New Jersey Water Resources Research Institute Annual Technical Report FY 2010

Introduction

The New Jersey Water Resources Research Institute (NJWRRI) supports a diverse program of research projects and information transfer activities. With oversight from the Advisory Council which sets the Institute's Research Priorities, the available funds are divided between supporting faculty with 'seed' projects or new research initiatives and supporting graduate students with developing their thesis research. The funding is intended to initiate novel and important research efforts by both faculty and students, and thus emphasizes new research ideas that do not have other sources of funding. We hope to support the acquisition of data that will enable further grant submission efforts, and, in the case of students, lead to research careers focused on cutting-edge research topics in water sciences.

Research projects span a wide range of water resources topics. In the first faculty project, Hazard studied the association between elevated salinity in freshwater habitats due to road deicers and decreased amphibian species diversity. This study used a physiological approach to water resource issues, which can provide insight into the causal mechanisms underlying species responses to environmental changes. In the second faculty project, Rector evaluated three methods of tracking cumulative implementation of BMPs at the subwatershed level. This investigation shows a causal relationship between stormwater BMP implementation and improvements in water quality. In the final faculty project, Wazne used an integrative approach to develop sustainable and green lignocellulosic anion exchange resins to remove nitrate and phosphate from water. The resins are used to augment nitrate and phosphate removal in biorention basins, and are then composted and recycled as fertilizer. This research has resulted in a patent application.

Graduate students have similarly carried out an impressive range of research. Bonventre and her advisor researched the effects of MTBE, ETBE and TAME, three common gasoline oxygenates, on the developing embryonic cardiovascular systems of zebrafish (Danio rerio). Results suggest the three oxygenates effect different genes and gene expression. Mumford and his advisor examined the role of arsenic reducing bacteria in the release of arsenic from bedrock shale and subsurface sediments into groundwater. Parsekian and his advisor used several hydrogeophysical techniques, such as ground-penetrating radar, to evaluate near-surface geology influencing local hydrology within canopy gaps in the New Jersey Pinelands. From this, he assessed the potential of paleo-channels to impact local and regional ground water flow. Punamiya and his advisor studied the potential use of iron and aluminum oxide drinking water treatment residuals as a green remediation method for veterinary tetracycline antibiotics, potentially reducing inputs of these antibiotics and their metabolites into water resources.

Additionally, Joshua Galster and Kirk Barrett of Montclair State University received funding from the NIWR/USGS National Competitive Grant Program for a study of the relationship between urbanization and stream baseflow. This two-year grant studies streams and watersheds in ten East Coast states. Interim findings of this research are reported.

The goal of our information transfer program is to bring timely information about critical issues in water resource sciences to the public, and to promote the importance of research in solving water resource problems. The information transfer program continues to focus on producing issues of the newsletter that provide a comprehensive overview of a particular water resource topic, as well as one issue a year that highlights water research occurring in New Jersey. The program continues to develop the NJWRRI website (www.njwrri.rutgers.edu) into a comprehensive portal for water information for the state. We also collaborate with other state and regional organizations in sponsoring and producing conferences and events.

Research Program Introduction

The New Jersey Water Resources Research Institute has had a policy, yearly reaffirmed by the NJWRRI Advisory Council, of using research dollars to promote new and novel directions of research. To this end, three projects directed by research faculty at institutions of higher learning around the state were selected, and four grants–in–aid were awarded to graduate students who are beginning their research. In both cases, we expect that the research is exploratory and is not supported by other grants. The intent is that these projects will lead to successful proposals to other agencies for further support. The larger goal of the research component of the Institute's program is to promote the development of scientists who are focused on water resource issues of importance to the state.

A NIWR/USGS National Competitive Grant Program project from New Jersey received continued funding this year. Additional findings from that research are reported here.

Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States

Basic Information

Title:	Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States
Project Number:	2009NJ210G
Start Date:	9/1/2009
End Date:	8/31/2011
Funding Source:	104G
Congressional District:	NJ 8th
Research Category:	Climate and Hydrologic Processes
Focus Category:	Surface Water, Water Quantity, Management and Planning
Descriptors:	None
Principal Investigators:	Joshua C. Galster, Kirk Barrett

Publication

1. Lopes, Jared, Joshua Galster, Kirk Barrett, 2010, A historical, empirical analysis of the relationship between imperviousness and stream baseflow in the eastern United States. Does urbanization impact baseflow during dry weather?: Geological Society of America Abstracts with Programs, v. 42 p. 241.

Problem and Research Objectives

Approximately half of the U.S. population depends on surface water (rivers and reservoirs) for their drinking water. During dry weather, rivers and reservoirs are fed by baseflow. Many of these areas are urbanizing, in some cases rapidly. Theoretically, urbanization will cause a decrease in baseflow, which means urbanization is a threat to water availability for about half of the population. Reduced baseflow can also negatively affect stream biota. The problem is that it is not clear if (and to what degree) this theoretical linkage between urbanization and decreased baseflow is actually experienced in the real world; there are several process associated with urbanization that could confound the theoretical relationship. This project will help resolve this relationship by conducting a large spatial and temporal scale, empirically-based investigation into the urbanization-baseflow relationship. The project will determine if (or how likely) there really is a relationship between urbanization and decreased baseflow in real watersheds.

This project will provide the most complete assessment about whether/how urbanization actually affects baseflow. Water supply managers and land development regulators can make use of this information to help better understand the effects of land development and manage it accordingly, especially in rural and water supply watersheds. The project results should be useful in assessing the threat posed by urbanization to dry-weather water availability and stream ecology.

Annual baseflow is an important metric for minimal stream flows, and is a critical value for water resource managers, wildlife interests and groundwater connectivity. One potentially significant but currently under-studied impact on baseflow is urbanization. We propose to analyze historic United State Geological Survey (USGS) gage data to determine trends in baseflow over time. Baseflow data would be collected for the states of New Jersey, Pennsylvania, Delaware, Maryland, and Virginia. We will use different timeframes and baseflow metrics for their utility in identifying trends. We propose to use four metrics of annual baseflow: 1) baseflow per unit drainage area (BF); 2) ratio of BF to precipitation (BF/P); 3) BF as a fraction of total flow (BF/TF); and, 4) the annual minimum daily average flow per unit drainage area (AMDAF). Trends will be indentified using the non-parametric Mann Kendal statistical test and the Sen slope estimator.

Methodology

We are empirically investigating the relationship between urbanization and stream baseflow by examining the stream discharge records maintained by the United States Geological Survey in ten states: NY below the Adirondacks, CT, NJ, PA, DE, MD, VA, NC, SC, and GA. We will select gages that have continuous records for at least 25 years and have had substantial changes in the amount of impervious surfaces within the watersheds. As a control, we will also analyze trends in ten gaged watersheds per state that showed near constant imperviousness. We will separate baseflow from other stream flows using a digital filtering method and aggregate daily baseflow to create an annual baseflow time series for each gage. We will then compute time series of three baseflow statistics: 1) annual baseflow per unit drainage area, 2) ratio of baseflow to precipitation and 3) baseflow fraction of total flow. We will have both undergraduate and graduate students assisting us with these tasks.

Stream discharge data for Maine, New Jersey and Georgia were gathered using the USGS National Water Information System under the condition that the data was continuous (>25 years), unregulated and of a drainage area <400mi^2. Gages that satisfied these parameters had their baseflow separated from the total flow using the "WHAT" Eckhardt digital filtering method. Based on available continuous data, a time series of three metrics was calculated: 1) baseflow per unit drainage area (BF, cm/yr), 2) ratio of baseflow to precipitation (BF/P), and 3) baseflow as a fraction of total flow (BF/TF). Each of the time series of the three metrics were analyzed for annual trends using the nonparametric Mann-Kendall test. The Mann-Kendall test states that if the (number of increases) > (number of decreases), then there is a statistically significant increasing trend. Watersheds of each gage were delineated using available digital elevation models in ArcGIS, and a historical population density time series from census data for each watershed was created and correlated with imperviousness. Utilizing the imperviousness data, a relationship between the three baseflow metrics and urbanization was examined.

Principal Findings and Significance

From the results shown in the table below, the number of positive significant trends is slightly higher than the number of negative base flow trends found using the baseflow metrics and Mann-Kendall statistical analysis. Percent imperviousness had significantly positive trends even in historically rural watersheds, while the BF/TF metric showed little to no change. Preliminary results from 31 unregulated gages in New Jersey agree with these new findings, where approximate numbers of cases with positive correlation were equal to that of cases of negative correlation for all baseflow metrics. The three metrics get sizably different results, but this is due to the fact that each metric is measuring something different. These initial results show no definitive answer, but show the complexity of baseflow and the systems such as urbanization influencing it. In order to determine a true relationship between imperviousness and baseflow, additional gages and states will need to be analyzed.

Our aim is to expand on these initial results by including other states with similar physiographic regions (e.g., Ridge and Valley, Piedmont, Coastal Plain, etc.) and also undergoing urbanization that places pressure on the local water resources. Students have been active in collecting and analyzing the USGS gages that meet the criteria above. Currently over 600 gages in the ten states have been determined to meet the criteria above, and will be analyzed for baseflow and precipitation trends. This work will continue in the summer of 2011.

Table 1 has selected results showing rivers and trends in baseflow metrics. Level of significance is calculated for detected trends and symbolized as (***) for 0.001 level of significance, (**) if a trend has 0.01 level of significance, (*) for 0.05 level of significance, and (+) for a trend with 0.1 level of significance. The significance level (***) 0.001 means that there is a 0.1% probability that the given values are from random distribution and that the existence of a monotonic trend is credible. The abbreviations are 1) baseflow per unit drainage area (BF, cm/yr), 2) ratio of baseflow to precipitation (BF/P), 3) (BF/TF) baseflow as a fraction of total flow, and 4) Percent impervious (%IMP).

Table 1. Selected results showing rivers and trends in baseflow metrics. Level of significance is calculated for detected trend. The abbreviations are 1) baseflow per unit drainage area (BF, cm/yr), 2) ratio of baseflow to precipitation (BF/P), 3) (BF/TF) baseflow as a fraction of total flow, and 4) Percent impervious (%IMP).

State	**River**	**Record**	**Area (sq mi)**	**BF**	**BF/P**	**BF/ TF**	**%IMP**
ME	MEDUXNEKEAG RIVER NEAR HOULTON	1941-1981	75	**+**, +	**+**,+	No trend	No trend
ME	GRAND LAKE STREAM AT GRANDLAKE	1962-2000	27	**-**, *	**-**, ***	No trend	**+**, ***
ME	CARRABASSETT RIVER AT CARRABASSET	1928-2000	53	**+**,+	**+**, *	No trend	**+**, ***
ME	LITTLE ANDROSCOGGIN RIVER NEAR SOUTH PARIS	1932-2000	3	No trend	No trend	No trend	**+**, ***
NJ	PASSAIC RIVER NEAR MILLINGTON	1940-2000	5.4	No trend	**+**, *	No trend	**+**, ***
NJ	WHIPPANY RIVER AT MORRISTOWN	1940-2000	9.4	**+**, *	**+**, ***	No trend	**+**, ***
NJ	ROCKAWAY RIVER AT BOONTON	1940-2000	16	No trend	No trend	No trend	**+,***
GA	BRUSHY CREEK NEAR WRENS	1959-2000	8	**-**, *	**-**,+	No trend	No trend
GA	TOBESOFKEE CREEK NEAR MACON	1949-2000	82	**-**,+	**-**,*	**-**, ***	**+**, ***
GA	CARTECAY RIVER NEAR ELLIJAY	1942-1976	34	**+**, +	No trend	No trend	No trend
GA	YELLOW RIVER NEAR SNELLVILLE	1943-1970	34	No trend	No trend	No trend	**+**, ***

Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues

Basic Information

Title:	Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues
Project Number:	2010NJ216B
Start Date:	3/1/2010
End Date:	2/28/2011
Funding Source:	104B
Congressional District:	NJ-008
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Ecology, Methods
Descriptors:	None
Principal Investigators:	Lisa Hazard

Publications

1. Hazard, L.C., K. Kwasek, E. Koelmel, and S. Gerges. 2010. Variation in behavioral aversion to road deicers in sympatric temperate zone amphibian species. American Physiological Society Intersociety Meeting- Global Change and Global Science: Comparative Physiology in a Changing World. Westminster, CO. (Poster.)
2. Hazard, L.C., K. Kwasek, E. Koelmel, and S. Gerges. 2011. Interspecific variation in behavioral aversion of sympatric temperate zone amphibians to road deicers. Society for Integrative and Comparative Biology Annual Meeting, Salt Lake City, UT. (Poster.)
3. Kwasek, K. and L. Hazard. 2011. Behavioral responses of the Eastern Newt to road deicers. Fifth Annual Student Research Symposium, Montclair State University. (Poster.)
4. Hazard, L., E. Koelmel and M. Gonzalez-Abreu. 2011. Behavioral aversion of four Ranid frog species to road deicers: Does terrestriality influence sensitivity? Fifth Annual Student Research Symposium, Montclair State University. (Poster.)
5. Hazard, L. and S. Gerges. 2011. Behavioral aversion of Ambystoma maculatum to road deicers: Are alternative deicers really more “environmentally friendly”? Fifth Annual Student Research Symposium, Montclair State University. (Poster.)
6. Kwasek, K. and L. Hazard. 2011. Behavioral responses of the Eastern Newt to road deicers. New Jersey Academy of Science Annual Meeting, Montclair, NJ. Second Place, Best Student Poster competition. (Poster.)
7. Hazard, L., E. Koelmel and M. Gonzalez-Abreu. 2011. Behavioral aversion of four Ranid frog species to road deicers: Does terrestriality influence sensitivity? New Jersey Academy of Science Annual Meeting, Montclair, NJ. (Poster.)
8. Kwasek, Kristen. 2011. Behavioral responses of the eastern newt to road deicers. MS Thesis, Department of Biology and Molecular Biology, College of Science and Mathematics, Montclair State University. 30 pages.
9. Hazard, L.C., K. Kwasek, E. Koelmel, and S. Gerges. 2011. Interspecific variation in behavioral aversion of sympatric temperate zone amphibians to road deicers. NJWRRI Grant Recipient Poster Presentation, Cook Campus Center, New Brunswick, NJ.

Problem and Research Objectives

Background and problem

Chemical road deicers (primarily rock salt, NaCl), used to improve winter road safety by reducing the freezing point of water on roadways, can be a significant source of salt contamination of inland waterways in cold climates (reviews in Forman and Alexander, 1998; Trombulak and Frissell, 2000). A recent study of northeastern U.S. watersheds showed that chloride concentrations in suburban and urban freshwater habitats may reach 25% the concentration of seawater, and exceed tolerable limits for freshwater organisms (Kaushal et al., 2005). Vernal pools, which typically lack an outlet, may be especially susceptible to accumulating salts. This is particularly problematic because these temporary ponds are critical breeding habitat for some organisms. Alternatives to rock salt (e.g. calcium chloride) are available, but their ecological effects are less well studied.

Amphibians are particularly sensitive to environmental salt levels due to their permeable skin and the need of most species for fresh water for breeding (Hillman et al., 2009). Amphibians that breed early in the spring, when salt levels due to winter deicers could still be high, may be especially vulnerable. In several studies of amphibian communities, chloride (measured as salinity, conductivity, or chloride concentration) has been shown to be a major determinant of presence or absence of individual species, and of overall amphibian species diversity. Agriculture-related salinization led to reduced amphibian species diversity in Australian wetlands (Smith et al., 2007), and in Nova Scotia, increased salinity of roadside ponds due to road salt runoff was associated with decreased species richness (Collins and Russell, 2009).

The proximate causes of the effect of salinization on amphibian populations are still unclear, and could be due to one or many of several different factors, including high mortality of adults, post-breeding mortality of eggs or larvae, or adult avoidance of unsuitable habitat. Most studies have focused on the effects of salts on the egg or larval stage. Elevated salt concentrations in freshwater lakes or ponds impact amphibian eggs and larvae, with main effects being dehydration, retarded development, and increased mortality (e.g. Dougherty and Smith, 2006; Sanzo and Hecnar, 2006; but see Karraker, 2007).

Unlike most aquatic organisms, amphibians have the potential ability to move out of unsuitable habitats, over land, to find more suitable habitat. This introduces a behavioral component to the response of amphibians to environmental changes, which has been little studied. Behavioral flexibility of adults could allow individuals to move to more suitable breeding pools if warranted. There is some evidence for this; chloride is the main environmental determinant of breeding habitat selection for one European salamander species (Sayim et al., 2009). However, many vernal pool-breeding species show strong site fidelity (Petranka, 1998), and might not have sufficient behavioral flexibility to move to a new pond if the environment changes. Some desert amphibian species have been shown to be capable of detecting variation in salinity to determine a solution's suitability as a source of water for rehydration (e.g. Hillyard et al., 1998; Maleek et al., 1999; Johnson and Propper, 2000; Sullivan et al., 2000), suggesting that temperate zone amphibians could have similar capabilities. Desert-adapted amphibians such as Couch's

Spadefoot Toads show strong behavioral aversion to increased environmental salt levels and will not remain in solutions of moderate to high concentrations (e.g. Johnson and Propper, 2000).

In pilot studies of New Jersey species, I found that while adult Eastern Newts show aversion to salts similar to what has been found in desert toads (Hazard and Vig, submitted), adult Wood Frogs appear to be insensitive to salinities as high as sea water (Hazard and Kwasek, 2009), and will remain in highly concentrated solutions even while losing substantial body mass due to osmotic flux of water. This raises questions about the tolerance of other temperate forest species, including close relatives of wood frogs (other Ranidae).

Amphibian species that are more terrestrial tend to have a greater tolerance for dehydration (increase in extracellular osmotic concentration) than aquatic species (Hillman et al., 2009); more terrestrial species should therefore also have greater physiological tolerance than aquatic species for high salinity. Because of this, I hypothesized that species which spend a greater portion of their lives tied directly to aquatic habitats (e.g. Eastern Newts, Bullfrogs) would show greater behavioral aversion to high salinity than species that only use aquatic habitats for breeding (e.g. Wood Frogs, Spotted Salamanders). However, I expected that more terrestrial species therefore may not avoid breeding in salinities that are tolerable to them, but detrimental to their offspring, while more aquatic species will avoid breeding in high-salt environments, leading to a higher probability of failure of reproduction in more terrestrial species.

Major objectives of this study

• Determine level of behavioral aversion to road deicers for adults of several focal species of New Jersey amphibians

- Correlate level of aversion with use of terrestrial/aquatic habitats.
- Evaluate responses to different deicers

• Monitor breeding activity by focal species at temporary and permanent bodies of water at varying distances from roads and test for a relationship between breeding success and concentrations of deicer-related ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-).

Methodology

Study Area and Study Species: The study focused on habitat within the northern Skylands conservation zones (zones 20-25; New Jersey Division of Fish and Wildlife, 2008); these zones within the state overlap substantially with the Northern NJ climate zone, which has the heaviest snowfall of the five state climate zones (Office of the New Jersey State Climatologist, 2009) and therefore the greatest use of deicers. However, the results are applicable throughout the state, as well as other northeastern states in which these species occur. Work centered around Montclair State University's New Jersey School of Conservation, located within Stokes State Forest. New Jersey has over 30 native amphibian species; most of them use wetlands for at least part of their life cycle. We targeted several common species of northwestern New Jersey (Table 2).

Table 1. Study species used

Family	**Species**	**Use of aquatic habitat by adults**
Ranidae (true frogs)	Wood Frog (*Lithobates (Rana) sylvaticus)*	breeding only (vernal pools)
	Green Frog *(Lithobates (Rana) clamitans)*	resident; semi-aquatic
	American Bullfrog (*Lithobates (Rana) catesbeiana)*	resident; semi-aquatic
	Northern Leopard Frog *(Lithobates pipiens)*	breeding; semiaquatic
Ambystomatidae (mole salamanders)	Spotted Salamander (*Ambystoma maculatum*)	breeding only (vernal pools)
Salamandridae (newts)	Eastern Newt (*Notophthalmus viridescens*)	some populations fully aquatic; some for breeding only

Research Questions and Experimental Approach

Measuring behavioral aversion

Animals were collected in the spring and summer or, in the case of leopard frogs, ordered from a commercial supply company, and kept at either the MSU campus or the NJ School of Conservation field station while trials were conducted. Animals were tested in choice trials in which they were placed in a chamber with two shallow compartments: one containing aged tap water (control) and one containing a deicer solution (using an ecologically relevant range of test concentrations; minimum of 8-10 trials per concentration). Location (test or control dish) and behaviors (moving, standing, or sitting) were recorded during 10-minute trials. Animals were weighed before and after the trial to evaluate water gain/loss. A repeated-measures design was used; each animal was exposed to each test concentration; animals were given at least three days to recover between tests.

