demonstrated a substantial link between the presence and quantity of AOB and the effluent quality from a full-scale municipal wastewater treatment facility. Even though the overall bacterial community from the aeration tank was quite complex, the AOB community was relatively simple and could be easily tracked by PCR-based analysis of either 16S rRNA gene fragments specific for AOB or of *amoA* gene fragments. Although the methods used to detect 16S rRNA genes are simpler, these techniques are more susceptible to amplification of non-AOB populations. The techniques used to detect *amoA* gene fragments were more specific and

quantitative, such that they offered numerous advantages. The principal limitation of this research is that a statistically relevant correlation between *amoA* gene copy number and effluent quality was not observed. Additional research could likely achieve this statistical correlation by analyzing more replicate samples on each date and by collecting samples more frequently.

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Publications associated with the project

None to date.

Students supported by the project -- graduate (MS, PhD) and undergradute

Sudeshna Ghosh (January 1, 2003 – date)
Department of Civil Engineering, University of Minnesota

Degree being sought: Doctor of Philosophy

Shelby Stanek
Department of Civil Engineering, University of Minnesota
Undergraduate

Awards and achievements resulting from your project
None to date.

Seminar or poster presentations resulting from your project
None to date.

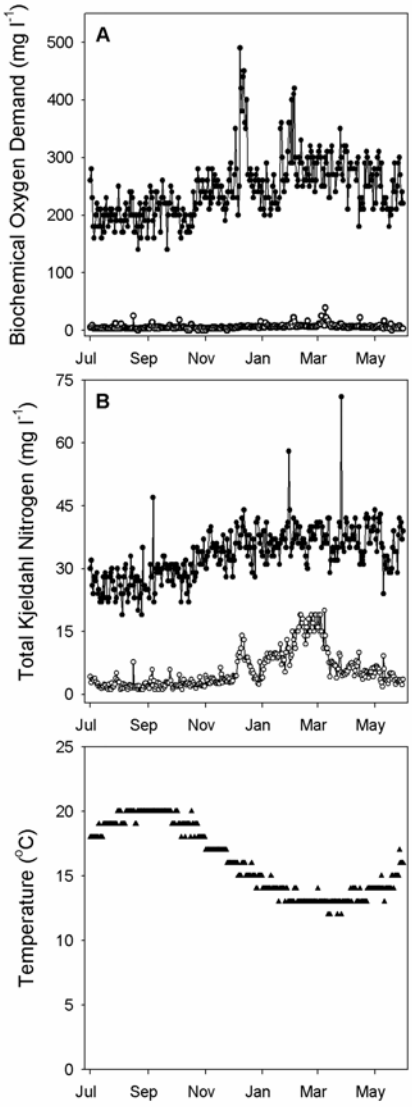

Figure 1. Effluent water quality data from the Metropolitan Wastewater Treatment Facility (St. Paul, Minn.) from 1 July 2002 to 31 May 2003. (A) Influent and effluent BOD5. (B) Influent and effluent TKN. (C) Temperature of the aeration tank. ˜ = influent concentration, ¡ = effluent concentration

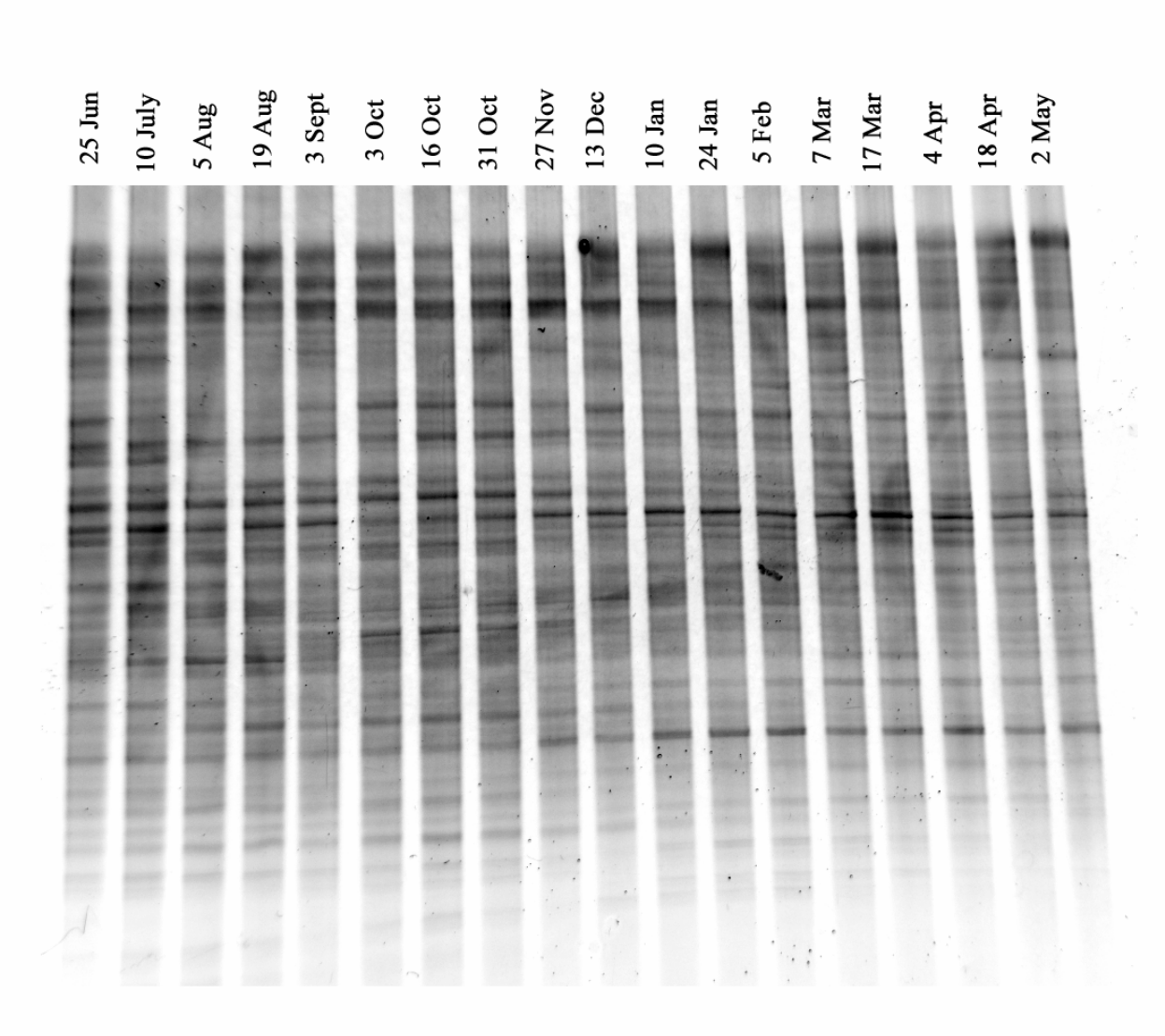

Figure 2. Fingerprints of the community structure of the bacteria growing in the aeration tanks of the Metropolitan Wastewater Treatment Facility between 25 June 2002 and 2 May 2003. Fingerprints were generated by PCR-DGGE of 16S rRNA gene fragments. Lanes are labeled to indicate the date at which the community fingerprint was determined.

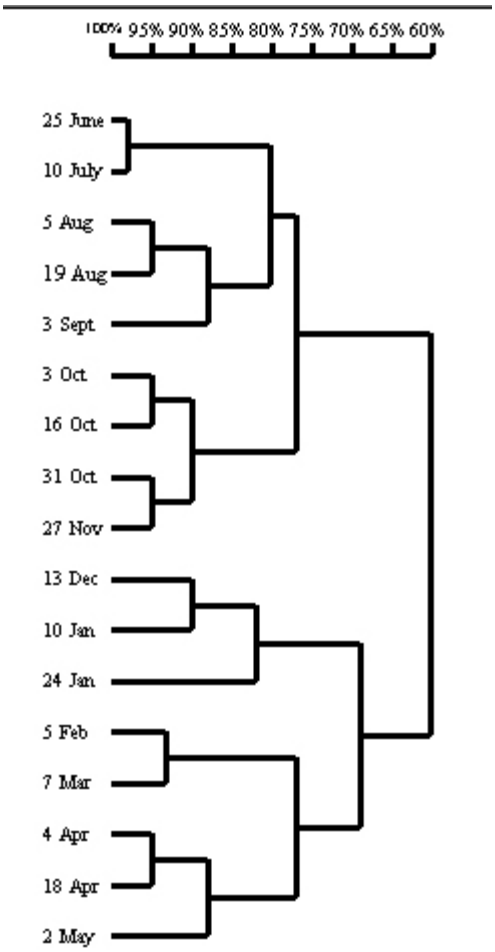

Figure 3. Dendrogram revealing the relatedness of PCR-DGGE fingerprints (Fig. 2) of the community structure of the bacteria growing the aeration tanks of the Metropolitan Wastewater Treatment Facility between 25 June 2002 and 2 May 2003.

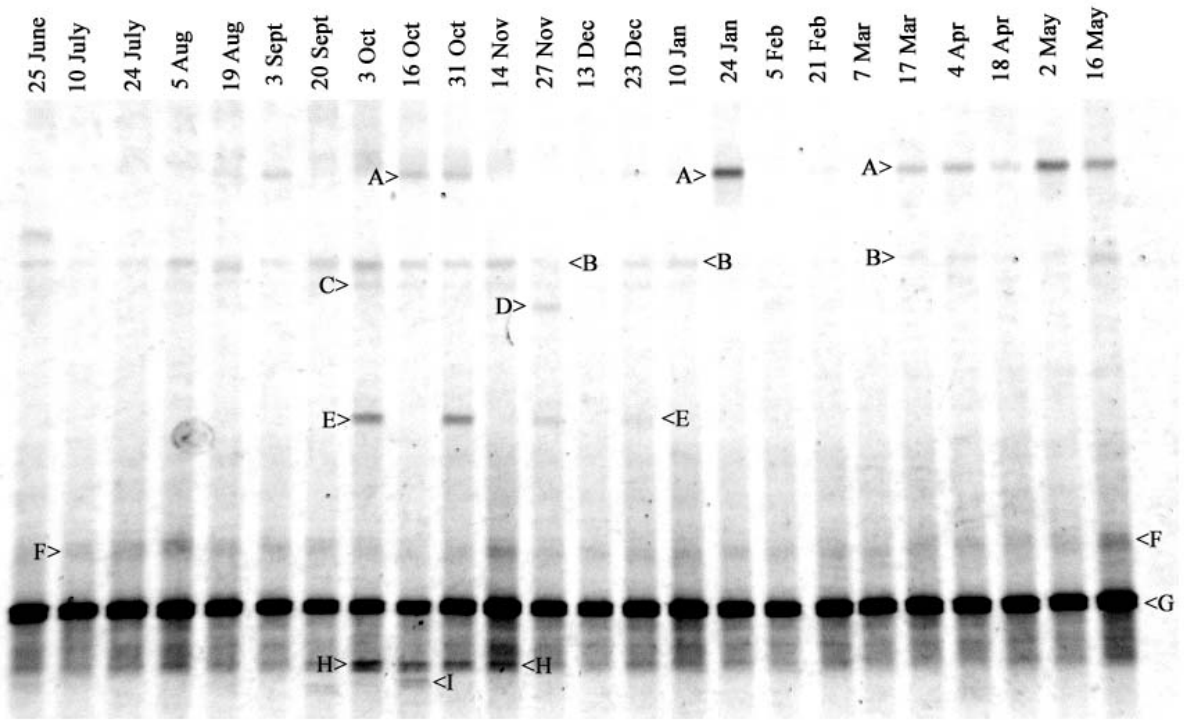

Figure 4. Fingerprints of the AOB community generated by nested PCR-DGGE of 16S rRNA gene fragments. The nested PCR was biased towards the ammonia-oxidizing bacteria within the Betaproteobacteria. Lanes are labeled to indicate the date at which the community fingerprint was generated. Specified bands were excised and sequenced (Table 1).

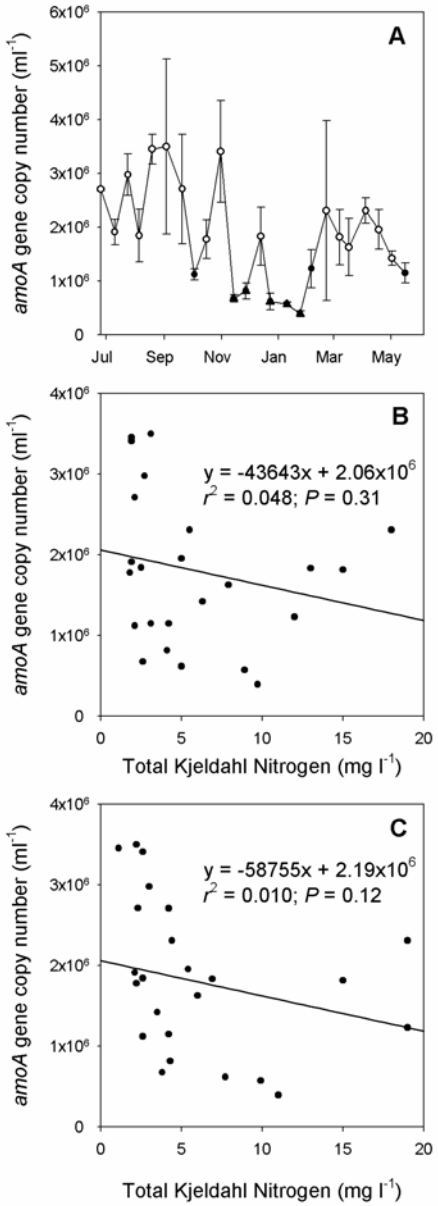

Figure 5. (A) The quantity of amoA gene fragments detected within the bacterial community growing in the aeration tanks of the Metropolitan Wastewater Treatment Facility between 25 June 2002 and 16 May 2003. Open circles (™) identify data points that were significantly higher than closed triangles (p). Statistical correlations between

amoA gene copy number and effluent TKN concentrations quantified: (B) on the same date that biomass samples were collected and (C) seven days after the biomass samples were collected.

Effects of riparian forest harvest on instream habitat and fish and invertebrate communities

Basic Information

Title:	Effects of riparian forest harvest on instream habitat and fish and invertebrate communities
Project Number:	2002MN2B
Start Date:	3/1/2002
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	7 and 8
Research Category:	Not Applicable
Focus Category:	Water Quality, Management and Planning, None
Descriptors:	
Principal Investigators:	Raymond Newman

Publication

Effects of riparian forest harvest on instream habitat and fish and invertebrate communities

Principal Investigators
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Funding Source: USGS-WRRI 104B National Grants Competition
Project Duration: March 1, 2003 through February 29, 2004

Summary
Stream riparian zones are critical to the health of stream fish and invertebrate communities. Forest harvest within the riparian zone may thus impact stream fish and invertebrate communities and the determination of the level of acceptable harvest within the riparian zone is important to balance forestry needs with stream biotic integrity. We have designed a manipulative experiment to determine the effects of no, low and high levels of riparian harvest on stream habitat and fish and invertebrate communities. Sites were selected, and pre-harvest sampling was done in summer 2003. Treatments were applied in winter 2004. There was considerable diversity in habitat and stream fish communities among the sites. Four sites contain brook trout and the other four sites are warm water streams. Post-harvest sampling will occur in summer 2004.

Introduction
Forest products are an important natural resource in the upper Midwest. In Minnesota, timber harvest has been increasing and will continue to increase in the near future (Anonymous 2001). Timber harvest activities have the potential to degrade water quality and aquatic resources and for this reason, best management practices (BMPs) or site-level forest management guidelines have been adopted to protect riparian and aquatic resources in Minnesota (MFRC 1999, Anonymous 2001). Although these best management practices are based on the best available scientific information, and implementation monitoring is being conducted (Anonymous 2001), they have not been evaluated for effectiveness at protecting aquatic resources. Most research on the effects of forest harvest on streams and the effectiveness of forest harvest BMPs has been conducted in more mountainous regions such as Tasmania (Davies and Nelson 1994), the Sierra Nevada's, the Pacific Northwest and the Appalachian East (e.g., Meehan 1991, Castelle and Johnson 2000). These results may not be directly applicable to the midwest (Perry et al. 1992).

Riparian zones provide many protective services to streams (Gregory et al. 1991, Castelle et al. 1994, Castelle and Johnson 2000). Determination of the necessary width of riparian buffers (e.g., Castelle and Johnson 2000) or the permissible level of harvest within a buffer is essential to adequately protect stream resources without removing a large portion of the basin from harvest. Most studies on the effectiveness of riparian buffers at protecting streams from upslope harvest have focused on the width of the buffer and have not considered harvest within the buffer zone (e.g., Barton et al. 1985, Castelle and Johnson 2000). Current Minnesota best management practices allow varying degrees of harvest within the riparian management zone (RMZ). Harvest within the zone may be

used to promote regeneration of shade intolerant species and thus it is important to know what level of harvest within the zone reduces its effectiveness at maintaining stream quality.

The objective of this project is to experimentally determine the effectiveness of various levels of riparian harvest at protecting in-stream resources. We will examine site-based effects associated with high, low and no riparian harvest (30m Riparian Management Zone, upland clearcuts) on aquatic habitat, macroinvertebrates and fish. Specifically, we will evaluate effects on fish and invertebrate habitat (temperature, sediment composition and embeddedness, depth, width, cover, bank stability, canopy coverage, woody debris, etc.), benthic macroinvertebrates and stream fish communities.

Methodology

Eight pairs of treatment sites (riparian control and one harvest treatment – low or high residual basal area) were located and harvest plots marked in 2003. The sites range across northern Minnesota and are located in Beltrami, Carlton, Cook, Lake, and St. Louis counties (Table 1). Riparian harvest treatments are high residual ($50ft^2$ basal area/acre remaining) or low residual ($20ft^2$ basal area/acre). Within each pair a riparian control (upland clearcut, no riparian harvest) and one riparian management treatment (low or high) were established to compare the effects of different residual basal area levels (e.g., 4 high basal area and 4 low basal area replicates). We were also able to establish a non-harvested control (both upland and riparian zone not harvested) at seven of the eight plots (beaver activity preclude a non-harvested control plot at one site).

All sites were sampled for habitat, fish and invertebrates in summer 2003 before harvest (control year) and all plots were harvested in Winter 2004, with the exception of one high basal area plot; neither the treatment nor riparian control were harvested at this site due to weather and logger complications. This site will be harvested in Winter 2005.

At each site, stream reaches were established in the no-harvest control, riparian control and riparian harvest plots. Within each plot, we sampled 100-meter reaches above the plot (upstream), within the plot (downstream most 100m) and below the plot (downstream) – these reaches provide internal upstream controls and allow for assessment of downstream effects. Thus at a given site, we generally sample nine 100-m reaches; up-, within and below at the non-harvested control, the riparian control and the harvest treatment. Due to spatial and habitat constraints, up and below reaches were not feasible for some plots.

Temperature loggers (Optic StowAway, Onset Computer, Pocasset, MA) were placed in all the reaches at each site in June 2003; they recorded temperature at 30 min intervals until removal in October. Water quality was recorded in the within reaches at each site: in the field, conductivity, dissolved oxygen, and pH were record with a Quanta Water Quality Monitoring System (Hydrolab Corporation); methyl orange alkalinity (mg $CaCO_3$) was determined by titration, and orthophosphate was determined by the PhosVer 3 (Ascorbic Acid) method with a Hach model DR/2000 spectrophotometer in the field

and nitrate was determined spectrophotometrically (APHA 1989) on HCl preserved samples with a Spectronic 1201 Dual Beam spectrophotometer in the laboratory.

In July, each 100-m reach was sampled for habitat characteristics following the methods of Merten (1999), which are modifications of methods given by Bailey et al. (1993). Variables measured include visual estimates of bank cover, channel stability, cover, woody debris, percent riffles, runs and pools, and aquatic plant coverage. Canopy coverage was determined in each reach with a spherical densiometer (Lemmon 1957). Streambed sediment and substrate type and size (e.g., percent silt, sand, gravel, cobble, etc.) and percent embeddedness were characterized (Platts et al. 1983) along 14 transects placed at regular intervals in each reach with a total of 56 measurements per reach. Mean depth, velocity and discharge were measured at the fourteen transects within each reach. Blow-down trees were also recorded in each reach.

Benthic macroinvertebrates were assessed in July following the family-level, composited, multi-habitat rapid bioassessment protocol (Barbour et al. 1999) in each of the upstream (internal control) and within-plot reaches for the control, riparian control and riparian harvest plots. Two composited samples of 20 kicks / jabs (each sample representing 50 m) were collected with d-net in each 100-m reach.

In August, the fish community was sampled in the up- (internal control), within- and downstream reaches at each treatment plot (including the control sites) with pulsed DC electrofishing (Wisconsin AbP-3 backpack shocker). Fish were identified to species, measured (total length), weighed and returned to the stream. Single-pass estimates of catch-per-effort were used to characterize the fish community (Simonson and Lyons 1995). Coldwater Index of Biotic Integrity (IBI) values will be calculated according to Mundahl and Simon (1998), and warmwater IBI values according to Karr et al. (1986) or Lyons (1992) to assess the environmental health of the stream fish communities. Species richness, species abundances and IBI scores will be analyzed for harvest treatment effects.

All sampling will be repeated in 2004, the first year post harvest, during the same times as the preharvest data were collected. We plan to continue data collection in 2005 to continue post-harvest assessment.

Table 1. Site locations and characteristics. Riparian harvest treatments are low harvest (high residual remaining = 50ft^2 basal area/acre) or high harvest (low residual remaining = 20ft^2 basal area/acre).

County	Stream Information				Preharvest RMZ basal area (ft.^{2}/acre)
	Treament	Name	Width (m)	Protection	
Beltrami	high rem	Shotley	4	None	125
Carlton	high rem	No name	1	None	113
Cook	high rem	Reservation tributary	3	Trout	90

Lake	low rem	W. Split Rock	4	Trout	60
Lake	high rem	Beaver	8	Trout	75
Lake	low rem	East Baptism	8	Trout	100
St. Louis	low rem	Cloquet tributary	3	None	100
St. Louis	low rem	No name	2.5	None	125

Results and on-going work

Pre-harvest sampling was completed in summer 2003 and the treatment sites were harvested in winter 2003-2004 (with the exception of site 3, a high residual basal area treatment site). Target riparian harvest levels were low remaining basal area (20 $ft.^2$/acre) and high remaining basal area (50 $ft.^2$/acre). Post-harvest data will be collected in summer 2004.

Macroinvertebrate samples have been sorted for each site and identified to Order; Family level identifications are currently being completed. Habitat data have been entered and collated for each site but have not been analyzed. Sites varied substantially in habitat characteristics. Mean widths ranged from <1m to > 10m and substrates ranged from primarily silt and sand to primarily cobble and boulder. Water temperatures varied among sites; some sites, primarily the trout streams, maintained temperatures ≤ 19 ˚C throughout the summer (range from 12-19), whereas other warmer water streams had summer maximums of 24-25 ˚C. Conductivity and alkalinity ranged from 40 µS/cm and 20 mg $CaCO_3$/L respectively (at 2 sites tributary to the St. Louis River) to 380 µS/cm and 245 mg $CaCO_3$/L (Beltrami site). Dissolved oxygen generally ranged from 8-11 mg/L and pH was > 7.5 at all sites. Orthophosphate ranged from 8 µg-P/L to 167 µg-P/L, although most sites ranged from 15-30 µg/L. Nitrate ranged from 0.45 mg-N/L to 0.88 mg/L. Nitrate was positively correlated with alkalinity and weakly negatively correlated with phosphorus.

Twenty-one species of fish were found among the 1600 fish collected. The fish communities also differed among streams, however. the streams could be grouped into three types. Four sites were trout streams, containing brook trout (*Salvelinus fontinalis*) and typically also black and longnose dace (*Rhinicthys cataractae*). With the exception of site 3, which had few fish, the other trout streams had 8-10 species, indicative of marginal trout waters. One site is a lake-outlet stream and contained a diverse array of warm and coolwater species (10 total species). The reaming sites are small minnow streams with low diversity and mudminows (*Umbra limi*), which may indicate poor winter or summer oxygen conditions. Indices of biotic integrity have not yet been computed and we likely will have to use both warm and coldwater IBIs to characterize the array of streams in the study.

Post-harvest data will be collected in summer 2004. Temperatures loggers will be placed at each site in late May, habitat and invertebrate samples will be collected in July and fish will be sampled in August.

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Publications associated with the project
None

Students supported by the project
Starting in June 2003:
Dickson Atuke (PhD), Fisheries and Aquatic Biology Track in Conservation Biology

Nick Schlesser (MS), Fisheries and Aquatic Biology Track in Conservation Biology – additional support from fellowships and LCMR Grant

Nat Hemstad (PhD), Water Resources Science – 3 month field assistant supported on LCMR Grant

Awards and achievements resulting from your project
None

Seminar or poster presentations resulting from your project
Effects of riparian forest harvest BMPs on instream fish communities and habitat. **R. Newman, N. Hemstad, and E. Merten**. 3rd Annual Forest And Wildlife Research Review, 15 January 2004, Duluth, MN,

Related grants submitted or funded as a result of this project
The Legislative Commission on Minnesota Resources funded the manipulation, travel, supplies and field assistance.

The Minnesota Forest Resources Council provided funds for some supplies and field assistance.

Vondracek, B. and R.M. Newman. Effects of riparian forest harvest on instream habitat and fish and invertebrate communities. Minnesota Department of Natural Resources, 6/15/04-6/30/05. $37,500: will fund travel, supplies, field assistance and one additional graduate student.

