

# **New Jersey Water Resources Research Institute Annual Technical Report FY 2011**

# 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 funding graduate students to develop their thesis research. The funding is intended to initiate novel and important research efforts by both faculty and students, thus emphasizing 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 topics in water resources. In the first faculty project, Bakacs evaluated the safety of rooftop runoff for rainwater harvesting. The results of this research will help to develop guidelines for water testing and best management practices for applying harvested rain water to backyard and community gardens. In the second faculty project, Deng studied the possibility of using ground tire rubber and wastewater treatment residuals as novel "green" sorbents to remove common metal pollutants from urban stormwater runoff. Initial results indicate that both sorbents are effective in remediating metal pollution. In the final faculty project, Rowe will examine the performance of organoclay as permeable pavement to remove hydrocarbon and metal pollutants from urban stormwater runoff. Results of this study are unavailable pending construction of the study site. She will continue her research through the summer.

Graduate students have similarly carried out an impressive range of research. Costello and her advisor researched the Green Frog (*Lithobates clamitans*) as a biological indicator of risk near Superfund sites in New Jersey. Her research focused on potential metal accumulation at the cellular level, and whether that is correlated with problems at upper levels of biological organization, such as feeding habits. Dolcemascolo and her advisor intended to assess the prevalence and intensity of *Batrachochytrium dendrobatidis* (Bd), a water-borne fungal disease that has recently been found in New Jersey. Instead, they documented the first cases of Ranavirus, an even more lethal disease than Bd, in New Jersey. Elwaseif and his advisor developed Ground Penetrating Radar and Electrical Resistivity processing techniques to improve the characterization of two aquifer types. Honarbakhsh and her advisor sought to distinguish the mechanism by which antibiotic resistant genes are acquired by native microbial populations in streams where treated wastewater is discharged. Reid and his advisor developed a push-pull technique to quantify both in-situ rates of biodegradation and volatilization of VOCs in shallow wetland sediments. Results of this study were incorporated into a research proposal to the National Science Foundation, which funded it with a \$310,000 grant. Walczak and her advisor studied the role of sulfide respiring microorganisms in the release of hazardous metals through the oxidation of reduced sulfide minerals into ground and surface waters. Results of this study could allow for advance modeling and mitigation strategies.

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 studied streams and watersheds in ten East Coast states. The final report on this research is presented here.

James A. Smith and colleagues from Princeton University received funding through the NIWR/USGS Program to develop statistical procedures for regional analysis of drought and flood in the eastern United States. This is the initial report of this two-year study.

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](http://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.

## **Research Program Introduction**

The New Jersey Water Resources Research Institute has a policy, yearly reaffirmed by the NJWRRI Advisory Council, of using research dollars to promote novel directions of water resources research. To this end, three projects directed by research faculty at institutions of higher learning around the state were selected, and six 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.

Two NIWR/USGS National Competitive Grant Program projects from New Jersey received funding this year. One of the projects is submitting a final report, while the other project is reporting initial research results.

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

## Basic Information

|                                 |                                                                                                                      |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Does urbanization decrease baseflow? A historical, empirical analysis in the coastal states of Eastern United States |
| <b>Project Number:</b>          | 2009NJ210G                                                                                                           |
| <b>Start Date:</b>              | 9/1/2009                                                                                                             |
| <b>End Date:</b>                | 12/31/2011                                                                                                           |
| <b>Funding Source:</b>          | 104G                                                                                                                 |
| <b>Congressional District:</b>  | NJ 8th                                                                                                               |
| <b>Research Category:</b>       | Climate and Hydrologic Processes                                                                                     |
| <b>Focus Category:</b>          | Surface Water, Water Quantity, Management and Planning                                                               |
| <b>Descriptors:</b>             |                                                                                                                      |
| <b>Principal Investigators:</b> | Joshua C. Galster, Kirk Barrett                                                                                      |

## Publications

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.
2. Lopes, Jared, Joshua Galster, 2011, A historical, empirical analysis of the relationship between urbanization and stream baseflow in the eastern United States: Geological Society of America Abstracts with Programs, v. 43, p. 472.

## **Project Summary:**

### **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 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 processes 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 determined the relationship between urbanization and baseflow in real watersheds.

This project provided the most complete assessment about 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 analyzed historic United State Geological Survey (USGS) gage data to determine trends in baseflow over time. Baseflow data were collected for the states of Maine, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland, Virginia, North Carolina, South Carolina, and Georgia. We used different timeframes and baseflow metrics for their utility in identifying trends. We used

three metrics of annual baseflow: 1) baseflow per unit drainage area (BF); 2) ratio of BF to precipitation (BF/P); and 3) BF as a fraction of total flow (BF/TF). Trends were identified using the non-parametric Mann Kendal statistical test, the Sen slope estimator, and through the use of dummy variables and panel data fixed effect models to determine baseflow trends for regions.

Post-urbanization results in increased impervious surfaces and increased water delivery systems. The impervious surfaces will decrease river baseflow by decreasing infiltration rates, while water delivery systems are notoriously leaky. However, the net results of these two counteracting forces is that the Appalachians and New England experience net WATER GAINS as they add more water through leaky systems than they lose from increased runoff. However, the Coastal Plain is the opposite, as it has net WATER LOSES as the impervious surfaces decrease infiltration to larger degree than water is added through leaky systems.

## **Methodology**

We empirically investigated 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 selected gages that have continuous records for at least 25 years and have had substantial changes in the amount of impervious surfaces within the watersheds. We separated baseflow from other stream flows using a digital filtering method and aggregated daily baseflow to create an annual baseflow time series for each gage. We then computed 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 had both undergraduate and graduate students assisting us with these tasks.

Stream discharge data for the ten states were gathered using the U.S.G.S. National Water Information System under the condition that the data was continuous (>25 years), unregulated, and of a drainage area <400mi<sup>2</sup>. 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) (BF/TF) baseflow as a fraction of total flow. 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.

Statistical analysis: Using population density as a proxy for urbanization, SPSS Statistics (IBM, v17.0) was used to run panel data fixed effect models to detect trends between the baseflow metrics and population density. We assume that individual heterogeneity such as climate, topographic index, and localized geologic influence exists at each watershed that may impact or bias the predictor outcome. When we use fixed effects models to explore the relationship of baseflow and urbanization, unmeasured values are considered constant over time.

## **Principal Findings and Significance**

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 (Table 1). 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 would be analyzed.

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).

The initial individual results were compiled and more advanced statistical analysis was completed on regional data. Population density was used as a proxy for impervious surfaces, and river gage data were grouped by physiographic province: Appalachian, Piedmont, Coastal Plain, Blue Ridge, and New England. A panel data fixed effect model using dummy variables was used to determine regional trends over time.

| 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 | +, ***   |

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).

| <b>RESULTS</b>                                     | <i>B Pop.<br/>Density</i> | Sig. <0.05 |
|----------------------------------------------------|---------------------------|------------|
| <b>BF metric</b> (baseflow per unit drainage area) |                           |            |
| Appalachian (13)                                   | 0.21                      | 0.013      |
| Coastal Plain (13)                                 | -0.105                    | 0.046      |
| New England (13)                                   | 0.138                     | 0.004      |
| Piedmont (13)                                      | 0.026                     | 0.446      |

TOTAL (52 Gages)

|                                                            |        |      |
|------------------------------------------------------------|--------|------|
| <b>BF/TF metric</b> (baseflow as a fraction of total flow) |        |      |
| Appalachian (13)                                           | 0.004  | 0.73 |
| Coastal Plain (13)                                         | -0.052 | 0    |
| New England (13)                                           | 0.012  | 0.12 |
| Piedmont (13)                                              | -0.081 | 0    |

**BF/P Metric** (Baseflow per unit drainage area divided by precipitation.  
**IE:** the percent of precipitation converted to baseflow)

|                    |        |       |
|--------------------|--------|-------|
| Appalachian (13)   | 0.163  | 0.026 |
| Coastal Plain (13) | -0.078 | 0.012 |
| New England (13)   | 0.064  | 0.095 |
| Piedmont (13)      | -0.005 | 0.831 |

Table 2. Regional results for the three baseflow metrics. The high-sloped Appalachian and New England regions GAINED water with urbanization, while the low-sloped Coastal Plain LOST water as a result of urbanization. Results shaded in green are statistically significant at 0.05 and yellow 0.10., while red shaded are not statistically significant.

# An Investigation of the Water Quality of Rainwater Harvesting Systems

## Basic Information

|                                 |                                                                       |
|---------------------------------|-----------------------------------------------------------------------|
| <b>Title:</b>                   | An Investigation of the Water Quality of Rainwater Harvesting Systems |
| <b>Project Number:</b>          | 2011NJ273B                                                            |
| <b>Start Date:</b>              | 3/1/2011                                                              |
| <b>End Date:</b>                | 2/29/2012                                                             |
| <b>Funding Source:</b>          | 104B                                                                  |
| <b>Congressional District:</b>  | NJ-006                                                                |
| <b>Research Category:</b>       | Water Quality                                                         |
| <b>Focus Category:</b>          | Water Quality, Toxic Substances, Methods                              |
| <b>Descriptors:</b>             | None                                                                  |
| <b>Principal Investigators:</b> | Michele Bakacs, Mike Haberland                                        |

## Publication

1. Bakacs, M. and M. Haberland. 2011. Preliminary Investigation of Roof Runoff Water Quality for Small Scale Rainwater Harvesting. Proceedings of the 96th Annual Meeting and Professional Improvement Conference, National Association of County Agricultural Agents, Overland Park, Kansas, p. 58.

## **Project Summary:**

### **Problem and Research Objectives –**

In New Jersey, small scale rain water harvesting using rain barrels has become a popular method for watering backyard and community gardens. This research will help increase our understanding of the chemistry of harvested water and the safety of using harvested rain water on vegetable gardens, for which little information currently exists. In a survey done at four residential “Build A Rain Barrel” workshops in Middlesex and Union Counties in Spring 2010, 57% of the survey participants (n=58) indicated that they would be using their rain barrel water to water a vegetable garden (M. Bakacs, unpublished data). Studies have shown that roof runoff can have high levels of pathogens, zinc, lead, and hydrocarbons (Bannerman, 1993; Van Metre, 2003). Many homeowners and community gardens harvest rooftop runoff with little to no protection from the first flush of runoff that has been shown to have the highest levels of contaminants. The results of this research will help to develop guidelines for water testing, results interpretation and best management practices for applying harvested rain water to backyard and community gardens.

This research has specific applications to New Jersey. Many communities around the state are promoting residential rain water harvesting as a tool to encourage drinking water conservation and reduce stormwater runoff with their constituents. Techniques for promoting outdoor water conservation have become increasingly important due to drinking water supply deficits in many regions of the state. Safe alternative water supplies are being sought that can help reduce our dependence on the drinking water supply. In urban areas such as Newark, Camden and New Brunswick, where the expense of infiltrating stormwater limits opportunities, harvesting rainwater is an inexpensive alternative that has the potential to greatly reduce the amount of stormwater entering overburdened storm drain systems. Furthering our understanding of how to safely utilize harvested rainwater in order to augment New Jersey’s limited water supply and reduce stormwater runoff can help to protect our state’s vulnerable water resources. This study examined levels of four contaminants in rain water draining off asphalt shingled roofs and collected by rain barrels: polycyclic aromatic hydrocarbons (PAHs), lead, zinc, and total coliform. In addition, we investigated whether there is a significant difference between levels of contaminants draining roofs in different land uses, specifically, suburban verses urban

communities. The results of this research will help to develop guidelines for water testing, results interpretation and best management practices for applying harvested rain water to backyard and community gardens.

### **Methodology –**

This project analyzed stormwater runoff from asphalt shingled roofs that was collected in rain barrels. Rain barrel water samples were collected from both urban and suburban locations in order to determine whether surrounding land use has an impact on water quality. Rain barrels were installed in two medium density housing communities in southern New Jersey, representing a suburban community, and high density housing in central New Jersey, representing an urban community. Sampling locations were chosen based on the NJDEP 2007 Land Use/Land Cover data, access to the site and permission from the property owner.

The experimental design for this study used clusters of three discrete rain barrels located at four locations for a total of 12 rain barrels. Two cluster locations were in an urban community of central New Jersey and two in a suburban community of southern New Jersey. A cluster approach was employed to better ensure that each cluster of three rain barrels captured the same rainfall event and are exposed to similar atmospheric deposition rates. The rain barrels within each cluster were located within ¼ mile of each other.

Samples were collected from each barrel over a four month period from July – October 2011. Six samples were collected in southern New Jersey and eight from central New Jersey for a total of 84 samples. Half of the central and half of the southern barrels were drained down completely prior to a rain event, while the other half were drained down by 50 percent. This was done to mimic system usage and test whether there is a buildup of bacteria when residual water is left in the barrel. Water samples were collected 3 – 7 days after a rain event in order to replicate the expected time frame that a homeowner/community gardener would wait before using the collected runoff.

Samples were analyzed for polycyclic aromatic hydrocarbons (PAHs), lead, zinc, and total coliform. After three rounds of sampling (18 samples), no PAHs were detected and PAH analysis was dropped. *Escherichia coli* testing was added to the rest of the samples due to the levels of total bacterial detected in the first three samples.

Samples for PAHs were collected in two 1-liter amber glass bottles and preserved at 4°C. Samples for lead and zinc were collected in one 250 ml plastic container, preserved with HNO<sub>3</sub> and chilled to 4°C. The total coliform samples were to be collected in a 125 ml plastic container, preserved with 0.008% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and chilled to 4°C.

All samples were submitted to New Jersey Certified Laboratories for analysis.

### Principal Findings and Significance -

#### Question 1: What is the water quality of rain barrels draining asphalt shingled roofs?

The water quality data was compared to the designated water quality standards (Table 1).

**Table 1: Water quality standards.**

| Parameter                         | NJ Surface Water Quality Standard*                                                                              | NJ Groundwater Quality Standard           | NJ Drinking Water Standard | EPA Irrigation Maximum Concentration** |
|-----------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------------------|----------------------------|----------------------------------------|
| Lead                              | 5.0 µg/L                                                                                                        | 5.0 µg/L                                  | 15.0 µg/L                  | 5.0 mg/L<br>(5,000 µg/L)               |
| Zinc                              | 7,400 µg/L                                                                                                      | 2,000 µg/L                                | 5.0 mg/L<br>(5,000 µg/L)   | 2.0 mg/L<br>(2,000 µg/L)               |
| Total Coliform<br>(counts/100 mL) | None                                                                                                            | Same as the<br>Drinking Water<br>Standard | 0/100 mL                   | None                                   |
| <i>E. coli</i><br>(counts/100 mL) | <i>E. coli</i> levels shall not exceed a geometric mean of 126/100 mL or a single sample maximum of 235/100 mL. | Same as the<br>Drinking Water<br>Standard | 0/100 mL                   | None                                   |

\* New Jersey Department of Environmental Protection Freshwater (FW2) criteria

\*\* Rutgers University Fact Sheet FS1165

The NJDEP’s Integrated Water Quality Monitoring and Assessment Methods advises that if the frequency of water quality results exceed the water quality criteria twice within a five-year period, then the waterway’s quality may be compromised (NJDEP, 2010). NJDEP has further stated that a minimum of eight samples collected quarterly over a two-year period are required to confirm the quality of waters (NJDEP, 2010). Therefore, if a waterbody has a minimum of eight samples collected quarterly over a two-year period and samples exceed the water quality criteria for a certain parameter twice, the waterbody is considered “impaired” for that parameter. By applying this rule to the rain barrel water quality data, it is possible to identify which sites are impaired for each parameter that has been identified as a concern for this project (i.e., lead, zinc and total coliform (Table 2).

*E. coli* data was not included in the analyses due to the low number of samples collected. (The standard for irrigation of leafy greens for *E. coli* for pre-harvest foliar applications is  $\leq 126/100$  ml for the geometric mean and  $\leq 235/100$ ml for any single sample. Non-foliar application is  $\leq 126/100$  ml for the geometric mean, and  $\leq 576/100$ ml for any single sample.)

**Table 2: Number of samples that exceed water quality standards.**

| Site           |         | Parameter |     |      |       |      |     |      |       |                |     |      |       |
|----------------|---------|-----------|-----|------|-------|------|-----|------|-------|----------------|-----|------|-------|
|                |         | Lead      |     |      |       | Zinc |     |      |       | Total Coliform |     |      |       |
|                |         | A*        | B** | C*** | D**** | A*   | B** | C*** | D**** | A*             | B** | C*** | D**** |
| South Jersey   | COLL-1  | 2         | 2   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | COLL-2  | 2         | 2   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | COLL-3  | 0         | 0   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | HOLLY-1 | 1         | 1   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | HOLLY-2 | 3         | 3   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | HOLLY-3 | 0         | 0   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
| Central Jersey | UNIT-SQ | 1         | 1   | 1    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | 51TH    | 5         | 5   | 2    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | 133RED  | 1         | 1   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | 183HA   | 1         | 1   | 1    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | 218HA   | 1         | 1   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |
|                | 263CO   | 0         | 0   | 0    | 0     | 0    | 0   | 0    | 0     | -              | 6   | 6    | -     |

\* A = NJ Surface Water Quality Standard

\*\* B = NJ Groundwater Quality Standard

\*\*\* C = NJ Drinking Water Quality Standard

\*\*\*\* D = EPA Irrigation Maximum Concentration

Blue cells are ‘urban’ sites.

### **Results for zinc analysis**

Zinc did not violate any of the water quality standards in either the suburban sites or the urban sites. The mean zinc concentration in the urban sites (89.4 µg/L) was more than double the suburban sites (37.1 µg/L).

### **Results for lead analysis**

Lead violated the surface water and groundwater quality standard in both urban and suburban sites. However, three of the urban sites violated state drinking water standards (UNIT-SQ, 51TH and 183HA). The main source of atmospheric lead is industrial processes (automobiles were an atmospheric source as well, but with unleaded gasoline, this is less the case today). Further testing of actual roofing shingles may determine if the source is atmospheric or leaching from the shingles themselves. The same association can be said for zinc.

### **Results for total coliform analysis**

Total coliform concentrations violated the groundwater and drinking water quality standards at all sites for all sampling events. Potential sources of total coliform are wildlife (birds, small mammals/squirrels) that have access to the monitored roofs or are living in trees adjacent to the homes. There is no surface water quality standard for total coliform.

Overall, water quality could be considered impaired for both lead and total coliform.

### **Question 2: Do we see an effect of land use in the water quality of rain barrel water?**

To answer this question, we ran a quick set of statistics (min/mean/max, t-tests and one-way ANOVA) in addition to Principal Component Analysis (PCA). Because the dataset was small, the results of the t-test can be used instead of ANOVA. (It turns out that the t-test and ANOVA results were similar.)

Because of the high number of non-detects (ND) in the dataset, some form of data correction was needed. For the total coliform data, we used substitution of half the detection limit for the ND

data (i.e. for each <1.0/100 mL, we used 0.5/100 mL in the analyses). This method was chosen since only 7% of the data values were below the detection limit.

For zinc and lead, we tried a variety of data correction methods. We used elimination where we removed the ND values completely and ran analyses; the substitution method where ND values were replaced with half the detection limit; and the third technique was the ‘trimmed mean’ method where a percentage of the lowest and highest measurements from the data set are removed to more accurately determine the mean value (USEPA, 2000). For environmental samples, 15% trimming is recommended and was used for these analyses (USEPA, 2000).

All analyses run on lead (1/2 the detection limit, removal of NDs and trimmed mean) showed no statistical difference between urban and suburban land uses. Analysis of water usage (barrel drained completely between samples vs. drained only 50%) also showed no statistical differences.

Zinc showed a statistically significant difference between urban and suburban land uses in all of the analyses performed. Zinc concentrations were much higher in the urban sites when compared to the suburban sites. Atmospheric zinc can come from tire dust and industrial processes. Traffic increases in urban areas and the siting of industrial processes involving metals near urban centers may be accounting for the differences seen in the data sets.

Principal Component Analysis was employed to determine if there were specific patterns among the various samples and different rain barrel locations (PRINCOMP, SAS Version 9.3; SAS Institute, 2006). PCA is a multivariate statistical procedure that transforms a large number of variables into a small number of uncorrelated variables (“Principal Components”). The first Principal Component accounts for the highest possible variance in the dataset, and each succeeding Principal Component explains as much of the remaining variability as possible. In a plot of Principal Components, samples that are similar will form a ‘cluster’ in ordination space. Conversely, dissimilar samples will be further away from each other.

PCA results for lead and zinc showed no discernable pattern among the sites monitored.

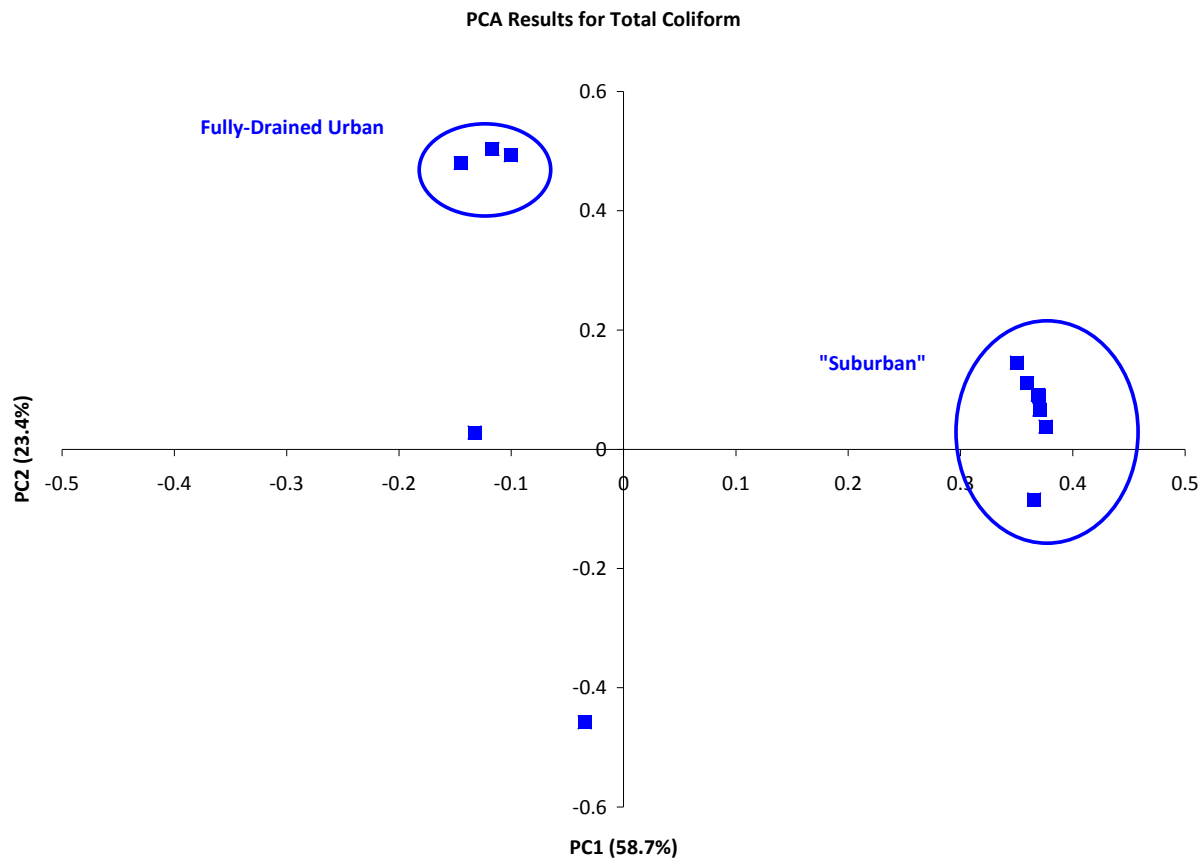
Total coliform levels showed no statistically significant differences between urban and suburban land uses. The geometric mean of total coliform in urban land uses, however, was more than double (366/100 mL) the suburban land uses (153.7/100 mL). The maximum total coliform measured was 9,000/100 mL for urban lands and 2,600/100 mL for suburban land uses.

### **Question 3: Does residual water left in the rain barrel impact pathogen levels?**

For total coliform, there is no statistical difference between the fully-drained and half-drained barrels in the suburban land uses and the urban sites. There were also no statistical differences between the fully-drained and half-drained total coliform levels within the land uses (full vs. half for suburban and full vs. half for urban).

PCA results for total coliform show that the fully-drained urban barrels are grouped separately from the other barrel treatments (Figure 1). This may be due to the fact that the fully-drained urban barrels had no non-detects.

**Figure 1: Principal Component Analysis (PCA) results for total coliform.**



The “Suburban” grouping in Figure 1 includes one urban site (218HA) with the entire suburban rain barrel sites, hence the quotations.

