New Jersey Water Resources Research Institute Annual Technical Report FY 2004

Introduction

The New Jersey Water Resources Research Institute supports a diverse program of research projects and information transfer activities. Under the continuing set of priorities enunciated by the Advisory Council, the available funds are split between supporting faculty in seed projects or new research initiatives and supporting graduate students who are beginning their thesis research. Priority goes for the former to junior faculty; the goal is to help new researchers establish research programs which will have long-term investment in New Jersey water resource problems. With the latter (graduate students), the priority is to fund emerging and promising young scientists with novel ideas but little initial support to develop those ideas.

Research projects emphasize studies of pollutant fate and transport, reflecting the primary pool of expertise in the state and the pervasive nature of pollutant problems in the state. Two projects address the microbial transformations of halogenated hydrocarbons; the ability of *Dehalococcoides* to debrominate polybrominated diphenyl ethers has been assessed, and the presence of these compounds in wastewater treatment plant residuals is being determined, and the ability of bacteria to dechlorinate polychlorinated dioxins was also studied. While the dechlorination process was found to proceed, debromination appears to be a much slower process. In another project, a novel method for tracking contaminant plumes in wetlands and water bodies has been developed. This method uses measurements of electrical conductivity to track changes in both surface and sediment-surface components, allowing contaminants and contaminanted water plumes to be detected without the need for invasive and expensive sampling. This exploratory project promises to have major applications in contaminated waters. A fourth project explored the feasibility of using stable isotope fractionation as a method for detecting transformations of mercury; the initial studies demonstrated for the first time that such a methodology is possible. This preliminary project has resulted in major funding from the National Science Foundation to expand the use of stable isotopes in understanding the biogeochemistry and microbiology of mercury. Another project addressed the state-mandated change in stormwater management regulations, which promote the use of bioretention basins to infiltrate stormwater. One major issue with this approach is the fate of fecal coliforms; a graduate student project used laboratory column experiments to demonstrate that bioretention sediments can be very effective at removing coliforms, thus supporting the use of these structures in stormwater management. Two final projects addressed the function of wetlands in urban areas. In one project, analyses of stream macroinvertebrate communities were undertaken in a set of wetlands spanning the urban/suburban environment in northeastern New Jersey; the sampling was designed to determine what environmental variables most strongly affect stream invertebrates. It was found that contrary to expectation, the hydrogeomorphic location of the wetland and the wetland area were not important predictors of invertebrate communities; rather, the flow characteristics of streams within wetlands (low flow, low oxygen, sandy substrate) exerted strong controls on the structure of the communities. In a second project, throughfall collectors were installed in the parallel set of 15 sites in order to determine the importance of atmospherically-deposited nitrogen in urban areas. Data collection is ongoing.

Information transfer activities in this program year were restricted by the inability of the University to continue significant funding support for the information transfer program. We continue to develop newsletters that focus on a particular issue; we have had strongly positive response from readers that these newsletters are highly valued. We have also extensively revised our website, adding numerous pages of links to useful information and information about meetings, publications, real-time data for New Jersey waters, educational resources and other water resource materials.

Research Program

Our research program attracts about three to four times as many proposals as can be funded. We are getting a higher proportion of proposals from institutions other than Rutgers, as state colleges develop a greater emphasis on research. We continue to encourage junior faculty and new graduate students to pursue research funds to start research programs addressing New Jersey water resource problems.

Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by Dehalorespiring Bacterial Cultures

Basic Information

Title:	Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by Dehalorespiring Bacterial Cultures	
Project Number:	2004NJ68B	
Start Date:	3/1/2004	
End Date:	3/1/2005	
Funding Source:	104B	
Congressional District:	6th	
Research Category:	Water Quality	
Focus Category:	Toxic Substances, Sediments, Treatment	
Descriptors:	, Polychlorinated dibenzo-p-dioxins/furans, Sediment	
Principal Investigators:	Fang Liu, Donna E. Fennell	

Publication

1. Poster Presentation: Liu, F.; E.-K. Son; D.E. Fennell. 2005. Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by a Dehalococcoides-Containing Culture. Poster Presentation in the 105th American Society for Microbiology General Meeting, Atlanta, GA, June 5-9, 2005.

Problem and Research Objectives

Dioxins and dioxin-like compounds (DLCs) are a group of planar chemicals some of which are very toxic to human beings and other organisms. This group includes polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and certain co-planar polychlorinated biphenyls (PCBs).

Dioxins are of great concern to the public because of their severe toxicity, lipophilic behavior—resulting in bioaccumulation in food chains, and their extraordinary chemical stability. They are found throughout the whole environment, including air, water, soil and sediment. Food contaminated with DLCs pose a threat to the public health.

Dioxin contamination is especially a severe environmental problem in New Jersey. The Passaic River is on EPA's list of contaminated watersheds because of its dioxin contamination. The contamination sources include a former pesticide manufacturing facility (Diamond Alkali) located in Newark which was designated as a Superfund site. A Remedial Investigation and Feasibility Study (RI/FS) is being conducted to evaluate the Passaic River Study Area for its potential long term remedies. Environmental dredging has been proposed for the most contaminated portions of the river.

In the meantime, related research investigations have been carried out to investigate other remediation strategies. *In situ* bioremediation has unique strong points compared with other remedies. *In situ* operation prevents the secondary contamination that may occur during the sediment dredging and disposal process.

We are using a mixed culture which contains *Dehalococcoides ethenogenes* strain 195 and a pure culture of *Dehalococcoides ethenogenes* strain 195 to investigate microbial dechlorination of PCDD/Fs. We are also trying to determine the effects of other halogenated co-substrates on the dechlorination processes.

Methodology

Microcosm study

We selected 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) and 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF) as target compounds. Seven sets of triplicate 60 mL serum bottles were set up in each set with pre-grown mixed culture. The mixed culture was pre-grown with tetrachloroethene (PCE) and butyric acid. PCE and 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) were added as halogenated co-substrates in some treatments.

The details were listed in Table1.

Table1. Experimental Setup					
Bottle Set	PCDD/F congener	Halogenated	Electron		
	(µM)	Co-substrates (µM)	Donor (µM)		
(1) 1,2,3,4-TeCDD Killed	1,2,3,4-TeCDD	None	None		
control	(31µM)				
(2) 1,2,3,4-TeCDD only	1,2,3,4-TeCDD	None	Butyrate		
	(31µM)		(440µM)		
(3) 1,2,3,4-TeCDD with	1,2,3,4-TeCDD	PCE (110µM)	Butyrate		
PCE addition	(31µM)		(440µM)		
(4) 1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-HxCDF	None	None		
killed control	(5µM)				
(5) 1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-HxCDF	None	Butyrate		
only	(5µM)		(100µM)		
(6) 1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-HxCDF	PCE (25µM)	Butyrate		
with PCE addition	(5µM)		(100µM)		
(7) 1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-HxCDF	1,2,3,4-TeCB	Butyrate		
with TeCB addition	(5µM)	(25µM)	(100µM)		

Analyses

Well-mixed samples were removed with a sterile, anoxic syringe and extracted with toluene/acetone (Fennell et al., 2004). PCDD/F congeners were analyzed by Gas Chromatography- Mass Selective Detector (Fennell et al., 2004). PCE was analyzed by Gas Chromatography- Flame Ionization Detector (Fennell et al., 2001).

Principal Findings and Significance (Progress Report)

We had previously reported the activity of D. ethenogenes strain 195 on a variety of chlorinated aromatic compounds. During previous studies we added tetrachloroethene as a co-substrate to ensure growth of D. ethenogenes strain 195 in case the aromatic chlorinated compounds tested (1,2,3,4-TeCDD, 1,2,3,4-tetrachlorodibenzofuran, 2,3,7,8-TeCDD, 2,3,4,5,6-pentachlorobiphenyl) were not supportive of growth. The mixed culture containing D. ethenogenes strain 195 was shown to dechlorinate 1,2,3,4-TeCDD both with and without the addition of PCE as a co-substrate. 1.2.3.4-TeCDD was dechlorinated 1.3to 1,2,4-trichlorodibenzo-*p*-dioxin (1,2,4-TrCDD)and dichlorodibenzo-p-dioxin (1,3-DCDD). Rates of daughter product formation were initially slower in PCE-amended cultures relative to cultures with no added PCE. At the end of the incubation, the extent of 1,2,3,4-TeCDD dechlorination was very similar in both treatments with and without PCE addition. It seemed that PCE addition did not affect the dechlorination of 1,2,3,4-TeCDD. We further transferred the pre-grown culture at 10% v/v ratio spiked with 1,2,3,4-TeCDD alone or together with PCE addition. The results also showed that 1,2,3,4-TeCDD was dechlorinated in both treatments at similar rates. The 1,2,3,4-TeCDD dechlorination and 1,3-DCDD formation did not show significant difference in the treatments. Although the first transfer results agree with that of the pre-grown culture, we have not yet confirmed that 1,2,3,4-TeCDD is a growth substrate for strain 195.

The dechlorination of 1,2,3,4,7,8-HxCDF was observed after one month of incubation. A penta-CDF congener was detected in all three active sets: the set spiked with 1,2,3,4,7,8-HxCDF as the only halogenated substrate, the PCE-amended set and the 1,2,3,4-TeCB-amended set. The most extensive dechlorination occurred in the 1,2,3,4-TeCB-amended set where the penta-CDF was further dechlorinated to two tetra-CDF congeners. We examined the dechlorination products and found no 2,3,7,8-substituted penta- or tetra-CDF congeners formed. This confirmed that the dechlorination process detoxified 1,2,3,4,7,8-HxCDF and formed non-2,3,7,8-substituted congeners. It shows the potential for use of the mixed culture to bioaugment contaminated sites.

Efficiency of Bioretention Systems to Reduce Fecal Coliform Counts in Stormwater

Basic Information

Title:	Efficiency of Bioretention Systems to Reduce Fecal Coliform Counts in Stormwater
Project Number:	2004NJ70B
Start Date:	3/1/2004
End Date:	3/1/2005
Funding Source:	104B
Congressional District:	6
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Water Quality, Water Quantity
Descriptors:	bioretention, water, sediments, precipitation, adsorption, ecological effects, chemicals, pollution prevention, treatment, pathogens, viruses, TMDL, total maximum daily load, nonpoint source pollution, runoff, water quality, water quantity, bmp, best management practice
Principal Investigators:	Gregory Rusciano, Christopher Obropta

Publication

 Rusciano, G.M., C.C. Obropta. 2005. Rusciano, G.M. Efficiency of Bioretention Systems to Reduce Fecal Coliform Counts in Stormwater. Proceedings of The North American Surface Water Quality Conference and Exposition, Orlando, Florida, July 18-25, 2005. Forrester Communications, Inc., Santa Barbara, CA.

EFFICIENCY OF BIORETENTION SYSTEMS TO REDUCE FECAL COLIFORM COUNTS IN STORMWATER

Gregory M. Rusciano and Christopher C. Obropta Graduate Assistant, Department of Environmental Sciences, greg.rusciano@rutgers.edu Assistant Professor, Department of Environmental Sciences, obropta@envsci.rutgers.edu

> Rutgers, The State University of New Jersey Cook College 14 College Farm Road New Brunswick, NJ 08901

ABSTRACT

Currently, 7,742 water bodies in the nation are impaired for pathogenic bacteria, viruses and/or parasites (14.4% of all reported water bodies), more than for any other impairment (USEPA, 2003). Impairments result in large part from nonpoint sources of pollution carried by urban and agricultural stormwater runoff. Fecal coliform (FC) counts are commonly used as an indicator of pathogens and are used by governmental agencies to help manage drinking water quality and recreational activities such as swimming, boating and fishing. The study seeks to evaluate the ability of bioretention systems to effectively reduce fecal coliform colony counts. Bioretention systems were modeled in the laboratory with columns with representative depths of gravel, sand and soil. Panicum virgatum, typically used in bioretention systems, was integrated into the columns. Typical rainfall conditions for New Jersey will be mimicked in the laboratory with regard to rainfall intensity and frequency and stormwater composition (bacterial colony counts). The drainage area received by a typical bioretention system was estimated to determine the appropriate flow rate of water input into the system. maximum percolation rate was observed to be approximately 37 mL/minute. Ponding occurred in the top of the column during every simulated storm event, although its maximum height never surpassed 12 inches. TSS removal was generally high with an average ratio of 92.3% and range of 82.5-99.4%. FC count reductions were generally high, with an average ratio of 87.8% and a range of 54.7-99.7%. The turbidity was observed to be significantly lower in leachate samples (see Figure 4). On average, the pH and temperature of the influent was 7.14 and 25.4 °C, respectively. The pH and temperature of the leachate was 4.71 and 22.9 °C, respectively. In addition to filtration and adsorption mechanisms, other mechanisms are responsible for acting directly on the bacteria regardless of their association with particulates. The primary mechanism is the pH. It is also likely that predation of FC bacteria by other microorganisms was a factor. Since bioretention is increasingly being implemented as a primary watershed management tool across the United States, this research will provide data to help optimize its effectiveness in the field and improve regulatory guidance for the future.

INTRODUCTION

Currently, 7,742 water bodies in the nation are impaired for pathogenic bacteria, viruses and/or parasites (14.4% of all reported water bodies), more than for any other impairment (USEPA, 2003). Impairments result in large part from nonpoint sources of pollution carried by urban and agricultural stormwater runoff. Runoff also contributes many other pollutants to receiving water bodies including suspended solids and heavy metals (Barrett *et al.*, 1998; Wu *et al.*, 1998; and Sansalone and Buchberger, 1997). Recent water quality studies investigating pathogens found high concentrations of fecal-indicator bacteria in water bodies receiving stormwater runoff from mixed land uses (Tufford and Marshall, 2002). Other sources of contamination include combined sewer over flows (CSOs), sewer leakages, septic fields, and publicly owned treatment works (POTWs) discharges (Marsalek and Rochfort, 2004). Burnes (2003) determined that the sources of fecal contamination originate from both humans and animals, including cattle, domestic, and wild species.

Fecal coliform (FC) counts are commonly used as an indicator of pathogens and are used by governmental agencies to help manage drinking water quality and recreational activities such as swimming, boating and fishing. While coliform bacteria themselves do not cause illness, they originate from the digestive tracts of warm-blooded animals and their presence suggests the occurrence of harmful

pathogens from the same origin. Other fecal-indicator organisms include enterococci, total coliforms, and *Escherichia coli*. Haile *et al.* (1999) reported epidemiological evidence that shows an increased risk of adverse health associated with swimming in recreational waters that are contaminated with untreated urban stormwater. The Beaches Environmental Assessment and Coastal Health Act (BEACH Act) was signed into law on October 10, 2000, and amends the Clean Water Act (CWA), incorporating provisions to reduce the risk of illness to users of the Nation's recreational waters. Total Daily Maximum Loads (TMDLs) for FC are developed to identify all point and nonpoint sources in impaired water bodies. Currently, five FC TMDLs have been established for water bodies in New Jersey, which may require the development of watershed management plans for the reduction of nonpoint sources of FC (NJDEP, 2004a).

Successfully meeting recent TMDL requirements will require some degree of on-site stormwater treatment (USEPA, 2001). A variety of structural best management practices (BMPs) are available to treat stormwater including extended detention basins, wet ponds, stormwater wetlands, bioretention systems, enhanced swales, prefabricated treatment devices, and riparian forest buffers. The selection of the most appropriate BMP depends on: (1) estimated pollutant removal capabilities of the BMP; (2) most appropriate land use conditions; and (3) treatment suitability of the stormwater (NJDEP, 2004b).



