Water Resources Center
Annual Technical Report
FY 2007
Introduction

The Minnesota WRRI program is a component of the University of Minnesota's Water Resources Center (WRC). The WRC is a collaborative enterprise involving several colleges across the University, including the College of Food, Agriculture and Natural Resource Sciences (CFANS), the University of Minnesota Extension, Minnesota Agricultural Experiment Station, and the University of Minnesota Graduate School. The WRC reports to the Dean of CFANS. In addition to its research and outreach programs, the WRC is also home to the Water Resources Sciences graduate major. The WRC has three co-directors, Professor James Anderson, Faye Sleeper, and Professor Deborah Swackhamer who share the activities and responsibilities of administering its programs. The WRC funds 3–4 research projects each year, and the summaries of the current projects are found in the rest of this report.
Research Program Introduction

In FY07, we funded 8 proposals under 104(B) and administered 2 proposal under 104(G).
Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain

Basic Information

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<td>Heiko L Schoenfuss, Larry B Barber, Matthew Julius</td>
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Publication

ASSESSING THE ECOTOXICOLOGY OF ALKYLPHENOL MIXTURES ACROSS THE AQUATIC FOOD CHAIN

FINAL REPORT, OCTOBER 2007

PROJECT NUMBER: 2005MN147G

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Start date: 9/1/2005
End date: 8/31/2007

Abstract
This study was designed to elucidate the effects of alkylphenols singularly and in mixtures on different levels of the aquatic food chain. Among estrogenic endocrine disrupting compounds, alkylphenolic surfactants stand out due to their ubiquitous presence in anthropogenically altered surface waters and their occurrence in complex mixtures. Although the parent compounds (nonylphenol and octylphenol) of most alkylphenol polyethoxylates are orders of magnitude less estrogenic than 17β-estradiol, they are also found in concentrations orders of magnitude greater than the natural estrogen in many treated wastewater effluents and receiving streams and rivers. In addition, the longer-chained alkylphenol polyethoxylates are altering the bioavailability of nonylphenol and octylphenol, thus potentially facilitating the uptake of the more potent parent compounds by aquatic organisms exposed to these alkylphenol mixtures. Furthermore, the chemical nature of surfactant raises the specter that organisms at different levels of the trophic cascade may experience differential effects that may be estrogen receptor independent (diatoms) or estrogen receptor mediated (daphnia and fathead minnow). As a consequence, we proposed to test the effects of an alkylphenol
polyethoxylate mixture, realistic in composition and concentration, on tiers of an abbreviated aquatic food chain: the primary producer community (diatoms); a primary consumer (Daphnia magna); and a secondary consumer (fathead minnow, Pimephales promelas). Our findings from the single organism mixture exposure experiments indicate a degrading effects of alkylphenols on the primary producer community, especially on diatoms which represent the preferred food source of many larval and fingerling fishes. In addition, we have found that diatoms are more sensitive to alkylphenolic compounds than they are to the potent estrogen 17b-estradiol suggesting that the effects of alkylphenolic compounds might disrupt receptor independent pathways at subsequent tiers of the trophic cascade (Julius et al. In 2007). As a consequence of the diatom exposure, the nutritional value of diatoms for D. magna and larval fathead minnows was greatly diminished. In contrast, we did not find adverse effects of alkylphenol exposure on daphnia at any environmentally relevant concentration. However, we did find a significant reduction in the reproductive ability of male fathead minnows exposed to environmentally relevant concentrations of 4- nonylphenol, the parent compound and most estrogenic alkylphenols (Schoenfuss et al. In Press). Furthermore, we established that alkylphenol mixtures have a more potent effect on larval fathead minnows than the parent compound nonylphenol alone (Bistodeau et al. 2006). Together, these results suggest that alkylphenols contribute significantly to the observed estrogenicity of many municipal wastewater effluents, adversely affect the primary producer community and may have profound reproductive effects on adult fishes.
**Introduction & research objectives**

Endocrine disrupting compounds have been detected in many anthropogenically altered surface waters in North America (Kolpin et al. 2002, EST 36: 1202-1211), and Europe (Desbrow 1998, EST 32: 1549-1558). Several classes of endocrine disrupting compounds are usually recognized, including natural/synthetic hormones (estrone, estradiol, ethynylestradiol), personal care products (i.e., the antimicrobial soap ingredient Triclosan) and alkylphenolic surfactants. The latter have been found almost ubiquitously in anthropogenically altered surface waters in part because they use is inherently water related. Alkylphenols are a group of compounds used in large quantities as industrial and household surfactants and have been found to be estrogenic (Hemmer et al. 2001, ETC 20:336-343). Alkylphenols are known to bind to the estrogen receptor of mammalian cells and disrupt the homeostasis of the internal milieu of the organism. Environmental estrogens such as alkylphenols are known to disrupt normal endocrine hormone that are central to maturation and reproduction in fishes, and the ubiquitous presence of these biologically active compounds in surface waters should be of environmental and human health concern. To date, alkylphenol studies have focused on 4-nonylphenol, the metabolic product of both aerobic and anaerobic microbial degradation of higher-chained alkylphenols and the US EPA has recently proposed effluent emissions criteria for this compound. However, mixtures of nonylphenol and higher chained alkylphenols are found routinely in effluents and their combined action is entirely unknown. In this study, we propose to examine the effects of alkylphenol mixtures on three tiers of the aquatic food chain: the primary producer community (diatoms), a primary consumer (*Daphnia magna*), and a vertebrate near the top of the food chain (the fathead minnow).

**Methodology & Results.**

We completed several rounds of diatom exposures (*M. varians*) to graded concentrations of 4-nonylphenol (NP) singularly and to mixtures of alkylphenolic compounds (Table 1) including NP, nonylphenol-1-ethoxylate (NP1EO), nonylphenol-2-
ethoxylate (NP2EO), nonylphenol-1-carboxylate (NP1EC), and nonylphenol-2-carboxylate (NP2EC). In addition, we exposed diatoms to 17β estradiol, a compound with known endocrine disrupting activity that served as a reference exposure for this study.

**Table 1:** Concentrations used in *M. varians* exposures for 4-nonylphenol (4-NP), 17-β estradiol (E2) and the alkylphenol mixture (AP).

<table>
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<tr>
<th>Treatment</th>
<th>Low Exposure Concentration (mg/L)</th>
<th>Medium Exposure Concentration (mg/L)</th>
<th>High Exposure Concentration (mg/L)</th>
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<tr>
<td>17β estradiol</td>
<td>3</td>
<td>30</td>
<td>300</td>
</tr>
<tr>
<td>4-nonylphenol</td>
<td>2</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Alkylphenol mixture*</td>
<td>74.5</td>
<td>373</td>
<td>746</td>
</tr>
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* sum of several alkylphenolic compounds (see Table 2).

**Table 2:** Alkylphenol compounds detected in the Metropolitan treated wastewater effluent (St. Paul, MN) and their environmental concentrations, used for determining experimental dose values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/L)</th>
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<tr>
<td>NP</td>
<td>2.11</td>
</tr>
<tr>
<td>NP1EO</td>
<td>3.536</td>
</tr>
<tr>
<td>NP2EO</td>
<td>6.987</td>
</tr>
<tr>
<td>NP1EC</td>
<td>25.201</td>
</tr>
<tr>
<td>NP2EC</td>
<td>33.618</td>
</tr>
<tr>
<td><strong>SUM</strong></td>
<td><strong>71.5</strong></td>
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For the diatom exposures, monocultures of *Melosira varians* were grown in sterile WC media, then exposed to pre-determined test chemical concentrations and incubated in diurnal growth chambers with a 12:12 light:dark cycle for a period of ten days. Procedures were as follows:

100 ml of homogenized culture aliquots were added to 900 ml of sterile media and allowed a period ≥ 24 hours to acclimate. Due to its affinity for binding to glass,
sterile polystyrene cell tissue rollers were used in the case of the 4-nonylphenol exposures, and 2 liter glass Florence flasks were used for the estradiol trials. Treatments consisted of control, low, medium and high exposure concentrations. Once treated, samples were taken on day one for cell count and chlorophyll-a analysis. Exposed cultures were then allowed to grow for a period of ten days, after which samples were once again obtained for chlorophyll-a and cell count. The chlorophyll-a content of the cells was measured using a fluorometer and averages for each treatment group were determined in order to quantitatively assess diatom health. Elevated chlorophyll A tissue concentration indicate a stress-effect caused by the exposure. In three exposure experiments using graded series of 17β-estradiol (experiment 1: 2μg/L, 20μg/L, 200μg/L; experiment 2: 4μg/L, 40μg/L, 400μg/L; experiment 3: 8μg/L, 80μg/L, 800μg/L) treatments at or above 80μg/L consistently found statistically significant (one way ANOVA, p<0.05) increases in chlorophyll A: lipid ratio indicating a reduced nutritional value of diatoms for higher levels of the trophic cascade. Clearly these 17β-estradiol concentrations are well beyond environmentally relevant concentrations and indicate that 17β-estradiol does not adversely affect exposed diatoms. In contrast, three exposure experiments using series of alkylphenols (experiment 1: 2μg/L, 20μg/L, 200μg/L; experiment 2: 4μg/L, 40μg/L, 400μg/L; experiment 3: 8μg/L, 80μg/L, 800μg/L) found statistically significant (one-way ANOVA, p<0.05) increases in the Chlorophyll A : lipid ratio at and above 40μg/L (Figure 1 a-d from Julius et al. 2007). This concentration of total alkylphenols has been exceeded in many treated wastewater effluents and indicates that the primary producer community is likely adversely affected by environmental concentrations of alkylphenols.
Figure 1. Alkylphenol exposures of diatoms result in significant differences in cell counts (A); chlorophyll A concentrations (mg/L) (B); total lipid content (mg/L) (C); and lipid : chlorophyll ratios (D).
Daphnia magna were the model organism for our mid-food chain tier exposures. Cultures of daphnia were obtained from US EPA laboratories and maintained following standard US EPA procedures (US EPA 2002). Exposure experiment utilized neonates less than 24 hours. Neonates were carefully transferred individually into 50mL beakers (10 beakers - 10 daphnia per treatment) and exposed using a static renewal assay with 50% of each medium being exchanged daily for 21 days. Number of offspring was recorded for each beaker starting on day 6 (7 day life cycle from neonate to maturity) and continued through day 21. All offspring at day 21 were observed under a light microscope to determine presence of morphological abnormalities. A series of alkylphenol exposures up to 500µg NP/L medium were performed and did not result in significant effects on daphnia survival or developmental defects.

Fathead minnows, Pimephales promelas, were exposed in this study at two stages of development: as larvae and as mature adult males. Larval fathead minnows were exposed for 67 days post-hatch to either nonylphenol singularly at an environmentally relevant concentration, or to a mixture of alkylphenols representing in composition and concentration a major municipal wastewater effluent (Table 3, Bistodeau et al. 2006).

Table 3. Fish exposure experiments and nominal dosing concentrations for the alkylphenol (APE) mixture and nonylphenol (NP) only exposures and total NP concentrations utilized in each exposure.

<table>
<thead>
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<th>Experiment Name</th>
<th>*Total NPE/OPE Concentration (µg/L)</th>
<th>Total NP (µg/L)</th>
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<tr>
<td>200% APE</td>
<td>148</td>
<td>4.2</td>
</tr>
<tr>
<td>100% APE</td>
<td>73.9</td>
<td>2.1</td>
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<tr>
<td>50% APE</td>
<td>38.1</td>
<td>1.1</td>
</tr>
<tr>
<td>NP</td>
<td>5</td>
<td>5.0</td>
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* APE mixture consists of NP, NP1EO, NP2EO, NP1EC, OP, OP1EO, and OP2EO in a ratio of 2.8:5.1:9.3:33.7:44.9:0.2:0.7:3.1 respectively.
Mortality was observed throughout the exposure and beyond the exposure where fish were reared in clear well water to maturity. Once mature, male fathead minnows from the exposures were paired with control males and allowed to compete for reproductive opportunities (Figure 2, Bistodeau et al. 2006).

![Bar chart showing % of Nest Sites Held for CONTROL and TREATMENT groups](image)

**Figure 2.** Competitive spawning results for (A) the 100% alkylphenol (APE) exposure experiment, (B) the 50% NPE/OPE exposure experiment, and (C) the 4-nonylphenol (NP) only exposure experiment. Bars indicate the percentage of nest sites held by either the control fish or the treated fish in each assay. (*p<0.005).

Our results indicate that reproductive competence is impaired in male fathead minnows that were exposed to the mixture for 67 days post-hatch at realistic concentrations (Fisher’s Exact test; p<0.05). In addition, secondary sexual characters and the gonadosomatic index are significantly reduced when compared to control males (Student t-test, p<0.05). Even at a mixture concentration representing 50% the mixture concentration measured in the treated effluent, reproductive competence was significantly reduced. In contrast, nonylphenol alone had an excitatory effect on nest holding ability (Fisher’s Exact test, p<0.05) that is likely the result of a priming effect of the low-concentration estrogenic compound. Detail results of the fathead minnow exposures are published in Bistodeau et al. (2006).

Our exposures of adult male fathead minnows further elucidated the effects of alkylphenols on exposed fishes and their reproductive potential. We exposed mature male fathead minnows for 28 days to graded concentrations of nonylphenol and
followed the exposure first with the same competitive spawning scenario employed in the larval exposure experiment and then assessed vitellogenin induction, secondary sexual characters, and histopathology (Schoenfuss et al. In Press).