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# Scrap Tire and Water Treatment Residuals as Novel "Green" Sorbents from Removal of Common Metals from Polluted Urban Stormwater Runoff

## Basic Information

|                                 |                                                                                                                                        |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Scrap Tire and Water Treatment Residuals as Novel "Green" Sorbents from Removal of Common Metals from Polluted Urban Stormwater Runoff |
| <b>Project Number:</b>          | 2011NJ274B                                                                                                                             |
| <b>Start Date:</b>              | 3/1/2011                                                                                                                               |
| <b>End Date:</b>                | 8/31/2012                                                                                                                              |
| <b>Funding Source:</b>          | 104B                                                                                                                                   |
| <b>Congressional District:</b>  | NJ-008                                                                                                                                 |
| <b>Research Category:</b>       | Water Quality                                                                                                                          |
| <b>Focus Category:</b>          | Methods, Water Quality, Non Point Pollution                                                                                            |
| <b>Descriptors:</b>             |                                                                                                                                        |
| <b>Principal Investigators:</b> | Yang Deng, Sudipta Rakshit, Dibyendu Sarkar                                                                                            |

## Publication

1. Morris, Ciapha; Sudipta Rakshit; Yang Deng; Pravin Punamiya; Edward Landa; Dibyendu Sarkar, 2012, Sorption of Toxic Metals from Polluted Urban Stormwater Runoff using Two Recycled Waste-Based Sorbents - Water Treatment Residuals and Tire Rubber. 2012 Spring Annual Meeting of Hudson/Delaware Chapter - Society of Environmental Toxicology and Chemistry, Montclair, New Jersey. April 26-27, 2012. (Poster presentation)

## **4. PROJECT SUMMARY**

### **4.1 Problem and Research Objectives**

#### **4.1.1 Problem statement**

United States is an extremely urbanized country. In 2000, over 75% of the total U.S. population was concentrated in more than 500 urban areas with a population density of  $> 390 / \text{km}^2$ . Among them, two-thirds lived in the 39 largest metropolitan areas (e.g. North Jersey-New York City Metro Area) with populations greater than 1 million. Despite many benefits, high urbanization has led to various environmental issues such as polluted urban runoff. Urban runoff is the surface runoff of stormwater created by urbanization. Urban areas are characterized by high densities of impervious surfaces, such as buildings, parking facilities, urban streets, highways, or walkways. These impermeable surfaces can provide an effective environment to collect and accumulate constituents from atmospheric deposition, vehicular traffic or other sources. As a result, the impervious surfaces significantly increase the flow of urban runoff that efficiently transports numerous water quality constituents, and increases the variety and amount of pollutants in urban runoff, which are eventually transported to receiving waters without proper management and treatment. Increased pollutant loads can harm fish and wildlife populations, kill native vegetation, degrade the quality of drinking water supplies, and make recreational areas unsafe. Thus, urban runoff has been recognized among the major nonpoint sources of groundwater pollution (USEPA, 2009).

Heavy metals are pollutants of great interest in urban runoff due to their non-biodegradability, accumulation in the environment and toxicity. Copper (Cu),

zinc (Zn) and lead (Pb) are the most frequently found, among which Pb is the most toxic and has consistently ranked #2 in ATSDR's most hazardous chemicals list. The reported ranges of the three metals in urban runoff are summarized in Table 1.

**Table 1.** levels of common heavy metals in urban runoff in literature (unit:  $\mu\text{g/L}$ )

| <b>Cu</b>  | <b>Zn</b>  | <b>Pb</b>   | <b>Ref.</b>            |
|------------|------------|-------------|------------------------|
| 34         | 144        | 160         | USEPA, 1983            |
| 27         | 114        | 154         | USEPA, 2006            |
| 390        | 160        | 1,300       | Duke et al., 1998      |
| 97-104     | 120-2000   | 11-525      | Gobel et al., 2007     |
| 0.06-1,410 | 0.7-22,000 | 0.57-26,000 | Makepeace et al., 1995 |

Stormwater best management practices (BMPs) are control measures taken to address the quantity and quality issues of urban runoff. To remove metals from urban runoff, many structural BMPs have been attempted, including bioretention, wet ponds, constructed storm water wetlands, dry wells, extended detention basins, infiltration basins, and manufactured treatment (NJDEP, 2004). Some of the conventional structural BMPs are almost ineffective for metals, and many others, such as infiltration basins, are often impractical to implement in urban environments. These limitations have generated modifications to existing structural BMPs or led to the design of new BMPs that can properly treat urban stormwater constituents. Therefore, innovative, viable, cost effective, low-impact treatment options for heavy metals in urban runoff are in demand to improve the living quality of urban environments and safeguard the public health.

#### **4.1.2 Research objectives**

The long term goal of this study is to develop an effective, low-cost, and “green” BMP to sustainably address the issue of metal pollution in urban runoff. The primary objective of this study is to evaluate the performance of two recycled wastes,

aluminum-based drinking water treatment residuals (Al-WTR) and Tire Rubber (TR), in the adsorption of three major runoff metals (Cu, Zn and Pb), and to assess potential leaching of metals from spent sorbents. Our central hypothesis is that Al-WTR or/and TR can effectively and irreversibly adsorb Cu, Zn and Pb from USW runoff under a variety of relevant environmental conditions. The specific objectives are:

**Objective I:** to physically characterize TR and Al-based WTR surfaces by SEM, XRD, BET, and zeta potential analyses.

**Objective II:** to determine sorption/desorption of Cu and Pb by TR, and Cu, Pb and Zn by Al-WTR as a function of solution pH, ionic strength, solid:solution ratio, and temperature.

**Objective III:** to determine sorption/desorption of Cu, Pb and Zn by a composite TR/Al-WTR matrix from single and mixed-metal systems.

**Objective IV:** to assess potential leachability of the spent TR and Al-WTR using Toxicity Characteristics Leaching Procedure (TCLP) tests.

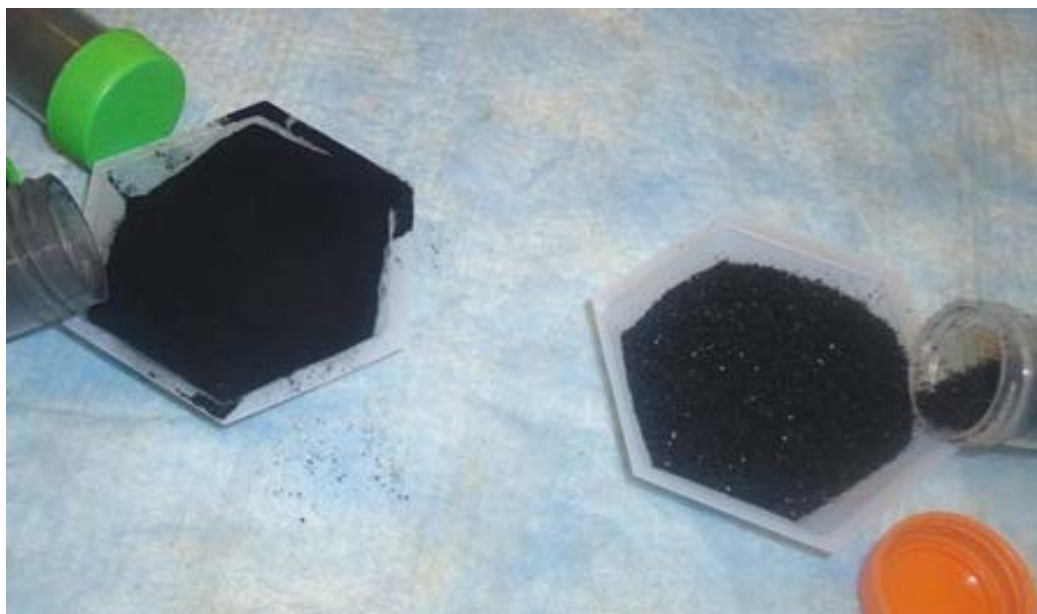
**Objective V:** to evaluate potential leachability of benzothiazole from TR and the effectiveness of Al-WTR in its retention.

## **4.2 Methodology**

### **4.2.1 Materials**

All the chemicals used are of analytical grade or above. Al-WTR and TR used in this study are shown in Figure 1. Al-WTR materials were collected from the Manatee County Water Treatment Plant in Bradenton, FL where they have been stockpiled following removal from storage lagoons. Once collected, the WTR was thoroughly mixed, air-dried, sieved through a 2-mm sieve, and finally ground into powder prior

to use. TR samples were provided from RubbeRecycle Inc., Lakewood, NJ. Prior to use, the TR was rinsed with Milli-Q water ( $18.2 \text{ M}\Omega\cdot\text{cm}$ ) twice and then air-dried.



**Figure 1.** Al-WTR (left) and TR (right) used in this study.

#### **4.2.2 Adsorption tests**

Bench-scale batch experiments were carried out with triplicate samples with appropriate analytical controls and standards. All the adsorption kinetics and isotherm experiments were conducted in 15 mL centrifuge tubes containing 15 mL simulated metal-contaminated urban runoff. The simulated runoff solution was prepared by addition of specific amounts of  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{NO}_3)_2$ , and  $\text{Pb}(\text{NO}_3)_2$  into Milli-Q water ( $18.2 \text{ M}\Omega\cdot\text{cm}$ ). The reactors were installed in a shaking water bath at  $25^\circ\text{C}$ . A rapid shaking speed ensured a completely mixing solution state. Initial solution pH was pre-adjusted to a desirable value with 1M NaOH and  $\text{HNO}_3$ , if needed. Solution pH was maintained during reactions using appropriate buffers such as PIPES. Desirable ionic strength was accomplished by addition of appropriate amount of  $\text{NaNO}_3$  into the simulated urban runoff. The adsorption reactions were initiated once certain amounts

of WTR and/or TR were added. In kinetics tests, at least three reactors were sacrificed at each designated sampling time. For adsorption isotherm experiments, shaking was stopped within a reaction time that allows the adsorption to reach a chemical equilibrium. Subsequently, 5 mL sample was collected from each reactor and then filtered through 0.45  $\mu\text{m}$  syringe membrane filter. The filtrate was stored in 2%  $\text{HNO}_3$  solution for metal analysis.

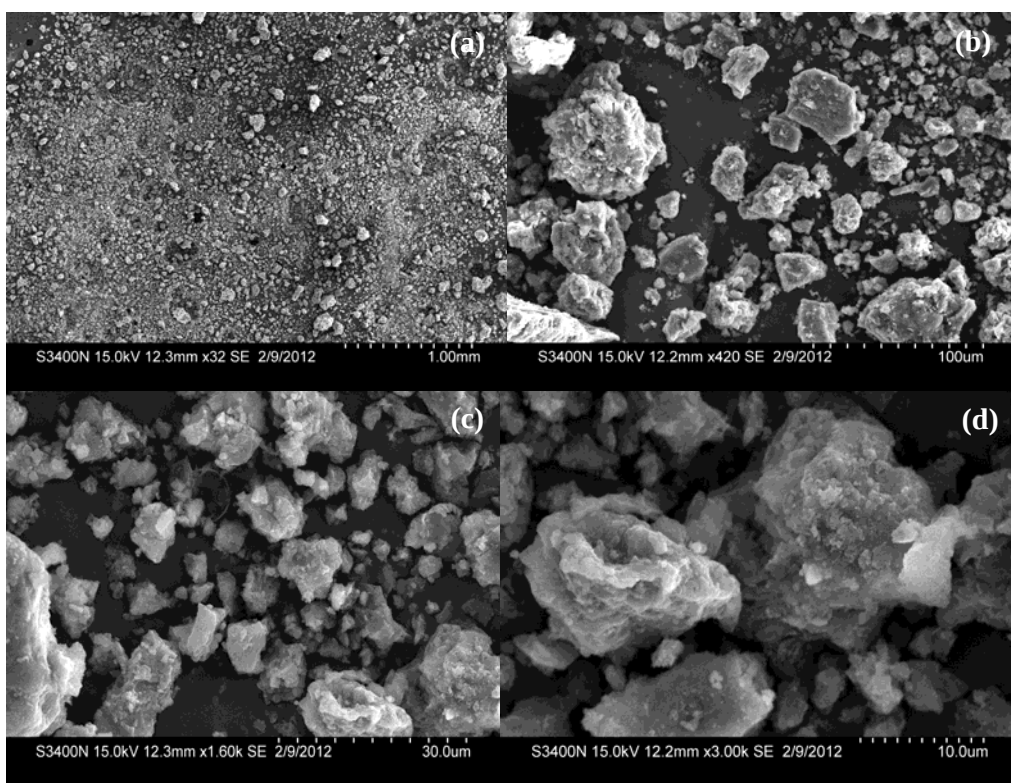
#### **4.2.3 Analytical method**

Surface morphology of WTR and TR samples was characterized by scanning electron microscopy (SEM) (Hitachi S-3400N). Crystallographic structure of the materials was determined by X-ray diffraction (XRD) (Philips, X'Pert). Cu, Zn and Pb in water were quantified using an inductively coupled plasma mass spectroscopy (ICP-MS) (Thermo, X Series II, XO 472). Solution pH was measured with a Thermo Fisher Scientific ORION 5-Star multiparameter meter.

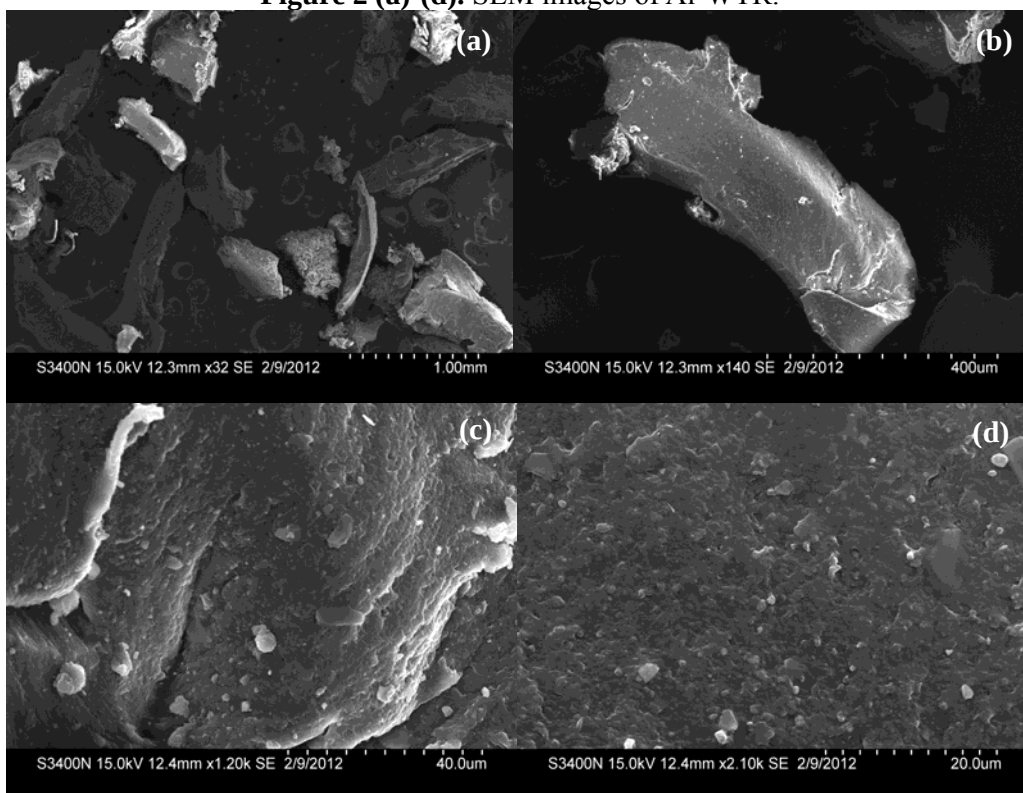
### **4.3 Principal Findings and Significance**

#### **4.3.1 Principal findings**

**4.3.1.1 Surface characterization.** SEM images of WTR and TR materials are shown in Fig. 2 and 3, respectively. Al-WTR was composed of amorphous aluminum oxides whose sizes broadly ranged on an order of a few  $\mu\text{m}$  to mm. The WTR particles usually had a rough surface. In contrast, TR particles had irregular shapes, their sizes were on the order of a few mm, and they had less rough surface than WTR.

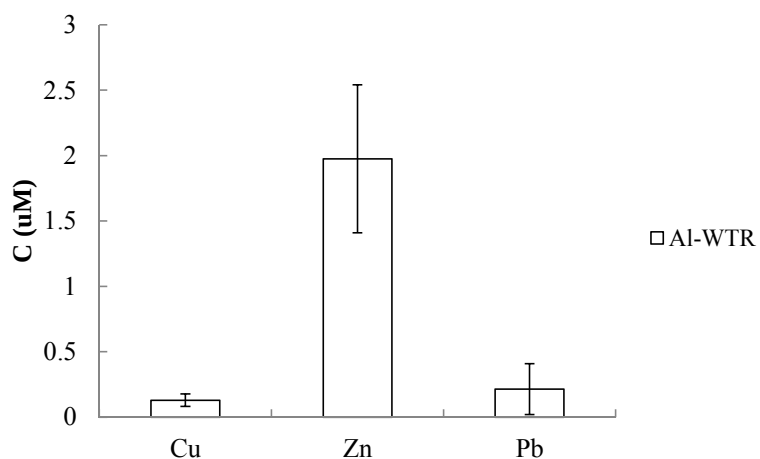


**Figure 2 (a)-(d).** SEM images of Al-WTR.

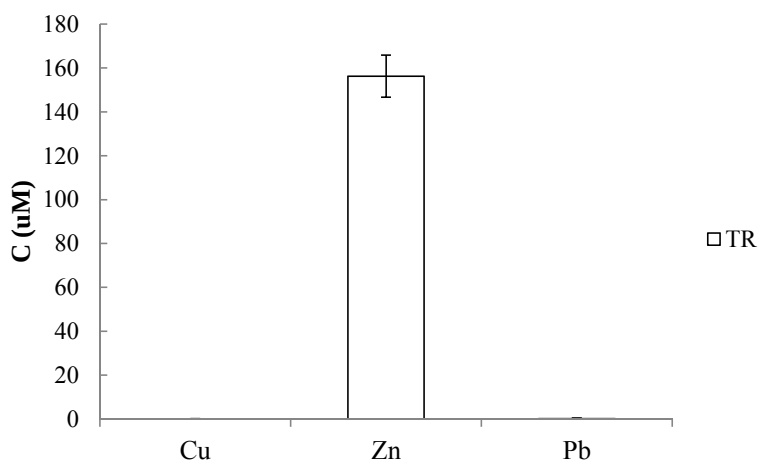


**Figure 3 (a)-(d).** SEM images of TR.

**4.3.1.2 Metal leaching from WTR and TR.** Metal leaching from 20 g/L WTR and TR in a Milli-Q water matrix is shown in in Fig. 4 and 5, respectively. Cu and Pb leaching from WTR and TR were negligible ( $< 0.2 \mu\text{M}$ ). The concentration of Zn released from WTR was also insignificant ( $2 \mu\text{M}$ ). However, Zn leaching from TR was noticeable. After 20 hours, Zn in 20 g/L TR solution reached  $156 \mu\text{M}$ .

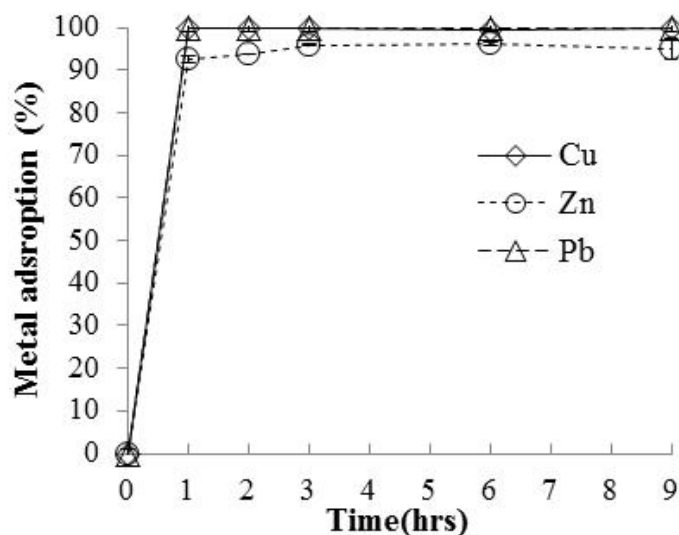


**Figure 4.** Leaching of different metals from Al-WTR in Milli-Q water (20 g/L WTR, reaction time = 20 hrs)



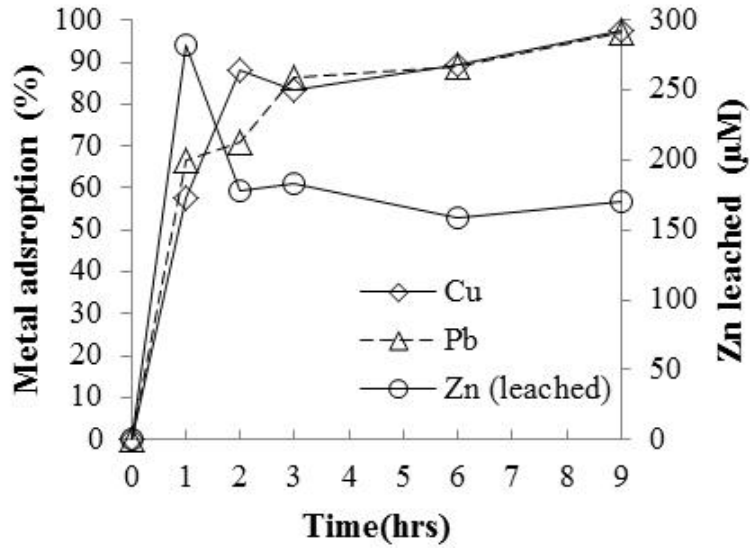
**Figure 5.** Leaching of different metals from TR in Milli-Q water (20 g/L TR, reaction time = 20 hrs)

**4.3.1.3 Kinetics tests .** Adsorption of Cu, Zn and Pb on WTR is shown in Fig.6. For all three metals, the adsorption reached equilibrium within one hour. Therefore, a removal efficiency of > 90% could be achieved within an hour. Within one hour, the removal efficiencies of Cu, Zn and Pb were 99.7%, 92.6% and 99.9%, respectively.



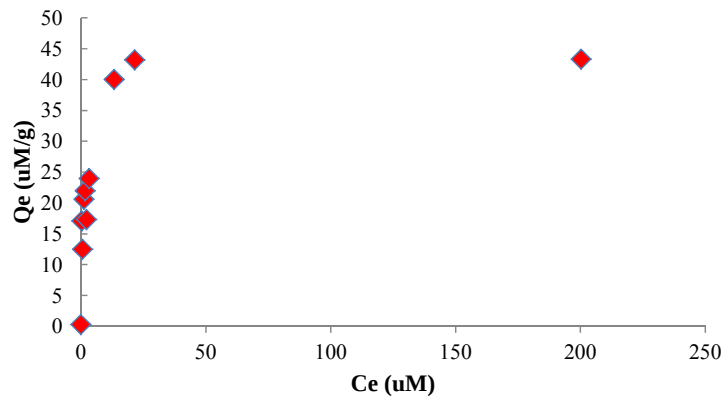
**Figure 6.** Metal adsorption by WTR with time (10 g/L WTR; initial Cu = 6  $\mu$ M, Zn= 6  $\mu$ M, and Pb = 2.45  $\mu$ M; initial pH 6.5)

Results of kinetic tests for adsorption of Cu and Pb on TR are shown in Figure 7. Adsorption efficiencies of Cu and Pb were gradually increased with time, and reached 97.4% and 97.2% after 9 hours, respectively. However, it is noted that Zn leached out from TR to enter the bulk solution, and the Zn concentration reached 170  $\mu$ M Zn within 9 hours.