Figure 1: Conceptual Bioretention System (NJDEP, 2004b)

The bioretention system is a structural stormwater BMP that is commonly used in suburban settings, especially for the treatment of parking lot runoff. The typical design for a bioretention system includes a sloped grass buffer strip, a ponding area with native vegetation (provides settling of suspended solids), a three-foot deep soil planting layer, and a one-foot deep sand layer. Some systems are equipped with gravel and underdrain piping where soils are not appropriate for groundwater recharge. The soil planting layer: (1) acts as a primary filter with attenuation of pollutants to soil particles, (2) provides rapid infiltration of stormwater runoff (complete infiltration within 72 hours to avoid mosquito breeding), and (3) sustains healthy vegetation at the surface. The soil

planting bed consists of a high sand content to achieve infiltration requirements. The sand layer acts as a secondary filter and transition between the soil planting bed and the under-drain system or underlying soil. A thin mulch layer can be applied to the top of the soil planting bed to retain moisture and attenuate pollutants. Water collected in the under drain can be retrofitted to a stormwater sewer system, which eventually discharges into surface waters. Systems without an under drain system are used to recharge groundwater through infiltration.

Plants in a bioretention system consist of native grasses, shrubs and trees that are intended to adapt well to the soil and climate of the region in which they are implemented. They must also tolerate pollutants and varied depths of water. The plants are intended to uptake water contaminated with excess nutrients and pollutants, however, plant roots may also provide pore spaces which will provide a habitat for microorganisms, thus promoting biological degradation of some pollutants and predation of other bacteria (Davis et al., 2001). Bioretention systems are intended to remove suspended solids, nutrients,

metals, hydrocarbons, and bacteria (NJDEP, 2004a); however they have not been investigated thoroughly for FC in the United States.

Research on stormwater-associated bacteria has been conducted for similar structural stormwater BMPs such as constructed wetlands and wet ponds. Birch *et al.* (2004) found a 76% removal of FC colony counts from constructed wetlands that received contaminated stormwater from four high-flow rainfall events. Kadlec and Knight (1996) reported an average 90% removal of coliform bacteria for constructed wetlands. Davies and Bavor (2000) reported constructed wetland removal efficiencies of 79% and 85% for thermotolerant coliforms and enterococci, respectively; and removal efficiencies of -2.5% and 23% for wet ponds. Bacterial removal was significantly less effective in the wet pond because of its inability to retain fine clay particles (<2 μ m) to which bacteria were predominantly adsorbed (Davies and Bavor, 2000; Baudart *et al.*, 2000). Davies and Bavor (2000) correlate increased vegetation with increased removal efficiency.

Since bioretention systems remove about 80% of the total suspended solids (TSS; NJDEP, 2004a), FC bacteria attached to sediments should be held up in the system. Simultaneous analysis of TSS and FC will be performed to test this hypothesis. We also hypothesize that as the system cycles through periods of wetness and dryness, aerobic and anaerobic microniches are formed. These conditions could support predatory bacteria. Thus, in addition to sediment entrapment and sorption as methods of removal, predation might also be important. Bioretention systems are an ideal candidate for managing pathogens in stormwater due to their manageable size, potential to remove sediments and their potential to induce predation. Bioretention systems can also be *engineered* for removal of pollutants through the choice of planting bed media. Thus, two different types of such media will be investigated. Three different concentrations of manure slurry will also be investigated to account for a possible variability in pollutant removal efficiency. Since bioretention is increasingly being implemented as a primary watershed management tool across the United States, this research will provide data to help optimize its effectiveness in the field and improve regulatory guidance for the future.

METHODS

Column construction

Pilot bioretention systems were constructed in the laboratory using six-inch diameter, clear PVC pipe cut into five-foot lengths (Harvel Plastics, Inc.). One end of the pipe was wrapped in perforated filter fabric and fitted with a six-inch to four-inch PVC reducer coupling (see Figure 2). The reducer coupling was filled with pea gravel (AASHTO M-43) and capped with a four-inch PVC cap which was drilled with a half-inch diameter hole for collecting leachate. The bottom twelve inches of the column was packed with clean medium aggregate concrete sand (ASTM C-33) at a bulk density of approximately 1.8 grams/cc. The next thirty-six inches of the column were packed with the soil planting bed media at a bulk density of approximately 1.3 grams/cc. The transparency of the clear PVC made packing easier. However, the



columns were wrapped in an opaque covering after packing to prevent algal growth. The soil planting bed consisted of three equal parts (by volume) of sphagnum peat, triple-shredded hardwood mulch and medium aggregate concrete sand. The mixture was blended homogeneously by hand before packing. An additional soil planting bed consisting of compost, top soil and medium aggregate sand will be investigated subsequently. A control column will be used and consists of soil-core samples of material taken from a New Jersey suburb. Five to seven two-inch plugs of switchgrass (Panicum virgatum) were planted at the top of the soil-planting bed. The switchgrass was watered regularly and permitted to grow for several months before experimentation. All stages of the experiment took place in a temperaturecontrolled greenhouse (21-27° C). Three columns were constructed identically and housed in a heavyduty wooden workbench (see Figure 3).

Preparation of manure slurry

Fresh horse manure (from animals not treated with antibiotics) was collected on experimentation days. A 200 gram equal-partsby-volume manure mixture (from three different horses) was added to 1800 mL of phosphate buffered dilution water (AWWA, 2001) in a 6000 ml Erlenmeyer flask. The mixture was then placed on a gyratory shaker for at least thirty minutes at 200 RPM. One liter of the supernatant was decanted and added to nine liters of dilution water. The total dilution was 100-fold. Ten- and thirty-fold dilutions will also be used to determine the differences in removal efficiencies of bioretention systems receiving different concentrations of pollutants (ASCE, 1999). All glassware was sterilized in a steam autoclave prior to use.

Experimental methods

Manure slurry was applied to the top of the column at a rate of 77 mL/minute for two hours using a peristaltic pump. This rate was based upon a 1.25-inch rainfall event over two hours, the storm event considered to be ideal for water quality research by

Figure 3: Photo of bioretention columns. the NJDEP. A rational method runoff coefficient of 0.8 was assumed, and the bioretention area was assumed to be 5% of the drainage area (Davis et al., 2001). Approximately fifteen simulated storm events will be conducted on each column by the end of the study. Each column will receive differentlydiluted manure slurry, as discussed earlier. Simulated storm events were conducted at least one week after each other to allow for complete drainage and drying (Davis et al., 2001).

The manure slurry was sampled before it was applied to the column. To determine a "background dieoff rate" of FC bacteria, two identical samples of the influent slurry were collected. One was left open to the atmosphere for a known time period while the other was plated and incubated immediately. Leachate samples were collected from the bottom spout at approximately one-hour intervals from the time of first appearance; leachate flow rate was also determined. The pH and temperature of all samples was measured using pH/temperature meter at the time of collection. Samples were stored in an ice-filled cooler during transport to the laboratory. Samples for TSS analysis were collected in 500 mL highdensity polyethylene jars. Samples for FC analysis were collected in sterile 15 mL glass culture tubes with sterile high-density polyethylene screw caps.

Samples were analyzed for FC using the delayed incubation method from Standard Methods (AWWA, 2001). Samples were filtered onto sterile 0.45 µm, 0.47 mm diameter gridded membranes by vacuum filtration. Membranes were plated into sterile 0.5 mm Petri dishes with adsorbent pads soaked with 2



mL of sterile FC broth (with rosolic acid). All instruments were steam-sterilized prior to use. Petri dishes were incubated for 24 hours in a 44.5° C water bath. All samples were plated in triplicate. Influent samples of the 100-fold dilution manure slurry were filtered in 0.1 and 1 mL volumes. Leachate samples were filtered in 1 and 10 mL volumes. All samples were simultaneously analyzed for TSS using *Standard Methods* (AWWA, 2001).

When all storm events are completed, the material from the columns will be sampled at different depths and analyzed for FC bacteria. A slurry will be prepared using each of these samples. The slurries will then be analyzed for FC using the delayed incubation method. This analysis will be compared with background data obtained on the soil before experimentation.

RESULTS

Results at the time of this conference paper are preliminary. To date, five simulated storm events were conducted using the 100-fold dilution manure slurry. A removal efficiency ratio was calculated for each simulated storm event by subtracting the influent concentration from the average leachate event mean

concentration (EMC). The EMC is defined as: $EMC = \frac{\sum_{i=1}^{n} V_i C_i}{\sum_{i=1}^{n} V_i}$, where V_i = the volume of flow during

period *i*, C_i =the concentration associated with period *i*, and *n* = the total number of measurements taken



Figure 4: Photo of an influent sample (left) and a leachate sample (right).

during an event. V_i was estimated using observed flow rate values (ASCE, 1999). TSS removal was generally high with an average ratio of 92.3% and range of 82.5-99.4%. FC count reductions were generally high, with an average ratio of 87.8% and a range of 54.7-99.7%. The turbidity was observed to be significantly lower in leachate samples (see Figure 4). On average, the pH and temperature of the influent was 7.14 and 25.4 °C, respectively. The pH and temperature of the leachate was 4.71 and 22.9 °C, respectively.

In general, it took one hour before leachate water was observed in the bottom spout. A curve was fitted to all leachate flow rate data versus time (Figure 5), and the maximum percolation rate was observed to be approximately 37 mL/minute. Ponding occurred in the top of the column during every simulated storm event, although its maximum height never surpassed 12 inches. NJDEP specifications require no more than 12 inches of ponded water for bioretention systems.

DISCUSSION

Currently the data is preliminary but looks consistent with regard to bioretention removal efficiency. Both FC and TSS were reduced by the bioretention column. This supports the idea that FC bacteria are associated with particles greater than or equal to $2\mu m$ in diameter. It is likely that a combination of filtration and adsorption is primarily responsible for FC and



TSS retention within the system. Bouwer (1984) reported that filtration generally occurs when the diameter of suspended particles is larger than 0.2 times the diameter of particles constituting the porous media. It is also likely that filtration is more effective during unsaturated conditions when transport takes preferential flow paths through the smallest pores (Stevik *et al.*, 2004). The presence of macropores or channeling in the media will have reduced the filtration capacity of the bioretention column. Water that flows along the sides of the bioretention column is also less effectively filtered. Macropores surrounding the mulch were observed through the clear PVC. In areas of the media where pore spaces are large, adsorption is the dominant physical mechanism for retaining FC and TSS (Sharma *et al.*, 1985). Adsorption of bacteria is influenced by physical, chemical and microbiological factors including the size and texture of porous media, presence of organic matter and biofilm, temperature, flow rate, ionic strength, pH, hydrophobicity, chemotaxis and electrostatic charge (Stevik *et al.*, 2004).

In addition to filtration and adsorption mechanisms, other mechanisms are responsible for acting directly on the bacteria regardless of their association with particulates. The primary mechanism is the pH. Bacterial survival decreases with non-neutral pH values (Sjogren, 1994). Sjogren (1994) reported negative survival of *E. coli* bacteria in more acidic soils. Considering the average observed pH value of the leachate was 4.71, a portion of FC bacteria did not survive the bioretention column. The peat portion of the bioretention soil planting bed media likely contributed to the acidity of the system. Increased temperature relates to a decrease in bacterial survival. Differences between observed influent and leachate temperature (i.e., 25.4 °C and 22.9 °C respectively) show that temperature was probably not a factor in reducing FC bacteria survival. It is also likely that predation of FC bacteria by other microorganisms was a factor. Protozoa are the main predators of bacteria (Acea and Alexander, 1988). FC bacteria may have also been negatively affected by competition for nutrients and inhibitory secretions from other microorganisms (Stevik *et al.*, 2003).

Clogging of the system with the accumulation of stable solids and bacterial biofilm build-up is likely to occur over time. This should enhance the filtration and adsorption capacity of the system by limiting pore space size (Stevik *et al.*, 2004). However, the benefits will later be surpassed by the system's inability to meet required percolation rate specifications.

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Soil Moisture Regimes and Nitrate Leaching in Urban Wetlands

Basic Information

Title:	Soil Moisture Regimes and Nitrate Leaching in Urban Wetlands
Project Number:	2004NJ71B
Start Date:	3/1/2004
End Date:	3/1/2005
Funding Source:	104B
Congressional District:	6
Research Category:	Not Applicable
Focus Category:	Wetlands, Acid Deposition, Nitrate Contamination
Descriptors:	urban ecology, urban hydrology, wetlands, nitrogen, nitrate, nitrate leaching, nitrogen deposition, throughfall
Principal Investigators:	Emilie Stander, Joan G. Ehrenfeld

Publication

Project Information

Problem and Research Objectives

Problem Nitrogen is one of the most widespread and pervasive pollutants present in surface waters throughout the United States. Excess nitrogen in surface waters can have drastic negative consequences for ecosystems. The zones of hypoxia in the Chesapeake Bay and the Gulf of Mexico are dramatic examples of the extent to which nitrogen excess can cause eutrophication in receiving water bodies. Also, because nitrate is a drinking water pollutant, elevated nitrate levels in surface waters pose a problem for human and wildlife health. These problems threaten to become more pervasive as more land is converted to urban land use.

Wetlands are increasingly being used as a management tool to combat the problem of excess nitrogen in urban watersheds. In New Jersey regional watershed management planning agencies throughout the state are emphasizing the protection and restoration of wetlands for water quality protection. This is based on the documented ability of treatment wetlands to remove nitrate in sewage effluent and of riparian buffer strips to remove nitrate from upland agricultural land use. Nitrate is removed through the process of denitrification, the microbially-mediated transformation of nitrate to nitrogen gas which is released to the atmosphere. This process requires anaerobic conditions which are found in the saturated soils of wetlands. Because of this ability to remove nitrate from upland land uses, wetlands function as nitrate sinks.

However, due to hydrological alteration resulting from urban land use, urban wetlands in northeastern New Jersey may experience lowered water tables, overall dryer conditions, and wet-dry cycles that may reduce nitrate removal capacity. In wetlands with lowered water tables, the biologically active zone of the soil where roots and microbial populations are located no longer experiences frequent saturation. As a result denitrification is inhibited. There is growing evidence that urban wetlands exposed to hydrological disturbance have a reduced ability to denitrify and thus remove nitrate before it reaches surface waters. Conversely, aerobic conditions in wetland soils, which are rich in organic matter, are well known to be conducive to high rates of nitrification. This results in the accumulation of high concentrations of nitrate and the potential for its movement through leaching to surface waters. This may cause New Jersey's urban wetlands to be acting as sources rather than sinks of nitrate, leading to elevated nitrate concentrations in receiving water bodies and associated impacts on the integrity of aquatic ecosystems.

Another issue which emphasizes the need to study nitrate removal in urban wetlands is the significant input of nitrogen into the system through atmospheric deposition. The density of urban development and amount of vehicular traffic in close proximity to many urban wetlands suggests that nitrogen deposition rates are significantly elevated, perhaps above regional averages, as documented along an urbanrural gradient from New York City to outlying suburbs. Several studies have explicitly linked atmospheric nitrogen deposition with eutrophication of coastal waters in the Northeast United States.

Objectives The aim of this study is to document nitrogen inputs and outputs in forested swamps along a gradient of urban to suburban conditions. This will allow me to

determine whether outputs are correlated with inputs (i.e., do sites with higher nitrogen inputs have higher nitrogen outputs). This study will also demonstrate whether nitrogen inputs are higher in areas with a higher intensification of urban land use. Also, using data collected from previous work partially funded by NJWRRI, I will be able to determine whether nitrogen outputs are higher in sites with altered hydrology and in sites with higher rates of nitrogen cycling processes such as nitrogen mineralization and nitrification.