In preliminary trials, time spent in the test concentration was bimodally distributed. Time was therefore converted to a binary variable (aversion/tolerance), and effect of concentration on aversion was analyzed using logistic regression. The EC_{50} (effective concentration, the concentration at which half of the individuals showed aversion) was calculated for each experiment as a means of comparing aversion across species and treatments.

We used these methods to address the following questions:

1. Is there interspecific variation in aversion to road deicers, and is it associated with the degree of aquatic habitat use?

Aversion to NaCl was measured in six NJ amphibian species (Table 2).

2. Does aversion differ depending on the kind of deicer used?

We measured aversion of spotted salamanders, a particularly sensitive species, to a variety of alternative deicers in addition to sodium chloride. (Table 3)

In addition to the laboratory aversion measurements, we conducted a field based study:

3. Preliminary evaluation of variation in salt content of vernal pools and impact on breeding activity

Water samples were collected from vernal pools and other bodies of water in northwestern New Jersey (primarily Sussex County), at varying distances from local roads. Samples were analyzed for conductivity, sodium, and chloride (the main components of the major road deicer, rock salt) using an Orion 4-Star multimeter and ion-selective electrodes. Samples were collected beginning in late winter just prior to emergence of early-breeding species, and then periodically throughout the breeding seasons of the study species. Pools were monitored for presence/absence of adults of the focal species, as well as breeding activity.

Principle Findings and Significance

1. Interspecific variation in aversion to road deicer aversion

The six species examined showed substantial differences in their responses to sodium chloride (Table 2). Spotted salamanders and eastern newts showed clear aversion to relatively low concentrations. Green frogs had similarly low tolerance (similar EC_{50}); however, the confidence interval is higher due to higher variability in the behavior of this species. Leopard frogs had higher tolerance than either spotted salamanders or eastern newts. For bullfrogs and wood frogs, there was no significant effect on concentration on aversion and it was not possible to calculate an EC_{50}. In the case of the bullfrogs, this was largely due to variability in behavior, leading to low statistical power to detect differences. There was a trend towards aversion at a level comparable to the leopard frogs, but it was not significant. Wood frogs clearly lacked aversion to salinities of up to 0.5 M (approximately sea water), remaining in the test concentration for 8 to 10 minutes regardless of concentration.

Table 2. Behavioral aversion of six New Jersey amphibian species to sodium chloride. EC_{50} is the concentration at which half of the individuals are predicted to show aversion, based on a logistic regression of aversion on concentration. Lower EC_{50} indicates lower tolerance/higher aversion. Treatments sharing a letter did not differ significantly (overlap of 95% confidence intervals).

Species	**N**	**EC_{50} (95% C.I.) (M)**
Wood frog	8	n/a
Green frog	10	0.219 (0.086-0.591)^{ab}
Bullfrog	8	n/a
Northern leopard frog	24	0.400 (0.334-0.526)^{b}
Spotted salamander	12	0.198 (0.145 – 0.283)^{a}
Eastern newt	28	0.205 (0.155 – 0.268)^{a}

2. Differential aversion to alternative deicers in a sensitive species, the spotted salamander.

Spotted salamanders showed differences in aversion to different deicing compounds (Table 3). In general, they had more aversion to chloride-containing compounds than to those lacking chloride, and more aversion to calcium and magnesium than to sodium compounds. However, the differences largely disappeared when concentrations were evaluated in terms of the estimated osmotic concentrations of the solutions rather than the molar concentrations. This suggests than osmolarity and chloride concentration are the main determinants of aversion in spotted salamanders.

Table 3. Behavioral aversion of the spotted salamander (N = 12) to several road deicing compounds. EC_{50} is the concentration at which half of the individuals are predicted to show aversion, based on a logistic regression of aversion on concentration. Lower EC_{50} indicates lower tolerance/higher aversion. Treatments sharing a letter did not differ significantly (overlap of 95% confidence intervals).

Deicer	**EC_{50} (95% C.I.) (M)**	**EC_{50} (95% C.I.) (osM)**
sodium chloride	0.198 (0.145 – 0.283)[bc]	0.395 (0.291 – 0.565)[ab]
sodium acetate	0.333 (0.264 – 0.468)[c]	0.668 (0.528 – 0.937)[b]
calcium chloride	0.109 (0.071 – 0.145)[a]	0.326 (0.212 – 0.434)[a]
calcium acetate	0.173 (0.129 – 0.231)[ab]	0.519 (0.388 – 0.694)[ab]
magnesium chloride	0.147 (0.127 – 0.170)[ab]	0.441 (0.380 – 0.510)[ab]
magnesium acetate	0.189 (0.153 – 0.243)[b]	0.568 (0.458 – 0.730)[b]
urea	0.632 (0.542 – 0.723)[d]	0.632 (0.542 – 0.723)[b]

3. Water quality and breeding success

Monitoring of water quality is still ongoing; preliminary results are presented here. Ion content was measured indirectly using conductivity as a proxy, and directly by measuring sodium and chloride content for some samples. Sodium and chloride were tightly correlated with conductivity (data not shown); therefore, conductivity alone was measured at most sites. Conductivity was low at all sites; however, sites within approximately 100 m of paved roads had higher conductivity than those further from roads (Table 4). There was no significant effect of date of sample collection (conductivity did not vary over time); however, there was low statistical power to detect any such differences. The funding period did not coincide with the breeding season for most species studied; however, we did observe reduced breeding or lack of breeding at two well- monitored roadside vernal pools compared to pools further from roads.

Table 4. Conductivity of water samples from vernal pools and other sites. Only sites for which multiple samples were collected are presented. Sites within 100 m of roads had significantly higher conductivity than those further from roads.

Site Code	Near Road?	Samples	Conductivity (μS/cm) (Mean ± S.E.)
206Sunrise	yes	2	79.3 ± 0.4
LCP	yes	3	116.1 ± 47.9
MBP	yes	3	545.8 ± 90.9
WPP	yes	4	116.1 ± 12.0
Spring	no	2	61.3 ± 9.5
VP1	no	3	23.6 ± 3.4
VP2	no	3	30.3 ± 9.7
VP6	no	5	59.2 ± 31.6
WFP	no	5	17.2 ± 3.2

Significance: This study provides critical information regarding the proximate causes of amphibian declines due to salinization of aquatic habitats, using a novel approach integrating conservation physiology and environmental science. This behavioral assay of amphibian salinity tolerance has not previously been applied to problems of water contamination, and provides a unique perspective on New Jersey water quality issues. The results will facilitate predictions of which species are more likely to be impacted by salinization due to either road salt runoff or saltwater intrusion. Based on our results, wood frogs in particular may face serious problems due to increased salinity. Adults show no aversion to even extremely high salinity, but tadpoles are extremely sensitive to increased salts, with an LC_{50} of approximately 0.05 M (Hazard, Kwasek and Conforti, unpublished data). This raises the possibility that wood frogs will stay and breed in ponds that are unsuitable for their offspring, leading to reproductive failure.

Followup research will be aimed primarily at (1) continuing to evaluate interspecific variation by testing additional species and (2) determining the physiological reasons underlying the variation in behavioral aversion. We have already begun to develop methods for examining physiological salinity tolerance to test the hypothesis that behavioral tolerance is associated with physiological tolerance in these species.

Additionally, this project complements existing studies at Montclair State University on New Jersey amphibian populations. Dr. Kirsten Monsen-Collar and I are currently collaborating with NJ Department of Environmental Protection to evaluate the distribution and prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) within New Jersey. All animals that were captured as a part of the road salt project were sampled (non-invasive skin swipes) and tested for *Bd* using a DNA assay for the fungus, thus allowing us to expand the scope of that project. DNA analysis for these animals is pending.

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Evaluation of three methodologies to document improvement of water quality through stormwater management measures in an urban subwatershed

Basic Information

Title:	Evaluation of three methodologies to document improvement of water quality through stormwater management measures in an urban subwatershed
Project Number:	2010NJ218B
Start Date:	3/1/2010
End Date:	2/28/2011
Funding Source:	104B
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Research Category:	Water Quality
Focus Category:	Water Quality, Non Point Pollution, Methods
Descriptors:	
Principal Investigators:	Pat Rector, Christopher Obropta

Publication

1. Rector, P., K.H. Klipstein, C. Obropta, R. O'Neill, B. Pearson, H. Barrett. Evaluation of three methods to determine impact of small Best Management Practices on Peters Brook, Somerville, NJ. In "2010 American Water Resources Association (AWRA) Annual Water Resources Conference". AWRA. Philadelphia, PA.

PROJECT SUMMARY

Problem and Research Objectives

This project is designed to evaluate three methods of tracking cumulative implementation of Best Management Practices (BMPs) on a subwatershed scale and determine the method that best documents water quality improvements. The criteria for determining the most appropriate methodology to document water quality improvement included: ease of use, cost, technical expertise necessary, and the ability to indicate the effects of cumulative BMPs in a subwatershed. Three methods were evaluated to document water quality improvement that resulted from implementation of BMPs. The three methods were: 1) modeling, 2) monitoring of flow to determine volume reductions, 3) monitoring of chemical and biological parameters. Additionally, "Build A Rain Barrel" Workshops were held and surveys were conducted to determine installation rates as well as the area of the home that was disconnected.

Methodology

1) Modeling: The Storm Loading and Management Model for Windows (WinSLAMM) was used to enter roof, driveway, street, and sidewalk data into a respective shape file in geographic information system (GIS) for two neighborhoods: one in Bridgewater Township and one in the Borough of Somerville (Fig. 1 and 2 respectively). The GIS was used to calculate the acreage of each of the land covers (Table 1), which was then entered into the WinSLAMM model as source areas for each neighborhood. The GIS also was used to determine soil types for each neighborhood. For this study it was assumed that driveways were connected impervious surfaces (i.e., they drain directly to the storm sewer system without passing over a pervious surface where runoff might infiltrate).

Rain barrels were modeled as bioinfiltration devices with a storage capacity of 50 gallons. It was assumed that each barrel was emptied twice a month. WinSLAMM was modeled with varied participation rates for both neighborhoods. Rainfall data from rain year 1993 at Newark International Airport (Fig. 3) was chosen to represent an average year of precipitation (42.5 in/yr) and the average storm during the simulated months was approximately 0.43 inches. This precipitation data would yield results valuable for our practice scenarios due to the small nature of each storm. At least 80% of the storms that will be modeled are less than 1 inch of total precipitation (Fig. 4). For smaller storms, volume reductions will be higher due to the limited storage capacity of the rain barrels.

Fig. 1. Aerial view Bridgewater Township study catchment.

Fig. 2. Aerial view the Borough of Somerville study catchment.

Table 1. Source areas and acreage for Somerville and Bridgewater neighborhoods

Somerville		
	Square Feet	Acres
Watershed	570,029.00	13.09
Roofs	80,395.48	1.85
Driveways	26,438.27	0.61
Streets	61,103.20	1.40
Sidewalks	16,977.10	0.39
Pervious	385,114.95	8.84
Bridgewater		
	Square Feet	Acres
Watershed	11,823,340.40	271.43
Roofs	512,644.68	11.77
Driveways	558,864.95	12.83
Streets	556,258.60	12.77
Sidewalks	22,068.90	0.51
Pervious	10,173,503.28	233.55

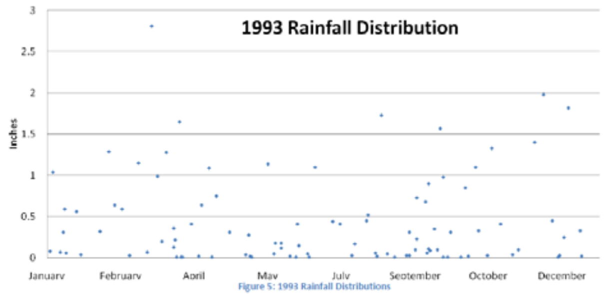

Fig. 3. 1993 Rainfall distribution Newark Airport. Total precipitation for 1993 was 42.5 inches.

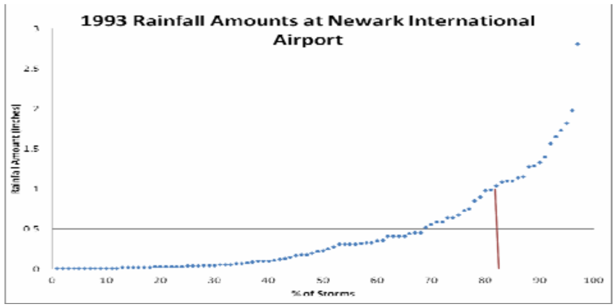

Fig. 4. Rainfall (inches) as percent of total rainfall at Newark Airport, 1993 Rain year.

The percent reduced under each scenario was calculated as:

$$\% \text{ Reduction} = \frac{R_b - R_{Sc}}{R_b} \times 100$$

where:

R_b = **Baseline Runoff based on the rainfall data at Newark International Airport without any BMPs installed**

R_{Sc} = **Scenario Runoff**

2) Monitoring of Flow: A flow monitor (Greyline Instruments Portable Stingray) level-velocity data logger with ultrasonic sensor was mounted in a storm sewer pipe in the catch basin within the Somerville neighborhood. Although it was originally planned to be mounted in an outfall pipe, sediment build-up within the pipe precluded this option. The meter sends an ultrasonic pulse and records the echo to determine the depth and velocity within the pipe when stormwater was flowing through the pipe.

Fig. 5. Storm sewer system Somerville Neighborhood. Circles indicate outfalls to Ross' Brook. Stingray flow sensor was placed in the catchbasin at Walnut Street (arrow).

3) Monitoring of Chemical and Biological Parameters: The study area was Ross' Brook, a tributary to Peters Brook in Somerville (Figure 6). Both neighborhoods are within this watershed. Peters Brook terminates at the Raritan River three miles above the confluence of a water supply intake.

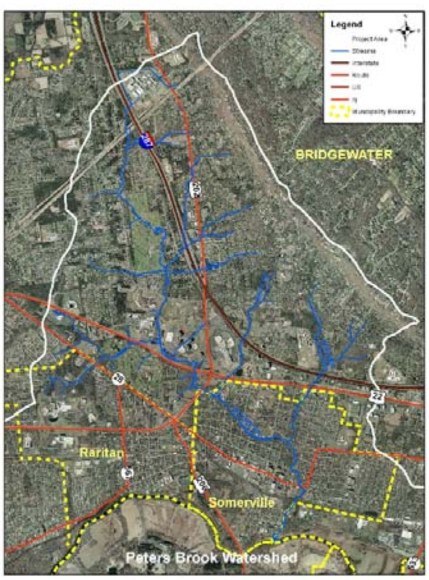

Fig. 6. Peters Brook Watershed.

Fig. 7. The Somerville Neighborhood

Sampling was conducted below the outfall of the Somerville neighborhood (Figure 7) at Walck Park, Somerville, New Jersey. A reach of approximately 50-m was selected to be sampled to remain upstream of the bridge as well as below the outfall. Visual assessments have been conducted on the stream and are included in Appendix A.

Previous biological data is included in Appendix B. Water quality sampling for total phosphorus (TP) and total suspended solids (TSS) was conducted on a monthly basis (Appendix C) to obtain baseline conditions, along with in-situ for temperature and dissolved oxygen. Flow measurements were taken during each sampling event (Appendix C).

Sampling was conducted from the downstream to the upstream section of the reach, using a 1-m kick net with three kicks collected, riffle single-habitat. Although the area consists of gravel, there was considerable sediment mixed with the gravel and stones. Due to the parameters of the study, the sampling area was only approximately 30m upstream of the bridge, not 100m as is usually the case when conducting biological monitoring. All stones were moved and cleaned within the 1-m sample area. The kick net was removed to shore and all individuals were identified in the field and then fixed in 95% ethanol and brought to the lab. Taxonomic identification was to Order or Family identification. In the case of leeches and aquatic worms, identification was only as generic as Class.

Rain Barrel Workshops and Surveys: Four “Build a Rain Barrel” workshops were conducted between June 2010 and August 2010 in the Borough of Somerville, the Borough of Raritan and the Township of Bridgewater. Rutgers IRB approved surveys (E11-031) were conducted in the fall of 2010 to determine installation rates and the area of the home that was disconnected.

<u>Significant Findings</u>

1) Modeling: When specific data regarding the neighborhood was entered into the WinSLAMM model, it was possible to identify the area best suited to show impact from installation of a Rain Barrel program as well as estimate the resulting reduction in roof runoff.

Three groups of scenarios were modeled. The first scenario was the "Baseline Scenario," which represented existing conditions where no rain barrels were installed. This Scenario was then run again with 10% of the homes having one rain barrel which captured runoff from 1/4 of their roof and the overflow from the barrel was discharged to a pervious, allowing the overflow water to be partially absorbed by this pervious surface. The number of homes with one rain barrel was then increased to 25%, 50% and 100%.

The second group of scenarios was the "Disconnection Scenario," which assumed that 100% of the roof was disconnected from flowing directly into the storm sewer. This scenario assumes that each home has four downspouts that discharge to a pervious surface such as a residential lawn. There are no rain barrels in this scenario. The scenario was then run assuming 10% of the houses in the neighborhood were completely disconnected. Then the scenario was run assuming 25%, 50% and 100% of the houses were completed disconnected in each neighborhood.

The third and final group of scenarios was the "Disconnection and Rain Barrel Scenario," which is the same as the second scenario except each of the four downspouts for each house has a rain barrel. This allows for more water to be detained for reuse and less water discharged to surrounding pervious surfaces. Therefore, this scenario provides the most reduction in runoff because the rain barrels keep additional stormwater from discharging to already saturated pervious surfaces.

Table 4. Results from model of rain barrels in Somerville and Bridgewater. Differences are due to Land Use and size of lots.

Somerville – 130 total houses		
	Roof Runoff	
Scenario	**cu. Ft.**	**% Reduction**
Baseline	75,300	-
10%	72,468	4
25%	68,254	9
50%	61,758	18
100%	39,807	**47**
100% Disconnection		
10%	70,360	7
25%	62,920	16
50%	50,558	33
100%	25,818	**66**
Disconnection and Barrels		
10%	68,787	9
25%	53,978	28
50%	43,114	43
100%	11,698	**84**

Bridgewater – 200 total houses		
	Roof Runoff	
Scenario	**cu. Ft.**	% Reduction
Baseline	305,411	-
10%	294,780	3
25%	284,441	7
50%	266,923	13
100%	134,191	**56**
100% Disconnection		
10%	278,509	9
25%	248,420	19
50%	198,252	35
100%	104,798	**66**
Disconnection and Barrels		
10%	275,418	10
25%	243,187	20
50%	187,811	39
100%	84,059	**72**

Although the lot sizes in Bridgewater were larger, allowing for more absorption of the disconnected runoff, Bridgewater also had larger roofs that generated more runoff. The results indicate that the increase in lot size was not substantial enough to compensate for the larger roof size. Therefore, the percent runoff reduction was not as high in Bridgewater as it was in Somerville. Somerville has less roof runoff and a greater ability for high rain barrel participation to capture a majority of runoff.

2) Monitoring of Flow: The Stingray Flow Monitor collected measurable data for each storm. The sensor constantly sits in 2.5" of water, or 0.2', measured and recorded for periods of dry weather. Limited to non-turbulent water, turbulence causes zero data points and gaps in the hydrograph. Data had to be filtered, and any measurements below 0.2' were removed. Despite these restrictions, the Stingray has shown promise to measure flow in the storm sewer pipes in a neighborhood system. This will allow for the measurement of impact of small BMPs on a small spatial scale, provided enough BMPs are installed to significantly reduce runoff. Monitoring is continuing under the generosity of the Rutgers Water Resources Program and we hope to have further data to calibrate this summer.

3) Monitoring of Chemical and Biological Parameters: Based on the expertise and funding available and the sampling conducted on Peters Brook already as part of New Jersey Water Supply Authority's Watershed Program, a few simple changes in protocol might allow for better discernment of changes in water quality based on impacts from installation of small BMPs without inordinate cost or expertise. Recommended suggestions included to increase the literature search for specific taxonomic information regarding macroinvertebrates identified and dominant orders or families. It is also suggested to maintain a scheduled sampling regime to allow for comparison of similar seasonality. Various small microscopes that can be attached to laptops may be of assistance for small investment of funds.

Rain Barrel Survey: The Rain Barrel workshop and survey found that although it was not as well attended in the specific neighborhoods identified, the overall installation rate was higher on a watershed basis (87%) than the statewide average (71%), presumably due to the community aspect and connection of the workshops to the neighborhood stream. A significant finding from the surveys was that although there was considerable educational focus on disconnection of the downspout leading to the driveway, the majority (76%) did not disconnect this downspout to their rain barrel.