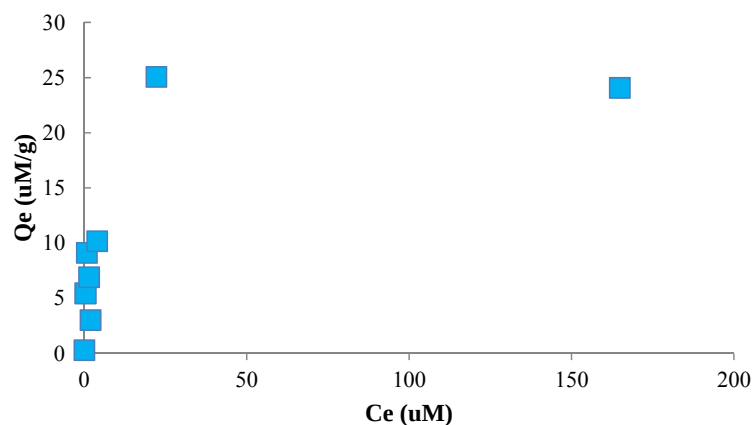


**Figure 7.** Cu and Pb adsorption onto and Zn leaching from TR with time (10 g/L TR; initial Cu = 6  $\mu$ M, Zn= 6  $\mu$ M, and Pb = 2.45  $\mu$ M; initial pH 6.5)

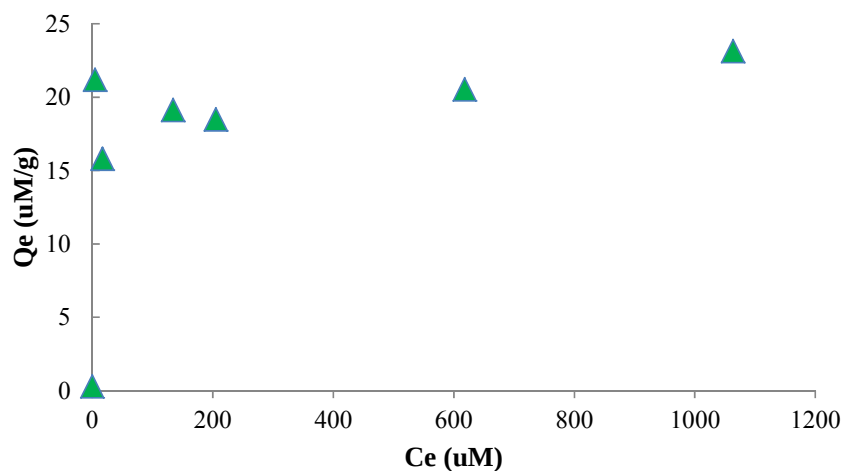
**4.3.1.4 Adsorption isotherm experiments with WTR only.** Adsorption isotherms of Cu, Pb and Zn during WTR adsorption of a simulated Cu, Pb and Zn mixed runoff are shown in Figures 8-10, respectively. The adsorption isotherms exhibited a two-phase pattern. At a low metal concentration ( $C_e$ ) in bulk solution, the metal mass adsorbed per unit mass of adsorbent (i.e. WTR) ( $Q_e$ ) was dramatically increased. However, when  $C_e$  was over a critical value, the increase of  $Q_e$  was almost marginal.



**Figure 8.** Cu adsorption isotherm on WTR (20 g/L WTR; initial pH 6.5)

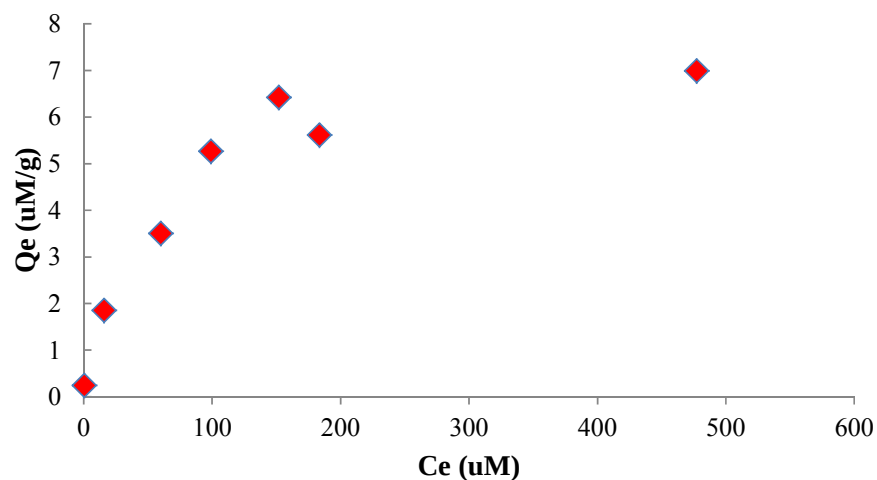


**Figure 9.** Pb adsorption isotherm on WTR (20 g/L WTR; initial pH 6.5)

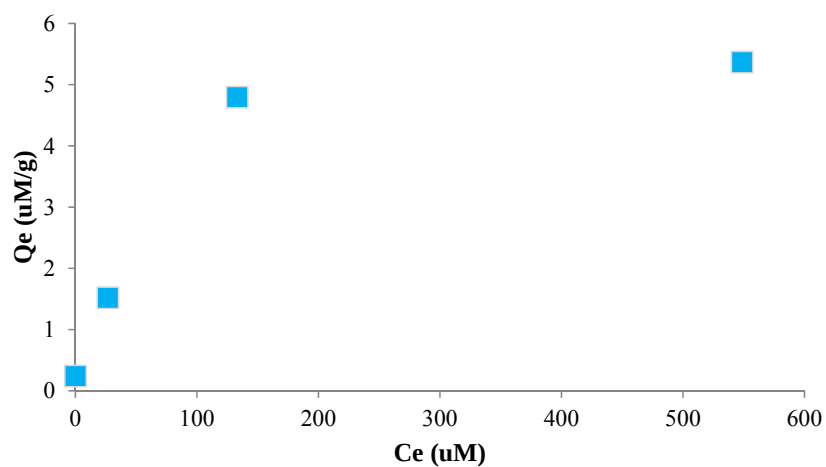


**Figure 10.** Zn adsorption isotherm on WTR (20 g/L WTR; initial pH 6.5)

**4.3.1.5 Adsorption isotherm experiments for TR only.** Adsorption isotherms of Cu and Pb during TR adsorption of a simulated Cu and Pb mixed runoff are shown in Figures 11 and 12, respectively. Similarly, the adsorption isotherms had a bi-phasic behavior. That is,  $Q_e$  was significantly increased with the increasing  $C_e$ , but plateaued at a high  $C_e$  level. Compared with WTR, TR exhibited a low adsorption capacity for both of the metals. For example, at  $C_e = 50 \mu\text{M}$ ,  $Q_e$  of the TR was  $\sim 3 \mu\text{M/g}$ , but  $Q_e$  of the WTR was  $\sim 45 \mu\text{M/g}$ .

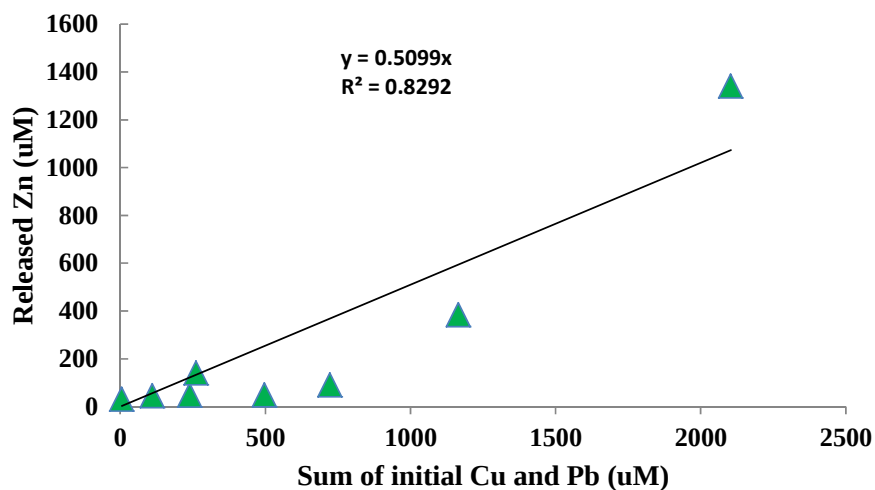


**Figure 11.** Cu adsorption isotherm on TR (20 g/L TR; initial pH 6.5)



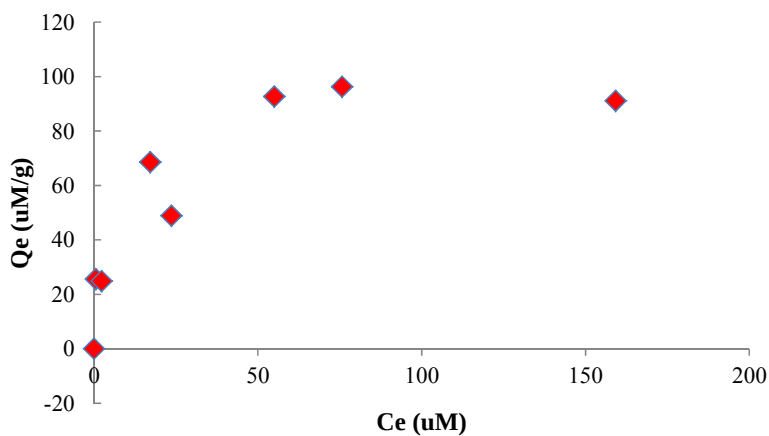
**Figure 12.** Pb adsorption isotherm on TR (20 g/L TR; initial pH 6.5)

The relationship of Zn leached out from TR and the initial combined Cu and Zn concentration is shown in Figure 13. In general, the level of leached Zn somewhat linearly increased with increasing combined Cu and Pb sorption.

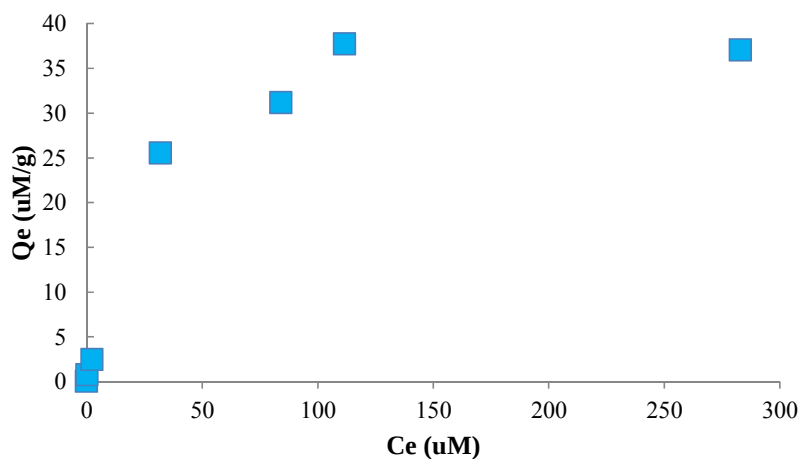


**Figure 13.** Sum of initial combined Cu and Pb vs. Zn leaching from tire during TR adsorption of Cu and Pb (20 g/L tire, pH = 6.5)

**4.3.1.6 Adsorption isotherm experiments for combined WTR and TR .** Adsorption isotherms of Cu and Pb during the combined WTR and TR adsorption of a simulated Cu and Pb mixed runoff are shown in Figures 14 and 15, respectively. The adsorption isotherms exhibited a two-phase pattern.

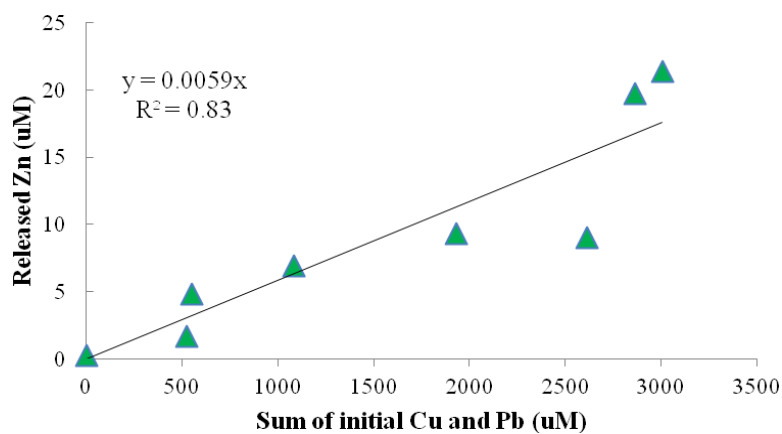


**Figure 14.** Cu adsorption isotherm on WTR and TR (20 g/L sorbents; mass WTR:TR = 1:1; initial pH 6.5)



**Figure 15.** Pb adsorption isotherm on WTR and TR (20 g/L sorbents; mass WTR:TR = 1:1; initial pH 6.5)

The relationship of Zn leached out from TR and the initial combined Cu and Zn sorption is shown in Figure 16. Likewise, the level of leached Zn somewhat linearly increased with increasing combined Cu and Pb sorption. Of note, the amount of Zn leached out was much less than that in the treatment by TR adsorption alone, thereby indicating that WTR played a critical role to control Zn release from TR.



**Figure 16.** Sum of initial Cu and Pb vs. Zn leaching from tire during combined WTR and tire adsorption of Cu and Pb (10 g/L WTR, 10 g/L tire, pH = 6.5)

#### **4.3.2 Significance**

Based on the current data, a few significant outcomes are obtained, including:

- WTR can rapidly adsorb Cu, Zn and Pb from water. Moreover, it has high adsorption capacities for the metal of concern. Therefore, WTR appears to be an excellent remediation material to address the metal pollution in urban runoff.
- TR also adsorbs Cu and Pb in water. However, compared to WTR, its adsorption rate is relatively slow. Likewise, its adsorption capacity is lower than that of WTR. Accompanied with Cu and Pb adsorption on TR, Zn in TR gradually leaches out into bulk solution, implying that Zn leaching might have been enhanced by Cu/Pb-Zn exchange in TR.
- A mixture of WTR and TR are able to quickly and effectively adsorb Cu and Pb. In addition, in the presence of WTR, less Zn is leached, thus indicating that WTR can adsorb Zn released from TR. This finding suggests that TR should be used in conjunction with WTR, because the latter can minimize Zn release from TR.

Currently, this project is in progress. More outcomes are expected by the summer of 2012 when the project will be officially completed.

#### **4.3.3 Plan to be completed by the summer of 2012**

Other research activities to be planned by the summer of 2012 include:

- Specific surface areas and zeta potentials of WTR and TR will be determined;

- XRD results will be analyzed;
- Effects of pH and temperature on WTR, TR, or combined WTR and TR will be evaluated;
- TCLP tests will be conducted to estimate the leaching of metals adsorbed onto WTR or TR during landfilling;
- Leachability of organic compounds from WTR and TR is assessed

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# Evaluation of organoclay in a permeable pavement system for removal of contaminants from urban stormwater runoff

## Basic Information

|                                 |                                                                                                                  |
|---------------------------------|------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Evaluation of organoclay in a permeable pavement system for removal of contaminants from urban stormwater runoff |
| <b>Project Number:</b>          | 2011NJ275B                                                                                                       |
| <b>Start Date:</b>              | 3/1/2011                                                                                                         |
| <b>End Date:</b>                | 8/31/2012                                                                                                        |
| <b>Funding Source:</b>          | 104B                                                                                                             |
| <b>Congressional District:</b>  | NJ-006                                                                                                           |
| <b>Research Category:</b>       | Water Quality                                                                                                    |
| <b>Focus Category:</b>          | Water Quality, Non Point Pollution, Water Supply                                                                 |
| <b>Descriptors:</b>             |                                                                                                                  |
| <b>Principal Investigators:</b> | Amy Rowe                                                                                                         |

## Publications

There are no publications.

**Project Summary:** This project has not started yet for a number of reasons. Late receipt of funding led to a delayed start, as well as last fall's extreme weather events. The experimental study site still has not been constructed. The project period has been extended to allow completion of the research.

### Problem and Research Objectives

Permeable pavement can not only reduce stormwater volume, but can filter some contaminants from runoff, as well. The addition of organoclay to the storage layer beneath the porous surface may adsorb typically hard-to-remove contaminants such as metals and hydrocarbons from infiltrating stormwater. It is expected that a permeable pavement system with an organoclay layer will remove hydrocarbons and metals more efficiently than one without any added organoclay. The pervasive presence of metals and hydrocarbons in urban runoff generally render it unusable, but the removal of these contaminants would possibly allow for the beneficial reuse of stormwater that would typically enter the sewer system.

The area underneath city sidewalks is generally an unexploited space with little to no value, and, at the same time, permeable pavement sidewalks have recently been gaining in popularity. These two tendencies could be combined to evaluate the possibility of a passive stormwater treatment system in an under-utilized piece of the cityscape. These treatment systems would not take up much space, would be underground, and would not spoil the aesthetic of the urban environment. These stormwater management systems would also be relatively easy to implement and cost-effective, as many cities have aging infrastructure and sidewalks that are in disrepair, so the installation could coincide with the regular sidewalk maintenance schedule. Finally, a permeable pavement sidewalk stormwater treatment system would not only reduce urban runoff volume, leading to a decreased burden on the combined sewer system, but the treated exfiltrate could be beneficially reused in community gardens or city landscape installations. It is expected that there would be many advantages to installing a permeable pavement sidewalk stormwater treatment system, but that there may be some unforeseen complications that may or may not outweigh those benefits.

### Specific study objectives

- Determine the removal efficiency of metals and hydrocarbons for a permeable pavement system cell with added organoclay compared to one without.
- Determine the practical feasibility of a permeable pavement sidewalk stormwater treatment system in terms of design and implementation.

### Methodology

No work has been done on this project at this time, but here is the intended methods description. Two side-by-side treatment cells will be installed below a pervious concrete surface. One cell will contain organoclay in the storage layer and the other will not. A perforated pipe will be placed at the bottom of each cell to allow for the capture of the exfiltrate, which will then be directed to the onsite rain garden or be sampled. This design will follow that of the U.S. Environmental Protection Agency (USEPA) permeable pavement demonstration site in Edison, NJ (Rowe et al., 2010). A curb cut will introduce stormwater runoff to the cells from Nye

Avenue, which is a relatively busy street that runs through a densely-populated residential area of Newark. The NJDEP stormwater quality design storm requires a rainfall depth of 1.25 inches with a total duration of 2 hours (NJDEP, 2004). The monitoring schedule will be weather-dependent, but it is expected that there will be at least one storm event per month that will satisfy the design storm requirements and will allow for sampling. Both the influent and effluent will be sampled in order to determine the pollutant removal efficiencies for the two cells. The samples will be analyzed for the following metals: copper, cadmium, lead, and zinc. Separate samples will be taken and analyzed for total petroleum hydrocarbons.

Principal Findings and Significance – no work has been done on this project at this time pending construction of the study site.

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Rowe, A.A.; Borst, M.; O'Connor, T.P.; Stander, E.K. Permeable pavement monitoring at the Edison Environmental Center demonstration site. *Conference proceedings of the ASCE/EWRI World Environmental and Water Resources Congress*, Providence, RI, May 16-20, 2010.

# Assessment of Green Frog, Lithobates clamitans, populations as biological indicators of risk to communities near Superfund sites

## Basic Information

|                                 |                                                                                                                                  |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Assessment of Green Frog, Lithobates clamitans, populations as biological indicators of risk to communities near Superfund sites |
| <b>Project Number:</b>          | 2011NJ276B                                                                                                                       |
| <b>Start Date:</b>              | 3/1/2011                                                                                                                         |
| <b>End Date:</b>                | 2/29/2012                                                                                                                        |
| <b>Funding Source:</b>          | 104B                                                                                                                             |
| <b>Congressional District:</b>  | NY-013                                                                                                                           |
| <b>Research Category:</b>       | Ecological Processes                                                                                                             |
| <b>Focus Category:</b>          | Toxic Substances, Ecology, Conservation                                                                                          |
| <b>Descriptors:</b>             | None                                                                                                                             |
| <b>Principal Investigators:</b> | Jennifer Costello, Richard Veit                                                                                                  |

## Publications

There are no publications.

## **Project Summary:**

### ***Problem and Research Objectives –***

Many ecosystems have been polluted by anthropogenic activities. While these activities are often associated with urban areas, suburban/rural ecosystems may also be impacted [1]. The Superfund Act is a Federal program established in 1980 to expedite remediation of soil, surface water and ground water at active and abandoned hazardous waste sites (CERCLA 1980). Hazardous substances, such as heavy metals, insecticides, volatile organic compounds (VOCs), phenolic compounds, polyaromatic hydrocarbons (PAHs) and inorganic compounds can negatively impact biological diversity by accelerating both local and global extinctions of numerous species as well as deteriorating human health [2-4].

Lone Pine Landfill is one of 141 superfund sites on the National Priorities List (NPL) in the state of New Jersey (EPA). These superfund sites are not isolated to highly industrialized areas with limited contact to NJ wildlife and communities, but are also found in residential and agricultural areas. Municipal, industrial and commercial wastes including chemical wastes from Scientific Chemical Processing Company were disposed of on-site throughout the landfill's operation, 1959-1979 [5]. Groundwater contamination at the site consists of metals in excess of background values, volatile organic compounds (VOCs), phenolic compounds, polyaromatic hydrocarbons (PAHs), and inorganic compounds [5]. Those potentially affected by the contamination include residents, wildlife of the Turkey Swamp Wildlife Management Area and surrounding areas, including the Manasquan River, for which groundwater from the site is a water source [5]. While remediation of the site is currently underway, an analysis of potential impacts is necessary to establish the current state of the ecosystem.

Focal species used to assay the environmental health of other sympatric species are termed biological indicators [6]. Fluctuations in indicator species should mirror fluctuations in the community as well as reflecting changes in the environment [7]. Recently, amphibians have been proposed as biological indicators due to the unique nature of the amphibian life cycle, which requires an aquatic juvenile stage and a terrestrial adult stage. This complete metamorphosis results in significant exposure to both aquatic and terrestrial environments. Furthermore, due to the permeable nature of amphibian skin, they not only have potential exposure to contaminants through ingestion, but also through dermal contact [8]. In addition, due to the numerous accounts of local and global extinction of frog species across six continents over the past 40 years, amphibian decline is a topic of interest [9,10].

Within a biological indicator species it may also be possible to establish early warning signs at the cellular or organism level that precede major population declines [11]. Due to this hierarchy, one would expect to observe changes at the cellular level prior to observing differences in the organism's behavior, which in turn may influence the overall population.

Elevated metal concentrations will be used as a measure of anthropogenic habitat degradation. Metals have been associated with sublethal cellular changes [12-14] and behavioral changes [9,11,13,15], as well as indirect mortality resulting from toxicity [9,16]. I will utilize techniques from several fields of study to assess if habitat degradation is affecting populations of the model organism, the green frog, *Lithobates clamitans*. This study will not only provide an understanding of the impact of degraded habitats on green frog populations and identify the processes by which these changes are occurring, but also predict changes in other organisms that share the same habitat.

The objective of this study is to determine if correlations exist between the presence of metals in the environment and observable differences in ultrastructure of tissues, feeding

behavior and population composition. Elevated metal concentrations at the site will be used as a measure of anthropogenic habitat degradation. Individuals from the site of interest, a pond adjacent to Lone Pine Landfill, N.J. will be compared to individuals from a pristine site in Ulster County, N.Y. The following hypotheses will be tested:

- 1) Does metal exposure correlate with cellular metal accumulation and ultrastructural differences in tissues?
- 2) Does metal exposure correlate with decreased prey capture efficiency?
- 3) Does metal exposure correlate with survival to adulthood?
- 4) Does a link exist between lower levels of biological organization, such as cellular abnormalities and metal accumulation, and upper levels of biological organization, such as feeding behavior and population composition?

### ***Methodology –***

Two sites were selected based upon proximity to potential sources of contamination. Turkey Swamp WMA, located in Freehold, N.J. was classified as potentially impacted site due to its proximity to Lone Pine Landfill. The Joy property, located in Ulster County, N.Y. was relegated as pristine due to the absence of any known environmental threats. Several abiotic factors in addition to heavy metals were monitored for each location to determine if any abiotic stressors correlated with the observed predator prey interaction. These data were collected during the 2011 field season (June – November).

#### *1) Soil and Water Analysis*

##### *Sediment processing*

Sediment samples were obtained from two locations at each site and kept at -20°C pending further processing. Sediment samples were thawed and filtered through 74µm mesh screen (Mc Master-CARR Supply Co.). Three replicates were taken from each sample to account for variability within the sample. Coarse and fine sediment samples are dried for 48-72 hrs at 65°C then weighed. Approximately 1g of ground fine sediment was reserved for acid digestion.

##### *Acid digestion*

Approximately 1g of sediment was placed in pre-weighed 20ml vials. Trace metal grade Nitric Acid (5ml) was added to each sample. Vials were topped with glass watch glasses and placed under the hood for 24 hrs. Samples were allowed to reflux on a hotplate for approximately 3-4 days, until clear. Watch glasses were removed and fluid was allowed to evaporate until samples were dry. The samples were then reconstituted in 2% Nitric Acid for 24 hrs. Each sample was then filtered through a 45µm filter (Millipore Corp.). Filtrate was collected and analyzed with a Perkin Elmer Elan DRC-e ICP-MS with HPLC and GC [17] in the Brooklyn College Environmental Sciences Analytical Center.

##### *Water Processing*

While water analysis for metals was not included in the initial proposal, a member of the grant committee had expressed interest in the collection of this additional data. Water samples were collected from each location. Samples were refrigerated until processing. Samples were processed by the associate laboratory director of the Interstate Environmental Commission located within the College of Staten Island. Samples were analyzed for metals by ICP.

### *On Site Water Analysis*

On site water sampling was conducted using both a YSI Professional Plus (ProPlus) multimeter and an IQ160 pH meter for collection of temperature, pH, dissolved oxygen, salinity, conductivity, and nitrate concentration data.

#### *2) Other site properties*

Water/Air temperature and Humidity were collected using an AcuRite Digital Weather Station and an MKA-38 Fisherman's Air & Water Temperature Sensor.