Methodology

Nitrogen inputs were measured weekly/monthly at three locations in eight sites using throughfall collectors. Sites were chosen to represent a gradient of urban-suburban conditions in forested, palustrine swamps adjacent to streams in northeastern New Jersey. The more urban end of the gradient is located in Cedar Grove and the Morristown area while the less urban end of the gradient is located in Griggstown. The throughfall collectors consist of 20 cm diameter funnels connected to four liter carboys that collect rain which has filtered through the forest canopy. Funnels are affixed one meter off the ground on PVC stakes. Glass fiber insulation filters are placed in the neck of each funnel to keep out particulate matter. Throughfall filters through the insulation and passes through tubing rinsed with deionized water into the carboys. Carboys are partially buried and exposed areas are covered with duct tape to keep out light and discourage microbial growth and activity. Acid washed funnels and carboys are placed in the field and collected one week later. No preservative or acid solution is used to prevent transformations in the carboys following standard protocols used by governmental monitoring programs like the National Atmospheric Deposition Program. Samples are transported on ice and are filtered through Whatman GF/F filters (less than 1 µm pore size -- most microbes are $\sim 3 \mu m$) immediately upon return to the laboratory. Filtered samples are then frozen in HDPE bottles until analyzed colorimetrically for nitrate and ammonium concentrations on an 8000 Series Lachat Flow Injection Analyzer (Hach Corp., Loveland, CO).

Nitrogen outputs were measured weekly/monthly using tension lysimetry. Tension lysimeters (Soil Moisture Equipment Corporation, Santa Barbara, CA) were installed in fall 2004 to a depth of 50 cm in three locations (adjacent to throughfall collectors) at the same eight sites. Lysimeters consist of a PVC tube with a porous, ceramic cup at one end and a cap with open tubing on the other end. A hand pump is used to pump 70 centibars (similar to the amount of pressure exerted by a plant root) of vacuum into the lysimeter through the open tubing a week prior to sampling. Water flows from the soil through the ceramic cup and is stored in the PVC pipe. Samples are pumped from the lysimeter directly into a glass scintillation vial. Samples are transported on ice and filtered immediately upon return to the laboratory same as above. Samples are also stored frozen in HDPE bottles until analyzed colorimetrically for nitrate and ammonium concentrations on the Lachat.

Principal Findings and Significance

Tension lysimeters were purchased last summer and installed in nine sites last fall. They require four to six months of fairly regular rains to equilibrate and establish good contact with the soil. The lysimeters equilibrated over the fall and winter. I began sampling the lysimeters weekly at the end of April. At the beginning of June, I initiated monthly sampling since there is not enough leachate produced for weekly collections, particularly during the summer when rain events are less frequent and abundant vegetation removes a significant portion of leachate before it can be collected. I intend to maintain a monthly collection schedule for a full year. I will also sample weekly during one month each season to capture variation on a finer temporal scale. Other funding sources have been obtained to extend this work.

Throughfall collectors were installed at eight sites at the end of April to coincide with the initiation of lysimeter collection. It was not possible to sample more than eight sites when following a weekly sampling schedule. The ninth site can be instrumented with throughfall collectors now that a monthly sampling schedule has been established.

Since sampling has just commenced, I do not yet have results to present in this report. Samples will be analyzed for nitrate and ammonium concentrations within several weeks of collection. A full data set will not be available for analysis until next summer. I expect nitrogen concentrations in throughfall to be higher in more urban sites (i.e., sites with higher road densities and higher numbers of road crossings over streams) than less urban sites. I will calculate these urban indicators using GIS data obtained through the Center for Remote Sensing and Spatial Analysis at Rutgers. I also expect nitrogen concentrations in leachate to be higher in sites with higher thoughfall so that these data sets should show some correlation. I also expect to find more nitrogen in leachate from sites with dry/flashy hydrology compared with sites with more normal hydrology. I have already collected two to three years of hydrology data at all of these sites. Lastly I expect to find more nitrogen in leachate from sites with lower nitrogen cycling rates. I have already collected one year's worth of nitrogen cycling data to use for this comparison.

Seed Dispersal Dynamics in Restored and Intact Salt Marshes: Implications for Restoration Success

Basic Information

Title:	Seed Dispersal Dynamics in Restored and Intact Salt Marshes: Implications for Restoration Success	
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Focus Category:	Ecology, Invasive Species, Wetlands	
Descriptors:	None	
Principal Investigators:	Polly L. Hicks, Jean Marie Hartman	

Publication

1. Seed Dispersal Dynamics in Restored and Intact Salt Marshes: Implications for Restoration Success

- Polly L. Hicks, Ph.D. student, Graduate Program in Ecology and Evolution, Rutgers University, 93 Lipman Drive, Room 113, New Brunswick, NJ 08904
 Tel: 732-932-4320 Fax: 732-932-1940 E-mail: phicks@aesop.rutgers.edu
- 3. Dr. Jean Marie Hartman, Graduate Program in Ecology and Evolution, Dept. of Landscape Architecture, Rutgers University, 93 Lipman Drive, Room 113, New Brunswick, NJ 08904 Tel: 732-932-6785 Fax: 732-932-1940 E-mail: jhartman@rci.rutgers.edu

4. Amount Requested: \$5,000

5. Priority Issues:

With the passing of the 1987 Freshwater Wetlands Protection Act, New Jersev established one of the most stringent and protective wetlands regulations in the United States. However, New Jersey is still experiencing a substantial decline in its wetlands with a loss of over 15,798 acres since 1986 (Balzano et al. 2002). Many of these losses are due to the apparent failure of wetland mitigation, which is the compensation for unavoidable negative impacts to wetland habitat through the restoration or creation of other wetlands. A federal study by the National Research Council (2001) and a state-wide study through the NJ Department of Environmental Protection (Balzano et al. 2002) have found that mitigation practices are not achieving the goal of preventing wetland loss. Mitigation failure is, in part, due to gaps in our ecological understanding of these valuable ecosystems and lack of rigorous testing of restoration practices (NRC 2001; Balzano et al. 2002). Information about the success rate of various restoration approaches is primarily contained in monitoring reports and other gray literature, which are difficult to obtain. Restoration failures are almost never reported. Therefore, although much work has been conducted in the field of restoration, as a whole we have not increased our ability to determine when a particular restoration approach is appropriate. To make restoration more successful, methodologies must be rigorously tested in different systems to determine under what conditions they can be effectively implemented (Zedler 2000; Roman et al. 2002). Only through achieving a higher level of predictability can we ensure that New Jersey's wetlands and the integrity of our aquatic-based resources are protected.

The research outlined in this proposal aims to critically examine one restoration approach (natural colonization) and its application to salt marsh restoration. Natural colonization is often incorporated into restoration activities because it helps to ensure the genetic diversity and health of a restored site. Starter populations from seeds or planted seedlings have the potential for reduced genetic diversity (Montalvo et al. 1997). Planting of horticultural seedlings has the greatest risk for lowered genetic diversity because the seedlings are raised in unnatural conditions that can shift the allele frequencies (Montalvo et al. 1997). Substantial genetic diversity is critical to the long-term survival of plant populations.

The main pathway for salt marsh colonization is thought to occur through secondary dispersal of seeds by tidal forces. Secondary dispersal is the additional movement of the seed from its initial substrate due to an outside force; primary dispersal is the release of a seed from the parent plant to a substrate, such as land or water. Neither the application of natural colonization for restoration activities nor the factors that influence secondary dispersal in salt marshes have been well studied (Bakker et al. 1996; Palmer et al. 1997; Zedler 2000). In my research, I will quantitatively examine seed input, germination and secondary dispersal to assess the influence of these important, but poorly understood, salt marsh dynamics on restoration success in the New York/New Jersey Harbor Estuary (Estuary). The methodologies developed and information collected in this project can be used by agency personnel and restoration practitioners to enhance their understanding of salt marsh restoration and the factors that influence success. This research therefore falls under the New Jersey Water Resources Research Institute's (NJWRRI) research priority I. Integrity of aquatic and water-associated ecosystems.

Salt marshes are characterized by low floral diversity and distinct vegetation zones that occur within specific tidal ranges (Mitsch and Gosselink 2000). Because of the relatively simple vegetation community, salt marshes are considered by some as one of the more predictable wetland habitats to restore (Zedler 1995; NRC 2001). However, these restorations still suffer from the trial and error approaches that plague all wetland restorations (Zedler, 1995). Typically for a restoration, tidal hydrology is restored and then either the site is left to be colonized, or only the dominant plant species, which is *Spartina alterniflora* in the mid-Atlantic region, is planted with the idea that seeds of other species will be carried in by the tide.

The incorporation of natural colonization has been successful for some restoration projects (Onaindia et al. 2001; Eertman et al. 2002; Thom et al. 2002; Williams and Orr 2002). However, natural colonization rates have not been carefully quantified or examined. In their comparison of the species composition of a 25 and 35-year-old restored salt marsh and a natural marsh, Onaindia et al. (2001) noted that the restored sites had only half of the native species contained in the natural system. They speculated that limited dispersal was the cause of the decreased species richness in the restored marshes. Kudoh and Whigham (2001) conducted a genetic analysis of standing populations of *Hibiscus moschuetos*, a salt marsh forb, in marshes close to and far from the main channel. They found that *H. moschuetos* populations in marshes adjacent to the channel were more closely related than the more isolated marshes because there was a greater mixing of seeds via secondary dispersal. This indicates that the location and connectedness of a restored marsh to seed sources is an important influence on its potential colonization.

Predicting the amount of secondary dispersal that is likely to occur at a restoration site is difficult because the basic ecology of secondary dispersal through tidal flushing in salt marshes has not been well researched. The few studies that have directly examined the issue of tidal transport in these marsh systems have produced conflicting results. While Koustaal et al. (1987) documented the long distance, mass transport of seeds from a marsh interior; Rand (2000) found little indication of secondary dispersal. Huiskes et al. (1995) conducted the most comprehensive examination of secondary seed dispersal via tidal flushing. They found that the direction and magnitude of secondary dispersal depended, in part, upon where in the marsh system the seeds were dispersed. Huiskes et al. also determined that although seeds moved out of the marsh in large numbers, very few seeds moved into the marsh system on the incoming tide. Seed bank studies of salt marsh systems have produced similar conflicting results. Several studies found well-mixed, species-rich seed banks that contrast the zonation patterns of standing vegetation indicating secondary dispersal (Hopkins and Parker 1984; Baldwin et al. 1996); however, others found seed banks to strongly reflect the standing vegetation (Hutchings and Russell 1989; Rand 2000; Egan and Ungar 2000).

Based on these dispersal and seed bank studies it appears that secondary dispersal of seeds by tides may, under certain conditions, allow for natural colonization of salt marshes. However, in order for restoration practices that rely, even in part, on natural colonization to be successful, secondary dispersal of seeds in a tidal system must be comprehensively studied under a variety of different landscape settings. In cases where natural colonization is relied upon as the sole means for vegetation re-establishment, lack of appropriate secondary seed dispersal might result in a failed restoration. On the other hand, if active planting is carried out for only one species the overall species diversity within a region may decline if other species cannot naturally colonize restored sites.

6. Specific Objectives of the Study:

The goal of this research is to critically examine natural colonization and the ecological processes that influence its successful implementation. More specifically, I will test the following hypotheses:

- Some secondary dispersal is occurring within restored and intact marshes.
- Species composition of incoming seeds from secondary dispersal processes is primarily determined by adjacent habitats and not the larger estuary.

To test these hypotheses, I will quantitatively examine seed input, germination and secondary dispersal in restored and intact salt marshes within the Estuary and assess how the surrounding community influences the species composition of the seed input. If natural colonization via secondary dispersal is to be successfully applied to restorations, it is insufficient to only determine whether or not secondary dispersal is occurring. One must determine what conditions, such as proximity to sources, enable seeds of desired species to disperse into a restored site. The methodologies developed and information collected in this project can be used by agency personnel and restoration practitioners to improve restoration success.

7. Research Methods, Experimental Design and Expected Results:

The proposed research will be conducted within two watersheds of the Estuary, including the Hackensack Meadowlands. Between 2002 and 2003, I examined secondary dispersal dynamics within a three-year-old restored marsh of the Hackensack Meadowlands. In order to determine if the findings of this earlier research result from specific site conditions or are indicative of larger seed dispersal dynamics, this research must be broadened to include more sites within different watersheds. The Estuary is an ideal setting for studying restoration practices because it is a heavily degraded system with a long history of human disturbance. An examination of natural colonization patterns is particularly important to the Estuary because a majority of the wetlands in this region are dominated by *Phragmites australis*, an invasive wetland plant species that forms dense monocultures in which few plant species can exist (Windham and Lathrop 1999; Keller 2000). Therefore, the native plant populations that are potential seed sources for restorations are greatly diminished and may be far removed. Currently, there are multiple programs working within the Estuary to restore salt marshes because these habitats have the potential to significantly improve the environmental integrity of this New Jersey region. By providing these programs with critical assessments of a common restoration practice, my work can help to increase the success of these important efforts.

Within the two watersheds, I will select four restored marshes that can be grouped into one of four landscape setting categories: intact marshes and a low level of adjacent urban habitat, intact marshes and a high urban component, *Phragmites* marshes and a low urban component, and *Phragmites* marshes and a high urban component. Intact marshes are defined as marshes with healthy populations of desired salt marsh species, such as *Spartina alterniflora, S. patens, Juncus gerardii* and *Distichlis spicata*. Comparing the seed input of restored marshes adjacent to intact and *Phragmites* marshes will allow me to examine whether natural colonization of desired species is influenced by proximity to seed sources. A comparison of restored sites surrounded by low and high levels of urbanization will provide important information regarding the effects of urban seed sources on plant establishment in restored marshes. These categories represent the majority of landscape setting influences natural colonization is vital to future restorations in the Estuary and along the coast of New Jersey. In addition to the restored marshes, one intact marshe will allow for an assessment of whether restored marshes function differently from undisturbed marshes.

To critically examine the hypotheses that secondary dispersal is occurring and that local landscape settings have a dominant influence on the species composition of the seed input, I will:

- characterize the seed input of each site,
- determine the influence of secondary dispersal and landscape setting on seed input, and
- experimentally investigate seed dispersal patterns within restored and intact marshes.

Seed Input Characterization: A characterization of seed input provides information regarding both primary and secondary dispersal and therefore can provide detailed information regarding the movement of seeds by tidal flow (Leck and Simpson 1994). At each site, six transects will be randomly established across the marsh surface, running perpendicular to the channel. Along each transect two permanent, 1-m² plots will be established with one plot in the low marsh and the other in the high marsh

zone. To ensure that the habitat zones among the individual marshes are comparable, the low and high marsh zones will be identified by elevation and flooding frequency. Sampling both the low and high marsh zones allows for the detection of differences in dispersal patterns across the elevation gradients.

To monitor seed input, a seed trap will be placed in the center of each permanent plot. Seed traps will be constructed of two 20 x 20 cm pieces of coarse burlap on top of a piece of weed exclosure cloth. The coarse burlap will trap seeds with hairs or hooks, while the exclosure material prevents small seeds from falling through the burlap. Traps will be secured to the marsh surface with wires. In June 2004, the traps will be placed in the sites, which is well before natural seed set of most marsh species. The traps will be replaced in October and collected in March before germination starts. Replacement of the traps maximizes seed input and minimizes deterioration of trap material. Collected seeds will be grown out for identification purposes. A majority of salt marsh plants require cold stratification to break dormancy (Baskin and Baskin 1998); therefore, to maximize germination, seeds will be cold stratified and then placed in a greenhouse for four months. Traps will be kept damp with freshwater because salt marsh plants have higher germination rates when raised under freshwater conditions (Bakker et al. 1985).

Influence of Secondary Dispersal: The standing vegetation found within each permanent 1-m² plot, will be sampled during the summer of 2004. Each species found within a plot will be identified and its percent cover recorded. The 2004 standing vegetation data will be compared against the seed input data using a similarity index to determine if the input is the result of local or wide spread dispersal patterns following similar analyses used by Hutchings and Russell (1989). If secondary dispersal is occurring, I expect to see a low similarity index between seed traps and standing vegetation. Multivariate analyses will be used to compare the composition of new species being brought into the sites via secondary dispersal to determine if landscape setting affects the composition of the seed input. Multivariate analyses will also be conducted using the 2004 standing vegetation data to determine how the sites are separating out and if differences among sites are due to differences in landscape setting.