Conclusions

Based upon this study, monitoring the flow appears to be the best option for measuring the impact of BMP installation on a watershed scale, provided a significant number of homes participate in the BMP program, in this case, the disconnection and rain barrel program. Due to the lack of sensitivity of the Stingray Flow Meter, it will be difficult to measure the reduction in post-BMP installation flows unless at least 10 to 20% of the homes participate. Additionally, a substantial database needs to be assembled so that pipe flow can be correlated with rainfall. Once the pre-BMP installation relationship is developed between flow and rainfall, it can easily be compared to a post-BMP installation relationship. This comparison would yield a measurement of flow reduction on a watershed scale that results from BMP installation. Until substantial data are collected, the WinSLAMM model appears to provide reasonable estimates of anticipated flow reductions for a disconnection program. Until the WinSLAMM model is calibrated with real flow data, the model can only be used to compare difference between various scenarios but this is important to determine the scale of BMP adoption (10%, 25%, 50%, or 100%) that is needed to begin to make a difference.

The funding provided by NJWRRI allowed for various methods to be tested and allowed the researchers to refine their experimental methodology. Data will continue to be collected to expand on these findings.

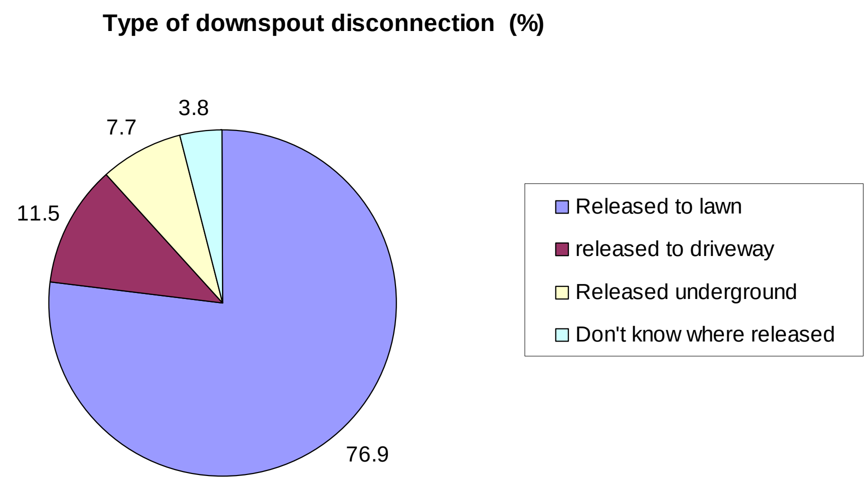

Fig. 8. Results of follow up survey conducted fall 2010.

Development of Sustainable Biosorbents to Recover Nutrients from Water

Basic Information

Title:	Development of Sustainable Biosorbents to Recover Nutrients from Water
Project Number:	2010NJ219B
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Focus Category:	Methods, Nutrients, Treatment
Descriptors:	None
Principal Investigators:	Mahmoud Wazne

Publications

There are no publications.

1. Introduction

Nutrient pollution, especially from nitrogen and phosphorus, is one of the main causes of water quality degradation in the U.S. In New Jersey, new stormwater rules were approved which state that new developments must include stormwater management measures to maintain 100% of the preconstruction groundwater infiltration and reduce the average annual total suspended solids (TSS) load in the development site's post-construction runoff by 80 percent (Stormwater Management Rules at N.J.A.C. 7:8). These measures must also reduce the nutrient load (consisting primarily of nitrogen and phosphorous) in the post-construction runoff by the maximum extent feasible. The recommended removal rates for total phosphorus and total nitrogen range from 20 to 60 percent for the former and 20 to 50 percent for the latter, depending on the removal method (NJDEP, 2004). The biosorbents developed in this research can be used to reduce nutrient loadings from new developments, small farms and other diffuse nutrient pollution hot spots.

This research aims to develop and test functionalized quaternary amine anion exchange resins consisting of waste lignoncellulosic materials to remove nutrients from water. The proposed route to the formation of these materials is by etherification of the cellulosic moiety of agricultural waste products using ammonium salts in the presence of an alkaline catalyst. The developed lignocellulosic anion resins can be placed in bioretention basins and trenches surrounding problematic areas to intercept nutrients from runoff. Upon saturation, the nutrient loaded biosorbents can be composted and recycled as fertilizer. If implemented, this practice would be a truly green and sustainable process, where lignocellulosic waste materials are used to recover nutrients that are otherwise pollutants and recycle them back to farm land.

2. Problem and research objectives

The objective of this research is to develop and test functionalized quaternary amine anion exchange resins consisting of waste lignoncellulosic materials to remove nutrients from water. The proposed route to the formation of these materials is by etherification of the cellulosic moiety of agricultural waste products using ammonium salts in the presence of an alkaline catalyst. Quaternary amines are reacted with the –OH functional groups in the cellulosic moiety to yield the resins. Anion selectivity is achieved by changing the side chain of the ammonium ion. The lignocellulosic anion resins are then used to remove nutrients from water. Upon saturation, the nutrient loaded biosorbent can be composted and recycled as fertilizer.

3. Methodology

3.1 Development of the biosorbents - Method I

Unmodified pine bark (UPB), unmodified cotton (UC) and unmodified rice husk (URH) were tested in this research as potential biosorbents to remove phosphate (PO_4^{3-}) from aqueous solutions. The raw biosorbents were also quaternized and tested for potential improved anion uptake. All raw materials were first milled in a Wiley mill

(Thomas Scientific, Swedesboro, NJ) and sieved to retain the 10–20 mesh fractions. The quaternization was conducted by using an ionic liquid analogue, based on a eutectic mixture of a choline chloride derivative and urea. The deep eutectic solvent (DES) was prepared by first adding 12.96 g of Chlorcholine chloride (ClChCl) to 10.27 g of urea and the mixture was heated at a Thermolyne Mirak hotplate, at 75°C and stirred occasionally for 30 minutes. The ionic liquid analogue was then allowed to cool to room temperature. The quaternization was commenced by adding 0.1 g of the raw material to 5 ml of DES followed by 0.392 g of Imidazole base. The mixture was heated in a Fisher Scientific HiTemp Oil Bath at various temperatures (90°C - 150°C) for 15 hours and then cooled to room temperature. The modified biosorbents were extracted from the eutectic solvent and washed with water.

3.2 Development of biosorbents with ion selectivity – Method II

Biosorbents with ion selectivity were developed by changing the side chain of the ammonium ion from trimethyl to dibutylmethyl ammonium ion. 20ml of N,N Dibutylethanolamine (100mmol) and 8 ml of Thionyl chloride (125mmol) were each dissolved separately in 60ml of dichloromethane solvent. The dissolved amino-alcohol was placed in a 250ml three neck round-bottomed flasks equipped with a magnetic stirring bar, a thermometer, an addition funnel, and an exit tube connected to a gas trap. With continuous stirring, the dissolved thionyl chloride was added drop wise, due to the exothermic nature of the reaction, to the dissolved alcohol for about 30 minutes under a hood to form N-(2-Chloroethyl) dibutylamine. An iced water bath was used, when necessary, to keep the temperature of the reaction below 40 °C to avoid the evaporation of the solvent. When the addition was complete, the addition funnel was closed with a glass stopper. The reaction was conducted at room temperature for three hours. After the completion of the reaction, the N-(2-Chloroethyl)dibutylamine product in the flask was distilled to remove dichloromethane at 50 °C and the excess of thionyl chloride at 100°C using an oil bath (Fisher scientific Hi temp bath). After completion of the distillation, the N-(2-Chloroethyl) dibutylamine product was reacted with methyl iodide to yield the chlorocholine chloride analogue. The reaction was conducted at 40 °C in the oil bath for 20 minutes. The product formed at the end of the reaction was recrystallized as chlorocholine chloride analogue using methanol.

Quaternization was conducted by reacting 10 g of the chlorocholine chloride analogue with 0.1 g of pine bark in the presence of 0.375g of sodium hydroxide. The mixture was heated at 90°C, in an oil bath, for 15 hours before being allowed to cool to room temperature. The modified pine bark was copiously washed with methanol then filtrated with a 0.45 μm filter. The retentate was dried under the hood for 24 hours.

3.3 Batch sorption tests

Anion sorption tests were performed using the biosorbents prepared by method I or method II. The biosorbents developed using method I are labeled with the number extension 1, whereas those developed using method II are labeled with the number extension 2. For example, modified pine bark, modified cotton and modified rice husks prepared by method I are labeled (MPB-1), (MC-1) and (MRH-1), respectively; whereas modified pine bark developed by method II is labeled as (MPB-2).

The batch adsorption experiments were carried out in 30ml plastic bottles. The tests were initiated by the addition of the biosorbents to a solution containing the dissolved anions. The bottles were then capped and mixed for 2 hours. Kinetic data (not presented) indicated that all uptakes reached equilibrium in less than 2 hours. After mixing, the solutions were withdrawn from the flasks and separated from the solids by filtration with 0.45μm filters. Solution pH was adjusted and measured with an Accumet AR20 pH–meter using sodium hydroxide (NaOH) solution (initial pH was approximately 3 for all prepared biosorbents), and the ionic strength was adjusted by to 0.01 M using of sodium chloride (NaCl). The initial and the residual concentration of the tested anion(s) for all samples were determined using spectrophotometry, whereas the concentrations of the cations were measured using inductively coupled plasma optical emission spectrometry (ICP-OES).

4. Results and discussion

4.1. Optimal temperature for quaternization

The quaternization of the biosorbents using method I was conducted at various temperatures ranging from 90°C to 150°C. The prepared biosorbents were then used in batch sorption tests to assess their uptake capacity for phosphate using PO_4^{3-} concentration of 100 mg/L. The tests results for MPB-1 and MC-1 are presented in Figure 1 below. The best phosphate uptake capacity was achieved for the biosorbents prepared at 150°C. Moreover, it appears that MPB-1 has better removal capacity than MC-1 for all temperatures tested. No PO_4^{3-} removal was achieved in the batch tests conducted with the raw materials; i.e., UPB and UC.

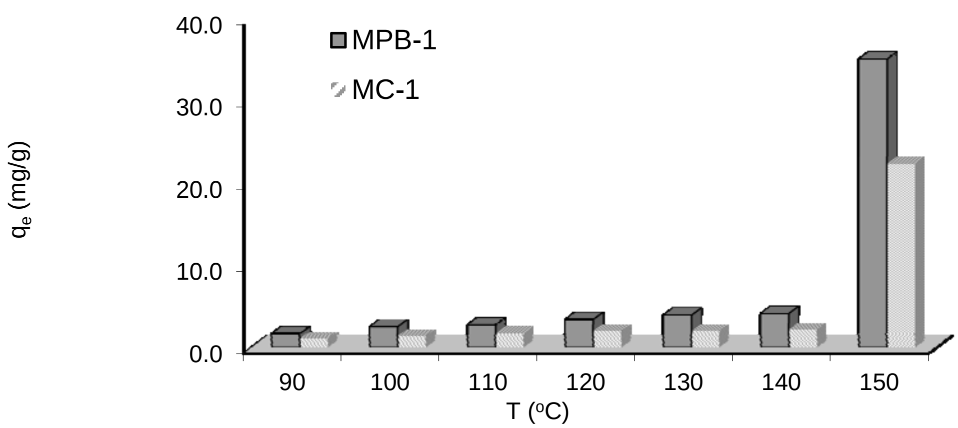

Figure 1. PO_4^{3-} - P removal from MPB-1 and MC-1

Realizing the best results were achieved at 150°C, the quaternization method was also tested using another agricultural waste residue, rice husk. The modified rice husk (MRH-1) was then tested for phosphate uptake from a solution containing 200 mg/L PO_4^{3-}. The uptake results are shown in Figure 2 which indicated a significant removal rate, but not as great compared to MPB-1.

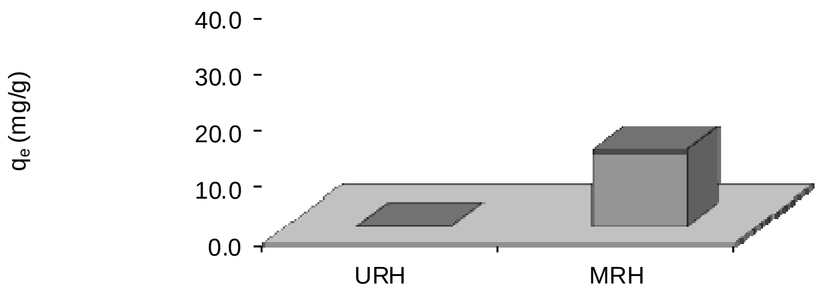

Figure 2. PO_4^{3-} - P removal from MRH-1

4.2. Adsorption isotherms

Batch tests adsorption isotherms were conducted to determine the PO_4^{3-} adsorption capacity of MC-1 at various equilibrium PO_4^{3-} concentrations. PO_4^{3-} synthetic solutions with concentrations ranging between 60-480 mg/L (PO_4^{3-}-P) were prepared and used in these tests. The results of the adsorption experiments are shown in Figure 3. The test results showed higher uptake PO_4^{3-} capacity at higher aqueous PO_4^{3-} concentrations.

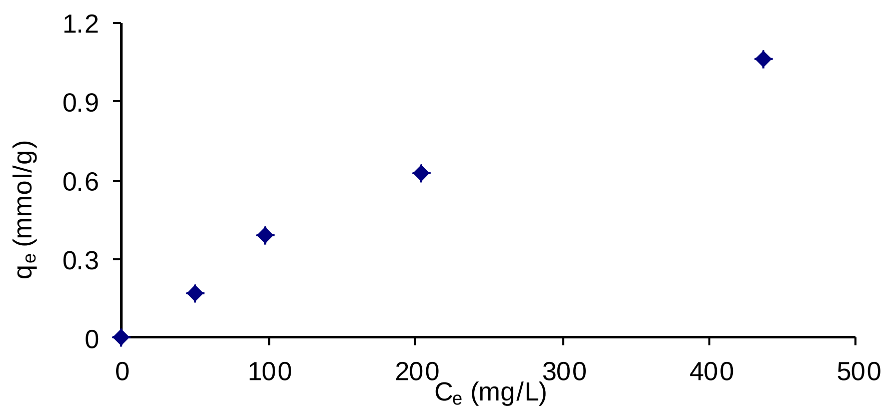

Figure 3. Adsorption isotherm of PO_4^{3-} - P by MC-1

4.3. Competitive ion adsorption

Competitive ion adsorption experiments between PO_4^{3-} and sulfate (SO_4^{2-}), and PO_4^{3-} and chromate (CrO_4^{2-}) were also conducted using MC-1 and MPB-1. The test results are shown in Figures 4 and 5, respectively. As observed for MC-1, the adsorption affinity is stronger for PO_4^{3-} than for SO_4^{2-}. Similarly, MPB-1 showed greater affinity for PO_4^{3-} than for CrO_4^{2-}. This indicates that the adsorption affinity is positively correlated with the ion valency.

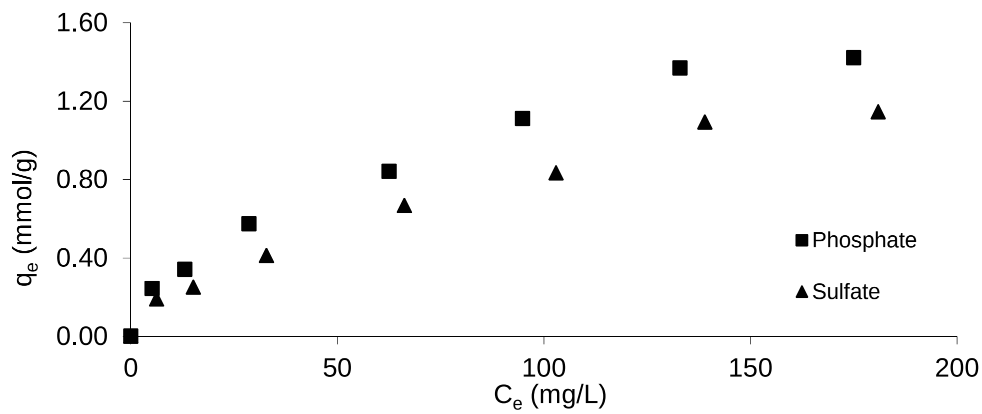

Figure 4. Isotherms of PO_4^{3-} and SO_4^{2-} co-exist with MC-1

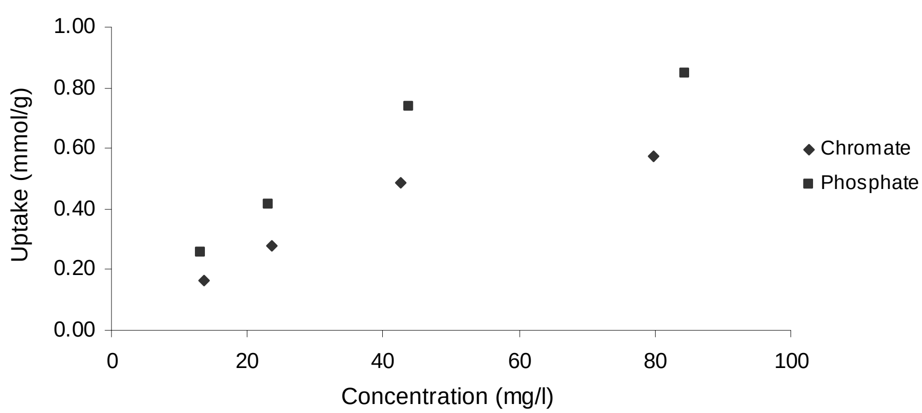

Figure 5. Isotherms of PO_4^{3-} and CrO_4^{2-} co-exist with MPB-1

4.4. pH effects

The effect of pH on PO_4^{3-} sorption capacity was investigated using MRH-1. The test results presented in Figure 6 indicated that greater removal rates were obtained at lower pH values. The accelerated decrease of PO_4^{3-} adsorption at higher pH values could be due to the competition between the hydroxide groups and the PO_4^{3-} anions for the adsorption sites.

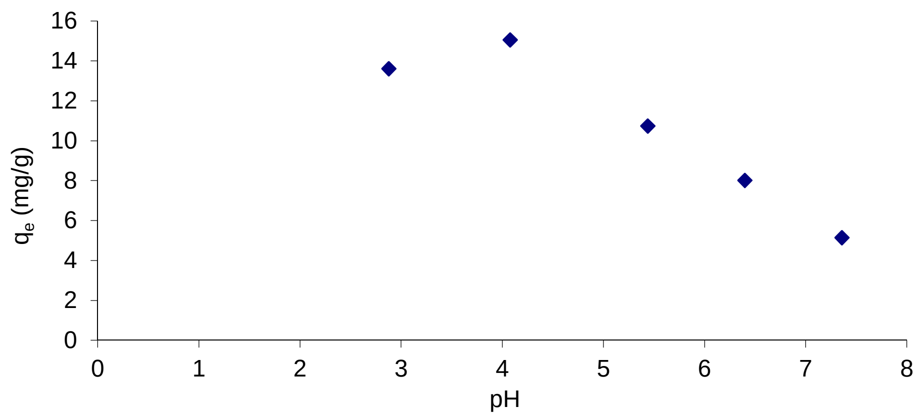

Figure 6. Effect of pH on the PO_4^{3-}uptake by MRH-1

4.5. Cations uptake

The cation uptake capacity of MPB-1 was tested using lead (Pb) as surrogate. A 200 mg/L Pb^{2+} solution was prepared and tested for MPB-1 uptake. The solution pH was adjusted to 3 in order to avoid Pb precipitation onto the biosorbent. The batch tests were conducted as described previously. The Pb analysis in the supernatant indicated that the biosorbent has no removal capacity for Pb^{2+} as expected.

4.6. Ion Selectivity

The ion selectivity tests with MPB-2 were conducted using separate PO_4^{3-} and NO_3^- solutions with a concentration of 200 mg/L each. The test results in Figure 7 indicated that MPB-2 has greater removal rates for PO_4^{3-} than for NO_3^-. Even though the developed resins were designed to provide better selectivity towards NO_3^-, the results indicated otherwise. More work is needed to improve the resins selectivity.