UV-B readings were collected with a Solarmeter model 6.2 UV meter. Measurements were collected from four approximately equidistant locations at each site between 12 and 1 p.m.

#### *3) Tissue/Cellular Analysis*

##### *TEM*

Both male and female frogs were obtained from ponds in New Jersey and New York. Five individuals from each study site were humanely sacrificed for Transmission Electron Microscopy (TEM) and Energy-dispersive X-ray Spectroscopy (EDS). Tissue samples from the brain and liver were processed following a modified electron microscopy procedure [18]. Images were obtained with a FEI Tecnai GZ Twin (2KX2K digital camera, AMT) and analyzed using an EDAX Si(Li) detector with Genesis X-ray microanalysis software.

##### *SEM*

Tissue samples were harvested from frogs euthanized for TEM analysis. Liver samples were stored in 3.7% paraformaldehyde, 2.5% glutaraldehyde in Millonig phosphate buffer at 4° C. Samples were dehydrated in an ascending ethanol series and dried at the critical point using liquid CO<sub>2</sub> transition fluid (Autosamdri-815A, Tousimis Research Corp.). The membranes were coated with approximately 10 nm of Carbon with a MED-020 coating system (Bal-Tec). SEM images were obtained with an AMRAY 1910 FE-SEM.

##### *ICP*

An additional five frogs from each site were sacrificed for total body concentrations of metals. Euthanized frogs were homogenized in Tris buffer solution and dried in an oven at 60 ° C for approximately 3-4 days. Samples were ground by mortar and pestle, and three subsamples (~.25g) were taken from each. Metal grade Nitric acid (5ml) was added to each subsample, and samples were digested by microwave acid digestion (Milestone EHTOS EZ Microwave Assisted Digested System). Once digested, a 1:10 dilution of sample to ultrapure water was analyzed by a 1 Perkin Elmer Elan DRC-e ICP-MS, with HPLC and GC [17] in the Brooklyn College Environmental Sciences Analytical Center.

#### *2) Behavioral Analysis*

Twenty frogs were captured per site for behavioral analysis. Frogs were observed and recorded for differences in prey capture efficiency. Prior to feeding trials, each individual was fasted for 24 hours to ensure individuals were motivated to participate. Trials were conducted on three consecutive days with an average efficiency taken across all days. Each subject was introduced at the same position in the chamber during the trials. Upon introduction, a recorded 10-minute habituation phase began. Trials were imaged using a Sony Handycam Vision DCR-SX44. Habituation periods were recorded to evaluate the subject's response to a novel environment [19]. Once the habituation phase was complete, five crickets were introduced

through tubing entering the chambers side. Again, a 10-minute recording period began. The recording chamber was cleaned and a new Labmat disposable liner used between sites. Efficiency was determined by number of attempts to capture prey items versus successful attempts, as well as the duration of time required to capture prey items, including latency between captures.

#### 4) Population Analysis

An overall population analysis was not conducted due to exceptional weather conditions that appear to have significantly hampered breeding during the 2011 season.

#### ***Principal Findings –***

The analyses of abiotic factors for water samples from each site are located in table 1. Factors measured include water temperature (°C), dissolved oxygen (DO %, mg/L), conductivity (uS/cm), salinity, pH, and nitrogen (mg/L). With the exception of dissolved oxygen, all values are within the normal ranges [20; 21; 22; 23; 24]. Dissolved oxygen levels for Turkey Swamp WMA (35%, 3.41 ml/L) are below the range indicative of a healthy stream or pond (~60% or higher, 4 mg/L or higher; [20]). This value is less than half of that observed in the Joy Property pond (85%, 9.08 mg/L).

**Table 1** Abiotic water measurements for both sites.

| Site               | Joy Property Ulster County, NY | Turkey Swamp WMA | Levels Considered Dangerous for Amphibians |
|--------------------|--------------------------------|------------------|--------------------------------------------|
| Date               | 10/4/2011                      | 10/4/2011        | –                                          |
| H2O Temp °C        | 13.5                           | 16.5             | –                                          |
| Barometer mmHg     | 755.8                          | 759.3            | –                                          |
| DO % Saturation    | 86.5                           | 35*              | <60%                                       |
| Do mg/L            | 9.08                           | 3.41*            | 2-4 mg/L                                   |
| Conductivity uS/cm | 36.4                           | 47.3             | >500 µS                                    |
| Salinity ppt       | 0.02                           | 0.03             | 6                                          |
| pH                 | 6.19                           | 6.93             | <4.50                                      |
| Nitrogen mg/L      | 0.13                           | 0.22             | >2.00                                      |

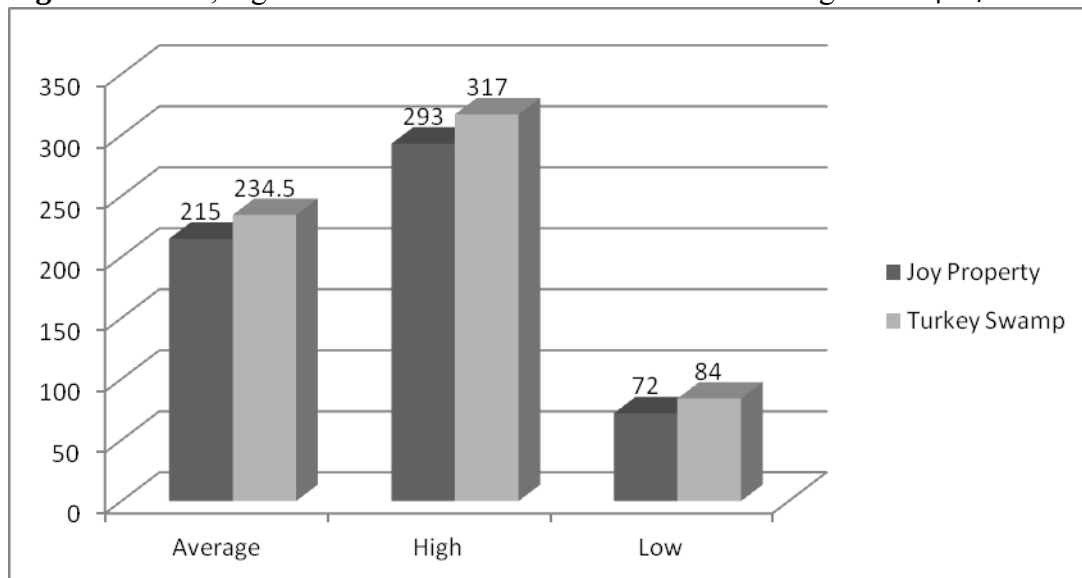
\* Indicate values outside normal range.

UVB measurements were also taken as abiotic indicators of habitat suitability. All measurements collected are located in table 2. Mean, high and low values for each site are located in figure 1. All measurements collected are located in table 2. UVB readings are in  $\mu\text{W}/\text{cm}^2$ , indicating the strength or intensity of the UVB rays. Measurements from each site are not significantly different ( $p = 0.793$ ).

**Table 2.** UVB measurements from both sites. UVB readings are in  $\mu\text{W}/\text{cm}^2$ .

| Site          | j.joy | Lone Pine |
|---------------|-------|-----------|
| Time          | 12:50 | 12:45     |
| UVB Reading 1 | 250   | 84        |
| UVB Reading 2 | 293   | 317       |
| UVB Reading 3 | 245   | 255       |
| UVB Reading 4 | 72    | 282       |

**Figure 1.** Mean, high and low values for each site. UVB readings are in  $\mu\text{W}/\text{cm}^2$ .



Sediment and water samples were collected and processed to compare to tissue concentrations of metals. Table 3 and table 4 display the data for both sites. Each reported frog value is an average of three subsample values. These data are fairly new and have yet to be analyzed completely.

**Table 3.** ICP analysis results for sediment, water and tissue samples from the Joy property. All values are in parts per million (PPM). Eastern USA Background values obtained from DEC Recommended soil cleanup objectives (mg/kg or ppm) for heavy metals [25].

\*\*New York State Background

\*\*\*\* Background levels for lead vary widely. Average levels in undeveloped, rural areas may range from 4-61 ppm. Average background levels in metropolitan or suburban areas or near highways are much higher and typically range from 200-500 ppm.

| <b>Metal</b> | <b>Eastern USA Background (PPM)</b> | <b>Joy-Sediment</b> | <b>Joy-Water</b> | <b>Joy Frog 1</b> | <b>Joy Frog 2</b> | <b>Joy Frog 3</b> | <b>Joy Frog 4</b> | <b>Joy Frog 5</b> |
|--------------|-------------------------------------|---------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| <b>Cr</b>    | 1.5-40**                            | 40.00               | 0.00             | 8.36              | 4.42              | 0.91              | 1.39              | 0.54              |
| <b>Mn</b>    | 50-5,000                            | 632.00              | 0.04             | 94.75             | 46.20             | 88.32             | 165.03            | 67.03             |
| <b>Co</b>    | 2.5-60                              | 9.00                | 0.00             | 0.71              | 0.17              | 0.21              | 0.33              | 0.11              |
| <b>Ni</b>    | 0.5-25                              | 26.00               | 0.00             | 2.39              | 1.99              | 2.00              | 2.06              | 1.45              |
| <b>Cu</b>    | 1-50                                | 12.00               | 0.00             | 13.00             | 11.55             | 11.49             | 12.02             | 10.59             |
| <b>Zn</b>    | 9-50                                | 79.00               | 0.00             | 64.16             | 64.68             | 72.51             | 78.50             | 51.05             |
| <b>As</b>    | 3-12**                              | 6.60                | 0.00             | 0.82              | 0.31              | 0.28              | 0.90              | 0.39              |
| <b>Cd</b>    | 0.1-1                               | 0.50                | 0.00             | 0.58              | 0.81              | 0.86              | 1.13              | 0.60              |
| <b>Ti</b>    | -                                   | 0.40                | 0.00             | 0.12              | 0.07              | 0.02              | 0.02              | 0.01              |
| <b>Pb</b>    | ****                                | 12.00               | 0.00             | 6.43              | 5.93              | 0.31              | 0.48              | 0.06              |
| <b>U</b>     | -                                   | 1.40                | -                | 0.05              | 0.00              | 0.01              | 0.16              | 0.00              |
| <b>Fe</b>    | 2,000-5,000                         | -                   | 0.06             | 10.35             | 1.89              | 2.75              | 4.04              | 1.39              |
| <b>Al</b>    | 33,000                              | -                   | 0.01             | 1576.65           | 61.25             | 169.36            | 301.94            | 47.85             |

**Table 4.** ICP analysis results for sediment, water and tissue samples from Turkey Swamp WMA. All values are in parts per million (PPM). Eastern USA Background values obtained from DEC Recommended soil cleanup objectives (mg/kg or ppm) for heavy metals [25].

**\*\*New York State Background**

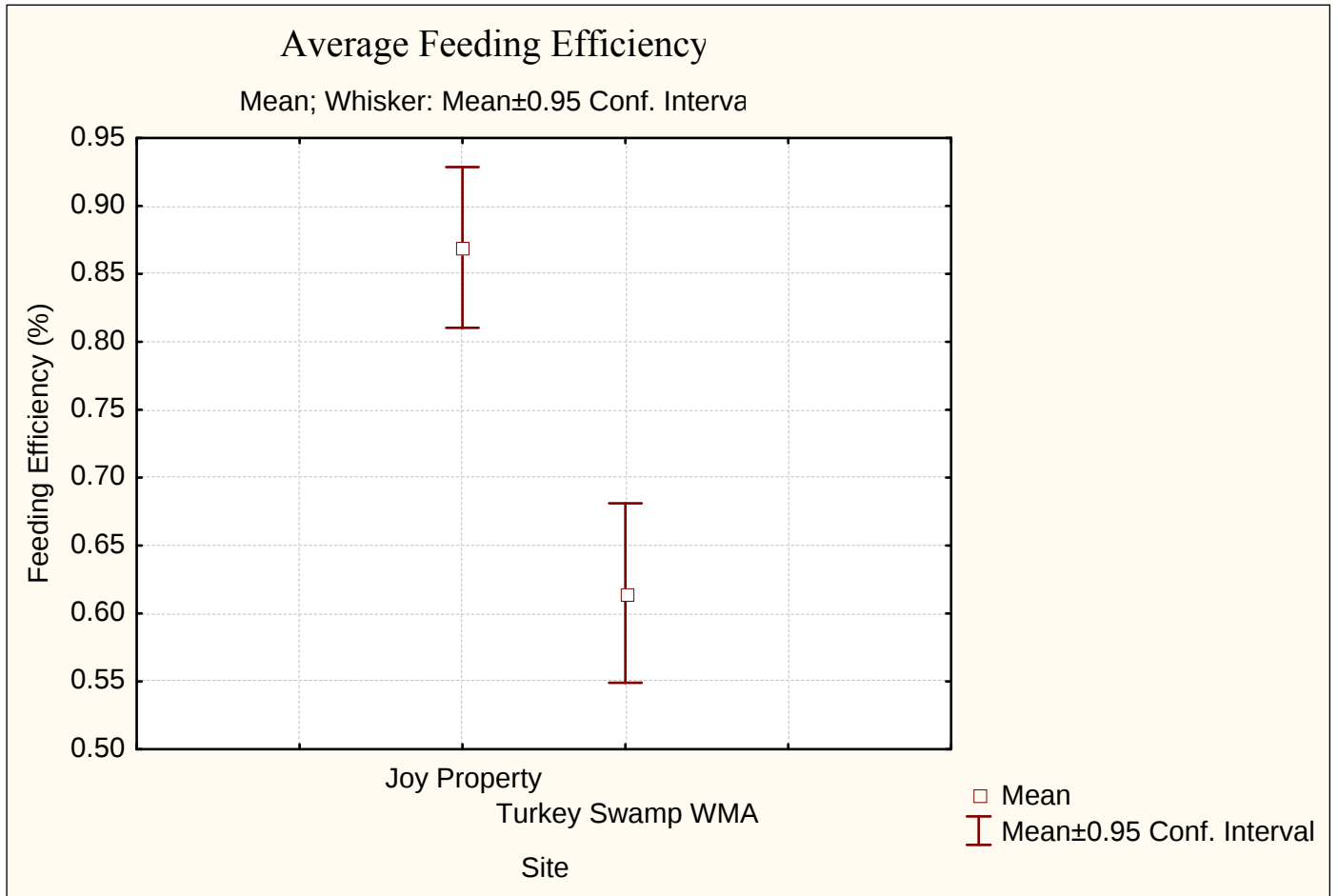
**\*\*\*\*** Background levels for lead vary widely. Average levels in undeveloped, rural areas may range from 4-61 ppm. Average background levels in metropolitan or suburban areas or near highways are much higher and typically range from 200-500 ppm.

| <b>Metal</b> | <b>Eastern USA Background (PPM)</b> | <b>TS-Sediment 1</b> | <b>TS-Sediment 2</b> | <b>TS-Water</b> | <b>TS Frog 1</b> | <b>TS Frog 2</b> | <b>TS Frog 3</b> | <b>TS Frog 4</b> | <b>TS Frog 5</b> |
|--------------|-------------------------------------|----------------------|----------------------|-----------------|------------------|------------------|------------------|------------------|------------------|
| <b>Cr</b>    | 1.5-40**                            | 122.00               | 528.00               | 0.00            | 1.43             | 1.17             | 0.85             | 0.86             | 1.05             |
| <b>Mn</b>    | 50-5,000                            | 109.00               | 40.00                | 0.00            | 8.39             | 16.59            | 12.52            | 14.21            | 4.95             |
| <b>Co</b>    | 2.5-60                              | 3.00                 | 2.00                 | 0.00            | 0.08             | 0.08             | 0.09             | 0.07             | 0.08             |
| <b>Ni</b>    | 0.5-25                              | 10.00                | 8.00                 | 0.00            | 1.74             | 1.96             | 2.46             | 2.35             | 2.02             |
| <b>Cu</b>    | 1-50                                | 28.00                | 13.00                | 0.00            | 24.92            | 15.37            | 26.25            | 17.85            | 7.72             |
| <b>Zn</b>    | 9-50                                | 90.00                | 65.00                | 0.01            | 65.40            | 76.47            | 67.95            | 62.37            | 90.60            |
| <b>As</b>    | 3-12**                              | 16.00                | 70.90                | 0.01            | 0.34             | 0.49             | 0.30             | 0.40             | 0.69             |
| <b>Cd</b>    | 0.1-1                               | 0.80                 | 0.50                 | 0.00            | 0.34             | 0.46             | 0.50             | 0.24             | 0.21             |
| <b>Ti</b>    | -                                   | 0.20                 | 0.30                 | 0.00            | 0.04             | 0.09             | 0.06             | 0.05             | 0.02             |
| <b>Pb</b>    | ****                                | 74.00                | 35.00                | -0.03           | 0.83             | 0.54             | 4.00             | 5.16             | 1.75             |
| <b>U</b>     | -                                   | 1.70                 | 3.50                 | -               | 0.02             | 0.00             | 0.00             | 0.06             | 0.02             |
| <b>Fe</b>    | 2,000-5,000                         | -                    | -                    | 2.17            | 1.82             | 1.93             | 1.97             | 1.91             | 1.67             |
| <b>Al</b>    | 33,000                              | -                    | -                    | 0.04            | -8.04            | 1.55             | -13.95           | -14.17           | -13.41           |

Both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were not sensitive enough to detect the small amount of metal present in the frog tissue. Both methods yielded no significant difference in elements present in tissue regardless of site. Further compounding the difficulty with TEM analysis was the inability to stain the tissue without risk of masking any minute amount of element present. This made capturing viable images impossible. Unfortunately, while time consuming, no usable information was acquired from either method.

Efficient prey capture was calculated by averaging feeding efficiency across three days with successful prey capture being used as an indicator of frog health. Frogs from the Joy property consumed crickets significantly more efficiently than frogs from Turkey Swamp WMA ( $p < 0.001$ ; Figure 2).

**Figure 2.** Average feeding efficiency (%) for frogs from both sites.



### ***Significance -***

Amphibians have permeable skin that has the potential to absorb harmful substances from their environment. Responses to these pollutants may be intensified by stressors present in their surroundings. Exposure to both stressor and pollutant are not limited to adulthood, but rather are continuous throughout their lifetime due to the unique nature of the amphibian lifecycle. The effects of such exposure may not be immediately lethal, but rather may result in sublethal responses. Decreased feeding efficiency is one such response. Preliminary findings in this study demonstrate correlations of at least one environmental stressor, low levels of dissolved oxygen (DO) in pond water critical for survival of eggs and tadpoles, with decreased feeding efficiency.

While no significant difference is immediately apparent between UVB readings from each site, previous studies have found UVB intensity readings as low as 93  $\mu\text{W}/\text{cm}^2$  to contribute to 93% mortality of larval amphibians [26]. While most intensity readings collected from both sites were two to three times greater than this value, vegetation cover and oviposition depth could minimize UVB impacts to both amphibian embryos and larvae. Limited cover from UVB radiation has been associated with developmental abnormalities such as delayed development and limb deformities.

Further analysis of correlations between metals present in the soil and sediment at each of these sites with feeding efficiency is anticipated to reveal similar relationships. In addition, continued analysis of the data is necessary to determine if any synergistic interactions are evident between abiotic factors and feeding efficiency.

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# Assessment of the water-borne pathogen, Batrachochytrium dendrobatidis, in New Jersey amphibians and their habitat

## Basic Information

|                                 |                                                                                                                    |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Assessment of the water-borne pathogen, Batrachochytrium dendrobatidis, in New Jersey amphibians and their habitat |
| <b>Project Number:</b>          | 2011NJ277B                                                                                                         |
| <b>Start Date:</b>              | 3/1/2011                                                                                                           |
| <b>End Date:</b>                | 2/29/2012                                                                                                          |
| <b>Funding Source:</b>          | 104B                                                                                                               |
| <b>Congressional District:</b>  | NJ-008                                                                                                             |
| <b>Research Category:</b>       | Ecological Processes                                                                                               |
| <b>Focus Category:</b>          | Ecology, Conservation, Invasive Species                                                                            |
| <b>Descriptors:</b>             | None                                                                                                               |
| <b>Principal Investigators:</b> | Paola Dolcemascolo, Kirsten Monsen                                                                                 |

## Publication

1. Monsen-Collar, Kirsten; Lisa Hazard; Paola Dolcemascolo. 2011. First Report of Ranavirus in New Jersey Amphibians. Northeast Partners in Reptile and Amphibian Conservation annual meeting, Millersville, MD, August 17-18, 2011. (poster presentation)

## **Project Summary:**

**Problem and Research Objectives** – The initial problem was to understand the prevalence and intensity of the water-borne fungus *Batrachochytrium dendrobatidis* (Bd) in New Jersey amphibians. The objectives related to addressing this problem were two-fold. **First**, to correlate water and air temperature to the prevalence and intensity of Bd. Other studies in the field and in the lab have shown that Bd is sensitive to both water and air temperature. No such study has been done in New Jersey. Our lab is the first lab to document Bd in the state (Monsen-Collar *et al.* 2010) and preliminary results show that Bd prevalence may be higher during cooler months. **Second**, to relate biotic factors to prevalence and intensity of Bd. I anticipated species-specific responses to Bd, with some species exhibiting more susceptibility to both infection and disease symptoms. Areas with significant populations of Bd-resistant organisms, such as bullfrogs and tiger salamanders, would have a higher prevalence of Bd.

While sampling for Bd and not finding any amphibians exhibiting symptoms of even a mild Bd infection, we were alerted to the presence of great quantities of dead tadpoles in Ocean County, NJ; tadpoles exhibited symptoms consistent with a Ranavirus disease. The site is being managed by Herpetological Associates, Inc. (Executive Director, Robert T. Zappalorti) for the endangered pine snake (*Pituophis melanoleucus*) and also harbors a population of Pine Barrens Treefrogs (*Hyla andersonii*). The Ranavirus disease is even more lethal than that caused by the Bd fungus, typically killing amphibians within a few days to a week of infection (Harp and Patrenka 2006). The presence of Ranavirus had never been documented in New Jersey prior to this occasion, and so we decided to focus our efforts on confirming, via molecular analyses, that the diseased tadpoles were indeed infected with Ranavirus and attempting to track the spread of the disease through the state.

**Methodology** – Animals were sampled from five ponds located in the vicinity of the Stafford Business Park, in Ocean County, NJ, within an area that is being managed for the benefit of pine snake (*Pituophis melanoleucus*) populations. Because of the mass mortality and severity of the disease outbreak, samples were collected from throughout the state of New Jersey and tested for Ranavirus in much the same way samples were collected from Ocean County. Other sites included Assunpink Wildlife Management Area, Green Creek and Lizard Tail Swamp (Middle Township), Tuckahoe pond (Upper Township), Great Swamp National Wildlife Refuge, Berkshire Valley Wildlife Management Area, Wanaque Wildlife Management Area, Weiss Ecology Center, Norvin Green State Forest and the NJ School of Conservation. Sites were visited multiple times.

At the site of mass amphibian mortality in Ocean County, dead and dying *Lithobates clamitans* tadpoles were collected by net, transported to the lab at Montclair State University on ice, and then frozen at -20° C for 24 hours before DNA extraction. A small piece of tail tissue was removed from dead tadpoles for DNA extraction using scissors sterilized in 95% EtOH. All asymptomatic *Anaxyrus fowleri* tadpoles were placed in 1.5 ml Eppendorf tubes and allowed to “swim” in the tube for approximately 30 seconds. These asymptomatic tadpoles were released back to the pond and the water remaining in the Eppendorf tubes they had been in were processed for DNA extraction.

Symptomatic *A. fowleri* tadpoles were collected in the same manner as symptomatic *L. clamitans* tadpoles. Entire *A. fowleri* metamorphs were collected in 1.5 mL Eppendorf tubes and frozen at -20° C until DNA extraction. A dead snapping turtle (*Chelydra serpentina*) tail as well as shed skin from a Northern water snake (*Nerodia sipedon*) and toe clips from adult Southern Leopard frogs (*Lithobates sphenoccephala*) and Green frogs (*L. clamitans*) were also collected. DNA was extracted from tissue using the Qiagen QIAamp DNA Mini Kit following the manufacturer's instructions. Tadpole water was processed for asymptomatic *A. fowleri* tadpoles, the entire tadpole for symptomatic *A. fowleri* tadpoles, a piece of tadpole tail from *L. clamitans*, and a rear leg from *A. fowleri* metamorphs. The DNA extraction isolated amphibian DNA as well as any viral or bacterial DNA on or in the tissue. DNA was also extracted from the dead *C. serpentina* tail tissue and the shed skin from *N. sipedon*.

**Traditional PCR-** Ranavirus-specific primers MCP4 and MCP5 designed by Mao *et al.* (1997) were used to amplify an approximately 530 bp fragment of the Ranavirus major capsid protein gene. We used 2 µl of DNA in 25 µl reactions that included the following components: 0.4 µM forward and reverse primers, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, and 0.1 U/µl taq polymerase. PCR reactions were run under the following conditions: 1 cycle of 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, followed by a final 7-min extension at 72°C. All reactions were run in a GeneAmp 9700 Thermalcycler (Applied Biosystems). Appropriate negative controls using water in place of DNA were included with each PCR run. PCR products were checked for the presence of the Ranavirus-specific fragment on 2% 1XTAE gels by SYBR Safe (Invitrogen) staining and UV illumination.