Secondary Dispersal Patterns: Within each watershed, one restored marsh will be randomly selected as a site for conducting a direct seed dispersal experiment. Both intact marshes will also be used in this experiment. At three different locations within the sites, I will release 10,000 *Spartina alterniflora* seeds in the high and low marsh zones. Seeds released in each marsh zone will be dyed a distinct color so that they can be traced back to their release point and will not be confused. The seeds will be placed in a small pile at the center of a plot during low tide. Each plot will have eight 2-m long transects running from its center, with adjacent transects at a 45° angle from each other. After two weeks, the number of seeds found every 20 cm along the transects will be recorded. From this data I will estimate the total number of seeds remaining within the plot and generate a dispersal shadow that will provide information about the directionality of secondary dispersal. This experiment will be conducted during the spring tides of September and October, which are the highest tides that occur during natural seed release. Data from the restored and intact marshes will be compared to determine if restored and intact marshes have similar seed dispersal patterns and whether secondary dispersal is occurring at the local or large scale level within these different marsh types. This experiment will provide critical information about how intact and restored marshes are functioning as potential seed sources for future restorations and for the Estuary.

In addition to these proposed activities, I will conduct a second round of vegetation monitoring in the summer of 2005. As with the 2004 data, the 2005 vegetation data will be compared to the seed input. The data produced from this comparison will provide important information as to how actual field conditions are influencing the vegetation community and establishment of new species at the sites. Additionally, by comparing the standing vegetation from year to year with the seed input data, I will be able to assess whether the seed input is reflective of and predictive for changes in the vegetation community. The research outlined in this proposal will provide novel information regarding the occurrence and influence of secondary seed dispersal in restored and intact salt marshes. Funding by the NJWRRI, will assist me in taking my research to the next level and produce valuable information that can help make restoration activities more successful and thus help protect the integrity of New Jersey's wetlands.

8. Progress Report Regarding Prior NJWRRI Funding:

I am a recipient of a 2003 Graduate Student Grants-in-Aid from NJWRRI. With this funding I was able to critically examine seed dispersal dynamics of a three-year-old restored salt marsh (the Site) in the Hackensack Meadowlands. Following similar protocols to my proposed research for FY 2004, I conducted an extensive characterization of the seed input, monitored the vegetation of plots before and after seed traps were released, and experimentally examined seed dispersal patterns within the Site. I have conducted a series of preliminary analyses and will be further analyzing the data over the upcoming months. Preliminary analyses indicate that secondary dispersal is occurring in the high and low marsh zones of the Site. However, the composition of seeds transported into the Site mainly reflects the communities adjacent to the Site and did not include many of the desired species that have populations in marshes located farther away from the Site, such as Spartina patens, Distichlis spicata and Juncus gerardii. The seed input and standing vegetation of the Site was primarily composed of annual species that are common but not dominant. Unlike an intact salt marsh of the mid-Atlantic region, the typical dominant clonal graminoids comprised a relatively small portion of the standing vegetation. Spartina alterniflora, which was repeatedly planted, did occur in scattered populations across the Site, but was not dominating the Site. In order to determine if these trends result from specific site conditions or are indicative of larger seed dispersal dynamics, this research must be broadened to include more sites within the NY/NJ Harbor Estuary.

I have made two presentations on my research supported by the 2003 Grant-in-Aid award. In October, I presented some of my results in a poster format at the Meadowlands Symposium held in Lyndhurst, NJ. Then in November, I gave an oral presentation regarding this research at the 2003 annual international conference of the Society for Ecological Restoration.

It should be noted that when I applied for the 2003 Grants-in-Aid award I was a Master's student at Rutgers University. I have recently decided to expand my research on seed dispersal in restored marshes by pursuing a PhD. This decision is based on strong encouragement from my committee and a desire to study these important dynamics at a more in-depth level.

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Use of stable isotope ratios of mercury to track and differentiate between sources of mercury pollution

Basic Information

Title:	Use of stable isotope ratios of mercury to track and differentiate between sources of mercury pollution	
Project Number:	2004NJ73B	
Start Date:	3/1/2004	
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Focus Category:	Methods, Toxic Substances,	
Descriptors:	Mercury, stable isotope fractionation, mercuric reductase	
Principal Investigators:	Krittee Krittee, Tamar barkay	

Publication

- 1. Kritee K., Bjorn Klaue, Tamar Barkay & Joel Blum, Mercury isotopic fractionation observed during the reduction of Hg(II) to Hg(0) by the bacterial mercuric reductase, presented at The 7th International Conference on the Mercury as a Global pollutant, RMZ Materials and Geoenvironment. Mercury as a global pollutant, Ljubljana, Slovenia (June 2004), Vol. 51, No: 2, 1154-55.
- 2. Kritee K., Bjorn Klaue, Joel D. Blum and Tamar Barkay, Biological Hg isotope fractionation presented at the 15th International Goldschmidt conference, Idaho. Geochimica et Cosmochimica Acta Vol. 69, Issue 10, Supplement 1, Page A708 (15 May 2005)

Document 2 Project Information: Ongoing project

Problem and Research Objectives:

Mercury (Hg) is a toxic and bioaccumulative trace metal with complex biogeochemistry. Total Hg deposition in the state of New Jersey (NJ) exceeds 600 Kg/yr. But the air emissions of mercury from within NJ do not appear to account for the majority of the deposition in the state. Elemental mercury (Hg⁰) has a half-life of about a year, it can travel long distances across the globe before its atmospheric deposition. Therefore, New Jersey Mercury Task Force (NJMTF, 2002) recommended that tools, which can be used to estimate of the relative contribution of in-state sources and out-of-state sources be maintained and enhanced. Once deposited, the speciation (and toxicity) of Hg depends on which transformations of mercury are dominant in that environment. Therefore, the task force also emphasized the need to develop tools to track the fate and transport of Hg in the environment. This project addresses the question: 'Can Hg stable isotope ratios be used to track the fate and transport of Hg in a given environment. This project addresses the question: 'Can Hg stable isotope ratios be used to track the fate and transport of Hg in a given environment. This project addresses the question: 'Can Hg stable isotope ratios be used to track the fate and transport of Hg at a given site, and distinguish between biological vs. non-biological transformations of Hg?'

Background

Toxicity: From among different species of Hg, methylmercury (CH₃Hg[I] or MeHg) is of the most concern to public health because of its ability to get biomagnified and bioaccumulated (upto 10^7 times) in fishes (Mason *et al.*, 1995; Barkay, 2000). Exposure to MeHg during fetal and neonatal periods effects motor skills such as walking and speech, and may cause mental retardation and so freshwater fish with Hg content of more than 1.5 ppm can should not be eaten (Brigham *et al.*, 2003).



Figure 1. The biogeochemical cycle of Hg. Solid arrows indicate uptake or transformation of Hg and hollow arrows indicate transport pathways. The width of hollow arrows reflects the relative importance of different fluxes. Scheafer *et al.*, 2002.

Need to identify sources of pollution: Since 69-80% of Hg deposition in NJ is contributed by human activities and can be controlled, the NJMTF recommended that NJ adopt strategies to achieve a 65% reduction in air emissions of Hg from within NJ by 2011 (NJMTF, 2002). But even if local input of Hg to our state's atmosphere is decreased, transport of Hg⁰ from global sources can continue to pose threat to NJ's ecosystems. Policy formulation and enforcement requires that we know which sources (geogenic vs. anthropogenic; biological vs. non-biological, in-state vs. out-of-state) and which processes (new deposition vs. mobilization of old Hg) are contributing to Hg pollution in a given environment (Fitzgerald, 1993; Rudd, 1995). Unfortunately, the existing probabilistic models for estimating relative contribution of local vs. global sources of Hg predict a highly variable (30-70%) contribution of local sources in NJ and elsewhere (NJMTF, 2002; Rob Mason, *personal communication*).

Need to study fate and transport of Hg: As depicted in Figure 1, once deposited into aquatic environments, Hg[II] is transformed to different species of Hg, both by biological and nonbiological processes (Fitzgerald, 1993; Barkay, 2000). Aerobic microorganisms which have capability to make an enzyme called mercuric reductase, reduce Hg[II] to Hg⁰. This process leads to loss of Hg from the immediate vicinity of the microorganism but adds to the global pool of gaseous mercury. Anaerobic sulfate reducing bacteria cause methylation of Hg [II] to MeHg by non-specific mechanisms (Barkay 2003). Reduction and methylation can also occur abiologically (Morel, 1998). Humic substances in the soil/sediment can methylate and reduce Hg[II] in the presence of catalysts like Fe and Mn or sunlight. But the relative importance of these non-biological transformations of Hg is a contested issue (Barkay, 2003). It can be extremely useful to determine which pathway (biological vs. non-biological) whether MeHg in a given environment is being synthesized biologically vs. non-biologically, thus helping to direct remediation efforts in an appropriate direction.



Potential use of Hg stable isotope ratios (HSIR): Stable isotope ratios of sulfur, carbon, nitrogen and lead have been used to recognize the source of pollutant or distinguish between manmade or biological source since the early 1970s. Hg has seven naturally (non-radioactive) occurring stable isotopes and this study intends to explore the possibility of using HSIR to differentiate track and between different sources of Hg. Klaue and Blum (2000) have developed a coldgeneration multi vapor collector

inductively coupled plasma spectrometry (MC-ICPMS) method, which has allowed them to get consistent high precision Hg isotopic ratio measurements. In order to successfully use HSIR to determine the source of Hg in a given sample, it needs to be determined how can the isotope ratio unique to a source be modified by bacterial processes. Stable isotope ratios can also be used for tracking the fate and transport of Hg in an environment. Determining isotopic fractionation (see below) by pure cultures of Hg transforming microbes will be an important

step in that direction. The knowledge of extent of fractionation by microbial communities can tell us how can the isotope ratios change during Hg's biologically mediated cycling.

Specific objectives: 1) Optimization of experimental setup to determine SIF during reduction of Hg[II] to Hg^0 by a pure culture of bacteria possessing mercuric reductase. 2) Determination of the effect of temperature, concentration of substrate mercurial, the extent of reaction completed and electron donors on SIF during the bacterial reduction 3) Determination of the extent of SIF during reduction of Hg[II] to Hg^0 in a contaminated natural water sample in NJ.

Methods





Hg(II) reduction by a pure culture NIST 3133 was used as a source of 3 µM (600 ppb) Hg(II). Hg⁰ volatilized during the growth of *E.coli*/pPB117 cells at 37°C (or 22°C) in M9-based minimal media and was purged into a trapping solution by air stripping (Fig. 2a & 2b). In order to determine the change in isotopic composition as a function of the extent of the reaction, traps were replaced every 30-40 min for a period of 320 min (and every 90 minutes for a period of 900 minutes for the experiment $22^{0}C$) at to collect products

corresponding to different stages of the reaction.

Hg reduction by naturally occurring bacteria : NIST 3133 was added to water samples from an uncontaminated source after a 4 day long pre-exposure and Hg⁰ produced was purged into a trapping solution (See Fig. 2b & 4). 250 ppb NIST was added to the control given no exposure.



MC-ICPMS analysis

Sample introduction: Cold vapor generation was employed using Sn(II) reduction. The cold vapor sample introduction has a >99% efficiency and generates a signal of ~600 mV/ppb at а sample consumption rate of 0.75 mL/min. Precision: Fractionation was measured relative to the NIST 3133 Hg standard run before and after each sample and data are presented as δ^{202} Hg/¹⁹⁸Hg (hereafter δ^{202} Hg). Typical in-run precision of better than ±0.05‰ (2 σ) and external reproducibility of δ^{202} between NIST 3133 and a secondary standard was ±0.08‰ (2 σ). The kinetic fractionation factor (α) was determined from the results of our experiments using the Rayleigh Distillation Equation: $R_{Vi}/R_{Lo} = (1/\alpha) f^{(1/\alpha - 1)}$

Principle findings and Progress report:

<u>At 37⁰C</u>, Hg(II) undergoes mass dependent Rayleigh fractionation (Fig. 3a) with fractionation factor (α) = 1.0006 +/- 0.00005 per amu during its reduction to Hg⁰ by *E. coli*.



<u>At 22^oC</u>, preliminary estimation indicates that $\alpha \sim 1.0015$

For Manipulated naturally occurring bacteria: When Hg^0 was produced after being preexposure to Hg(II) conc. of 250 & 175 ppb: 100% of surviving bacterial cells were Hg resistant & $\alpha \sim 1.0006$ (similar to pure culture) was observed. But at low or no pre-exposure: Much lower % of total cells (10%) were Hg resistant & lower extent of fractionation (Fig. 5a and 5b) was observed.

Conclusions:

- Systematic Hg stable isotope fractionation does happen, both in pure cultures of bacteria and naturally occurring bacterial consortia!
- Hg is the heaviest metal for which biological fractionation has been detected to date. In spite of the reduced % mass spread of its isotopes and increased molecular weight, the extent of fractionation found lies in the same range as for much lighter elements (Table 1).

	Avg. Mol. Weight	% mass spread	Maximum Range of δ (‰/amu ⁾	Maximum reported α/amu
Fe	56	7	2**	1.0015**
Se	80	10	3#	1.003#
Мо	96	8	1.7##	1.002##
Hg	200	4	2	1.0015

Table 1. Comparison^{*} of the extent of fractionation observed for Hg with other redox-sensitive elements undergoing fractionation^{5,6}.

 \ast This is a crude comparison & does not include fractionation due to amplifying processes such as iterative distillation or chromatgraphy.

** Maximum range of isotopic variation (relative to standard) reported for low temperature processes occurring either in nature & under laboratory conditions. Eg. $\delta^{56/54}$ Fe in natural samples varies from ~-3 to +1 making the max. range ~2‰/amu⁵. Max. α for $^{56/54}$ Fe is 1.003 for non biological redox eqm. of Fe(III) and Fe(II)⁵.

Max. ϵ ~13‰ for $^{80}Se/^{76}Se$ during various Se transformations (or 3.25‰/amu). ϵ = 1000*(α -1)(Johnson & Bullen⁶).

$\delta^{97/95} \text{Mo}$ varies between -0.9 to +2.5 for natural samples (Anbar6).

- Use of Hg isotope ratios for identifying sources and sinks, in situ pathways leading to its toxicity, and/or the nature and evolution of redox reactions in both modern and paleo environments is plausible.
- Future work will determine how the change in physico-chemical parameters (T, pH, edonor etc.) can change the extent of fractionation during Hg(II) reduction and other Hg transformations.

HIgh resolution geophysical imaging as a novel method for noninvasive characterization of contaminated wetlands: application to Kearny Marsh

Basic Information

Title:	HIgh resolution geophysical imaging as a novel method for noninvasive characterization of contaminated wetlands: application to Kearny Marsh
Project Number:	2004NJ74B
Start Date:	3/1/2004
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Funding Source:	104B
Congressional District:	10
Research Category:	Water Quality
Focus Category:	Wetlands, Solute Transport, Water Quality
Descriptors:	Wetlands, Landfills, Geophysics, Electrical Resistivity, Magnetics, Contamination
Principal Investigators:	Lee Slater

Publication

- 1. Mansoor, N., Slater, L. and Artigas, F., 2005, Case history: High-resolution geophysical characterization of shallow-water wetlands, Geophysics, Submitted 01/20/05
- Mansoor, N. and Slater, L., 2004, Integrating high-resolution geophysical technologies with a GIS-based decision support system into evaluation and management of wetlands, 2004 Joint Assembly, American Geophysical Union (AGU), Canadian Geophysical Union (CGU), Society of Exploration Geophysicists and Environmental and Engineering Geophysical Society, May 17-21, Montreal, Canada, Abstract NS13A-02
- Mansoor, N., 2005, High-resolution geophysical characterization of shallow-water wetlands, Meadowlands Environmental Research Institute Spring 2005 Seminar Series, May 4 2005, http://meri.njmeadowlands.gov/2005seminar.html

PROJECT INFORMATION

Problem

Geophysical technologies permit non-invasive assessment of physical properties of the subsurface. These physical properties are a function of the pore fluid composition and hence sensitive to contaminant concentration. Wetland sediments and pore waters are often cumbersome to sample directly. Geophysical technologies are increasingly used on land for rapid, non-invasive, environmental contamination assessment. I propose to develop data acquisition, processing, interpretation, and integration protocols to permit effective transfer of state-of-the-art geophysical technologies to the study of shallow water wetlands. I argue that geophysical technologies, hitherto rarely utilized by wetlands scientists (primarily ecologists, geochemists and hydrologists), can significantly improve understanding of shallow-water wetland environments.