Exposure resulted in no discernable effects on the fishes gonadosomatic or hepatosomatic index. Secondary sexual characters were also not affected and vitellogenin induction was inconsistent. However, the competitive spawning assay revealed a marked decline in the exposed males ability to hold nests sites when nonylphenol concentrations exceeded 5 µg Np/L (Figure 3, Schoenfuss et al. In Press).

**Figure 3.** Nest holding ability of nonylphenol exposed male fathead minnows in direct competition with control males in (a) experiment 1 and (b) experiment 2. [Numbers in parentheses indicates total observations of nest holding; p values are presented for each treatment, Fisher’s exact test].
PUBLICATIONS RESULTANT FROM NIWR FUNDING


SEMINARS & PRESENTATIONS

(* indicates student presentation)

Schoenfuss, HL and TJ Bistodeau. 2006 Midwest SETAC Meeting, St. Cloud, MN

Gable, C*, A. Gkineh and ML Julius. 2006 Midwest SETAC Meeting, St. Cloud, MN

Allen, AK*, T Loes and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN
March 20-22, 2006 - poster presentation.

Grove, KJ*, RA Cediel and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN
March 20-22, 2006 - poster presentation.

Koch, JK*, M Minger and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN
March 20-22, 2006 - poster presentation.


Schoenfuss, HL, Bistodeau, TJ, Society for Environmental Toxicology and Chemistry, Montreal, Canada, November 2006 - oral presentation.
Julius, ML, Society for Environmental Toxicology and Chemistry, Montreal, Canada, November 2006 - poster presentation.

STUDENTS SUPPORTED BY THE PROJECT
Kent Grove (MS expected Fall 2007)
Jason Koch (MS expected Spring 2007)
Nathan Jahns (MS expected Spring 2008)
Roberto Cediel (MS expected Summer 2007)
Carolyn Gamble (MS expected Fall 2007)
Angela Allen (undergraduate project Summer 2006)
Tim Loes (undergraduate project Summer 2006)
Bradley Sivanich (undergraduate project Summer 2007)
Josh Stepanek (undergraduate project academic year 2006-07)
Factors Affecting Revegetation Success in Lakeshore Restorations

Basic Information

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Publication
Factors Affecting Revegetation Success in Lakeshore Restorations

Principal Investigator
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Research Assistant
Dana Vanderbosch, Graduate Program in Water Resources Science, UMN

Project Number: 2006MN153B

Start Date: 3/1/2006
End Date: 2/29/2008

Abstract

Revegetation of littoral zones is crucial to the overall success of a lakeshore restoration. Efforts to improve fish habitat and reverse shoreline erosion both depend upon the re-establishment of emergent macrophyte beds. Despite their central role in lakeshore restoration, most littoral plantings fail. The reasons for this are poorly understood, in part due to limited information on the effects of planting time, water depth, and root/rhizome development on the survival of emergent macrophyte plantings. To address this, we planted 3.8 liter pots and 28 x 28 cm prevegetated mats of *Scirpus validus* at two different water depths (0-30 cm and 31-60 cm of water depth) in five lakes each month between May and September 2006, to evaluate the effect of planting month, water depth, rootstock type and plant condition on the survival of transplanted softstem bulrush (*Scirpus validus* Vahl). Survival of plantings was assessed three times after planting. Overall survival decreased from 73% at 30 days after planting to 15% one year later. The results of our study indicate that the robustness of the stock used for the restoration effort, as well as planting date, are critical factors influencing bulrush survival. Our evaluation of the two different bulrush rootstocks indicates that pots generally outperform mats when planted early- to mid-summer. Water depth is only important immediately after planting, after which time its influence on successful establishment diminishes. Survival of bulrush planted later in summer is poor, regardless of the rootstock type used, and should be avoided. Overall, our research indicated that key choices made by the restoration practitioner can improve the likelihood that transplants establish. That said, lake environment effects, such as herbivory and wave impacts, also contributed to transplant mortality. Research into these lake effects could further increase the success of littoral revegetation efforts.
Introduction

Aquatic macrophytes are an important component of lake and wetland littoral zones. In particular, the emergent macrophyte zone is the region of greatest productivity (Wetzel 1990). Loss of aquatic macrophytes results in reduced structural complexity and diminished habitat for invertebrates, and reduced food supply, spawning/nesting habitat and cover for fish, muskrats (*Ondatra zibethicus*), and waterfowl (Wilcox and Meeker 1992). The littoral zone is influenced by stressors originating from the lake, as well as from the adjacent shoreland area (Crowder et al. 1996). Recent research links increased lakeshore development to decreased aquatic macrophyte abundance (Bryan and Scarnecchia 1992; Jennings et al 1999; Radomski and Goeman 2001; Elias and Meyer 2003; Radomski 2006). For example, in a study of 100 lakes (Minnesota, USA), Radomski (2006) observed the abundance of emergent and floating leaved macrophytes decreased by as much as 17% between 1939 and 2003, with the most developed lakes experiencing the greatest losses. While littoral wetland restorations have been attempted in the north central USA for over twenty years, the number of projects has risen recently with the development of regional lakeshore and shoreland restoration programs. For example, since 2003, the Minnesota Department of Natural Resources’ (DNR’s) Shoreland Habitat Restoration Grant Program has initiated approximately 20 projects of ~3,100 meters in length on average each year.

Though some guidelines for littoral revegetation exist, the protocols have not been methodically evaluated. Currently, preferred planting practices are those that have been adopted through trial and error; however, planting survival is inconsistent enough that it is difficult to determine the role these practices have played in the revegetation outcome. Without systematic evaluation, ineffective planting strategies may be promoted. Therefore, experimental research to elucidate which aquatic planting practices will most reliably result in the survival of littoral revegetation is needed.

We conducted a study of *S. validus* establishment in urban lakeshore restorations to determine the importance of several environmental factors and planting practices on revegetation success. Specifically, we sought to determine: 1) whether establishment is
affected by time of planting, rootstock type and condition, and water depth, and 2) the optimal combination of factors to maximize littoral revegetation establishment. We investigated these questions by planting two rootstock types of *S. validus* at various water depths on five lakes in the Minneapolis/St. Paul Metropolitan Area (Minnesota, USA). The results of this study were intended to provide resource managers with practical planting strategies to increase the success of littoral revegetation projects.

**Study Site Selection**

The general study region, the seven metropolitan counties (Ramsey, Hennepin, Dakota, Washington, Anoka, Carver, and Scott) surrounding St. Paul and Minneapolis, is characterized by glaciated terrain with numerous natural, shallow lakes. Several organizations offer funding for lakeshore restorations projects within this 7-county area. The Minnesota DNR initiates most of the projects providing funding through its Shoreland Habitat Restoration Grant Program to local units of government (LUGs) implementing lakeshore restoration projects. According to DNR records (Hiebert 2008), the typical lakeshore restoration project is approximately 200 meters in length and consists of restoring native vegetation to the upland, transition and aquatic portions of lakeshore. The restoration goal for the littoral zone is to replace emergent vegetation that has been completely removed.

We selected study sites that represented the shoreland conditions typical of restoration projects funded by the DNR. We limited the lake size range to ensure sites were located on water basins large enough to support recreational activities typical of most Minnesota lakes, yet small enough to avoid high energy wave impacts. Sites were considered for the study if they: 1) were located on lakes between 4 and 500 hectares; 2) were at least 100 meters in length to provide adequate space for the experimental plantings; 3) were located on property owned by a willing cooperator; 4) contained no emergent vegetation; and 5) contained lakeshore that has been modified from its natural state. Based on a review of the DNR’s records and on interviews with LUGs that had either received restoration grants in the past or were applying for them in 2006, we identified 26 sites that potentially met our site selection criteria. From this initial pool,
Four sites were eliminated from the study because lakeshore length was inadequate for the experimental planting. Field reconnaissance revealed that fifteen sites already contained emergent vegetation, had been riprapped, or were located in undesirable locations (i.e., near stormwater inlets, exposed to high impact waves, on very small lakes, etc). Two other potential sites were eliminated because the property owners were disinterested in the project.

Five lakes best met the selection criteria and were included in the study. All were located on urban lakes ranging in size from 24 to 162 hectares. The lakeshore of all five lakes had been modified from its historic state either directly (e.g., deliberate removal of emergent vegetation) or indirectly (e.g., altered hydrologic regimes, recreational activity that has eroded the shoreline). Four of the sites are located in public parkland and one on private property. None of the selected sites contained emergent vegetation prior to the study, though four of the five did contain submersed or floating-leaved vegetation.

**Experimental Design and Implementation**

We used a randomized split-split plot design with three factors to determine the effects of planting practices on transplant success. Each research site was divided into five adjacent plots, each approximately 20 meters long. Bulrush was planted in one of the five randomly chosen plots each month between May and September 2006 to test whether seasonality influences the survival of planted emergent vegetation. Experimental plantings occurred 4-5 weeks apart. Each plot was further divided into a shallow and deep water zone to test the effect of water depth on the bulrush plants. The shallow water zone was defined as the area between 0-30 cm of water depth; the deep water zone between 31-60 cm of water depth. The depth zones were determined in May 2006, when lakes were at their maximum water level. Last, to test whether or not the depth and overall development of rhizomes and roots affects planting survival, bulrush seedlings were grown as two different types of rootstock, 3.8-liter (1-gallon) pots 5-cm deep and in 28 x 28-cm loosely woven coconut fiber biodegradable mats 15-cm deep, and planted in equal numbers in the each depth zone. Both pots and mats are commonly used in lakeshore restoration. Each treatment combination was replicated 25 times in the shallow water zone. Because bulrush planted in the deeper water zone were expected to
have a lower overall survival rate, each rootstock treatment combination was replicated fifty times in the deep water zone so lower probability events could be detected with greater certainty.

**Data Collection and Analysis**

The growth and development of the bulrush was assessed prior to planting. The individual seedlings in each pot and mat were assessed and classified prior to planting using three different measurements to gauge the overall condition of the remaining bulrush. First, the height of the tallest stem from each seedling was measured. Next, the number of stems per plant was approximated using a visual estimate, and categorized in one of three classes: 1) 0-10; 2) 11-20; and 3) >21. Lastly, the overall condition of the plants was categorized as: 1) most senescing; 2) mix of vigorous and senescing or 3) all, or nearly all, vigorous. A stem was classified as senescing when it was chlorotic and browning, and considered dead when it was yellowed or over half its length was brown.

We recorded survival of individual bulrush plants three times after planting: 30 days after planting, prior to lake freeze (November 2006), and after winter (May - June 2007). From this information, mean total survival was calculated by dividing the number of plants that had survived by the total number of bulrush planted for each treatment combination. For those plants that survived, we also estimated the shoot number and recorded the condition of each emergent clump using the same abundance classes as the pre-planting assessments. Post-planting shoot abundance was estimated for the entire emergent clump because it was not always possible to distinguish individual plants after 30 days of growth. The mean total number of plants that survived was compared among lakes, depth levels, rootstock types and planting month using analyses of variance (ANOVA). All main factors and two and three-way interactions were included in the model. The error term for the model was calculated from the highest order (4-way – lake*depth*rootstock*month) interaction term. Where ANOVA tests indicated significance (month*rootstock interaction), pairwise means comparisons were performed with Tukey HSD tests to evaluate which means were significantly different. Survival was square root transformed to standardize normality. Using the procedure described above, data were analyzed at all three timesteps: 30 days following planting, the end of
the first growing season, and post-winter. We also used ANOVA to compare mean biomass by rootstock types and planting month. Incorporating the pre-planting condition data (height, number of shoots and shoot condition) in an analysis of covariance did not improve the model, and those results are not presented. The software program R (Version 2.5.1, 2007) with a significance level of $\rho \leq 0.05$ was used for all statistical analyses.

**Results**

Total mean plant biomass of stock used for this experiment varied widely from month to month, with mean biomass greatest in June (20.15 g) and lowest in September (2.19 g). Mean belowground biomass was significantly different between planting months ($F = 30.23, p < 0.001$), ranging from 13.5 g in June to 0.9 g in September. In addition, the type of rootstock used was important ($F = 5.98, p = 0.019$); mean belowground biomass was 28% greater for mats than pots. Mean shoot biomass also differed by planting month ($F = 41.52, p < 0.001$). Although shoot biomass was not generally greater for mats or pots, shoot biomass of mats was 83% greater than pots in August. Mean belowground biomass was as much as 40% greater (June) for mats than pots in every month except the month of May. In May, the mean belowground biomass of pots exceeded that of mats by 11%.

A comparison of survival at three times after planting (30 days after planting, prior to winter and post-winter) shows an incremental decrease in survival (Figure 1). Thirty days after planting, the average survival was 73%. By the end of the first growing season, fewer than half (40%) of the bulrush were still alive. Only 563 (15%) of the 3,750 total bulrush planted were alive post-winter. Post-winter survival ranged from 4% to 31% among lakes.