**RT-PCR-** RT-PCR was used to screen each *A. fowleri* DNA sample that came up negative for Ranavirus using traditional PCR. We used 2 µl of DNA in 25-µl RT-PCR reactions that included the following components: 0.4 µM forward and reverse primers, 12.5 µl of Brilliant II SYBR Green qPCR Master Mix (Agilent Technologies), and H<sub>2</sub>O to a final volume of 25 µl. Positive control reactions were run using 2 µl of *L. clamitans* tadpole DNA that tested positive for Ranavirus using traditional PCR and negative control reactions using water in place of DNA under the same conditions. PCR reactions were subjected to 1 cycle of 95°C for 10 min followed by 40 cycles of 95°C for 45 sec, 50°C for 30 sec, and 72°C for 30 sec. All RT-PCR reactions were run on a Stratagene Model Mx 3000P Thermalcycler (Stratagene Technologies). All positive samples were re-screened in triplicate using RT-PCR to verify the results.

**Principal Findings and Significance** – Tadpoles at the Ocean County site displayed signs of Ranaviral disease including lethargy, swelling, and red skin lesions. Disturbingly, *Anaxyrus fowleri* tadpoles sharing the same body of water with the infected tadpoles were observed consuming the carcasses of dead *L. clamitans* tadpoles.

PCR results: Of the 27 *L. clamitans* samples from the Ocean County site that were subjected to traditional PCR, 20 (all tadpoles) tested positive for the presence of Ranavirus DNA. Results were confirmed by sequencing. Of the 38 *A. fowleri* tadpoles collected, three tested positive for Ranavirus using traditional PCR; after rerunning these samples with RT-PCR one additional sample came up positive for Ranavirus. One adult *L. sphenoccephala*, which was found dead on site, tested positive for Ranavirus using

traditional PCR. With regards to the other sites throughout NJ, one *A. fowleri* tadpole from Tuckahoe pond in Upper Township has tested positive for Ranavirus.

To our knowledge, this is the first reported case of Ranavirus in the state of New Jersey. What is disturbing is the significant level of mortality associated with an outbreak of Ranavirus and the rapidity with which it spreads through a population. Furthermore, at this time it seems as if Ranavirus is located in the southern part of NJ, which is home to numerous amphibians and reptiles of special concern, such as the Pine snake, the Pine Barrens Treefrog and the Southern Gray Treefrog (*Hyla chrysoscelis*). Ranavirus has been known to affect a wide variety of amphibians and reptiles, and so such a deadly disease could pose a significant threat to NJ's herpetofauna. Additionally, results from this work have shown that while traditional PCR is apparently effective at detecting Ranavirus in heavily infected individuals (such as the dead and dying tadpoles at the Ocean County site), its sensitivity may be insufficient for detecting Ranavirus during the initial stages of infection. Indeed, one sample that came up negative using traditional PCR then tested positive when RT-PCR was used.

This work is ongoing and sites visited in 2011 will be re-visited to track the progression of the disease over multiple years and seasons. Results are being shared with NJ Division of Fish and Wildlife to implement wide-scale sampling for Ranavirus throughout the state and to create educational materials for wildlife managers in order to help document outbreaks and contain the spread of the disease.

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# Advancing the characterization of sandstone and fractured limestone aquifers using surface electrical geophysical methods: application to water resources evaluation at two sites in New Jersey

## Basic Information

|                                 |                                                                                                                                                                                                 |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Advancing the characterization of sandstone and fractured limestone aquifers using surface electrical geophysical methods: application to water resources evaluation at two sites in New Jersey |
| <b>Project Number:</b>          | 2011NJ278B                                                                                                                                                                                      |
| <b>Start Date:</b>              | 3/1/2011                                                                                                                                                                                        |
| <b>End Date:</b>                | 2/29/2012                                                                                                                                                                                       |
| <b>Funding Source:</b>          | 104B                                                                                                                                                                                            |
| <b>Congressional District:</b>  | NJ-010                                                                                                                                                                                          |
| <b>Research Category:</b>       | Ground-water Flow and Transport                                                                                                                                                                 |
| <b>Focus Category:</b>          | Methods, Hydrology, Water Quantity                                                                                                                                                              |
| <b>Descriptors:</b>             | None                                                                                                                                                                                            |
| <b>Principal Investigators:</b> | Mehrez Elwaseif, Lee Slater                                                                                                                                                                     |

## Publications

There are no publications.

## **Abstract**

The primary objective of this research is to advance surface Ground Penetrating Radar (GPR) and Electrical Resistivity (ER) processing techniques for characterizing sandstone and limestone subsurface aquifers at two sites in New Jersey. Our research explores the use of time – frequency analysis to process GPR data in order to better characterize sandstone and limestone aquifers. We have applied three popular time frequency analysis tools (short time Fourier Transform, Wavelet Transform and S-Transform) on a synthetic non-stationary signal to identify the optimum approach to use for the GPR processing. In addition, we have tested the performance of our suggested approach, S-Transform, on synthetic GPR data representing variety of fractured limestone and sandstone model scenarios. The S-Transform results show that a frequency shift occurs when the GPR signals transmit through mediums that have different dielectric permittivity values. Furthermore, our developed approach facilitates mapping particular targets of interest (e.g. water filled fractures) based on their observed frequency shift, and ignoring all the remaining features within the data. We also describe a resistivity inversion strategy that is more appropriate for defining layers and for reconstructing the conductivity distribution within those layers.

## **1. Introduction**

Mapping the soil moisture content with direct methods is time consuming, invasive and has a limited subsurface spatial sampling density. In addition, borehole geophysics is expensive and often requires a network of boreholes, which is not available in most field sites. On the other hand, quantifying the moisture content with surface geophysical methods, such as ER and GPR, is often difficult as it requires a detailed knowledge about the variations of water distribution and petrophysical parameters of subsurface materials (e.g., Deiana et al., 2008). Empirical relationships between geophysical parameters (such as dielectric permittivity and electrical resistivity) and changes in hydrological parameters such as Volumetric Water Content (VWC) are used for calibrating hydrologic flow and transport models. The objectives of the research were: (1) developing a time-frequency analysis technique for GPR data sets to predict the changes in the amplitude of GPR signals associated with the subsurface aquifers, which subsequently can be used to estimate the VWC using a derived empirical relationship based from the proposed laboratory experiments, (2) developing an inversion code that is more appropriate for predicting boundaries between electrical layers and also solves for the optimum resistivity distribution inside those individual layers, (3) applying the developed approaches on data collected at two sites in NJ, which contains sandstone and fracture-dominated systems. A time frequency analysis tool, based on S-Transform, which is efficient in estimating frequency shift and amplitude of non-stationary signals, and mapping particular features of interest was developed. In addition, a Matlab based 3D resistivity inversion strategy that is more appropriate for inverting hydrogeophysics data was developed by modifying an existing code available for academic use. We still plan to run the lab experiments and apply the proposed approaches on field data.

### **3. Geophysical methods**

#### **3.1 Electrical Resistivity**

The resistivity method is used to predict buried targets that have a detectable electrical resistivity contrast relative to the host medium. The success of the method in imaging targets is based on employing both an appropriate data collection setup and an appropriate inversion strategy honoring the characteristics of the subsurface environment. The data collection setup should take into account a number of parameters such as subsurface geology, depth and dimensions of investigated targets (e.g., Loke, 2004). Such parameters would determine the amount of injected current to the ground, array configuration and electrode spacing. The choice of an appropriate inversion strategy is based upon the availability of *a priori* subsurface information from geological logs or information from other geophysical methods, along with the characteristics of the edge of suspected targets [i.e., diffuse vs. sharp]. In the absence of *a priori* subsurface information on the geometry and distribution of buried targets, the smoothness constraint inversion is utilized to produce smooth resistivity images that represent the minimal structure required to satisfy the data.

#### **3.2 GPR method**

The GPR method is a high resolution, non-invasive near-surface geophysical imaging technique, routinely employed to obtain an image of subsurface structures and objects having detectable dielectric permittivity contrast relative to a host medium. The success of the method in imaging targets, assuming the applicability of the low loss assumption, is based upon the frequency of the used antennae, physical properties of subsurface materials, the depth and dimensions of the investigated targets (e.g., Annan, 2006; Franseen et al., 2007). When the spacing between consecutive targets is below the minimum resolution of GPR, significant scattering of electromagnetic waves occurs. Furthermore, the reflected energy from individual fractures stacks together such that those targets will not be individually resolved as discrete reflected pulses in the time series. Studying the spectral content of the GPR time series potentially can help in characterizing targets separated by distances smaller than the tuning thickness.

### **4. Description of the proposed approaches**

#### **4.1 GPR data**

The GPR method has been extensively applied to characterize fracture density, orientation and pore-fluid properties (e.g., Seol et al., 2004; Liu, 2006). The minimum vertical resolution of GPR commonly is approximated as being equal to one-quarter wavelength of the transmitted signal ( $z_{min}$ ) and is a function of both antenna frequency and dielectric permittivity of subsurface materials (e.g., Zeng, 2009). Studying the spectral content (i.e., changes of amplitude, phase, and frequency

content of a signal with time) of the GPR time series potentially can help in characterizing targets separated by distances smaller than  $z_{min}$ . Such an approach has been applied extensively to seismic data sets (e.g., McIvor, 1964; Dilay and Eastwood, 1995).

#### **4.1.1. Short time Fourier Transform (STFT) vs. Wavelet Transform (WT) vs. S-Transform (ST)**

To better identify the optimum approach to perform the time-frequency analysis for GPR data, we have created a synthetic signal and applied the STFT, WT and ST techniques on it in order to demonstrate recovery of the frequency content and amplitude of that signal. The signal is non-stationary, where its frequency and amplitude changes with time. It has four different frequencies of 25, 50, 100, and 120 Hz, and is characterized by four different amplitude values of 0.5, 1, 1.5 and 2 (Figure 1a). The results of applying the three approaches on the signal are shown in Figure (1). The performance of the STFT method depends on the window length (user defined), where different time lengths gives different resolution. As shown in Figure (1b), although the STFT approach captures the correct frequency content of the signal, it overestimates the length of the shortest signal in time (probably due to using large time widow); in addition it does not recover the amplitude of the signal accurately. In contrast to the STFT, the WT determines the optimum window length automatically. However, the method tends to attenuate high frequency signals, meaning the recovered amplitude information of the signal is meaningless as shown in Figure (1c). The S-transform (Figure 1d) gives the optimum resolution in terms of recovering both the frequency content and amplitude of the signal.

#### **4.1.2 Characterizing fractured-limestone aquifers using the S-Transform**

Elwaseif et al. (2010) propose applying the S-Transform to GPR traces in order to characterize fracture density and specify the pore fluid content of fractures. Figure (2) shows 1D fracture models, with fractures spaced within each model at distances both considerably greater than  $z_{min}$  and less than  $z_{min}$ . In the first model, all fractures were assumed to be filled with air, whereas they are assumed to be filled with water in the second model. On the other hand, the fractures in the third model are filled with both air (widely spaced set) and water (closely spaced set). All individual fractures have a length of 0.25 m and a width of 0.01 m. The results of applying the S-Transform technique on the three models are shown in Figure (3). The S-Transform sections of the air-air and water-water fracture models do not show a frequency shift between the widely spaced and closely spaced fractures, indicating that spacing between fractures has little effect on the frequency shift of radar signals. On the other hand, the S-Transform section of the air-water model clearly shows a frequency shift between the two fracture sets, indicating that the dielectric permittivity contrast has a significant effect on changing the frequency content of transmitted radar signals.

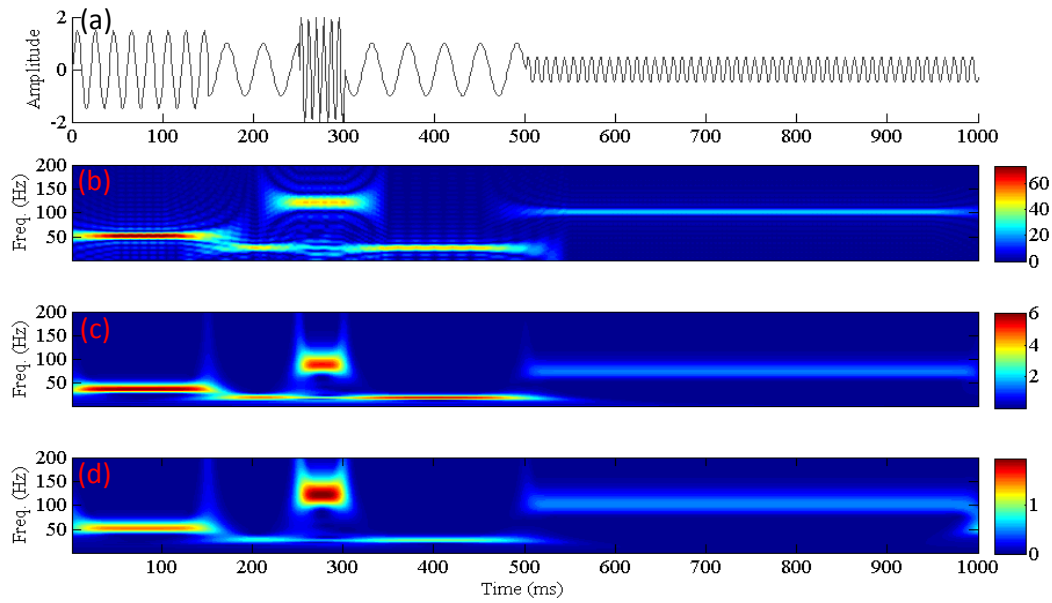


Figure 1: Time – Frequency analysis for a synthetic signal (a) time series, (b) STFT section, (c) WT section and (d) ST section

Figure (4) shows the results of applying the S-Transform on a 2D fracture model (Figure 4a). The migrated radargram of the synthetic model is shown in Figure 4b. As expected, the first four sets of widely spaced fractures were resolved better than the fifth fracture groups. In contrast, resolution of individual fractures as distinct events in the time domain becomes increasingly challenging as the fracture density increases, as a result of the constructive interference between reflected wavelets from multiple reflection events. A useful technique for displaying variations in the S-transform results across 2D space is to plot variations in amplitude for a specific frequency of interest identified in the frequency spectrum of the 1D S-transform. For this model, the 1D S-transform analysis revealed that water-filled fractures exhibit peak amplitudes in the frequency spectrum at a frequency of approximately 300 MHz (Fig. 3c). The plot of the amplitude of the S-transform spectrum across the 2D model space at a frequency of 300 MHz is shown in Figure 4c. As expected, known water-filled fractures show higher amplitudes than do air-filled fractures.

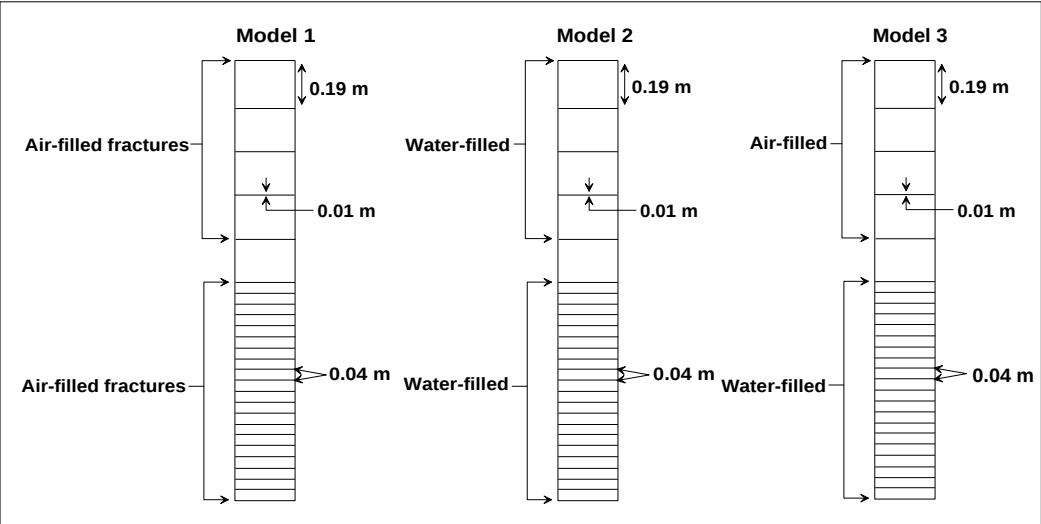


Figure 2: Synthetic subsurface 1D fracture models examined in this study

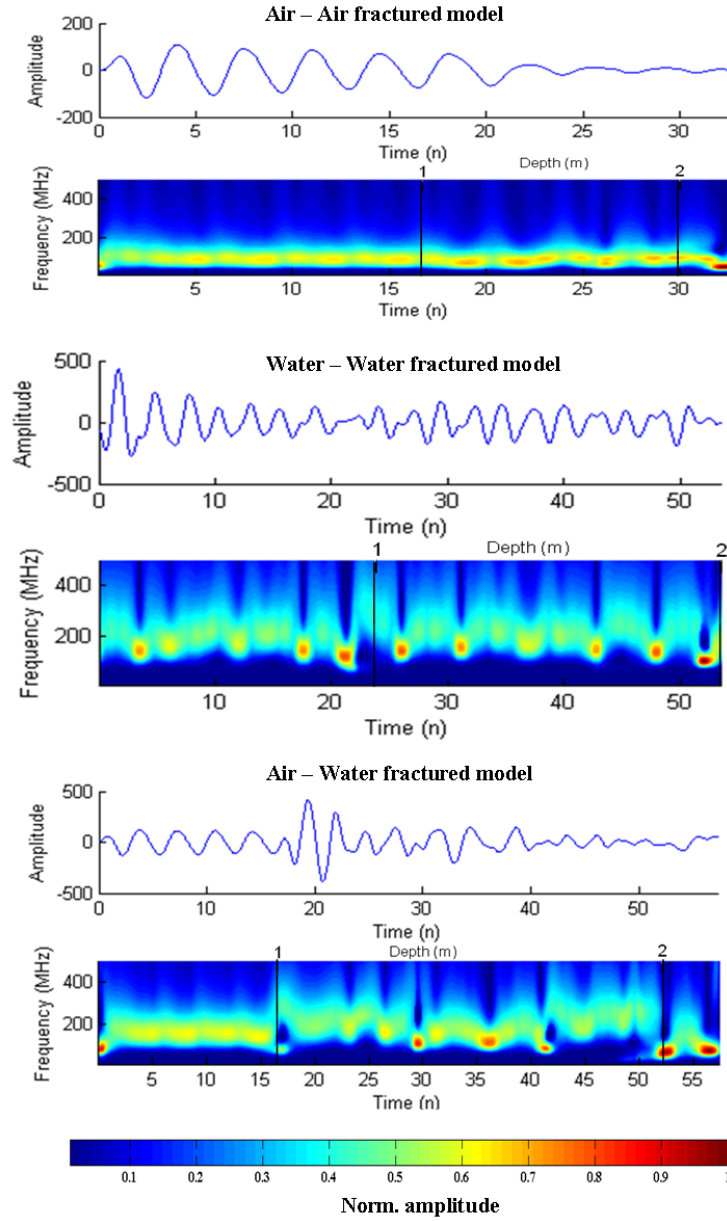


Figure 3: S-Transform analyses for the 1D fracture models: (a) and (b) time series and S-Transform section for model 1, (c) and (d) time series and S-Transform section for model 2, (e) and (f) time series and S-Transform section for model 3.

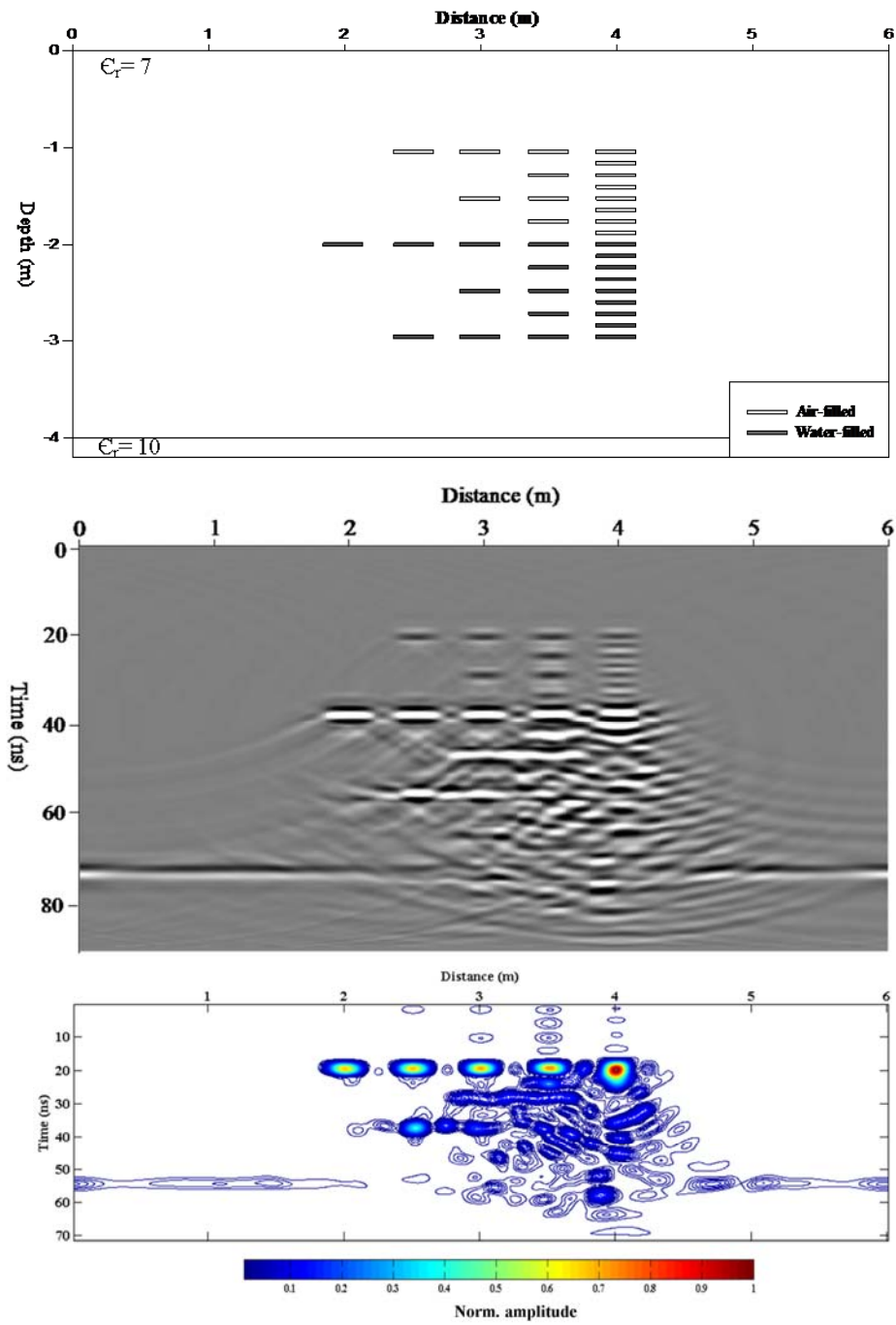


Figure 4: Results of applying S-Transform to the 2-D fracture model for a 300 MHz frequency (a) Synthetic model (b) Migrated radargram (c) 2D S-Transform section. The water-filled fractures (showing high amplitudes) are distinguished from the air-filled fractures.

### 4.1.3 Characterizing sandstone aquifers using the S-Transform

Figure (5) shows 1D synthetic sandstone models, having different degrees of water saturation and containing simulated clay lenses. This model was selected to examine the potential of the S-transform approach for identifying changes in lithology and degree of water saturation. Each model consists of dry sandstone and wet sandstone layers each having a thickness of 2 m, followed by a clay layer at a depth of 4 m. In all models, the dry and wet sandstone zones, and the clay layer are assigned dielectric permittivity value ( $\epsilon_r$ ) of 6, 15 and 20. In addition, to simulate real conditions, the clay lens in the dry sandstone is assigned an  $\epsilon_r = 10$ , whereas  $\epsilon_r = 20$  in the wet sandstone zone. The dry and wet sandstone zones in the first model are clean (i.e., clay free). The second model contains a 0.2 m thick clay lens only in the wet sandstone zone, whereas the third model (Model A3) has the same clay lens in both dry and wet sandstone zones. The results of applying the S-Transform on the three models are shown in Figure six. As expected, the resulting time series of all models (Figures 6a, 6c and 6e) exhibits distinct reflections at the interface between the dry and wet sandstone zones. In addition, the clay lenses show distinct reflections in the dry sandstone at time  $\sim 17$  ns and in the wet sandstone at time  $\sim 58$  ns (e.g., Figure 6e). However, the magnitude of the reflections resulting from the clay lenses is similar to that observed at the boundary between the dry and wet sandstone zones (e.g., Figure 6c), since they both have close reflection coefficients. That similarity might cause ambiguity in the interpretation of time series data, e.g. when observing lithology changes or locating the depth to water table. The clay layer at depth 4 m exhibits distinct reflections in the clean sandstone model at time  $\sim 80$  ns, but it progressively exhibits less distinct reflections in other models since clay lenses cause more energy loss.