Research Objectives

The project has four primary research objectives designated A-D below. A. Advancement of the implementation of geophysical technologies in wetland environments from shallow-water boats: We propose the use of a four person paddle boat as used for recreation on small lakes/ponds as a "research vessel" for geophysical studies in wetlands. Advantages of these boats include: (a) very shallow draft permitting operation in less than 1 ft standing water (b) adequate space for two persons plus high accuracy GPS unit, geophysical instrumentation, surface water quality probes, and laptop (c) all plastic construction minimizing interference of the boat with geophysical measurements (d) hands-free control permitting operation of geophysical instruments whilst surveying.

B. Development of a protocol for the integration of geophysical datasets within a 3D and 4D spatial GIS framework: High data density obtainable with state-of-the-art geophysical instrumentation will result in spatially extensive 3D and 4D datasets. Geographical information system (GIS) databases are appropriate for managing and visualizing multiple types of data, including high-resolution geophysical data as to be obtained in this study. We will determine how spatially and temporally extensive geophysical data can be organized within a wetland GIS to integrate information into a coherent georeferenced framework suitable for analyses and decision making.

C. Implementation of high resolution geophysical imaging for monitoring solute release from landfills fringing on wetlands: We will investigate how electrical imaging can noninvasively delineate and temporally monitor contaminant plumes entering wetlands. D. Concept application and testing in Kearny Marsh, Hackensack Meadowlands, New Jersey (Fig. 1): We will adopt this integrated geophysical/GIS approach to (a) evaluate the primary sources contributing to pollution of Kearny Marsh; (b) determine the distribution of these pollutants within the marsh; (c) assess seasonal/hydrological controls on pollutant fluxes. Geophysical data interpretation will be constrained by direct sampling of sediments and pore waters, surface water chemistry and hydrogeological measurements of the groundwater surface around primary contaminant source zones.



Figure 1: Site map delineating Kearny Freshwater Marsh and showing identified potential contamination source zones. The yellow lines define the 2D electrical imaging lines and the blue grid outlines the proposed 3D resistivity monitoring experiment.

Methodology

The work to date has involved five primary tasks: (1) reconnaissance geophysical and surface water surveys of Kearny Marsh to rapidly assess likely contaminant source zones; (2) water and sediment sampling and geochemical analyses; (3) laboratory electrical measurements to determine the sensitivity of electrical parameters to heavy metal contaminants in the marsh sediments; (4) time-lapse electrical resistivity imaging along a number of monitoring transects adjacent to suspected contaminant source zones; (5) integration of geophysical datasets within a GIS framework for data management and visualization.

Reconnaissance geophysical and surface water surveys:

We utilized an all-plastic (excluding the steering mechanism) four-person paddleboat, typically used for recreation on small lakes/ponds, for rapid acquisition of geophysical data in shallow water wetlands (Fig. 2). The shallow draft (approximately 0.3 m) of this boat is ideal for operation in such wetlands. The paddleboat was equipped with the following instrumentation: a high precision Trimble[®] differential GPS unit (location accuracy = \pm 25cm), digital surface water quality probe (temperature, pH, electrical conductivity, salinity, total dissolved solids, dissolved oxygen, turbidity and water depth), digital magnetic gradiometer or digital terrain conductivity meter and a waterproof laptop.

All instrumentation was programmed to automatically record a measurement every two seconds during surveys. Geophysical data acquisition rates exceeded 10 km of line (+ 12,000 measurements) per eight-hour field day.



Figure 2: Paddleboat in operation on Kearny marsh showing on-board instrumentation. Note: both the magnetic gradiometer and the terrain conductivity meter are shown for illustration purposes only; Data are collected on separate surveys to avoid interference between instruments.

The EM31 terrain conductivity meter was mounted to the paddleboat with a fixed orientation perpendicular to the survey direction (Fig. 2). Due to the shallow water depth, the EM31 response primarily reflects the electrical conductivity of the sediments, as we shall show. A Scintrex EnviTM gradiometer was employed with the sensors mounted 1.5 m behind the boat from a PVC frame in order to avoid interference from the metallic parts of the steering mechanism and other equipment in the boat. Surface water parameters were measured simultaneous to geophysical measurements using a HydrolabTM probe mounted to the front of the paddleboat. The measured parameters were surface water electrical conductivity, pH and water depth. The electrical conductivity of the surface water helps constrain any dependence of the EM31 terrain conductivity measurement on the surface water layer. All terrain conductivity, gradiometry and surface water chemistry data were recorded every two seconds whilst in survey. Data from each survey period were spatially referenced using the synchronized time stamps provided by each instrument and the GPS unit.

Sampling and geochemical analyses:

We collected twenty eight surface water and bottom sediment samples to investigate the cause of terrain conductivity variation recorded in our reconnaissance surveys. The sediments samples were obtained using an AMS extendible lake sediment corer equipped with a drop hammer without rotary operation to minimize disturbance. The samples were collected directly into plastic liners placed into the coring device and then sealed with plastic end caps to prevent water evaporation. Depth of sampling ranged between 0-60 cm from the top of the marsh sediments. Sample locations were selected to investigate trends in the terrain conductivity data. Pore waters were extracted from the samples by centrifuging and filtering the liquid with a pressure vacuum.

The bottom sediment samples were prepared and analyzed using the MERI labs during October and November (2004) in accordance with the EPA sampling and preparation standards. Major heavy metals as well as major anions and cations were analyzed for both the sediments and the surface water. Furthermore, the pore water extracted from the sediment samples were also analyzed for major cations and anions as well as iron content. Pore water extraction was performed by centrifuging each sample and then by applying a high pressure vacuum for water extraction. The sediment samples were acid-digested in an OI-7295 microwave digestion system. A Varian SpectrAA-220 fast sequential Atomic Absorption Spectrometer was used for all chemical analysis. Quality control was performed by analyzing the reagents, blanks, standards and duplicate samples for each sampling preparation event. Recovery percent for the sediments samples ranged from 96 and 103% and with a maximum error of $\pm 3.5\%$. Tables 1 through 4 represent the detailed results of the chemical analysis of the different matrices.

Laboratory electrical measurements:

We measured the low-frequency (complex conductivity) electrical properties of the twenty eight sediment samples obtained. Four electrode measurements were conducted with a dynamic signal analyzer (DSA). Current was injected at the stainless steel mesh electrodes and sample voltage recorded using Ag-AgCl electrodes. A pre-amplifier was used to boost the input impedance on the voltage channel to approximately 10⁹ ohms in order to avoid current leakage into the external electrical circuit. Any residual frequency and sample resistance dependent phase response resulting form the external circuitry was removed by performing calibration measurements of pure electrolyte solutions. The phase shift and conductivity magnitude for the samples were determined relative to a reference resistor for forty measurement frequencies spaced at equal logarithmic intervals from 0.1 to 1000Hz.

Time-lapse electrical imaging

We established six transects for time-lapse electrical imaging designed to investigate solute transport from suspected contaminant source zones (Fig. 1). The first dataset was collected in September, 2004 and subsequent datasets were collected at biweekly intervals. This work is still in progress. Electrical imaging is performed by towing a floating electrode array from the back of the paddleboat. A state-of-the-art ten channel Syscal Pro (Iris Instruments, France) resistivity imaging system is used for data acquisition. We utilize the 'Sysmar' marine data acquisition software developed by Iris Instruments for continuous (in survey) electrical imaging. In this mode, 10 four electrode measurements are collected every two seconds as the survey progresses. A high precision Trimble® differential GPS unit (location accuracy = ± 25cm) is used for spatial location. Water depth and surface water conductivities are also collected during resistivity surveys. The electrical datasets are inverted for a two dimensional distribution of resistivity along the survey line using the RES2DINV software that has a built in option for processing continuous marine datasets collected from floating electrode arrays. The RES2DINV allows also in integrate the bottom sediment profile (elevation) as well as the conductivity of the surface water layer minimizing artifacts in the data inversion.

GIS integration and visualization

We used a GIS database to manage, process, visualize and interpret the high-resolution geophysical data. This database incorporates previous data, maps, aerial photographs and geophysical data into an analytical environment, permitting easy modification and updating with the acquisition of additional data. The database defines several spatial themes such as landuse zones, transportation networks and industrial facilities. Existing surface water and sediment geochemical data were converted to database tables and imported into our GIS framework.

Spatial image creation for the terrain conductivity and the measured surface water parameters was performed in GIS ArcEditor® 9.0 using the Inverse Distance Weight (IDW), a deterministic interpolation tool that weights the distance and magnitude of the surrounding points and determines the smoothness of the resulting surface. The best results from IDW are obtained when sampling density is high, as in the case of our geophysical/surface water quality datasets. The IDW method attenuates the relative influence of distant data points on the local interpolation. The barrier option in GIS, defining linear features with no z value, was used to specify the location of features known to interrupt the surface continuity and appropriately limit the selected set of input sample points used for the interpolation. Barrier features were created by outlining the Phragmites Colomiza within the study area as well as the boundary of the marsh.

Principal Findings and Significance - Progress Report

Reconnaissance geophysical surveys

Our surveys reveal a detailed patterning in both the surface water chemistry and geophysical measurements of the subsurface of Kearny Marsh. The surface water conductivity image reveals highest values in the central and northern parts of the marsh and shows no evidence for a tidal connection to the marsh in the east/northeast corner of the marsh as suggested based on hydrological measurements. The surface water measurements also suggest that there is no significant surface water plume associated with the Keegan landfill.

The terrain conductivity image exhibits a distinctly different pattern from that apparent in the surface water image and illustrates that the geophysical measurement is sensitive to the sediment properties (Fig. 3a). Most significant is the region of high terrain conductivity mapped in the northeast corner of Kearny Marsh. This may reflect the presence of a groundwater plume emanating from the 1E landfill or it may alternatively indicate a lithological change with electrically conductive sediments towards the northeast. Available lithologic data do not indicate any significant lithologic change in the upper 3 m of sediment. Pore water conductivities measured from samples collected close to the metal junkyard and the baseball field are between 0.900 and 0.125 S/m, whereas sediment samples collected from the northeast corner of the marsh close to the 1E landfill exhibit the highest pore water conductivities ranging between 0.200 and 0.300 S/m (Fig. 3a). We therefore conclude that the high terrain conductivity values are likely due to the presence of subsurface contamination from landfill leachate, and not to lithological variations. The terrain conductivity data also indicate a possible groundwater plume from Section C of the Keegan landfill but restricted to the east (Fig. 3a). The pore

water samples collected around the Keegan landfill have conductivities ranging from 0.125 S/m west of the landfill to about 0.175 S/m to the east.



Figure 3: a) A spatial image showing: (1) the terrain conductivity (in S/m) generated from the highdensity geophysical data measurements, and (2) conductivity of pore water (in S/m) from 28 sediment samples, (b) magnetic gradiometer spatial image (in nT/m) generated from the point-based measurements taken within Kearny Marsh.

Sampling and geochemical analyses

The most significant results from the geochemical analyses on the samples and pore waters pertain to the heavy metal analyses. Consistent with previous studies in Kearny Marsh, our work has revealed high levels of heavy metal contamination in the marsh sediments. Our datasets suggest that the heavy metal concentration increases towards the 1E Landfill in the northeast corner of the marsh.

Laboratory electrical measurements

The laboratory electrical measurements have been completed but modeling of these datasets remains a work in progress. The initial results suggest that there is a relationship between the magnitude of the interfacial polarization of the sediments determined from Cole-Cole relaxation modeling and the total heavy metal concentration. However, the variation in the iron concentration between samples is a factor that influences the electrical measurements and complicates interpretation. This aspect of the work will be fully detailed in our final report.

Time-lapse electrical imaging

This work remains in progress. Our preliminary findings indicate that the resistivity images obtained along the established monitoring transects are consistent with our EM31 reconnaissance datasets. However, the resistivity images provide additional information on vertical resistivity structure that may relate to the shape of solute plumes. Furthermore, we hope that the electrical resistivity imaging at various time intervals will also reveal subsurface contaminant fluxes in response to hydrologic forcing. This aspect of the work will be fully detailed in our final report.

GIS integration and visualization

The GIS database and visualization has proven highly effective for managing and interpreting our geophysical datasets. A good example of its effectiveness is the evaluation of the magnetic gradiometry datasets collected as part of the reconnaissance geophysical surveys. This survey mapped the distribution of buried metallic debris within Kearny Marsh that reflects a legacy of land misuse and environmental degradation (Fig. 3b). Aerial photographs from 1969, when the landfill was operational, support this concept. These photographs were precisely rectified and spatially registered into the GIS environment for comparison with the geophysical data. The old boundary of the Keegan landfill and old access roads were identified and outlined. The gradiometer data were then overlain on the 1969 photograph. The resulting image shows that the gradiometer data appear to map the maximum operational extent of the Keegan landfill (Fig. 4). Water levels within Kearny marsh have increased since 1969, covering the edges of the Keegan landfill and changing its morphology as well. We also find that the gradiometer anomalies mapped peripheral to the Belleville turnpike and along the southern parts of the Keegan landfill are in alignment with access roads that existed in 1969. The gradiometer data therefore seem to reflect historical dumping associated with these access roads. Illegal dumping is also associated with marshland immediately proximal to the metal junkyard and the baseball field.



Figure 4: A multi layer spatial image showing (1) aerial view of Kearny Marsh in 1969, (2) the extent of the Keegan landfill during operational time and the trend of old roads are outlined, (3) magnetic gradiometer image with 50% transparency, and (4) current dry and vegetated areas within Kearny Marsh.

Fate of Brominated Flame Retardants in New Jersey Wastewater Treatment Facilities

Basic Information

Title:	Fate of Brominated Flame Retardants in New Jersey Wastewater Treatment Facilities
Project Number:	2004NJ75B
Start Date:	3/1/2004
End Date:	3/1/2005
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Congressional District:	6
Research Category:	Water Quality
Focus Category:	Waste Water, Treatment, Toxic Substances
Descriptors:	Flame retardants, polybrominated diphenyl ether, dehalogenation, sludge, wastewater
Principal Investigators:	Donna E. Fennell, Uta Krogman, Lisa Totten

Publication

- 1. Fennell, D.E.; F. Liu; M. Giacalone. 2004. Dehalogenation of polyhalogenated aromatics in cultures and enrichments. Proceedings of the American Chemical Society, Division of Environmental Chemistry, 228 th ACS National Meeting, Philadelphia, PA, August 22-26, 2004.
- 2. Zarnadze, A.; L. Totten; D.E. Fennell, D.E.; M. Giacalone; and U. Krogmann. 2004. PBDEs in the NY/NJ Harbor estuary. Proceedings of the American Chemical Society, Division of Environmental Chemistry, Poster presentation at the 228th ACS National Meeting, Philadelphia, PA, August 22-26, 2004.
- 3. Fennell, D.E.; F. Liu; E.-K. Son; A. Zarnadze; U. Krogmann; L.Totten. 2005. Biotransformation of halogenated contaminants in sludges and enrichments from municipal anaerobic digesters. Submitted to the SETAC North America 26th Annual Meeting, Baltimore, MD, November 13 17, 2005.

Problem and Research Objectives

Wastewater treatment facilities (WWTF) are called upon to prevent macropollutants such as organic compounds measured as biochemical oxygen demand, nitrogen and phosphorous from entering aquatic systems. Increasingly they are also expected to remove trace persistent, bioaccumulative and toxic chemicals (PBTs). This class of chemicals includes emerging pollutants such as the brominated flame retardants, polybrominated diphenyl ethers (PBDEs) (Betts, 2002) (Figure 1).