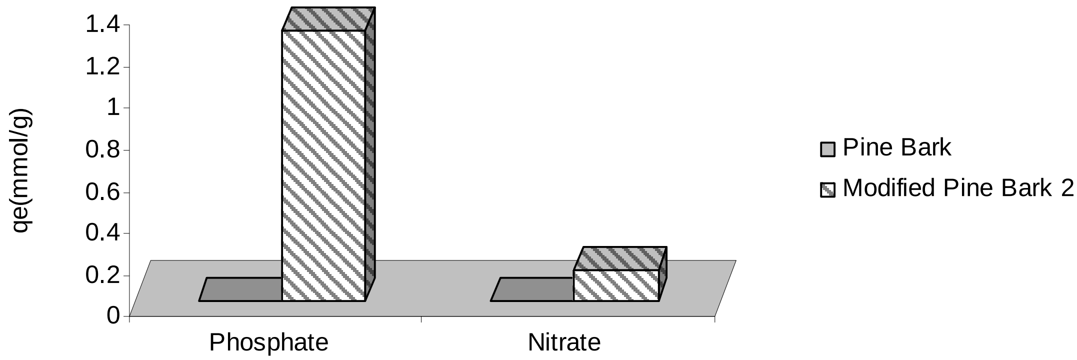

Figure 7. PO_4^{3-}-P and NO_3^- -N removal capacities by MPB-2.

5. Significance

This research was successful in developing biosorbents from lignocellulosic waste product. The developed resins have shown excellent removal capacity for anions including phosphate and nitrate. However, more work is needed to further develop the ion selectivity.

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Comparative toxicological assessment of gasoline oxygenates MTBE, ETBE and TAME, and their metabolites, in the zebrafish cardiovascular system

Basic Information

Title:	Comparative toxicological assessment of gasoline oxygenates MTBE, ETBE and TAME, and their metabolites, in the zebrafish cardiovascular system
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Principal Investigators:	Josephine Bonventre, Keith R Cooper

Publications

1. Bonventre, J.A., L.A. White and K.R. Cooper, 2011, Early disruption of angiogenesis-related genes by methyl tert-butyl ether (MTBE) leads to vascular specific lesions in the zebrafish (Danio rerio). Poster Abstract #2548, The Toxicologist: Vol 120, Number S-2, Society of Toxicology Meeting, 6-10 March 2011, pg 545.
2. Bonventre, J.A., L.A. White and K.R. Cooper, 2010, Embryonic exposure to MTBE, ETBE and TAME in zebrafish leads to chemical specific lesions and altered expression of angiogenesis related genes. Poster Abstract #MP143, SETAC North America 31st Annual Meeting, 7–11 November 2010, pg 229.
3. Bonventre, J.A., L.A. White and K.R. Cooper, 2010, Increased lesion occurrence and decreased gene expression following embryonic exposure to MTBE, ETBE, and TAME in the zebrafish (Danio rerio). Poster. MASOT Regional Chapter Spring Meeting, May 2010.
4. Bonventre, J.A., L.A. White, K.R. Cooper, 2011, Methyl tert butyl ether targets developing vasculature in zebrafish (Danio rerio) embryos. Aquatic Toxicology. In press, doi:10.1016/j.aquatox.2011.05.006
5. Bonventre, J.A., L.A. White and K.R. Cooper, Embryonic exposure to MTBE, ETBE and TAME in zebrafish leads to chemical specific lesions and altered expression of angiogenesis related genes. NJWRRI Grant Recipient Poster Presentation, Cook Campus Center, New Brunswick, NJ, February 10, 2011.

Project Summary:

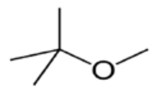
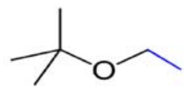
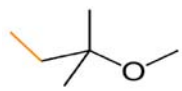
I. Problem and Research Objectives

The research conducted here addressed the first priority of the Water Resources Research Act regarding consequences of potential contaminants to water supplies and toxicological impacts on aquatic species. This project compared the toxicological effects of common gasoline oxygenates, methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) on developing zebrafish (*Danio rerio*), using morphological and molecular endpoints. The developing cardiovascular system is often a target for chemical toxicants and the pathways affected are conserved across species (Heideman *et al*., 2005). Our preliminary studies on MTBE, ETBE and TAME toxicity to developing zebrafish demonstrated that acute low dose exposure of zebrafish embryos to nominal concentrations of the individual gasoline oxygenates (0.625mM to 10mM) yielded a dose-response relationship for various lesions associated with vascular development, including pooled blood in the common cardinal vein, cranial hemorrhages, abnormal intersegmental vessels, and pericardial edema. The preliminary studies also suggested that though ETBE and TAME induced lesions in embryos similar to those that occurred with MTBE, lesions occurred at lower doses, were more severe and were less targeted to the developing vasculature. We hypothesized that MTBE-targeted toxicity to developing vasculature resulted from a disruption in the vascular endothelial growth factor (VEGF) pathway, a mediator of blood vessel development. Angiogenesis, the development of blood vessels from preexisting blood vessels, is a conserved developmental process and important to proper growth and development of all species. In addition, we hypothesized that ETBE and TAME would significantly alter expression of other non-vascular specific genes that were not affected by MTBE.

MTBE [$CH_3OC(CH_3)_3$] and other oxygenates have been added to gasoline since the 1970s, first as a replacement for lead as an octane enhancer, and later in the form of "reformulated" and "oxygenated" fuels (Ahmed, 2001). The Clean Air Act Amendment of 1990 (US EPA, 1990) brought about a surge in MTBE production, but evidence of MTBE presence and persistence in surface and groundwater supplies, along with increasing complaints of ill effects from people in cities using MTBE, generated a concern for adverse human health and wildlife effects as a result of exposure (Beller and Middaugh, 1992; Moolenar et al., 1994; Morgan et al., 2005). As of August 2007, 25 states, including New Jersey, have enacted either complete or partial bans on the use of MTBE in gasoline (US EPA, 2007). New Jersey allows up to 0.5% (v/v) of MTBE in gasoline. While MTBE use in the US has declined since its peak in the mid 1990s, its use as a gasoline additive worldwide continues (Rosell et al., 2006; vanWezel et al., 2009). Production of MTBE remains high, averaging just over 1 million barrels per month in 2009 and 2010 (DOE-EIA, 2010). Limitations on the use of MTBE in the United States have led to the use of structurally related chemicals ETBE [$CH_3CH_2OC(CH_3)_3$] and TAME [$CH_3OC(CH_3)_2CH_2CH_3$] (Coons, 2009; van Wezel et al., 2009), despite the similarities in chemical characteristics with the banned chemical [Table 1].

Other than acute lethality, MTBE's effects on aquatic vertebrates are largely uninvestigated. In a review of early invertebrate and vertebrate LC50 studies, Werner et al. (2001) reported that environmental levels of MTBE were unlikely to cause acute toxic effects in freshwater organisms. However, embryonic studies in the African catfish (*Clarias gariepinus*) and the Japanese medaka (*Oryzias latipes*) indicated that sublethal concentrations of MTBE resulted in increased embryonic lesions and decrease survival (Moreels et al. 2006; Longo, 1995). Far fewer studies on the effects of ETBE and TAME exist in the literature. Our studies investigate the specificity of MTBE to the developing vasculature and the increased toxicity of the MTBE substitutes, ETBE and TAME, to the aquatic embryo.

Table 1: Structurally similar gasoline oxygenates MTBE, ETBE and TAME

Chemical	Structure	Molec. formula	Molec. Weight (g/mol)	Solubility (g/L)
Methyl tert-butyl ether (MTBE)		$C_5H_{12}O$	88.15	48 g
Ethyl tert-butyl ether (ETBE)		$C_6H_{14}O$	102.18	12 g
Tertiary amyl methyl ether (TAME)		$C_6H_{14}O$	102.18	12 g

II. Methodology

A. Model organism

The zebrafish has emerged as a powerful model for understanding vertebrate development (Spitzbergen and Kent, 2003). Transgenic zebrafish *fli1*-EGFPs (Fli1s), which express enhanced green fluorescent protein driven by the fli1 promoter in all vascular endothelial cells, were used for all experiments (Lawson and Weinstein, 2002). Expression of EGFP in all endothelial cells of Fli1s allows for *in vivo* examination of vasculature morphology in live embryos.

B. LC50 Studies

Exposures were performed in triplicate at static, non-renewal nominal concentrations ranging from 6.25 to 200 mM MTBE, 2.5 to 40 mM ETBE, and 2.5 to 35 mM TAME. Treatments were performed in sealed glass scintillation vials to avoid volatilization of the chemical (N = 3 vials per concentration, 15 embryos each vial). The studies were repeated twice. Exposure began at 3 hours post fertilization (hpf) (512 cell stage) and embryos were observed daily for mortality until 120 hpf. LC50 and 95% confidence intervals were calculated at 120 hpf using the Litchfield-Wilcoxon method (Litchfield and Wilcoxon, 1949).

C. Dose Response Studies

Following OECD 212 guidelines, embryos were exposed individually (N = 20 per treatment) in sealed 4 ml glass vials to static, non-renewal concentrations of 0.625, 1.25, 2.5, 5, and 10 mM of MTBE, ETBE or TAME, and observed under light and fluorescence microscopy. Embryos were observed daily for the 5-day developmental period and at 3 days post hatch (dph). EC50 and 95% confidence intervals for lesion occurrence were determined using the Litchfield-Wilcoxon method (Litchfield and Wilcoxon, 1949). The MTBE, ETBE and TAME dose response studies were each repeated 3 times.

D. Critical Period Studies

A staggered exposure regime was used to determine the beginning (Study A) and end (Study B) of the critical window for MTBE-induced specific lesions (Fig. 2). In Study A, embryos (N = 25) were exposed in individual glass vials to static nonrenewal treatments of 10 mM MTBE at an initial time point: 15, 24, 30, or 48 hpf. In Study B, embryos (N = 25) were treated in individual vials to static nonrenewal treatments of 10 mM MTBE at 3 hpf (time zero) and treatment was discontinued at 15, 24, 30, or 48 hpf. In the third study (Study C, N = 25) embryos were exposed to 10 mM MTBE during the critical period (15 hpf to 30 hpf; 6-somites to prim-5), determined by Study A and B (N = 20 per treatment). Embryos were observed daily under light and fluorescence microscopy for 5 days. Studies A, B and C were each repeated twice.

E. Real-time Polymerase Chain Reaction

Embryos were exposed to a static non-renewal treatment of 0 (control) or 5 mM MTBE, ETBE or TAME until 24 hpf (21-somites) in glass scintillation vials to determine the effect of the chemical on the mRNA expression of key genes associated with the VEGF pathway and embryonic development. Approximately 50 embryos were used per treatment, each treatment was performed in triplicate, and the experiment was repeated three times. Descriptions of the genes of interest are listed in Table 5.

III. Principal Findings and Significance

The calculated LC50s and confidence intervals (CI) based on nominal test concentrations for MTBE, ETBE and TAME are reported in Table 2. The similarities in LC50s were expected since MTBE, ETBE and TAME differ by only one methyl group. However, TAME had the lowest LC50 and the steepest mortality curve, as determined by the range of the CIs. Based on LC50s, TAME and ETBE are more toxic than MTBE.

Table 2. Calculated LC50s for MTBE, ETBE and TAME

	LC50 Study I	LC50 Study II
MTBE	19 mM [CI = 15.9 - 22.3]	20 mM [CI = 17.9 - 22.7]
ETBE	16 mM [CI = 12.0 - 22.0]	12 mM [CI = 8.0 - 18.0]
TAME	11.2 mM [CI = 9.0 - 14.1]	8.7 mM [CI = 7.0 - 11.1]

The concentrations used for the dose response studies were based on sub-lethal concentrations of MTBE (0.625 - 10 mM). The aims of these studies were to (A.) characterize the specific vascular lesions associated with MTBE exposure observed in preliminary studies, and (B.) determine if the replacement oxygenates, ETBE and TAME, had similar toxicities to MTBE. The lesions observed with exposure to MTBE were consistent with the disruption of cardiovascular development including pooled blood in the common cardinal vein (CCV), cranial hemorrhages, abnormal intersegmental vessel (ISV) formation, pericardial edema, and reduced circulating RBCs. There was a dose dependent increase in the occurrence and severity of these lesions (Figure 1), while no effect was observed on the heart or other organ system development. This suggested that MTBE specifically targets angiogenesis in the developing zebrafish.

ETBE exposed embryos exhibited dose dependent vascular lesions similar to those of MTBE exposed embryos (Figure 1). However, ETBE induced hemorrhages in different parts of the body, specifically in the medial caudal region, that were not observed in MTBE exposed embryos. ETBE also effected cardiac development: hypertrophic cardiac cells, enlarged hearts, and decreased heart rate at the 10mM. Severely edematous embryos were also observed with 10mM ETBE, which may have resulted from the effect on the heart. At the same nominal concentrations, however, TAME affected embryonic development more severely. Within 24 hpf, embryos exposed to 5 and 10 mM TAME were 2 and 9 stages behind control embryos (18-

somites and 12-somites, respectively verses 21-somites in controls). By 48hpf, 5 mM TAME treated embryos no longer appeared to be at a delayed stage, while 10 mM TAME embryos were significantly smaller, lacked any formation of blood cells and circulation, and exhibited craniofacial abnormalities such as smaller inter-occular distance and diminished or absent lower jaw formation. Similar to ETBE embryos, TAME embryos had a significantly increased percentage of severely edematous embryos at 5 and 10 mM MTBE. However, at 2.5 mM TAME, lesions appeared more targeted toward the vascular system, and was similar to the toxicity observed with 5 and 10 mM MTBE (Figure 1). The LOAELs and EC50s for the major lesions observed with embryonic exposure to MTBE, ETBE or TAME are presented in Table 3 and 4. The data for the LC50 and dose response studies suggests the order of toxicity to developing zebrafish embryos for the three oxygenates to be TAME > ETBE > MTBE.

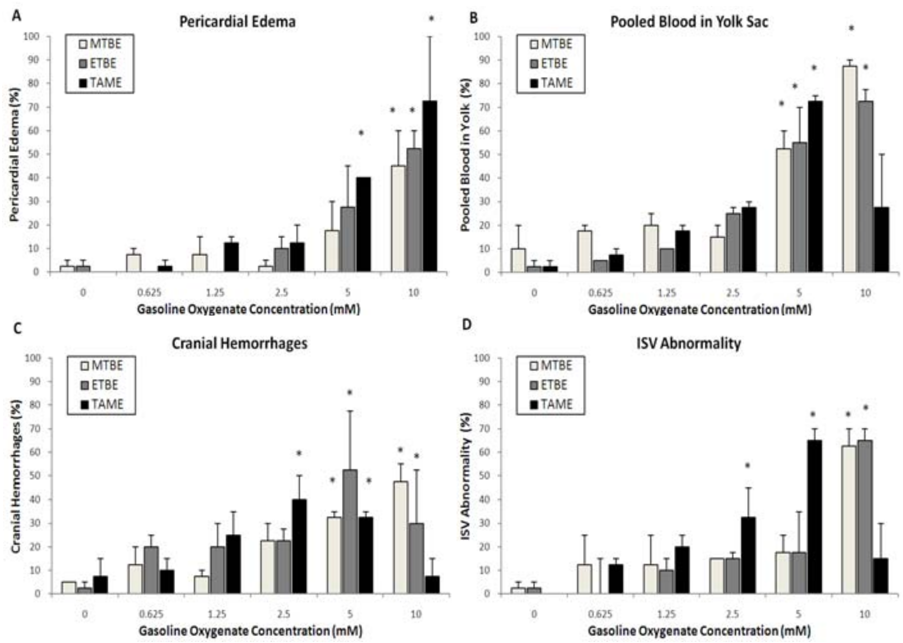


Figure 1. The percent of embryos exhibited MTBE-specific vascular lesions in the three oxygenates: MTBE [white bars], ETBE [gray bars] or TAME [black bars] (A) Pericardial edema, (B) Pooled blood in yolk sac, (C) Cranial hemorrhages, (D) ISV abnormalities. Percentages are based on averages of repeated studies; error bars represent range. *Indicates a statistical difference between control and treated groups. Fischer's Exact Test was used to determine significance, $p<0.05$.

Table 3. LOAELS for MTBE, ETBE, or TAME

	MTBE (mM)	ETBE (mM)	TAME (mM)
Total Lesion	5	2.5	2.5
Mortality by 120 hpf	-	10	10
Hatched by 120 hpf	-	10	5
Survival to 3 dph	-	10	10
Pericardial Edema	5	5	5
Pooled blood in CCV	5	2.5	2.5
Cranial Hemorrhage	5	5	2.5
ISV Abnormality	5	5	2.5
Yolk Edema	-	2.5	2.5
Yolk Dysmorphogenesis	-	5	5
Craniofacial abnormalities	-	-	5

Table 4. EC50s for individual lesions occurring over 5 days of development

	MTBE (mM)	ETBE (mM)	TAME (mM)
Pericardial Edema	20.0 [9.6 - 41.6]	9.5 [6.1 - 14.8]	6.0 [4.1 - 8.8]
Pooled Blood in CCV	3.2 [2.2 - 4.7]	4.2 [2.9 - 6.1]	4.1 [3.1 - 5.5]
Cranial Hemorrhage	11.0 [5.9 - 20.5]	10.0 [4.9 - 20.6]	4.8 [2.8 - 8.2]
ISV Abnormality	14.5 [6.5 - 32.4]	8.5 [5.4 - 13.3]	3.8 [2.2 - 6.4]
Yolk Edema	not observed	7.5 [5.1 - 11.1]	9.0 [5.9 - 13.8]

A critical period for MTBE toxicity in zebrafish embryos was established to further understand the specificity of the chemical, and the sensitivity of the embryonic vascular system Figure 2). In Study A, the embryos exposed to MTBE between 15-120 hpf or 24-120 hpf exhibited significantly more lesions ($p \leq 0.05$) than control embryos, while the number of lesions observed in embryos exposed to MTBE between 30-120 hpf or 48-120 hpf were not significantly different from controls. In Study B, all embryos exposed from 0-30 hpf and 0-48 hpf had significantly more ($p \leq 0.05$) lesions than control embryos or than the embryos exposed from 0-15 hpf and 0-24 hpf. Together, these studies suggest that the critical period for MTBE induced vascular disruption is between 15 and 30 hpf (the 6 somites and Prim-5 stages). To directly test the proposed critical period, embryos were exposed to 10 mM MTBE from only 15 to 30 hpf (the 6 somites to Prim-5 stages). Embryos exposure during the critical period, exhibited a significant increased in vascular lesions compared to control. On developmental days 2, 3 and 4, embryos were no longer in 10 mM MTBE, but 36%, 40%, and 38% of embryos exhibited the MTBE-specific vascular lesions that were present in the embryos treated for the entire developmental period. The critical period for MTBE induced vascular disruption was determined to be between 15 and 30 hpf, which corresponds to the 6 somites and Prim-5 stages.

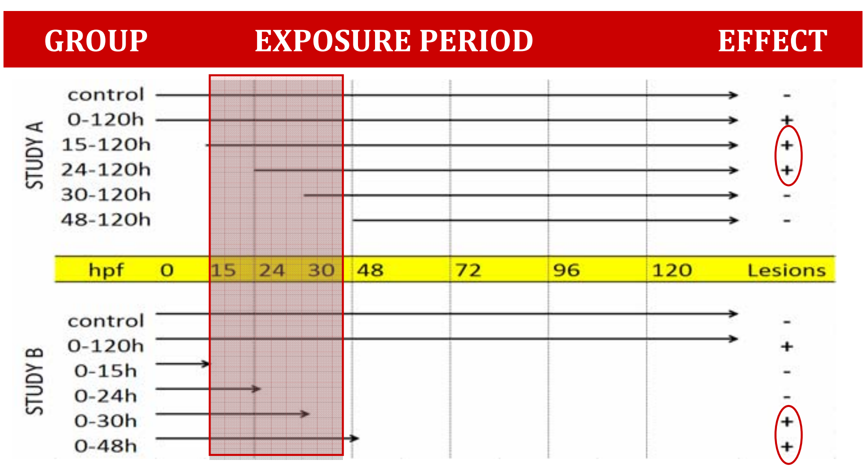


Figure 2. Embryos were exposed to 10 mM MTBE for staggered exposure periods to determine the critical window. The arrows represent the length of treatment and the + indicates treatment groups that exhibited a significant increase in vascular lesions as compared to control (Chi-Squared, $p < 0.05$). A third study (C) exposed embryos from only 15 to 30 hpf (red box); these embryos exhibited significantly increase vascular lesions. Each study was repeated 2x.