The S-Transform sections of all models show a strong local increase in the frequency content of the radar signals at the transition between the dry sandstone and the wet sandstone zones at time 31 ns, whereas the transition between the wet sandstone and the clay layer at approximate time of 81 ns shows a local decrease in frequency content due to the significant  $\epsilon_r$  contrast between these zones (e.g., Benedetto, 2010; Elwaseif et al., 2010). Furthermore, the resolution of the frequency content across the wet sandstone zone is better than the dry sandstone zone, since more energy is reflected back from that wet zone. Figure 6b shows the results of S-transform analysis for Model A1 (clean sandstone model). The frequency content across the dry sandstone and wet sandstone zones shows little variations especially at the beginning of the dry sandstone zone probably due to modeling errors and noise in the data. Similarly, the amplitude of the radar signals across both dry and wet sandstone zones is nearly constant but higher in the wet sandstone zone since it has a higher  $\epsilon_r$  value.

Figure 6d shows the S-transform analysis for model A2. Similar to what is observed in Model A1, the frequency content and the amplitude of the radar signals throughout the dry sandstone zone shows little variations and a higher amplitude is observed across the wet sandstone zone. Additionally, between approximate arrival times of 58 to 65 ns, the frequency content and amplitude of the radar signals across the wet sandstone zone shows a local decrease most likely due to the clay lens in this zone (20 cm thick). We notice that the predicted location of the clay lens on the S-transform section in Figure 2d do not occur at the exact arrival times (i.e., between 58 to 61 ns

based from the real model); this is most likely due to contamination of the S-transform frequency spectrum by noise.

Figure 6f show the S-transform analysis for model A3. The frequency content of the radar signals throughout the dry sandstone zone shows a local increase between times 15 to 25 ns, whereas the amplitude shows a local decrease between times of 14 to 18 ns as a result of the clay lens in this zone (20 cm thick). Similarly, the frequency content and amplitude of the radar signals throughout the wet sandstone zone shows a local decrease approximately between 58 to 65 ns because of the clay lens in this zone (20 cm thick). The frequency content at the approximate location of the clay lens within the dry sandstone zone shows significant distortions, presumably due to less Rayleigh scattering in this zone compared to the wet sandstone zone.

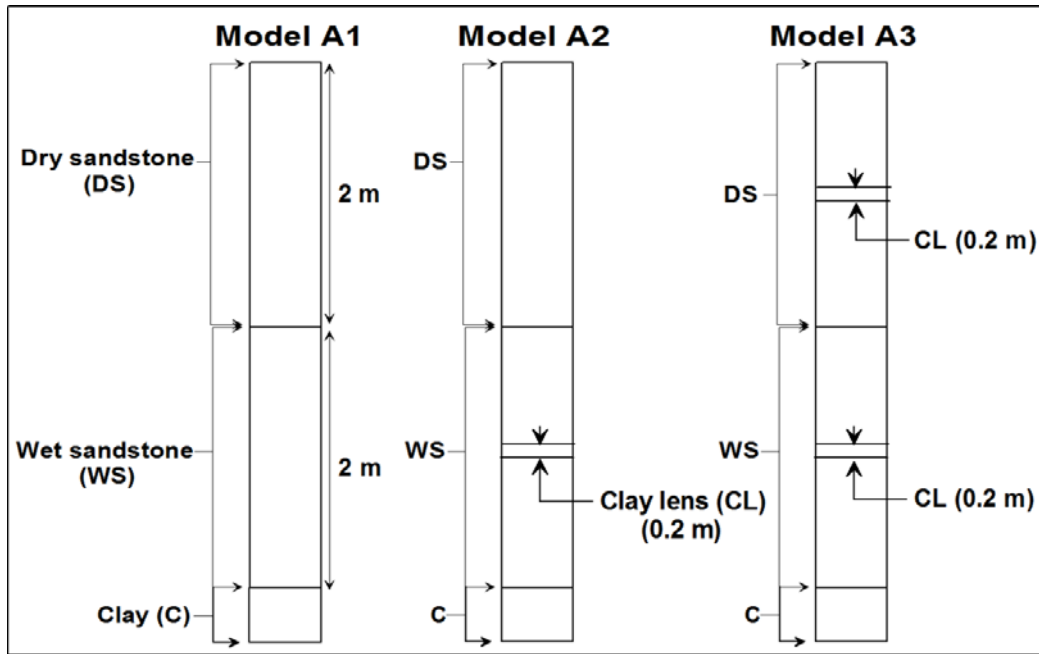


Figure 5: Subsurface 1D sandstone models.

#### 4.2. Resistivity inversion for hydrogeophysical data

The smoothness constraint inversion is not appropriate for inverting hydrogeophysical data, especially when there is a sharp change in resistivity between subsurface layers. Recent inversion approaches requires either *a priori* information about the subsurface (e.g. disconnect inversion) or requires two or more geophysical datasets to be collected at the same site (e.g. joint inversion). We propose applying an approach that: [i] doesn't require *a priori* information or other geophysical data, and [ii] is more appropriate for defining sharp boundaries and for reconstructing the conductivity distribution within those boundaries. Our approach combines: [i] after each iteration, an edge detector method is applied to the produced trial model in order to predict edges within that model, and [ii] the roughness matrix is then updated such that the model space consists of regions with different weights. The inversion can be adjusted

to produce smooth or rough conductivity distributions within each region through giving different regularization parameters for each region. This approach has been applied recently on archaeological applications (Elwaseif and Slater, 2012), and it appears to work best when the subsurface consists of different electrical units or contains sharp boundary targets.

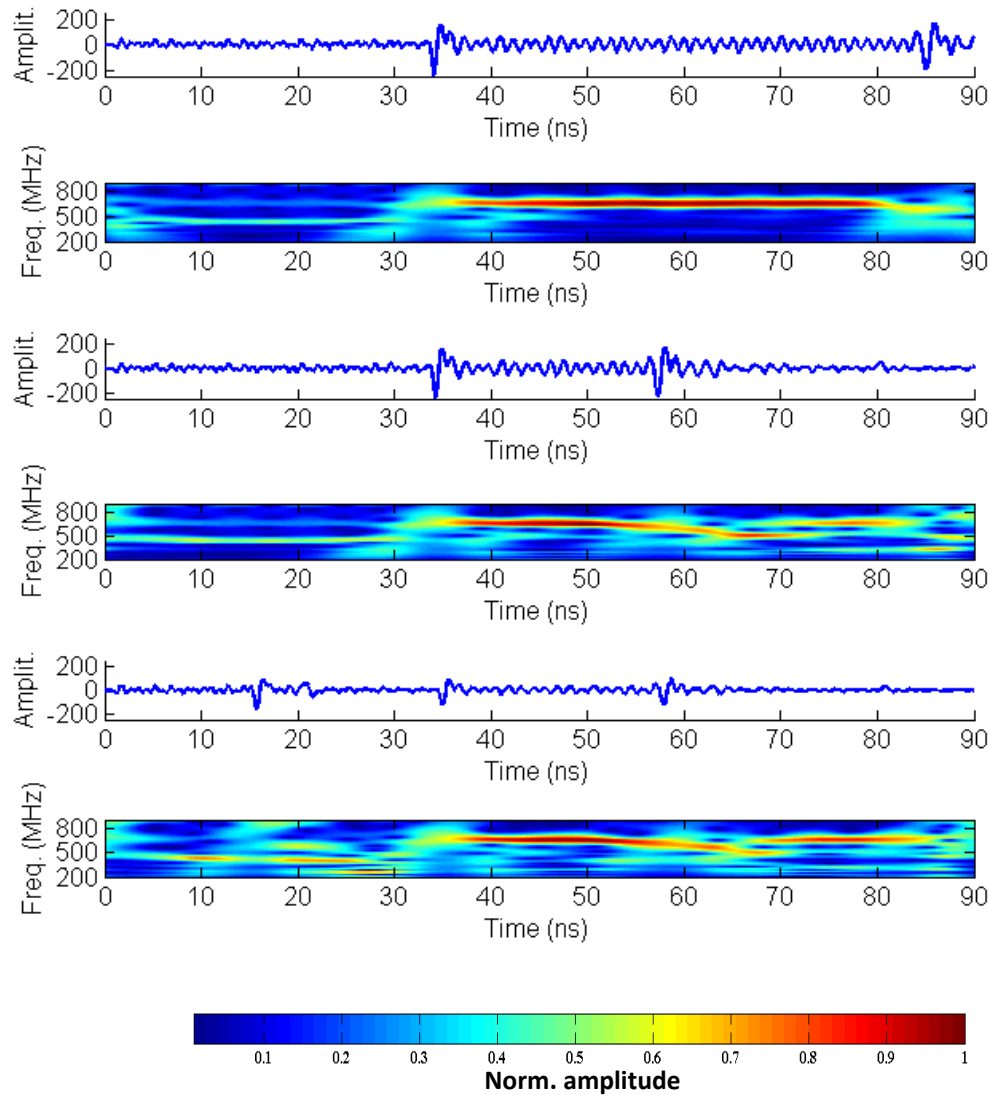


Figure 6: S-Transform analyses for the 1D sandstone models (Model A): (a) and (b) timeseries and S-Transform section for model A1, (c) and (d) timeseries and S-Transform section for model A2, (e) and (f) timeseries and S-Transform section for model A3.

## Conclusions

The proposed GPR and resistivity processing strategies appear theoretically suitable to achieve the remaining objective of this project. Based on the synthetic studies, processing GPR data using time-frequency analysis improves the imaging of particular target of interest, in addition it could help to distinguish between features that shows close amplitude values in the time domain. The results presented encourage performing the planned GPR lab experiments and applying the approach on field data. The proposed resistivity strategy could be applied as well to wide range of applications, such as in engineering geophysics, where targets with sharp resistivity contrasts are suspected.

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# Identification and Characterization of Novel Antibiotic Resistance Genes from Wastewater Effluents and Surface Waters

## Basic Information

|                                 |                                                                                                                       |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Identification and Characterization of Novel Antibiotic Resistance Genes from Wastewater Effluents and Surface Waters |
| <b>Project Number:</b>          | 2011NJ279B                                                                                                            |
| <b>Start Date:</b>              | 3/1/2011                                                                                                              |
| <b>End Date:</b>                | 2/29/2012                                                                                                             |
| <b>Funding Source:</b>          | 104B                                                                                                                  |
| <b>Congressional District:</b>  | NJ-006                                                                                                                |
| <b>Research Category:</b>       | Water Quality                                                                                                         |
| <b>Focus Category:</b>          | Water Quality, Wastewater, None                                                                                       |
| <b>Descriptors:</b>             | None                                                                                                                  |
| <b>Principal Investigators:</b> | Maryam Honarbakhsh, Elisabetta Bini                                                                                   |

## Publications

1. Honarbakhsh M., Z. Rosario-Cruz, C. Zhu, D. Sannino, and E. Bini. Comparative analysis of structure and 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)
2. Honarbakhsh, Maryam. 2011. Impact of wastewater effluents on the structure and antibiotic resistance of native microbial communities, "MS Dissertation," Department of Biochemistry and Microbiology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 58 pages.

## **Project summary**

### **Problem and Research Objective**

The proposed project fits within a broader study currently carried out in the lab, and is concerned with the impact of antibiotic pollution on the establishment of emerging diseases. The rationale for the proposed work is provided by preliminary data that I have generated during the preparation of my master thesis. I have compared the structure of microbial communities at different sites upstream and downstream the Somerset Raritan Valley Sewage Authority in New Jersey. I have also investigated the occurrence of antibiotic resistance in bacterial isolates from the final effluents of the plant, and from two locations downstream the sewage discharge. All the isolates resulted resistant to elevated concentrations of amoxicillin, regardless of the site of sampling, and many showed azithromycin and trimethoprim resistance. Based on these results, I made the hypothesis that resistance to specific antibiotics observed downstream the discharge is due to lateral transfer of resistance genes from wastewater effluents to freshwater bacteria. Alternatively, resistance might have derived from the selection of pre-existing resistant genes in the native community as a consequence of prolonged exposure to antibiotic pollutants. To distinguish between these two hypotheses, gene sequences would be analyzed and compared to establish if antibiotic resistance is mediated by lateral gene transfer (sequences have a common origin), or if it emerged by selection of rare pre-existing resistant genes in the native community (multiple or polyphyletic origins). To enable the detection of unknown and novel antibiotic resistance genes encoded by plasmids or chromosome encoded, I proposed the following approach:

*Aim #1. Detection of antibiotics resistant genes encoded by plasmids.* Plasmids of cultured microorganisms and metagenomic DNA isolated from the WTP effluents and the surrounding area were targeted for sequencing to detect novel antibiotics resistance genes.

*Aim #2. Detection of antibiotics resistant genes chromosome-encoded.* Gene expression libraries were prepared using metagenomic DNA, and screened for their ability to confer specific antibiotic resistance to sensitive strains. The sequences of clones identified this way were determined.

The sequences obtained through aim #1 and #2 were analyzed using phylogenetic methods, including distance and maximum parsimony analyses, to assess the relationship among sequences from different sites and track their origin.

### **Methodology**

*Detection of antibiotic resistant genes encoded by plasmids (from culturable microorganisms).* Plasmids were “captured” using a transposon containing an *E. coli* origin of replication. Plasmids were extracted from cultures of environmental isolates grown with amoxicillin or trimethoprim, and treated using the Epicenter EZ-Tn5 kit, including a transposon carrying the R6Ky origin of replication, and a transposase that catalyzes the random transposition of the transposon within the plasmids. After treatment, plasmids were transferred into *E.coli* DH5 $\alpha$  by routine heat-shock transformation, and grown on selective medium. Recovered plasmids were further characterized by sequencing, through fragmentation, subcloning, and sequencing of the

subclones. Open reading frames (ORFs) identified within the plasmid were compared with sequences deposited in the NCBI database for gene identification.

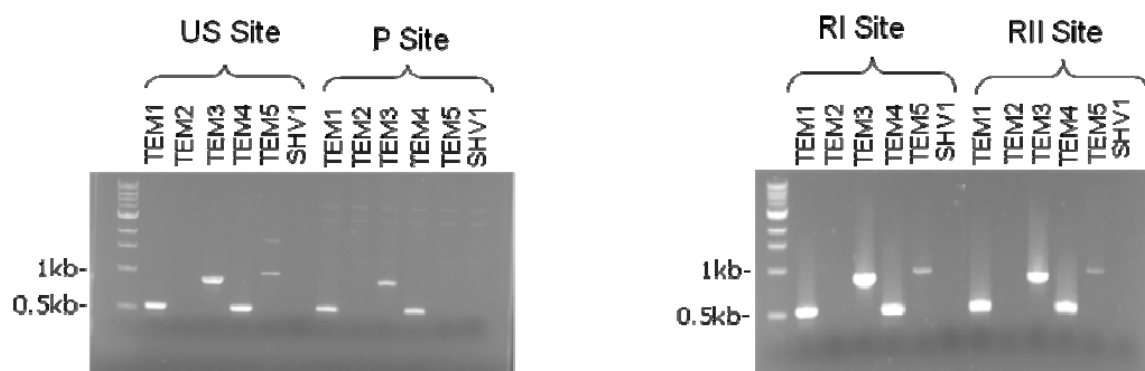
#### *Detection of antibiotics resistant genes chromosome-encoded*

A clone library of metagenomic DNA was constructed to identify antibiotic resistance genes that are located on chromosomes. The DNA, purified by cesium chloride gradient, was digested with different restriction enzymes that cut frequently and generate blunt ends, such as AluI. The time and amount of enzymes had to be optimized to find the conditions for obtaining fragment in the range 5-15Kb. Fragmented DNA was cloned in the pCR- BluntII TOPO vector (Invitrogen) and transformed in *E.coli*. Plasmids were first selected for kanamycin resistance, to select for clones carrying the recombinant plasmids. Next, recombinant clones were replica-plated on solid media containing either amoxicillin, trimethoprim or other antibiotics, at concentrations that are lethal to *E.coli*.

#### Principal Findings and Significance

Samples were collected from the final effluents of the wastewater treatment plant (P) and from two sites along the receiving stream (RI and RII). A fourth site, upstream of the discharge (US), was used as a control. Most isolates were resistant to elevated concentrations of amoxicillin. In addition, resistance to multiple classes of antibiotics was detected among the isolates. Given that amoxicillin resistance was the most frequent type of antibiotic resistance observed in all the sites of sampling, we wanted to find out how many different variants of the  $\beta$ -lactamase gene responsible for resistance were present, and whether these variants were prevalent at specific sites or equally distributed in the aquatic environment.

Plasmids were extracted from combined cultures of isolates for each of the four sites. Each plasmid mixture was tested using all of the seven pairs of primers. Only four of the seven pairs of primers produced a PCR product. All of the isolates resistant to amoxicillin carried the TEM 4 gene on their plasmid, as demonstrated by the presence of an amplification band (Fig. 1). Furthermore, the comparison of the *bla* gene sequence from the isolated plasmids showed that all the plasmids considered shared highly similar  $\beta$ -lactamase gene. The fact that isolates from different sites, belonging to different taxonomic groups, carried highly similar  $\beta$ -lactamase genes on their plasmids strongly support the hypothesis that the same resistance gene has spread to species sharing the same environment.

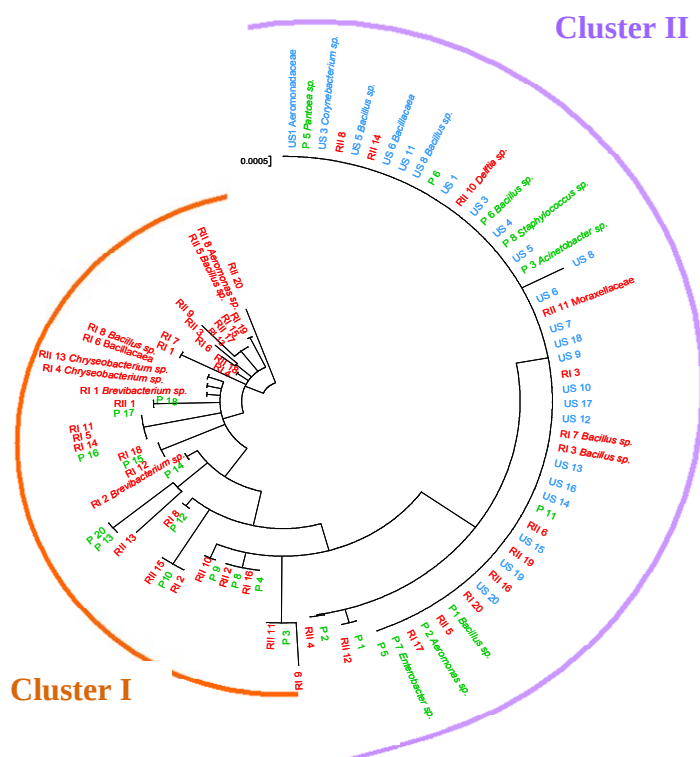


**Fig. 1. Presence of the *bla* gene in the combined plasmids from each site.** Variants of the *bla* gene were detected by PCR assays using the primers indicated at the top of the gel. Isolates from each site were grown in the presence of amoxicillin and combined before plasmid extraction. The amplification products from the sites US, P, RI, and RII were visualized on 0.8% agarose gel.

*Distribution of  $\beta$ -lactamase resistance genes at the four sites.* In order to evaluate the distribution of *bla* TEM4 gene variants within our isolates and the whole community, a library of the *bla* TEM4 was constructed for each of the four sites of sampling. The gene was also amplified from plasmids extracted from individual isolates, which allowed us to link the gene to specific genera and antibiotic resistance. The TEM4 primer pair amplifies about 500-bp of the  $\beta$ -lactamase gene, and the fragments obtained provides sufficient information to look at the distribution of variants of the  $\beta$ -lactamase gene at the four sites of sampling.

The nucleotide sequence of a total of 80 clones was determined for all four sites. To find a possible correlation between the clone library of the  $\beta$ -lactamase gene sequences and their phylogenetic affiliation, we used as reference the TEM4 sequences from known strains that were isolated from the four sites. The phylogenetic relationships among the *bla* TEM4 sequences from the gene libraries and environmental isolates are depicted in Fig. 2. The tree revealed two major clusters of the *bla* TEM4 gene. The first cluster consists of sequences that are exclusively found at the sites P, RI and RII and were not detected at the upstream site US, whereas the second cluster contains sequences found at all sites (Fig. 2). The nucleotide sequence alignment of the second cluster was shown to be highly similar among the sequences except for one nucleotide position.

**Conclusions.** In this work, we investigated the impact of antibiotic resistant bacteria from final effluents of a sewage discharge on the selection of antibiotic resistance in natural bacterial communities in sites downstream the treatment plant. The data obtained from the antibiotic profiling of the isolates showed resistance to high concentrations of  $\beta$ -lactam antibiotics, including amoxicillin, and multi-drug resistance, which are regarded as common features of isolates from wastewater (Costanzo et al., 2005). However, the analysis of the distribution of



**Fig. 2. Distribution of the TEM4 genes.** Phylogenetic analysis of TEM4 fragments of the  $\beta$ -lactamase genes amplified from community DNA and plasmids from environmental isolates. The tree was obtained with Mega 5, using the neighbor-joining method.

## Reference

Costanzo, S.D., Murby, J. and Bates, J.: Ecosystem response to antibiotics entering the aquatic environment. *Mar Pollut Bull* 51 (2005) 218-23.

plasmid-encoded *bla* TEM genes (Fig. 1) of the isolates suggests that the spread of resistance genes might also occur through HGT among different genera, and that isolates not previously reported in the literature may indeed contribute to the spread of resistance. By looking at the distribution of the *bla* TEM gene within our communities, it was found that genes grouping within cluster 1 (Fig. 2) are remarkable in that they are exclusively found in the plant, RI and RII sites, but they were not detected in the US site. We can speculate that *bla* TEM genes specific to the community of the plant effluent moved to the community of receiving body of water, which would explain the particular distribution of the *bla* TEM genes belonging to cluster 1. In contrast, *bla* TEM genes belonging to cluster 2 are ubiquitous, and do not seem to be prevalent at any specific site.

# Development of a Push-Pull Technique to Simultaneously Characterize Volatilization and Biodegradation Rates of VOCs in Shallow Wetland Sediments

## Basic Information

|                                 |                                                                                                                                                  |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Development of a Push-Pull Technique to Simultaneously Characterize Volatilization and Biodegradation Rates of VOCs in Shallow Wetland Sediments |
| <b>Project Number:</b>          | 2011NJ280B                                                                                                                                       |
| <b>Start Date:</b>              | 3/1/2011                                                                                                                                         |
| <b>End Date:</b>                | 2/29/2012                                                                                                                                        |
| <b>Funding Source:</b>          | 104B                                                                                                                                             |
| <b>Congressional District:</b>  | NJ-012                                                                                                                                           |
| <b>Research Category:</b>       | Engineering                                                                                                                                      |
| <b>Focus Category:</b>          | Methods, Wetlands, Toxic Substances                                                                                                              |
| <b>Descriptors:</b>             | None                                                                                                                                             |
| <b>Principal Investigators:</b> | Matthew Reid, Peter Jaffe                                                                                                                        |

## Publications

1. Reid, Matthew C. and Peter R. Jaffe. 2011. A Push-Pull Test to Measure Volatilization Fluxes of Organic Pollutants without Flux Chambers. AGU Fall Meeting, San Francisco, CA, December 2011. (poster presentation)
2. Reid, Matthew C. and Peter R. Jaffe. 2011. Measurement and Modeling of Volatilization through Wetland Macrophytes. Joint Meeting of the Society of Wetland Scientists, Wetland Biogeochemistry Symposium, and Wetpol. Prague, Czech Republic, July 2011. (poster presentation)

## Project Summary

### *Problem and Research Objectives:*

New Jersey's industrial history has resulted in a legacy of surface and groundwater resources contaminated with volatile organic compounds (VOCs). 44% of New Jersey's community water supply wells were classified as "highly susceptible" to VOC contamination, and 19% of wells with available data met or exceeded the maximum contaminant limit for trichloroethylene (TCE). TCE has been identified in 55 contaminated sites in New Jersey, benzene at 56 sites, and vinyl chloride (VC), a degradation product of TCE and a known human carcinogen, has been identified at 28. These VOCs are highly toxic to humans and their removal from contaminated groundwater is a major priority for the protection of New Jersey's water resources.