During the first growing season, factors affecting survival were similar over time. Bulrush survival initially (30 days post-planting) varied by planting month ($F = 6.79, p = 0.001$) and water depth at planting ($F = 9.44, p = 0.006$). Mean survival of bulrush planted in the deep zone (80%) was greater than that of bulrush planted in the shallow zone (68%). The 30-day post planting mean survival of bulrush was highest in June (90%) and lowest in August (42%). Mats and pots showed different patterns of survival.
by month and water depth ($F = 12.08, p < .001$ and $F = 10.35, p = .003$, respectively) at
this time. Survival of bulrush mats was lower than that of pots in every month except for
August. Though the mean survival of pots in the shallow zone (72%) was similar to that
of pots in the deep zone (76%), mats in the shallow zone experienced lower mean
survival (63%) than did mats planted in the deep zone (83%). In general, the mean
bulrush survival patterns observed 30 days after planting persisted through the end of the
growing season; however, by autumn, water depth was less important to survival than it
had been earlier in the summer.

Patterns of post-winter survival were similar to those found during the 30-day and
pre-winter timesteps. Post-winter mean survival of bulrush differed significantly
depending on the planting month ($F = 5.058, p = 0.0079$), with highest mean survival
(29%) for bulrush planted in the month of June and lowest (3%) for bulrush planted in
September (Figure 1). Post-winter survival of bulrush planted in August (Tukey HSD, $p$
= 0.024) and September (Tukey HSD, $p = 0.011$) was significantly lower than that of
bulrush planted in May, June, or July. More than 95% of the bulrush planted in August
and September had died by the spring following planting. The interaction between
planting month*rootstock was the only combination of factors that proved significant for
bulrush survival post-winter ($F = 3.245, p = 0.0214$). Pots planted in June yielded the
highest overall mean post-winter survival (35%). Mean post-winter survival was lowest
for pots planted in August (3%) and for mats planted in September (3%). Survival of
mats and pots differed ±4% for most months. Survival of June pots, however, was 12%
greater than survival of mats planted that same month. Post-winter survival varied
greatly among lakes, ranging from 4 to 31% (Table 1).

Discussion

Our study indicates that, surprisingly, water depth was relatively unimportant to the
establishment of aquatic plantings, whereas the robustness of the stock, planting
month, and type of rootstock were found to be key drivers. These findings suggest that
planting choices made by the restoration practitioner can improve the likelihood that
plantings will establish, with planting potted *Scirpus* in early summer providing the optimal conditions for littoral transplant success.

The planting month plays a critical role in the success of the revegetation effort. We observed the greatest survival from bulrush planted early in the growing season (June) and the greatest mortality from those planted at the end of the growing season (September). Likewise, aquatic *Carex* (also Cyperaceae) survive best if transplanted early in the growing season (Yetka and Galatowitsch 1999, Steed and DeWald 2003). Sedge shoots grow rapidly in spring, due to both the translocation of materials from belowground rhizomes and photosynthesis (Bernard 1975, Bernard and Solsky 1977). Belowground nutrient and carbohydrate levels in sedges are low in early summer due to high demand by aboveground tissues for growth and inflorescence development, and are gradually replenished during summer and early fall in preparation for winter dormancy (Roseff and Bernard 1979, Bernard 1990). Yetka and Galatowitsch (1999) indicated that inadequate underground reserves were a primary factor responsible for low survival rates of *C. lacustris* fall transplants. Steed and DeWald (2003) similarly suggested that the lower belowground reserves of summer sedge transplants may have been offset by above- and below-ground plant growth prior to fall that replenished those reserves. Fall transplants have limited carbohydrate, nutrient, and water reserves on which to survive the winter before initiating new growth in spring.

Mortality differences observed among planting months corresponded to the size and robustness of rootstock. Stock planted in May, June and July was started in the greenhouse in early spring and summer when temperatures were relatively cool, and these plants were the largest. Stock for August and September plantings were much smaller and likely heat stressed during production; greenhouse temperatures often cannot be adequately regulated during summer. The difference in size and robustness of stock produced for our experiment allowed us to compare pre-planting belowground biomass to plant survival. Plant survival trended with pre-planting belowground biomass; the more robust the roots and rhizomes, the more likely the plant was to survive. Transplant survival of aquatic species (e.g., *Carex* spp.) and terrestrial species (e.g., *Calamagrostis rubescens*) has been known to be linked to transplant size (Davies et al. 1999; Steed and
DeWald 2003; Page and Bork 2005). Steed and DeWald (2003) suggested that the smaller *Carex* transplants had smaller and less developed root systems as compared to larger *Carex* transplants, which contributed to greater mortality of smaller plants after transplant. Further research is needed to establish the connection between belowground biomass and planting performance. Though belowground biomass appears to be an important determinant of planting success, we also observed differences in bulrush survival that could not be explained by plant biomass. For instance, in June the mean mat belowground biomass was greater than that for pots; however, survival of June pots was greater on average than that of June mats. This suggests other factors that influence plant survival can over-rule rhizome development.

Our study found that prevegetated mats fail to establish more often than pots of bulrush. The survival of May pots was 5% greater than that of mats, and 12% greater than that of June mats. The survival of mats and pots later in the summer was similar, suggesting that mortality is not only caused by failure to store carbohydrates prior to winter, but also by an inability of mats to anchor to the lake substrate efficiently. Though prevegetated mats were each fixed to the lake bottom with two 10 cm biodegradable stakes, they were not adequate to counteract the effects of wave activity. This seemed to be a particular problem at sites with coarse substrates. Wilson and Keddy (1988) examined the affect that waves have on emergent macrophyte zonation and found that biomass accumulation varied significantly with position on a lakeshore gradient of exposure to wave action, and tended to be greatest on sheltered shores. We suggest that the higher mortality of mats was caused by frequent wave action created by wind and recreational activities, which prevented the plants from anchoring to the lake substrate.

Bulrush survival in the shallow water zone was lower than the deep water zone the month following planting, but the influence of water depth diminished over time. By the spring following planting, water depth was no longer a significant factor affecting transplant growth and development. Although deeper water levels are well known to limit emergent plant growth (Harris and Marshall 1963; Lieffers and Shay 1981; Shay and Shay 1986; Shipley et al. 1991), we observed higher mortality in shallower conditions. This high mortality in shallow water was unexpected because although our
experiment did not seek to test the tolerance limits of emergent macrophyte plantings, they were planted at depths well within the known tolerance limit for *S. validus*. Lieffers and Shay (1981) observed higher *S. maritimus* shoot survivorship when seedlings were grown at or above the water surface. Likewise, Budelsky and Galatowitsch (2000, 2004) found *C. lacustris* and *C. stricta* seedling survival was highest when the soil surface was moist to saturated, and declined under conditions of extended flooding. Prolonged inundation of wetland plants reduces shoot growth, rhizome expansion and seed production for newly established plants by reducing soil aeration. Our prediction that water depth would also have a significant effect on *S. validus* plantings was not fully realized, but our observation that water depth was unimportant once plantings had established was also reported by Budelsky and Galatowitsch (2004). In contrast to these wetland studies, our lake plantings would likely have experienced high wave energy, and the greater transplant mortality we observed in the shallow water zone appeared to be caused by a variety of lake environment factors, including early summer wave activity that uprooted shallow water plantings (particularly mats), shading from riparian trees and shrubs, repeated herbivory, and burying of plantings by sand and submerged vegetation (i.e. *Potamogeton crispus* and *Myriophyllum spicatum*).

Our study indicates that optimal planting practices can improve revegetation success; however, these practices only increased transplant survival one year after planting to a maximum of 35%. Further research into planting choices likely will not yield much greater increases in aquatic planting survival for restorations located on highly developed lakes. These restorations are subject to factors that cannot be controlled at the site scale and must be addressed as whole lake issues. Herbivory is a key lake-scale factor; muskrats are one of the dominant vertebrate herbivores of aquatic macrophytes, and they have potential to significantly impact plant communities (Errington 1963; Weller 1994; Szalay and Cassidy 2001). In our study, 21 (84%) of the research plots experienced some level of muskrat damage, though each plot was fenced and a trapper employed. Other important lake factors include water-level regulation and stormwater inputs that create anomalous hydrologic regimes resulting in altered species composition and reduced structural diversity of aquatic macrophyte communities (Wilcox and Meeker 1991; Wilcox and Meeker 1992; Reinelt et al. 1998).
established aquatic beds are vulnerable to uprooting by wave impacts (Elias and Meyer 2002). Aquatic recreation amplifies natural wave activity, and heavily developed lakes lack coarse woody debris (Christensen et al. 1996) which serves as a natural wave break. In general, highly degraded sites located within a highly degraded landscape have low probability of full restoration (National Research Council 1992). With this understanding, major gains in littoral revegetation on developed lakes will only be realized when restoration is pursued at multiple scales.
Figure 1. Overall percent of *Scirpus validus* alive during the three post-planting assessment periods: 30 days after planting, at the end of the first growing season, and the summer following planting.  N = 750 individuals per planting (month).
Table 1. The number and percent of *S. validus* still alive by lake the summer following planting.

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<th>Survived</th>
<th>Total Planted</th>
<th>Percent survival (%)</th>
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<tr>
<td>Snail Lake</td>
<td>154</td>
<td>750</td>
<td>21</td>
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<tr>
<td>Island Lake</td>
<td>108</td>
<td>750</td>
<td>14</td>
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<td>Lake Calhoun</td>
<td>32</td>
<td>750</td>
<td>4</td>
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<tr>
<td>Lake Harriet</td>
<td>231</td>
<td>750</td>
<td>31</td>
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<tr>
<td>Sweeney Lake</td>
<td>38</td>
<td>750</td>
<td>5</td>
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Student worker planting bulrush (*Scirpus validus*)

Bulrush about to be planted
Lakeshore restoration showing nicely vegetated upland, but the emergent revegetation has not established.
Publications, Presentations, or Published Abstracts:
None to date.

Students and Post-Doctoral Students supported by this project:
Dana Vanderbosch
Graduate Program in Water Resources Science, University of Minnesota
Master of Science

Awards and Special Recognition:
None to date.
Ecological Stoichiometry and Microbial Biodiversity Effects on Water Quality in Minnesota Lakes

Basic Information

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<td>Principal Investigators:</td>
<td>James B Cotner, Timothy Michael LaPara</td>
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Publication
Ecological Stoichiometry and Biodiversity as Controlling Factors in Nutrient Biogeochemistry in Minnesota Lakes

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Project Number: 2006MN155B

Start date: 3/1/2006
End date: 2/29/2008

Abstract

Prokaryotic heterotrophs are extremely abundant and have large impacts on global biogeochemistry and ecosystem processes such as nutrient regeneration and productivity (Cotner and Biddanda, 2002). Ecological stoichiometry examines the balance of energy and chemical elements in living systems (Sterner and Elser, 2002). In the work discussed here, the importance of microbial diversity and ecological stoichiometry to biogeochemical processes was being examined in aquatic systems.

Microbial stoichiometry and diversity interact to affect nutrient regeneration; stable interactions are promoted when decomposers are limited by organic carbon and the stoichiometry of decomposers is similar to that of autotrophs. Furthermore, biodiversity promotes redundancy and reliability in ecosystem function. However, the relationships among microbial stoichiometry, diversity, and ecosystem function have not been explored. This study helps clarify whether microbial diversity promotes stability in ecosystem function by providing increased stoichiometric diversity with subsequent effects on nutrient regeneration and productivity.

Hypotheses being tested are that (a) individual strains of bacteria are strongly homeostatic and (b) variable microbial community stoichiometry is achieved through variability...
in community composition. It is further hypothesized that (c) the efficiency at which nutrients are re-mineralized by the microbial community is directly dependent on the diversity present in a given lake/ecosystem.

The first outcome of our research was to demonstrate that the bacterial communities in lakes are substantially variable with respect to the ratio of their carbon-to-phosphorus and less so with respect to carbon-to-nitrogen. This result is critical, because our hypothesis is that individual strains are restricted with respect to their carbon-to-phosphorus; natural oscillations in carbon-to-phosphorus, therefore, would reflect adaptations within the structure of the bacterial community. The second outcome of research was to quantify the degree of homeostasis from eight different bacterial strains. These organisms all exhibited some degree of homeostasis, though less than previously reported for *E. coli*. Finally, we compared bacterial diversity in lakes of differing trophic status in the Twin Cities and lakes of similar trophic status in and near Itasca State Park. Bacterial community diversity in the Twin Cities was linked to primary productivity. Similarly, bacterial community diversity in Itasca State Park exhibited a hump-shaped profile as a function of the carbon-to-phosphorus ratio. In conclusion, our research supports our hypotheses that the principles of ecological stoichiometry can be used to better understand bacterial community diversity and nutrient biogeochemistry in Minnesota lakes.

**Introduction**

The field of ecological stoichiometry is a rapidly developing area of biology that attempts to understand reciprocal interactions between organisms and their chemical milieu (especially carbon [C], nitrogen [N], and phosphorus [P]) under the constraints of limited energy and materials (Sterner and Elser 2002). The utility of ecological stoichiometry is in explaining patterns in the composition of organisms and their impacts on the environment, an important tool in this age of rapid human-induced biogeochemical change (Vitousek et al. 1997).

One of the cornerstones of ecology is understanding relationships between biodiversity and ecosystem function (BEF) (Kinzig et al. 2002; Loreau et al. 2002; McGrady-Steed and Morin 2000; Morin and McGrady-Steed 2004; Naeem and Li 1998; Naeem and Wright 2003). These studies typically show that diversity can have strong effects on ecosystem processes (Tilman et al. 1997; Knops et al. 2001; Kennedy et al. 2002), but the relative importance of diversity vs. the presence/absence of particular functionally important species is contested (Givnish 1994; Grime
Many of these studies have been done in grasslands with few performed in aquatic systems (Naeem and Wright 2003), the focus of the current proposal.