Figure 1. Chemical structure of polybrominated diphenyl ether (PBDE). The compound may contain up to 10 bromine substituents.

WWTF are the first line of defense in preventing contamination of aquatic systems by these compounds. WWTF influents commonly contain a multitude of PBTs, however little is known about their fate in the facilities. Like polychlorinated biphenyls (PCBs) and chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDD/Fs), PBDEs are hydrophobic, associated with organic matter, and are resistant to aerobic degradation. Thus these compounds tend to accumulate in wastewater treatment sludges (Hale et al., 2001; NRC, 2002; Litten et al., 2003). After these wastewater treatment sludges are treated to reduce pathogens and to stabilize them, in New Jersey, 41.3% are beneficially used in-state (e.g., in agriculture, in top soils distributed to the public and in landfill covers), and 30.9% are used beneficially out-of-state (NJDEP, 2003). Sludge treatment processes used in New Jersey include anaerobic digestion, aerobic digestion, lime stabilization, advanced alkaline stabilization, composting, pelletization and wet air oxidation. Little is known how the different sludge treatment processes affect the fate of PBDEs. Few data are available on concentrations of PBDEs in sewage, sludges and biosolids (treated sewage sludge) however, those that are available suggest a significant presence (Hale et al., 2001; Litten et al., 2003). We intend to document the presence and level of PBDEs in New Jersey sewage, sludges and biosolids from selected WWTF.

Anaerobic bacterial dehalogenation has been shown to be an effective method of removal or detoxification of halogenated environmental pollutants in groundwater, soils and sediments. Exploitation of dehalogenating bacteria for detoxification during anaerobic digestion of municipal wastewater treatment sludges may also be possible. We will determine whether environmentally relevant congeners of PBDEs are transformed or detoxified during one sludge treatment process—anaerobic digestion.

The specific objectives of the project are:

(1) Document the presence and level of PBDEs in New Jersey sewage, sludges and biosolids at selected WWTF.

(2) Document the ability of anaerobic digestion to dehalogenate/detoxify selected environmentally relevant congeners of PBDEs.

(3) Prepare a full proposal to the EPA and/or the National Science Foundation for a broader assessment of the life cycle of halogenated PBTs, including PBDEs in the wastewater treatment process from influent to final disposal.

Methodology

Analysis of PBDEs in Sewage, Sludge and Biosolids. Sewage influent, finished effluent, anaerobic digester sludge, and processed biosolids were obtained from four regional wastewater treatment facilities. The participating facilities treat various combinations of domestic and industrial wastewater with differing potentials for PBDE content. The samples were collected by WWTF personnel using in-place compositing protocols specific to each facility. Samples were collected over (at most) a 24-hour period and were stored at 4°C until pickup by Rutgers personnel. Samples were then stored at 4°C until processing. A detailed sample processing and extraction protocol (see description of the newly developed methods in **Principal Findings and Significance** section) was developed based on published methods for determination of PDBEs in sewage, sludges and biosolids (*Hale et al., 2001; Hyotylainen and Hartonen, 2002*).

Dehalogenation of Spiked PBDE in Anaerobic Digester Sludges. We monitored the transformation of PBDEs in batch studies of digester sludge from the NJ wastewater treatment facilities and in a highly enriched mixed culture originally started from, digester sludge from the municipal anaerobic digester in Ithaca NY and containing a known dehalogenating bacterium, *Dehalococcoides ethenogenes* strain 195 (*Fennell et al., 2004*). DecaBDE, one of the most commonly used PBDE formulations was used to spike sludge inoculated enrichments at levels slightly elevated over those that have been observed in sludges (*Hale et al., 2001; Litten et al., 2003*) to allow observation of activity.

Culture set up. Both the sludge inoculated bottles and the *D. ethenogenes*containing culture were set up for deca-BDE transformation using methods described previously for polychlorinated dibenzo-p-dioxins and furans (*Vargas et al., 2001*). Briefly, for sludges, triplicate sterile 160 mL serum bottles containing about 0.5 g of dry, ground, sterile sludge were prepared for each treatment. A 0.07 mL volume of toluene stock solution containing 2660 μ mol/L of decaBDE (0.5 μ mol deca-BDE) was added to each bottle in order to completely coat the sediment. For *D. ethenogenes*-containing culture triplicate sterile 50 mL serum bottles containing about 0.25 g of fine dry, sterile sediment were prepared for each treatment. A 0.5 mL volume of stock solution containing 1000 μ mol/L of decaBDE (0.5 μ mol deca-BDE) was added to each bottle in order to completely coat the sediment. All bottles were purged overnight with sterile anoxic nitrogen to remove the toluene carrier.

After bottle preparation, the NJ sludge enrichments were developed by diluting 10 mL sludge into 90 mL (i.e., 10% v/v dilution) of an anaerobic minimal salts medium (*Fennell et al., 2004*) into the bottles. The highly enriched mixed culture containing *D. ethenogenes* strain 195, was grown at 25°C as described previously (*Fennell et al., 2004*) on PCE and butyric acid. The mixed culture contained, through stoichiometric estimation based upon chloride release and the hydraulic (solids) retention time, approximately 16 μ g *D. ethenogenes* protein/mL (*Fennell et al., 2004a*) or about 10⁸ cells/mL. The culture (35 mL) was added to the prepared 50 mL serum bottles. In addition to decaBDE, some bottle sets were amended with alternate halogenated compounds tetrachloroethene (PCE) or 1,2,3,4-tetrachlorobenzene (TeCB). A mixture of organic acids and yeast extract were added periodically as a carbon/energy/hydrogen source. **Table 1** shows the bottle set up protocol for assessing decaBDE transformation.

	Treatments						
Bottle Set	Dehalococcoides-containing mixed culture						
Dottie Set	Electron Donors		Other Electron	Trace Nutrien	deca-BDE		
	(µM)		Acceptors (µM)	Thee Public	(μΜ)		
1 (killed)	None		None	None	13		
2	Butyrate (440) None		Yeast extrac	t 13			
3 (plus co-substrate)	Butyrate (440) PCE (110)		PCE (110)	Yeast extrac	t 13		
	NJ anaerobic digester sludges (10 % v/v)						
1 (killed)	None		None	None	5		
2	Lactate (100)/	None		Veast extract	5		
2	Butyrate (100			i cust extract	5		
3 (plus co-substrate)	Lactate (100)/	PCF (25)		Yeast extract	5		
5 (plus co-substrate)	Butyrate (100		101(25)	i cust extract	5		
4 (plus co-substrate)	Lactate (100)/	1	234-TeCB (25)	Veast extract	5		
	Butyrate (100	1,2,3,4-10CD (23)		i cast extract	5		

 Table 1. Enrichment protocol for assaying PBDE biotransformation by anaerobic enrichments.

Culture sampling. 1- or 2-mL samples of well-mixed bottle contents were withdrawn periodically using a sterile, anoxic glass syringe for PBDE analysis. 0.1 ml samples of gas headspace were withdrawn from the bottles periodically and analyzed for methane production and transformation of alternate halogenated compounds PCE and TeCB to monitor health of the enrichments.

Molecular analysis. NJ anaerobic digester sludges were examined for the presence of *Dehaloccoides* using polymerase chain reaction (PCR) analysis. Briefly, 1 mL of sludge was centrifuged at 15,000 g and the supernatant was discarded. Microbial community DNA was extracted from the pellet using a soil DNA extraction kit (MoBio Laboratories, Inc.). The community DNA was subjected to PCR using universal bacterial PCR primers (*Ahn et al., 2003; Fennell et al., 2004b*) and PCR primers specific for the 16S rRNA gene of *Dehalococcoides (Maymo-Gattel et al., 1997)*. The PCR product obtained using the universal bacterial 16S rRNA primers was subjected to re-amplification using the *Dehalococcoides* specific primer in a nested PCR procedure (*Fennell et al., 2001*).

Principal Findings and Significance (Progress Report)

The on-going project seeks to document both presence of PBDEs in wastewater and sludges, but also to document transformation capacity of anaerobic microbial communities. Primary results to date are development of a sampling, extraction and analysis protocol and initial results for microbial enrichments.

Sample and extraction protocols. Development of standard operating protocols (SOPs) for extraction and analysis of PBDEs has been completed. We modified published and in-house methods to create PBDE extraction and analysis SOPs tailored to microbial enrichments and WWTF samples. The draft SOPs are shown in **Figure 2**. Analysis of samples is on-going and will be reported at the conclusion of the project.

Microbial enrichments. We examined the biotransformation of halogenated pollutants by microorganisms in anaerobic digester sludges and microbial enrichments from sludges. An anaerobic digester mixed culture enrichment containing *D. ethenogenes* strain 195, did not dehalogenate decaBDE over a six month incubation period. In a related NJWRRI-funded project, the organism did not dechlorinate octachlorodibenz-*p*-dioxin however; it did dehalogenate a hexachlorodibenzofuran congener to penta- and tetra dibenzofuran daughter products. The tests with NJ sludges have been on-going for about six months. Tetrachloroethene added to the sludges was dehalogenated to trichloroethene and dichloroethene with no formation of vinyl chloride or ethene. On-going experiments are being analyzed to determine whether decaBDE is being dehalogenated by these sludges.

Molecular analysis. Dehalococcoides-like bacteria were not detected by direct or nested PCR analysis of community DNA using *Dehalococcides*-specific primers. The observed dechlorination pattern with PCE (trichloroethene and dichloroethene with no formation of vinyl chloride or ethene) suggests the presence of dehalogenating bacteria other than *Dehalococcoides*.

Discussion. Several dehalogenating bacterial isolates were originally obtained from anaerobic digesters. Our results to data suggest that one—*D. ethenogenes* strain 195—does not dechlorinate deca-BDE to any detectable extent in a 3 month incubation period. Although *Dehalococcoides* sp. was not detected in the four NJ WWTF sludges, initial activity on PCE suggests the presence of other dehalogenating strains. Results from on-going microbial dehalogenation tests and examination of PBDEs in WWTF samples will be reported at the conclusion of the project.

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Figure 2. PBDEs (and PCBs) Wastewater Treatment Plant Draft Sample SOP (working draft).

PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP A. Important Notes

- All procedures should be performed under low light. Wrap vials, flasks or separatory funnels in aluminum foil whenever possible.
- All glassware should be baked at 450°C overnight.
- All other surfaces that come into contact with the sample should be rinsed with either hexane or dichloromethane just prior to performing the procedure.

B. Wastewater Effluent Samples

1. Preparation

- 1) Rinse separatory funnels with dichloromethane (DCM, methylene chloride) and let dry under hood. Bake at 450°C overnight.
- Bake sodium sulfate (450°C for 4 hours). 2)
- 3) Open each triplicate I-Chem Jar and remove a well-mixed sample (100 to 150 mL) for determination of Total Suspended Solids and Volatile Suspended Solids according to Standard Methods 2540D and 2540E, respectively. [Note, volume for solids measurements is estimate, it may need to be adjusted to achieve 2.5 to 200 mg dried residue.]

2. Extraction:

- 1) Add surrogate standard ¹³C 183 PBDE (total PBDE measured previously in US effluent is approximately 29,023 pg/L ± 1490 (North, 2004), thus we should have about 18 ng PDBEs in the total sample (800 mLs) to be extracted) directly to sample jar using protocol in Table 1.
- Add PCB surrogates using protocol in Table 1 directly to sample jar.
- Transfer entire remaining sample (e.g., 950 mL 150 mL = 800 mL) to a 2-L separatory funnel. [Note, give the values 3) expected from North (2004) we should have about 18 ng PDBEs in the total sample to be extracted].
- 4) Add the same volume of dichloromethane (DCM) as sample to the empty sample jar to rinse, and then pour into the separatory funnel.
- Shake funnel every few minutes for 1/2 hr. 5)
- Collect extracted aqueous fraction and discard.
- Add 60 mL of milliQ water saturated with NaCl to separatory funnel and back extract to remove polar compounds from the DCM-shake every few minutes for 1/2 hr.
- Add 1 gram of sodium sulfate to empty rotovap flask (round bottom flask).
- Discard aqueous fraction from the separatory funnel. It is better to remove all water (and possibly lose a small amount of 9) DCM) than have residual water in the sample.
- 10) Remove DCM fraction to the rotovap flask containing the sodium sulfate.
- 11) Rinse separatory funnel 3 times with a small amount of hexane and allow all three washings to go into the rotovap flask with the extract and sodium sulfate.

3. Rotovap (Follow the Totten Lab Rotovap SOP in the PCBs SOPs document, note rotovap flask = round bottom flask):

- 1) Rinse empty rotovap flasks with hexane.
- Carefully pour extract and hexane rinse from original rotovap flask from extraction into new rotovap flask. If Teflon beads 2) and sodium sulfate are present in flask, do not let them reach the new flask during pour.
- 3) Rinse original rotovap flask 3 times with a small amount of hexane and transfer all three washings into the new rotovap flask (again do not let sodium sulfate or Teflon beads reach the new flask).
- Rotovap DCM extract to about 5 mL. (see SOP for rotovapping).
- Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial. 5)
- Rinse rotovap flask 3 times with hexane and add all three washings to the 12-mL glass amber vial containing the extract. 6)
- 7) Blow down the extract in the amber vial under nitrogen to about 1 mL.
- Store in vial in -20°C freezer until cleanup. 8)

4. Cleanup:

- 1) Perform clean up in accordance with PCBs SOPs on "Alumina Cleanun"
- Collect Fraction 1 (hexane, about 12 mLs) separately from Fraction 2 (hexane:DCM, about 12 mLs) in amber glass vials of 25 2) to 50 mL volume (covered with foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].
- DO NOT blow down the fractions. 3)
- 4) Store at -20°C until analysis.

5 GC/MS analysis

- 1) Spike Fraction 1 and Fraction 2 amber collection vials (containing about 12 mLs) with PBDE internal standard solution according to Table 1. [We will decide what to do about PCBs in these samples later.]
- 2) Transfer about 0.5 mL of each fraction to separate GC vials for analysis for PBDEs (GC-MS).

PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP (Continued)

C. Wastewater Influent

1. Preparation:

- 1) Rinse separatory funnels with DCM and let dry under hood. Bake at 450°C overnight.
- 2) Rinse filter apparatus with DCM and let dry under hood. Bake glass parts at 450°C??? overnight (not sure what the stoppers on the bottom portion of the filter apparatus can withstand).
- 3) Remove a well-mixed sample (15 to 20 mL) from each jar and determine Total Suspended Solids and Volatile Suspended Solids according to Standard Methods 2540D and 2540E, respectively. [Note, volume for solids measurements is estimate, it may need to be adjusted to achieve 2.5 to 200 mg dried residue.]
- 4) Bake glass fiber filters for collection of solids samples for subsequent extraction at 450°C overnight.

2. Collection of Solid/Liquid Fractions:

- 1) For each triplicate jar, remove triplicate 20 mL samples and filter through a 0.7 um, 47 mm glass fiber filters, let air pull through for a little while to dry off excess water. [Note: total PBDEs previously measured in US influent was 640 ng/L (North, 2004) thus triplicate samples of 20 mL should yield about 40 ng PBDEs]. Filter the triplicate volumes into the same flask. Wrap triplicate filters in foil together and freeze at -20°C until extraction.
- Save filtrate and carry through liquid-liquid extraction as described for effluent samples (See Section B and Section C.5 below).