Plant-enhanced remediation of contaminated sediments and groundwater, or "phytoremediation," is an attractive alternative to traditional environmental remediation methods due to its energy-efficiency and cost-effectiveness. Vegetated soils act to remove organic contaminants through biodegradation, sorption, and plant uptake. Of these three processes, plant uptake remains the least understood. In particular, the extent to which organic pollutants are transported through plants and volatilized to the atmosphere ("phytovolatilization") is highly uncertain due, in part, to difficulties in the measurement of these processes. Volatilization can be an effective remediation mechanism because it transports VOCs to the atmosphere, where they are degraded to nontoxic products on the order of days relative to months to years by natural attenuation in groundwater. Effective phytoremediation strategies require estimates for volatilization rates, however, and the subsequent times scales for phytoremediation applications. Phytovolatilization rates will be highly site-specific, and at present there are no good field measurement techniques for assessing rates of phytovolatilization. The objective of this project was to develop a push-pull test (PPT) to simultaneously quantify in-situ rates of biodegradation and volatilization of organic contaminants in shallow wetland sediments.

### *Methodology:*

Over the past year, we have worked to optimize the experimental push-pull technique in greenhouse meso-scale experiments and have developed an analytical model framework with which to interpret the data.

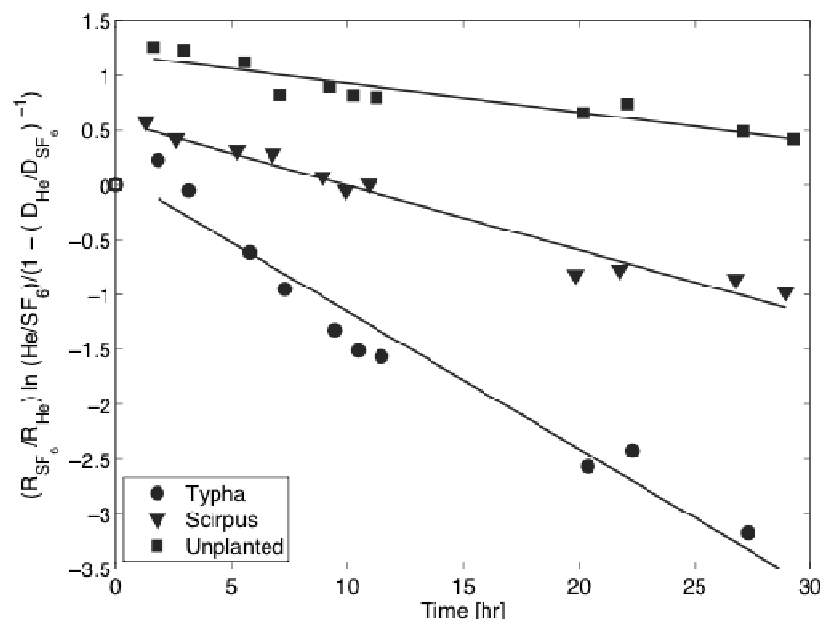
The experimental technique is similar to the traditional PPT: a test solution consisting of a nonreactive (typically bromide) tracer and one or more reactive tracers is prepared and injected into a saturated subsurface formation. In our experiments, we have used buckets filled with sand and planted with cattails or rushes as physical models for wetland systems. The reaction of interest in this case is volatilization, so a variety of volatile (gaseous) tracers were used in these experiments. Sulfur hexafluoride ( $\text{SF}_6$ ), helium, argon, CFC-12, methane, ethane, propane, and butane have all been experimented with as potential volatile tracers. NJWRRRI funding was used to purchase many of these gases and the gas chromatographic materials necessary to measure these gases. We have determined that  $\text{SF}_6$  and helium are the two best

chemicals to measure, based on a combination of their physiochemical properties and relative ease of measurement, analytical precision, and dynamic range.

A set of bench-scale hydroponic experiments were also conducted in parallel with the PPT development. The purpose of these small-scale, well-controlled experiments is to understand how to scale a volatilization rate measured with a tracer to a rate for a pollutant of interest. This is necessary since most field applications will not allow direct injection of a toxic compound, so it is necessary to infer a volatilization rate for a pollutant based on the measured rate of a nontoxic tracer. Using these experiments, we have developed a scaling relationship based on the diffusion coefficient of the chemical in water.

#### *Principal Findings and Significance:*

We have developed two analytical frameworks with which to interpret the data from these tests. The first is the “tracer normalization” technique, which involves normalizing the concentration of the reactive tracer by the nonreactive tracer to determine a dilution-adjusted volatilization rate constant. This is the standard analysis method for push-pull test results, but it was found to be inadequate in cases where the trapped gas bubble volume in soils was large. This is due to the fact that volatile tracers partition into these bubbles while bromide (or other nonreactive, nonvolatile tracers) does not. Because bromide and the volatile tracers are subject to different transport processes, bromide cannot be used to accurately account for dilution processes. Another potential problem with bromide is that it is taken up by plants, though evidence for this process was not displayed in our experiments.



**Figure 1: Results from Volatilization Push-Pull Test using Dual Tracer Analysis Method, in mesocosms with two different vegetation types and an unplanted control. The tracer-normalization method was not appropriate in these cases because of a relatively large trapped gas bubble volume.**

The second method is the “dual volatile tracer” method, and does not rely on a nonvolatile tracer like bromide to account for dispersive mixing processes (Figure 1). This method is used in chemical oceanography to measure gas exchange rates in the open ocean and estuaries, and this is the first time, to our knowledge, that it has been applied to gas transfer processes in subsurface environments. A thorough description of this method is found in Wanninkhof et al<sup>1</sup>. This technique used measurements of the concentrations of two volatile tracers, and a known ratio of their volatilization rates, to determine the volatilization rate constant of each tracer (Table 1). The ratio of the volatilization rates is based on results from the hydroponic experiments, described in the “Methodology” section. Because this technique uses measurements of two gas tracers, the errors introduced by tracer partitioning into gas bubbles is minimized.

We are currently in the planning stages of a field-scale PPT measurement to be carried out this summer. NJWRRRI funds have been used to purchase a robust, battery-powered pump to be used in these experiments.

**Table 1: Volatilization Rate Constants ( $k_r$ ) estimates from dual tracer PPT. Note that rates follow expected trends: Volatilization is fastest in the *Typha latifolia* mesocosm and slowest in the unplanted mesocosm.**

| <b>Helium <math>k_r</math></b> |                                                              |
|--------------------------------|--------------------------------------------------------------|
| <b>Plant</b>                   | <b><math>k_r</math> (hr<sup>-1</sup>)</b>                    |
| <b>Typha</b>                   | <b>0.126</b><br><b><math>\pm 7.76 \times 10^{-3}</math></b>  |
| <b>Scripus</b>                 | <b>0.0588</b><br><b><math>\pm 3.76 \times 10^{-3}</math></b> |
| <b>Unplanted</b>               | <b>0.0261</b><br><b><math>\pm 3.51 \times 10^{-3}</math></b> |

In summary, the principal findings of this research project are:

- (a) The push-pull test, modified to include volatile tracers, provides novel quantitative information on the rates of transport and volatilization processes in near-surface saturated soils.
- (b) The effect of trapped gas bubbles on measuring rates of processes involving volatile substances can be (partially) overcome using the dual volatile tracer method. This is a novel application of a measurement technique adapted from chemical oceanography.
- (c) A basic model describing the ratio of the volatilization rates of two substances as a function of the ratio of the diffusivities of the two compounds has been shown to be appropriate for modeling phytovolatilization rate constants. More work needs to be

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<sup>1</sup> R. Wanninkhof et al. (1993). Gas Transfer Experiment on Georges Bank Using Two Volatile Deliberate Tracers. J. Geophys. Res. 90, 11, 20,237-20,248.

done to determine which diffusivities are most appropriate – the diffusivity in water, or in some type of polymer?

## Release of Hazardous Metals into Surface and Groundwater by Microbial Oxidation of Sulfide Minerals

### Basic Information

|                                 |                                                                                                     |
|---------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>Title:</b>                   | Release of Hazardous Metals into Surface and Groundwater by Microbial Oxidation of Sulfide Minerals |
| <b>Project Number:</b>          | 2011NJ281B                                                                                          |
| <b>Start Date:</b>              | 3/1/2011                                                                                            |
| <b>End Date:</b>                | 2/29/2012                                                                                           |
| <b>Funding Source:</b>          | 104B                                                                                                |
| <b>Congressional District:</b>  | NJ-006                                                                                              |
| <b>Research Category:</b>       | Water Quality                                                                                       |
| <b>Focus Category:</b>          | Groundwater, Non Point Pollution, Toxic Substances                                                  |
| <b>Descriptors:</b>             | None                                                                                                |
| <b>Principal Investigators:</b> | Alexandra Walczak, Lily Young                                                                       |

### Publications

1. Walczak, Alexandra B., Nathan Yee, Lily Y. Young, 2011, Bosea sp. str. WAO Oxidizes Lead Sulfides at Neutral pH, Gordon Research Conference: Applied and Environmental Microbiology. Mount Holyoke College, South Hadley, MA. July 10-15, 2011. (Poster Presentation)
2. Walczak, Alexandra B., Nathan Yee, Lily Y. Young, 2012, Bosea sp. str. WAO Oxidizes Lead Sulfides at Neutral pH, Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ. February 2-3, 2012. (Poster Presentation)

## **Project Summary:**

### **Priority Issues addressed by this project:**

New Jersey's dense population and long manufacturing and industrial history has left a legacy of pollution. There are approximately 100 active National Priority List (Superfund) sites contaminated with metals in New Jersey<sup>1</sup>. Of these superfund sites, 20 have groundwater migration statuses that are unknown or uncontrolled. New Jersey is also home to the largest manufacturing and industrial centers in the eastern United States, which surround the Newark Bay<sup>2</sup>. In the Newark Bay Estuary system (Passaic River, Hackensack River, Arthur Kill, and Kill van Kull), high metal concentrations have been measured in the sediment. Lead concentrations were highest in the Passaic River, with an average of 706.5ppm, and lowest in the Hackensack River, with an average of 180ppm<sup>2</sup>. Lead is released into the environment from various anthropogenic sources including landfills, mining operations, and industrial smoke stacks<sup>3</sup>. In New Jersey industrial processing sites' wastewater is combined with municipal wastewater and accounts for two-thirds of the volume<sup>4</sup>. Wastewater treatment plants and combined sewer overflows (CSO) account for 57% of the metal discharged into the Newark Bay Estuary<sup>2, 5</sup>. Sediment sampled around CSO discharge sites was found to contain As, Cd, and Pb<sup>4</sup>. These CSOs discharge untreated waste directly into the waterways of New Jersey during 50% of all rainfall events<sup>4</sup>.

Current research on metal distribution in New Jersey waterways in the Newark Basin has largely focused on aquatic chemistry, hydrology, and on aquatic organisms such as fish and birds. A crucial link remains to be understood regarding the fate of these metals, namely the effects of microorganisms. Once metals such as lead are mobilized the likelihood of human exposure increases and can result in well-documented health effects. Lead has been found to be especially harmful to children because they are still in the crucial stages of development. In adults it can cause reproductive problems, high blood pressure, hypertension, nerve disorders, memory and concentration problems, and muscle and joint pain<sup>6, 7</sup>. Lead has also been shown to reduce photosynthesis in phytoplankton; increase mortality of polychaetes, bivalves, and crustaceans; affect development of fish embryos; and cause neurological disorders in birds<sup>5</sup>.

This study will focus on determining the effect microbial activity has on releasing Pb from the sediments. *Bosea* sp. str. WAO was isolated from the Lockatong formation based on its ability to oxidize and mobilize arsenic and sulfur from arsenopyrite and will be used as the experimental model organism in this study<sup>8</sup>. The metal sulfide galena (PbS) will be used as an energy source for this chemolithotrophic organism to grow at mesophilic temperature and circumneutral pH. The rate and extent of oxidation of the mineral will be monitored by measuring the release of sulfate and metal ion by ion chromatography and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), respectively. The oxidation process will also be monitored visually using confocal microscopy. This study will demonstrate WAO's ability to oxidize metal sulfides releasing soluble metal ions and sulfate at mesophilic temperature and circumneutral pH. This research can be applied to advanced modeling and mitigation strategies.

### **Specific Objectives:**

The overall objective of this study is to determine the role of microorganisms in the oxidation of sulfidic minerals and how they affect the fate and transport of metals in aquatic sediments. This study specifically looks at oxidation of the metal sulfide galena (PbS) by the

model organism WAO. The oxidizing activity of the organism will be monitored by chemical and microscopic analysis.

## **Methodology**

### **Experimental Design:**

1. PbS oxidation by WAO in high phosphate minimal salts medium at pH 7, 30°C with shaking.

#### **Experimental Conditions**

- a. Active Cultures in Triplicate- containing 2 mM PbS and WAO inoculum to determine biotic oxidation.
- b. Media Control in duplicate- media and 2 mM PbS to determine abiotic oxidation.

#### **Sampling**

- a. 0.8mL culture filtered and frozen for ion chromatography to monitor sulfate production.
- b. Three sample treatments for ICP-OES to monitor soluble metal ion release and total dissolved sulfur.
  - 0.8 mL filtered immediately
  - 1.0 mL acidified to 0.1% nitric acid and then filtered
  - 1.0 mL pelleted and supernatant removed and pellet resuspended in 10% nitric acid, then filtered

#### **Data Analysis**

- a. Measure the release over time of the sulfate and metals.
  - b. Calculate if the release of metal and sulfate is stoichiometric.
2. PbS oxidation by WAO in low phosphate minimal salts medium at pH 7, 30°C with shaking.

#### **Experimental Conditions**

- a. Active Cultures in Triplicate- containing 2mM PbS and WAO inoculum to determine biotic oxidation.
- b. Media Control in duplicate- media and 2mM PbS to determine abiotic oxidation.
- c. Killed Cell Control in duplicate- triple autoclaved WAO inoculum and 2mM PbS to determine if oxidation due to cell presence but not activity.

#### **Sampling**

- a. 0.8mL culture filtered and frozen for ion chromatography to monitor sulfate production.

#### **Data Analysis**

- a. Calculate the rate of release over time of the sulfate.
3. Study of the colonization of PbS by strain WAO over time.

#### **Experimental Conditions**

- a. Minerals embedded on microscope slides.
- b. Slides incubated in the presence of strain WAO.
- c. At each time point cells fixed with gluteraldehyde, and fluorescently stained.
- d. Cells and minerals visualized with epifluorescence and confocal microscopy.

4. X-ray diffraction analysis of PbS mineral transformation.

#### **Experimental Conditions**

- a. Active Cultures - containing 2mM PbS and WAO inoculum to determine biotic oxidation effects on mineral composition.
- b. Media Control - media and 2mM PbS to determine abiotic oxidation effects on mineral composition.

#### **Sampling**

- a. After color change of mineral from black to grey/white cultures and controls were filtered down to collect precipitate.
- b. Precipitates dried and then ground to a fine powder.

#### **Data Analysis**

- a. Determine the diffraction pattern for the abiotic versus the biotic experimental conditions.
  - b. Compare diffraction pattern to mineral standards to determine chemical composition.
5. Genome sequencing and analysis of *Bosea* sp. str. WAO

#### **DNA Preparation**

- a. Genomic DNA extracted from WAO using MoBio Power soil DNA extraction kit.
- b. Purified DNA sent out for sequencing and assembly.

#### **Data Analysis**

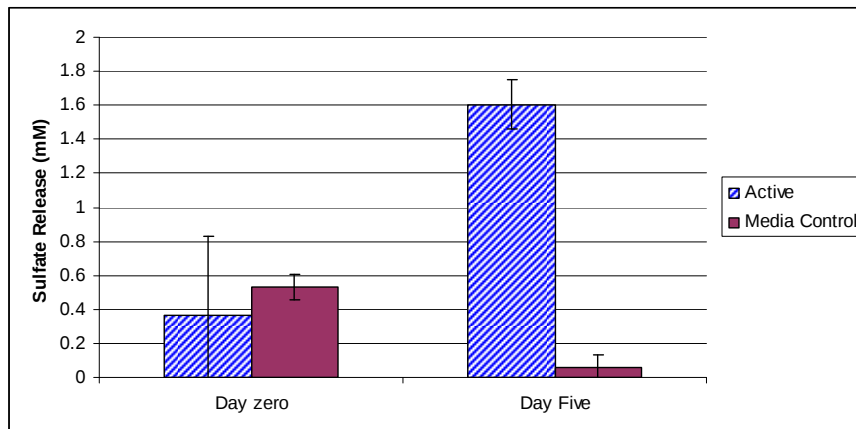
- a. Metabolic pathways for sulfide oxidation will be mapped.

### **Principle Findings and Significance**

#### **Mineral Oxidation by WAO:**

##### *High Phosphate Conditions*

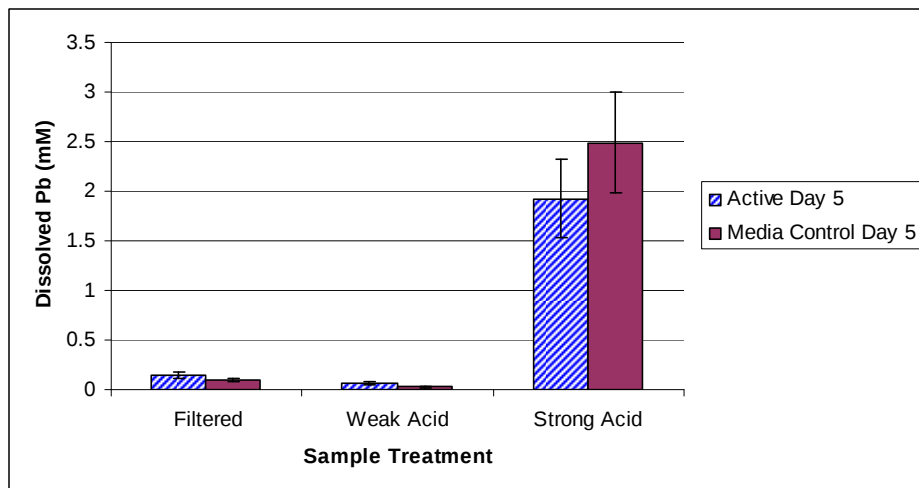
Microbial oxidation of 2mM PbS was studied in a minimal medium buffered by 40 mM phosphate. Triplicate active cultures were inoculated with *Bosea* sp. str. WAO and the oxidizing activity of the organism was monitored for sulfate release as measured by ion chromatography. The duplicate media control contained only mineral and media and was used to determine abiotic oxidation during the experimental time period. The data in figure 1 shows the media control did not have an increase in sulfate concentration over the five day time course whereas the active cultures with WAO had a release of ~1.6 mM sulfate from the 2 mM PbS. This demonstrates that the oxidation of the sulfide is due to microbial processes.



**Figure 1. Sulfate Release due to the oxidation of PbS by WAO.** The active culture shows an increase in sulfate between day zero and day five. The media control remains low indicating the mineral is not abiotically oxidizing during the experimental period. Sulfate was measured using ion chromatography.

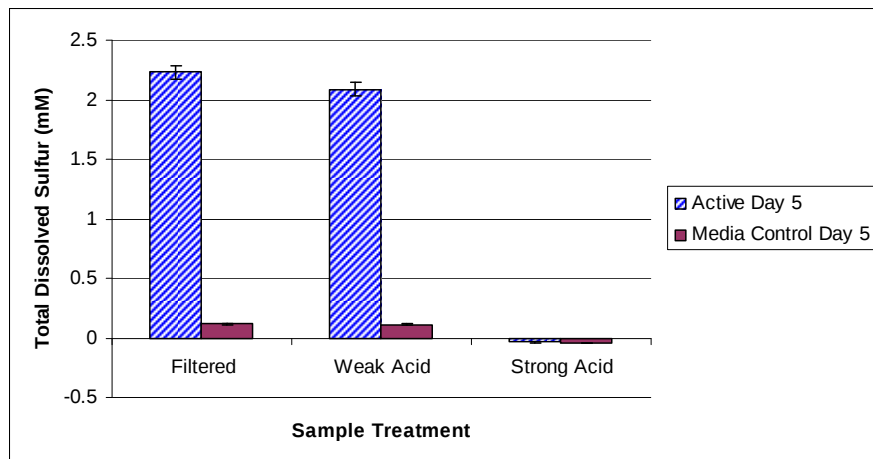
The release of Pb and the total dissolved sulfur (TDS) from the oxidation process was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Samples for ICP-OES were prepared using three treatments. Samples were (1) immediately filtered, (2) treated with a weak acid (0.1% nitric acid) then filtered, and (3) pelleted and then resuspended in a strong acid (10% nitric acid). These treatments were to extract Pb and TDS that might be

bound to other components in the medium such as cells or re-precipitated on mineral surfaces. The filtered treatment measures the soluble Pb/TDS, the weak acid measures weakly associated Pb/TDS with insoluble components in the medium, and the strong acid treatment measures the Pb/TDS associated with the insoluble fraction that can be dissolved in a strong acid. Figure 2 shows that Pb was less than 0.1 mM in both the filtered and weak acid treatment for the active and the media control, whereas ~2 mM Pb was measured in the strong acid treatment. This indicates that the Pb is being retained in the solid phase but is acid extractable. Therefore, although oxidation of the sulfide bound in the PbS is occurring and releasing sulfate the Pb is being retained in a precipitate.



**Figure 2. Dissolved Pb in active cultures and media control.** Errors bars represent instrumental variability within a single sample. Filtered samples were immediately filtered. Weak acid samples were acidified to 0.01% nitric acid before filtering. Strong acid samples were pelleted and then resuspended in 10% nitric acid before filtering. Pb concentration was low in both the filtered and weak acid treatment for both the active culture and the control. For the strong acid treatment ~2 mM of Pb was observed in both the active and the media control.

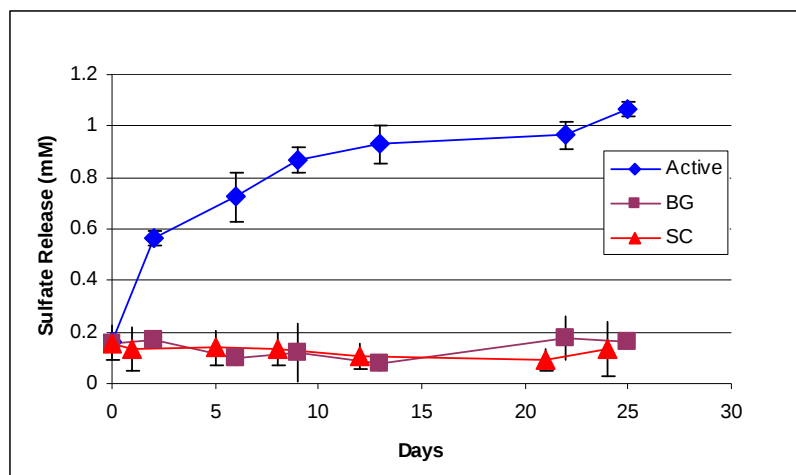
In figure 3, ~2 mM TDS was observed in the active cultures in both the filtered and the weak acid treatments. This concentration matches the sulfate concentration obtained by IC suggesting all available TDS is being oxidized to sulfate. In the strong acid treatment the TDS was below the detection limit. This indicates that TDS is associated with the soluble components in the medium and not with the pelleted fraction. For the media controls less than 0.1 mM TDS was observed in the filtered and weak acid treatment. This matches the low values that for sulfate release that was measured with IC. However the low level observed in the strong acid for the media control indicates that remaining ~1.9 mM of S that should be present was not acid extractable.



**Figure 3. Total dissolved sulfur in active cultures and media controls.** Total dissolved sulfur is all oxidation states of sulfur that are soluble in the sample. Errors bars represent instrumental variability within a single sample. Filtered samples were immediately filtered. Weak acid samples were acidified to 0.01% nitric acid before filtering. Strong acid samples were pelleted and then resuspended in 10% nitric acid before filtering. TDS concentration was ~2 mM in both the filtered and weak acid treatment for the active culture. For the media control less than 0.1 mM was observed in the filtered and weak treatment. For the strong acid treatment TDS was below the detection limits.

### *Low Phosphate Conditions*

Low phosphate experiments were set up due to the Pb remaining in the pelleted fraction of the above experiment even though the associated sulfide was being oxidized to sulfate. One possibility for the immobilization of Pb is the reaction of lead (Pb) and phosphorus-containing compounds. This can cause the formation of pyromorphite-type mineral phases<sup>9</sup>. Microbial oxidation of 2 mM PbS was studied in a minimal medium with 0.5 mM phosphate compared to 40 mM in the previous experiment. Triplicate active cultures were inoculated with *Bosea* sp. str. WAO and the oxidizing activity of the organism to release sulfate was monitored by ion chromatography. The duplicate media control contained only mineral and media and was used to determine abiotic oxidation during the experimental time period. Duplicate sterile controls contained triple autoclaved kill cells, 2 mM PbS, and media where used to indicate if the presence but not activity of the cells caused oxidation. Figure 4 shows that a release of 1 mM sulfate was observed in the active culture but that the level of sulfate in the media control and sterile control remained constant. This shows that cells need to be alive and present for oxidation of the mineral to occur. The release of only 1 mM sulfate instead of a stoichiometric concentration of 2 mM may indicate that at lower phosphate levels the Pb has a toxic effect since it is not being bound by the phosphate or that the phosphate is actually mediating the release of sulfide for the cells to access.