A proposal for longer-term continuation of this project has been submitted to the Legislative Commission on Minnesota Resources.

Biodiversity in Urban Ponds and Lakes: Human Effects on Plankton Populations

Basic Information

Title:	Biodiversity in Urban Ponds and Lakes: Human Effects on Plankton Populations
Project Number:	2002MN7B
Start Date:	3/1/2002
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	Four
Research Category:	None
Focus Category:	Water Quality, Surface Water, Management and Planning
Descriptors:	None
Principal Investigators:	Robert Warner Sterner

Publication

Biodiversity in urban ponds and lakes: human effects on plankton populations

Principal Investigator
R.W. Sterner, Professor, Department of Ecology, Evolution and Behavior, University of Minnesota

Funding Source: USGS-WRRI 104B National Grants Competition

Project Duration: March 1, 2002-February 29, 2004

Summary
The objectives of this project were to explore the diversity of planktonic algae and zooplankton in small inland lakes in and around a densely populated urban area. We employed stratified random sampling using GIS as a tool and identified 100 sampling sites within the seven-county Twin Cities metropolitan area. We then sampled each of these sites three times during the 2002 growing season for phytoplankton and zooplankton communities, as well as basic limnological parameters including chlorophyll and phosphorus. We found differences in biodiversity between urbanized and non-urbanized regions, with lower biodiversity in the inner urban regions than in the outer regions. Based as they are on a rigorous, random, site selection process, we believe these to be the first results documenting an effect of urbanization on planktonic organisms.

Introduction
This project addressed two sorely neglected aspects of water resources research: 1) urban habitats and 2) biodiversity of small, inconspicuous species. There is wide recognition that biodiversity is an important aspect of water quality, yet on several critical fronts, we lack sufficient scientific underpinning to incorporate biodiversity into most assessments of water quality. First, there is little scientific data on effects of different environmental factors on biodiversity of small aquatic organisms. Second, there has been little attention paid to the water resources closest to the large proportion of today's society that lives in urban environments. This project sought to establish whether urbanization deleteriously affects the majority of the biodiversity within those habitats.

The sustainability and integrity of our water resources are threatened in many ways. Twenty six percent of total terrestrial evapotranspiration and fifty four percent of runoff that is geographically and temporally accessible are used by humans (Postel et al. 1996). Important contributing factors to reduced water quality due to human effects – well known to all who have an interest in water quality – include increased nutrient loading and sedimentation, acid rain, and contamination by heavy metals and other toxicants. Though much is left to learn about these factors, significant scientific advances have been made on all of these fronts. However, it is becoming increasingly clear that reduced biodiversity ranks among the most critical problems in management of aquatic ecosystems (Naiman et al. 1995), and here our knowledge base is much weaker.

Most discussions of biodiversity center on terrestrial habitats such as rain forests. However, biodiversity is even more threatened in aquatic ecosystems than in terrestrial

ecosystems (Naiman et al. 1995). Approximately 20% of the world's species of freshwater fish have declining abundance or are already extinct (Moyle and Leidy 1992). The Environmental Defense Fund has estimated that 30-70% of several major aquatic groups, such as mollusks and fishes, are threatened. Maintenance of aquatic biodiversity has been identified as a freshwater research priority second only to restoration and rehabilitation of aquatic habitats (Naiman et al. 1995). Local and global extinction of aquatic species may come about through overt habitat change or loss, such as river impoundment, excessive nutrient loading, and drainage of aquatic ecosystems. Although habitat loss and degradation are probably most damaging to aquatic biodiversity, other potentially important threats include exploitation of commercial species and introduction of exotic species. Biodiversity loss may also be caused by chronic introduction of substances such as sediment or nutrients that alter the habitat. Further, it may occur due to introduction of novel predators, such as the Nile Perch in Lake Victoria, or due to highly successful exotic species that capitalize resources, such as Eurasian Water Milfoil or the zebra mussel.

Shifts in human demographics affect water resource pressures. The human population is becoming increasingly urbanized. Approximately 41% of the world's human population now lives in urban areas. There are 411 cities worldwide with over 1 million human inhabitants. However, until recently ecologists have avoided urban areas for research (McDonnell and Pickett 1991), and only recently have they begun serious examination of urban habitats as unique, important ecosystems. Examining species relationships along rural to urban gradients can be extremely useful, because doing so address practical, applied questions while also providing insight on basic questions regarding the structure and function of ecosystems (McDonnell and Pickett 1991). Although limnologists have not shied away from addressing important practical problems, the bulk of their research has been conducted on non-urban sites. As the human population continues to encroach on and urbanize habitats, the importance of understanding the effects of human disturbance on aquatic species and ecosystems will increase.

Methodology

We split the seven-county Twin Cities, Minnesota Metro Area into three zones: an urban core, a surrounding, less urban ring, and the nonurban outskirts; these zones were based on land use and percent impervious surface (Fig. 1). We then combined the data from the two urban zones into a single zone, and we have contrasted the inner urbanized area with its surroundings. To choose sites, we randomly distributed points within each zone and then we used a combination of GIS and ground searching to identify the nearest permanent (containing water year-round) pond or lake to those random points (Fig. 2). Fifty ponds or lakes were located within the urban habitat and 50 were located in the nonurban habitat. These lakes and ponds ranged in size (0.003 – 5667 ha) and productivity (as measured by phosphorus levels, 0.126 – 21.4 µM TP). We found that lake size and TP did not differ significantly with urbanization. Some of these lakes were surrounded by parking lots while others are in protected areas. During the 2002 ice-free season we sampled each lake 3 times: in the early spring, mid summer, and late fall. At each sampling we took standard limnological measurements such as chl a, total phosphorus, dissolved phosphorus, seston phosphorus, and seechi depth. We also

preserved composite algal and zooplankton samples. Phytoplankton samples were preserved with Lugol's iodine and stored at 4 °C in the laboratory. For species identification, we took a 10 ml subsample from each of the preserved samples and let the phytoplankton cells settle overnight in a 10 ml settling chamber. The samples were then observed under an inverted light microscope at x 400. Magnification of 1000x was also used when further details were needed for species identification. Phytoplankton cells larger than 5 microns in any dimensions were identified at least to the genus level and to the species whenever possible, and their counts were recorded for each of the six 250 µm x 250 µm fields of view, which were randomly selected. Filamentous algae, however, were counted if a trichome was longer than 5 µm, and the number of cells within a trichome was estimated by dividing the length of the trichome by the representative length of each cell. Some of the samples had a large number of cells and/or debris of algal or macrophytic origin, making accurate cell counts difficult. In those cases, the subsamples were diluted tenfold with deionized water before settling. We enumerated the zooplankton samples to species. To avoid bias associated with sampling effort we invested a similar quantity of effort in each sample. One 1 mL Sedgwick-grafter cell was counted per lake. During 2004, we returned to each site and collected detailed information on macrophytes and on the physical and biological characteristics of the shoreline.

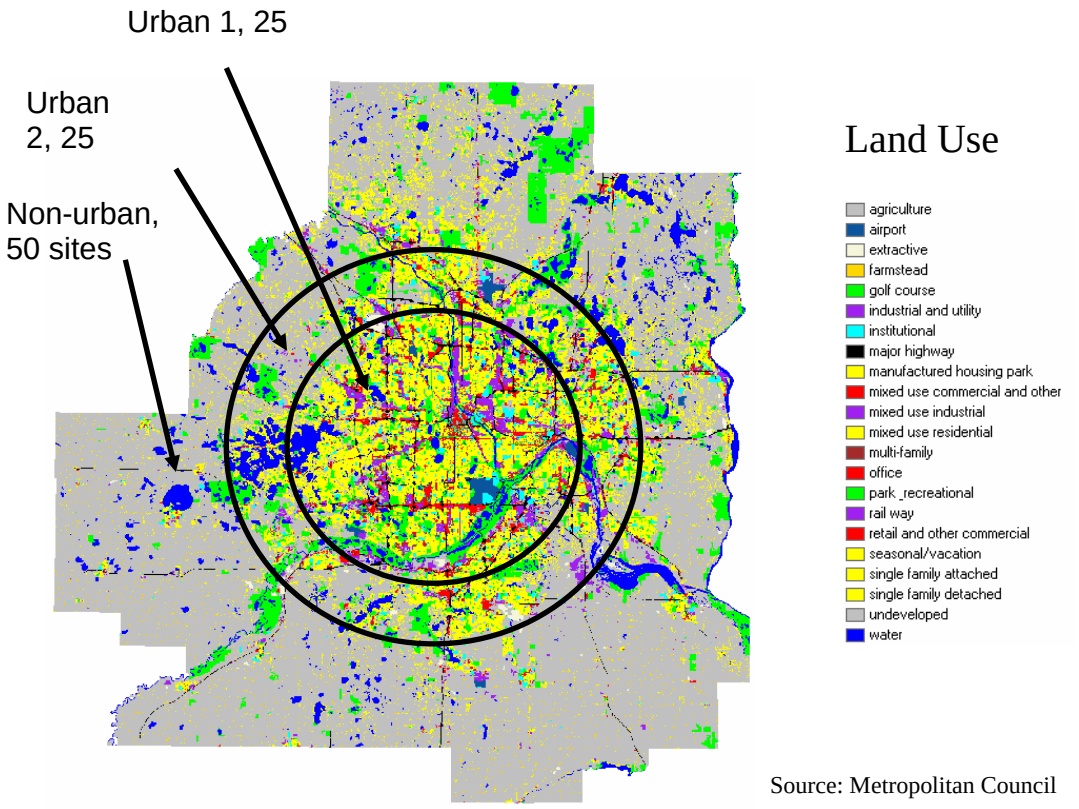


Figure 1. *Major land use types and zones defined for this project.*

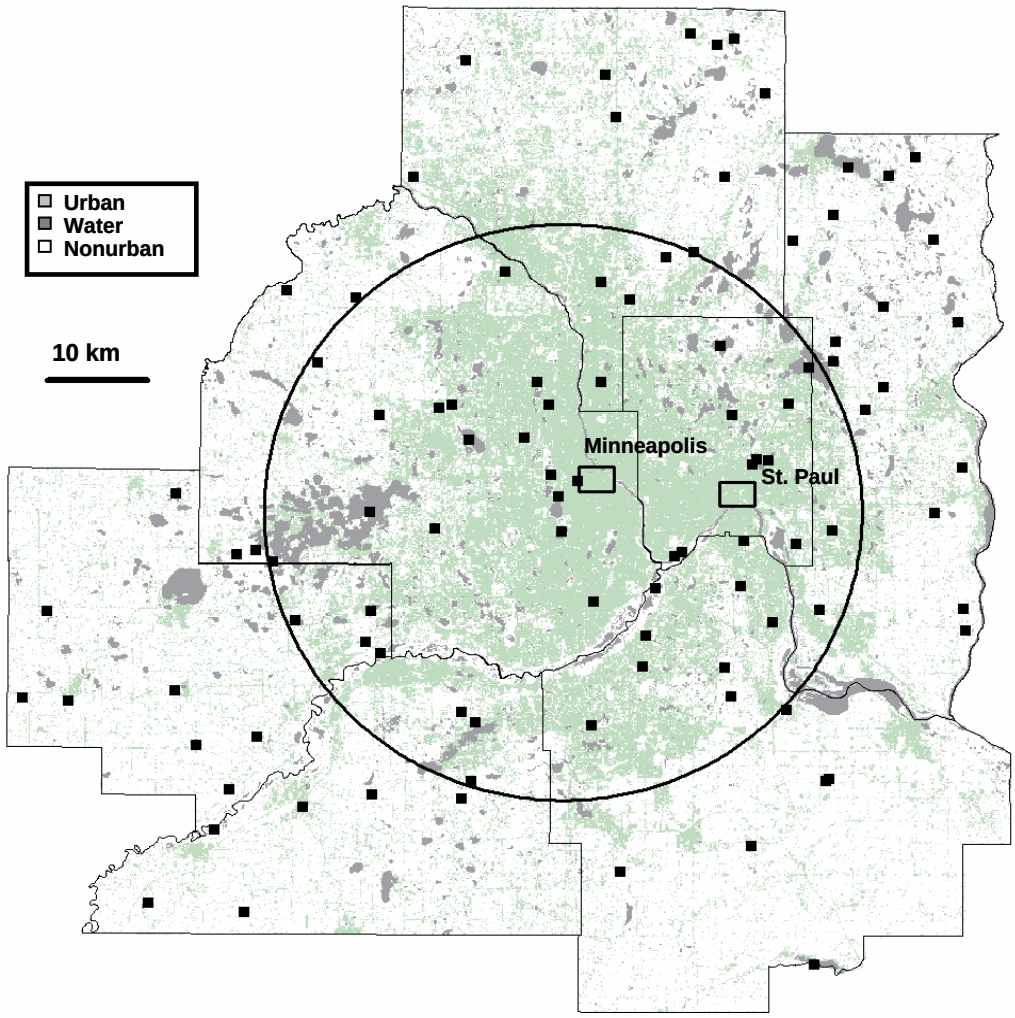
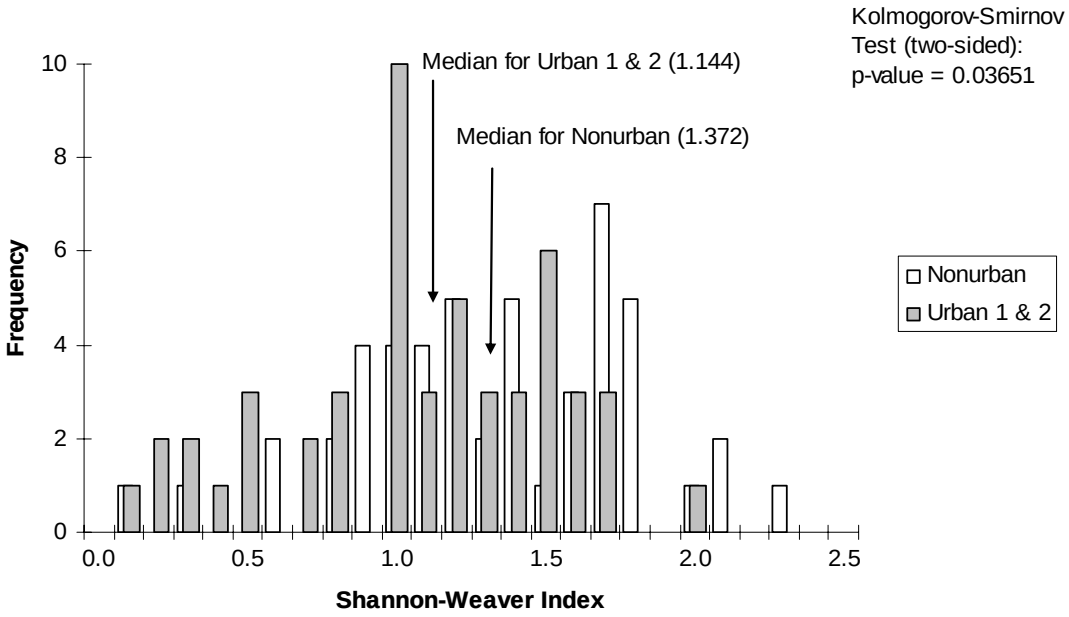


Figure 2. *Locations of study systems within the Metro Area.*

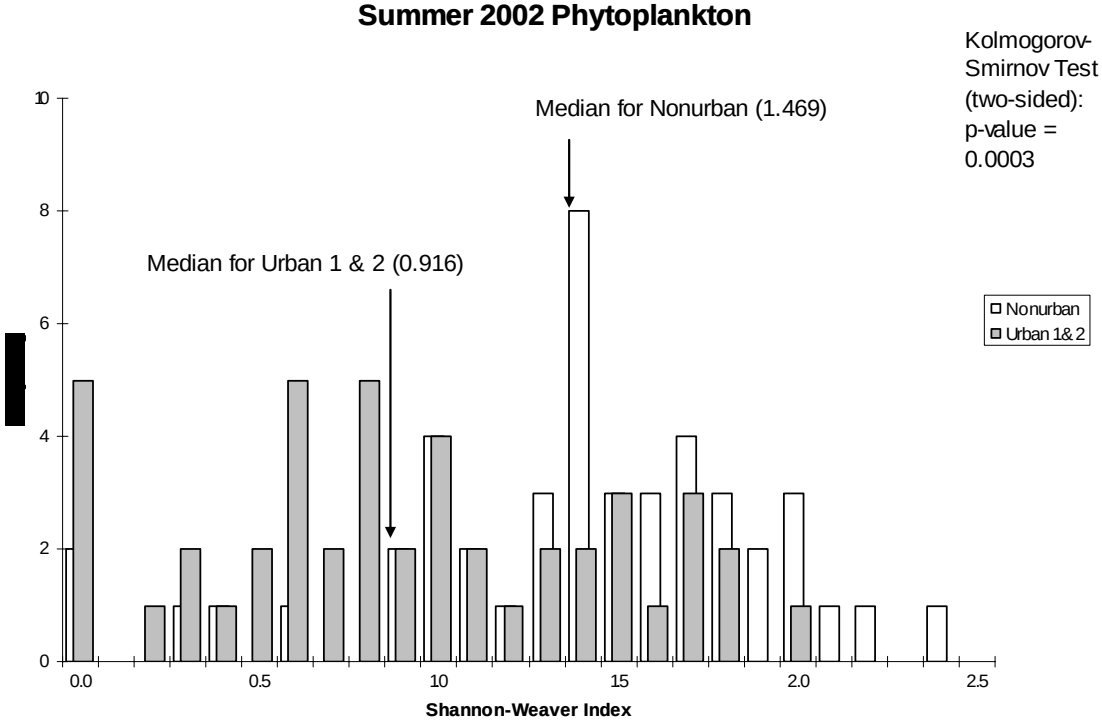
Results and on-going work

For both phytoplankton and zooplankton, we calculated the Shannon diversity index to evaluate species diversity for each sample. We observed land-use effects on biodiversity both for phytoplankton and for zooplankton. For phytoplankton, both the spring and summer communities showed noticeably lower biodiversity in the urban zones (Fig. 3).

Spring 2002 Phytoplankton



Summer 2002 Phytoplankton



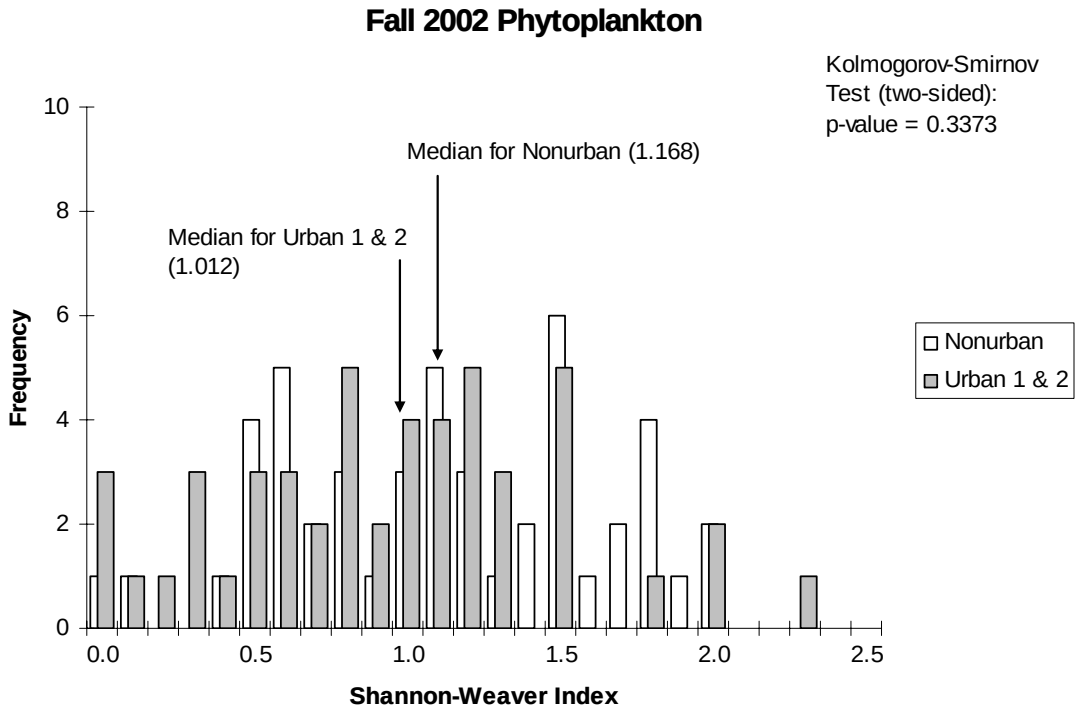
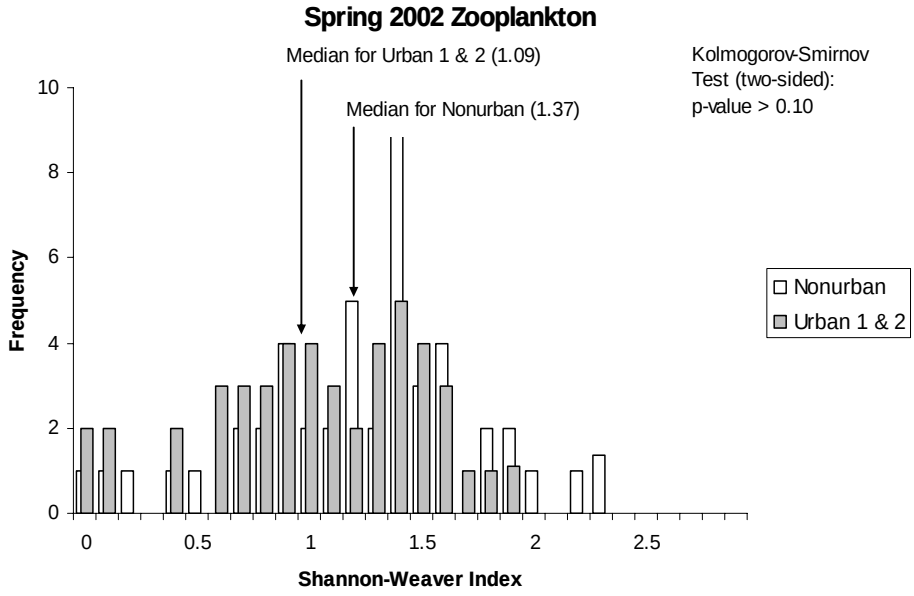


Figure 3. *Phytoplankton biodiversity as a function of urbanization. Algal biodiversity is significantly lower in the urban region during spring and summer. The differences in biodiversity are especially noticeable in the summer, during time of maximal human contact.*



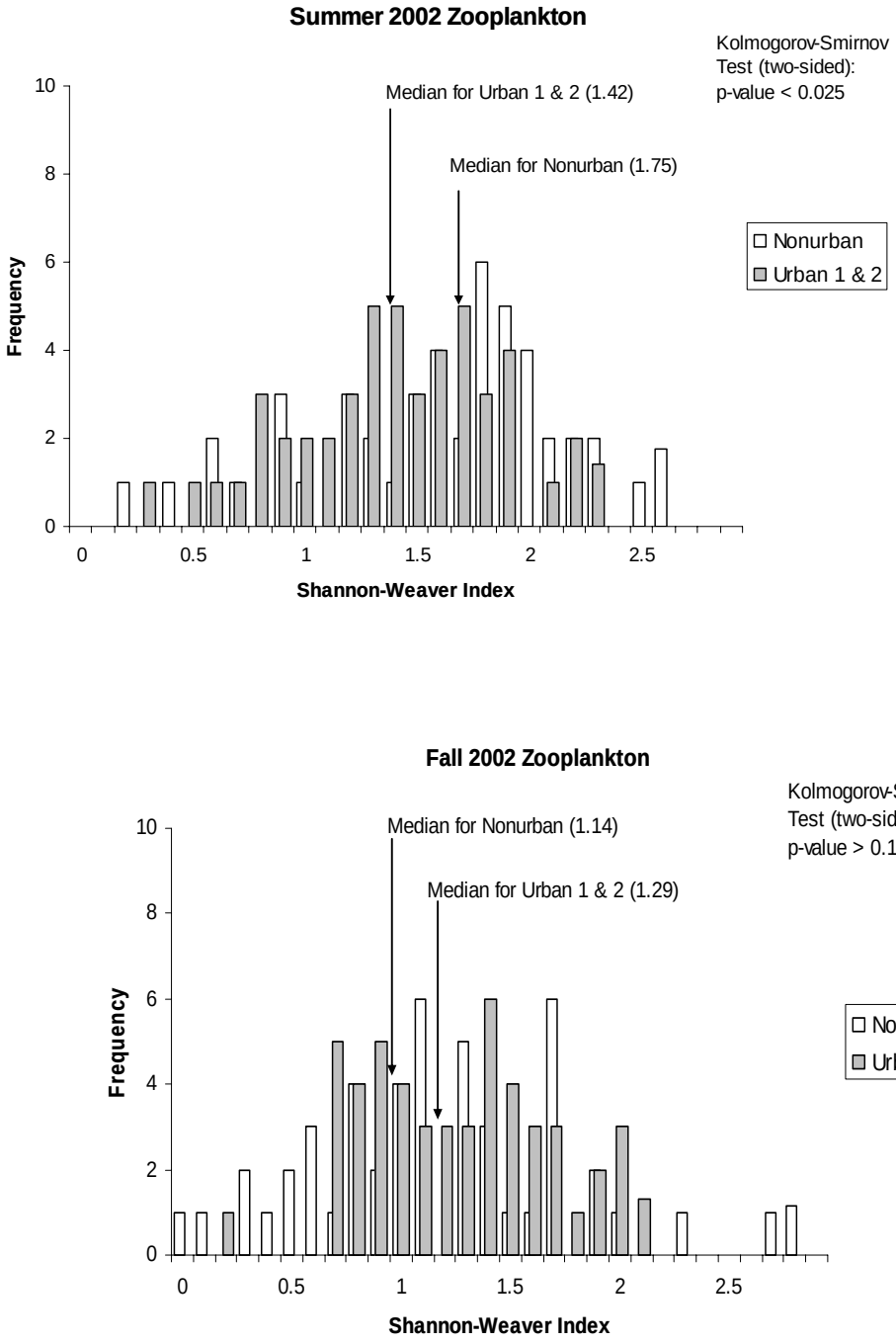


Figure 4. *Zooplankton biodiversity as a function of urbanization. Zooplankton biodiversity is significantly lower in the urban region during summer.*

For zooplankton, our results indicate that urbanization does decrease zooplankton diversity during the summer (K-S test: $p < 0.05$, Fig. 4). However, our urban and nonurban categories do not explain the variance in spring or fall zooplankton diversity.