The mRNA transcript levels of a number of genes associated with the VEGF pathway were determined for MTBE, ETBE and TAME at a time point within the critical period, 24hpf. This time period is associated with the formation of the major descending and ascending vessels, required for microvascular angiogenesis (Kimmel et al., 1995; Isogai et al., 2003; Herbert et al., 2009), and an increase in zebrafish *vegfa* mRNA expression (Liang et al., 1998). The different effects of MTBE, ETBE and TAME on gene expression during the critical period are presented in Table 5. MTBE was the only chemical to decrease both isoforms of VEGF tested, as well as the primary VEGFR, *vegfr2*. The decrease in expression of both VEGF isoforms by MTBE may explain the targeted effect on developing vasculature. In contrast to MTBE, ETBE decreases *mmp9, wnt3a* and *β-actin*, which are critical to overall embryogenesis, but not restricted to vascular development. TAME affects the most genes on our list, but this may be a result of the overt toxicity exhibited by this chemical at 5 mM. The genes selected for this investigation were tailored to understand the underlying mechanism of MTBE's specificity to the developing vascular system. Other genes that may play a more critical role in the toxicity of ETBE or TAME should be examined in order to determine a target pathway.

Table 5. The effect of MTBE, ETBE and TAME on key genes involved in angiogenesis

GENE	FUNCTION	MTBE	ETBE	TAME
vegfa	Primary isoform, associated with microvascular formation in zebrafish	✐50%	not sig	not sig
vegfc	Regulates lymphangiogenesis	✐70%	not sig	✐40%
vegfr2	Tyrosine kinase receptor, primary receptor for VEGFa, upregulated by VEGF	✐40%	not sig	✐50%
hif1-!	Transcription factor for hypoxic regulation, upregulates *vegf* transcription	not sig	✐50%	not sig
ve-cadherin	Intercellular junction proteins required for vascular stability	✐70%	not sig	✐50%
mmp2	Gelatinase, breaks down extracellular matrix, activates MMP9	not sig	not sig	✐70%
mmp9	Gelatinase, breaks down extracellular matrix, is upregulated by VEGF	not sig	✐30%	✐70%
wnt3a	Migration of cells during development, increases *vegfa* transcription	✐60%	✐50%	✐60%
"actin	Cytoskeletal structure, lined to VE-Cadherin to stabilize endothelial cell junctions	✐60%	✐50%	✐70%

In summary, short term embryo-sac fry toxicity studies (OECD 212) with nominal concentrations of MTBE (0.625 mM to 10 mM) yielded a dose-response relationship for lesions related to abnormal vascular development, including pericardial edema, pooled blood in the common cardinal vein, cranial hemorrhages, and abnormal intersegmental vessels (ISVs) of the dorsal muscles (Bonventre *et al*., *2011*). At the same nominal concentrations, ETBE and TAME significantly induced lesions at lower concentrations than MTBE, and do not specifically target developing vasculature. At equal molar concentrations, ETBE and TAME are more toxic than MTBE based on the determined LC50s and EC50s.The critical period for vascular disruption by MTBE is between 15 and 30 hours of zebrafish development. During the critical period, the expression of critical genes involved in angiogenesis are down regulated by exposure to MTBE, ETBE and TAME. However, each chemical affects different genes, which was expected due to the different lesions observed with the three chemicals.

The addition of a methyl group does not drastically alter the chemical characteristics between MTBE, ETBE and TAME. However, the toxicity of the three oxygenates on developing zebrafish is different. While MTBE, ETBE and TAME elicit some of the same vascular lesions, the addition and placement of a methyl group in ETBE and TAME results in more toxic compounds with less specific target organ systems. These compounds are currently used in New Jersey and may pose a threat to groundwater and surface water quality. The toxicological studies presented here may be helpful in risk assessment and developing regulations for gasoline oxygenate use in the future.

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Investigation into the Role of Arsenic Reducing Bacteria in the Mobilization of Arsenic into Ground Water in New Jersey

Basic Information

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Descriptors:	None
Principal Investigators:	Adam Mumford, Lily Young

Publications

1. Mumford, Adam C., Barringer, J., Reilly, P., Young, L.Y. 2010. Potential Role for Bacteria in Arsenic Release to Groundwater. In Abstracts of 2010 Goldschmidt Conference, Knoxville, TN. June 13-18, 2010. (Poster Presentation)
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Priority issues addressed by this work:
Groundwater accounts for 15% of the public water supply used in northern New Jersey, and 85% of the public supply in southern New Jersey (12). The presence of arsenic in groundwater used for human consumption was identified as early as the 1930's, and a large body of evidence has been amassed demonstrating that the consumption of water contaminated with even low levels of arsenic places individuals at a higher cancer risk (11). As a result of these findings, the US EPA, in accordance with the World Health Organization, has set the maximum contaminant level for arsenic at 10μg/L (11). The New Jersey Department of Environmental Protection has adopted an even more stringent level of 5μg/L. Arsenic in excess of the 5μg/L limit has been found in approximately 25% of the drinking water wells in Readington and Raritan Townships in Hunterdon County, and 3% of the wells in this area were found to have arsenic levels in excess of 10μg/L (6).

It is recognized that there are both anthropogenic and geological sources of arsenic to the environment of New Jersey (1, 10). While the application of arsenical pesticides has been found to be responsible for the contamination of surface soils with arsenic, it has been suggested that this is not a major source of arsenic into the groundwater (7). However, high levels of arsenic have been found in the sediments and bedrock of the state. The shale bedrock of the Lockatong and Passaic Formations has been found to have arsenic levels of up to 240mg/kg, and groundwater pumped from these formations has been shown to have arsenic levels of up to 215μg/L (10). Deep sediments within the Coastal Plain have been found to have average arsenic concentrations of 15-23μg/L (3, 4).

The interplay of physical, geochemical and biological factors must be studied in order to understand the release of arsenic to the groundwater. In this study, we propose to examine the role of microbial activity on the mobilization of arsenic from subsurface sediments into the groundwater. Towards this end, we will analyze groundwater and sediment samples from high-arsenic sites on Crosswicks Creek and the Millstone River for the presence of the arsenic respiratory reductase gene, *arrA*, as well as the presence of arsenic reducing bacteria. Earlier studies in New Jersey have been focused on the geochemical factors involved in arsenic mobilization, and it is hoped that this study addressing the microbiology of arsenic transformations will provide a more complete understanding of how arsenic is released into the groundwater.

Research Objective:

The overall objective is to determine the role of arsenic active bacteria in the mobilization of arsenic into groundwater.

The specific objective of this study is to identify the presence of arsenic reducing bacteria in the field sites and to relate the presence of these bacteria to the mobilization of arsenic into groundwater. The presence of arsenic reducing bacteria will be identified by amplification of the arsenic reductase gene, *arrA*, along with the development of arsenic reducing microcosms using site water/sediment as an inoculum.

Methodology:
Study Sites:

Three sites in New Jersey were studied as models for their respective regions. Crosswicks Creek Site C6 in Upper Freehold, New Jersey was studied in collaboration with the U.S. Geological Survey (USGS), and has been found to have groundwater arsenic levels in excess of 50µg/L (13). The Crosswicks Creek site is within the Coastal Plain physiographic province, and receives groundwater through shallow aquifers in glauconitic sediments which have been found to have arsenic concentrations in the range of 7-15mg/kg (3). Two tributaries of the Millstone River, Six Mile Run and Pike Run in Somerset County, NJ, were sampled in collaboration with the USGS. Additionally, a NJDEP monitoring well several hundred meters from the Six Mile Run site was sampled in collaboration with the USGS. The Millstone River lies, in part, in the Piedmont Physiographic Province of New Jersey, an area noted for elevated arsenic concentrations in groundwater pumped from black shale aquifers (10).

Research Methods:

1. **Collection of field samples**:
    a. Crosswicks Creek Site C6:
        i. 1 L of groundwater was filtered through 0.22µM filters. The filters were then stored on dry ice for transport and later analysis.
        ii. 1 L of groundwater was obtained and stored on ice for transport.
        iii. 1 L of surface water was collected and stored on ice for transport.
        iv. A 1M core of the surface sediment was obtained and stored on ice.
    b. Millstone River:
        i. Groundwater was sampled at Six Mile Run and Pike Run as described for Crosswicks Creek Site C6.
        ii. Surface water was sampled at Six Mile Run and Pike Run as described for Crosswicks Creek site C6.
        iii. We were unable to obtain usable sediment cores for either Six Mile Run or Pike Run
        iv. Groundwater was sampled from NJDEP MW114, a monitoring well approximately 100M from Six Mile Run, and handled as described for Crosswicks Creek site C6.
2. **Laboratory analysis of samples**

    Molecular analysis
    a. DNA extraction from filters and/or sediments
    b. PCR amplification of the 16s RNA gene to confirm presence of bacteria in samples
    c. Cloning and sequencing of the 16s RNA gene to elucidate microbial community structure
    d. PCR amplification of the *arrA* gene, used as a biomarker for arsenate respiration
    e. Cloning and sequencing of the *arrA* gene for confirmation of amplification and elucidation of arsenic reducing organisms

Enrichment culturing

a. Inoculate site groundwater and/or sediment into enrichment media designed to select for arsenic respiring bacteria
b. Monitor arsenate reduction by HPLC
c. Extract DNA and amplify the 16s RNA and *arrA* genes from active enrichments

Data Analysis

a. Comparison of microbial communities between sites based on similarity of the 16s RNA gene
b. Comparison of arsenic reducing microbes between sites based on similarity of the *arrA* gene
c. Correlation of site arsenic concentrations to presence or absence of the *arrA* gene and arsenic reducing microbes.
d. Comparison of 16s and *arrA* gene sequences from enrichment cultures to those obtained without culturing.

Principal Findings and Significance:

Sampling Sites:

Groundwater and surface water samples were collected from gaining reaches at the Crosswicks Creek, Pike Run and Six Mile Run sites, and groundwater only was collected at NJDEP MW114 (MW114). While we had initially planned to obtain sediment from the Pike Run and Six Mile Run sites, it was found that the Six Mile Run streambed was too stony to obtain a core, and the Pike Run streambed was too unconsolidated to successfully recover a core sample. A summary of the groundwater and surface water chemistry can be found in Table 1.

Site	Date	DO (mg/L)	DOC (mg/L)	Nitrate (mg/L)	Sulfate (mg/L)	Iron (mg/L)	Arsenic (µg/L)
MW114 GW	10/28/10	0.1	2.7	BDL	0.15	2500	20.2
6 Mile Run GW	8/5/10	2.8	2.6	BDL	5.54	20700	27.4
6 Mile Run SW	8/5/10	7.4	2.8	0.3	28.1	132	1.6
Pike Run GW	8/6/10	4.9	1.1	5.48	20.9	197	2.1
Pike Run SW	8/6/10	5.0	4.4	8.08	111	156	1.3
Crosswicks GW	8/18/09	2.2	16.5	BDL	1.23	48100	16.6
Crosswicks SW	8/18/09	7.3	7.3	0.035	19.7	3200	1.4
Crosswicks Sediment	8/18/09	n/a	n/a	n/a	n/a	87000	25

Table 1. Summary of site water and sediment chemistry.

It was noted that elevated levels of iron appeared to correlate to elevated concentrations of arsenic, and that despite elevated concentrations of arsenic in shallow groundwater at Crosswicks Creek (16.6µg/L) and Six Mile Run (27.4µg/L), surface water was not found to have high levels of arsenic, suggesting that arsenic may not cross the oxic/anoxic interface of the streambed.

Microcosm Studies:

Crosswicks Creek at Ellisdale (USGS 01464485)

Sediment microcosms were prepared by inoculating 10 g of composited sediment from the 15-30 cm depth into 30 mL of defined anaerobic medium. Groundwater microcosms were prepared by inoculating 10 mL of groundwater into 30 mL of defined anaerobic medium. Each microcosm was set up in triplicate, with duplicate sterile controls prepared by autoclaving, and unamended background controls were also prepared. Microcosms were amended with 2.5mM As(V) as a terminal electron acceptor, and 5mM sodium acetate as a carbon source. These microcosms were monitored by HPLC to observe the reduction of As(V) to As(III).

Following inoculation of the sediment microcosms, it was found that all of the amended As(V) was immediately sorbed to the sediment, presumably to iron minerals (Figure 1). A second amendment of 5mM As(V) was added, and it was noted that approximately 0.5mM of this was reduced to As(III), while the remainder was sorbed (Figure 1). The As(III) persisted in solution (Figure 1). A 1:10 dilution was performed, and near stochiometric reduction of 7mM As(V) was observed within 28 days (Figure 2).

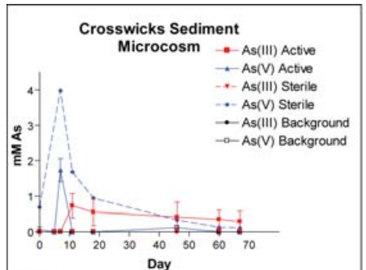

Figure 1.
Initial 2mM spike of As(V) was immediately sorbed to sediment in the microcosm. A second 5mM As(V) spike was partially reduced, but reduction appeared to stop once all As(V) was sorbed. No reduction was observed in killed controls

Groundwater microcosms were prepared by inoculating 30mL of defined minimal media with 10mL of groundwater obtained from the site. The triplicate active microcosms were amended with 5mM As(V) as a terminal electron acceptor and 5mM sodium acetate as a carbon and energy source. Duplicate killed controls were prepared as described for the active microcosms, and sterilized by autoclaving. Background controls were prepared by inoculating groundwater into unamended minimal media.

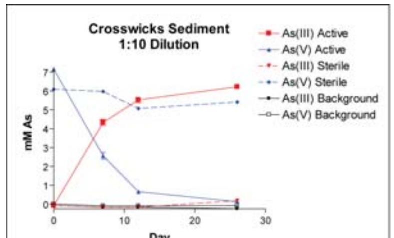

Figure 2:
The sediment microcosm was diluted 1:10 to reduce the amount of sedment available for As(V) sorption. Near-stoichiometric reduction of 7mM As(V) was observed. No reduction of As(V) was observed in killed controls.

Reduction of As(V) to As(III) was observed within 24 days of incubation, with complete reduction of As(V) to As(III) observed after 70 days of incubation (Figure 3).

Based upon these findings, it can be summarized that microbes capable of As(V) reduction are present at the Crosswicks Creek at Ellisdale site. Further, based upon the persistence of As(III) in the liquid phase in the sediment microcosms, it appears that microbially reduced As(III) is much more mobile than As(V).

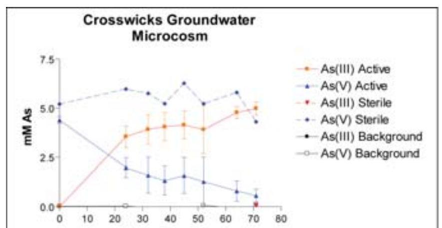

Figure 3:
As(V) reduction was seen within 24 days of incubation, with complete reduction of 5mM As(V) observed in 72 days. No reduction was observed in killed controls.

Six Mile Run at Blackwells Mills:

Groundwater microcosms were prepared by inoculating 10mL of groundwater from the Six Mile Run at Blackwells Mills site (USGS 01401900) into 30mL of defined minimal media amended with 1mM As(V) as a terminal electron acceptor and 5mM sodium acetate as a carbon source. Active microcosms were prepared in triplicate, and duplicate killed controls were prepared by autoclaving inoculated microcosms. As(V) reduction was initially observed after 14 days of incubation, with complete reduction of As(V) to As(III) observed after 28 days of incubation (Figure 4). From these results it can be summarized that microbes capable of respiratory reduction of arsenate are present.

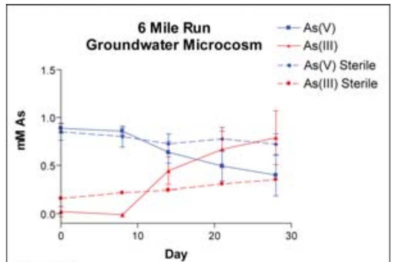

Figure 4:
Reduction of 1mM As(V) was observed in the 6 Mile Run groundwater microcosm. The observed reduction rate was greater in active microcosms than in killed controls.

New Jersey DEP Monitoring Well 114 (NJDEP MW114):

This site is a monitoring well screened at approximately 20 feet, located approximately 100M from the Six Mile Run at Blackwells Mills site. Groundwater microcosms were prepared by inoculating 10mL of groundwater from monitoring well 114 (MW114) into 30mL of defined minimal media amended with 5mM As(V) as a terminal electron acceptor and 5mM sodium acetate as a carbon source. Active microcosms were prepared in triplicate, and duplicate killed controls were prepared by autoclaving inoculated microcosms. As(V) reduction was initially observed after 35 days of incubation, with complete reduction of As(V) to As(III) observed after 75 days of incubation in two of the three active microcosms (Figure 5). A 1:10 dilution of this series of microcosms was performed, and initial reduction of As(V) to As(III) was observed within 7 days, and complete reduction was observed within 24 days (Figure 6). From these results we summarize that microbes capable of respiratory reduction of arsenate are present.

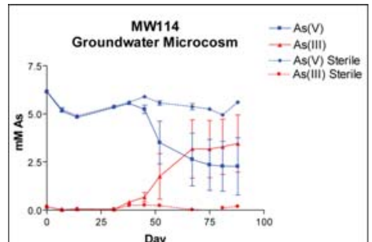

Figure 5:
As(V) reduction was observed to begin after 28 days of incubation. No As(V) reduction was observed in killed controls.

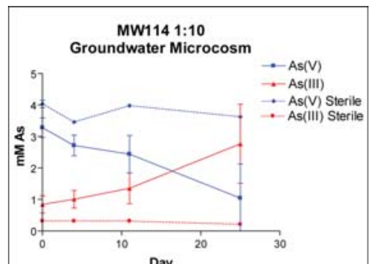

Figure 6:
Reduction of 4mM As(V) was observed within 28 days. No As(V) reduction was observed in killed controls

Pike Run at Rocky Hill (USGS 01401700):

This site was sampled due to the fact that while it is relatively near (8km) the Six Mile Run and NJDEP MW114 sites, it has been found to have low levels of arsenic (Table 1). Groundwater microcosms were prepared by inoculating 10mL of groundwater from beneath the streambed of Pike Run into 30mL of defined minimal media amended with 5mM As(V) as a terminal electron acceptor and 5mM sodium acetate as a carbon source. No reduction of arsenate was observed after 90 days of incubation (Figure 7). Based upon these results

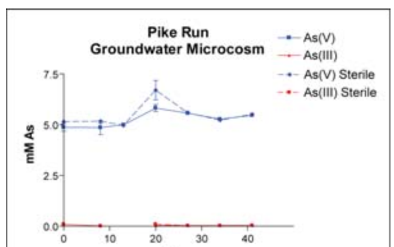

Figure 7:
No As(V) reduction was observed in Pike Run groundwater microcosms. Reduction was not observed after 90 days incubation (data not shown)

we summarize that if microbes capable of the respiratory reduction of arsenic are present, they are only present at extremely low numbers, or that the culturing methods used were not appropriate for their cultivation.

Microbial Community Analysis:

Crosswicks Creek at Ellisdale:

DNA was successfully extracted from sections of filters used to collect microorganisms from ground and surface waters, as well as from sediments. The 16S rRNA gene was amplified, cloned and sequenced from this DNA in order to characterize the microbial community at the site. Analysis of the sequences using ARB (5, 8) and mothur (9) indicated that the groundwater hosted a diverse anaerobic microbial community (Figure 8). Comparison to the SILVA 16S rRNA database (8) suggested the presence of iron reducing microorganisms as well as an arsenic-oxidizing microorganisms (2).

A similar analysis was performed for sediment collected at the Crosswicks Creek site. Analysis indicated that the microbial community of the sediment was less diverse than the community in the groundwater, perhaps due to the perturbation of the subsurface environment caused by the pumping of groundwater (Figure 9).

Phylogenetic analysis of the *arrA* gene was performed in order to determine the relationship of any arsenic reducing bacteria found at the Crosswicks site to known arsenic reducing bacteria. The *arrA* gene was successfully amplified and cloned from site groundwater. *arrA* was also successfully amplified from sediment, however, cloning was unsuccessful. Comparison of cloned *arrA* sequences uncovered three distinct groups, related most closely to the *arrA* of *Geobacter uraniireducens* Rf4 (2) (Figure 10).

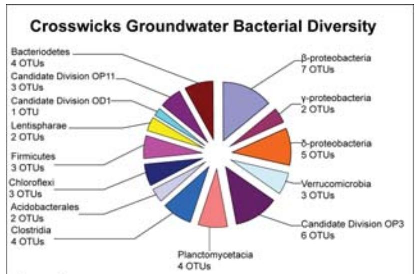

Figure 8:
51 clones of the 16S rRNA gene were sequenced, giving 49 Operational Taxonomic Units based on a 97% similarity cutoff.