3. Extraction of Solids Captured on Filters:

- Shove triplicate filters into Soxhlet apparatus.
- 2) Place a scoop of sodium sulfate and a scoop of Teflon chips in the bottom of each round bottom (rotovap) flask.
- Add surrogate standard ¹³C 183 PBDE to the Soxhlet according to the protocol shown in Table 1 (total PBDEs previously 3) measured in US influent was 637 ng/L (North, 2004), thus we expect a mass of PBDE of roughly 40 ng in our sample).
- Add PCB surrogates (PCB solution containing PCBs 23, 66, and 165) directly to Soxhlet according to Table 1. 4)
- Soxhelet extract with dichloromethane (DCM) for 24 hrs (see PCBs SOPs for Soxhlet protocols). 5)

4. Rotovap:

- 1) Rinse empty rotovap flasks with hexane.
- Carefully pour extract and hexane rinse from rotovap flask from Soxhlet extraction into new rotovap flask. If Teflon beads 2) and sodium sulfate are present in flask, do not let them reach the new flask during pour.
- 3) Rinse original rotovap flask 3 times with hexane and transfer all three washings into the new rotovap flask.
- Rotovap DCM extract to about 5 mL. (see PCBs SOPs for rotovapping). 4)
- Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial.
- Rinse rotovap flask 3 times with hexane and add all three washings to 12-mL amber vial containing the extract.
- 7) Blow down the extract in the amber vial under nitrogen to about 1 mL.
- Store in vial in -20°C freezer until cleanup. 8)

5. Cleanup

- Perform clean up in accordance with PCBs SOPs on "Alumina Cleanup" **1**)
- Collect Fraction 1 (hexane) separately from Fraction 2 (hexane:DCM) in amber glass 25 to 50 mL amber vials (covered with 2) foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].
- 3) DO NOT blow down the fractions.
- 4) Store at -20°C until analysis.

5. GC/MS analysis:

- 1) Spike Fraction 1 and Fraction 2 collection vials (containing about 12 mLs each) with PBDE internal standard solution according to Table 1. [We will decide what to do about PCBs in these samples later.]
- 2) Transfer about 0.5 mL of each fraction to separate GC vials for analysis for PBDEs (GC-MS).

7. Extraction of Liquid Fraction Generated by Filtration:

- Add surrogate PBDE standard 13C 183 PBDE and surrogate PCB standard (mass in filtrate would probably be about 1/100th of 1) that in the total sample, see section C.3.3 above) to filtrate in filter flask according to Table 1.
- Transfer entire sample (15 to 50 mL collected as filtrate during filtration of each of the influent aliquots) to a 500-L separatory 2) funnel
- Add 50 mL dichloromethane (DCM) to the filter flask. Swish it around
- Transfer DCM from filter flask to separatory funnel and perform extraction and cleanup as described above for Wastewater 4) Effluent in Section B. Add internal standard for PBDEs in the amount shown in Table 1.

Figure 2. PBDEs (and PCBs) Wastewater Treatment Plant Draft Sample SOP (continued).

PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP (Continued)

D. Sludges and Biosolids

1. Preparation: Make sure to do this under LOW LIGHT.

- Rinse mortars and pestles with dichloromethane (DCM, methylene chloride) and let dry under hood. Bake at 450°C overnight.
 Remove triplicate well-mixed samples (25 to 50 g) and determine Total Solids and Volatile Solids according to Standard
- Remove triplicate well-mixed samples (25 to 50 g) and determine Total Solids and Volatile Solids according to Standard Methods 2540G.
- 3) Weigh out ~5 g of biosolids into a mortar.
- 4) Add¹³C BDE 183 as surrogate to the biosolids/sludge according to Table 1 (total PBDEs previously measured in US sludges/biosolids was about 4000 µg/kg (North, 2004), thus we expect about 20 µg available for extraction in each sample).
- Add PCB surrogates according to Table 1 (250 µL of PCB solution containing PCBs 23, 65, and 166) directly to biosolids/sludge.
- 6) Add 5 g pre-baked sodium sulfate.
- 7) Mix gently using a pestle until a powder.

2. Extraction of Dried Sludges/Biosolids:

- 1) Transfer sodium sulfate dried sample to Soxhlet apparatus.
- 2) Place a scoop of sodium sulfate and a scoop of Teflon chips in the bottom of each round bottom rotovap flask.
- 3) Soxhelet extract with dichloromethane (DCM) for 24 hrs (see PCBs SOPs for Soxhelet protocols).

3. Rotovap:

- 1) Rinse empty rotovap flasks with hexane.
- Carefully pour extract and hexane rinse from rotovap flask from Soxhlet extraction into new rotovap flask. If Teflon beads
 and sodium sulfate are present in flask, do not let them reach the new flask during pour.
- 3) Rinse original rotovap flask 3 times with hexane and transfer all three washings into the new rotovap flask.
- 4) Rotovap DCM extract to about 5 mL. (see PCBs SOPs for rotovapping).
- 5) Transfer remaining extract (now mostly hexane) into a 12-mL amber glass vial.
- 6) Rinse rotovap flask 3 times with hexane and add all three washings to 12-mL amber vial containing the extract.
- 7) Blow down the extract in the amber vial under nitrogen to about 1 mL.
- 8) Store in vial in -20°C freezer until cleanup.

4. Cleanup:

- Label separate amber Fraction 1 and Fraction 2 vials for each sample. Add foil and cap to each vial. Get an empty tare weight for each vial.
- 2) Perform clean up in accordance with PCBs SOPs on "Alumina Cleanup"
- Collect Fraction 1 (hexane) separately from Fraction 2 (hexane:DCM) in amber vials (covered with foil and then capped). [Fraction 1 contains PCBs and PBDEs, Fraction 2 contains PBDEs].
- 4) After fraction collection, weigh each vial containing fraction plus foil and cap.
- <u>DO NOT</u> blow down the fractions.
- 6) Store at -20°C until analysis.

5. GC/MS analysis:

- Remove an aliquot of approximately 500 µL from each fraction collection vial using a pre-baked glass pipette and place each aliquot in a separate GC vial. Cap GC vial.
- 2) Quickly recap Fraction 1 and Fraction 2 amber collection vials and reweigh.
- 3) Calculate the percentage of the mass of analyte in the original fraction that the aliquot represents.

mass in allquot% = weight of fraction collection vial before aliquot removal - weight of fraction collection vial after aliquot removal x100 weight of fraction collection vial before aliquot removal

 Add the PBDE internal standard solution to the GC Vial containing the aliquot according to Table 1. [We will decide what to do about PCBs in these samples later.]

PBDEs (and PCBs) Wastewater Treatment Plant Sample SOP (Continued)

E. Laboratory Blanks

- 1. Fill Amber I-Chem jars with 800 mL of milliQ water (same source of water as used for any handling of the samples or reagents), cap and shake a little.
- 2. Carry through liquid-liquid extraction exactly as described for Wastewater Effluent (starting at Section B.2)

F. Standard Additions Protocol

Table 1. Surrogate and Internal Standard Additions for PBDE and PCB Extractions from wastewater treatment plant effluent, influent and sludges/biosolids.

Standards->	PBDE Surrogate Standard (¹³ C BDE 183)	PCB Surrogate Standard (PCBs 23, 66, 165)	PBDE Internal Standards (BDE 75)	*PCB Internal Standards (PCBs 30, 204)
Standard Stock Concentration for Influent and Effluent Samples→	50 ng/mL	200 ng/mL	20 ng/mL	200 ng/mL
Samples	Volume	Volume	Volume	Volume
Influent (residue on triplicate filters)	40 µL	10 µL	20 µL (F1, F2 no blowdown)	10 μL (F1)
Influent (filtrate collected from triplicate samples)	5 μL	5 µL	5 µL	5 μL
Effluent (liquid-liquid extraction)	40 µL	10 µL	20 μL (add to both F1 and F2 which have NOT been blown down)	10 μL (F1)
Standard Stock Concentration for Sludges/Biosolids→	5 µg/mL	200 ng/mL	20 ng/mL	200 ng/mL
Samples	Volume	Volume	Volume	Volume
Sludge/Biosolids	40 μL	250 μL	200 μL (add to an aliquot of F1 and F2 which have NOT been blown down)	250 μL (F1)

* To be determined

Wetlands in urban regions: connections among wetland structure, wetland function and regional water quality.

Basic Information

Title:	Wetlands in urban regions: connections among wetland structure, wetland function and regional water quality.	
Project Number:	2002NJ16G	
Start Date:	10/1/2001	
End Date:	7/31/2004	
Funding Source:	104G	
Congressional District:	6th	
Research Category:	Biological Sciences	
Focus Category:	Non Point Pollution, Water Quality, Ecology	
Descriptors:	denitrification, nitrogen retention, stream macrobiota, wetland function, water quality	
Principal Investigators:	Joan G. Ehrenfeld, Jonathan G. Kennen	

Publication

Wetlands in Urban Regions: Connections Among Wetland Structure, Wetland Function and Regional Water Quality"

Significance and Regional Importance:

The New Jersey Department of Environmental Protection has made watershed management the primary process for the protection of water resources throughout the state. Of the 20 watershed management units that have been designated, nearly half are primarily urban in land-use (www.state.nj.us/dep/gis/), reflecting the largely urban/suburban nature of the east coast, as well as many regions throughout the country. Watershed management requires knowledge of the functional relationship between landscape elements and water quality and quantity. While extensive data is available to demonstrate the deleterious effects of urban land-use on water resources, there is much less information concerning the ameliorative effects of natural areas, particularly wetlands, within urban regions. In fact, there is remarkably little known about the biotic integrity or functional capacity of urban wetlands, as well as their role in protecting water quality.

Analysis of data collected as part of the Long Island-New Jersey Study Unit of the NAWQA Program demonstrate clearly that stream health, as indicated by both benthic invertebrate-based indices of biological integrity (Kennan 1998, 1999) and measurements of pollution by nutrients and pollutant chemicals (O'Brien 1997, O'Brien et al. 1997, Stackelberg 1997, Reiser and O'Brien 1999) is highly correlated with the amount of wetland in the basin. While the ability of wetlands to improve water quality is known in general, especially for agricultural landscapes, there is little information available to evaluate the effectiveness of different qualities and locations of wetlands within urban basins to improve or protect water quality. Indeed, there is little data permitting direct comparisons of wetland structure and function, especially for urban wetlands. Management of urban/suburban watersheds, as well as the restoration of these wetlands, requires better knowledge of the functional role of wetlands in protecting stream water. While the proposed study specifically addresses the urban watersheds of the LI-NJ NAWQA, the results will be widely applicable to urban/suburban watersheds throughout the country.

The research is producing quantitative and predictive relationships between the biological and chemical measures of surface water quality obtained through the NAWQA program and quantitative measures of the structure (invertebrate-based indices) and function (nitrogen removal capacity) of wetlands. Furthermore, the two measures of wetland quality will be analyzed for wetlands of different sizes and landscape positions relative to surface waters; the results will be integrated with spatial data on the extent and position of wetlands within the selected watersheds to yield predictive relationships between landscape structure within a watershed and downstream water quality.

These results will be of use to a variety of different groups. First, the results will be directly usable by land, water and watershed managers, in both the governmental and private sectors, seeking to protect and manage both wetlands and surface waters. For example, three current controversies in the Rahway River watershed (one of the proposed study areas) involve applications to destroy forested wetlands (one, to construct a sports complex, the other to construct a housing/commercial development) and a request for state funds to restore wetlands on a previously filled portion of the floodplain. Local and state officials are seeking scientific information on both the function of urban wetlands, and the connection of these wetlands to riverine water quality, in trying to resolve these situations, but the necessary data do not exist. Wetland protection and restoration in urban/suburban regions has taken on an extreme urgency, and is a high priority for government agencies from the federal to the municipal levels, as well as for landmanagement NGOs; our discussions with land managers in all these sectors indicate that the results will be immediately useful and highly valued by them. Second, the data will be useful to scientists trying to understand the linkage between terrestrial land-use and water quality, as models currently rely simply on total areal extent of wetlands, rather than specific placement, size and internal characteristics of wetlands within a basin. Third, there is an extraordinary lack of information about the functions and qualities of wetlands in urban landscapes; the data will thus provide wetland scientists with important data on a class of wetlands that are not well understood but which are critical for the management of water resources in urban environments. As over half the population of the US now lives in urban/suburban regions, the data will be widely useful. Fourth, the data will complement and extend the NAWQA data, thus improving the usefulness of this extensive research effort. Thus, the results will be directly useful to managers, but also useful to a wide variety of scientists studying the determinants of surface water quality.

Progress to date:

The fifteen sites selected for the study were grouped into hydrogeomorphic classes (riverine, flat-riverine, and mineral flat); within each class, we selected at least one site that served as a 'control' – minimally urbanized. No truly undisturbed sites, with no urban development in the basin, exist within the region. The sites also were grouped into three size classes; one set of sites were 10-20 ha in area, one set 95-120 ha, and two were larger (nearly 200 and >3000 ha). Within each site, we have installed (1) an RDS well for continuous monitoring of water table levels, and (2) three piezometers arrayed along a transect perpendicular to the stream and crossing the study area for soil function. The piezometers have been monitored on a bi-weekly basis while water is present, and the recording wells are being downloaded regularly. Data analysis of the wetland hydrographs and piezometric heads is ongoing. Preliminary analyses show that, as expected, most of the highly urbanized sites have 'flashy' hydrology (Fig. 1a), whereas the sites within less urbanized areas have more typical hydrology (prolonged flooding during fall, winter, and spring, with slow drawdown during the summer (Fig. 1b).





Nitrogen processes

At each site, sequential incubations of core have been conduction to measure nitrogen cycling processes. These measurements have been made since the soils rewetted when the drought ended last fall (sampling was physically impossible during the summer, due to the extremely hard condition of the dry, clayey soils). Every three weeks, two sets of samples are taken at five points across the sample wetland area of each site, using a soil probe with plastic inserts; one set is returned to the lab for extraction of mineral and dissolved organic N fractions, and the other sample capped and returned to the hole for field incubation until the next sampling episode. The samples returned to the lab are measured for denitrification activity (using acetylene inhibition) prior to extraction. Basic soil properties (moisture, pH, organic matter content) are also determined for each sample. Initial analysis of the nitrogen mineralization/ nitrification data show that while HGM class does not appear to have any effect on N production rates, there is a striking difference between the more urban and the reference sites (Fig. 2). There is a large net production of nitrate in the more urban sites, suggesting that these site may be sources of nitrate to surface waters, rather than sinks.



Fig. 2. Patterns of N mineralization and net nitrification in urban wetlands.

Analyses of denitrification rates is ongoing; because of instrumentation problems, analyses of the samples was delayed and is now being completed. We anticipate that all data will be collected and analyzed by the end of summer 2005.

Stream invertebrates and litter decomposition

The stream work accomplished includes the following:

1. Quantitative sampling of the macro-invertebrate community, by microhabitat type, was conducted through a 200-meter reach in spring and fall. Collected organisms were identified to genus or species, with the assistance of local specialists.

2. Stream habitats have been quantitatively described (sediment texture, stream crosssectional profiles at 10 m intervals through the study reach, sinuosity, diversity of microhabitats). In addition, water quality data have been collected reach each reach, at the time of both the invertebrate and the litterbag collections.

3. A litter decomposition experiment was conducted to provide data on ecosystem function of these streams. Litter containers with a 3 mm mesh were prepared, containing two standard substrates in separate compartments (popsicle sticks, a standard wood substrate, and *Phragmites* stems, a standard herbaceous plant m,aterial substrate). Approximately 500 litterbags were constructed and deployed in the streams, and were retrieved at monthly intervals.

4. Artificial substrates (Hester-Dendy collectors) were prepared and deployed in the streams to supplement the kick-neck collections.

Finally, the GIS analysis of the study sites, to characterize land-use in the watersheds upstream of each study site, has been completed, and the results are being integrated with the nitrogen and invertebrate data.

Stream invertebrate and stream characterization results

The stream segments were remarkably uniform; no differences among HGM classes or among size categories were detected. All the streams had very low flow rates (<0.05 m³/sec), little sinuosity, predominantly sandy-textured bottoms with little coarse material, little rooted vegetation, but a variable amount of coarse woody debris. These physical characteristics were paralleled by predominantly low dissolved oxygen concentrations (20-40% saturation in most measurements), low redox values (100-300 mv), but high nitrate concentrations (4-14 mg/L). The low flow and sandy textures clearly reflect the physical qualities of the adjacent wetlands: there is no gradient across these wetlands, and the soil sampling revealed a sandy substratum in most of the sites at the depth at which the stream bottoms were observed.