More detailed examination of land use effects on plankton biodiversity are ongoing. We have constructed detailed information on land use in the surroundings of each of our study sites, looking at several different scales of integration of land around the sites (Fig 5). The GIS analysis is complete and statistical analysis is underway.

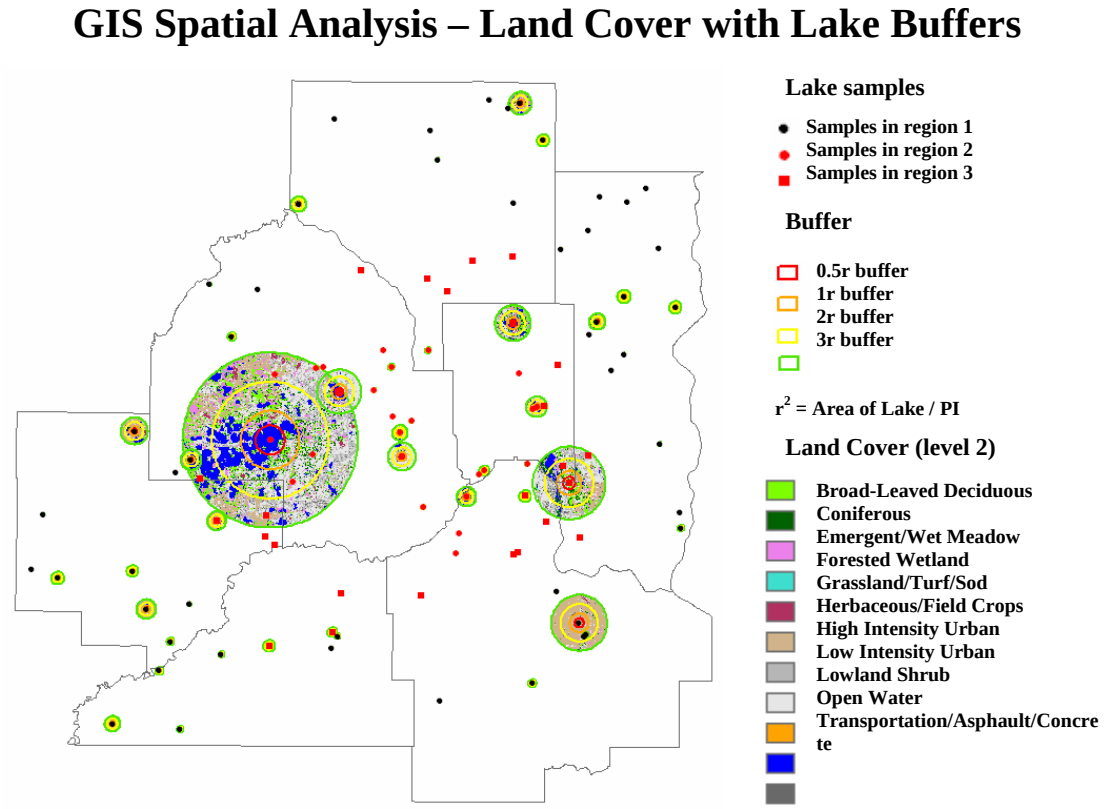


Figure 5. *Land surround each lake has been characterized using GIS, by taking circular areas of successively larger radius (0.5, 1, 2, and 3X lake radius calculated from lake area).*

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McDonnell, M.J., and S.T.A. Pickett. 1991. Comparative analysis of ecosystems along gradients of urbanization: opportunities and limitations. Pages 351-355 *in* S. Findlay, editors. Comparative Analysis of Ecosystems: Patterns, Mechanisms, and Theories. Springer-Verlag, New York.

Moyle, P.B., and R.A. Leidy. 1992. Loss of biodiversity in aquatic ecosystems: evidence from fish faunas. Pages *in* S. K. Jain, editors. Conservation Biology: The Theory and Practice of Nature Conservation. Chapman and Hall, New York.

Naiman, R.J., J.J. Magnuson, D.M. McKnight, and J.A. Stanford, eds. 1995. The Freshwater Imperative. Washington: Island Press.

Postel, S.L., G.C. Daily, and P.R. Ehrlich. 1996. Human appropriation of renewable fresh water. Science 271:785-788.

Publications associated with the project
None to date. First manuscript expected to be submitted by August, 2004.

Students supported by the project
James Hood, Kiyoko Yokota, Fei Yuan

Awards and achievements from your project
None

Seminar or other presentations resulting from your project
Presented Seminar to the Minnesota Pollution Control Agency, February 10, 2004, St. Paul, MN. Seminar entitled: An assessment of zoo- and phytoplankton biodiversity in Twin Cities lakes and ponds: Effects of urbanization on pelagic communities

Presented seminar at the University of Minnesota, Department of Ecology, Evolution and Behavior on April 23, 2004. Presentation entitled: The tale of the city rotifer and the country rotifer.

Related grants submitted or funded as a result of this project
None.

Arsenic in Minnesota Groundwater and its Impact on the Drinking Water Supply

Basic Information

Title:	Arsenic in Minnesota Groundwater and its Impact on the Drinking Water Supply
Project Number:	2003MN29B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	2 and 7
Research Category:	Not Applicable
Focus Category:	Groundwater, Water Use, Toxic Substances
Descriptors:	
Principal Investigators:	Randal J Barnes

Publication

1. Erickson, M.L., K. Peterson, and R.G. Soule. Got Arsenic? Site Investigation as an Innovative Compliance Option, submitted February 2003 to Journal of the American Water Works Association.
2. Erickson, M.L, and R.J. Barnes. Arsenic in Ground Water: Recent Research and Implications for Minnesota. CURA Reporter. 34[2]. May 2004.
3. Erickson, M.L, and R.J. Barnes. Arsenic in Ground Water: Recent Research and Implications for Minnesota. Minnesota Ground Water Association Newsletter. 23[1]. March 2004.
4. Erickson, M.L. and R.J. Barnes. 2004. Arsenic in Groundwater: Recent Research and Implications for Minnesota. CURA Reporter. University of Minnesota Center for Urban and Regional Affairs. Minneapolis, MN.

Arsenic in Minnesota groundwater and its impact on the drinking water supply

Principal Investigator
Randal J. Barnes, Department of Civil Engineering, University of Minnesota

Funding Source: USGS-WRRI 104B

Project Duration: March 1, 2003 to February 29, 2004

Summary
Arsenic contamination in upper Midwestern ground water is widespread, naturally occurring, and associated with the lateral extent of northwest source Late Wisconsin (Des Moines lobe) till. Arsenic concentration in ground water is not directly related to arsenic concentration in sediment.

In west-central Minnesota, private wells that have relatively short screens set close to the upper confining unit are more likely to have elevated arsenic concentrations than otherwise comparable private wells. The variability of arsenic concentrations over time in newly constructed wells is similar to concentration variability observed in older wells; there is no temporal trend.

Reductive desorption is the mechanism proposed to explain observed important temporal changes in water quality in two Minnesota public water supply wells.

Two procedures have been developed and tested for screening low-cost compliance options for public water systems with elevated arsenic. A 'site investigation' evaluates the option of drilling a new well. The procedure can identify low-arsenic aquifers at different elevations and/or different locations. Sampling a well several times over a period of a few hours provides the necessary information to evaluate the option of changing well operations. Changing well operations may be a viable option for communities with arsenic concentrations that predictably fluctuate around 10 μg/l.

Introduction
Arsenic exposure from contaminated drinking water at 50 μg/l is a significant environmental cancer risk, similar to the cancer risk associated with environmental tobacco smoke and home radon exposure (Smith et al. 1992). In response to reports by the National Research Council (1999; 2001) and others about risks from arsenic in drinking water at 50 μg/l, the U.S. federal drinking water standard, or Maximum Contaminant Level (MCL), was recently changed from 50 μg/l to 10 μg/l. Public water systems must comply with the new MCL by January 2006. In Minnesota, USA, over 100,000 people are estimated to use a public drinking water system with arsenic concentrations over 10 μg/l.

Welch, et al. (2000) made the association between glaciated areas in the upper Midwest and high arsenic in ground water. Statewide arsenic sampling in Minnesota indicates that a significant area of the state has detectable concentrations of arsenic in ground water (Centers for Disease Control 1994; Minnesota Pollution Control Agency 1995), with approximately 14% of sampled wells exceeding 10 μg/l. In Minnesota, 150,000 to 250,000 people are estimated to obtain their

drinking water from private wells with arsenic concentrations exceeding 10 μg/l (Soule 2004). A recent study of arsenic occurrence and exposure in western Minnesota found that over 50% of the 900 sampled private drinking water wells had arsenic over 10 μg/l (Minnesota Department of Health 2001). However, even in this high-arsenic area, arsenic concentrations in water had significant variability.

State and local governmental agencies are evaluating potential low-cost ways for public water systems to meet the new MCL (Erickson et al. 2004; Erickson and Barnes 2004a; Erickson and Barnes 2004b). Low-cost options include drilling a different well or changing well operation practices. However, very little is known about the mechanisms that cause the observed high-arsenic concentrations and the significant spatial and temporal variations in arsenic concentrations. Without a concrete understanding of the mechanisms that cause arsenic release from solids and into ground water, a public water supplier cannot implement a low-cost option with any assurance that it will be a long-term solution. Overall, lack of mechanistic understanding prohibits prediction of ground water arsenic concentration, interferes with the formulation of sound public policy, and inhibits the development of effective regulation.

The overall objective of this research project was to better understand arsenic concentrations in upper Midwestern glacial aquifers. Two keys to meeting this objective are 1) understanding the geochemical mechanisms governing arsenic in ground water, and 2) understanding the relationship between the geology/hydrogeology and arsenic concentration in ground water. Joint evaluation of geochemical mechanisms and potential geology/hydrogeology controls is a new approach to the upper Midwest's arsenic problem. The results of this approach are permitting better characterization of spatial variability of arsenic in ground water, as well as modeling and prediction of temporal variability of arsenic in ground water. Results are also providing information to governmental units for the development of sound regulations and guidance regarding drilling and using drinking water wells in high arsenic areas. The results may be applicable to other areas throughout the world that have reduced aquifers (e.g. Inner Mongolia, Vietnam, Romania, and Hungary (Smedley and Kinniburgh 2002)).

Methodology

The key components of the research project involved creating a useful database from existing data, collecting ground water and sediment samples, analyzing ground water and sediment samples, and data analysis/model building.

A comprehensive database of measured arsenic concentrations in the upper Midwest has been compiled. Public water supply, state well sampling, well construction (as available), and surficial geology data were obtained from various state agencies in MN, SD, ND, and IA, as well as from the U.S. Geological Survey (Centers for Disease Control 1994; Iowa Environmental Protection Division 2003; Minnesota Department of Health 2001; Minnesota Department of Health 2002; Minnesota Geological Survey and Minnesota Department of Health 2004; Minnesota Pollution Control Agency 1995; North Dakota Department of Health 2003a; North Dakota Department of Health 2003b; North Dakota Water Commission 2004; South Dakota Drinking Water Program 2003; South Dakota Geologic Survey 2003). This is the first time a comprehensive regional map of arsenic concentrations in upper Midwest groundwater and surficial geology has been compiled.

Water samples were collected from selected private wells in Minnesota and analyzed for arsenic and other analytes of interest. Sediment and water samples were collected from two monitoring wells drilled in June 2003 in northwestern Minnesota using the rotasonic method. Water samples were analyzed for arsenic and other analytes of interest. Sediment samples were analyzed for arsenic and other elements of interest. Sequential extractions were performed according to Keon (2001) to quantify the amount of labile arsenic present in sediment.

Results were analyzed using univariate statistics, indicator analysis, multivariate statistics, and geostatistics. Geochemical modeling was performed using MINEQL+.

Results and ongoing work

Arsenic contamination in upper Midwestern ground water is widespread, naturally occurring, and associated with the lateral extent of northwest source Late Wisconsin (Des Moines lobe) till. Although Late Wisconsin till does not have particularly high arsenic concentrations, it does have specific physical characteristics (fine-grained matrix and entrained organic carbon (Harris et al. 1995; Harris 1999; Matsch 1972; Parkin and Simpkins 1995; Patterson (née Jennings) 1999; Simpkins and Parkin 1993)) that create a geochemical environment favorable to regional scale mobilization of arsenic (Kim et al. 2002; Korte 1991; Smedley and Kinniburgh 2002; Warner 2001). Although it was originally hypothesized that ground water arsenic concentrations in the upper Midwest are associated with sediment arsenic concentrations, this hypothesis was not supported. In samples collected during this and related studies, arsenic concentrations in ground water are not directly related to arsenic concentration in sediment.

In west-central Minnesota, private wells that have screens less than 8 feet long set within 4 feet of the upper confining till unit have an average arsenic concentration of 20 µg/L, with 58% of wells exceeding 10 µg/l. Private wells with longer screens set further from the upper confining unit average only 12 µg/L arsenic, and 40% of wells exceed 10 µg/l.

In newly constructed wells, the variability of arsenic concentrations over time, from the date of construction over a period of one to two years, is similar to concentration variability observed in older wells; there is no temporal trend.

Two Minnesota public water supply wells have notable arsenic concentration variability over time. The arsenic concentration change is notable because the arsenic concentration is less than 10 µg/l initially, increases to more than 10 µg/l over a pumping period of one hour, and then decreases again after the well stops pumping. During the same pumping time, both the iron and sulfur concentrations decrease. After a period of no pumping, the iron concentration increases again, and the sulfur concentration remains lower. The Eh, which is a measure of redox potential, decreases significantly over the period of time that the well pumps, indicating that the redox state of the water is higher before the well is pumped. The mechanism of reductive desorption is proposed to explain the observed water quality changes in these two wells as they are pumped. Arsenic species measurements and geochemical modeling results support this proposed mechanism.

Two procedures have been developed and tested for evaluating low-cost compliance options for public water systems with elevated arsenic. A 'site investigation' evaluates the option of drilling a new well. The procedure can identify low-arsenic aquifers at different elevations and/or different locations. Sampling a well several times over a period of a few hours provides the necessary information to evaluate the option of changing well operations. Changing well operations may be a viable option for communities with arsenic concentrations that predictably fluctuate around 10 µg/l.

Additional data analysis and modeling efforts are ongoing.

Publications associated with the project

Erickson, M.L., K. Peterson, and R.G. Soule. Got Arsenic? Site Investigation as an Innovative Compliance Option, submitted February 2003 to *Journal of the American Water Works Association.*

Erickson, M.L, and R.J. Barnes. Arsenic in Ground Water: Recent Research and Implications for Minnesota. *CURA Reporter*. 34[2]. May 2004.

Erickson, M.L, and R.J. Barnes. Arsenic in Ground Water: Recent Research and Implications for Minnesota. Minnesota Ground Water Association Newsletter. 23[1]. March 2004.

Additional journal article manuscripts are currently being prepared.

Students supported by the project

Melinda L. Erickson, Water Resources Science Ph.D. candidate, was provided with a 50% Research Assistant position for approximately 1.5 semesters by this grant.

Awards and achievements resulting from the project

The Water Resources Science program (University of Minnesota) awarded a $500 travel grant to graduate student Melinda Erickson for travel to the November 2003 annual meeting of the Geological Society of America to present a poster of research results for the project.

The Albert Howard Fellowship (University of Minnesota) for 2003-2004 academic year was awarded to graduate student Melinda Erickson to augment research funds for continuing work on the project.

Seminar or poster presentations resulting from the project

Erickson, M.L. October 2, 2003. Arsenic in Minnesota Groundwater. Invited conference presentation at the American Water Works Association Minnesota Section Meeting, Moorhead, Minnesota.

Erickson, M.L., R.G Soule, and K. Peterson. October 28, 2003. Got Arsenic? Site Investigation as an Innovative Compliance Option. Poster presentation at the 36th Water Resources Conference sponsored by the University of Minnesota. Minneapolis, Minnesota.

Erickson, M.L. & R.J. Barnes. November 5, 2003. Measured and Modeled Arsenic Species Variability in Midwestern Ground Water. Poster presentation at the Geological Society of America Annual Meeting and Exposition. Seattle, WA.

Erickson, M.L. December 16, 2003. Arsenic in Minnesota Groundwater. Invited seminar presentation at the Minnesota Geological Survey, Minneapolis, Minnesota.

Erickson, M.L. January 14, 2004. Arsenic in Minnesota Groundwater. Invited seminar presentation at the Minnesota Department of Health, St. Paul, Minnesota. Seminar was telecast statewide to six MDH regional offices.

Erickson, M.L. & R.J. Barnes. March 23, 2004. Arsenic Contamination in Minnesota Groundwater: Recent Research and Regulatory Implications. Poster presentation at Water 2004. Minneapolis, Minnesota.

Erickson, M.L. April 26, 2004. Arsenic in Minnesota Groundwater. Invited seminar presentation for Minnesota State University – Moorhead, North Dakota State University, and Concordia College. Hosted by Minnesota State University – Moorhead. Moorhead, Minnesota.

Erickson, M.L. & R.J. Barnes. May 5 – 7, 2004. Late Wisconsin Till and Arsenic Contamination in Upper Midwest Groundwater. Poster presentation at the Institute of Lake Superior Geology Annual Conference. Duluth, Minnesota.

Related grants submitted or funded as a result of this project

Minnesota Department of Health funded three related projects: Summer 2003, $9,000 summer RA salary; June 2003 – June 2004, $6,250 water analytical contract; Summer 2004, $18,000 summer RA salary and RA travel expenses.

A proposal was submitted to the US Geological Survey March 1, 2004, in response to a Request for Proposal. The proposal requested funding for three years of additional work on arsenic in the upper Midwest. The proposed research would be conducted in partnership with ND State University and Minnesota Geological Survey researchers. Approximately $250,000 was requested, primarily for post-doc salary, field work costs, and analytical costs. As of June 7, 2004, no word has been received either way about this proposal. If awarded, the project would start September 2004.

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In Situ measurement of denitrification in agricultural streams

Basic Information

Title:	In Situ measurement of denitrification in agricultural streams
Project Number:	2000MN9G
Start Date:	9/1/2000
End Date:	8/31/2001
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Congressional District:	5
Research Category:	Water Quality
Focus Category:	Nitrate Contamination, Non Point Pollution, Methods
Descriptors:	denitrification, agriculture, surface drainage, isotopes
Principal Investigators:	Patrick L Brezonik, Lorin Kent Hatch

Publication

In Situ measurement of denitrification in agricultural streams

Principal Investigators
P. L. Brezonik Ph.D., Department of Civil Engineering, College of Engineering; and L. K. Hatch Ph.D., Water Resources Center, College of Natural Resources, University of Minnesota.

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Summary/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 research compared several methods used to assess *in situ* denitrification rates in agricultural streams and evaluated their accuracy. We evaluated how variations in key environmental factors affect the importance of this process as a nitrogen sink. Agricultural managers at local to national levels need this information in order to (i) prescribe nitrate mitigation techniques to lessen downstream impacts and (ii) predict how changes in best management practices in agricultural landscapes will affect nitrogen export at the drainage basin scale.

Contamination of ground water and streams by nitrate is a problem in many agricultural areas. Impacts associated with this pollution range from local (contamination of wells used for drinking water) to national (e.g., hypoxia in the Gulf of Mexico). Excess nitrogen loading, principally in the form of nitrate from the Mississippi River, is considered to be the cause of a large hypoxic zone in the nearshore Gulf of Mexico (Goolsby et al., 1999; Rabalais et al., 1999), and a large fraction of this nitrogen is thought to be derived from non-point agricultural sources in the Corn-belt region of the Upper Mississippi River Basin. The UMRB generates roughly one-third of the nitrate loads reaching the Gulf of Mexico, while the Ohio River basin generates roughly one-fifth of the nitrate reaching the Gulf. The Midwest region thus is responsible for roughly half of the non-point source loads of nitrate entering the Gulf of Mexico. These loads have been attributed to heavy precipitation on intensively row-cropped soils that have extensive networks of surface ditches and subsurface tile drains, are high in organic matter content, and receive high rates of inorganic and organic nitrogen amendments (Randall and Mulla, 1998). Nitrogen applied to the soil surface or mineralized from soil organic matter can be delivered in the nitrate form to surface waters by leaching and drainage through subsurface tile drains after heavy precipitation.

The Minnesota River Basin (MNRB, Figure 1) is typical of tributary basins of the Upper Mississippi and Ohio River Basins that contribute to hypoxia in the Gulf of Mexico (Randall and

Mulla, 1998). The MNRB (Mulla and Mallawatantri, 1997) has moderate to heavy annual precipitation (56-79 cm/yr), intensive row-cropping (92% of the land), extensive tile drainage (>40% of the land), extensive soils with high organic content (>80% of the land has an organic content exceeding 4%), and high rates of nitrogen application via fertilizer (county-wide averages up to 12.5 t km^{-2} yr^{-1}) and manure (county-wide averages up to 4.5 t km^{-2} yr^{-1}). As a result, the 4.0 million ha MNRB has a mean annual nitrate-N yield of 3.1 kg km^{-2} d^{-1} (11.2 kg ha^{-1} yr^{-1}), and its Le Sueur watershed has a mean annual nitrate-N yield of 6.4 kg km^{-2} d^{-1} (Randall and Mulla, 1998). These values are similar to the mean annual nitrate yields for other Midwest rivers; e.g., 5.5, 4.8, and 3.5, kg N km^{-2} d^{-1}, respectively, for the Iowa, Illinois, and Wabash Rivers (Goolsby et al., 1999).

Much of the nitrate exported from agricultural lands in the Cornbelt is transported through drainage ditches, but we have only a poor understanding of the nitrogen transformation processes in these water courses. Depending on physical circumstances, denitrification could be a significant pathway for nitrate removal from these systems, enhancing water quality and reducing health hazards downstream. Several methods are available to measure denitrification, but they usually are conducted using sediment core experiments in the laboratory, where handling and incubation conditions are not conducive to obtaining accurate estimates of *in situ* rates. Even when rates are measured using field incubations, ambient hydrodynamic conditions are not replicated in the incubated samples, and correspondence between measured rates and *in situ* values is problematic.

Our main goal was to assess *in situ* denitrification rates in agricultural streams and determine how these rates vary with stream stage, flow, and temperature. We compared *in situ* results obtained by ^{15}N tracer, ^{15}N natural abundance ratios, acetylene block, and mass balance methods. Part of our field work used in-stream mesocosms for assessments. We made measurements over a range of discharge and stage. Field work was conducted in the eastern Minnesota River Basin, an agricultural area with high nitrate levels in its streams and rivers.

Methodology

We selected two primary sites (Waseca Stream, in Waseca County, MN; and Beauford Stream, in Blue Earth County, MN). The Beauford site is ~ 20 miles west of the Waseca site (Figure 1). We installed three piezometers adjacent to each of these locations. In 2003, we included two additional streams (Buffalo Creek and Seven Mile Creek, Figure 1) in our study because of their unique characteristics (e.g., forested areas, significant groundwater contribution, trout stream designation).

We found it necessary to apply different denitrification assessment methods based on specific objectives. For example, we used mass balance and stable isotopes to assess large-scale denitrification rates, while we used N-tracer and sediment core analyses to assess small-scale sediment/stream interactions. Details are given below.

(A) *Assessment of Stream Size, Flow, and Temperature Effects on Denitrification.* We measured denitrification rates for this component using mass balance (Bachmann et al., 1991) and stable isotope methods in the three systems. These two approaches allowed us to assess denitrification rates under differing flow and temperature regimes. During late summer (July/August, 2001 and 2002) we collected water samples in the Beauford and Waseca systems (first order, stations 400

m apart). In 2003, we focused on sampling Buffalo and Seven Mile Creeks (second and third order, stations 400 m apart).

Stream and ground water analyses (APHA 1998) included nitrate, ammonium, and total dissolved and total nitrogen for the mass balance method. The dual isotope method was employed in 2002 (Beauford and Waseca systems) concurrently with the mass balance sampling to assess denitrification losses using stable isotopes, following the prodedures of Revesz et al. (1997). These samples were analyzed by the University of Waterloo, Canada.