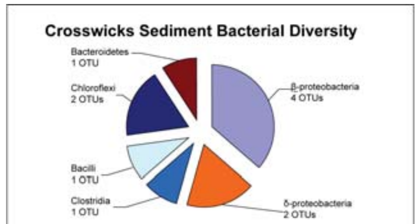

Figure 9:
44 clones of the 16S rRNA gene were sequenced, giving 11 OTUs based on a 97% similarity cutoff. The sediment contained diverse anaerobic bacterial communities. No close relatives of known arsenic reducing genera were detected. Much higher diversity was noted in groundwater than in sediment.

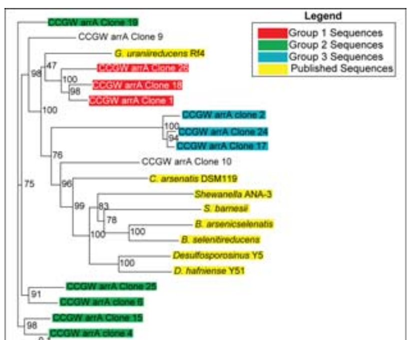

Figure 10:
ClustalX neighbor-joining tree of *arrA* sequences cloned from Crosswicks groundwater. Bootstrap values based on 1000 replicates. Groupings are based on an 85% similarity cutoff. Additionally, *arrA* was successfully amplified from sediment microcosms (data not shown).

Comparison of Microbial Communities between Sites:

The 16S rRNA and *arrA* genes were successfully amplified, cloned and sequenced from groundwater sampled at Six Mile Run and Pike Run. Too little biomass was recovered on filters from MW114 for successful amplification of either gene, however the 16S and *arrA* genes were successfully amplified and cloned from a microcosm inoculated with water from MW114. This allowed for comparison of

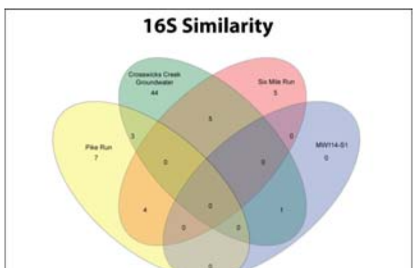

Figure 11:
Venn diagram illustrating different 16S sequences recovered. 16S sequences from clone libraries were aligned to the Silva 102NR database in ARB. Operational taxonomic units were assigned based on a 97% similarity cutoff in Mothur.

the microbial communities between of the sites using mothur (9) (Figure 11). The microbial communities were found to be distinct at each site; however, there were some shared organisms.

A similar analysis of the arsenic-reducing microbial community based on the *arrA* gene was also performed (Figure 12). Each site appeared to have a distinct group of arsenic reducing microorganisms, however, some sequences were shared between Pike Run and MW114. It is interesting to note that while *arrA* sequences were recovered from Pike Run, we were unable to develop an arsenic-reducing microcosm from this site.

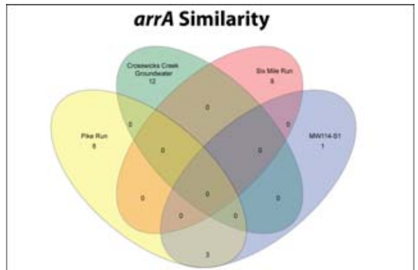

Figure 12:
Venn diagram illustrating different *arrA* sequences recovered. Arsenic respiratory reductase (*arrA*) gene sequences from clone libraries were aligned to known *arrA* sequences from Genbank using Clustal within ARB. Operational taxonomic units were assigned based on a 97% cutoff.

Major Findings:

- Microbial arsenic reduction was observed in microcosms inoculated with material from sites with elevated arsenic, while no arsenic reduction was observed in microcosms from a site lacking elevated arsenic.
  - This suggests the potential for microbial involvement in arsenic mobility in groundwater.

- Different microbial communities were found at each site. However, some microorganisms were common between sites.

- Microbially reduced As(III) persisted in sediment microcosms after all As(V) was bound.
  - This indicates that microbially reduced arsenic may be more mobile in groundwater.

- Despite the presence of *arrA*, Pike Run groundwater microcosms did not reduce As(V).
  - This underlines the importance of culture studies to confirm activity.

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Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersy Pinelands

Basic Information

Title:	Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersy Pinelands
Project Number:	2010NJ230B
Start Date:	3/1/2010
End Date:	2/28/2011
Funding Source:	104B
Congressional District:	NJ-010
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrogeochemistry, Water Supply, Groundwater
Descriptors:	None
Principal Investigators:	Andrew Parsekian, Lee Slater

Publications

1. Parsekian, A, C Cimiluca, AE Gates and I Calderon, 2010. Retention of Information as a Function of Lesson Design for Middle School Studies of Wetlands in New Jersey. Abstract ED31B-0636, presented at 2010 Fall Meeting, AGU, San Francisco, Calif., 13-17 Dec. (poster)
2. Parsekian, A and L Slater, 2011. Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersey Pinelands. NJWRRI Grant Recipient Poster Presentation, Cook Campus Center, New Brunswick, NJ, 10 February. (poster)
3. Parsekian, A, 2011. New Student Chapter Organizes a Hydrogeophysics Workshop. The Leading Edge, March, 30(3) p. 352-353.

Problem and Research Objectives

The New Jersey Pinelands Biosphere Reserve (Pinelands) is an ecologically sensitive area (as delineated by UNESCO) managed with the goal of preserving the local natural resources, protecting the large drinking water aquifers from contamination and enabling appropriate human usage of the land. The management plan bears all of these facets in mind and acknowledges the integral nature of the biosphere, geosphere and hydrosphere. In order for this system to continue to function as a viable ecosystem and anthropogenic resource, each part must be equally managed (Walker and Solecki, 1999). For example, the health and efficiency of the aquifers is reliant on the presence of the undisturbed pine forest on the surface. Therefore, it is necessary to have a complete understanding of the dynamics of forest development and regeneration for a greater ecological understanding and to facilitate appropriate management practices (Walker *et al.,* 2008). The growing population of New Jersey is putting increasing pressure on groundwater resources from aquifers underlying the Pinelands for drinking water and agriculture. Ensuring a clean, reliable water resource for the citizens is essential for the future of the state.

The nature of the vernal pond canopy gaps is such that they are seasonal wetlands and therefore sensitive ecologically, and potentially harbor niche endangered species within the Pinelands. The similarly sized "spung" *[colloquial term]* canopy gaps, characterized by modest peat accumulations (<90cm), are functionally different from the vernal ponds, though they may be influenced by similar hydrogeology. Previous research aimed at determining the origin of these canopy gaps by investigating ecological and chemical factors was inconclusive (Ehrenfeld *et al.*, 1995; French *et al.,* 2001).

The objectives of this study are three-fold: 1) estimate properties of the near-surface geology (i.e. clay content, particle texture) which may influence local hydrology within individual canopy gaps, 2) make comparisons of the physical properties of the subsurface between vernal pond canopy gaps and peat-filled "spungs" and 3) assess the potential of paleo-channels to impact local and regional ground water flow.

Methodology

During the spring, summer and fall of 2010, ground-penetrating radar (GPR) surveys were employed as the primary means for investigating the subsurface stratigraphic structure below two vernal pool wetlands and two spung wetlands. Each wetland was investigated with a series of transects located through passable areas that required minimal disturbance of the vegetation. This method records data in the form of vertical profiles of the subsurface sediments.

In the fall of 2010, terrain conductivity was investigated using a GEM2 electromagnetic instrument. This survey used multi-frequency electromagnetic induction technology to estimate the electrical properties of the subsurface in a 2.5D manner by conducting a series of transects in and around the spungs and vernal pools. This instrument records two-dimensional "map view" datasets that can have an associated depth estimated.

Ten peat and sediment cores were collected along the lines at depths up to 235 cm below the ground surface using manual coring tools. Two to five samples were collected from each core totaling 40 samples. Samples were analyzed for soil chemical parameters (UMASS Soil Testing Lab) and for particle size (Rutgers University). Two samples of peat extracted from the deepest organic layers of the spungs were radiocarbon dated to estimate the time at which they began to accumulate peat.

Principal Findings, Discussion and Significance

Vernal Pools

The principal finding of this study was data supporting our hypothesis that subsurface strata with low permeability are present below the investigated wetlands. An example demonstrating this is shown in Figure 1. It was possible to identify a stratigraphic anomaly at approximately 2 m below the surface after first completing a geophysical transect across the wetland. Additionally, the reduced signal return below the anomaly is indicative of elevated clay content that typically leads to electromagnetic energy attenuation. With this information to guide sampling, a series of hand auger cores were installed to characterize the subsurface.

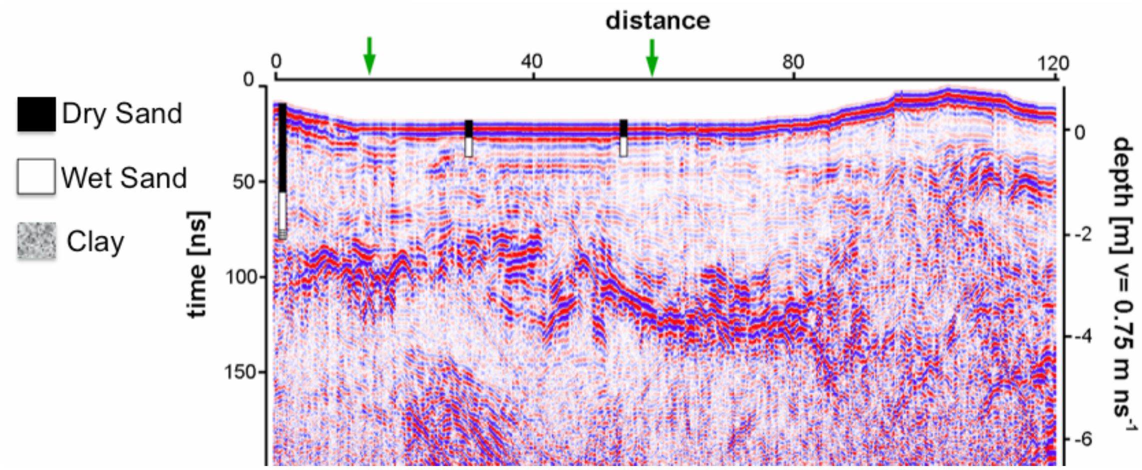

Figure 1. Ground penetrating radar profile with core data superimposed from Vernal Pool 1. Note the anomalous reflection at 2 m below the surface. This was confirmed as clay-rich through core sampling just adjacent to the wetland.

Initial attempts to install borings were unsuccessful due to highly saturated conditions just below the surface of the wetland. A layer was finally sampled at 2.35 m below the surface at a location <10 m beyond the edge of the wetland. This sediment was found to have clay particle size content of >30% and was highly different in physical character than overlying sediments containing >97% sand (Figure 2).

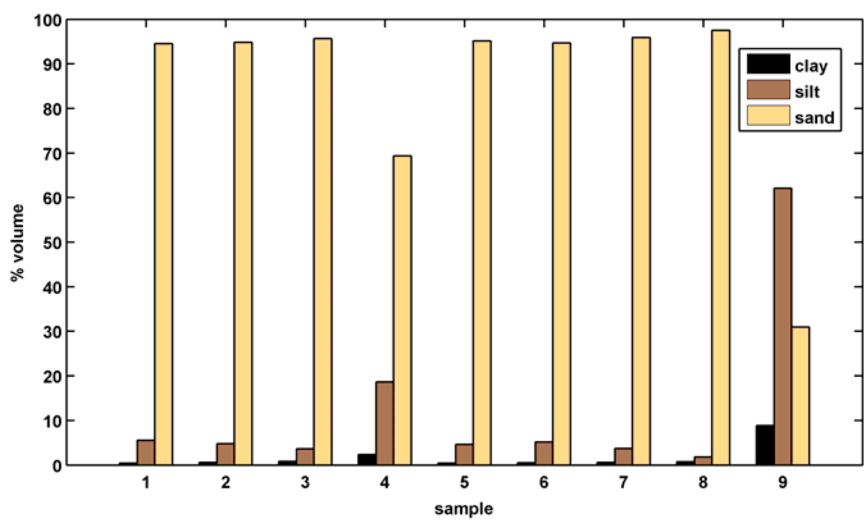

Figure 2. Grain size analysis for samples in and around the vernal pool. Sample 4 is from just below the surface in the center of the wetland and sample 9 is from the clay layer 2.35 m below the surface. All other samples are from outside the wetland.

An electromagnetic geophysical survey was conducted in an effort to determine if the low permeability clay-rich layers were spatially extensive. This investigation was highly effective at identifying a conductivity anomaly in the sediment that approximately corresponds with the extent of the wetlands (Figure 3).

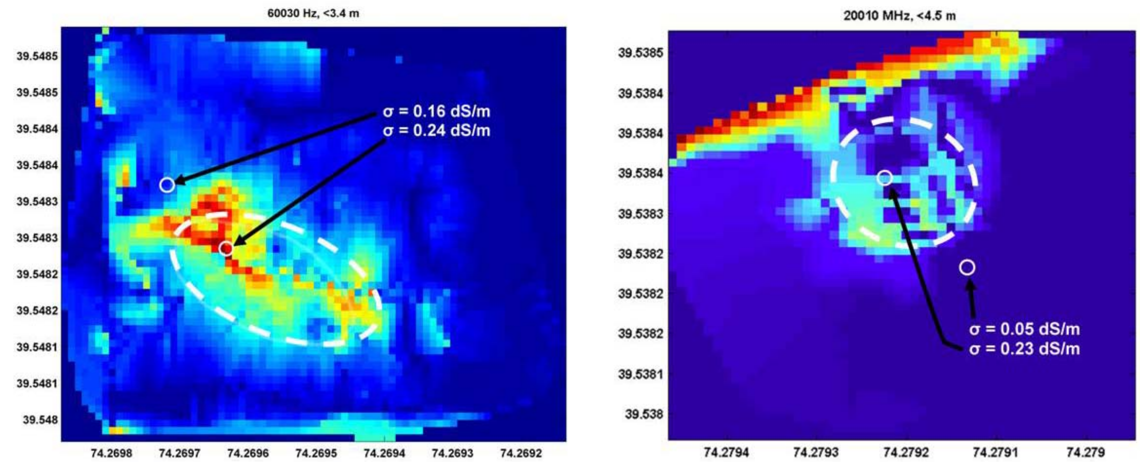

Figure 3. Results of electromagnetic surveys conducted over vernal pool 1 (left) and vernal pool 2 (right). Warm colors indicate relatively higher conductivity, cool colors indicate relatively low conductivity.

The estimated depth of investigation for these surveys is <3-4 m which would coincide well with the observed clay-rich layers. However, it is also possible that near-surface conductivity variations associated with the wetland chemistry could be influencing the signal. Therefore it is not possible to conclusively say that the electromagnetic results highlight the extent of the subsurface clay layers.

Spungs

Similar ground penetrating radar profiles were acquired across two peat filled spungs. These radargrams clearly image the thickness of the peat soil to about 0.9 m and also resolve an area of high signal attenuation on the western edge of the wetland (Figure 4).

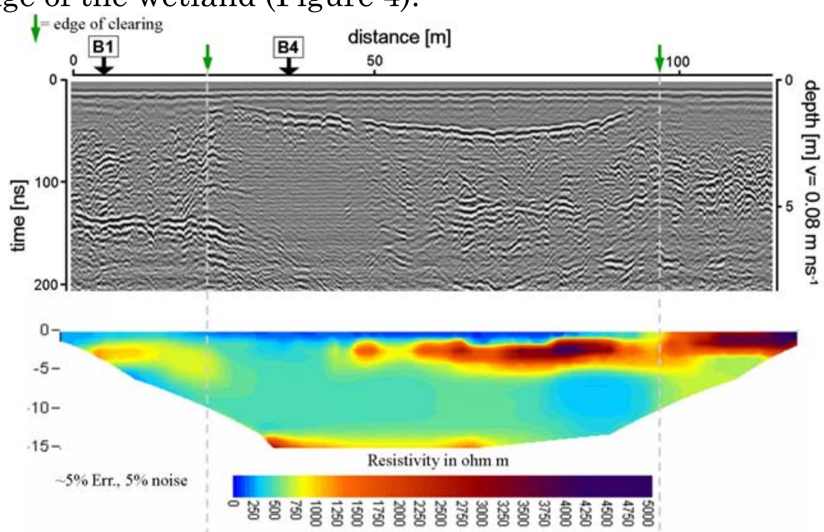

Figure 4. Ground-penetrating radar profile across Spung 1 (top) showing the peat thickness as a curved reflection near the center of the image. The resistivity distribution below the same area is presented in the lower panel.

The resistivity profile (resistivity is the inverse of conductivity, so warm colors in this case are the opposite of the electromagnetic data) indicates that the sediment below the western edge of the spung is more conductive than the dry sands to the east of the wetland. Additionally, this investigation revealed highly resistive sediments below the eastern portion of the wetland that may indicate hydraulic isolation from the overlying peat.

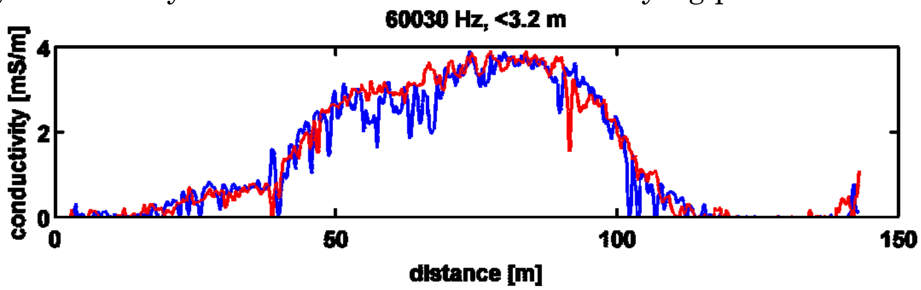

Figure 5. Electromagnetic survey conducted across Spung 1.

An electromagnetic survey line was acquired at the Spung 1 study site that revealed elevated bulk conductivity across the peat-filled portion of the wetland (Figure 5). In this case, the instrument is likely responding primarily to the peat sediment as the elevated conductivity signal corresponds to the uppermost layer of the resistivity profile (Figure 4) and correlates poorly with the highly resistive sediments below the wetland.

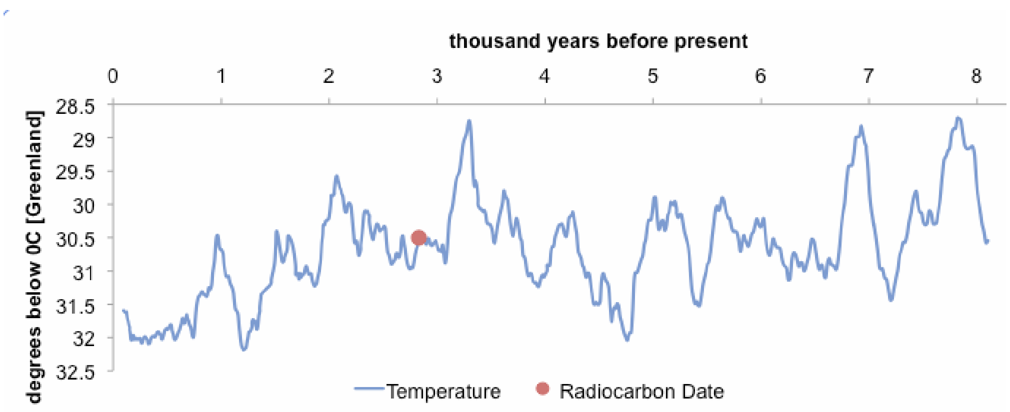

Figure 6. Radiocarbon date from Butterworth spung and temperature record from Greenland ice sheet (Alley, 2000).

A sample of the organic matter just above the mineral sediment contact at 0.76 m was extracted from a nearby spung known as Butterworth. This sample was analyzed for radiocarbon age and found to be 2,830 ±25 years old. This date indicates that the peat accumulated at approximately 0.27 m per 1,000 years. To put the age of peatland initiation into context, the date is placed on the temperature record determined from an ice core in Greenland on Figure 6 (Alley, 2000). Peat growth in Butterworth spung apparently began shortly after an abrupt drop in temperature at 3.2 ka following the warmest period in the past 8 ka.

Clay materials found below vernal pools were likely deposited as part of fluvial channels, slackwater fill and interchannel deposits during the Miocene. Weathering and aeolian redistribution left a layer of coarse sandy sediment on the surface. This surfical sand has been reworked through the Quaternary into a generally flat but irregular landscape with variations in topography from one to tens of meters (Forman, 1998). These results suggest that there are clay layers underlying the isolated wetlands. Clay lenses are common throughout the northern pinelands soils, at various depths below the surface (Walker *et al.,* 2004). It is likely that as the surface sediments were reworked, enough sediment was removed from certain areas to minimize the distance to sporadic clay layers. Water could then collect and be retained seasonally resulting in a range of water features (ponds) and wetlands (peatland 'spungs' and vernal pools).