Prokaryotic heterotrophs are perhaps the most dominant biogeochemical force in aquatic systems (Cotner and Biddanda 2002; Karl 2002) and recent work in freshwater ecosystems (Zwart et al. 2002) indicates that these communities harbor an amazing diversity of flora, even over very small (10s to 100s of meters) spatial scales. The factors affecting microbial diversity seem to be similar to those impacting diversity of macro-organisms such as productivity (Kassen et al. 2000; Yannarel and Triplett 2004; Horner-Devine et al. 2003), temperature (Yannarel and Triplett 2004; Ravenschlag et al. 1999), and predation (Massana and Jurgens 2003).

**Methods**

**Sample Collection**

We established three different “study sites” within the state of Minnesota. We have been collecting water samples from ~10 lakes within and near Itasca State Parks (sampled May 2006, August 2006, May 2007, and August 2007). We have also collected samples from 8 different lakes within the Twin Cities Metropolitan region, sampled numerous times (10-12 times per lake) from June 2006 through June 2007. We also collected samples from ~10 different lakes within and near the Boundary Waters Canoe Area Wilderness (BWCAW) (sampled June 2007 and August 2007). Water samples were collected from the mixed layer of the lakes. Samples were collected with a Van Dorn water sampler into acid-cleaned, sterilized containers.

**Isolation and characterization of microbial strains**

We isolated over 50 strains of Bacteria from the lakes that we have sampled using the streak plate technique. Strains were isolated using several different types of media to increase the potential for greater metabolic and genetic diversity. These isolates were identified by determining their 16S rRNA gene sequence and comparing it with sequences available in the GenBank database.

**Community analysis and biodiversity quantification**

The composition of lake water bacterial communities were characterized by extracting genomic DNA from water samples and then by performing PCR targeting a portion of the
ribosomal RNA operon. Automated ribosomal intergenic spacer analysis (ARISA; Fisher and Triplett, 1999) was used to generate fingerprints of bacterial community composition using primers ITSF and ITSReub (Cardinale et al., 2004) or primer 1522F and 132R (Ranjard et al., 2001), which target the region between the 16S rRNA gene and the 23S rRNA gene. PCR products were resolved via denaturing capillary electrophoresis (ABI 3130xl Genetic Analyzer).

ARISA fingerprints were statistically analyzed by creating a matrix of fragment length versus normalized peak area (i.e., the peak area of an individual fragment divided by the sum of all peak areas within a fingerprint). Fragments less than 121 bp were excluded because this is less than the theoretical minimum for amplification with these primers. Relative peaks areas were then ranked and fragments beyond 80% of the cumulative proportion were excluded. Fingerprints were then statistically analyzed via non-metric multidimensional scaling using R ver. 2.6.1. Diversity values were computer using the Shannon-Weaver Index using the following equation:

\[
H' = -\sum_{i=1}^{S} p_i \ln p_i
\]

Where \( p_i \) is the relative abundance of each bacterial population in the ARISA profile, calculated as the proportion of area of an individual peak in the electropherogram to total peak area within the electropherogram and \( S \) is the total number of individuals within each electropherogram.

**Chemostats Experiments to Determine Bacterial Homeostasis**

Chemostat experiments were performed on individual bacterial strains that have been isolated from many of the lakes in the studies described above. These bacteria were grown under nutrient-limited conditions imposed by a chemostat while modulating the ratio of carbon-to-phosphorus, allowing us to observe the effects on strain homeostasis and elemental composition.

**Results**

A fundamental tenant of our research is that bacterial communities in lakes are affected by the elemental composition of the nutrient supply. That is, as the ratios of the elemental composition of the nutrients composition changes within a lake, bacterial populations will be selected due their niche differentiation as a function of nutrient stoichiometry. Furthermore, the growth rate hypothesis suggests that bacteria modulate their ribosome content (rich in
phosphorus), such that the carbon to phosphorus ratio would be the most critical parameter within lakes.

The first goal of our research, therefore, was to measure the elemental composition of the bacteria within numerous lake water samples to determine its variability (Fig. 1). These results support the growth rate hypothesis in that the carbon-to-nitrogen ratio is reasonably consistent, especially compared to the carbon-to-phosphorus (or nitrogen-to-phosphorus ratio) that is considerably more variable.

Previous research showed that *E. coli* was largely homeostatic (H’ >> 1) (Makino et al., 2003); that is, *E. coli* was able to modulate the elemental composition of its cell at a relatively constant value even while the elemental composition of the available substrates (particularly: carbon, nitrogen, and phosphorus) varied by several orders of magnitude.

The second goal of our research, therefore, was to determine if this result in *E. coli* was consistent among bacteria isolated from lake water (Fig. 1). We selected a group of bacteria representing relatively diverse phylogeny. Our results reveal that these organisms exhibit mixed degrees of homeostasis, depending on their taxa. That is, our strain from the *Gammaproteobacteria* (Fig. 1E; most closely related to *E. coli*) was the most homeostatic (H’ = 9.0) whereas some of our other strains were not as capable of modulating their nutrient composition independent of the nutrient supply (e.g., Fig. 1A, where H’ = 2.1).

A third goal of this research was to connect bacterial community diversity to primary productivity and nutrient composition with lakes. In the lakes in the Twin Cities, therefore, we compared bacterial community diversity among all of our samples with primary productivity measured as chlorophyll A (Fig. 3A). In this plot, there appears to be an asymptotic relationship, such that bacterial community diversity initially increases with primary productivity but then plateaus with further increases in chlorophyll A. Comparing the mean chlorophyll levels within each lake, however, suggests that there is a relationship between primary productivity and the bacterial community diversity.

We have also directly compared the ratio of carbon-to-phosphorus in the lakes in and near Itasca State Park with bacterial community diversity. The majority of these lakes were oligotrophic or mesotrophic, such that there seemingly no connection between primary productivity and bacterial community diversity (Fig. 4A). In contrast, bacterial community diversity exhibited a hump-shaped profile as a function carbon-to-phosphorus (Fig. 4B). This
latter result is consistent with our original hypothesis in that the elemental composition of the available nutrients in a lake should be a pertinent factor in bacterial community diversity.

**Summary of Findings**

The first outcome of our research was to demonstrate that the bacteria in lakes are substantially variable with respect to the ratio of their carbon-to-phosphorus. This result is critical, because our hypothesis is that individual strains are restricted with respect to their carbon-to-phosphorus; natural oscillations in carbon-to-phosphorus, therefore, would reflect adaptations within the structure of the bacterial community. The second outcome of research was to quantify the degree of homeostasis from eight different bacterial strains. These organisms all exhibited some degree of homeostasis, though less than previously reported for *E. coli*. Finally, we compared bacterial diversity in lakes of differing trophic status in the Twin Cities and lakes of similar trophic status in and near Itasca State Park. Bacterial community diversity in the Twin Cities was linked to primary productivity. Similarly, bacterial community diversity in Itasca State Park exhibited a hump-shaped profile as a function of the carbon-to-phosphorus ratio. In conclusion, our research supports our hypotheses that the principles of ecological stoichiometry can be used to better understand bacterial community diversity and nutrient biogeochemistry in Minnesota lakes.

**References**


Fig. 1. The ratio of the concentrations of key nutrients in Minnesota lakes.
Fig. 2. Ratio of the carbon-to-phosphorus in the biomass (ordinate) versus the carbon-to-phosphorus ratio in the substrate (abscissa) for eight different bacterial isolates.
Fig. 3. Bacterial community diversity as a function of primary productivity in eight lakes in the Twin Cities. (A) all samples. (B) Mean annual values.
Fig. 4. (A) Bacterial community diversity in 11 lakes in/near Itasca State Park as a function of: (A) primary productivity, and (B) the elemental ratio of carbon-to-phosphorus.
Publications, Presentations, or Published Abstracts:

Publications
None to date (several manuscripts are in preparation)

Presentations (* indicates student presentation)


Students and Post-doctoral Research Associates supported by this project:

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Post-doctoral research associate

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Department of Civil Engineering, University of Minnesota
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Andre M. Amado (Summer 2006-Spring 2007)
University of Rio de Janeiro
Graduate student who worked on this project during his ‘sandwich’ program.

Awards and Special Recognition:
None to date
Development of a DNA Marker Gene System to Determine Sources of Fecal E. coli in Watersheds

Basic Information

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Publication

Development of a DNA Marker Gene System to Determine Sources of Fecal *E. coli* in Watersheds

**Principal Investigator**  
Michael J. Sadowsky, Professor, Department of Soil, Water, and Climate, UMN

**Research Assistants**  
Matt Hamilton, Department of Microbiology, UMN  
Charlie Sawdey, Graduate Program in Water Resources Science, UMN

**Project Number:** 2006MN161B

**Start date:** 3/1/2006  
**End date:** 2/29/2008

**Project Report**

Many of Minnesota's rivers, lakes, and streams do not meet the Clean Water Acts “swimmable” goal due to elevated numbers of fecal coliform bacteria. Sources of fecal coliform bacteria include runoff from feedlots and manure-amended agricultural land, wildlife, inadequate septic systems, urban runoff, and discharge from sewage systems. Moreover, high levels of fecal bacteria in Minnesota’s watersheds threaten the use of these water resources for recreational use and drinking. In this study, we used pooled genomic tester and driver DNAs in suppression subtractive hybridizations to enrich for host source-specific DNA markers for *Escherichia coli* originating from cows, pigs, and humans. For human specific marker DNAs, three separate subtractive hybridizations were done using 5-60 human *E. coli* strains as tester DNAs and 20-50 *E. coli* from other animals as driver DNAs. This generated 576 potential marker genes specific for human *E. coli*. These 576 marker genes were screened by dot-blot Southern hybridization for reactivity to *E. coli* from humans, and 146 reacted with human *E. coli* control DNA. The 146 DNA fragments were also screened by restriction enzyme analysis and the majority were found to be clonal, 44 fragments were unique. The 44 probes were tested for specificity in hybridization reactions with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Results of these analyses indicated that 21 cloned DNA fragments showed some hybridization specificity to DNA from *E. coli* isolated from humans, whereas the
remaining 23 probes cross-reacted with *E. coli* from all the animal sources. While our best probes identified greater than 50% of the 210 human *E. coli* strains tested, they also cross hybridized to a significant numbers of non-human strains. Using the same overall strategy, but with different tester and driver strains, we were able to isolate potential DNA probes that detect *E. coli* strains originating from pigs/turkeys, and animals in general. One set of probes developed in this project identified ~65% of all of the tested pig *E. coli*. However, they cross-react with ~8% of *E. coli* from Turkeys.

Over the past decade, several microbial source tracking (MST) methods have been intensively investigated, leading to the development of a wide variety of potential methods. Most methods to date, however, have suffered from low discriminatory power. In contrast, several genotypic-based methods have been found to be highly efficient in discriminating amongst bacteria originating from different animal hosts. We have developed a genetic marker based detection system (using DNA probes) for host-specific traits that are ecologically meaningful with respect to the microorganism studied. We have been using a multi-strain, genomic comparison approach to identify DNA fragments unique to *E. coli* strains isolated from a particular type of host source. Using this approach we have successfully developed DNA probes specific for *E. coli* strains originating from Canadian geese and ducks.

In our current studies we focused our efforts on the development of marker probes for *E. coli* strains originating from cows, humans, and pigs. The prioritization of these three types of host sources was mainly due to their predominance as contributors to agricultural- and urban-derived fecal contamination in watersheds. To achieve our goals, we used the technique of subtraction suppressive hybridization (SSH) in an attempt to identify DNAs that are specific for *E. coli* originating from humans, cows and pigs.

We used a multi-strain genomic comparison approach for the identification of host-specific DNA markers. The suppression subtractive hybridization (SSH) technique was used to enrich for DNA fragments unique to *E. coli* from each type of host sources. The *E. coli* strains used in SSH and subsequent specificity analyses were obtained from a library of unique isolates previously isolated from the feces of 12 known animal host sources (cats, chickens, cows, deer, dogs, ducks, geese, goats, horses, pigs, sheep, and turkeys), and humans. Suppressive subtractive hybridizations were done using the
CLONTECH PCR-Select™ Bacterial Genome Subtraction kit (BD Biosciences CLONTECH, Mountain View, CA). In initially, three different subtraction hybridizations were done; Human subtraction 1 used 5 human *E. coli* strains as tester DNA, and 5 goose *E. coli* strains as driver DNAs. Following transformed of subtraction products, 192 clones were picked. These were screened by dot-blot hybridization and 11 probes were found to be tester specific (all 11 were confirmed as specific by Southern Hybridization). All 11 probes were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. However, none of the probes reacted with a large number of human strains and cross hybridization with strains from other hosts was pronounced. This suggested that a larger number of tester and driver DNAs were needed.