(B) *Assessment of Sediment Core-Based Denitrification Measurement Methods.* We evaluated the importance of surface sediment nitrification as a nitrate source by measuring nitrate concentrations in pore water profiles of cores obtained across the stream cross-section at various locations along all four stream systems. Cores were extruded into 1-2 cm thick segments in the laboratory, and pore water for chemical analysis was obtained by centrifugation (if the sediments were sufficiently unconsolidated) or dilution/extraction with deionized water (if the sediments were too firm for centrifugation). Acetylene block analyses were performed on 2003 core samples (Buffalo, Seven Mile, and Waseca systems). *In situ* mesocosms (clear plexiglass 14.6 cm diameter cylinders) with battery-powered stirrers were used in ^{15}N-NO_3 addition experiments during the mass balance sampling periods as well (Waseca and Seven Mile only). Denitrification rates for this method were calculated according to Nielsen (1992). These samples were analyzed by Aarhus University, Denmark.

Results

Mass Balance Studies

The results of several upstream-downstream (400 m) mass balance studies during July and August of Year 2001 and Year 2002 in the Beauford and Waseca Streams showed that the two systems varied with regards to the composition of total dissolved nitrogen (TDN). Waseca Stream had nitrate-N, dissolved organic N (DON), and ammonium-N (NH_4) average concentrations of 11.75, 1.86, and 0.04 mg/L, respectively. Comparable values for Beauford Stream were 4.48, 4.03, and 0.06 mg/L, respectively. In general, nitrate concentrations were higher in the Waseca system than in the Beauford system, although elevated nitrate-N concentrations were present at Beauford during the August 28, 2002 sampling event.

Nitrate-N loss rates for Waseca Stream and Beauford Stream ranged from 0-0.59 mg/L and 0.31-1.08 mg/L, respectively. Calculation of areal loss rates were achieved by assuming a zero order loss rate in the advective-dispersion equation assuming dispersion transport is negligible. The resulting areal loss rates for Waseca Stream and Beauford Stream ranged from 0.12-0.39 mg/L/hr and 0.07-0.44 mg/L/hr, respectively. Using the combined data for these two streams, there was a strong correlation ($r^2 = 0.65$) between stream flow (L/s) and nitrate-N loss (mg/L): $N0_3$-N loss = -0.28 log flow + 1.07. There was a poor relationship ($r^2 = 0.21$) between stream temperature (C) and nitrate-N loss (mg/L): $N0_3$-N loss = 0.11 temperature – 1.67.

Stable Isotopes

During our Year 2002 mass balance studies, we collected composite samples at both the upstream and downstream ends of the 400 m stream reaches in Beauford and Waseca Streams. These samples were analyzed at the University of Waterloo (Canada) where both the ^{15}N and ^{18}O

isotope ratios were measured on the same nitrate molecule. Averaged results are presented for locations within the two systems (Figure 2). Overall, the data shows a general positive relationship between the N and O isotopes, with the exception of a value from Waseca groundwater from the upstream site (0.81, 12.83). This trend supports the prediction of Kendall and McDonnell (1998) that fractionation (towards heavier isotopes) will occur because microbial processes will favor the lighter isotopes. Thus, increasing values of the N and O isotopes suggest that denitrification has occurred. However, examination of the upstream-downstream values for the two streams shows little change in either of the isotope values. For example, Waseca upstream-to-downstream values of N and O isotopes changed from (9.22, 8.81) to (9.44, 9.54), indicating little fractionation of either isotope. Comparable values for Beauford Stream were (13.52, 9.13) and (13.52, 9.26), respectively.

Isotope values for tile lines that discharge into the streams were in the same range of range seen for the creek waters themselves. For example, the Beauford tile value for the N and O isotopes was (6.06, 7.45), which is slightly less that the instream values. Values for the Waseca tile were (13.78, 11.96), again in a similar range to the instream values. This suggests that tile lines may be a significant source of nitrate to the stream since similar isotope values occur in each pool.

Interestingly, values of N and O isotopes for groundwater showed very wide variability. It was previously noted that a Waseca groundwater site (located near the 0 m mass balance station) had low and high O values (0.81, 12.83), which did not follow the linear trend of most data. However, the Waseca groundwater site located at the end of the stream reach (400 m) showed two relatively high values of N and O isotopes (22.66, 14.23; 26.59, 15.39). These results suggest that I) there is a large variability of N and O isotope values in groundwater in the Waseca system, and ii) significant denitrification may be occurring within the groundwater system (at least at the downstream groundwater site). This reasoning is in line with a significant body of research that indicates there is a significant amount of dentrification occurring in riparian groundwater zones.

Sediment Cores

In Year 2002 we took stream bottom sample cores after completion of each mass balance study for both Beauford and Waseca Streams; Year 2003 samples were taken from Buffalo and Seven Mile Creeks. Sediment cores were sectioned into 0-1 cm, 1-2 cm, and 2-3 cm slices for analysis. Overall (combined data for cores slices) values for Waseca nitrate-N and ammonia-N were 1.38 and 0.04 mg/L, respectively. The nitrate-N value was much lower than that seen in the streamwater (11.75 mg/L), but the ammonia-N value was the same as that seen for the streamwater (0.04 mg/L). Nitrate-N and ammonia-N values for Beauford sediments were 1.48 and 0.04 mg/L, respectively. The nitrate-N value was again lower than that seen in the streamwater (4.48 mg/L), but the ammonia-N value was slightly lower than that seen for the streamwater (0.06 mg/L). These overall results suggest that although Beauford and Waseca Streams are located relatively close to one another in an intensive row-crop agricultural region, N processing may be taking place at different rates in each of these systems.

Sediment core pore-water values for Buffalo and Seven Mile Creeks were in general lower for nitrate-N and higher for ammonia-N than values seen for Beauford and Waseca Streams. Buffalo Creek values for nitrate-N and ammonia-N were 0.095 and 0.095 mg/L, respectively;

comparable values for Seven Mile Creek were 0.051 and 0.116 mg/L, respectively. Again, although the Beauford-Waseca systems and Buffalo-Seven Mile systems are reasonably close to one another (Figure 1), significant variation in N processing is apparently taking place.

Acetylene Block Analyses

Results from the acetylene block analyses indicate a wide range of denitrification potential between three of the study creeks. Mean denitrification (ng-N/g*day) rates for Buffalo Creek, Seven Mile Creek, and Waseca Stream were 145 (st. dev. 194), 851 (st. dev. 1007), and 1593 (st. dev. 1544), respectively. Significant variation, as indicated by the standard deviation values, is also present in these systems with respect to denitrification potential.

15N Nitrate Mesocosm Additions

Utilizing *in situ* mesocosms inserted into the stream sediments, we added slightly over 1 mg/L $^{15}N\text{-}NO_3$ to four mesocosms in Waseca Stream for a 4-hour incubation (August 2003). Stream nitrate-N concentrations varied from 0.97-1.05 mg/L over the course of the experiment, while ammonia-N varied from 0.11-0.13 mg/L. Mesocosms A and B were placed over bare sediment, while Mesocosms C and D were placed over actively-growing alga and sediments. Results are given in Table 1.

Table 1. Waseca mesocosm study results

Mesocosm	Nitrate-N Decrease (mg/L)	Denitrification Rate (micromoles N/m^2*hr)
A	0.33	211
B	0.12	365
C	0.21	1436
D	0.56	1626

Results indicate that denitrification was taking place in Waseca Stream, and that the denitrification rate was significantly higher when algae were noticeably present. This result supports the idea that algal oxygen production can expand the area of coupled nitrification/denitrification in the sediments, thus enhancing denitrification potential.

Conclusions

The high concentration of nitrate in agricultural streams is a difficult problem for water managers to address. The methods examined in this study involve the whole-reach and the sediment-core spatial scales. Here we address the implications of our results.

Reach-Scale Methods

For the mass balance method to provide useful information about nitrate retention, the stream reach must be long enough (i.e., have a long enough travel time for significant nitrate loss to occur) to ensure a statistical difference between upstream and downstream concentrations. This technique only gives information on nitrate retention and not the individual process mechanisms occurring; there are many physical, chemical, and biological transformations taking place in streams, and our ability to discern the contribution of each is very problematic.

The stable isotope provides information as to the processes involved in nitrate retention. Fractionation of the isotopes of N and O of the nitrate molecule in up and downstream pools can be used to infer what types of biological processes are occurring. However, the stream water can be comprised of many sources (i.e. groundwater, surface runoff, precipitation) so there is the potential to have a mixture of isotope signals. If there is a strong input of these other sources, the utility of this method in measuring denitrification is questionable because the mixing water (e.g., tile line water) will swamp out the signal (e.g., Kellman and Hillaire-Marcel, 1998).

For these two reach-scale methods, distance between sampling stations (and stream water packet travel time) is key to achieving useful results. Since the majority of stream water runoff and hence nitrate export occurs during the spring snowmelt/rain season in Upper Midwest agricultural areas, the stream distances necessary to measure significant statistical differences would most certainly be large. Additionally, drainage networks are very dense in this area of the country; dilution of a given stream reach by an intercepting ditch occurs frequently. Hence, use of these two methods to assess denitrification during the time of the year when the majority of nitrate export occurs is not recommended. These methods have a greater probability of useful results during lower flow periods the remainder of the year (when in-stream processes dominate, i.e., little tile line input occurs), although only a small portion of the annual nitrate export occurs during these periods.

Sediment Core-Based Methods

The acetylene block examines denitrification directly. It is not an *in situ* method but can be used to assess the spatial variability in denitrification potentials. The acetylene block method gives a volumetric denitrification rate, not a spatial rate: it is difficult to extrapolate results to the whole-reach scale, which would involve a number of tentative assumptions. There also tends to be high variability in the results, as denitrification may be dependent of the sediment texture and physical and chemical conditions. Hence acetylene block is useful as a screening technique for denitrification potential, but scaling-up to the stream-reach or watershed level using this method is wrought with difficulties.

Use of the dual isotope method using *in situ* mesocosms remedies some of the shortcomings of the acetylene block technique. The method gives a flux measurement and can technically be classified as an *in situ* measurement. However, even if the mesocosm is mixed to mimic the stream flow, hydrodynamic reality is lost in that the same parcel of water is in continuous contact with the sediments in a mesocosm, which is not the case in a stream. Problems with scaling-up to the stream-reach and watershed level are similar to that of the acetylene block method.

Final Comments

For the purposes of determining the impact of denitrification on nitrate loss in Upper Midwest agricultural streams, the four methods analyzed in this study appear to be most useful during low-flow stream conditions. During high-flow runoff conditions, the reach-scale methods are not sensitive enough to detect nitrogen-species changes; hence physical conditions are largely controlling our ability to measure denitrification. Core-based methods may not be realistic because stream water packet contact time with the bottom sediments is minimal. During low-flow conditions, biological conditions may be playing a significant role in dentrification. Reach-scale methods can be sensitive enough to detect changes in nitrate, but again they are not

measuring denitrification directly. Core-based methods utilized during these conditions may be strongly impacted by the presence or absence of biota (e.g., in-stream and/or stream-bank macrophytes, algae).

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2000-2001 Annual Report.

2001-2002 Annual Report.

Minnesota Water 2002 & Minnesota Lakes and Rivers Conference, April 17-20, 2002, St. Cloud, MN. Ben L. O'Connor, Melissa M. Atkinson, Lorin K. Hatch, Patrick L. Brezonik. Denitrification in agricultural streams.

American Society of Limnology and Oceanography 2003 Aquatic Sciences Meeting, Salt Lake City, UT. Hatch, L., B. O'Connor, and P. Brezonik, P. Denitrification in agricultural streams: a comparison of in situ methods.

Frontiers in Assessment Methods for the Environment (FAME) Symposium, August 10-13, 2003, Minneapolis, MN. Ben O'Connor, Lorin Hatch, Miki Hondzo, and Patrick Brezonik. Nitrate dynamics in streams: methods and scales for examining a complex process.

Students Supported by Grant
Ben O'Connor, Melissa Atkinson
Note: Mr. O'Connor is continuing to analyze the field data collected during this project, especially with regard to synthesizing the results from the different phases and methods and evaluating environmental conditions under which the various methods are appropriate to use. He also is continuing to conduct laboratory-scale measurements in a flume to evaluate the influence of stream hydrodynamic conditions on denitrification rates. The field studies conducted as part of this WRRI project will form the basis for two chapters in Mr. O'Connor's dissertation, which is expected to be completed in late 2005.

Achievements and Awards
n/a

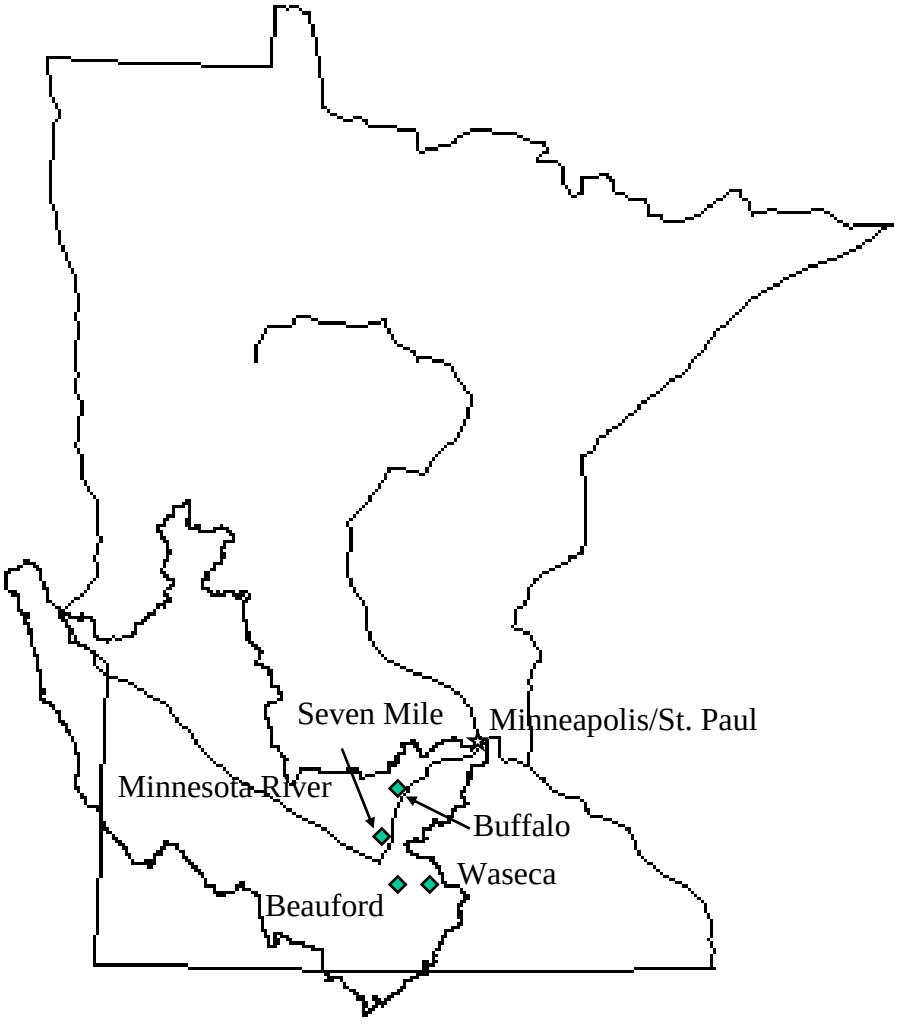

Figure 1. The Minnesota River Basin and research sites.

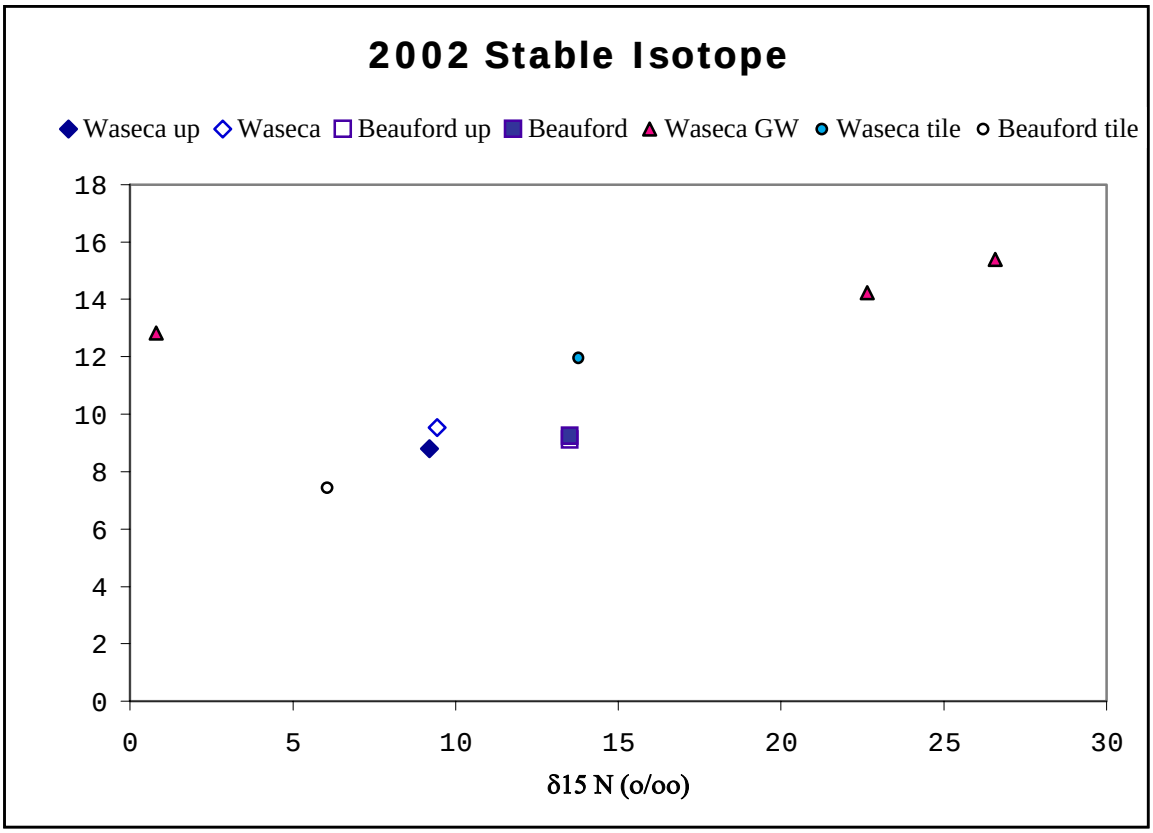

Figure 2. Nitrate dual stable isotope values for Beauford and Waseca Streams, Year 2002.

Photochemical fate of pharmaceutical compounds discharged and detected in natural waters

Basic Information

Title:	Photochemical fate of pharmaceutical compounds discharged and detected in natural waters
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Publication

1. Latch, D.E.; Stender, B.L.; Packer, J.L.; Arnold, W.A. ; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. Environmental Science and Technology, v. 37(15), pp. 3342-3350.
2. Latch, D.E.; Packer, J.L.; Arnold, W.A.; McNeill, K., 2003. Photochemical conversion of triclosan to 2,8 dichlorodibenzo p dioxin in aqueous solution. Journal of Photochemistry and Photobiology A: Chemistry, v. 158(1), pp. 63-66.
3. Packer, J.L.; Werner, J.J.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen. Aquatic Sciences, v. 65(4), pp. 342-351.
4. Boreen, A.L.; Arnold, W.A., McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: a review. Aquatic Sciences, v. 65(4), pp. 320-341.
5. Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J; Arnold, W.A.; K. McNeill, 2004. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin and oligomerization products, Environmental Toxicology and Chemistry, in review.
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environment: sulfa drugs containing five-memberred heterocyclic groups. Environmental Science and Technology, in review.

Photochemical fate of pharmaceutical compounds discharged and detected in natural waters

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Summary
Recent studies have detected numerous pharmaceuticals and personal care products (PPCPs) in US surface waters. The potential environmental impact of these chemicals will be dictated by their persistence in the environment and the biological activity of any degradation products. One potential loss process for pharmaceuticals and personal care products is photodegradation. In this work, the direct photolysis and indirect photolysis (hydroxyl radical mediated and singlet oxygen mediated) of selected PPCPs was investigated. To date, the fate of antacids (cimetidine and ranitidine), non-steroidal anti-inflammatory drugs (naproxen, ibuprofen, diclofenac, and mefenamic acid), a lipid regulator (clofibric acid), and an antimicrobial compound (triclosan) have been studied. Preliminary work with 5 members of the sulfa antibiotic class was also performed. All the compounds studied react with hydroxyl radical at nearly diffusion limited rates, but given the low concentration of hydroxyl radical in natural waters, other processes appear to be more important for most of the compounds studied. The heterocyclic groups in cimetidine, ranitidine, and the sulfa drugs are susceptible to attack by singlet oxygen. Ranitidine and the sulfa drugs are subject to direct photolysis while cimetidine is not. Direct photolysis occurs rapidly for triclosan when present in the deprotonated, phenolate form, and reaction with singlet oxygen can also be important under appropriate conditions. Naproxen and diclofenac are rapidly transformed via direct photolysis. Even though it has a larger solar spectral overlap integral than diclofenac, the direct photolysis rate of mefenamic acid is quite slow due to a low quantum yield. Mefenamic acid, however, is destroyed via reaction with photoexcited (triplet) natural organic matter. Clofibric acid reacts via a combination of direct photolysis and radical mediated indirect photolysis. Reaction with radicals is the only photochemical loss process observed for ibuprofen. An important finding of this study is that the products of photolysis are not always benign. Specifically, photolysis of triclosan leads to the formation of 2,8-dichlorodibenzo-*p*-dioxin and 2,4-dichlorophenol, two priority pollutants.

Introduction
Pharmaceuticals and personal care products (PPCPs) are a class of chemicals that are continuously released into the environment through human activities, and even though they have known biological effects, have received minimal attention until recently (1,2). Examples of PPCPs include antibiotics, lipid regulators, psychiatric drugs, over the counter medications, and antimicrobial compounds. Most of these chemicals are introduced into the sewage system

through their normal course of use. Once in the sewage system, many PPCPs are not completely removed at treatment plants (3) and thus, there is continuous introduction of these compounds to the environment. Numerous PPCPs have been detected in both ground and surface waters throughout the United States and Europe (2,4-12).

The impacts of PPCPs on the environment are unknown. Undesirable effects on non-target aquatic organisms and damage to sensitive ecosystems are possible (2). Furthermore, antibiotic drugs and antimicrobial agents in the environment may aid in the development of resistant bacteria (2,13). The lifetimes of the PPCPs in aquatic systems will partially determine the magnitude of the effects and potential threats to drinking water supplies. Loss processes such as photolysis, therefore, will play an important role in the environmental impact of these compounds. This includes not only direct and indirect photolysis loss processes, but also, where possible, identifying intermediates and products that are formed through photolysis as transformation products may still have biological activity.

The research objective of this study is to determine the importance of both direct photolysis and indirect photolysis as loss processes for common PPCPs, including over-the-counter medications, antimicrobial compounds, and antibiotic medications. Additionally, the research aims to identify major products resulting from the photolysis experiments.

Methods

Direct and natural water photolysis experiments

Photolysis experiments were performed outdoors under natural sunlight or indoors under medium pressure Hg-vapor lamps. Sample solutions were contained in quartz test tubes (OD = 13 mm, ID = 11 mm, V = 10 mL) which were arranged on a turntable apparatus to ensure equal irradiation for all of the samples. For kinetic analyses ~ 0.5 mL samples were withdrawn from the quartz tubes at predetermined intervals and analyzed on an 1100 Series Hewlett Packard HPLC or on a Waters LC module 1 plus both equipped with UV-absorbance detection and a computer driven data acquisition system. In experiments designed to probe for pH effects, various buffer solutions were employed to set the pH values. Quantum yields were calculated by comparing the rate constant for the disappearance of the PPCPs with the rate constant for the disappearance of a *p*-nitroanisole actinometer as described in ref. 14.

Natural water photolysis experiments were performed in 0.2 μm filtered Mississippi River (MRW) or Lake Josephine (LJW) water. To determine which pathways were responsible for the photodegradation, various quenchers were added to the water samples (sodium azide or DABCO for $^1O_2$, isopropanol for radicals, isoprene for excited triplet species) and the substrate was also photolyzed in DI water in a separate tube.

Hydroxyl radical

The second-order rate constant for the reaction of PPCPs with hydroxyl radical was determined using Fenton's reagent. Serum bottle reactors contained a 100 μM solution of the PPCP of interest, 100 μM acetophenone, 0.2 mM Fe^{2+}, and 5 mM hydrogen peroxide adjusted to pH 3 with sulfuric acid (15). Samples were withdrawn at predetermined intervals and mixed with an equivalent volume of methanol to quench the reactions (16). HPLC analysis for both the PPCPs and the acetophenone was performed.

The hydroxyl radical rate constant was determined using competition kinetics according to:

$$k_{\cdot OH}^{S} = \frac{\ln([S_t]/[S_0])}{\ln([R_t]/[R_0])} k_{\cdot OH}^{R}$$

where *S* is the substrate (the PPCP) and *R* is the reference compound with a known hydroxyl radical rate constant (acetophenone, $k_{\cdot OH} = 5.9 \times 10^9$ M^{-1} s^{-1}, 17).

Singlet oxygen

Singlet oxygen reaction kinetics were measured in one of two ways, directly by laser flash photolysis (LFP) or indirectly by steady-state photolysis (SSP). In both types of experiment the substrate (typically at micromolar concentrations) and 40 μM Rose Bengal (RB), 100 μM Eosin Y, or 100 μM perinaphthenone, three well-defined singlet oxygen sensitizers, were dissolved in aqueous buffer solutions. In the LFP experiments, a pulse of laser light excites the sensitizer, which then produces singlet oxygen after the excited-state sensitizer is quenched by dissolved molecular oxygen. A sensitive Ge-photodiode detector then monitors the phosphorescence emission from singlet oxygen. The rate of disappearance of the singlet oxygen phosphorescence signal is a measure of a substrate's activity toward singlet oxygen. The resulting total quenching rate constant (k_{tot}) is the sum of the chemical reaction and physical quenching rate constants.