Summary Remarks

Geophysical methods have been highly successful at characterizing the subsurface in and around isolated wetlands in the New Jersey Pinelands. This study effectively identified clay layers located beneath vernal pools and peat-filled spungs. These subsurface units may control surface hydrology and, as a result, vegetation patterns. Evidence suggests that the wetlands were initiated in conjunction with existing low permeability strata perhaps corresponding with a dramatic climatic shift in the time period since the Wisconsin glaciation.

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Green Remediation of Tetracycline in Soil-Water Systems

Basic Information

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Project Number:	2010NJ231B
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End Date:	2/28/2011
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Focus Category:	Non Point Pollution, Water Quality, Treatment
Descriptors:	None
Principal Investigators:	Pravin Punamiya, Dibyendu Sarkar

Publications

1. P. Punamiya, Sarkar, D., Datta, R. 2011. Green Remediation of Tetracyclines in Soil-Water Systems. NJWRRI Grant Recipient Poster Presentation, Cook Campus Center, New Brunswick, NJ, February 10, 2011.
2. P. Punamiya, Sarkar, D., Datta, R. (2011) Evaluate the potential of Al-based drinking-water treatment residuals as a “green” sorbent for tetracycline: A comprehensive batch sorption study. HDC-SETAC Conference Proceedings, Wilmington, DE. April 27-28, 2011. Poster presentation.

Problem and Research Objectives –

Veterinary antibiotics are used in large amounts in concentrated animal feeding operations (CAFOs) to treat disease and protect the health of animals, and as feed supplements to improve growth rate, thereby allowing animals to be brought to market much faster and at lower cost [1]. VAs and other compounds of wastewater origin have been observed throughout the U.S. including New Jersey in surface waters mainly impacted by urban activities. The presence of VAs in aquatic and terrestrial environments is of concern because, even at ng/L levels, these molecules are biologically active and can affect critical development stages and endocrine systems of aquatic and terrestrial organisms. Also, the widespread use and frequent detection of VAs in the environment have raised concerns over proliferation of antibiotic-resistant bacteria, decrease in the effectiveness of medical antibiotics, and other potential adverse human health and ecological effects [2, 3]. Kumar et al. [4] observed that as much as 90% of the VAs administered orally passed through the alimentary canal of cattle unchanged. Once excreted in urine and manure, VAs can enter into soils, surface water and/or groundwater via manure applied soils or via sludge storage at CAFOs.

Among the VAs commonly used in the livestock industry is the tetracycline antibiotics (TCs) [e.g. tetracycline (TTC), oxytetracycline (OTC), and chlorotetracycline (CTC)], which ranks second in production and usage worldwide. A survey carried out by Pawelzick et al [5] in sandy soils fertilized with liquid manure reported concentrations of up to 270 μg kg^{-1} (OTC), 443 μg kg^{-1} (TTC), and 93 μg kg^{-1} (CTC) in a manure impacted surface soil. Winckler and Grafe [6] found TCs to persist in agricultural soils at concentrations of 450–900 μg kg^{-1}. Other studies have reported detection of TCs at around 0.15 μg L^{-1} in groundwater and surface waters [7], 86-199 μg kg^{-1} in soils, 4.0 mg kg^{-1} in liquid manure [8], and 3 μg L^{-1} in farm lagoons [9].

Tetracyclines are complex organic compounds with unique chemical characteristics and behaviors. Their structures contain connected ring systems (lettered A through D from right to left) with multiple ionizable functional groups. Tetracyclines have three pK_a's and hence, can exist as cationic, zwitterionic or anionic species under acidic, moderately acidic to neutral, and alkaline conditions, respectively. The ionization behavior can be expected to significantly influence tetracycline sorption to soil components and other sorbents. The high polarity (e.g., log K_{ow} = -1.97 to -0.47) and consequently high aqueous solubility (0.52−117 mM) of TCs portends that TCs could be highly mobile in soils. Thus, effective means of immobilizing TCs in soils and manure need to be employed to reduce the potential health hazards that could result from the presence of TCs in the soil environment, and consequently in surface water bodies.

In recent times, considerable efforts have been made to understand the sorption mechanisms of TCs in various natural and synthesized sorbents, including soils, iron/aluminum hydroxides, clay minerals, humic substances, and clay-humic complexes [11-13]. These studies revealed that sorption of TCs is mainly controlled by cation/anion exchange, cation bridging, and surface complexation (e.g., H-bonding) between the functional groups of tetracyclines and the respective charged/polar sites of sorbents, while hydrophobic effect is only a minor factor contributing to the overall sorption. Cheng and Karthikeyan [14] showed ability of TCs to form strong complexes with Al and Fe hydrous oxides. Both Al and Fe hydrous oxides demonstrated high affinity for TC.

The lack of widespread use of a Fe/Al-based technique to immobilize TCs in manure piles or in soils may be the cost associated with the use of synthesized hydrous oxides of Fe/Al. We have identified an inexpensive surrogate of Fe and Al hydro(oxides) in the form of drinking-water treatment residuals, (WTRs) that can be potentially utilized to immobilize and stabilize TCs in manure and TC-enriched manure amended soils. The WTRs are waste products of water purification process that, by virtue of their composition and reactivity, have the potential for environmental remediation as a soil amendment [15, 16]. The WTRs are primarily sediment, metal (Al, Fe) oxide/hydroxide, activated C, and polymers removed from the raw water processed during the water purification process [17]. There are thousands of drinking-water treatment plants in the United States that use metal salts as coagulants for efficient removal of particulate solids and colloids from surface water supply, generating more than 2 million metric tons of WTR daily [18].

The **central hypothesis** is that the nanoparticles of Fe/Al-oxides and organic C in WTRs, with their high specific surface area, pH-dependent surface chemistry and high reactivity have high adsorptive capacity for OTC. We expect to answer the following research **questions**:

- Is it possible to bind OTC and its metabolites by amending manure with Fe- and Al-WTR thereby reducing potential availability/mobility?
- What is the geochemical fate of OTC and its degradation products/metabolites?
- What is the specific mechanism of retention of these various species?
- What are the effects of relevant environmental factors on the retention process?
- What is the possibility of the bound OTC undergoing desorption with time?

Answering these basic questions will let us evaluate if it is possible to model/extrapolate the bench-scale laboratory data to a larger scale to design an effectively implement a field-scale OTC removal technology? The hypothesis will be evaluated, and the research questions will be answered by pursuing the following **objective**:

Objective: Determine the retention-release mechanism and characteristics of OTC and its metabolites on WTRs as a function of environmental factors.

Methodology –

***Experimental approach*:** Two types of WTRs were used – Fe-based (Fe-WTR) and Al-based (Al-WTR) (Table 3). The WTRs were obtained from Florida. The manure was obtained from Cook Campus, Rutgers NJ. The WTRs and the manures were extensively characterized for their physico-chemical properties and metals/nutrients content using standard methods (USEPA, 2000; Standard Method 2320). TCLP analyses were performed to determine the potential of contaminants leaching from the WTRs following standard EPA protocols.

Objective: Adsorption-desorption characteristics and mechanisms of OTC on WTRs

Laboratory batch sorption experiments were performed to study Fe and Al WTRs effectiveness in immobilizing OTC, in manure and manure amended soils as a function of pH, sorbate-to-sorbent ratio, ionic strength, reaction time, and equilibration time. Both mechanistic (generating adsorption envelopes as a function of solution pH followed by chemical modeling of potential adsorption mechanisms) and non-mechanistic (generating adsorption isotherms at a given pH but varying OTC concentration, followed by empirical modeling to quantify degree of adsorption) approaches were adopted to obtain a comprehensive picture of the retention behavior of OTC.

Seven different solid:solution ratio (SSR) ranging from 1 g L^{-1} to 100 g L^{-1} (Fe/Al WTR:OTC) were employed to study the effect of the SSR on OTC sorption. The SSR's ranges were based on the values from the previous studies on various contaminants. OTC solution was prepared by dissolving tetracycline hydrochloride in 0.01 M KCl (background solution) to achieve the concentration of 0.1, 0.5 and 1.0mM respectively. The concentrations were chosen based on the mean concentration in the soil and water environment and 5X and 10X higher than that concentration. Sorption of OTC was initialized by mixing Fe and Al WTR with 50 ml of OTC solutions in 50-mL polypropylene centrifuge tubes to provide the desired SSR's. This resulted in a matrix of 2 WTRs x 7 SSR's x 3 OTC concentrations x 3 replicates and respective controls. The pH was maintained at pH 6.0 ± 0.2, value based on previous reports with Fe and Al hydroxides with 0.1 M PIPES buffer. Sorbed OTC was inferred from the difference between the concentration of OTC added in the initial solution and the concentration of OTC in the solution at equilibrium.

Effect of contact and reaction time on sorption of OTC on Fe and Al-WTR was examined at different time intervals ranging from 5 min to 96 h (5min, 10 min, 20 min, 30 min, 1 h, 2 h, 5 h, 10 h, 24 h, 48 h, and 96 h). Fe and Al-WTR and OTC solutions (0.1, 0.5, and 1 mM OTC prepared in 0.01 M KCl) were added in to each test tube to make up the optimum SSR determined from the previous SSR experiment. The samples were withdrawn at desired time intervals, centrifuged and filtered. Filtered solutions collected at each time interval were then analyzed for OTC using HPLC. After the sorption kinetics experiment, desorption studies was conducted on the above OTC reacted samples for times ranging from 5 min to 96 h.

Effect of pH and ionic strength on sorption of OTC on Fe and Al-WTR were assessed using sorption edge experiment. The optimized SSR (20 and 40 g L^{-1} for Al and Fe-WTR respectively) and equilibration time (24 h) were used from the above sorption experiments. Effect of pH on sorption was studied by determining the amount of the OTC adsorbed within the pH range of 3-11 (1 unit increment). The suspensions/mixtures in the tubes were adjusted to the desired pH values by adding 0.1 M HCl/0.1 M NaOH. The volume of the acid/base used during the adjustment was recorded and added to the total volume for the use of final calculations. PIPES (0.1 M) was used as non-interfering buffering agent. Stock OTC solution was added to obtain an initial concentration of 0.1, 0.5 and 1.0 mM respectively. This experiment protocol was repeated to investigate the effect of ionic strength on the sorption efficiency of Fe and Al-WTR. KCl was chosen as a background solution. Three ionic strengths of KCl - 0.01M, 0.1M, and 0.5M were employed for this experiment. Optimized SSR, equilibration time, and pH (6.0 ± 0.2) were used.

Sorption isotherms were obtained to evaluate OTC distribution between the Fe and Al-WTR and the OTC solution as a function of Fe and Al-WTR concentration. Equilibration time, SSR's, pH, and ionic strength were selected on the basis of the above sorption experiments. Batch sorption experiment was conducted using similar protocol used in the sorption edge experiments. Sorption isotherms were obtained by mixing a fixed amount of Fe and Al-WTR (20 and 40 g L^{-1} for Al and Fe-WTR respectively) with varying OTC concentration (0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mM) solutions prepared in optimum pH (6.0 ± 0.2) and varying ionic strength (0.01, 0.1, and 0.5 M KCl).

Principal Findings and Significance –

Findings:

- Sorption of OTC on Al-WTR increased with increasing pH up to pH 7 (stable behavior for Fe-WTR), above which it started decreasing in the range of 8 to 11.
- A concentration of 20 and 40 g L^{-1} was deemed as optimum SSR for Al-WTR and Fe-WTR respectively, where more than 95% (Al-WTR) and 90% (Fe-WTR) of the OTC was sorbed and equilibrium was reached in 2h and 5h respectively.
- The reaction kinetics data best fit pseudo second order model (r^2=0.99) for both Al and Fe-WTR sorption on OTC.
- Sorption isotherms were plotted to evaluate the effect of ionic strength *IS* on OTC sorption.
- Best data fits were obtained using the linearized form of the Freundlich isotherm (r^2=0.98 Al-WTR and r^2=0.99 Fe-WTR).
- No significant effect (p>0.05) of *IS* on sorption of OTC was observed between 0.05-0.5 mM. However, at higher initial concentration (>1 mM), *IS* dependence of OTC sorption was observed for both WTRs.

Conclusions:

- Both Al- and Fe-based WTRs exhibited high OTC sorption affinity, exhibiting minimal OTC desorption.
- Sorption of OTC by the WTRs was rapid and equilibrium was reached within 5h regardless of the initial OTC concentration.
- pH-dependent sorption behavior of both Al and Fe-WTR was observed for all SSR and initial OTC concentration tested.
- No significant effect (p>0.05) of ionic strength on sorption of TCs was observed between 0.1-1 mM. However at higher initial concentration of OTC (1 and 2 mM), ionic strength dependence was observed.
- Results from the current sorption study suggest that both Al and Fe WTR can be used as an excellent low-cost and green sorbent for OTC removal from aqueous medium.
- The research will also provide a cost-effective medium for OTC immobilization in wastewater treatment facilities as well as in lagoons at concentrated animal feeding operations.
- The current research finding will also help us to develop a "green remediation" technique to immobilize and stabilize OTC in lagoons, storage at CAFO's, and manure and manure-amended soils rich in OTC.

Future Studies

- Spectroscopic evidence and molecular simulations are needed to further elucidate the actual mechanism of the sorption by Al and Fe-WTR.
- Further studies are needed to document WTR efficacy in OTC remediation under green house and field conditions.
- Future studies aims to develop a full scale green house and field study where dynamic interaction of manure, soil, water, and plants will be evaluated under natural environmental conditions.

Tables and Figures:

Table 1. Physicochemical properties of Al and Fe-based WTRs. Data are expressed as the mean of three replicates ± one standard deviation.

	Al-WTR	Fe-WTR
Source	Bradenton, FL	Tampa, FL
pH	6.1 ± 0.2	6.4 ± 0.3
Electrical conductivity (dS m^{-1})	0.84 ± 0.1	0.14 ± 0.1
Solid organic matter (%)	33 ± 1.4	40 ± 1.2
Carbon (g/kg)	184 ± 4.2	211 ± 6.4
Nitrogen (g/kg)	6 ± 0.5	10 ± 0.7
(Al+Fe) Total (g/kg)	125.3 ± 2.3	269.6 ± 4.2
(Al+Fe) Oxalate extractable (g/kg)	109.2 ± 1.5	79 ± 2.4

Table 2. Freundlich Isotherm model* parameters for OTC sorption of Al and Fe-WTRs. pH was maintained at 6.0 ± 0.1, SSR 20 and 40 g L^{-1} for Al and Fe-WTR respectively, and reaction time 24h.

Sorbent (WTR)	Ionic Strength (KCl)	K_f	n	r^2
Al-WTR	0.01 M	0.178	1.81	0.99
	0.1 M	0.166	1.45	0.98
	0.5 M	0.130	1.61	0.98
Fe-WTR	0.01 M	0.150	1.27	0.99
	0.1 M	0.110	1.44	0.99
	0.5 M	0.068	1.68	0.99

*Freundlich isotherm: $q_e = K_f \times C_e^n$; where q_e is the amount of tetracycline sorbed onto the Al and Fe-WTR in mol g^{-1}; C_e is the equilibrium tetracycline concentration in mM; and K_f and n are dimensionless Freundlich isotherm constants.

Table 3. Pseudo-second-order reaction rate constants in WTRs suspensions after a 0.5 and 1.0 mM OTC initial concentration, pH 6.0 ± 0.1, *IS* 0.01M KCl, SSR 20 and 40 g L^{-1} for Al and Fe-WTR respectively and contact time ranged from 0.083 to 48 h.

Source	WTR Form	OTC mM	1^{st} order rate fit (r^2)	2^{nd} order rate fit (r^2)
Bradenton, FL	Al-Based	0.5	0.86	0.99
Bradenton, FL	Al-Based	1.0	0.83	0.99
Tampa, FL	Fe-Based	0.5	0.52	0.99
Tampa, FL	Fe-Based	1.0	0.53	0.98

Table 4. Desorption (%) of OTC by various treatments on the sorbed OTC after equilibration time (24 h). Data are expressed as the mean of three replicates ± one standard deviation.

Desorption treatments	Al-WTR	Fe-WTR
DI water	>MDL**	>MDL**
Methanol	3.6 ± 0.3	5.5 ± 0.4
1M KCl (pH 6.0 ± 0.1)	4.8 ± 0.2	7.6 ± 0.3
0.25M EDTA (pH 6.0 ± 0.1)	14.1 ± 0.5	17.4 ± 0.4

** Below method detection limit (1 x 10^{-3} mM).

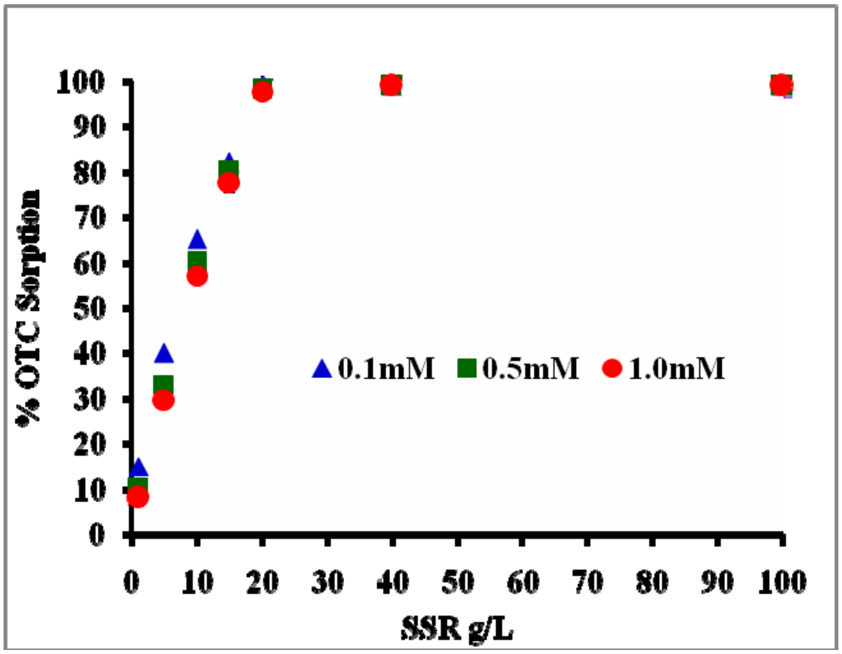

Figure 1: Effect of SSR on the amount of OTC sorbed by Al-WTR as a function of different initial OTC concentration (0.1, 0.5, and 1.0 mM OTC). Reaction time 24 h, ionic strength 0.01 M KCl, and pH 6.0 ± 0.1. Data are expressed as mean of three replicates ± one standard deviation.

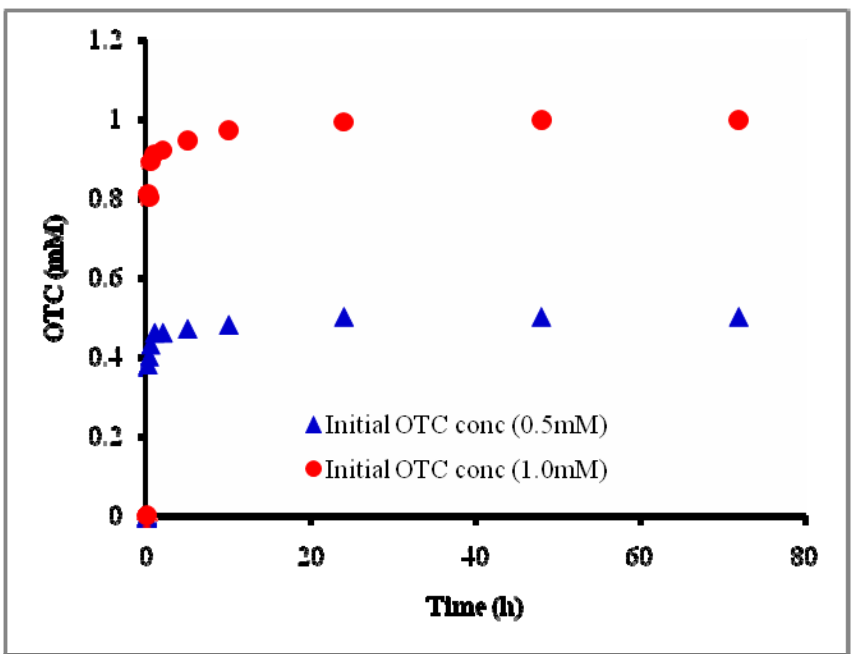

Figure 2: Effect of reaction time on the amount of OTC sorbed by Al-WTR (as a function of different initial OTC concentration (0.5 and 1 mM OTC). SSR 20 40 g L^{-1}, pH 6.0 ± 0.1, and ionic strength 0.01 M KCl. Data are expressed as mean of three replicates ± one standard deviation.