In subsequent analyses, 20 human *E. coli* strains were used as tester DNAs and 20 *E. coli* from animals (5 cows, 5 geese, 5 pigs, 1 chicken, 1 dog, 1 cat, 1 horse, and 1 sheep) were used as driver DNAs. Following transformed with the subtraction mixture, we picked 480 clones and screened 75 of these by dot blot Southern hybridization. All 75 clones had strong hybridization signal when probed with pooled DNAs from the tester strains (Figure 1) and only weakly hybridized when probed with driver strains. Sixty-four of these clones were confirmed as tester specific by Southern hybridization and restriction enzyme digestion analysis showed there were 41 unique probes. Of these 15 were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Nine probes were shown to react predominately with human strains, but only about 10% of the human strains reacted and the same human strains reacted with probes. One probe reacted with 26 of 210 human strains and only 2 chickens, 2 horse, and 1 sheep strain. No further colony hybridizations were attempted because the same human strains were identified with almost all 15 probes.

Consequently, we tried an additional subtraction using 60 human strains as tester DNAs and same 20 animal strains as driver DNAs as discussed above. Following transformation of the subtraction products, we picked 576 clones and screened these by dot blot Southern hybridization. Of these clones, 74 were selected as being tester specific
and 71 of the 74 were confirmed as being tester DNA specific by Southern hybridization. Twelve of these were tested by colony hybridization with same strains as discussed above, and all 12 tested hybridized with greater numbers of human strains than probes from the first or second human subtractions. Some of these 12 clones identified greater than 50% of the 210 human strains. Unfortunately, they also cross hybridized with significantly greater numbers of non-human strains, compared to probes from the first human subtraction (10-30% for several hosts). In one case, nearly 60% of cat strains cross reacted with the tested probes.

We also used the SSH technique in an attempt to isolate marker genes specific for cows. We used DNA from 20 E. coli cow strains as tester and 20 DNAs from non-cow strains as driver (E. coli from 5 humans, 5 pigs, 5 chickens, 2 horses, 2 sheep, and 1 goose). The cow, human, pig and chicken strains were selected by HFERP dendrogram analysis, and the horse, sheep and goose strains were selected randomly. Subtraction products were cloned and 576 clones were picked for the subtraction library. Of these, 288 clones were tested by dot blot hybridization for specificity for cow E. coli – 68 were found to be tester specific. Furthermore, 60 of 68 were also confirmed to be tester specific by southern blot hybridization analysis. Restriction enzyme analysis on the 60 confirmed cow specific clones showed that the clones contained 25 different insert DNAs. Colony hybridizations were done using 14 of the 25 different inserts against an array of E. coli from 13 animal hosts and humans. These arrays included E. coli strains from the following sources, with the number of strains in parentheses, cat (48), chicken (96), cow (189), deer (96), dog (96), duck (81), goose (135), goat (42), horse (78), human (210), pig (218), sheep (60), and turkey (96). However, none of the insert DNAs tested were specific and results showed that inserts cross hybridized with isolates from many different animal hosts. Inserts hybridized with considerable numbers (>15%) of isolates from source groups not represented or poorly represented in the driver sample. The remaining 11 inserts were not tested, since the isolated probes appeared not be specific for cows.

To potentially increase the specificity of the probes, we chose to modify the subtraction reaction – by both increasing the diversity of strains present in the driver sample and increasing the time of the primary hybridization. To do this, DNAs from 25
cow *E. coli* strains were used as tester and DNAs from 40 non-cow strains used as driver in SSH reactions. The driver sample consisted of 5 strains from each of the following source groups: chickens, goats, geese, horses, humans, pigs, sheep, and turkeys. All strains used in the subtraction were selected by HFERP DNA fingerprint dendrogram analysis. Subtraction products were cloned and 384 clones were initially picked for the subtraction library. We initially analyzed 192 clones by dot blot hybridization to tester and driver DNAs and 35 were found to be tester specific (for cows). Of these, 26 of the 35 were confirmed as tester specific by southern hybridization analysis. Gel electrophoresis analysis of cloned DNA fragments suggested that 10 of the 26 clones contained the same DNA insert. In total, it appears that there are 12 different fragments. It is possible that these inserts have different sequences and nearly the same size. Further analysis through restriction analysis and/or hybridization will be necessary to determine the exact number of different inserts. This is currently ongoing. We also tested 6 cloned insert DNAs by hybridization to a panel of *E. coli* strains from 12 animal hosts and humans. Results of these analyses indicated that 5 of the inserts had a significant percentage of cross-hybridization with non-cow strains, approx 15-25% of all isolates (including cow isolates) hybridize with these inserts. However, 1 insert hybridized only with cow strains, although it only recognized 11 of 189 strains tested. This insert was sequenced and found to be nearly identical to the colicin-N gene of *E. coli*

We also performed SSH reactions to isolate DNA clones specific for pigs. The DNAs from 21 *E. coli* strains from pigs were chosen as tester and 30 non-pig strains (10 cows, 10 humans, 5 chickens, 1 dog, 1 cat, 1 goat, 1 goose, 1 turkey) were chosen as driver DNAs. All strains used in the subtraction were selected by HFERP DNA fingerprint dendrogram analysis. Subtraction products were cloned and 576 clones were picked for the subtraction library. All 576 clones were tested by dot blot hybridization and 50 were found to be tester (Pig) specific. Of these, 12 of the 50 clones were confirmed as tester specific by Southern blot hybridization to genomic DNAs. Seven of the cloned insert DNAs were tested by colony hybridization. However, none of the inserts were specific to pig isolates, although one insert hybridized predominately with pigs (~40%) and turkeys (~30%). Isolates from other host species cross-hybridized with the probes at <15%, suggesting that this insert may be useful to identify Pig
contamination in waterways not impacted by turkeys. The remaining 11 inserts were not tested, instead we chose to modify the subtraction as described above, by both increasing the diversity of strains present in the driver sample and increasing the time of the primary hybridization. To do this, DNAs from 21 pig strains were chosen as the tester and DNAs from 40 non-pig strains (chickens, cows, goats, geese, horses, humans, sheep, and turkeys) were used as driver DNAs. All strains used in the subtraction were selected by HFERP dendrogram analysis. Subtraction products were cloned and 192 clones were picked for the subtraction library. All 192 clones were screened by dot-blot hybridization to the tester and driver DNAs, and 22 were found to hybridize specifically to the tester (pig) DNAs. Southern blot analysis indicated that 10 of 22 clones were pig specific.

Colony hybridizations to all *E. coli* strains in our library indicated that 8 hybridization probes had the ability to detect pig *E. coli*, together they detect ~65% of the tested pig *E. coli*. However, they cross-react with ~8% of *E. coli* from Turkeys. The basis for the cross-reaction is not known. One of the isolated probes proved to be very interesting, it reacted with Deer, Pigs, Sheep and Goat *E. coli*, but with very few (~1%) of human isolates. This may be useful as a more general animal probe. Moreover, one of the probes reacted with the following percentage of tested isolates as follows: Humans - 1.4%, Horses - 16%, Goats - 2.4%, Sheep - 8.2%, Pigs - 10%, and turkeys 0.08%. Since this probe reacted mostly with horses, this probe may also have use for a general probe for non-human related contamination.
Publications, Presentations, or Published Abstracts:

Publications

Presentations (* indicates student presentation)


Sadowsky, M.J. 2006. Alternate source and sinks of pathogens in the environment. Oral presentation at the Annual Meetings of the American Society of Agronomy (ASA), Crop Science Society of America (CSSA), and Soil Science Society of America (SSSA), November 12 – 16, Indianapolis, IN.


Students and Post-doctoral Research Associates supported by this project:
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Master of Science

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Master of Science
Additional Funding:
Funding from this current project was used to leverage additional support from the Minnesota Department of Agriculture (MNDAG) for a project entitled: Development of a DNA Marker Gene System for *E. coli* from Cows, Pigs, and Turkeys and Using Small Watersheds to Monitor Bacteria Loadings and Effects of Mitigation Practices. This project was awarded to James Anderson (PI) and Michael Sadowsky (co-PI) for the period March 15, 2007 to June 30, 2009. The MNDAG project is currently supporting the salaries of two graduate students (Charlie Sawdey and Daniel Norat), who continue to work on this project.

Awards and Special Recognition:
None to date
Application of Wireless and Sensor Technologies for Urban Water Quality Management

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Publication
Application of wireless and sensor technologies for urban water quality management

Principal Investigators
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Christine Wennen, Graduate Program in Water Resources Science, UMN

Project Number: 2006MN187G

Start Date: 9/01/2006
End Date: 8/31/2009

Abstract

The water quality of streams draining watersheds has been degraded by increasing urbanization. The general symptoms of this degradation include more frequent large flow events, reduction in channel complexity, reduced retention of natural organic matter, and elevated concentrations of nutrients. Newly emerging urban water quality threats, including insecticides, herbicides, pharmaceuticals, and estrogens, are known or suspected to damage the health of humans and ecosystems. The restoration and management of streams have traditionally attempted to improve the hydrological and water quality conditions in-stream or in riparian zones. Recent studies have indicated the portion of a watershed covered by impervious surfaces and connected to the stream by stormwater drainage is the primary degrading process of stream ecology and health. These findings suggest that the sustainable restoration and management of stream water quality require quantification of hydrological, chemical, biological, and geomorphological processes, and that these processes must be assessed across a range of scales. Furthermore, interactions among biogeochemical processes across watersheds are either non-linear processes or linear processes dependent on non-linear drivers. The monitoring of such a system inherently requires a change in traditional field sampling strategies. We propose to transform traditional and very limited (in terms of spatial and temporal resolution) field
measurements through the integration of multi-scale, spatially-dense, high frequency, real-time, and event-driven observations by a wireless network with embedded networked sensing.

The goals of the proposed research are to assess the benefits of stormwater best management practices in mitigating the pollutant loads from urban and peri-urban sources, to evaluate the effectiveness of traditional grab sampling in calculating pollutant loads, and to develop correlations to predict the concentrations of non-sensed chemical or biological pollutants. These goals will be achieved by establishing a wireless sensor network capable of monitoring fundamental water quality parameters at high spatial and temporal resolution. It is hypothesized that sensed fundamental water quality parameters can be used for predicting the presence of emerging chemical contaminants in urban streams. It is also hypothesized that the water quality in streams draining similar impervious urban areas is controlled by the mean and variance of effective stormwater residence time. The mean and variance of water residence time, the time it takes urban runoff to travel between the impervious urban land and a receiving aquatic body, will be characterized by radio frequency identification technology (RFID), which will augment the proposed wireless network. Ultimately, data generated from such a monitoring network will enable mechanistically-based scaling and forecasting of water quality in urban streams and rivers. This will transform urban planning practices and management of water quality in streams draining urban land.

Progress

In 2007, the wireless sensor network for water quality monitoring was expanded to include five individual stations, each equipped with a datalogger and radio antenna. In addition to the two existing sensor bundles, three HydroLab sondes (Hach Environmental), two grab samplers (ISCO), and two MicroLab nutrient analyzers (EnviroTech LLC) were added to the network. The network is now capable of measuring temperature, pH, conductivity, dissolved oxygen, turbidity, depth, and nitrate on a continuous basis. Computer code was developed to run the equipment and record data automatically. A wireless cell modem was used to upload the data nightly to a computer located at St. Anthony Falls Laboratory. Remote communications were enabled, which allowed the user to make changes to the sensor network from the laboratory.
Unfortunately, the spring, summer, and fall in 2007 were unusually dry in the Twin Cities area. Thus, with essentially no storm events to monitor, we were unable to deploy along our targeted stream (Shingle Creek). This allowed us extra time, however, to pinpoint our site for BMP testing and monitoring by coordinating with USGS and the consultants responsible for the Single Creek watershed. For the 2008 field season, we have selected to monitor at a golf course in Brooklyn Center, MN. The ponds on the golf course collect stormwater from the surrounding neighborhood and mall parking lots and direct it into Shingle Creek. This site is just upstream from the USGS gauging station for Shingle Creek.

From Nov. 2007-March 2008 various quality assurance exercises were performed in controlled environments to quantify sensor reliability and errors. Within a 20 meter flume, two instrument layouts were studied; first all five instruments were grouped together and second each instrument was longitudinally spaced within the flume. Each set up was subjected to pulse and step inputs of turbidity and conductivity and the observed results were analyzed. Based on flow characteristics of the flume and the implemented inputs, errors associated with each measurement were subsequently quantified. The MicroLab analyzers were also put though a series of calibration exercises and tests.

In the spring, the network was deployed in Shingle Creek to allow continuous monitoring of water quality and BMP performance. Grab samples for the target pesticides, fecal coliforms, and chloride will be taken, as well as samples for sensed variables for verification. These data will be used to determine relationships between sensed parameters and those measured via grab samples. Efforts will be coordinated with activities of the USGS and the local watershed district.
Publications, Presentations, or Published Abstracts:

Publications

Presentations (* indicates student presentation)


Students and Post-doctoral Research Associates supported by this project:
Jeremiah Jazdzewski (awarded in 2007)
Department of Civil Engineering, University of Minnesota
Master of Science

Michael Henjum, (expected 2009)
Department of Civil Engineering, University of Minnesota
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Christine Wennen (expected 2009)
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Master of Science

Additional Funding:
Funding has been obtained for related or complimentary projects from the National Science
Foundation/Consortium of University for the Advancement of Hydrologic Sciences Inc. (as part
of the test-bedding activities for the WATERS Network; www.watersnet.org) and from the
Mississippi Water Management Organization/Minnehaha Creek Watershed District.