In SSP experiments, the samples are photolyzed continuously and small aliquots are removed for analysis by HPLC. In this case, the disappearance of the PPCP is monitored (as decreases in peak area), rather than the singlet oxygen signal. This allows for the determination of the chemical reaction rate constant (k_{rxn}) for the PPCP with singlet oxygen.

Triplet natural organic matter

The role of excited triplets was explored using natural water (MRW), Suwannee River fulvic acid (SRFA), or model sensitizers (3'-methoxyacetophenone, 2-acetonaphthone, and perinaphthenone). Quartz bottles (70 mL) containing an aqueous solution of the sensitizer and the target pharmaceutical compound were irradiated with an Hg-vapor lamp, and the loss of the parent compound was monitored via HPLC. Bottles without sensitizer present were used to quantify direct photolysis. The effect of the addition of the triplet quencher isoprene, or the removal of oxygen (also a triplet quencher) via argon sparging on the loss rate of the target compound were used to assess the role of excited triplet species in the observed loss of the parent pharmaceutical compound.

Product identification

To analyze the products of various photoreactions, GC-MS, LC-MS, and NMR spectroscopy were employed. An Agilent Technologies 6890 Gas Chromatograph with Mass Selective

Detector was used to obtain mass spectra of various reaction mixtures. Photolysis samples run in organic solvents were analyzed by GC-MS to identify products. Product peaks were compared to mass spectral libraries to aid in their identification. Authentic samples of the likely products were then run under identical conditions to compare to the photolysis samples. Proper retention times and mass spectra indicated that a peak from the reaction mixture matched the standard solutions.

Results

Cimetidine and ranitidine (Latch et al., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. Environmental Science and Technology, v. 37(15), pp. 3342-3350.)

The anti-ulcer drugs cimetidine and ranitidine both rapidly decayed in MRW, but with disparate mechanisms (see Figure 1). In MRW, cimetidine rapidly photodegraded, and the addition of 1 % isopropanol did not alter the rate. Addition of 10 mM sodium azide, a $^1O_2$ quencher, however, drastically diminished the photodegradation rate. The photodegradation rate of the azide spiked sample matched that of a dark control and a direct photolysis sample (in which cimetidine was dissolved in DI water with no sensitizer present). These results indicate that cimetidine does not undergo any direct photolysis, but is rapidly removed from natural waters due to its rapid reaction with $^1O_2$. Ranitidine, however, readily photodegrades in DI water, indicating that it undergoes direct photolysis. The photodegradation rate in MRW is slightly faster than it is in DI water, though, indicating a competing indirect photolysis mechanism. Quencher studies were used to determine that direct photolysis is the primary loss process in MRW, while reaction with $^1O_2$ causes a slight increase in the decay rate (as evidenced by the rate retardation upon addition of the $^1O_2$ quencher DABCO and the lack of effect when isopropanol was added).

To better assess the environmental fate of ranitidine and cimetidine, studies were performed in aqueous samples buffered to different values. As a compound that is active toward direct photolysis, ranitidine (pK_a = 8.2) was photolyzed in DI water buffered to pH 6 and pH 10. At these values, ranitidine is > 99 % in its conjugate acid form and 98 % in its free base form, respectively. At these two end member pH values the degradation rates were nearly identical, with quantum yields (relative to a p-nitroanisole actinometer) of $5.3 \pm 0.1 \times 10^{-3}$ at pH 6 and $5.5 \pm 0.1 \times 10^{-3}$ at pH 10.

The pH dependence of reaction of cimetidine (pK_a = 7.1) with $^1O_2$ was determined using SSP. Photolysis experiments were performed using RB or perinaphthenone as sensitizer in buffered samples spanning from pH 4 to 10. The bimolecular reaction rate constants (k_{rxn}) were found to be highly pH dependent, with a value of $3.3 \pm 0.3 \times 10^6$ M^{-1} s^{-1} at pH 6 and $250 \pm 20 \times 10^6$ M^{-1} s^{-1} at pH 10. At intermediate pH values, experimentally determined rate constants agreed well with those calculated from the end member rates and the speciation of the compound.

For ranitidine, the pH dependence of its reaction with $^1O_2$ was determined by LFP to minimize the competing direct photolysis reaction. Rose Bengal or Eosin Y were used as sensitizers for these experiments and D_2O buffer solutions were used as solvent due to the intrinsically longer lifetime of $^1O_2$ in D_2O than H_2O. The total quenching rate constant (k_{tot}) was determined at pD 6.4, 7.5, and 9.8. A fourfold difference in rate constants was observable across this range: 1.6 ±

0.2×10^7 M^{-1} s^{-1} at pD 6.4, $2.65 \pm 0.07 \times 10^7$ M^{-1} s^{-1} at pD 7.5, and $6.4 \pm 0.4 \times 10^7$ M^{-1} s^{-1} at pD 9.8.

The experiments performed with cimetidine and ranitidine allow for predictions regarding their fate in surface waters. The fate of cimetidine is expected to be highly variable and controlled primarily by the pH and $[^1O_2]_{SS}$ of the water body. Its environmental half-life is expected to vary between 53 min (in pH 8 water bodies with $[^1O_2]_{SS} = 10^{-12}$ M) and 900 h in pH 6 water bodies.

The loss of ranitidine from natural waters is expected to be due primarily to direct photodegradation. Based on calculated quantum yields and differences in seasonal and longitudinal solar irradiation, the half-life is expected to range from 65 min to 6 h and be insensitive to pH. In waters that contain a high $[^1O_2]_{SS}$, the half-life is expected to decrease slightly due to the competing reaction with $^1O_2$.

Triclosan

(Latch et al., 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry,* v. 158(1), pp. 63-66; and Latch et al., 2004. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-*p*-dioxin and oligomerization products, *Environmental Toxicology and Chemistry,* in review.)

Triclosan was found to decay rapidly in MRW. The photodegradation in MRW matched that in DI water and added quenchers did not slow the degradation rate in MRW, indicating that there was little indirect photolysis occurring. This finding is consistent with what other researchers have found (18, 19). Both 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) and 2,4-dichlorophenol (2,4-DCP) are observed as products of triclosan photodegradation. The 2,8-DCDD and 2,4-DCP are also photolabile and thus are intermediates. The yields for 2,8-DCDD and 2,4-DCP range from 3–12 % depending on the conditions employed. When triclosan is photolyzed in the presence of Suwannee River fulvic acid, a portion of the initial mass is recovered as insoluble material. It is postulated that photolysis in natural waters leads to the majority of triclosan being coupled to humic matter based on experiments in which the formation of insoluble material was monitored with photolysis time. The pathways of triclosan photolysis are summarized in Figure 2. Triclosan also reacts rapidly with both singlet oxygen ($k_{rxn} = 1.07 \pm 0.03 \times 10^8$ M^{-1} s^{-1} in pH 10 water) and hydroxyl radical ($k_{\cdot OH} = 5.4 \pm 0.3 \times 10^9$ M^{-1} s^{-1}). 2,4-DCP is an intermediate in the singlet oxygenation of triclosan, with a yield of 79%. Only at high steady state singlet oxygen concentrations (i.e. 10^{-12} M) and pH > 8, however, will indirect photolysis pathways be important in environmental systems.

Pharmaceuticals containing carboxylic acid functionalities: naproxen, diclofenac, ibuprofen, clofibric acid, and mefenamic acid

(Packer et al., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen, *Aquatic Sciences,* v. 65(4), pp. 342-351; and Werner et al., 2004. Environmental photodegradation of mefenamic acid, *Chemosphere*, in review.)

The photolysis of naproxen in Milli-Q water and in MRW by natural sunlight is shown in Figure 3. In Mississippi River water, naproxen was photodegraded slightly more slowly than in

deionized water ($k_{rel} = k_{MRW}/k_{DI} = 0.78$). The rate constant for the interaction of singlet oxygen with naproxen was determined by LFP to be $1.1 \pm 0.1 \times 10^5$ M^{-1} s^{-1}, too slow to be of environmental significance for this compound. The radical inhibitor IPA reduced the photodegradation rate in Mississippi River water ($k_{rel} = k_{IPA}/k_{MRW} = 0.43$). Subsequent work with 1% IPA in Milli-Q water showed similar inhibition. The quenching of the reaction with IPA was not consistent with the reaction rate with hydroxyl radical determined with Fenton's reagent. Another possibility for the observed effect of IPA is the quenching of a radical form of the naproxen itself. Support for this possibility arises from previous phototoxicity work, in which the direct photolysis products of naproxen were identified (20-22). The first step is conversion of the carboxylate (COO-) group to a carboxyl radical (COO·). Decarboxylation, resulting in carbon dioxide and a benzylic radical, then occur giving a variety of products. If a portion of the carboxyl radical is quenched by IPA, then the resulting product would be naproxen and the overall degradation rate of naproxen would be slower in the presence of IPA. The overall effect on reaction rate would be dependent on the relative rates of quenching by IPA and decarboxylation. The participation of other radical species (*e.g.,* peroxy radicals, DOM radicals), however, cannot be ruled out. Note that such a quenching effect by the DOC in the MRW (16 mg/L) would be minimal compared to that by 1 % IPA (≈4700 mg/L DOC).

Sunlight photolysis of diclofenac in natural water proceeded at a rate that is equivalent to that in Milli-Q water ($k_{rel} = k_{MRW}/k_{DI} = 1.00$). The radical inhibitor IPA, however, increased the photodegradation rate in Mississippi River water ($k_{rel} = k_{IPA}/k_{MRW} = 1.68$). Further study revealed that IPA also increased the photodegradation rate of diclofenac in deionized water by a similar amount ($k_{rel} = k_{IPA}/k_{DI} = 1.45$). The quantum yield found in this study, 0.094, is very close to that reported in the literature (0.12-0.2) (12, 23). The acceleration of the reaction in the presence of IPA occurred in both Milli-Q water and in MRW, indicating that either a reaction with or mediated by IPA takes place. The results of this study and of previous researchers (12, 24, 25), however, indicate that direct photolysis in the dominant degradation mechanism for diclofenac.

In Figure 4, the photolysis of clofibric acid in sunlight is depicted. The quantum yield was found to be 0.002, similar to that found in ref. 25. The rate of disappearance in MRW is 2.9 times faster than in Milli-Q water. IPA slows the photolysis rate in MRW ($k_{rel} = k_{MRW\text{-}IPA}/k_{MRW} = 0.39$) and the resulting rate is the same as that in Milli-Q water. Addition of IPA to the Milli-Q water resulted in a photolysis rate 1.7 times that observed in Milli-Q water alone. This is attributed to experimental variation as only 8-10% of the clofibric acid was degraded during the experiments. Both the more rapid reaction observed in MRW versus Milli-Q water and the quenching by IPA in MRW in this work suggest a role indirect photochemical processes. At a typical, upper-range value of 10^{-16} M for ·OH, ~20% of the quenched transformation can be attributed to hydroxyl radical based on the second-order rate constant determined for clofibric acid ($4.7 \pm 0.3 \times 10^9$ M^{-1} s^{-1}).

Because limited transformation was observed under sunlight, the photolysis of ibuprofen was studied using Hg-vapor lamps (Figure 5). Ibuprofen was negligibly degraded in Milli-Q water (with or without IPA). In MRW, however, transformation did occur, and this reaction was quenched by the addition of IPA. Based on the k_{OH} value for ibuprofen and the observed degradation rate that can be attributed to radical processes ($k_{MRW} - k_{MRW\text{-}IPA} = 3.5 \times 10^{-4}$ min^{-1}), the calculated steady state hydroxyl radical concentration would be 5.4×10^{-14} M in the

irradiated MRW. This value is 5-10 times greater than that obtained previously using this Hg lamp for similar waters collected in Minnesota (26, 27). It is likely that photo-generated radicals other than hydroxyl radical are also involved in the transformation of ibuprofen. This analysis indicates that indirect photolysis mediated by radicals is the primary photolysis process for ibuprofen in surface waters.

The direct solar quantum yield of mefenamic acid was observed to be $1.5 \pm 0.3 \times 10^{-4}$, much lower than that of the structurally similar diclofenac. Significant photosensitization was observed in solutions of Suwannee River fulvic acid and Mississippi River water, as well as for the model photosensitization compounds 3'-methoxyacetophenone, 2-acetonaphthone and perinaphthenone. Quenching, sparging and light-filtering experiments suggested a transfer of triplet energy (or oxidation without destruction of the sensitizer) as the sensitization mechanism (Figure 6). An upper limit of 186 kJ mol^{-1} for the triplet energy of mefenamic acid was determined. Due to its low quantum yield, the loss of mefenamic acid in sunlit natural waters is expected to depend on both direct and indirect photodegradation processes.

Antibiotics: sulfa drugs

The sulfa drugs sulfamethoxazole, sulfamethizole, sulfathiazole, and sulfisoxazole were found to photodegrade in LJW at varying rates, and the degradation of all four matched that observed in DI H_2O, indicating the degradation is due solely to direct photodegradation. To further investigate the direct photolysis of the sulfa drugs, photolyses were performed at a range of pH values using buffered solutions. The direct photodegradation was found to by highly pH dependent for each of the sulfa drugs investigated.

The sulfa drugs are subject to reaction with hydroxyl radicals. The second order rate constants for these compounds are shown in Table 1. In addition to the reaction rate constants, environmental half-lives were calculated based on the range of hydroxyl radical concentrations expected in natural waters, which may range from 10^{-16} M in agriculturally impacted waters containing high nitrate levels to 10^{-18} M in pristine waters (26, 27).

The interaction of the sulfa drugs with $^1O_2$ was monitored in two stages. First, the total quenching rate constant (k_{tot}), the sum of the physical quenching and chemical reaction rate constants of substrate with $^1O_2$, for the interaction between the sulfa drugs and $^1O_2$ was determined using laser flash photolysis. The values obtained were found to vary greatly within the class of sulfa drugs, and are shown in Table 2.

Table 1. Hydroxyl radical reaction rate constants and environmental half-lives for selected sulfa drugs.

Sulfa Drug	$k_{rxn,\ \cdot OH}$ ($M^{-1}s^{-1}$)	$t_{1/2}$ $[\cdot OH]_{SS}=10^{-16}$ M	$[\cdot OH]_{SS}=10^{-18}$ M
Sulfamethoxazole	$5.77 \pm 0.06 \times 10^9$	13.9 days	3.9 years
Sulfathiazole	$7.13 \pm 0.06 \times 10^9$	11.3 days	3.1 years
Sulfamethizole	$4.87 \pm 0.04 \times 10^9$	16.5 days	4.6 years
Sulfisoxazole	$6.59 \pm 0.06 \times 10^9$	12.2 days	3.4 years

Table 2. Total quenching rate constants for the interaction of selected sulfa drugs with $^1O_2$.

Sulfa Drug	k_{tot} ($M^{-1}s^{-1}$) pD ~ 9.5
Sulfamethoxazole	$2.3 \pm 0.4 \times 10^{4*}$
Sulfathiazole	$5.6 \pm 0.3 \times 10^{7}$
Sulfamethizole	$3.6 \pm 0.2 \times 10^{6}$
Sulfamoxole	$3.0 \pm 0.2 \times 10^{8}$
Sulfisoxazole	$6.5 \pm 0.2 \times 10^{7}$

* Measured in acetone.

The pH dependence on the total quenching rate constant was investigated for sulfamoxole and was found to exhibit a sigmoidal relationship, with elevated rate constants observed at more basic pH values.

Next, the specific chemical reaction rate constant was measured for both sulfathiazole and sulfisoxazole using steady-state photolysis. The reaction rate constants observed at pH 10 were $5.8 \pm 0.7 \times 10^7$ $M^{-1}s^{-1}$ and $4.27 \pm 0.06 \times 10^7$ $M^{-1}s^{-1}$, respectively, indicating that the majority of the k_{tot} for both sulfa drugs is due to chemical reaction with $^1O_2$. The degradation was verified to be due to reaction with $^1O_2$ through the observed rate suppression upon addition of NaN_3, a known $^1O_2$ quencher, as well as by the rate enhancement observed in D_2O due to the $^1O_2$ kinetic isotope effect.

Ongoing work

With the additional funding obtained (see below), we are now investigating the photochemical fate of a variety of antibiotic and estrogen compounds. Additionally, we are developing assays to determine if photoproducts retain antibacterial or estrogenic activity.

Summary of findings

The primary photodegradation mechanism for the antacid cimetidine is reaction with singlet oxygen while that for ranitidine is direct photolysis. These results reveals that minor structural changes can give rise to disparate environmental loss mechanisms. Direct photolysis occurs rapidly for triclosan when present in the deprotonated, phenolate form. For triclosan, an important finding of this study is that the products of photolysis are not always benign. Specifically, photolysis of triclosan leads to the formation of 2,8-dichlorodibenzo-*p*-dioxin and 2,4-dichlorophenol, two priority pollutants. Direct photolysis is the dominant loss process for naproxen and diclofenac. For clofibric acid, direct photolysis and radical mediated indirect processes are of equal importance. Mefenamic acid has a surprisingly low quantum yield for direct photolysis and is subject to reaction with excited organic matter. Reaction with radicals is the only photochemical loss process observed for ibuprofen. The sulfa drugs are susceptible to indirect photodegradation by hydroxyl radical and singlet oxygen and direct photolysis. Direct photolysis is the dominant pathway, and the rates are highly pH dependent, revealing the importance of environmental conditions on photodegradation.

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List of publications & presentations resulting from this project

Peer Reviewed Publications

Latch, D.E.; Stender, B.L.; Packer, J.L.; Arnold, W.A. ; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. *Environmental Science and Technology*, v. 37(15), pp. 3342-3350.

Latch, D.E.; Packer, J.L.; Arnold, W.A.; McNeill, K., 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry,* v. 158(1), pp. 63-66.

Packer, J.L.; Werner, J.J.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquatic Sciences,* v. 65(4), pp. 342-351.

Boreen, A.L.; Arnold, W.A., McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: a review. *Aquatic Sciences,* v. 65(4), pp. 320-341.

Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J; Arnold, W.A.; K. McNeill, 2004. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-*p*-dioxin and oligomerization products, *Environmental Toxicology and Chemistry,* in review.

Werner, J. J.; McNeill, K.; W.A. Arnold, 2004. Environmental photodegradation of mefenamic acid, *Chemosphere*, in review.

Invited Presentations

W.A. Arnold

Photochemical Fate of Pharmaceuticals in the Environment. Department of Geography and Environmental Engineering, The Johns Hopkins University, Baltimore, MD, December 12, 2003.

Photochemical Degradation of Triclosan in the Environment. Recycling Association of Minnesota/Solid Waste Association of North America Land of Lakes Chapter 9th Annual Fall Conference & Show, St. Louis Park, MN, November 4-6, 2003.

Photochemical Fate of Pharmaceuticals in the Environment. St. Anthony Falls Laboratory , University of Minnesota, Minneapolis, MN October 29, 2003.

Photochemical Fate of Pharmaceuticals in the Environment. Presented at the Water Resources Science seminar series, University of Minnesota, St. Paul, MN April 8, 2003.

Photochemical Fate of Pharmaceuticals in the Environment. Presented at the Department of Environmental Engineering Science, Washington University in St. Louis, St. Louis, MO, March 14, 2003.

K. McNeill

Photochemical Fate of Pharmaceutical Pollutants. Gordon Research Conference, Environmental Sciences: Water, June 27 – July 2, 2004, *Forthcoming*.

Environmental Fate of Pharmaceutical Pollutants. University of Wisconsin-Eau Claire, October 25, 2002.

Environmental Fate of Pharmaceutical Pollutants. Hamline University, October 16, 2002.

Environmental Fate of Pharmaceutical Pollutants. Macalester College, October 9, 2002.

Singlet Oxygen and the Fate of Pharmaceuticals in the Aquatic Environment. 34th American Chemical Society Great Lakes Regional Meeting, Minneapolis, MN, June 4, 2002.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Grinnell College, April 24, 2002.

Environmental Photochemistry of Pharmaceuticals, University of Minnesota Undergraduate Society for Lectures in Chemistry, November 28, 2001.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Portland State University, October 26, 2001.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Reed College, October 25, 2001.

Singlet Oxygen and Environmental Photooxidations. University of Minnesota, Duluth, September 21, 2001.

Featured Speaker: Singlet Oxygen and Environmental Photooxidations. American Chemical Society, Winona-La Crosse Section Meeting, September 18, 2001.

Conference Presentations

McNeill, K.; Arnold, W.A. Contribution of photochemistry to the fate of pharmaceuticals and personal are products in surface waters. Paper to be presented at the 228th American Chemical Society National Meeting, Philadelphia, August 22-27, 2004.

Werner, J.J.; McNeill, K.; Arnold, W.A. Kinetics of the environmental photodegradation of mefenamic acid. Poster to be presented at the 228th American Chemical Society National Meeting, Philadelphia, August 22-27, 2004.

Boreen, A.L.; Arnold, W.A.; McNeill, K., 2004. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. Paper presented at the Minnesota Water 2004 conference, Minneapolis, MN, March 23, 2004.

Werner, J.J., McNeill, K., Arnold, W.A., 2004. Photochemical fate of pharmaceuticals in the environment. Poster presented at the Minnesota Water 2004 conference, Minneapolis, MN, March 23, 2004.

Latch, D.E.; Packer, J.L.; Arnold, W.A.; McNeill, K., 2003. The photochemical fate of triclosan. Paper presented at the Midwest Environmental Chemistry Workshop, Iowa City, IA, October 11-12, 2003.

Boreen, A.L.; Arnold, W.A.; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. Poster presented at the Midwest Environmental Chemistry Workshop, Iowa City, IA, October 11-12, 2003.

Werner, J.J., McNeill, K., Arnold, W.A., 2003. Chemical loss processes for various pharmaceuticals in the environment. Poster presented at the Midwest Environmental Chemistry Workshop, Iowa City, IA, October 11-12, 2003.

Boreen, A. L.; Arnold, W. A.; McNeill, K. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. Paper presented at the 226th National Meeting of the American Chemical Society, New York, NY, September 2003.

Arnold, W.A; Boreen, A.L.; Latch, D.E.; McNeill, K.; Packer, J.L.; Werner, J.J., 2003. Photochemistry of pharmaceuticals in the environment. Poster presented at the AEESP Frontiers in Assessment Methods for the Environment symposium, Minneapolis, MN, August 10-13, 2003

Latch, D. E.; Packer, J. L.; Arnold, W. A.; McNeill, K. The Photochemical Fate of Cimetidine and Ranitidine Hydrochloride. Paper presented at 225th National Meeting of the American Chemical Society, New Orleans, LA, March 2003.

Packer, J.L.; Latch, D.E.; McNeill, K.; Arnold, W.A. Photochemical fate of pharmaceuticals in the environment: Naproxen, Ibuprofen, Diclofenac, and Clofibric Acid. Poster presented at American Chemical Society National Meeting, New Orleans, LA, March 23-27, 2003.

Packer, J.L.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2002. Photochemical Fate of Triclosan in the Environment. Poster presented at the 17th Annual Conference on the Environment, Bloomington, MN, November 14, 2002.

Latch, D. E.; McNeill, K. The Photochemical Fate of Triclosan. Poster presented at 34th Great Lakes Regional Meeting of the American Chemical Society, Minneapolis, MN, June 2002.

Boreen, A. L.; McNeill, K. Kinetics of the interaction between
singlet oxygen and sulfa drugs. Poster presented at 34th Great Lakes Regional Meeting of the American Chemical Society, Minneapolis, MN, June 2002.

Latch, D.E., W.A. Arnold, and K.McNeill (2002) Singlet Oxygen and the Photochemical Fate of Triclosan. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

Packer, J.L., K.McNeill, and W.A. Arnold (2002) Direct and Indirect Photolysis of Triclosan. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

K. McNeill, D. E. Latch, B. L. Stender and J. VanOverbeke, Singlet Oxygen and the Photochemical Fate of Triclosan. Paper presentation at 223rd ACS National Meeting, Orlando, FL, April 2002.

Latch, D. E.; McNeill, K. Singlet Oxygen and the Photochemical Fate of Ranitidine and Cimetidine. Poster presented at 24th Annual Midwest Environmental Chemistry Workshop, Minneapolis, MN, October 2001.

Latch, D. E.; Stender, B. L.; McNeill, K. Singlet Oxygen and the Photochemical Fate of Pharmaceuticals. Poster presented at 222nd National Meeting of the American Chemical Society, Chicago, IL, August 2001.

Statement of related grants submitted or funded as a result of this project

Dr. Arnold and Dr. McNeill have continued to apply for funding to continue this avenue of research. Drs. McNeill, Arnold and Swackhamer (Division of Environmental Health Sciences) obtained funding from the 2003 USGS-WRRI 104G competition to study the degradation and biological activity of antibiotics and estrogen mimics. This team also received a grant from the Camille and Henry Dreyfus Foundation in 2002 to hire a postdoctoral researcher to study the fate of pharmaceuticals in aquatic systems. Dr. Arnold has received funding from the Center for Urban and Regional Affairs (University of Minnesota) to investigate the photodegradation of selected antibiotics in Minnesota waters in 2003-2004. Dr. Arnold is also a co-investigator on a United States Department of Agriculture grant (2003-2006) to investigate the loss of veterinary antibiotics in soil systems.