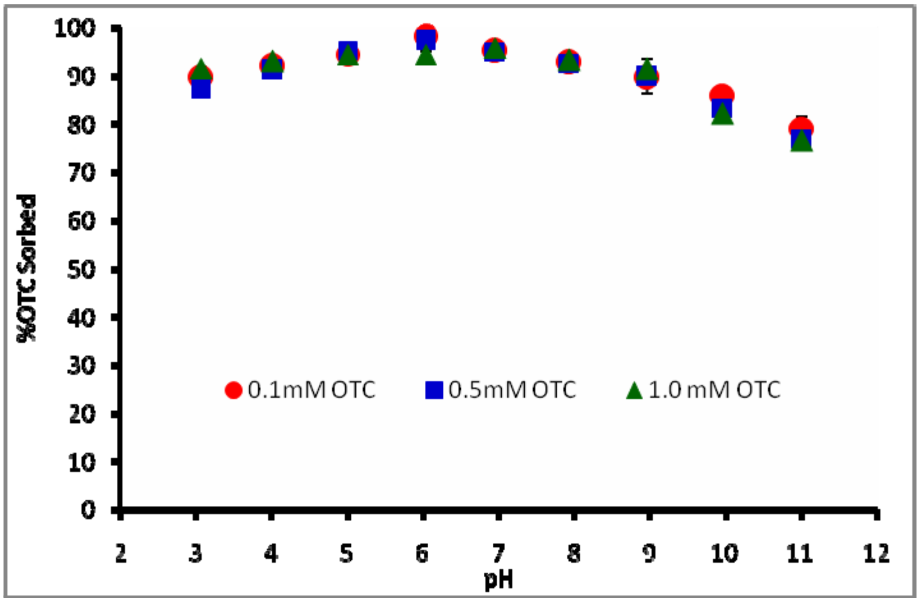

Figure 3: Effect of pH on the amount of OTC sorbed by Al-WTR as a function of different initial OTC concentration (0.1, 0.5, and 1.0 mM OTC). Reaction time 24 h, ionic strength 0.01 M KCl, and SSR 20 g L^{-1}. Data are expressed as mean of three replicates ± one standard deviation.

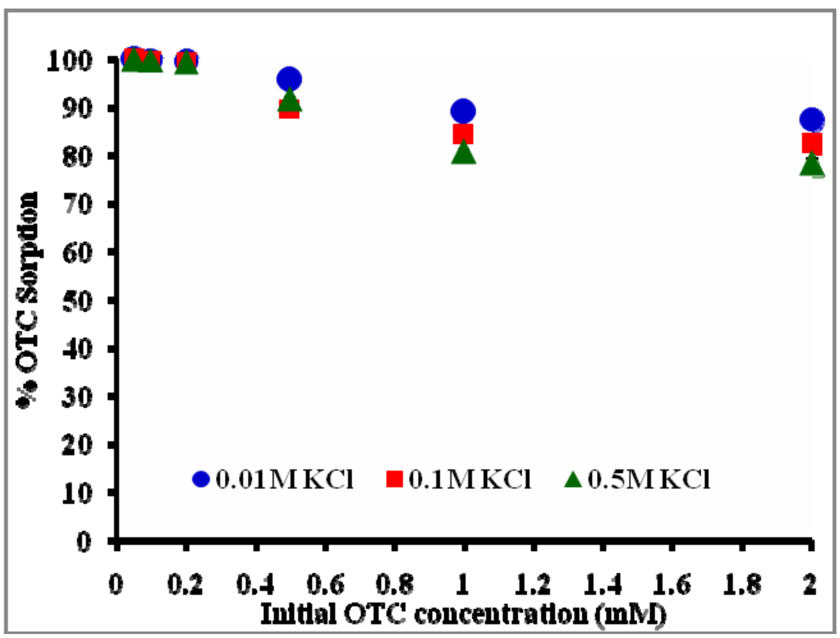

Figure 4: Effect of ionic strength (0.01, 0.1 and 0.5 M KCl) on the amount of OTC at different as a function of different initial OTC concentration . Reaction time 24 h, pH 6.0 ± 0.1, and SSR 20 g L^{-1}. Data are expressed as mean of three replicates ± one standard deviation.

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Information Transfer Program Introduction

The information transfer program serves an important purpose to the state's water resource community. The goal is to bring timely information about critical issues in water resource sciences to the public, and to promote the importance of research in solving water resource problems. The program accomplishes this goal through a variety of means. One focus is on producing a newsletter that provides an in-depth overview of current water resource issues. The program continues to develop the NJWRRI website (www.njwrri.rutgers.edu) into a comprehensive portal for water information for the state. We also collaborate with other state and regional organizations in sponsoring and producing conferences.

Information Transfer Program

Basic Information

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Project Number:	2010NJ232B
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Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	None
Principal Investigators:	Christopher Obropta, Diana Morgan

Publications

There are no publications.

Information Transfer Program

This year, the information transfer program has emphasized the presentation of conferences and other events to educate and inform the water resources community in New Jersey. We continue to develop the website and e-based communications with stakeholder groups. We have also focused on the production of a substantive newsletter addressing specific water resource issues as an effective way to communicate information to the public.

NJWRRI sponsored and co-sponsored several conferences, meetings and other events during this fiscal year. On January 27, 2011, NJWRRI was the lead sponsor for the conference “Managing Stormwater Runoff from Imperious Surfaces: Green Infrastructure Solutions for New Jersey” in New Brunswick, NJ. (http://www.water.rutgers.edu/Conference2011/Jan2011.html) Despite a severe snowstorm, the conference was attended by representatives from state and local government, universities, private industry and consulting, and non-profit groups. Presentations by regional experts focused on five topics relating to stormwater management: rain water harvesting, bioretention systems, green roofs, pervious pavement, and the first impervious cover-based TMDL.

NJWRRI also sponsored a series of sustainability workshops in fall 2010 with Rutgers Cooperative Extension and New Jersey Sea Grant. “Water Resources Solutions for New Jersey’s Sustainable Communities” provided opportunities and resources for Green Teams, Environmental Commissions, and Township Committees to engage residents in making their communities sustainable. (http://www.njwrri.rutgers.edu/pdfs/Final_Water%20Resources%20Solutions%20to%20Sustainable%20Jersey%20Workshops_11%209%202010.pdf)

A poster session featuring research conducted by NJWRRI grant recipients from FY 2006-2010 was held on February 10, 2011 at the Cook Campus Center of Rutgers University. Ten posters were presented, and abstracts from those posters were used for an issue of the New Jersey Flows newsletter. NJWRRI also held a seminar called “How to Write a Grant Proposal” on October 27, 2010 to help graduate students from around New Jersey improve their grant writing skills. Approximately 30 students attended.

NJWRRI co-sponsored the “Summit Meeting on the Role of Nutrient Management in Urban and Suburban Landscapes in Nutrient Loading of Surface and Groundwater,” held May 13, 2010 at the Rutgers Ecocomplex, Bordentown, NJ. (A summary report can be found here: http://www.njwrri.rutgers.edu/pdfs/Summary%20of%20Summit%20Meeting%20on%20Nutrient%20Management.pdf) The meeting presented the current understanding of nutrient fate and transport from suburban and urban landscapes. The audience and participants consisted of scientists, environmental agency representatives, industry leaders, policy makers, and other stakeholders from New Jersey. NJWRRI was also a platinum-level co-sponsor of the 4th Passaic River Symposium held June 22, 2010 at

Montclair State University (http://www.csam.montclair.edu/pri/conferences/index.html). We provided small scholarships for students and non-profits to attend the symposium.

One issue of the newsletter was produced this year, and a second is in progress. The first issue featured the information transfer and outreach activities performed by NJWRRI during FY2010. The primary focus was a poster presentation session featuring NJWRRI grant recipients from FY 2006-2010. The newsletter can be found online: http://www.njwrri.rutgers.edu/newsletters/Spring%202011%20Newsletter.pdf. The upcoming issue in production is the annual research issue. It is intended to showcase NJWRRI-funded research from the most recent fiscal year, and also to illustrate the importance of research in solving water-related problems. Each issue of the newsletter is approximately eight pages. It is primarily distributed via our e-mail lists to approximately 2,000 people throughout the state, and as paper copies to all members of the New Jersey legislature and Congressional delegation.

Our website (www.njwrri.rutgers.edu/) has been continually updated with information on water resource events and information in New Jersey, the U.S. and around the world. A new page, "Past NJWRRI Events," was added so that handouts and Powerpoint presentations from previous events can be archived and accessible to the public (www.njwrri.rutgers.edu/past_njwrri_events.html). The home page and events pages are regularly updated to highlight upcoming events, publications and other water–related news. The website is our primary means of information transfer to the water community and the public, and we will continue to update and improve its functionality with new pages and greater content.

We continue to expand and use targeted, group-specific e-mail lists to bring relevant information to specific audiences. Targeted lists include a list of scientists/principal investigators, water resource managers, non-governmental organizations and people affiliated with NGOs, and policy-makers. The lists are continuously updated and expanded, and are used to keep these groups informed of events, conferences, publications, and funding opportunities. These lists enable us to initiate and maintain frequent contact with stakeholder groups. We believe these lists are an excellent method of keeping the water-related public aware of NJWRRI, as well as informed about water-related news and information.

We also continue to participate in the New Jersey Water Monitoring Council, a statewide body representing both governmental and non-governmental organizations involved in water quality monitoring.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	7	6	0	0	13
Masters	3	2	0	0	5
Ph.D.	5	0	0	0	5
Post-Doc.	0	0	0	0	0
Total	15	8	0	0	23

Notable Awards and Achievements

For project 2010NJ216B, "Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues," the following student presentation award is reported: Kwasek, K. and L. Hazard. 2011. Behavioral responses of the Eastern Newt to road deicers. New Jersey Academy of Science Annual Meeting, Montclair, NJ, April 9, 2011. Second Place, Best Student Poster competition.

For project 2010NJ230B, "Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersey Pinelands," a student presentation award is reported for the following oral presentation: Parsekian, A, L Slater, KVR Schäfer and D Gray, 2011. Subsurface hydrologic controls on sensitive wetland ecosystems in the New Jersey Pinelands. MAESA Ecological Society of America meeting, Montclair, NJ, April 9, 2011.

For project 2010NJ222B, "Investigation into the Role of Arsenic Reducing Bacteria in the Mobilization of Arsenic into Ground Water in New Jersey," the following awards are reported: Mumford, Adam C. and Young, Lily Y. 2011. Role for Bacteria in Arsenic Mobility in New Jersey Shallow Groundwater. 2011 New Jersey Water Environment Association Meeting, Atlantic City, NJ. May 11, 2011. First Place, Ph.D. Student Research Poster Session.

A travel grant was awarded for the poster presentation: Mumford, Adam C., Barringer, J., Reilly, P., Young, L.Y. 2010. Potential Role for Bacteria in Arsenic Release to Groundwater. In Abstracts of 2010 Goldschmidt Conference, Knoxville, TN. June 13-18, 2010.

For project 2010NJ221B, "Comparative toxicological assessment of gasoline oxygenates MTBE, ETBE and TAME, and their metabolites, in the zebrafish cardiovascular system," the following award is reported: SETAC Student Travel Award - PhD Level ($390) to present a poster of this work at the National Society of Environmental Toxicology and Chemistry Meeting in Portland, OR in November 2010.

For project 2010NJ231B, "Green Remediation of Tetracyclines in Soil-Water Systems," the following awards are reported: 2nd Prize in Student Oral Presentation Competition for the presentation "Interaction and effect of solution chemistry on sorption of oxytetracycline on Fe-based drinking-water treatment residuals." 2010 Annual Meeting of the Hudson-Delaware Chapter of the Society of Environmental Chemistry and Toxicology, April 21-22, 2010 in Stockton, NJ.

A Student doctoral research award was received from Montclair State University, College of Science and Mathematics.

For project 2010NJ219B, "Development of Sustainable Biosorbents to Recover Nutrients from Water," a patent disclosure was filed at Stevens Institute of Technology for the functionalization process of the quaternized resins which was partially funded by this grant.

For project 2010NJ218B, "Evaluation of three methodologies to document improvement of water quality through stormwater management measures in an urban subwatershed," the following application was reported: The Bridgewater, Raritan and Somerville, NJ Environmental Commissions, based upon recommendations in the final report and the success of the Pilot Rain Barrel Workshops, have jointly applied for a grant to fund more Rain Barrel Workshops in their communities. This was a team effort and fully supported by the three municipalities based on the educational output of this research.

For project 2009NJ198B, "Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents," the following award is reported: Society of Environmental Toxicology and Chemistry Student Travel Award ($390) to present this research at the 2011 Annual Meeting.

Publications from Prior Years

1. 2004NJ71B ("Soil Moisture Regimes and Nitrate Leaching in Urban Wetlands") - Book Chapters - Stander, E. K. and J. G. Ehrenfeld. “Urban Riparian Function.” Chapter in J. Aitkenhead-Peterson and A. Volder, eds., Urban Ecosystem Ecology. ASA-CSSA-SSSA, Madison, WI. Pp. 253-276.
2. 2004NJ71B ("Soil Moisture Regimes and Nitrate Leaching in Urban Wetlands") - Book Chapters - Ehrenfeld, J. G. and E. K. Stander. “Habitat Functions in Urban Riparian Zones.” Chapter in J. Aitkenhead-Peterson and A. Volder, eds., Urban Ecosystem Ecology. ASA-CSSA-SSSA, Madison, WI. Pp. 103-118.
3. 2005NJ86B ("Microbial degradation of MTBE in anaerobic environments") - Articles in Refereed Scientific Journals - Youngster LKG, Rosell M, Richnow HH, Häggblom MM (2010) Assessment of MTBE biodegradation pathways by two-dimensional isotope analysis in mixed bacterial consortia under different redox conditions. Appl. Microbiol. Biotechnol. 88:309-318. (doi:10.1007/s00253-010-2730-0)
4. 2006NJ101B ("Female Hormones in Surface Water of Central/Northern New Jersey: Impacts of Combined Sewer Overflows versus Treated Wastewater Discharge") - Dissertations - Kim, Il Yum. 2009. The sorption, biotransformation, and detection of hormones in the environment. "Ph.D. Dissertation." Dept. of Environmental Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 131 pages.
5. 2006NJ102B ("Cranberry Agriculture as Wildlife Habitat in the Pine Barrens Wetland Ecosystem") - Dissertations - Wen, Ai. 2010. Ecological functions and consequences of cranberry (Vaccinium macrocarpon) agriculture in the Pinelands of New Jersey. Ph.D. Dissertation. Graduate Program of Ecology and Evolution, School of Environmental and Biological Sciences, Rutgers University—New Brunswick, New Brunswick, New Jersey. 117 pages.
6. 2007NJ144B ("Using assimilated C-DNA to fingerprint active microorganisms in methylmercury demethylation by stable-isotope probing") - Articles in Refereed Scientific Journals - Yu, Ri-Qing, Isaac Adatto, Mario Montesdeoca, Charles Driscoll, Mark E. Hines, and Tamar Barkay. 2010. Mercury methylation in Sphagnum moss mats and its association with sulfate reducing bacteria in an acidic Adirondack forest lake wetland. FEMS Microbiology Ecology 74(3): 655-668.
7. 2007NJ139B ("Phosphate and Thermal Stabilization of Dredged Sediments for Reuse as Construction Material") - Other Publications - Ndiba, P. K.; Axe, L. Risk to groundwater assessment of phosphate and thermal treated dredged sediments. Poster session, Third Passaic River Symposium, Montclair State University, Montclair, NJ, October 16, 2008.
8. 2007NJ146B ("Biogeochemistry of Pb transformations mediated by phosphate-releasing bacteria") - Other Publications - Walczak A., Yee N., Young L., (2010) Bosea sp. WAO Oxidizes Metal Sulfides at Neutral pH, Geochimica et Cosmochimica Acta 74: A1092, Suppl.2010. Goldschmidt Conference, Knoxville, TN.
9. 2008NJ161B ("Microbial Mobilization of Arsenic and Selenium Oxyanions in Subsurface Aquifers") - Other Publications - Rauschenbach I, Yee N, Häggblom MM, Bini E (2010) Energy metabolism and multiple respiratory pathways revealed by genome sequencing of the arsenate and selenate-respiring Desulfurispirillum indicum strain S5. Abstract Q-2341, American Society for Microbiology 110th General Meeting, San Diego, CA, May 23-27, 2010.
10. 2008NJ161B ("Microbial Mobilization of Arsenic and Selenium Oxyanions in Subsurface Aquifers") - Articles in Refereed Scientific Journals - Rauschenbach, I., Narasingarao, P., and Haggblom, M.M. (2011) Desulfurispirillum indicum sp. nov., a selenate- and selenite-respiring bacterium isolated from an estuarine canal. Int J Sys Evol Microbiol 61: 654-658.
11. 2009NJ194B ("Innovative Research and Development for Environmental Protection and Sustainable Waste and Wastewater Management System Design") - Dissertations - Babson, David. 2010. Enhancing Energy Recovery from Biomass Waste Streams – from Mega-landfill and Biorefineries to Microbial Communities, “Ph.D. Dissertation”, Department of Environmental Sciences, School of

Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, 250 pages.

12. 2009NJ198B ("Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents") - Other Publications - Bugel, S.M., Cooper, K.R. 2010. Altered gonadal development in Newark Bay organisms. Passaic River Institute of Montclair State University, Fourth Passaic River Symposium. Montclair, NJ. June 22, 2010.
13. 2009NJ198B ("Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents") - Articles in Refereed Scientific Journals - Bugel, S.M., White, L.A., Cooper, K.R., 2011. Decreased vitellogenin inducibility and 17 ^{2}-estradiol levels correlated with reduced egg production in killifish (Fundulus heteroclitus) from Newark Bay, NJ. Accepted (March 23): Aquat. Toxicol. doi: 10.1016/j.aquatox.2011.03.013
14. 2009NJ198B ("Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents") - Other Publications - Bugel, S.M., White, L.A., Cooper, K.R., 2010. Decreased reproductive capacity of killifish (Fundulus heteroclitus) from Newark Bay, NJ, was correlated with decreased estrogen sensitivity and vitellogenin production. Society of Environmental Toxicology and Chemistry, North American 31st Annual Meeting. Nov 7-11, Portland, OR.
15. 2009NJ198B ("Development of two in vivo fish assays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents") - Other Publications - Bugel, S.M., White, L.A., Cooper, K.R., 2011. An acute 1 hour exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) inhibited vitellogenin (zfVTG 1-3 induction by 17 ±-ethynylestradiol in zebrafish (Danio rerio). Society of Toxicology, 50th Annual Meeting. March 6-10, Washington, DC.
16. 2009NJ199B ("Application of molecular and metabolic biomarkers of anaerobic hydrocarbon degradation as evidence for natural attenuation in New Jersey groundwater samples") - Articles in Refereed Scientific Journals - Oka, Amita; Craig Phelps; Xiangyang Zhu; Diane Saber; L.Y. Young, 2011. Dual biomarkers of anaerobic hydrocarbon degradation in historically contaminated groundwater. Environmental Science and Technology, (45), 3407-3414.
17. 2009NJ188B ("Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities") - Other Publications - Honarbakhsh M., Z. Rosario-Cruz, C. Zhu, D. Sannino, and E. Bini. Comparative analysis of structure ad composition of bacterial communities in wastewater final effluents and receiving stream. 5th Annual Mini-Symposium on "Microbiology at Rutgers University: Cultivating Traditions, Current Strength, and Future Frontiers". New Brunswick, NJ, February 3-4, 2011. (poster presentation)
18. 2009NJ188B ("Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities") - Other Publications - Honarbakhsh M., Z. Rosario-Cruz, C. Zhu, D. Sannino, and E. Bini. Comparative analysis of structure ad composition of bacterial communities in wastewater final effluents and receiving stream. Meeting in Miniature of the Theobald Smith Society, NJ Branch of the ASM. New Brunswick, NJ, April 21, 2011. (poster presentation)
19. 2009NJ188B ("Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities") - Other Publications - Honarbakhsh M., Z. Rosario-Cruz, C. Zhu, D. Sannino, and E. Bini. Comparative analysis of structure ad composition of bacterial communities in wastewater final effluents and receiving stream. 111th General Meeting, American Society for Microbiology, New Orleans, LA, May 21-24, 2011. (poster presentation)