Awards and Special Recognition:
None to date.
Triclosan and triclosan–derived dioxins in the Mississippi River sediment record

Basic Information

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<td>Kristopher McNeill, William Alan Arnold</td>
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Publication

Triclosan and triclosan-derived dioxins in the Mississippi River sediment record

Principal Investigators:
Kristopher McNeill, Associate Professor, Department of Chemistry, University of Minnesota;
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Research Assistants
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Matthew L. Grandbois, Department of Chemistry, UMN
Peter O. Steen, Water Resources Science, UMN

Project Number: 2007MN203B

Start Date: 3/1/07
End Date: 2/29/09
Report Duration: 3/1/07-6/30/08

Project Summary

This project is focused on establishing whether triclosan has been and continues to be a source of dioxins to the aquatic environment. It is hypothesized that triclosan, a widely used antimicrobial found in consumer products, is transformed into toxic dioxin compounds through chlorination of triclosan-containing wastewater and sunlight exposure in rivers that receive chlorinated wastewater. It is further hypothesized that triclosan and its products will associate with the sediment downstream of point of discharge and their release to the environment thus will be recorded in the sediment record. To determine the historical inputs of triclosan and its products in to the Upper Mississippi river from Minnesota’s largest wastewater treatment plant, the Metro Plant, in St. Paul, sediment cores from Lake Pepin will be analyzed. It is expected that triclosan and its products will not be found in pre-1960 sediment, it will be at low levels between 1960 and 1990 when its use was limited, and will be at the highest levels after 1990 following its widespread use in liquid handsoap and toothpaste. The results of this study will further our understanding of micropollutants in wastewater and will provide specific information about the appropriateness of chlorination disinfection for triclosan-containing waters.
Progress Report

Methods

Triclosan analytical method development

A liquid chromatography (LC) method with quadrupole time-of-flight mass spectrometry (Qq-TOF-MS) detection was developed to determine triclosan and its chlorinated derivatives in extracts of sediment and wastewater samples. An 1100 Series Agilent HPLC with a Bruker microTOF-Q detector was used for this analysis. Electrospray ionization (ESI) was carried out in negative mode. Mass calibration was performed using sodium formate, and extracted ion chromatograms were generated with a ± 0.005 amu mass tolerance for each analyte. Wastewater samples were collected in pre-washed glass bottles, filtered through 0.2 µm filters to remove particulate matter, adjusted to pH 2 for preservation, and stored at 4 °C in the dark until analysis. 250 mL wastewater samples were solid-phase extracted using Oasis HLB cartridges followed by a washing step with 50:50 water:methanol to remove interfering dissolved organic matter (DOM). The cartridges were eluted with acetonitrile. The acetonitrile extracts were concentrated under nitrogen to a minimal volume (~ 200 µL) and analyzed by LC-Qq-TOF-MS.

Synthesis of chlorinated derivatives of triclosan

The synthetic approach was inspired by the route taken by Marsh et al. (1) Starting with either commercially available or readily synthesized chlorinated phenols, ortho-directed formylation followed by phase-transfer catalyzed methylation of the aromatic hydroxyl group yielded 2-methoxy-chlorinated benzaldehydes. Baeyer-Villager oxidation of the resulting aldehydes yielded the respective phenols. The chlorinated triclosan precursors underwent basic coupling with 2,2’,4,4’-tetrachlorodiphenyliodonium iodide to form the diphenylether backbone, which was then deprotected to yield the target triclosan derivatives. The synthetic scheme for the chlorinated triclosans is shown in Figure 1.

Triclosan and chlorinated triclosan photochemistry

Aqueous solutions of triclosan and chlorinated triclosans were photolyzed using a Suntest CPS+ solar simulator equipped with a Xe-arc lamp and a UV special glass filter which mimics...
the solar spectrum. Photolysis experiments were also performed under natural sunlight on clear
days in late spring at 45° N latitude. Samples were photolyzed in quartz test tubes (OD = 13
mm, ID = 11 mm, V = 10 mL). In kinetic experiments, sub-samples were periodically taken and
analyte concentrations were determined by HPLC analysis using an 1100 Series Hewlett Packard
HPLC with UV-absorbance detection. Quantum yields of photodegradation were determined
using a p-nitroanisole/pyridine actinometer system. Because the photolysis of the substrates was
expected to be highly pH dependent based on the protonation state of the phenolic moiety,
quantum yields were measured at acidic pH, where each analyte is fully protonated, as well as
basic pH, where each analyte is fully deprotonated. Acidic photolyses were carried out in a pH 4
solution of 80:20 acetate buffer:methanol, while basic photolyses were run in pH 10 borate
buffer.

Product Identification

Various detection methods were used to elucidate the identity of the photochemically
produced products. Concentrated photolysis solutions were directly injected onto a Agilent 1100
Series HPLC equipped with photodiode array (PDA) detection and coupled with a Bruker Micro-
TOF Q mass spectrometer utilizing atmospheric pressure chemical ionization (APCI) in negative
mode. A Hewlett Packard (HP) 5890 Series GC system equipped with a HP 5972 series mass
spectrometer was also used for product identification. Standard material was purchased as
available to confirm the identification of photoproducts.

Results to date

Triclosan analytical method development

A liquid chromatographic method has been developed to effectively separate the triclosan
and its chlorinated derivatives, including two mono-chlorinated isomers. The injection volume
and mass spectrometer parameters have been optimized to maximize sensitivity while
maintaining satisfactory chromatographic resolution. A method detection limit on the order of 1
ng/L has been realized. The recovery of the solid-phase extraction pre-concentration step was
maximized by optimizing the cartridge type, sample pH, washing step, and elution volume. The
Oasis HLB cartridge was found to provide the greatest recovery. The amount of interfering
dissolved organic matter (DOM) was reduced by increasing the pH of the sample. However, to keep the analytes fully protonated to aid in their extraction efficiency, a pH of 4 was chosen to extract the samples. Washing the cartridge with 3-5 mL aliquots of 50:50 methanol:water solution prior to elution was found reduce the amount of interfering DOM in the extract by approximately an order of magnitude. Elution with 2-6.5 mL aliquots of acetonitrile provided maximum recovery. Greater than 75% recovery was obtained for every analyte. Wastewater samples have been collected quarterly from the Metropolitan Plant in St. Paul, MN for 1.5 years both prior to chlorination and after the chlorination step to determine the extent of formation of chlorinated triclosan derivatives during chlorine disinfection.

Synthesis of chlorinated derivatives of triclosan and HO-BDE-47

The synthesis of 5,6-dichloro-2-(2,4-dichlorophenoxy)phenol was completed in 5 steps to yield 1.670 g as a white solid. The overall synthetic yield for this derivative was 26%. The synthesis of 4,5-dichloro-2-(2,4-dichlorophenoxy)phenol did not follow the general scheme presented above, due to difficulty purifying several intermediates. An alternative approach utilizing regioselective chlorination of guaiacol followed by basic coupling/deprotection mentioned above yielded 1.051 g as a white solid over 3 steps. The overall synthetic yield for this derivative was 18%. The synthesis of 4,5,6-trichloro-2-(2,4-dichlorophenoxy)phenol required an additional synthetic step not mentioned in the above scheme. 2,3,4-Trichloroaniline was diazotized and then subjected to water to synthesize the corresponding phenol. This was then subjected to the above mentioned synthetic scheme to yield 0.107 g as a white solid over 6 steps. The overall synthetic yield for this derivative was 5%.

All synthesized compounds have been characterized and found to match by nuclear magnetic resonance (\(^1\)H and \(^{13}\)C), mass spectrometry, and melting point when literature values have already been reported.

Triclosan and chlorinated triclosan photochemistry

The solar quantum yield of degradation under basic conditions reflected a strong dependence on the chlorine substitution pattern of the analyte. Specifically, the quantum yield was significantly depressed with chlorine substitution in the 2-position on the phenol ring. While triclosan (TCS) and 4-Cl-TCS exhibited relatively high quantum yields (0.39 and 0.29, respectively), the
quantum yields of 2-Cl-TCS and 2,4-Cl-TCS were much lower (0.06 and 0.07, respectively). As expected, the rate of photodegradation for all four analytes was substantially higher under basic compared to acidic conditions. Triclosan and its three chlorinated derivatives all degraded at least two orders of magnitude faster at pH 10 in their deprotonated state than at pH 4 in the protonated form under natural sunlight. Thus, the environmental photochemical fate of triclosan will be highly dependent on the pH of the natural water body. Identification of the photoproducts is ongoing.

Sediment coring and analysis
Sediment cores will be collected in June 2008. The cores will be dated via magnetic profiling and then sectioned. The sections will be extracted and analyzed for triclosan, chlorinated triclosan derivatives, and the photochemically produced dioxin products.

Reference

Figures:

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\( X = \text{H, Cl} \)

**Figure 1. Synthesis of Chlorinated Triclosan Derivatives.** Reaction conditions: a) MgCl\(_2\), paraformaldehyde, TEA, CH\(_3\)CN; b) (C\(_4\)H\(_9\))\(_4\)NOH, NaOH, Mel, CH\(_2\)Cl\(_2\), H\(_2\)O; c) 1. H\(_2\)O\(_2\), (CF\(_3\))\(_2\)O, KH\(_2\)PO\(_4\), CH\(_2\)Cl\(_2\), 2. MeOH, HCl; d) K\(_2\)CO\(_3\), 18-crown-6, 2,2',4,4'-tetrachlorodiphenyliodonium iodide, DMAC; e) BBr\(_3\), CH\(_2\)Cl\(_2\).
Publications, Presentations, or Published Abstracts:

Book Chapters

Invited Presentations

Conference Presentations

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Doctoral Student

Peter O. Steen
Graduate Program in Water Resources Science, University of Minnesota
Master of Science and Doctoral Student

Matthew L. Grandbois
Department of Chemistry, University of Minnesota
Doctoral Student

Additional Funding:
This project is complemented by a project from the National Science Foundation (2006-2009) to study the photolysis of triclosan and polybrominated diphenyl ethers in both the laboratory and in the field.

Awards and Special Recognition:
Jeff Buth, 2006 EPA STAR Fellowship
Jeff Buth, 2008 ACS Graduate Student Award in Environmental Chemistry
The Role of Local Stakeholders in Water Resource Management: Characterization and Diffusion of Minnesota Lake Improvement Districts

Basic Information

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Publication
The Role of Local Stakeholders in Water Resource Management: Characterization and Diffusion of Minnesota Lake Improvement Districts

Principal Investigators
Dennis R. Becker, Assistant Professor, Department of Forest Resources, UMN

Research Assistant
Kaitlin Steiger-Meister, Department of Natural Resource Science and Management, UMN

Project Number: 2007MN204B

Start Date: 6/01/2007
End Date: 5/30/2009

Progress Report

The research investigates the development of policy tools to enable and coordinate water quality management actions at the community level. It assesses the effectiveness of existing Minnesota programs empowering citizens to affect water quality solutions. In particular, the research will assess use and diffusion of Lake Improvement Districts (LIDs), where local units of government organize to enhance water quality by securing grants and taxing landowners to support mitigation activities within a lake district.

In order to characterize existing LIDs including funds secured, staff resources, partnerships formed, and accomplishments relative to state priorities, all 24 LIDs in the state were contacted, and of those in-depth semi-structured interviews were conducted with representatives from 14 LIDs during the summer and fall of 2007. Interviews are in the process of being transcribed, which will then be coded to identify barriers to the diffusion of the LID program.

Researchers also met with the LID coordinator from Minnesota’s Department of Natural Resources to learn about the agency’s role in the program and collect education and outreach materials focused on LIDs. The research assistant is currently in the process of interviewing county water planners, select a subset of applicable case study locations for further analysis, and conducting a legal analysis of the LID legislation. The research assistant also attended the annual meetings of three LIDs and conducted participant observation for how the meetings operated. Follow-up thank you letters were sent to all involved stakeholders in the winter 2007.
Currently, the research assistant is in the process of carrying out a comparative study between Minnesota’s LIDs and Wisconsin’s Lake Districts. She is examining the institutional arrangements surrounding Lake Districts, as well how they operate at the local level to accomplish water quality objectives. The research assistant will complete her dissertation oral exams this summer culminating in her dissertation research on this topic.

Publications, Presentations, or Published Abstracts:

**Presentation**

Students and Post-doctoral Research Associates supported by this project:
Kaitlin Steiger-Meister
Department of Natural Resource Science and Management, University of Minnesota
Doctoral Student

Additional Funding:

Upon recommendation from the review panel for this project, the study was expanded to include a comparative analysis of Minnesota’s Lake Improvement Districts with Wisconsin’s Lake Districts. Steiger-Meister successfully secured competitive funding in the amount of $5,654 through the 2008 University of Minnesota Consortium on Law and Values in Health, Environment & the Life Sciences (project title: *Building clean water communities: Understanding how environmental policies can promote and coordinate community participation in the long-term management of local freshwater resources*). The research will supplement the project by examining Wisconsin Lake Districts and the Wisconsin Lakes Partnership.

Additional funding in the amount of $100 was provided by the International Association for Society and Resource Management for Steiger-Meister to present a paper on the project at the 14th International Symposium on Society and Resource Management.