Dr. Arnold and Dr. McNeill also have a proposal pending with the Legislative Commission on Minnesota Resources.

Description of student training provided by project:

Name: Jennifer L. Packer
Program: Department of Civil Engineering, University of Minnesota
Degree earned: M.S. (completed 2002)

Name: Douglas E. Latch
Program: Department of Chemistry, University of Minnesota
Degree earned: M.S. (completed 2002)
Degree being sought: Ph.D.

Name: Anne L. Boreen
Program: Department of Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Jeffrey J. Werner
Program: Water Resources Science, University of Minnesota
Degree earned: M.S. (completed 2004)
Degree being sought: Ph.D.

Name: Jennifer L. VanOverbeke
Program: Dept. of Chemistry, Univ. of Minnesota Summer Undergraduate Research Program
Degree earned: B.S. (Northwestern University)

Achievements and Awards
2003 1st Place Montgomery-Watson-Harza Consulting Engineers/AEESP Master's Thesis Award for Jennifer L. Packer's Thesis, *Photochemical Fate of Pharmaceuticals in the Environment*

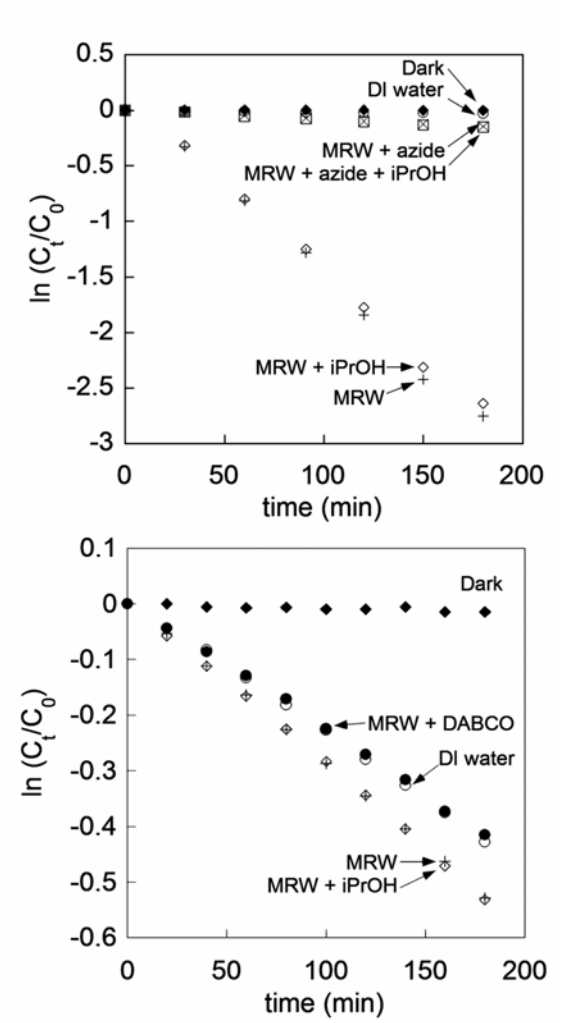

Figure 1. Degradation of cimetidine (top panel) and ranitidine (bottom panel) in various water samples.

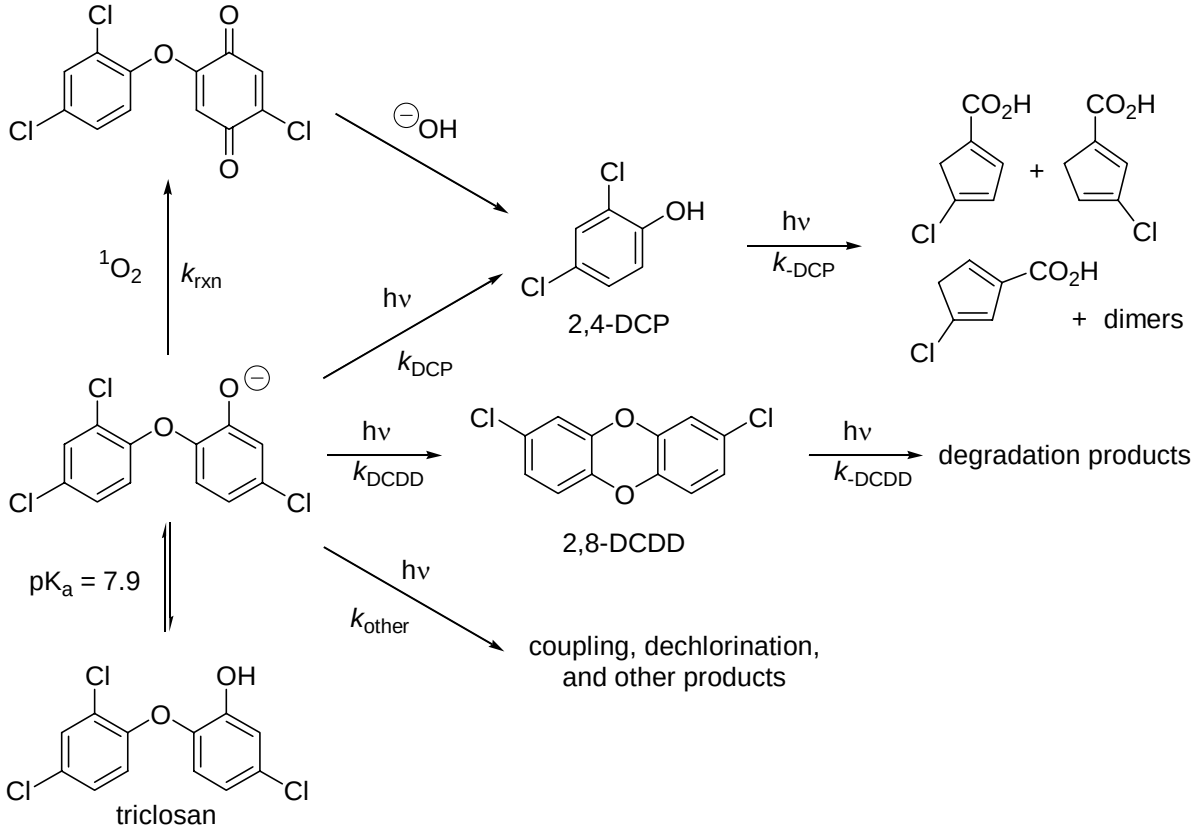

Figure 2. Photochemical reaction pathways of triclosan in water.

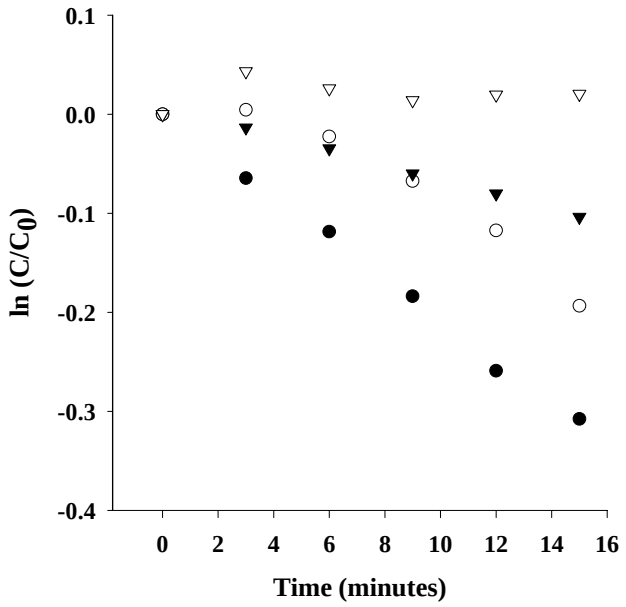

Figure 3. Direct photolysis of naproxen in H_2O in sunlight. Conditions are as follows: ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), ▼ = MRW with 1 % isopropanol, ▽ = dark control.

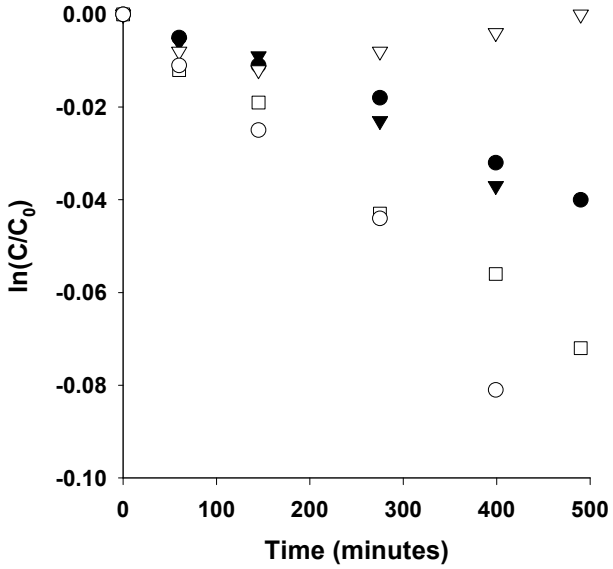

Figure 4. Direct photolysis of clofibric acid in H_2O in sunlight. Conditions are as follows: ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), □ = Milli-Q water + 1 % isopropanol, ▼ = MRW with 1 % isopropanol, ▽ = dark control.

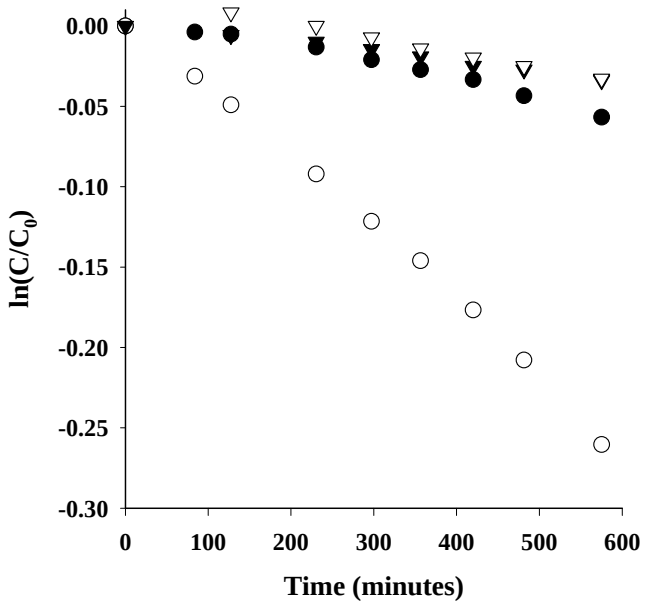

Figure 5. Direct photolysis of ibuprofen in H_2O by a Hg-vapor lamp. Conditions are as follows: ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), ▼ = Milli-Q water with 1 % isopropanol, ▽ = MRW with 1 % isopropanol.

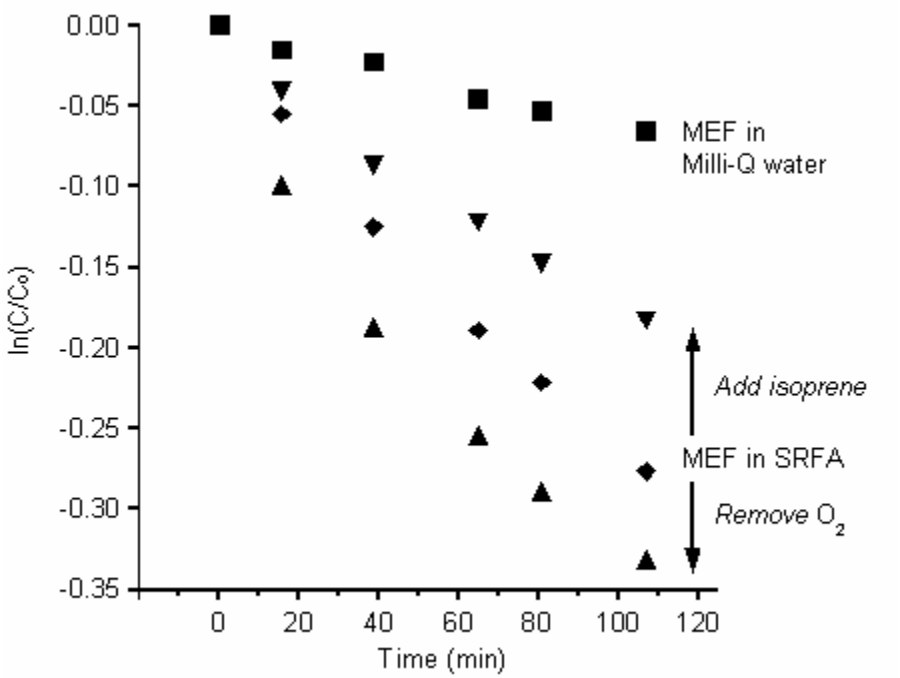

Figure 6. First-order kinetic plot for the Hg-vapor lamp photolysis of mefenamic acid in various aqueous solutions: Milli-Q water (■), SRFA (♦), SRFA with added isoprene (▼), and SRFA sparged with Ar gas (▲).

Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields

Basic Information

Title:	Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields
Project Number:	2001MN1041G
Start Date:	9/1/2001
End Date:	8/31/2003
Funding Source:	104G
Congressional District:	4th
Research Category:	Not Applicable
Focus Category:	Agriculture, Non Point Pollution, Water Quality
Descriptors:	
Principal Investigators:	Satish C. Gupta, Ashok Kumar Singh

Publication

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

Antibiotic losses in runoff and drainage from manure-applied fields

Principal investigators
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Anita Thompson, Yogesh Chander, Department of Soil, Water, & Climate; University of Minnesota

Research Assistant
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Wade Hammer, Department of Soil, Water, & Climate; University of Minnesota

Funding Source: USGS-WRRI 104G National Grants Competition
Project Duration: 9/01/2001-8/31/2003

Executive summary

Antibiotics are commonly used as feed additive in animal production. Recently, there have been concerns regarding the effect of this practice on development of antimicrobial resistance in the environment. Most of the antibiotics fed to animals are excreted in urine or manure. Once excreted these antibiotics can enter surface and/or ground waters through non-point source pollution from manure-applied lands. Potentially this is one of the pathways for the spread of the antibiotics and the antimicrobial resistance into the wider environment. This study deals with assessing the effect of land application of antibiotic laced swine manure on antibiotic losses in surface runoff and tile drainage. Two antibiotics studied are chlortetracycline and tylosin. Field studies showed very little transport of chlortetracycline and tylosin through Webster clay loam soil into tile drainage. There was also no transport of dissolved chlortetracycline in surface runoff. However, 0.07% of the applied tylosin was transported as dissolved tylosin in surface runoff. Because of the difficulty of extracting soil-adsorbed antibiotics, the extent of antibiotic losses with sediment is unknown. Breakthrough and adsorption studies also showed that these two antibiotics are tightly adsorbed in high clay and high organic matter soils. Further bioassays studies showed that antibiotics adsorbed on clays were biologically active and that the activity depended upon the antibiotic concentration in the sediment. Antibiotic resistance characterization of soil microbes at the experimental site also showed an increase in resistance of soil microbes to tylosin, monensin but not to tetracycline after 5 years of manure application. Questions raised by this study are: (1) Does these trends in small antibiotic losses also apply to low clay and more permeable soils? (2) Whether trace levels of antibiotics in soil and water promote antibiotic resistance among their bacterial population and if the trends are the same irrespective of the soil's clay content? and (3) To what extent antibiotic resistant microbes leach through the soil or move with the runoff water? We have received some funding from the USDA to address some of these questions.

Introduction

Since their discovery, antibiotics have been instrumental in treating infectious diseases that were previously known to kill humans and animals. However, it has now become clear that widespread use of antibiotics is not without problems (Halling-Sørensen et al., 1998; Jørgensen and Halling-Sørensen, 2000). The major concern is the development of antibiotic-resistant microorganisms, which are difficult to treat with existing antibiotics (Ford, 1994, Herron et al., 1997). Increasingly more microorganisms are becoming resistant to multiple antibiotics (Goldburg, 1999).

According to one estimate, two million pounds of antibiotics were produced in the U. S. in 1954 compared to more than 50 million pounds being produced each year currently (Environmental Media Services, (EMS) 2000). Although most of these antibiotics are used for the treatment of infections in humans and animals, a significant portion is used as a supplement in animal feed to promote growth of food-producing animals. According to EMS (2000), more than 40% of the antibiotics produced in the U.S are used as feed supplements. The use of antibiotics in animal feed helps increase the animal's ability to absorb feed and thus reach market weight on time. In addition, supplementing antibiotics in animal feed helps counteract the effects of crowded living conditions and poor hygiene in intensive animal agriculture (EMS, 2000).

Antibiotics commonly used as feed additive for animals include aureomycin, bacitracin, bambermycins, erythromycin, lincomycin, monensin, oleandomycin, oxytetracycline, penicillin, tylosin, and virginianmycin (Church and Bond, 1982). The antibiotic dose varies from 1 to 200 g per ton of feed depending upon type and size of the animal and the type of antibiotic. Most of the antibiotics added to animal feed are excreted in urine or manure. In some cases, as much as 80% of the antibiotic administered orally may pass through the animal unchanged (Levy, 1992).

Once excreted in urine and manure, these antibiotics may enter surface and/or ground waters through non-point source pollution from manure-applied lands. Land application of manure is a common practice in many parts of the U.S. In the northern tier of the country, manure is applied even during winter over snow. Manure is applied to land because of its value in supplying nutrients to crops as well as a means of disposing unwanted waste. Although it is strongly recommended that application rates of manure be based on the nutrient status of the soil and crop needs, this recommendation is not always followed and thus the manure applications have been higher than the recommended rate. The goal of this study is to determine whether or not there are losses of any antibiotics from manure-applied fields either in surface runoff or through subsurface drainage. Specifically, the objectives of this research are:

1. Quantify the effects of liquid swine manure application on antibiotic losses in surface runoff and subsurface drainage under a conventional (moldboard plowing) and a conservation (chisel plowing) tillage system.
2. Quantify the degree of antibiotic adsorption both in batch and flow through studies on a major soil in the upper Midwest.
3. Quantify the degree of antibiotic degradation at lower temperatures.

The field study was conducted in the Minnesota River basin where artificial drainage is common. Besides subsurface tile drainage, farmers often install surface tile inlets that drain depressional areas in the field to subsurface tile drains. These tile inlets allow transport of sediment and

surface-applied chemicals to subsurface tiles, which ultimately flow to surface waters including the Minnesota River. The manure applied at the site is swine manure from a finishing pig operation.

Methods

Field Experiment

Antibiotic losses were monitored in both surface runoff and tile drainage from a field experiment at Lamberton, MN. The drainage plots are 18.2 m long and 9.1 m wide (Fig. 1). Each plot is isolated to a depth of 1.8 m by trenching around plot borders and installing a 0.3 mm plastic sheet (Zhao et al., 2001). A perforated plastic tile drain, 10 cm in diameter, is installed at 1 m depth and 1.5 m away from the plot boundary along its width. This arrangement drains 16.7 m (18.2 m minus 1.5 m) length of the plot, one-half side of tile drains that may be 33.4 m apart. Tile drains empty into a monitoring well. Surface inlets are located at the lowest point in the plots and also drain surface runoff into the monitoring well.

The tillage treatments are conventional (moldboard plowing) and conservation tillage (chisel plowing). Manure and urea application rates are based on the University of Minnesota recommendations corresponding to a yield goal of 150 bushel/acre. Both surface runoff and subsurface tile drainage are measured by tipping bucket devices that are connected to CR-10 data loggers. Volume-distributed (composite water sample over a certain number of tips) runoff samples from surface inlets are taken by automated ISCO® samplers. Time-distributed (composite water sample over a certain time interval) subsurface drainage samples are collected manually once a day. The other details of sampling set-up and protocol are given in Zhao et al. (2001) and Thoma (2003).

For the 2001-2002 crop year, primary tillage was done October 4, 2001 and subsequently liquid hog manure was injected on November 5, 2001 in half of the plots @ 45,794 L/ha. This corresponds to N application of 56 kg/ha. Two passes of secondary tillage were made on May 1, 2002. In the remaining half of the plots urea was applied at an equivalent of 161 kg-N/ha just before the secondary tillage. Corn was planted on May 1, 2002 right after secondary tillage.

For the 2002-2003 crop year tillage was done on 18 October 2002 and subsequently liquid hog manure was injected on the same day in half of the plots at 36,400 L/ha. Two passes of secondary tillage was done April 23, 2003 and corn planted the same day.

Currently, there are no standard methods for analysis of antibiotics in soil and water samples. Therefore, most of our effort this year has gone in the development of analytical methods for antibiotic in manure, water, and soil samples. The farmer supplying manure for our experiment mentioned that he is mixing aueromycin (chlortetracycline) and tylosin in swine feed. Therefore, our methods development was geared towards quantification of chlortetracycline and tylosin.

Development of ELISA Test

Subsequent analysis of runoff and tile line samples showed that both concentrations of chlortetracycline and tylosin were too low to be detected with HPLC. Therefore, a new method based on immuno assay (ELISA-Enzyme-Linked Immunosorbent Assay) was developed to analyze runoff samples. Two commercially available enzyme-linked immunosorbent assay

(ELISA) kits for tylosin or tetracycline residues in meat and milk were adapted for ultratrace analysis of these antibiotics in surface and ground waters. The ELISA test is based on solid phase immunoassay technology. Antibiotics containing standards or water samples are added to microtiter wells coated with high affinity capture antibody to tetracycline or tylosin. The antibiotic enzyme conjugate competes with antibiotic in the sample for binding sites on the capture antibody. After a wash step, a substrate is added which reacts to any bound enzyme, creating a different color. Antibiotic in samples blocks the binding of enzyme conjugate to the capture antibody, resulting in little or no color development depending on the amount of antibiotic in the sample. Results are quantified by measuring optical density values (450 nm) of both standards and samples after stopping the reaction with a stop solution in a microplate reader. The optical density is inversely proportional to antibiotic concentration in the sample.

Adsorption of antibiotic on soil and their subsequent extraction
Our other efforts in this project have gone in characterizing the adsorption characteristics of tetracycline, chlortetracycline and tylosin on two different soil types (Webster clay loam and Hubbard sandy loam). Adsorption studies were done both in batch (Fig. 2) and in flow through (Fig. 3) set-up. The surface samples of Webster clay loam soil were taken from urea plots of our field experiment at Lamberton. Hubbard sandy loam is a glacial outwash soil and represents a major soil group in Central Sands of Minnesota.

We have also been working on extraction procedures to quantify the amount of adsorbed antibiotics on soil/sediment. These procedures involve (1) using various extracting agents in trying to replace antibiotics on the exchange complex, and (2) testing the survival of microbes of a defined resistance level.

We used the following extractants in our desorption study: MeOH; MeOH-0.01M EDTA pH6.6; 1 N HCl; and 0.1 M Na_2 EDTA- McIlvaine buffer pH 4.0. The procedure included fortifying 1 g of soil sample with a given antibiotic of concentrations varying from 50-100 $\mu g\ g^{-1}$ soil, allowing the soil and antibiotic to equilibrate for 4 hrs, and then using various extractants to evaluate antibiotic recovery.

Antimicrobial activity of soil adsorbed antibiotics:
Antimicrobial activity of soil bound antibiotics was determined against three bacterial strains namely *Salmonella* sp. (resistant), *Salmonella* sp. (sensitive) and *E.coli* ATCC 25992 on Webster clay loam and Hubbard sandy loam soils. The procedure involved, mixing microbial culture to 0.5 gm of soil, incubating the soil-bacteria mixture at 37^0C under both static and dynamic conditions, and then enumerating the bacterial growth. For static conditions, the soil was inoculated with 0.1 ml of 0.5 McFarland adjusted inoculums and incubated in a stationary condition. For dynamic conditions, the soil was inoculated with 0.5ml of Tryptic soy broth (TSB, Beckton and Dickenson,) containing 1% of 0.5 McFarland adjusted bacterial inoculum and incubated on a shaker at 37^0C (200 rpm).

After 24 hrs of incubation, 10-fold serial dilutions were made in Buffered peptone water (BPW, pH 7.0) and appropriate dilutions were plated on solidified agar media. For enumeration of *Salmonella* sp. Brilliant Green Agar medium (Beckton and Dickenson) and for enumeration of *E.coli* MacConkey Agar medium (Beckton and Dickenson) were used. Plates were incubated at

37^0C and examined for number of colony forming units (cfu) after 24 hrs of incubation. Percent decline in number of colonies forming units in antibiotic adsorbed soil was calculated relative to the control (no antibiotics present).

Bioassay Analysis:
Surface soil samples were collected on 15 April 2004 from the experimental field at Lamberton, MN to characterize the antibiotic resistance of soil microbes after 5 years of hog manure application. All samples were screened for the presence of antibiotic resistant bacteria (ARB) using Agar dilution technique as per the NCCL (1997) recommended standards. Briefly, the procedure involved suspending 1.0 g of the soil sample in the 9.0 ml of Buffered peptone water (BPW, pH 7.0), homogenizing the suspension by vortexing, making ten-fold serial dilutions with BPW and then plating the appropriate dilutions on solidified Muller-Hinton Agar (MHA) medium supplemented with antibiotic. Samples were screened on three different antibiotics namely Tetracycline, Tylosin and Monensin at concentrations 20.0 $\mu g\ ml^{-1}$, 10.0 $\mu g\ ml^{-1}$ and 6.0 $\mu g\ ml^{-1}$ respectively. For control, samples were simultaneously plated on plate without antibiotic. After inoculation plates were incubated at 37^0C. After 24hrs of incubation, number of colony forming units (CFUs) in antibiotic containing plates as well as in control plate was counted visually. Resistance was calculated as a ratio of the colonies forming units in the antibiotic containing plates to the control plates.