Stream invertebrate densities (number per sampling event) ranged from 1 to nearly 600; most stream samples had 200-400 individuals, representing 10-12 families (representative data in Table 1). Diversities and densities were very low, compared to literature values of typical upland streams. However, multivariate analyses of these data, comparing them with physical and chemical characteristics of the stream segments, showed that there was significant variation mostly related to the physical qualities of the streams. Thus, it appears that stream properties resulting from the characteristics of the wetlands independent of their urban landscape placement are the most important factors structuring invertebrate communities. However, it should be noted that the data did suggest some differences between the riverine sites and the other HGM classes.

Litter decomposition

Litter decomposition in the streams was used as an index of ecosystem function related to both the nutrient content of the water and the macroinvertebrate communities. Surprisingly, there were no differences in litter decomposition rate among the sites. The *Phragmites* stem material decomposed relatively slowly, with 60-80% of the material remaining at the end of the 6-month period, and there was virtually no decomposition of the wood substrates (Fig. 3, Table 2). These results suggest that the predominantly low dissolved oxygen and low redox of the stream waters have a controlling influence on decomposition, exerted in part through the low densities of invertebrates. Again, wetland area and HGM class appeared to have little effect on decomposition rates.

Conclusions

Final data analyses and comparison of the nitrogen and invertebrate sampling is continuing, and will be completed by the end of the summer. The invertebrate studies form the substance of a Ph. D. thesis (scheduled for defense in July 2005), which will contain the complete analyses including GIS data on the watersheds of the 15 study sites. Initial conclusions suggest that there is poor correlation between nitrogen retention, which varies strongly between more and less urbanized sites and which is low in all sites, and the invertebrate communities, which appear to reflect more strongly the geomorphic and geological character of the wetlands than the characteristics of the surrounding urban environment.

Order	Family	C H	E N	E S	G B	M 5	M 6	M T	O R	P N	P S	R L
Collembola	Isotomidae					Х			Х			Х
Collembola	Poduridae											
Diptera	Chironomidae	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х
Diptera	Tabanidae	Х		Х			Х	Х		Х		Х
Diptera	Sciomyzidae										Х	
Diptera	Empididae										Х	
Diptera	Tipulidae		Х						Х			
Diptera	Ceratopogonidae								Х			
Trichoptera	Hydropsychidae		Х						Х		Х	
Trichoptera	Hydroptilidae								Х			
Odonata	Coenagrionidae				Х							
Odonata	Libellulidae				Х					Х		
Coleoptera	Psephenidae											
Coleoptera	Dytiscidae				Х			Х				
Coleoptera	Elmidae				Х							
Coleoptera	Chrysomelidae											
Heteroptera	Corixidae											
Amphipoda	Gammaridae				Х	Х	Х	Х	Х	Х	Х	Х
Isopoda	Asellidae				Х	Х	Х	Х	Х	Х	Х	Х
Decapoda	Cambaridae							Х				
Harpacticoida	Unknown Family				Х							
Cyclopoida	Cyclopidae		Х		Х					Х	Х	
Veneroida Bassommatophor	Sphaeridae	Х	Х		Х		Х	Х			Х	Х
a Descommentanhan	Planorbidae				Х				Х	Х	Х	
a	Physidae	Х	Х		Х		Х	Х	Х	Х	Х	Х
Bassommatophor a	Unknown Family				Х							
Bassommatophor	Anoulidaa				v							
a Bassommatophor	Ancynuae				л							
a Bassommatophor	Hydrobiidae				Х							
a Bassommatophor	Pleuroceridae				Х							
a Bassoniniatophor	Viviparidae				Х							
Bassommatophor	D:4h				v							
d Hanlatarida					Λ							
Наріотахіца	Tubilicia Family											
Hanlotaxida	Family	x	x	x		x	x	x	x	x	x	x
Rhynchobdellida	Glossinhoniidae	X	X	X	x	~	X	X	X	X	X	X
Rhynchobdellida	Piscicolidae	X			2 b	х	X			X		X
inclusion and										••		••
	Total Families	7	8	4	19	6	9	10	10	11	12	10

Table 1. Presence and absence of benthic macroinvertebrate families during Spring 2004 sampling season.

Fig. 3. Representative litter decomposition curves from two sites. Squares – popsicle stick (wood substrate) decomposition; circles – *Phragmites* stem decomposition.



Table 2. Litter decomposition coefficient (*k*) day ⁻¹ of popsicle sticks (Stix) and *Phragmites* (Phrag) and benthic macroinvertebrate abundance and family-level diversity. HGM classes are: (FLAT) mineral flat, (RIV) riverine, and (FLAT-RIV) mineral flat and riverine. (Size) denotes size classes: (S) small, (M) medium, and (L) large. M6 was omitted due to not enough litterbags being recovered.

HGM Class	Site(Size)	Stix Decomposition Coefficient (<i>k</i>)	Phrag Decomposition Coefficient (<i>k</i>)
FLAT	PS	0.00005	0.0010
RIV	EN	0.00008	0.0005
RIV	ES	0.00010	0.0012
RIV	MT	0.00007	0.0012
RIV	OR	0.00006	0.0011
FLAT-RIV	СН	0.00020	0.0008
FLAT-RIV	M5	0.00008	0.0006
FLAT-RIV	PN	0.00006	0.0012
FLAT-RIV	RL	0.00004	0.0011

Information Transfer Program

No information transfer project proposals were submitted to the annual 104B competition, and thus there are no specific IT projects to report. Funds supplied by Rutgers University during the past three years to enhance the information transfer program were no longer available to us to support an expanded information transfer program. Therefore, our information transfer activities focused on the newsletter and the web site.

1. Our newsletter has been expanded to 12 pages in order to include more information about research and water-related activities at academic institutions throughout the state. In addition to an issue summarizing water resource research around the state, we prepared two issues that have been widely circulated by other organizations. In one, we featured a series of articles about the new stormwater management regulations adopted by the NJ DEP; articles explained the nature of the rules and presented perspectives on their impact on water and development by a variety of stakeholders. In another issue, we featured a large multi-institutional water resource research project that is ongoing in south Jersey, the Kirkwood-Cohansey Project. In this project, researchers from the New Jersey Pinelands Commission, Rutgers University, the Water Science Center of the NJ District Office, US Geological Survey, and the US Fish and Wildlife Service are collaborating to determine the potential effects on aquatic ecosystems of increased water withdrawals from the Kirkwood-Cohansey aquifer system. This five-year research project will have major repercussions for water use in the southern half of the state. The newsletter was widely circulated by other organizations involved with Pinelands land management and conservation. A final issue reports on the WRRI-supported research that is ongoing. We also moved to a web-based system for newsletter distribution, supplementing and expanding the distribution of hard copies by regular mail. Currently, about 2,000 people around the state receive the newsletter.

2. We have expended considerable effort in revising, expanding, and updating the website. Our goal has been to make the NJ WRRI website a one-stop-shopping portal for water information for as broad an array of New Jersey citizens as possible. Newly developed pages include 1) pages reporting on USGS-NJ research activities, NJ DEP research activities, other water-related research (non WRRI-supported) at Rutgers and water research at other academic institutions in NJ, with the goal of communicating the importance of research for problem-solving; 2) links to NJ DEP water-related offices, including links to publications, permit forms, hot-lines, etc., 3) links to many federal and international water resource agencies and organizations, 4) links to all NJ watershed organizations, 5) a page of educational resources for students and teachers, 6) links to newsletters of other water-related organizations and water publications that are freely available, 7) access to real-time water data from the USGS and New Jersey agencies, and links to pages for on-line mapping and spatial information 8) a regularly updated listing of meetings and conferences, and 9) pointers to sources of funding (including RFPS for WRRI-supported grants programs. We plan to widely publicize the new page and invite other organizations to link to our site.

3. We continue to participate in the New Jersey Water Monitoring Council, a statewide body representing both governmental and NGO organizations involved in monitoring. As a result of this activity, we anticipate managing a grant from the NJDEP dudring the next fiscal year to organize a major conference on volunteer monitoring, and to support a student to assist with water monitoring data collection and organization.

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4. We participated the following meetings: US Geological Survey Water Science Center Review of 5-Year Plan; Planning meeting, Upper Raritan Watershed Association, Watershed Stewardship Project; Fourth Stakeholders Work Session for the Hackensack Meadowlands; New Jersey Chapter, AWRA Planning meeting for Annual Meeting.

Student Support

Student Support									
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total				
Undergraduate	2	0	0	0	2				
Masters	2	0	0	0	2				
Ph.D.	5	0	0	0	5				
Post-Doc.	4	0	0	0	4				
Total	13	0	0	0	13				

Notable Awards and Achievements

Rutgers Board of Trustees Research Fellowship for Scholarly Excellence awarded to PI Lee Slater in April 2005, in recognition of research activities prior to tenure

Rusciano, G.M. July 22, 2004 (abstract recently accepted). Efficiency of Bioretention Systems to Reduce Fecal Coliform Counts in Stormwater. The North American Surface Water Quality Conference and Exposition, Orlando, Florida.

Publications from Prior Projects

- 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid herbicides (CAHs) in water") - Articles in Refereed Scientific Journals - Xiaoyan Wang, Chutarat Saridara, Somenath Mitra, Microfluidic Supported Liquid Membrane Extraction, Analytica Chimica Acta, 543 (2005), 92- 98.
- 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid herbicides (CAHs) in water") - Articles in Refereed Scientific Journals - Xiaoyan Wang, Somenath Mitra, Development of a total analytical system (TAS) by interfacing membrane extraction, pervaporation and high-performance liquid chromatography, Journal of Chromatography A, 1068 (2005), 237-242.
- 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid herbicides (CAHs) in water") - Articles in Refereed Scientific Journals - Dawen Kou, Xiaoyan Wang, Somenath Mitra, Supported liquid membrane microextraction with high-performance liquid chromatography-UV detection for monitoring trace haloacetic acids in water, Journal of Chromatography A, 1055 (2004), 63-69.
- 4. 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid

herbicides (CAHs) in water") - Other Publications - Abstract: Xiaoyan Wang, Chutarat Saridara, Somenath Mitra, Microfluidic Supported Liquid Membrane Extraction, 229th ACS National Meeting, March 13-17, 2005, San Diego, CA.

- 5. 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid herbicides (CAHs) in water") - Other Publications - Abstract: Xiaoyan Wang, Somenath Mitra, Development of a total analytical system (TAS) by interfacing membrane extraction, pervaporative concentration and detection, 43rd Annual Eastern Analytical Symposium, Nov. 15-18, 2004, Somerset, NJ.
- 6. 2003NJ43B ("Development of Supported Liquid Membrane Micro-Extraction (SLMME) followed by Ion-Pair Chromatography (IPC) for analysis of halo-acetic acids (HAAs) and chlorinated acid herbicides (CAHs) in water") - Other Publications - Abstract: Somenath Mitra, Xiaoyan Wang, Dawen Kou, Supported liquid membrane micro-extraction (SLMME) for monitoring trace acidic analytes, 227th ACS National Meeting, March 28-April 1, 2004, Anaheim, CA.
- 2001NJ941B ("Factors controlling methylmercury degradation in Pine Barrens lakes and the Meadowlands") - Articles in Refereed Scientific Journals - Schaefer, J.K., J. Yagi, J. Reinfelder, T. Cardona, K. Ellickson, S. Tel-Or, and T. Barkay. 2004. The role of the bacterial organomercury lyase in controlling methylmercury accumulation in mercury contaminated natural waters. Env. Sci. Technol. 38:4304-4311.
- 2001NJ941B ("Factors controlling methylmercury degradation in Pine Barrens lakes and the Meadowlands") - Conference Proceedings - Ní Chadhain, S. M., S. Hicks, J. Schaefer, T. Barkay, G. J. Zylstra. Novel Mercuric Reductase Genes Found in Anaerobic Communities of Mercury Contaminated Sediments. 104th Annu. Meet. Am. Soc. Microbiol. New Orleans, May 19 - 23, 2004.
- 9. 2001NJ941B ("Factors controlling methylmercury degradation in Pine Barrens lakes and the Meadowlands") - Conference Proceedings - Schaefer, J. K., J. Yagi, T. Cardona-Marek, K. Ellickson, S. Tel-Or, J. Reinfelder, and T. Barkay. The role of the bacterial enzyme, organomercurial lyase, in controlling methylmercury accumulation in mercury contaminated natural waters. 7th International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia, June 27 - July 2, 2004.
- 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Stream biomonitoring using macroinvertebrates in the New Jersey Pinelands: Consistency with water chemistry and landuse. Mid Atlantic Chapter of the Ecological Society of America Conference on Sustainable Landscapes. Lancaster PA, March 2004. William Cromartie, Stockton University (with L. Maun, J. Akers, T. Johnson, J. Gliddon,
- 11. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Using Stream Insects on Woody Debris to Assess Water Quality in the New Jersey Pinelands, at the NJ Academy of Sciences' annual meeting, April 2004, Fairleigh Dickinson University, Madison NJ. William Cromartie, Ph. D. (with L. Maun, J. Akers, T. Johnson, J. Gliddon, J. Grimes And D. Monzo)
- 12. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Adams Branch Restoration: Macroinvertebrates and Water Chemistry. Poster session. Joint meeting, American Entomological Society and Entomological Society of Pennsylvania. Newark, DE. 20 October 2004. William Cromartie, Ph. D. (with Lynn Maun, Julie Akers, Denis Cummings, Justine Cook, Jordan Leckenbush, and Bob Fromtling)

- 13. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Aquatic Macroinvertebrates in the Great Egg Harbor River Watershed. Newark Entomological society, New Brunswick, NJ. 23 October 2004. Poster session. William Cromartie, Ph. D. (with Jason Gliddon, Tamica Johnson, Lynn Maun, Julie Akers, Denis Cummings, Justine Cook, Jordan Leckenbush, and Bob Fromtling)
- 14. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Biomonitoring pinelands rivers using snag-dwelling insects. William Cromartie, Ph. D. Invited speaker, American Entomological Society, at the Academy of Natural Sciences, Philadelphia PA, 10 November 2004.
- 15. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Macro-invertebrates, landuse and chemistry in an urbanizing watershed. Poster session. Mid-Atlantic Region, Ecological Society of America, Baltimore MD, 12 March 2005. William Cromartie, Ph. D. (with Julie Akers, Justine Cook, Denis Cummings, Jordan Leckenbush and Graeme Millar)
- 16. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Undergraduate Research Project of Prof. William Cromartie, Stockton University Conference Presentation Macro-invertebrates, landuse, hydrology and chemistry in an urbanizing watershed. Poster session. Delaware Valley Chapter, Society for Conservation Biology, Rowan University, 9 April 2005. William Cromartie, Ph. D. (with Julie Akers, Justine Cook, Denis Cummings, Bob Fromtling, Jordan Leckenbush and Graeme Millar)
- 17. 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Orlins, J., Gallagher, K., and Ross, S. (2005). Hydraulic Analysis Of Kandle Lake Dam and Downstream Inundation Due To Dam Breach. Engineering submitted to Bureau of Dam Safety and Flood Control, New Jersey Department of Environmental Protection.
- 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Other Publications - Orlins, J., Miller, D., Hasse, J., Everett, J., Jahan, K. (2004) Watershed Assessment Through Undergraduate Research, 2004 EWRI World Water and Environmental Resources Congress, Salt Lake City, Utah.
- 2002NJ-ADMIN Program Administration ("2002 Undergraduate Interns' Research and Education") -Conference Proceedings - Orlins, J., Miller, D., Hasse, J., Everett, J., Jahan, K. (2004). Watershed Assessment With Undergraduate Research Students Abstract Proc. of the American Water Resources Association 2004 Annual Conference, Orlando, FL. American Water Resources Association.