Awards and Special Recognition:
None to date.
The Influence of Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems

Basic Information

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Publication
The Influence of Drainage on Biogeochemical Cycling of Carbon in Agricultural Ecosystems

Principal Investigators:
Jennifer King, Assistant Professor, Department of Soil, Water, and Climate, UMN
David Mulla, Professor, Department of Soil, Water, and Climate, UMN
Jacques Finlay, Assistant Professor, Department of Ecology, Evolution, and Behavior, UMN
Gary Sands, Associate Professor, Department of Bioproducts and Biosystems Engineering, UMN

Research Associate:
Brent Dalzell, Department of Soil, Water, and Climate, UMN

Project Number: 2007MN205B
Start Date: 3/1/2007
End Date: 2/29/2009

Abstract

A broad suite of global climate models predict that precipitation and subsequent surface runoff will increase in many areas of the Midwestern United States. Current trends in increasing precipitation and predicted climatic influences are superimposed over a suite of agricultural land management practices that also contribute to increasing water yield from landscapes to streams and rivers. In this context, a field-scale study is being conducted to investigate dissolved carbon export from agroecosystems in response to changing hydrology. A series of field-scale plots located at the University of Minnesota Southern Research and Outreach Center (located in Waseca, MN) have been modified with subsurface tile drainage treatments of varying intensity.

Preliminary data do not show a positive correlation between flow of drainage water and DOC concentrations (contrary to watershed-scale results from other studies); although these data do show increases in concentration during low-flow periods immediately following flow events. In general, plots with more intense drainage exhibit higher DOC concentrations and greater annual water yields than plots with standard drainage, resulting in greater annual DOC export from plots with more intense drainage. Based on 8 years of flow data, annual DOC export from field scale plots ranged from approximately 1 to 5 kg ha-1 yr-1. Plots with more intense drainage treatments export from 60 to 100% more DOC annually than plots with standard drainage at this site in south-central Minnesota. These differences are primarily due to drainage influences on
water yield with secondary influences on DOC concentrations. Monitoring of dissolved inorganic carbon (DIC) reveals that plots with more intense drainage treatments have greater DIC concentrations than plots with conventional drainage. This difference is maintained throughout the year despite overall variations in DIC concentrations. These results provide insight into how altered hydrology in agricultural landscapes may be influencing weathering of soil carbonates and help to explain broader long-term trends that have been identified in larger river basins.

Taken together, preliminary results from this study demonstrate that drainage practices in agricultural landscapes are capable of producing changes in biogeochemical cycling and export of both organic and inorganic dissolved carbon. These changes appear to be the result of drainage influences on water yield as well as actual C export from the fields. In addition to an additional year of monitoring DOC and DIC concentrations from these plots, ongoing work includes characterizing the molecular weight distributions and bioavailability of dissolved organic matter exported from these agricultural fields.

**Progress Report – Preliminary Findings and Ongoing Work**

**Introduction and Overview**

Carbon cycling in agricultural ecosystems has received much attention in recent years due to the loss of carbon (C) that resulted from land use conversion from native vegetation to agriculture. This deficit in soil carbon pools is widely viewed as the potential sink for C sequestration following adoption of alternative agricultural management practices such as reduced tillage in row-cropping systems common in the Midwestern United States (Lal et al., 1999; see however, Baker et al., 2007). Studies that focus on C cycling in agricultural ecosystems are often centered on measurements of changes in soil carbon pools as well as CO2 fluxes from the landscape (Reicosky et al., 2002).

One aspect of C cycling that has not received much attention, however, is the export of dissolved carbon from agricultural landscapes (in the forms of dissolved organic carbon and dissolved inorganic carbon, DOC and DIC, respectively). The goal of this study is to quantify and characterize dissolved C export from experimental field plots in an effort to develop a more complete understanding of all aspects of C cycling in agricultural ecosystems. More specifically, this research focuses on fields that have been modified with subsurface tile drainage systems – a
common practice in row-crop producing regions of the Midwest. To address the issues of subsurface drainage systems on dissolved carbon cycling, we are quantifying and characterizing dissolved carbon export from experimental drainage plots located at the University of Minnesota Southern Research and Outreach Center (SROC) located at Waseca, MN. This is a 2-year project that extends from spring 2007 through early 2009. A summary of research efforts and presentation of preliminary results is provided below.

**Summary of work completed for the reporting period from March 1, 2007 through February 29, 2008.**

*Field Sampling* - Flow monitoring from the subsurface tile drainage plots occurred nearly continuously throughout the growing season (subsurface drainage doesn’t flow in the winter when soils are frozen). Exceptions occurred when flow monitoring equipment (tipping bucket flow meters) was being updated with newer acoustic Doppler flow velocimeters (this disruption occurred during a period of low/no flow, however). In addition to flow monitoring from the field plots, water samples were collected at roughly weekly intervals (when flow was present) from April through November 2007. In all, 218 water samples were collected from the 12 research plots and one wetland. These samples represent a range of flow conditions representing: (1) early season drainage from snow melt and spring rains, (2) baseflow, primarily during early summer, during which plant evapotranspiration was high and rainfall inputs were low, and (3) late summer storm flow initiated by a large rainfall event and followed by several smaller events (Fig 1). All samples were analyzed for concentrations of DOC and DIC on a Shimadzu TOC Vcpn carbon analyzer and related to corresponding flow data in order to determine annual dissolved carbon flux from the study sites.

**Preliminary Results**

*Flow* - The field plots at the U of MN SROC were designed to accommodate drainage rates of 13mm day-1 (standard drainage practice common in this region) or 51mm day-1 (more intense drainage). As has been reported previously (Sands et al., 2006), the more intense drainage treatments produced a greater annual water yield. For 2007, the average water yield from plots with more intense drainage treatments was 44% greater than from plots with standard drainage;
the majority of this difference occurred as a result of multiple large flow events following intense late-summer rainfall (Fig. 2.)

*Dissolved Organic Carbon* – While there was considerable variability in DOC concentrations among treatments, the plots with intense drainage had, on average, greater DOC concentrations than the plots with standard drainage. Differences in DOC concentrations between treatments occurred primarily during low flow conditions and these differences became negligible during periods of high flow (Figs. 3 and 4). We speculate that these differences between treatments during low flow conditions may be due to longer flow paths (and longer water travel times) in plots with standard drainage; allowing more opportunities for DOC removal from soil pore water via either microbial degradation and/or physical removal through sorption to deeper mineral soils. Ongoing experiments are planned to determine if biodegradation rates occur on time scales conducive to this explanation.

Dissolved organic carbon concentrations from all field plots decreased with increasing discharge (Fig. 4). This relationship suggests a relatively constant source of DOC that becomes diluted with increasing flow. This result is somewhat surprising when viewed in light of watershed-scale results that show a positive correlation between stream flow and DOC concentrations in agricultural watersheds (Dalzell et al., 2007; Royer and David, 2005). This suggests that fundamentally different processes may be acting to influence DOC export at the field vs. watershed scale. In order to determine if the positive correlation described in other agricultural watersheds exists in this area, 2008 field sampling will be augmented with additional samples of the ditches and streams occurring downstream of the SROC plots.

Based on average DOC concentrations from 2007 field work and annual water yields, total annual DOC yields from the field plots at the SROC ranged from approximately 1 to 5 kg ha⁻¹ yr⁻¹ (Fig. 5). These values are near the low end of the range of annual DOC yields that would be expected based on the situation of the study area near the interface of grassland and deciduous forest ecosystems (Aitkenhead and McDowell, 2000). It is important to note that plots with more intense drainage treatments export from 60 to 100% more DOC annually than plots with standard drainage. If this observation is applicable at the scale of larger watersheds, then these results indicate that annual DOC export from agricultural watersheds in the Midwestern United States (where subsurface drainage is common) is influenced by varying land management
practices. This is an important consideration for future work that considers biogeochemical carbon cycling in waters draining landscapes that have been influenced by anthropogenic activities.

**Dissolved Inorganic Carbon** – Measurements of dissolved inorganic carbon from the study plots showed that DIC concentrations were consistently greater in plots with the intense drainage treatments (Fig. 6). This is an interesting result when viewed in light of recent research on long-term trends in alkalinity in the Mississippi River basin (Raymond and Cole, 2003; Raymond et al., 2008). In particular, the authors in those studies showed a positive correlation between the amount of agricultural land used and the amount of DIC export. Currently, the mechanism by which alkalinity export is increasing from agricultural watersheds is unknown although Raymond and Cole (2003) and Raymond et al. (2008) speculate that it may be related to perturbations in the hydrologic cycle (including drainage) or mechanical soil disturbance. Results from this study are important in that they show that drainage practices do have a significant impact on DIC export and annual fluxes from more intensely-drained plots have greater DIC yields through both (1) increased concentrations and (2) increased total water yield.

**Ongoing field work and lab experiments planned for the remainder of the project.**

2008 Field Sampling – For the 2008 sampling season, flow measurements and water sampling began in April. Sampling included event-related samples collected by autosampler during periods of increased discharge. As of the preparation of this report, approximately 180 samples have been collected for DOC and DIC analyses. Final estimates of DOC and DIC export from the SROC plots will be based on data collected over the 2007 and 2008 growing season. These data will be used to determine broader estimates of annual C export from Midwestern agricultural landscapes.

Molecular Weight Determination - In addition to quantifying DOC and DIC from these plots, ongoing sample collection is occurring for molecular weight distribution analysis via high performance size exclusion chromatography (HP-SEC). Samples for molecular weight analysis have been collected from SROC field plots and a nearby wetland as well as sites downstream from SROC on agricultural ditches and the LeSueur River. Additional sampling has been
conducted with finer time resolution over the course of a single flow event from one field plot. The calibration curve for molecular weight determination has been developed from a suite of polystyrene sulfonate standards and samples are currently being processed to isolate dissolved organic matter and prepare them for HP-SEC analysis.

**Biodegradability of DOC** – A series of laboratory experiments will be conducted to determine the bioavailability of DOC exported from field plots and compare it against DOC collected from other local sources. For these experiments, bioavailability will be determined through measurements of DOC loss and O2 consumption. These experiments are planned to begin in summer 2008.

**References**


Figures

**Figure 1.** Summary of tile drainage and sampling dates for 2007. Precipitation events are shown along the top of the figure. Drainage is shown for one plot from the study area for illustration purposes. The amount of flow varied with drainage intensity; however, the overall shape of the hydrograph is similar between treatments.
Figure 2. Cumulative water yield from field plots with differing drainage treatment. Water yields were determined by calculating area-normalized discharge for each plot and then determining average daily discharge for plots grouped by drainage intensity (conventional drainage vs. intense drainage).
Figure 3. Dissolved Organic Carbon concentrations measured from experimental drainage plots over the course of 2007. Error bars represent variability amongst different plots with the same drainage treatment. Daily average flow data from one selected plot is shown to indicate general hydrologic conditions over the course of the growing season.
Figure 4. The influence of mean daily discharge on DOC concentrations from experimental drainage plots. The data show a relationship of decreasing concentrations during periods of high discharge. Plots with more intense drainage treatments generally produced the greatest discharge during high flow events as well as the greatest concentrations during low flow events.
Figure 5. Estimated annual DOC export from different drainage treatments over the past 8 years. Annual estimates are based on average DOC concentrations from 2007 and flow data collected over the period from 2001 to 2007.
Figure 6. Dissolved Inorganic Carbon concentrations from field plots over the course of 2007. Plots with more intense drainage treatments had greater DIC concentrations than plots with standard drainage. Daily average flow data from one selected plot is shown to indicate general hydrologic conditions over the course of the growing season.
Publications, Presentations, or Published Abstracts:

**Presentation**

**Students and Post-doctoral Research Associates supported by this project:**
Brent Dalzell
Department of Soil, Water, and Climate
Post-doctoral research associate

**Awards and Special Recognition:**
None to date
Enhanced Contaminant Remediation: Fermentation as a Method to Enhance Dissolution of Hydrophobic Compounds

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Publication
Enhanced Contaminant Remediation: Fermentation as a Method to Enhance Dissolution of Hydrophobic Compounds

Principal Investigator
Paige Novak, Associate Professor, Department of Civil Engineering, UMN

Research Assistants
Denice Nelson, Department of Civil Engineering, UMN

Project Number: 2007MN215B

Start date: 3/1/2007
End date: 2/29/2008

Executive Summary

The mechanisms of enhanced mass transfer stemming from fermentation of carbon (molasses) were identified through this research. Several partitioning experiments were performed including the use of non-aqueous phase liquid (NAPL) and soil aged with trichloroethene (TCE) to evaluate how both fresh and fermented molasses affect the partitioning of TCE between NAPL, sorbed and aqueous mass. These experiments indicated a >200% increase in TCE solubility could be achieved through addition of fresh molasses solution. Increased solubility leads to increased mass flux of TCE NAPL to aqueous phase liquid. Fermented fluid did not increase solubility of TCE but did interact with the NAPL to form an emulsion. Increased surface area also leads to increased mass transfer from NAPL to aqueous phases. Accordingly, increased mass transfer from NAPL was caused by both fresh and fermented molasses solution, through two separate mechanisms. In addition, soil partitioning appeared to be affected by fermented fluid indicating fermented molasses could also enhance transfer of sorbed mass to the aqueous phase.