Results and Discussion
Antibiotics concentrations in manure
Analysis of the 2001 hog manure from the supplier lagoon showed presence of chlortetracycline (5.0 mg/L of manure slurry) and tylosin (5.6 mg/L of manure slurry). At 45,794 L/ha, this is equivalent to 229 gm/ha of chlortetracycline and 256 gm/ha of tylosin.

Analysis of the 2002 hog manure from the supplier lagoon showed presence of chlortetracycline (5.47 mg/L of manure slurry) and tylosin (4.52 mg/L of manure slurry) and oxytetracycline (1.31 mg/L of manure slurry). At 36,400 L/ha, this is equivalent to 199 g/ha of chlortetracycline and 165 g/ha of tylosin and 48 g/ha of oxytetracycline. Antibiotic analysis in manure sample was done on HPLC (High Performance Liquid Chromatography).

Antibiotic losses in runoff and tile line flow
We have completed the analysis of all 2002 surface runoff and tile line samples for presence of chlortetracycline using ELISA test. None of the samples showed any presence of chlortetracycline. Furthermore, there was no presence of tylosin in the tile line water. This is consistent with our laboratory batch adsorption and flow through studies that show strong tendency of chlortetracycline and tylosin to adsorb on the Webster clay loam soil. Tylosin losses in surface runoff for four storm events in 2002 amount to 168 mg/ha for the manure treatment compared to 41 mg/ha for the urea treatment (Table 1). These amounts translate to tylosin losses of about 0.07% of the tylosin applied in manure. Presence of tylosin in the urea treatment is possibly due to cross contamination of plots during tillage. Most of these losses are of dissolved tylosin. It is unknown how much of these antibiotics remain adsorbed on the soil and to what extent these antibiotics are transported with sediment losses.

There were not very many major events in 2003 that has generated runoff from these plots. However, we have collected many tile line samples and we are in the process of analyzing those samples.

We also completed the analysis of nutrient and sediment losses from the field experiment. In 2002 there was a significant difference ($p=0.054$) by nutrient source treatment for surface losses of NO_3-N and combined NO_3+NH_4-N. Losses of surface NO_3-N and NO_3+NH_4-N from urea treated plots were 0.58 kg/ha and 0.71 kg/ha respectively, while losses of surface NO_3-N and NO_3+NH_4-N from manure treated plots were 0.24 kg/ha and 0.34 kg/ha respectively (Table 2). However, these losses are relatively small compare to the losses by tile drainage. There was no difference in NO_3-N and NO_3+NH_4-N losses in tile drainage between the nutrient source treatments in 2002. The lower losses from manure compared to urea plots suggest slow but continuous release of manure organic N that is taken up by the crop more efficiently. Additionally, the inorganic fertilizer was not incorporated as deeply as the injected liquid hog manure. This may have left it more susceptible to surface transport, especially in a year like 2002, which had more intense storms than previous years as indicated by the greater surface runoff losses.

There was no significant difference between nutrient source (manure and fertilizer) or tillage (moldboard plowing, chisel plowing) treatments in terms of total P (TP), dissolved molybedate reactive phosphorus (DMRP), and total solids (TS) losses in surface runoff (Table 2). A complete report summarizing 4 years of data on sediment and nutrient losses from the field experiment is given in Thoma (2003).

Adsorption of antibiotics on soils:
Batch experiments showed that tetracycline and chlortetracycline are strongly adsorbed on both soils than tylosin. Among the soils, Webster clay loam has higher adsorption capacity than the Hubbard sandy loam. The differences in soil types are due to differences in clay and organic matter content of soils. Webster clay loam is higher in both clay and organic matter contents (34% & 4.4%) than the Hubbard sandy loam (10.4% & 2.2%). Flow through experiment with Hubbard sandy loam showed results consistent with the batch experiment i.e. chlortetracycline and tetracycline are more strongly adsorbed on the soil than tylosin.

Linear sorption coefficients (K_d) of chlortetracycline, tetracycline and tylosin on Webster clay loam were 2386, 2370, and 92 L/kg as compared to 1280, 1147, and 66 L/kg for Hubbard sandy loam. Thus at saturation, the retardation coefficient of chlortetracycline, tetracycline and tylosin in Webster clay loam will be 6083, 6042, 236 as compared to 4466, 4002, 231 for Hubbard sandy loam. The higher the retardation value, the greater is the adsorption potential of that chemical for a given soil. This number also reflects the quantity of water needed to displace a chemical through soil to the same distance as the non-adsorbing chemical. In other words, chlortetracycline will need 6083 times more water to displace than chloride or nitrate in a Webster clay loam at saturation. In other words, if it takes chloride or nitrate one year to reach a given depth then it will take 6083 years for chlortetracycline to reach the same depth. The variation in K_d values reduces when it was normalized with clay or organic carbon contents, thus suggesting that clay and organic may be the primary adsorption sites for these antibiotics.

Extraction of adsorbed antibiotics from soil particles:
Our best extraction was with 0.1 M Na_2 EDTA- McIlvaine buffer pH 4.0 but that recovery was only 41 to 67% (Table 3). The recovery was a bit higher for Hubbard sandy loam than Webster clay loam. This is expected because of high clay and high organic matter content of the Webster than Hubbard soil. At present there are no procedures available in the literature for full recovery of antibiotics from soil or sediments. The question at hand is whether the antibiotic on the soil is active or passive (in terms of its effect on microbial survival). We are testing an indirect method to assess the activity of soil-adsorbed antibiotics. The procedure involves use of microbes of a defined resistance level and testing their survival when mixed with soil that contains adsorbed antibiotics.

Development of ELISA Tests
We have further completed the work on the enzyme-Linked immunosorbent assay (ELISA) method for ultratrace determination of antibiotics in aqueous samples**.** Two commercially available enzyme-linked immunosorbent assay (ELISA) kits that are commonly used for tylosin or tetracycline residues in meat and milk were adapted for ultratrace analysis of these antibiotics in surface and ground waters. Both ELISA techniques were found to be highly sensitive and selective for the respective antibiotics with detection limits of 0.10 and 0.05 $\mu g\ L^{-1}$ for tylosin and tetracycline, respectively. The recovery of both tylosin and tetracycline from spiked samples of lake waters, runoff samples, soil saturation extracts, and nano-pure water was close to 100%. Tetracycline ELISA was highly specific for tetracycline and chlortetracycline but not for other forms of tetracycline (oxytetracycline, demeclocycline, and doxycycline). These results indicate that both ELISA kits can be useful tools for low cost screening of tylosin, tetracycline and chlortetracycline in environmental waters. Furthermore, both ELISA procedures are rapid, portable, and easily adaptable to testing multiple samples simultaneously. The ELISA techniques reported here are inexpensive (approx. $5 per sample for tylosin and $15 per sample for tetracycline) and rapid, require a small sample volume (< 100 μl), are field portable, and work at very low but environmentally significant concentrations (>0.10 $\mu g\ L^{-1}$). The results of this study are published in a Journal of Environmental Quality article (Kumar et al., 2004).

Antimicrobial activity:
Figures 4 and 5 show examples of the percent decline (compare to control, no adsorbed antibiotics) in number of Cfu as a function of antibiotic solution concentrations at which two soils were equilibrated. Following are some general observations on the trends:

1. The decline in Cfu due to the soil adsorbed antibiotics was greater under dynamic than static conditions. This may be because the agitation under dynamic condition is (a) helping to increase diffusion of soil adsorbed antibiotics into the solution, and/or (b) increase the contact between bacteria and the soil adsorbed antibiotics.

2. With an increase in antibiotic concentration, there was a greater decline in the growth of a bacterial species. However after a given concentration, the percent decline in bacterial growth was relatively constant. These threshold values depended upon the combination of growth conditions (static vs. dynamic), soil type (clay loam vs. sandy loam), and bacterial species (sensitive vs. resistant, *salmonell* sp. vs. *E. coli*).

3. Percent decline in growth of any specie was much greater for Hubbard sandy loam soil than Webster clay loam. This possibly reflects the strong adsorption ability of the Webster clay loam than the Hubbard sandy loam (Table 1) due to higher clay and organic contents in the former.

4. For any given soil and a specie, percent decline in Cfu was greater for tetracycline than tylosin. This possibly reflects the greater potency of tetracycline than tylosin in killing given bacterial specie.

Bioassay Analysis:
Bioassay analysis of surface soil samples showed that there was a significant increase in antibiotic resistance of soil bacteria from 5 years of hog manure application (Table 4). This increase was much higher for tylosin than monensin. There was no effect of manure application on antibiotic resistance due to tetracycline in soil bacteria. This may be possibly due to strong adsorption of tetracycline by high clay soils.

Ongoing work
We will continue characterizing antibiotic losses from our field site. Other questions include the effects of temperature on antibiotic losses in soil. We are planning to undertake incubation studies over the next month or so on temperature effects. One of the concerns in the incubation study is the recovery of soil-adsorbed antibiotic. We will continue with our test on the use of microbes to characterize the activity of soil-adsorbed antibiotics.

Summary of findings
Dissolved chlortetracycline and tylosin losses from manure-applied fields in the Minnesota River Basin will be relatively small both in surface runoff and in tile drainage. This is because of strong adsorption characteristics of these two antibiotics on high clay soil. However, it is unknown as to the extent of antibiotic losses with sediment. Since there is more potential for sediment losses from clay soils, this component could be important. It is also unknown whether or not there is an increased potential of antibiotic leaching from low clay soils especially in presence of preferential flow paths. Recently, the PIs received a grant from the USDA to further investigate antibiotic losses from a shallow silt loam soils underlain with fractured bed rock and a sandy outwash soil with relatively shallow water table.

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List of publications & presentations resulting from this project

Gupta, S.C. 2004. Role of Antibiotics Feeding in the spread of Antibiotic Resistance in the Environment. Winter Field day Presentation at the West Central Research and Outreach Center, Morris, MN. 13 February 2004

Kumar, Kuldip, A. Thompson, A.K. Singh, Y. Chander, and S.C. Gupta. 2004. Enzyme-Linked Immunosorbent Assay for Ultratrace Determination of Antibiotics in Aqueous Samples. J. Environ. Qual. 33: 250-256.

Gupta, S.C. and Y. Chander. Antibiotic losses from manure applied fields. 30 Minutes question answer show on KDHL Radio Station at Fairbault. 16 April 2003.

Gupta, S., Kuldip Kumar, Ashok Singh, Anita Thompson, and David Thoma. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Pork Producers Association, Mankato. 27 June 2002.

Gupta, S., Kuldip Kumar, Ashok Singh, Anita Thompson, David Thoma, Yogesh Chander, and Wade Hammer. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Pork Producers Association, New Ulm. 27 March 2003.

Gupta, S., Kuldip Kumar, Ashok Singh, Anita Thompson, David Thoma, Yogesh Chander, and Wade Hammer. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Turkey Growers Association, Buffalo, MN. 13 May 2003.

Kumar, Kuldip, A. Thompson, A.K. Singh, and S.C. Gupta. 2002. Adsorption of antibiotics on soils. Agronomy abstract.

Chander, Y., K. Kumar, S.C. Gupta, A.K. Singh, and S.M. Goyal. 2003. Antimicrobial activity of soil bound antibiotics. Agronomy Abstract S11-Chander 633887.
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Web site: http://www.misa.umn.edu/

Statement of related grants submitted or funded as a result of this project

USDA-Impact of swine manure application on phosphorus, nitrate-N, bacteria, and antibiotics concentration in surface runoff and subsurface drainage water. (Gupta, Singh, Kumar). $12,346. 2002-2003.
Minnesota Pork Producers Association. Antibiotic and nutrient losses in runoff and drainage from swine manure application. (Gupta, Singh, & Kumar). $60,000. 2002-2004.
NCSARE- The occurrence of antibiotic resistant bacteria in manure, manure applied fields, and pets on the farm. (Gupta, Goyal, Singh, Kumar, Murray). $99,835. 2002-2004.
U of M Grants-in-Aid. Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields. Ashok Singh, Satish Gupta, and Kuldip Kumar. $25,000. 2002-2004.
USDA-NRI- Antibiotic losses and development of antimicrobial resistance from manure application. (Gupta, Goyal, Singh, and Kumar). $310,000. 2003-2006.
LCMR-The role of animal manure in spreading antibiotic resistance in the environment. (Goyal, Gupta, Singh, Kumar, and Murray) $410,000 . NOT FUNDED.

Description of student training provided by project:

Name: David Thoma
Program: Department of Water Resource Science, University of Minnesota
Degree being sought: Ph.D.

Name: Erica Sherry
Program: Technical Writing
Degree being sought: B.S.

Name: Anne Marsdren
Program: Computer Science
Degree being sought: B.S.

Table 1: Tylosin losses via surface runoff in 2002.

Event	Manure (mg/ha)	Urea(mg/ha)
30 July	46.6	0
4 August	4.3	0.8
9 August	113.8	39.5
22 August	3.8	1.2
Total	168.5	41.4

Table 2. Average annual loads in runoff and tile drainage for the duration of the study.

	Surface runoff							Tile drainage			
Year	Flow (cm)	NH_4	NO_3	NO_3+NH_4	TP	DMRP	TS	Flow (cm)	NH_4	NO_3	NO_3+NH_4
		(kg/ha)							(kg/ha)		
1999¶	0.2	0.0	0.1	0.1	0.1	0.0	66.8	N£	0.7	N£	N£
2000	2.8	0.0	0.6	0.6	0.8	0.1	691.7	3.7	0.1	6.3	6.4
2001	7.2	1.0	1.8	2.8	3.8	0.3	2615.5	T£	0.6	27.1	27.3
2002¶	3.7	0.1	N£	N£	0.9	0.1	1666.6	2.5	0.0	2.3	2.3

¶1999 includes precipitation events between 5 May and 31 December. 2002 includes precipitation events between 1 January and 22 August.
£ (N) Nutrient source effect only or (T) tillage effect only
TP=Total phosphorus, DMRP=Dissolved molybdate reactive phosphorus, TS=Total solids

Mean recovery (%) of tylosin and tetracycline antibiotics from fortified soils.

Extractant	Hubbard				Webster			
	Tyl	OTC	TC	CTC	Tyl	OTC	TC	CTC
MeOH	62	22	27	21	49	11	17	15
MeOH-0.01 M EDTA, pH 6.6	69	33	47	35	55	21	23	19
1 N HCl	15	40	42	50	5	32	30	27
0.1 M Na_2 EDTA- McIlvaine buffer pH 4.0	61	59	67	49	52	43	49	41

Tyl – Tylosin; OTC – Oxytetracycline; TC – Tetracycline; and CTC – Chlortetracycline.

Table 4: Effect of hog manure application on percent increase in antimicrobial resistant bacteria at Lamberton, MN plots.

Treatment	% Increase in Resistant Bacteria		
	Tetracycline	Tylosin	Monensin
Manure	0	34.8	27.0
Urea	0	7.0	6.0
Manure never applied	0	0.3	0.7

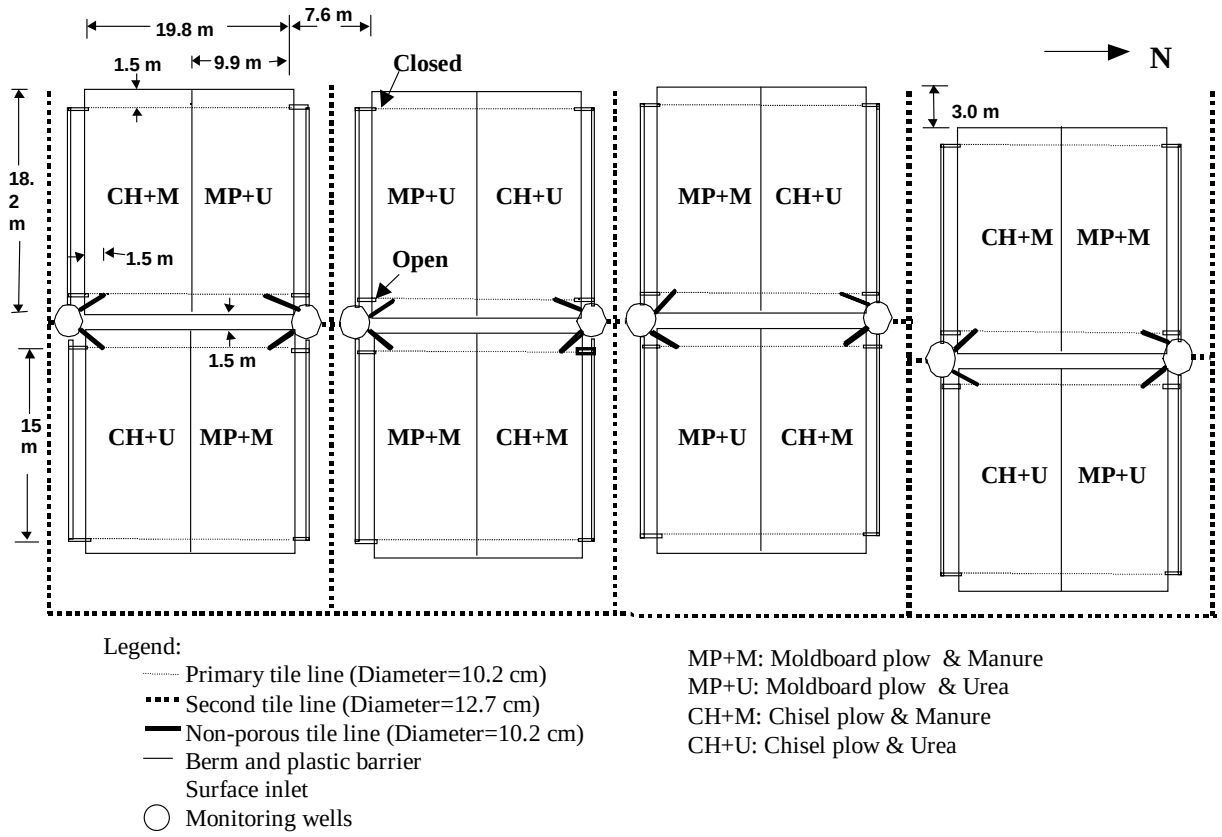

Fig. 1: Surf-n-sub plot lay out at the Southwest Research and Outreach Center in Lamberton, MN

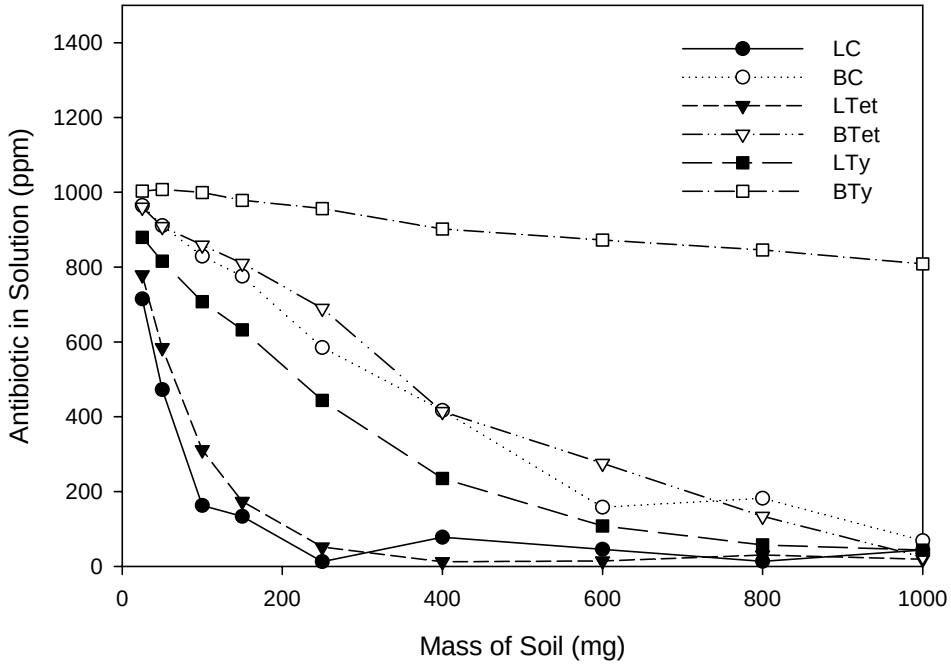

Figure 2. Antibiotic remaining in Solution after shaking 1000 ppm of antibiotic solution with various amounts of soil. L= Webster clay loam, B=Hubbard sandy loam, C=chlortetracycline, Tet=tetracycline, Ty=Tylosin. Top three curves are for Hubbard sandy loam soil whereas bottom three curves are for Webster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

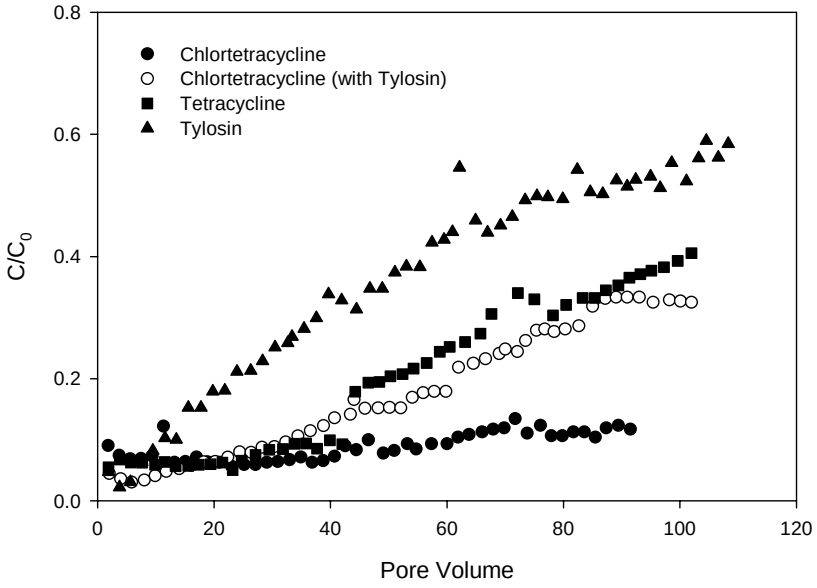

Figure 3. Breakthrough Curves for the Hubbard sandy loam soil for three antibiotics. Each Data Point is average of three replicates. As shown by batch adsorption studies, chlortetracycline and tetracycline are strongly adsorbed on the Hubbard sandy loam soil than tylosin.

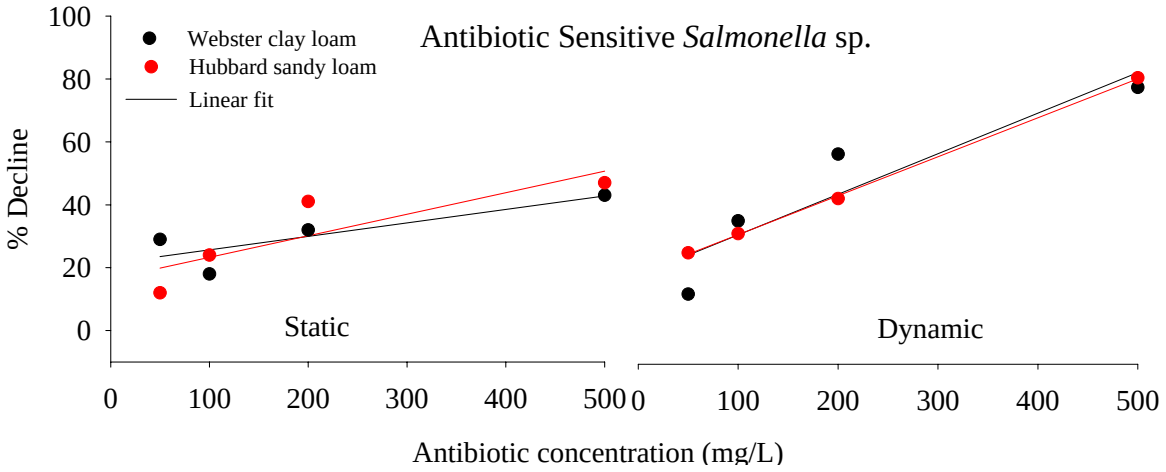

Figure 4: Effect of soil adsorbed tertcycline on percent decline in number of colony forming units of antibiotic sensitive *Salmonella* sp. both in static (in the absence of a nutrient solution) and dynamic systems (in presence of a nutrient solution). Concentrations on the x-axis are solution concentrations at which soil was equilibrated.

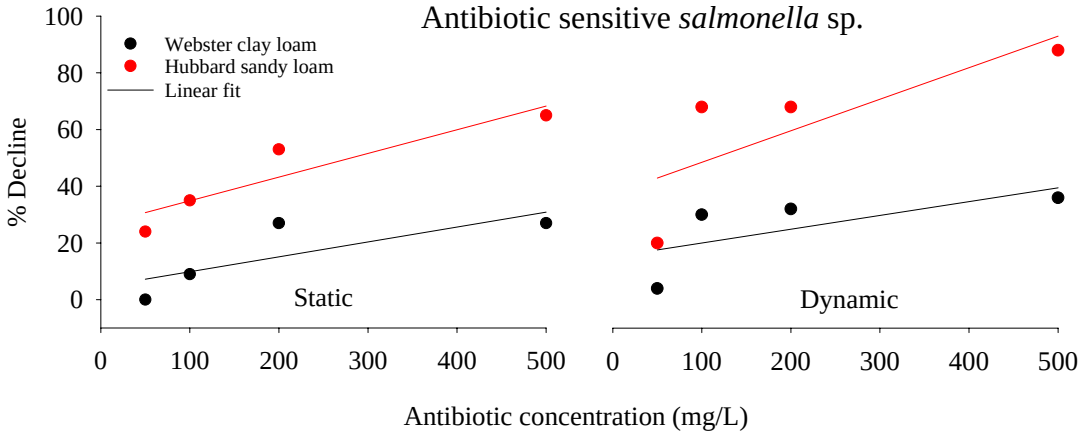

Figure 5: Effect of soil adsorbed tylosin on percent decline in number of colony forming units of antibiotic sensitive *Salmonella* sp. both in static (in the absence of a nutrient solution) and dynamic systems (in presence of a nutrient solution). Concentrations on the x-axis are solution concentrations at which soil was equilibrated.

Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency

Basic Information

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2. A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups, Environ. Sci. Technol., 2004, Accepted for publication.

Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency

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Project Duration: 9/01/2003-8/31/2005

Summary
Antibiotics and estrogens are two classes of wastewater contaminants that have been detected in US surface waters. The potentially adverse effects of these pollutants on water quality are unknown, but will be determined, in part, by their persistence and the biological activity of both the parent compound as well as the degradates. Photolysis is one possible loss process, and the direct and indirect photolysis of ten members of the sulfa drug antibiotic class and triclosan has been investigated. The structure of the R-substituent on the sulfa drugs controls the reactivity; those containing five-membered heterocyclic substituents degrade via direct photolysis in natural waters, and those containing six-membered substituents degrade through both direct photolysis and reaction with triplet dissolved organic matter. Triclosan degrades by direct photolysis and forms the toxic compounds 2,8-dichlorodibenzo-*p*-dioxin, 2,4-dichlorophenol, in addition to oligomerization and dissolved organic matter-coupled products. Preliminary results suggest that the products of photolysis of sulfa drugs containing five-membered heterocyclic substituents are not toxic to bacteria while intermediates produced during triclosan photolysis may retain some biological activity.

Introduction
Reports of pharmaceuticals and personal care products (PPCPs) in natural waters have recently appeared with increasing frequency.[1-5] Two important subclasses of these emerging contaminants are particularly worrisome due to their potential to adversely affect surface waters: antibiotics and environmental estrogens. Estrogenic compounds have a demonstrated ability to interfere with the development of aquatic organisms,[5,6] while there is concern that the presence of antibiotics in natural waters will lead to an increase of antibiotic resistant bacteria.[7,8] These compounds are released into surface waters as a result of human use, through discharge of treated and untreated wastewater. An additional, major source of antibiotics comes from their wide use in the production of food animals and in fish farming. [1-5]

The magnitude of the effects and potential threat to water quality due to antibiotics and hormones is, in part, determined by the compounds' persistence in aquatic systems. The principle goal of this proposed study is to understand one aspect of their persistence—their degradation by photochemical processes. Based upon our work[9-12] and that of others,[11,13-19] we believe that photodegradation may be a major loss process for these compounds in sunlit waters. Thus, it is important to understand the photochemical processes that degrade these chemicals in surface waters, to identify intermediates and products that are formed, and to assess the biological activity of these products.

Methods

Direct and natural water photolysis experiments

Photolysis experiments were performed outdoors under natural sunlight or indoors under medium pressure Hg-vapor lamps. Sample solutions were contained in quartz test tubes (OD = 13 mm, ID = 11 mm, V = 10 mL), which were arranged on a turntable apparatus to ensure equal irradiation for all of the samples. For kinetic analyses approximately 0.5 mL samples were withdrawn from the quartz tubes at predetermined intervals and analyzed on an 1100 Series Hewlett Packard HPLC equipped with UV-absorbance detection and a computer driven data acquisition system. In experiments designed to probe for pH effects, various buffer solutions were employed to set the pH values. Solar quantum yields were calculated by comparing the rate constant for the disappearance of the PPCPs under natural sunlight with the rate constant for the disappearance of a *p*-nitroanisole actinometer.

Natural water photolysis experiments were performed in 0.2 μm filtered Lake Josephine (LJW) water or Lake Superior (LSW) water. To determine which pathways were responsible for the photodegradation, various quenchers were added to or removed from the water samples (sodium azide or DABCO for $^1O_2$, isopropanol for radicals, oxygen and isoprene for triplet DOM) and the substrate was also photolyzed in DI water in a separate tube.

Singlet oxygen

Singlet oxygen reaction kinetics were measured in one of two ways, directly by laser flash photolysis (LFP) or indirectly by steady-state photolysis (SSP). In both types of experiment the substrate (typically at micromolar concentrations) and 40 μM Rose Bengal (RB), 100 μM Eosin Y, or 100 μM perinaphthenone, three well-defined singlet oxygen sensitizers, were dissolved in aqueous buffer solutions. In the LFP experiments, a pulse of laser light excites the sensitizer, which then produces singlet oxygen after the excited-state sensitizer is quenched by dissolved molecular oxygen. A sensitive Ge-photodiode detector then monitors the phosphorescence emission from singlet oxygen. The rate of disappearance of the singlet oxygen phosphorescence signal is a measure of a substrate's activity toward singlet oxygen. The resulting total quenching rate constant (k_{tot}) is the sum of the chemical reaction and physical quenching rate constants.

In SSP experiments, the samples are photolyzed continuously and small aliquots are removed for analysis by HPLC. In this case, the disappearance of the PPCP is monitored (as decreases in peak area), rather than the singlet oxygen signal. This allows for the determination of the chemical reaction rate constant (k_{rxn}) for the PPCP with singlet oxygen.

Product identification
Mass spectra of photolyzed solutions were obtained on a ThermoFinnigan LCQ Advantage ion trap MS/MS equipped with an orthogonal ESI and APCI source operated under Xcalibur software. The spectra obtained were compared to those of authentic standards of suspected products for identification.

Biological activity
The ability of the antibacterial compounds and their photolysis products to inhibit bacterial growth was tested using *E. coli* DH5α. The bacteria were maintained on agar plates and grown up overnight on Iso-Sensitest broth (ISB) (Oxoid, Inc.) prior to testing. One mL of antibacterial compound or photolysis product and 100 µL of *E. coli* were added to test tubes containing nine mL of ISB prepared in a pH 7 phosphate buffer (9.7 g KH_2PO_4 and 19.4 g Na_2PO_4 per liter deionized water). The solutions were incubated in the dark at 37 °C while being shaken. Bacterial growth was assessed after 8 hours by measuring optical density at 600 nm (OD_{600}).

The antibacterial compounds and their photolysis products were also tested for their ability to inhibit bacterial respiration. The respiration assay used was based on the ability of the bacteria to reduce iodonitrotetrazolium chloride. *E. coli* (400µL) was added to 40 mL of ISB and incubated at 37 ºC. Once the OD_{600} of this solution had reached 0.4 (in the exponential phase of the growth curve), 1 mL aliquots were centrifuged at 19,000g for five minutes. The supernatant was decanted, and 0.5 mL of antibiotic or photolyzed antibiotic was added. The bacterial pellet was resuspended, and the tubes were then incubated in the dark at 37 °C while being shaken. After one hour of incubation (approximately one generation time), 0.5 mL of a 5 mM solution of the tetrazolium salt was added and the tubes were incubated for an additional hour. The tubes were then centrifuged, the supernatant decanted, and 1 mL of an organic solution (1:1 dimethylformamide: ethanol) was added to the bacterial pellet to extract the formazan. The pellet was resuspended, and the tubes were incubated in the dark at room temperature for one hour. After centrifuging, the absorbance of the supernatant was measured at 464 nm to quantify the amount of formazan formed.

Results to date
Photodegradation of the Sulfa drugs
Sulfa drugs with five-membered heterocyclic substituents
The direct photolysis rate constant of the sulfa drugs containing five-membered heterocyclic substituents (sulfamethoxazole, sulfamoxole, sulfamethizole, sulfathiazole, and sulfisoxazole; Figure 1) systematically varies with pH, therefore, the quantum yield (Φ) of direct photodegradation for each of the three protonation states, SH_2^+, SH, and S^-, was determined through matrix deconvolution of both the absorption spectra and direct photolysis rate constants. The pH dependent direct photolysis rate constants, as well as the deconvoluted spectral overlap integral, direct photolysis rate constants, and quantum yields are listed in Table 1.

Comparison of HPLC retention time and mass spectral data with authentic standards has shown both expected products (sulfanilic acid and the amino-R substituents) arising from cleavage of the sulfonamide linkage in photolysate solutions and appears to be the dominant cleavage pathway. Cleavage at this position accounts for 35 % to 79 % of the degradation. Cleavage of the R-group was also observed, as a small peak coincident with an authentic standard of

sulfanilamide was detected in the photolysis solutions, although to an appreciably lesser extent. The pH of the photolysis solutions appeared to affect the rate of product appearance, but not their identity.

The photolysis rates in the two natural water samples, Lake Josephine (DOC = 5.9 mg/L) and Lake Superior (DOC = 2.5 mg/L), matched the photodegradation rates in DI H_2O run concurrently, thus implicating direct photolysis as the dominant photochemical loss process in these natural waters.

Sulfa drugs with six-membered heterocyclic substituents

The photolysis rates of the sulfa drugs containing six-membered heterocyclic substituents (sulfachloropyridazine, sulfadiazine, sulfamerazine, and sulfamethazine; Figure 1) in Lake Josephine (DOC = 5.9 mg/L) water were enhanced by a factor of 1.4-2.6 relative to the photodegradation rates in DI H_2O. The enhancement in the natural water has been attributed to reaction of the sulfa drugs with excited triplet dissolved organic matter due to the enhancement of the degradation upon eliminating oxygen from the system and suppression of the degradation upon addition of isoprene. The natural water photodegradation of sulfadimethoxine matched the degradation in DI H_2O, and the degradation was thus attributed solely to direct photolysis. The direct photolysis of sulfadimethoxine is pH dependent, and is explained by differing reactivity of the protonation states. The remaining sulfa drugs' direct photolysis and triplet-sensitized degradations are not pH dependent over the pH range 6-9.

Photodegradation of triclosan

2,4-dichlorophenol formation.

2,4-Dichlorophenol (2,4-DCP) was identified as a photoproduct of triclosan after extracting the aqueous photolysate into ethyl acetate and analyzing the extract by GC-MS. This result was confirmed by comparing the retention time and mass spectrum (and also the LC retention time) to that of an authentic 2,4-DCP sample. The yield for this process was determined to be 3.1 % at pH 8. 2,4-DCP was also found to be photoreactive, however, and the photolysis of triclosan will lead to only small steady state concentrations of 2,4-DCP in natural waters.

Oligomerization and dissolved organic matter (DOM)-coupled products

Triclosan was also found to form oligomerization products upon photolysis, as evidenced by the formation of insoluble particles and the growth of larger molecular weight species (as determined by size exclusion chromatography) when triclosan was photolyzed in basic DI water. These products formed in experiments when as little as 10 μM triclosan was photolyzed. In natural waters, however, the concentration of triclosan is much lower, and it is unlikely that a photoexcited triclosan molecule would couple to another triclosan molecule. Given the relatively high concentration of DOM (typically 1-20 mg C/L) in natural waters, coupling to DOM (Suwanee River fulvic acid, SRFA) was proposed to be a major sink for triclosan photodegradation. This hypothesis was confirmed in experiments in which triclosan (10 μM) was photolyzed in the absence and in the presence of SRFA. The photolysates of these samples were filtered through tared 0.2 μm filter membranes, and the amount of insoluble material was measured gravimetrically. A negligible amount of insoluble material was collected on the filter membrane when triclosan was photolyzed alone, while a significant amount (7 %) of the initial starting mass (triclosan + SRFA) was collected as insoluble particles when SRFA was present in

the photolysis solution. A control experiment in which SRFA was photolyzed alone followed by filtration yielded no insoluble products.

Biological activity

Figure 2 compares the effects of triclosan and photolyzed triclosan on bacterial growth. The data in the photolyzed triclosan series represents the remaining parent triclosan concentration at the time the sample was obtained. Any photolysis products generated at that point and present in the sample in addition to the triclosan shown would be responsible for deviations from the unphotolyzed triclosan data series. As shown in the figure, the points at which growth is completely inhibited or not inhibited are essentially the same for the two series, suggesting that the presence of the photolysis products does not substantially increase toxicity of the solution. That is, it appears that most of the toxicity of the photolyzed solution comes from the remaining triclosan; once the triclosan concentration has decreased below its effective level the ability of the compounds to inhibit bacterial growth has been eliminated. Photolysis products may retain some antibacterial activity; the concentration at which partial growth inhibition is observed appears to be slightly lower for the photolyzed solutions. Any enhancement in growth inhibition is small, however, and appears to be transient, disappearing once triclosan has been photolyzed. Effects on respiration are similar.

For sulfathiazole and sulfamethoxazole (sulfa drugs containing five-membered heterocyclic substituents) all of the toxicity of the photolyzed solutions was attributable to the parent sulfa drugs suggesting that photolysis of these compounds results in complete loss of biological activity.

Ongoing work

Current work is underway in determining the products of the photodegradations of the sulfa drugs with six-membered substituents using preparative HPLC coupled with MS and NMR as well as further characterization of the reaction between the sulfa drugs and triplet DOM. The antibacterial activity of selected sulfa drugs with six-membered substituents is being assessed. The photodegradation of three other classes of antibiotics, nitrofurans, tetracyclines, and fluoroquinolines is also underway. The goals for these experiments are to determine the major photolysis processes and to asses the antibacterial activity of the photolysis products. Experiments are also being designed to better quantify the apparent activity of the triclosan photolysis intermediates.

Summary of findings

The primary photodegradation mechanism for the five-membered sulfa drugs is direct photolysis while that for the six-membered sulfa drugs is both direct photolysis and reaction with triplet dissolved organic matter. These results reveal that minor structural changes can give rise to disparate environmental loss mechanisms. The direct photolysis of the five-membered sulfa drugs is highly pH dependent, revealing the importance of environmental conditions on photodegradation. Triclosan degrades by direct photolysis and forms the toxic 2,4-DCP, oligomerization, and DOM-coupled products. For the compounds examined to date, photodegradation leads to a decrease in or elimination of antibacterial activity as the parent antibacterial compound disappears. Photolysis of the sulfa drugs containing five-membered

heterocyclic substituents results in products with no observable biological activity; some intermediate products of triclosan photolysis may retain some activity.

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List of publications & presentations resulting from this project

Peer Reviewed Publications

D.E. Latch, J.L. Packer, B.L. Stender, J. VanOverbeke, W.A. Arnold, K. McNeill, Aqueous photochemistry of triclosan: Formation of 2,4-dichlorodibenzo-*p*-dioxin and oligomerization products, *Environ. Toxicol. Chem.*, Submitted for publication.

A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups, *Environ. Sci. Technol.*, **2004**, Accepted for publication.

Invited Presentations

K. McNeill**.** Photochemical fate of pharmaceuticals pollutants. **Gordon Research Conference, Environmental Sciences: Water**, June 27 - July 2, 2004, *Forthcoming*.

Conference Presentations

K. McNeill, W.A. Arnold. Contribution of photochemistry to the fate of pharmaceuticals and personal care products in surface waters. *Oral Presentation.* ENVR, 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

A.L. Boreen, W.A. Arnold, K. McNeill. Photochemical fate of sulfa drugs in the aquatic environment. *Oral Presentation*. ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

K.H. Wammer, K. McNeill, T.M. LaPara, W.A. Arnold, D.L. Swackhamer. Changes in potency of antibacterials in the environment due to photochemical transformations. *Poster Presentation.* ENVR , 228th **ACS National Meeting**, Philadelphia, PA, August 2004.

A.L. Boreen, W.A. Arnold, K. McNeill. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. *Oral presentation*. 9th Biennial **MN Water Conference**, Minneapolis, MN, March 2004.

D.E. Latch, J.L. Packer, W.A. Arnold, K. McNeill. The Photochemical Fate of Triclosan. *Oral Presentation.* 26th Annual **Midwest Environmental Chemistry Workshop**, Iowa City, IA, October 2003.

A.L. Boreen, W.A. Arnold, K. McNeill, Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. *Poster presentation*. 26th annual **Midwest Environmental Chemistry Workshop**, Iowa City, IA, October 2003.

Statement of related grants submitted or funded as a result of this project
Dr. Arnold and Dr. McNeill have continued to apply for funding to continue this avenue of research. Dr. Arnold has received funding from the Center for Urban and Regional Affairs (University of Minnesota) to investigate the photodegradation of selected antibiotics in Minnesota waters in 2003-2004. Dr. Arnold is also a co-investigator on a United States Department of Agriculture grant (2003-2006) to investigate the loss of veterinary antibiotics in soil systems.

Description of student training provided by project:
Name: Anne L. Boreen
Program: Department of Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Betsy L. Edhlund
Program: Depatrment of Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Douglas E. Latch
Program: Department of Chemistry, University of Minnesota
Degree being sought: Ph.D.

Name: Jeffrey J. Werner
Program: Water Resources Science, University of Minnesota
Degree earned: M.S. (2004)
Degree being sought: Ph.D.

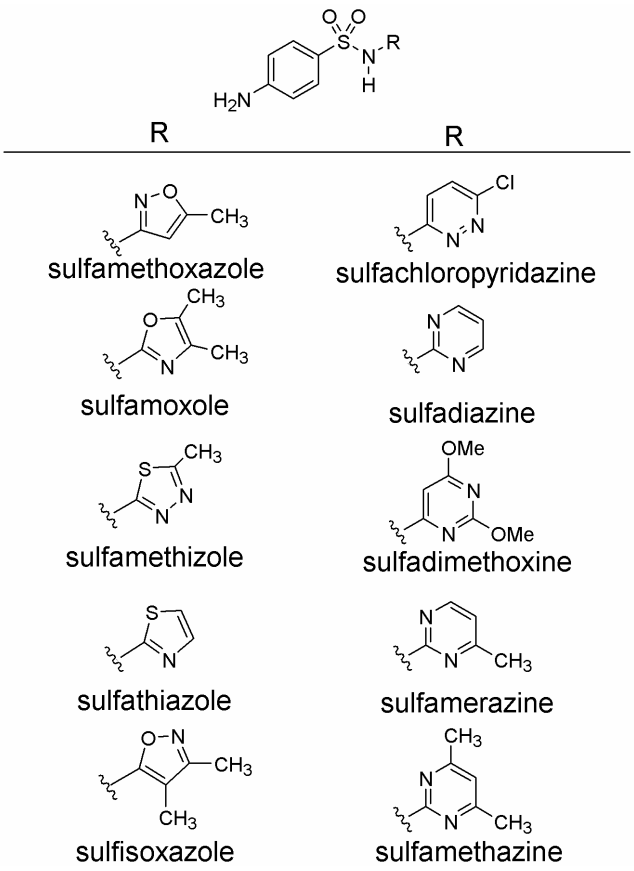

Figure 1. Structure of the ten sulfa drug antibiotics studied.

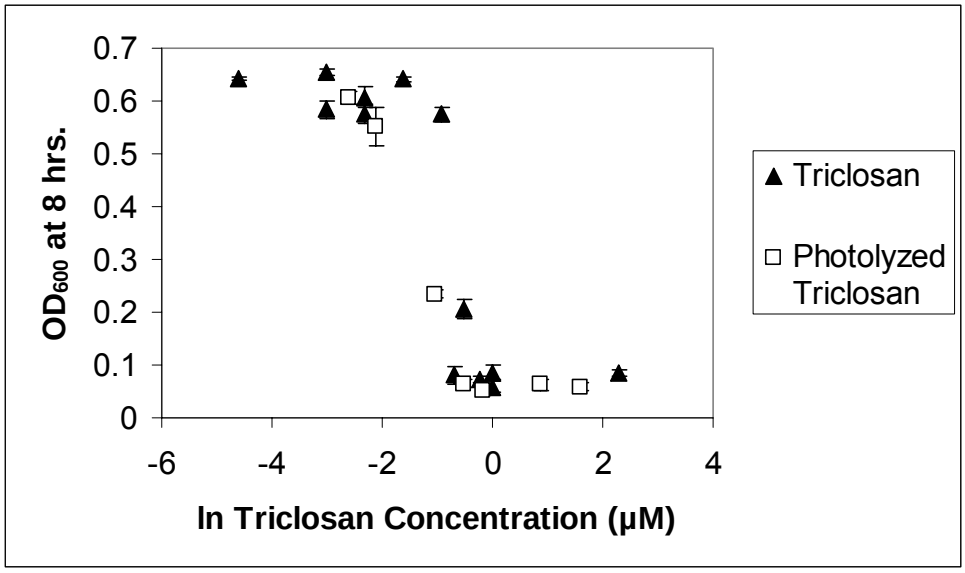

Figure 2. Optical density at 600 nm (OD_{600}) after 8 hours of growth for *E. coli* DH5α in the presence of triclosan (closed triangles) and triclosan plus photolysis products (open squares).

Table 1. Direct photolysis rate constants for sulfamethoxazole, sulfisoxazole, sulfamethizole, and sulfathiazole in various buffered H_2O solutions measured under natural sunlight. Spectral overlap integral, natural sunlight direct photolysis rate constant, and direct photolysis quantum yield data calculated for the three components of these sulfa drugs using matrix deconvolution of measured data.

Compound	pH	k_{direct} /10^{-5} (s^{-1}) [a]	Protonation State	$\Sigma\varepsilon_{295\text{-}340nm}L_\lambda$ (mE cm^{-3} M^{-1} day^{-1} nm^{-1}) [b]	k_{direct} calculated /10^{-5} (s^{-1}) [c]	Φ [c]
sulfamethoxazole	2.6	5.1 ± 0.9	SH_2^+	0	≤ 0.3	0
	4.1	6 ± 1				
	5.3	5.1 ± 0.8	SH	7.8	6 ± 1	0.50 ± 0.09
	6.9	1.3 ± 0.3	S^-	4.6	0.8 ± 0.2	0.09 ± 0.01
	10.8	0.6 ± 0.1				
sulfisoxazole	2.5	7 ± 2	SH_2^+	7.0	11 ± 5	0.7 ± 0.3
	4.1	6 ± 1				
	5.2	4 ± 1	SH	27.9	7 ± 1	0.17 ± 0.03
	6.8	2.5 ± 0.5	S^-	19.9	2.1 ± 0.5	0.07 ± 0.02
	10.7	1.7 ± 0.4				
sulfamethizole	2.4	≤ 0.3	SH_2^+	8.7	≤ 0.3	≤ 0.01
	3.2	≤ 0.3				
	4.0	≤ 0.3	SH	51.6	≤ 0.3	≤ 0.005
	5.2	0.5 ± 0.1				
	6.7	1.3 ± 0.3	S^-	15.9	1.3 ± 0.3	0.05 ± 0.01
	8.6	1.3 ± 0.2				
sulfathiazole	2.5	2.3 ± 0.4	SH_2^+	21.1	0.6 ± 0.6	0.02 ± 0.02
	3.9	2.3 ± 0.4				
	4.9	2.5 ± 0.5	SH	66.9	3.1 ± 0.6	0.07 ± 0.03
	6.3	5.7 ± 0.8	S^-	27.2	14 ± 1	0.40 ± 0.04
	8.4	13 ± 1				

[a] Errors represent the 95 % confidence levels. [b] L_λ values obtained from Leifer[20] averaged for 45 °N noon, mid-spring sunlight, mE = millieinstein. [c] Error values were estimated through a sensitivity analysis.

Information Transfer Program

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	1	0	0	1
Masters	3	6	1	0	10
Ph.D.	6	6	0	0	12
Post-Doc.	0	3	0	0	3
Total	9	16	1	0	26

Notable Awards and Achievements

The following faculty and students were honored for their achievements in the past year. Dr. Kristopher McNeill was named as a 2004 McKnight Land-Grant Professor. Recipients are honored with this title, a special award they will hold for two years. The award consists of a $25,000 research grant in each of two years, summer support, and a research leave in the second year. The winners were chosen for their potential for important contribution to their field; the degree to which their past achievements and current ideas demonstrate originality, imagination, and innovation; the potential for attracting outstanding students; and the significance of the research and the clarify with which it is conveyed to the non-specialist. Dr. Kris McNeill received funds under 104G.

Jennifer L. Packer won the 2003 1st Place Montgomery-Watson-Harza Consulting Engineers/AEESP Master's Thesis Award for her MS thesis, Photochemical Fate of Pharmaceuticals in the Environment. Jennifer worked with Dr. William Arnold on this grant which was funded this year under 104B.

Dr. Michael Sadowsky was named a Distinguished McKnight University Professor in 2004. Recipients are honored with this title, which they will hold for as long as they remain at the University of Minnesota. The grant associated with the Professorship consists of $100,000 to be expended over five years. The winners were chosen on the merit of their scholarly achievements and the potential for greater attainment in the field; the extent to which their achievements have brought distinction to the University of Minnesota; the quality of their teaching and advising; and their contributions to the wider community. Dr. Mike Sadowsky has received funds under 104G.

Dr. Partick L. Brezonik, former Co-Director of the Water Resources Center, was named the 2003-2004. Fesler-Lampert Chair of Urban and Regional Affairs. Dr. Brezonik has received funds under 104B and 104G.

Publications from Prior Projects

1. 2001MN1041G ("Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields") - Articles in Refereed Scientific Journals - Kumar, K., A. Thompson, A.K. Singh, and S.C. Gupta. 2004. Enzyme-linked immunosorbent assay for ultra trace determination of antibiotics in aqueous samples. Journal of Environmental Quality 33:250-256.
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