Introduction

Recently researchers have been working to develop innovative technologies to address both sorbed phase contaminants and non-aqueous phase liquid (NAPL) source areas in situ. Among these technologies are Surfactant-Enhanced Aquifer Remediation and cosolvent flooding. Both of these technologies involve the injection of chemicals into
an aquifer in a manner designed to “flood” the impacted zone, thereby mobilizing sorbed contaminant mass and NAPL (ITRC, 2002). The mobilized contaminant is subsequently extracted and treated ex situ. Although effective, these methods depend on our ability to adequately contact the contaminants with surfactants or cosolvents. This can be a challenge, and is typically addressed by adding large quantities of chemical in the hope that this will facilitate contact. A novel, and perhaps better method of cosolvent and/or biosurfactant delivery is through the stimulation of naturally occurring organisms that produce various cosolvents and biosurfactants in situ through fermentative processes. These processes can be stimulated by addition of readily degradable carbon sources (i.e. sugars) to the aquifer. Multiple fermentation pathways exist, each yielding a particular set of end products, including alcohols, ketones, volatile fatty acids and gases. Our hypothesis for this research was that the combination of cosolvents and biosurfactants produced in situ during fermentation could contribute to the release of sorbed contaminants and aid in the dissolution of NAPL present within the aquifer. We proposed that an accelerated cleanup of lingering source areas could be achieved by optimizing the production of cosolvents and biosurfactants via fermentation processes.

The experiments described within this report were designed to evaluate how fermented carbon (molasses) affects the partitioning of a hydrophobic compound, trichloroethene (TCE), within an aqueous system. Long-term aging of soil to promote sorption of TCE began in February 2007 to prepare for soil partitioning experiments. Two fill and draw reactors were initiated in June 2007, and consisted of one control reactor containing a minimal groundwater media (no carbon amendment) and a fermentation reactor containing minimal groundwater media combined with 10% molasses by volume (vol/vol). In addition, a 20% (vol/vol) molasses-fed culture was also started using a 500-mL batch reactor with approximately 400 g soil and 250 mL of 20% molasses in minimal groundwater media.

Three different types of partitioning experiments were performed. The first set of experiments began in March 2007 and consisted of TCE NAPL and individual cosolvents commonly produced during fermentation, consisting of acetone, ethanol, methanol, 2,3-butanediol, and volatile fatty acids in the protonated form (lactic, acetic, butyric, succinic, formic, propionic, valeric and caproic) at varying fractions up to 20% vol/vol. These were
initiated to determine whether low levels of cosolvents could individually enhance solubility of TCE. The second set of partitioning experiments were initiated in December 2007, and included the use of the fermentation and control reactors and NAPL to determine whether fermented liquid affected NAPL and TCE solubility. The third set of experiments was initiated in February 2008, and utilized the TCE aged soil (aged approximately 12 months) to determine whether fermented fluid had an affect on sorbed TCE.

Aqueous phase TCE from partitioning and sorption experiments, and alcohols and ketones from the fermentation reactors were analyzed using a Hewlett Packard (HP) 5820 Series II gas chromatograph equipped with a flame ionization detector. For the sorption experiments, TCE was extracted with hexane and analyzed using an HP 5890A GC equipped with an electron capture detector. Extraction efficiencies for TCE in saturated soil averaged 108% with a standard deviation of 7%. Volatile fatty acid analysis was performed using high performance liquid chromatography (Shimadzu LC-19 AT) equipped with a SPD-10A UV-Vis detector (210 nm wavelength) and an Aminex HPX-89H column. Total Organic Carbon from the reactors was analyzed using a HACH High Range (100 to 700 mg/L) Total Organic Carbon Test ‘n Tube Reagent set. An Orion Model 420 digital pH meter equipped with a combination electrode was used for pH measurements. Surface tension measurements were obtained using a Kruss DSA10 Drop shape analysis system. Soil was sent to the University of Minnesota Research Analytical Laboratory for textural analysis via sieving and hydrometer analysis, and fraction of organic matter (foc) analysis by the Loss on Ignition method.

Results

Table 1 summarizes the results of all tested cosolvents in the NAPL partitioning experiments, and Figure 1 demonstrates the increases in TCE solubility resulting from the addition of different fractions of selected cosolvents. As shown, fractions of 0.2 or higher were generally required to enhance TCE solubility through cosolvency, if enhancement occurred. Methanol was determined to enhance solubility at a lower fraction of 0.05, and several compounds did not affect TCE solubility at fractions of 0.2 or lower. Cosolvents that enhanced solubility did so at concentrations higher than would...
be expected to be produced during fermentation processes (Jones and Woods, 1986; Ren et al., 2007).

In addition to the three-phase NAPL partitioning experiments, the fermented liquid from each reactor were evaluated to determine their effect on TCE solubility. In addition, non-fermented molasses (both 10% and 20% vol/vol) were evaluated for TCE solubility effects. As shown in Figure 2, the solutions with the greatest impact on TCE solubility were the fresh molasses solutions. Approximately a 2.3- and 2.7-fold increase in TCE solubility was observed with the fresh 10% and 20% molasses solutions, respectively. The fermented 10% molasses solution (after 234 days of fermentation) did not have an impact on solubility. The fermented 20% molasses solution (after 239 days of fermentation) did, however, increase TCE solubility (Figure 2). The solubility enhancement with fresh molasses solutions is similar to that observed by Macbeth et al. (2006), where up to a 6.7-fold increase in TCE solubility was observed when in the presence of a fresh 10% whey solution. Macbeth and coworkers attributed the solubility enhancement to the protein fraction (10 to 13%) present in the whey powder. The blackstrap molasses used in these experiments contained approximately 3% crude protein, which may have resulted in the solubility enhancement with fresh molasses.

Both the liquid in the fermenting reactors and field samples were analyzed for compounds that could potentially serve as cosolvents to determine whether cosolvency was the reason for solubility enhancement in the fermented 20% molasses reactor. High levels of volatile fatty acids (VFAs) and ethanol were detected in the reactors undergoing molasses fermentation (data not shown). To determine whether cosolvency contributed to the solubility enhancement observed in the fermented 20% molasses reactor samples, the highest detected concentration of each cosolvent from the analysis was combined and used in a NAPL partitioning experiment to determine the effect on TCE solubility. The combination of cosolvents did not enhance solubility (Figure 2), further supporting that the solubility enhancement shown in the 20% fermented reactor is due to a degradable solubilizing component within the raw molasses.

While solubility was not substantially increased by the fermentation products present in the fermented 10% molasses reactor, the NAPL phase surface area was visibility altered. As shown in Figure 3A, the NAPL formed a microemulsion with the
fermented 10% molasses solution (234 days of fermentation) that continued to be stable >100 days after settling, while the control resumed two distinct phases (NAPL and aqueous) immediately after mixing. Fresh molasses (10%) also formed a microemulsion immediately following removal from the rotator, but the emulsion was not stable and returned to two distinct phases after 48 hours of settling (Figures 3.B1 and 3.B2).

The effect of fermented molasses on soil partitioning was evaluated by assessing the soil distribution coefficient ($K_d$) of TCE while in the presence of fermented fluid. Data were used to calculate $K_d$ for each sample by dividing the mass of TCE sorbed to the soil by the equilibrium concentration of TCE in the liquid phase (Eqn 1). An averaged value of triplicates was used for each dataset. Fermented 10% molasses (after 234 days fermentation), fresh 10% molasses and media from the control reactor were used in the experiments.

\[
K_d = \frac{C_{sorb}}{C_{eq}}
\]

Where $C_{sorb}$ [M/M] is the mass of contaminant sorbed per mass of soil, $C_{eq}$ [M/V] is the equilibrium concentration of contaminant present in the liquid phase, and $K_d$ is an experimentally derived soil distribution coefficient (Watts, 1998).

As shown in Figure 4, the fermented molasses sample appeared to result in a decreased $K_d$ value when compared to the control. This difference, however, was not significant at the 95% confidence level ($p = 0.12$). Accordingly, more work is needed to determine whether the $K_d$ value is truly affected by the products generated during the fermentation of organic carbon.

In conclusion, our studies indicate that enhanced dissolution of TCE NAPL can occur through the addition and subsequent fermentation of dilute molasses solutions. The mechanism by which this occurs is different depending on whether the solution is fresh or has been allowed to ferment. Enhanced mass transfer occurs through an increased concentration gradient when fresh molasses is present, as the solubility of TCE is increased greater than 2-fold. Data indicate that cosolvency of TCE does not occur with fermented products; therefore, after the liquid has fermented, solubility enhancement does not contribute to enhanced mass transfer. Biosurfactants appear to be generated
during the fermentation process, as evidenced by the stable emulsion formed when the NAPL and fermented aqueous phases were mixed. The resulting increase in surface area could greatly affect the mass transfer of TCE NAPL into the aqueous phase if mixing could be accomplished in the subsurface. In addition, fermented carbon may have an affect on the soil partitioning coefficient, indicating that fermented liquid may also enhance the dissolution rate of sorbed mass into the aqueous phase.

References


Table 1. Summary of results from 3-phase cosolvent partitioning runs.

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<td><img src="image" alt="Structure" /></td>
<td>No change ≤ 20%</td>
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<td>Valeric Acid (Pentanoic)</td>
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<td>Caproic Acid (Hexanoic)</td>
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a Change in concentrations considered statistically significant at \(p=0.05\) using the Student’s t-test.
b Solubility increase normalized to average of unamended controls (triplicate run) within same experimental set.
c Percent based on volume per volume (vol/vol) basis
d Cosolvent fraction at which solubility increase observed.
Figure 1. TCE solubility in presence of selected (A) ketones, (B) volatile fatty acids, and (C) alcohols. Data points represent average of triplicate runs with error bars showing standard deviation. Dashed line represents TCE solubility (1.1 g/L) in water (Pankow and Cherry, 1996).
Figure 2. Concentrations of soluble TCE in vials containing a NAPL phase and media only (control), media amended with unfermented molasses (Fresh), media amended with molasses that was allowed to ferment (Ferm) for 234 days (10% molasses), and 239 days (20% molasses). TCE solubility in water (1.1 g/L) is indicated by the dashed line. Data points represent the average of triplicate runs, with error bars showing the standard deviation.

Figure 3. Photographs showing changes in NAPL surface area as affected by fermented versus non-fermented substrate and emulsion behavior over time. (A1) TCE emulsion resulting from mixing NAPL-phase TCE with 10% fermented molasses solution, shown 118 days after mixing, (A2) NAPL surface area after mixing with control reactor media. (B1) TCE emulsion immediately following mixing with fresh non-fermented molasses (<24 hrs), (B2) NAPL >48 hours following mixing with fresh molasses where return to solid state indicates initial emulsion was not stable. Note: Glass beads are visible on the bottom of vials in (A2) and (B2).
Figure 4. $K_d$ values for TCE in soil partitioning experiments with vials containing media only (control), media amended with unfermented 10% molasses (Fresh), and media amended with 10% molasses that was allowed to ferment (Ferm) 234 days. Data points represent the average of triplicate runs, with error bars showing the standard deviation.
Publications, Presentations, or Published Abstracts:

Publications
The paper detailing the results of this research has been finalized and will be submitted to the Journal of Environmental Engineering by June 30, 2008.

Presentations (* indicates student presentation)


Students and Post-doctoral Research Associates supported by this project:
None

Additional Funding:
Denice Nelson received a Sommerfeld fellowship from the University of Minnesota’s Department of Civil Engineering to conduct this research. We have submitted a proposal to the National Science Foundation for follow-up work.

Awards and Special Recognition:
None to date
Information Transfer Program Introduction
## Student Support

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Notable Awards and Achievements

Shahram Missaghi received the 2007–2008 Butler and Jessen Water Resources Science Fellowship. Mr. Missaghi is a PhD student in the Graduate Program in Water Resources Science at the University of Minnesota. He is supported by Dr. Miki Hondzo (Department of Civil Engineering).

Martin Tsui received the Chris Lee Award for Metal Research from the Society of Environmental Toxicology and Chemistry International Copper (SETAC/ICA) Association. Mr. Tsui is a PhD student in the Graduate Program in Water Resources Science at the University of Minnesota. He is supported by Dr. Jacques Finlay (Department of Ecology, Evolution, and Behavior and Graduate Program in Water Resources Science).

Martin Tsui received a University of Minnesota Graduate School Thesis research grant. Mr. Tsui is a PhD student in the Graduate Program in Water Resources Science at the University of Minnesota. He is supported by Dr. Jacques Finlay (Department of Ecology, Evolution, and Behavior and Graduate Program in Water Resources Science).

Jeffrey Werner received an Honorable Mention for the Universities Council on Water Resources (UCOWR) PhD Dissertation Award in Natural Sciences and Engineering. He was supported by Drs. Bill Arnold (Department of Civil Engineering and Graduate Program in Water Resources Science) and Kris McNeill (Department of Chemistry and Graduate Program in Water Resources Science).

Jeffrey Buth received a 2006 EPA STAR Graduate Fellowship. Mr. Buth is a graduate student in the Department of Chemistry at the University of Minnesota. He is supported by Kris McNeill (Department of Chemistry and Graduate Program in Water Resources Science).

Jeffrey Buth received a 2008 American Chemical Society Graduate Student Award in Environmental Chemistry. Mr. Buth is a graduate student in the Department of Chemistry at the University of Minnesota. He is supported by Kris McNeill (Department of Chemistry and Graduate Program in Water Resources Science).

Dr. Mike Sadowsky was awarded the 2008 College of Food, Agricultural, and Natural Resource Sciences Distinguished Diversity and Inclusion Award. The award recognizes his “significant efforts to assure broad access to University programs for students of color thorough a host of active and collaborative recruitment and retention efforts.” Dr. Sadowsky is a professor in the Department of Soil, Water, and Climate and the Graduate Program in Water Resources Science at the University of Minnesota.
Publications from Prior Years