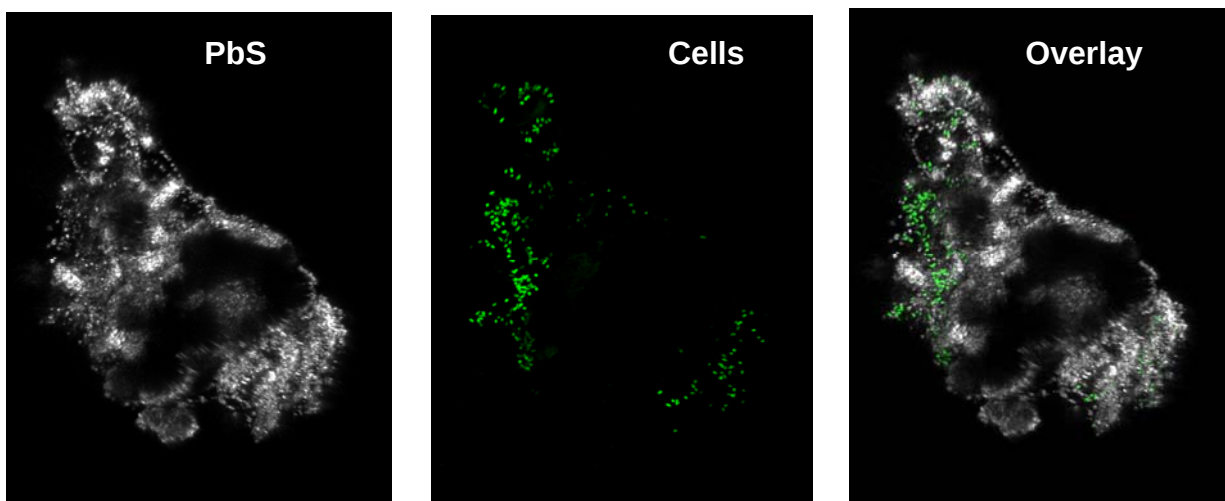


**Figure 4. Oxidation of PbS in low phosphate media.** The active culture shows the release of 1 mM sulfate during incubation while the sterile control (SC) and media control (BG) show no increase in sulfate during the 25 day incubation period.

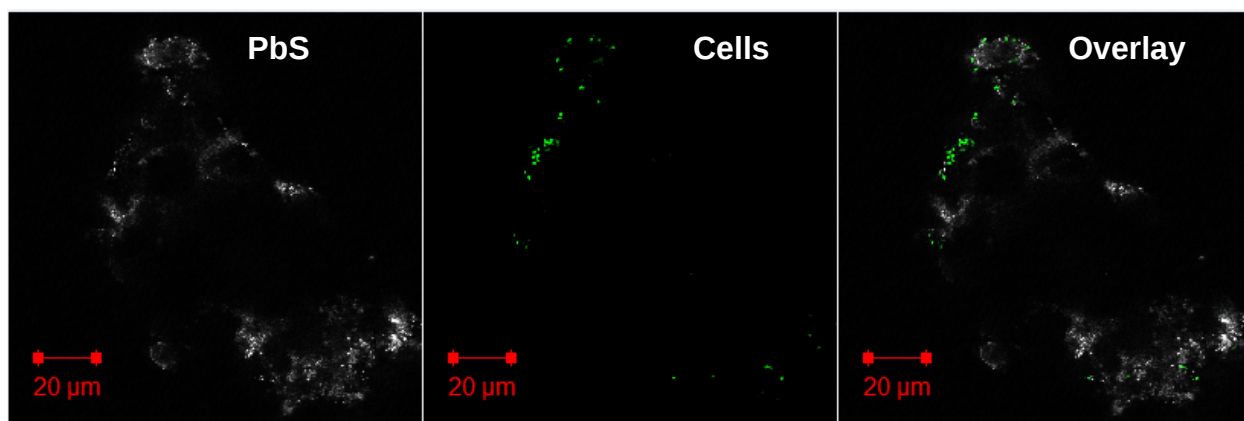
### PbS Mineral Colonization by WAO:

#### *Confocal Microscopy*

Confocal microscopy was performed on slide cultures to determine if the cells were closely associated with the mineral surface. Images were obtained with a Zeiss LSM510 Meta Confocal Laser Scanning Microscope at 630X with water immersion. The PbS image was obtained by collecting the reflection of the 488 nm argon laser source from the mineral surface. WAO was imaged by exciting DAPI with a 364 nm laser source. Figure 5 shows in (a) a 3D rendering of thirty 0.5  $\mu\text{m}$  z-stack slices of PbS (white/grey) and WAO (green) as separate channels and then an overlay of the two channels. This shows that cells are only present where mineral is also present. In figure 5 (b) is a 0.5  $\mu\text{m}$  single slice of mineral, WAO, and then overlay. Multi-track consecutive imaging of PbS surface and WAO indicates that cells and mineral surface are colocalized to within 0.5  $\mu\text{m}$  of one another to be in focus in the same z-stack slice. The lack of cells away from the mineral suggests that cells are in close association with the mineral surface for growth.



**a. 3D Reconstruction**

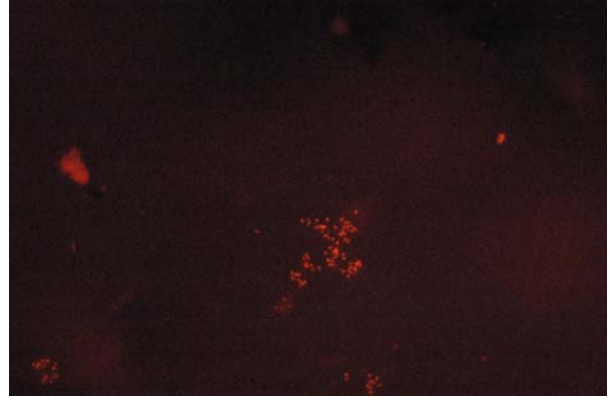


**b. Single z-Stack Slice**

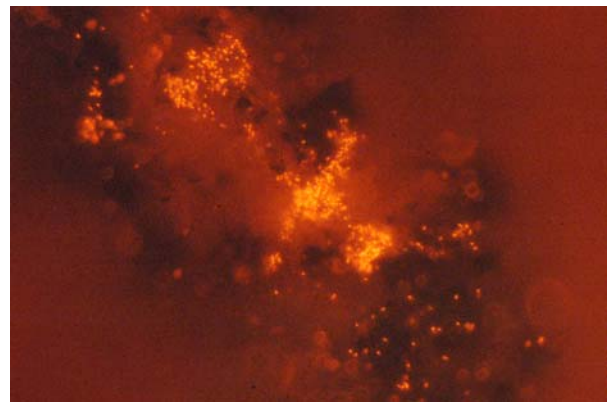
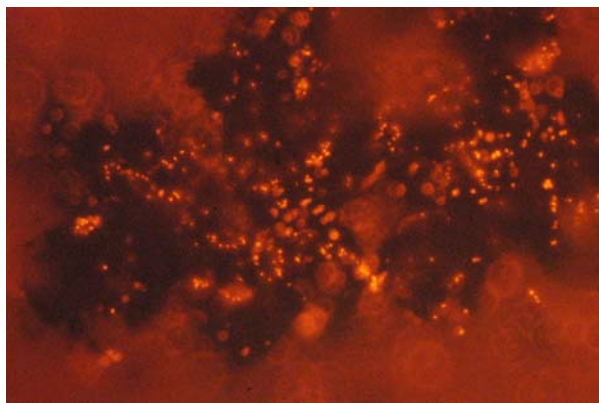
**Figure 5. Confocal imaging of WAO on PbS particle surface.** a.) A 3D rendering of thirty 0.5  $\mu\text{m}$  z-stack slices of PbS (white/grey) and WAO (green) separate channels and then an overlay of the two channels. b.) 0.5  $\mu\text{m}$  single slice of mineral, WAO, and then overlay. Multi-track consecutive imaging of PbS surface and WAO indicates that cells and mineral surface are colocalized to within 0.5  $\mu\text{m}$  of one another to be in focus in the same z-stack slice.

#### *Epifluorescence Microscopy Time Course*

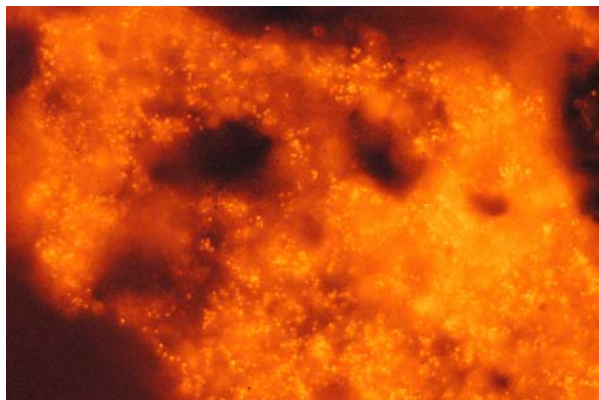
WAO was incubated with replicate slides embedded with PbS mineral to determine colonization of mineral surface over time. At each time point a slide was sacrificed and stained with ethidium bromide. Multiple fields were imaged for each day at 400X, with two fields at each time point shown in figure 6. Day 1 after inoculation, very little biomass (orange) is associated with the dark mineral surface. Day 4 an increase in biomass is clearly observed on the PbS even after several washing and staining bath transfers. Day 6 the surfaces of some PbS particles are completely covered with cells. The increase in biomass associated with the mineral surface over time suggests that the cells are not just settling on the mineral surface but are also growing.



**Day 1**



**Day 4**

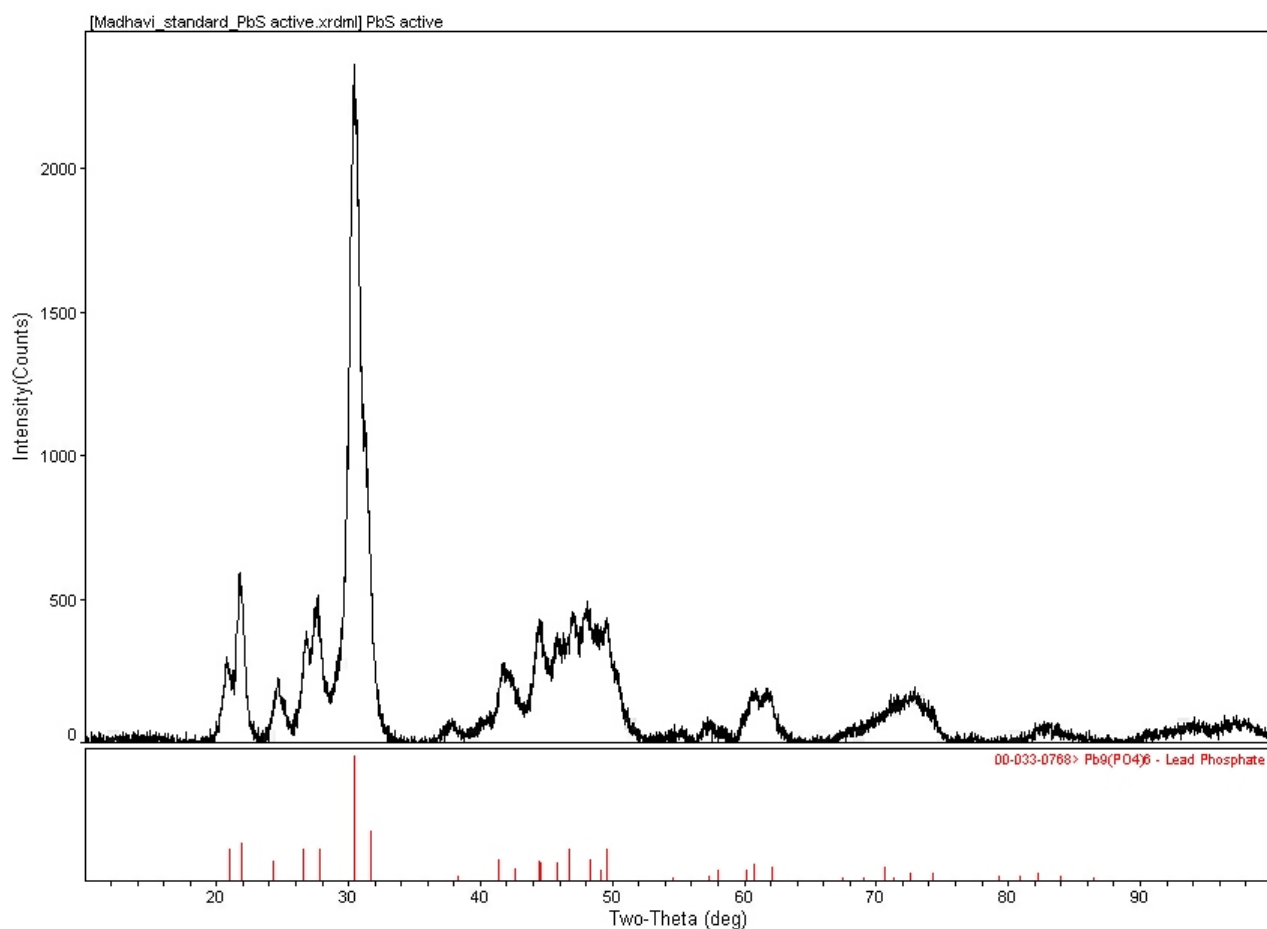


**Day 6**

**Figure 6. Epifluorescence microscopy of WAO on PbS overtime.** At each time point a slide was sacrificed and stained with ethidium bromide. Multiple fields were imaged for each day at 400X, with two fields at each time point shown above. Day 1 after inoculation, very little biomass (orange) is associated with the dark mineral surface. Day 4 an increase in biomass is clearly observed on the PbS even after several washing and staining bath transfers. Day 6 the surfaces of some PbS particles are completely covered with cells.

### X-ray powder diffraction of PbS mineral Transformation:

X-ray powder diffraction of the reacted mineral was performed to determine what insoluble compound was present after a stoichiometric oxidation of sulfide to sulfate from PbS was observed. Ten active replicate flasks of 2 mM PbS with WAO and 10 replicate media control flasks of 2 mM PbS were filtered to collect precipitate after complete oxidation was observed in the active flasks. The filters were then dried and the precipitate collected and ground. The samples were analyzed by X-ray diffraction to determine the mineral composition. The diffractogram of the biotic flasks is shown in figure 7. The black major peaks of the sample match the red peaks of the  $\text{Pb}_9(\text{PO}_4)_6$  standard. This indicates that the mineral has been transformed from lead sulfide to lead phosphate.

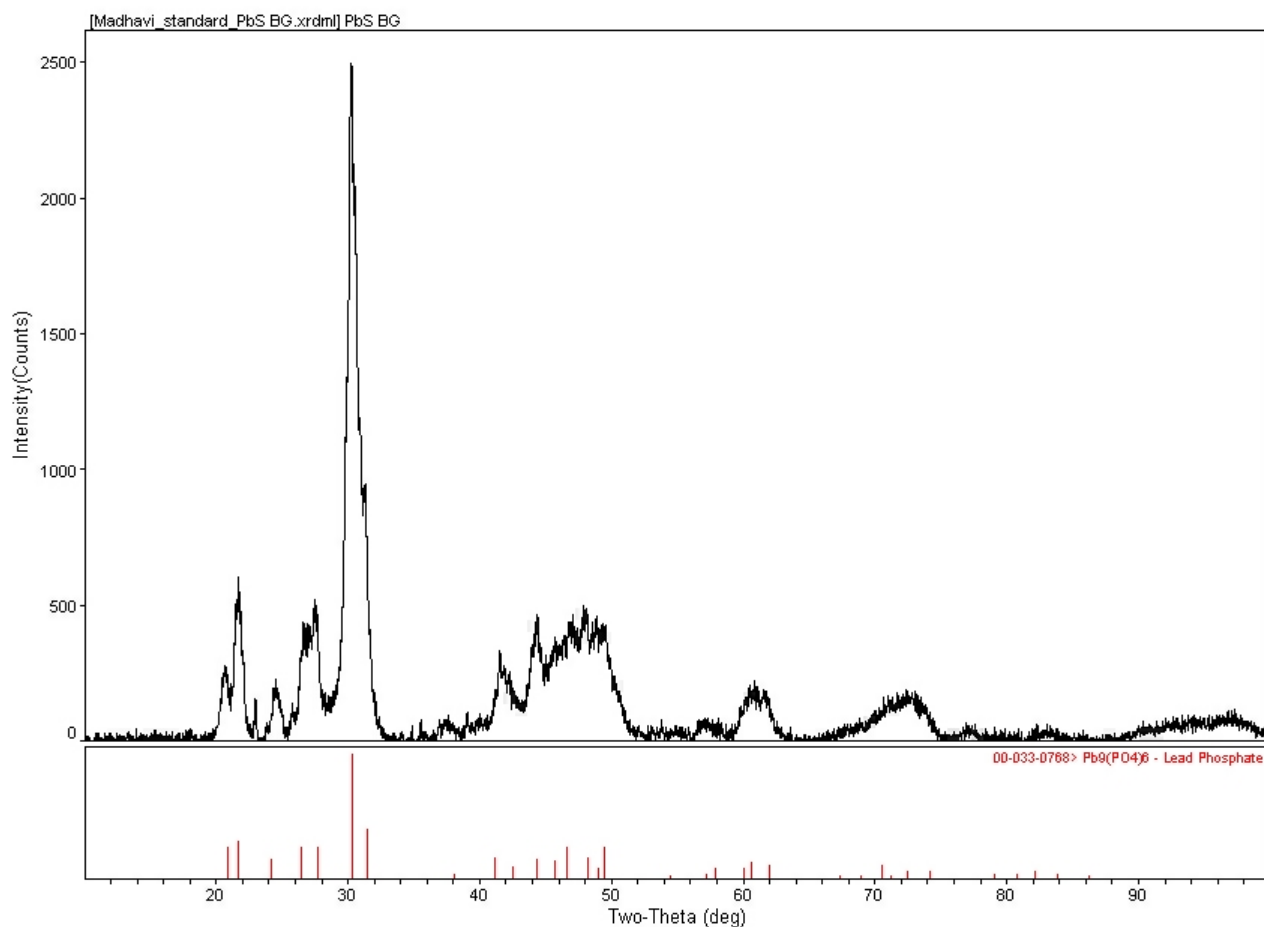


Materials Data, Inc.

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**Figure 7. Diffractogram of biotic mineral precipitate.** Insoluble fraction of experimental culture of 2 mM PbS incubated with WAO. Black line indicates the diffraction pattern of the collected precipitate. The red peaks are the closest standard with major peaks matching. The major peaks of the sample matches the  $\text{Pb}_9(\text{PO}_4)_6$  standard indicating that the initial PbS mineral has been transformed to lead phosphate.

The diffractogram of the abiotic precipitate is shown in figure 8. The black major peaks of the sample match the red peaks of the  $\text{Pb}_9(\text{PO}_4)_6$  standard. This indicates that the mineral has been transformed from lead sulfide to lead phosphate. In both cases whether cells are present or not the PbS is being transformed by the phosphate in the medium to  $\text{Pb}_9(\text{PO}_4)_6$  indicating that this is not a cell mediated process. However according to previous sulfate data the sulfide present in PbS is only being oxidized in the presence of cells.



Materials Data, Inc.

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**Figure 8. Diffractogram of abiotic mineral precipitate.** Insoluble fraction of experimental control of 2 mM PbS incubated in media. Black line indicates the diffraction pattern of the collected precipitate. The red peaks are the closest standard with major peaks matching. The major peaks of the sample matches the  $\text{Pb}_9(\text{PO}_4)_6$  standard indicating that the initial PbS mineral has been transformed to lead phosphate.

### Genomic Analysis of WAO:

Sequencing the genome of an organism can allow for the elucidation of metabolic pathways that may otherwise be difficult to obtain using traditional molecular techniques. In this case the functional genes of the sulfide oxidation pathway will be explored. Once the genes of interest are identified primers can be developed to screen for this activity in the environment. As of this report's deadline the genome has been sequenced and is currently being annotated and assembled.

### Overall Conclusions and Significance:

- Oxidation of sulfide bound to PbS only occurs in the presence of *Bosea* sp. str. WAO and not with killed cells or when incubated with only media, therefore this is a microbially mediated process.
- Metal analysis shows there is not a release of Pb into the bulk solution even with sulfide oxidation, suggesting that secondary reactions are taking place to sequester the Pb.
- *Bosea* sp. str. WAO remains in close association with the surface of the PbS mineral, suggesting that it must be in contact for oxidation to occur.
- The concentration of phosphate has an effect on the amount of sulfide that can be oxidized from PbS. Reduced phosphate levels showed a reduction in the concentration of sulfate released.
- X-ray diffraction of reacted mineral shows the remaining precipitate has been transformed from PbS to  $\text{Pb}_9(\text{PO}_4)_6$  in both abiotic and biotic conditions indicating that this reaction is abiotically occurring. This abiotic reaction is sequestering the Pb from the media and can serve as a remediation technique; however, the release of sulfide for microorganisms to oxidize can potentially acidify the environment.
- Sequencing and analysis of WAO's genome will allow for the elucidation of its sulfide oxidation pathway.

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# Drought and Flood in the Eastern US

## Basic Information

|                                 |                                          |
|---------------------------------|------------------------------------------|
| <b>Title:</b>                   | Drought and Flood in the Eastern US      |
| <b>Project Number:</b>          | 2011NJ292G                               |
| <b>Start Date:</b>              | 9/1/2011                                 |
| <b>End Date:</b>                | 8/31/2013                                |
| <b>Funding Source:</b>          | 104G                                     |
| <b>Congressional District:</b>  | 12th                                     |
| <b>Research Category:</b>       | Climate and Hydrologic Processes         |
| <b>Focus Category:</b>          | Drought, Floods, Water Supply            |
| <b>Descriptors:</b>             | None                                     |
| <b>Principal Investigators:</b> | James Smith, Justin Sheffield, Eric Wood |

## Publications

There are no publications.

## **Project Summary:**

### **Problem and Research Objectives -**

We propose to develop statistical procedures for regional analyses of drought and flood in the eastern US based principally on USGS stream gaging data. We will focus on the interrelationships between drought and flood, with a particular emphasis on water supply systems for large urban centers of the eastern US. Procedures will exploit mixture distribution representations of flood and drought variables; these representations center on tropical cyclones, which are major rainfall and flood agents during summer and fall, and extratropical systems, which are major rainfall and flood agents during spring and fall in the eastern US. We adopt a regional approach covering the eastern US due to the scale of the weather and climate systems at play. Special emphasis will be placed in this study on the Delaware River basin (water supply for New York City), the Potomac River basin (water supply for Washington D. C.), the Catawba River basin (water supply for Charlotte, North Carolina) and the Chattahoochee River basin (water supply for Atlanta, Georgia). The procedures that we will develop are designed for broad use by USGS National and District offices for water resource assessment studies. Additional users will include river basin planning and management agencies (including the Delaware River Basin Commission and Interstate Commission on the Potomac River Basin), states and local municipalities.

The procedures will be used for regional assessments of drought and flood frequency, short-term (seasonal to interannual) characterization of drought and flood occurrence and long-term trend assessment of drought and flood variables. These procedures will provide capabilities for regional water resources analyses covering the eastern US. Detailed analysis capabilities will be developed for the Delaware, Potomac, Catawba and Chattahoochee River basins, providing information on drought and flood frequency for major urban centers of the eastern US.

The broad objective of this study is to develop statistical tools for characterization of water resources and flood hazards based on USGS streamflow records. The specific objectives of the study are to develop statistical procedures for: 1) assessing non-stationarities of drought and flood variables (in terms of change-points, slowly varying trends and long-term persistence), 2) characterizing spatial extremes of drought and flood and 3) characterizing the interrelationships between drought and flood, including their relationships to climate indices. The project will be carried out over a two year time period. Development of data sets and statistical procedures will be largely completed during year 1. Implementation of procedures for assessing non-stationarities in drought and flood variables will be completed in year 1. Other project tasks will be initiated during year 1. In the second year of the project, we will complete analyses of the interrelationship of drought and flood for the eastern US, analyses of spatial extremes and analyses of drought and flood occurrence in terms of climate indices. We will also synthesize analyses for the Delaware, Potomac, Catawba and Chattahoochee River basins during year 2.

### **Methodology -**

During the first year of the project, we have developed data sets and statistical methods for examining drought and flood. For the drought analysis, we have focused on two topics: a) the

impact of tropical cyclones on drought and b) the climatology of low flows over the eastern US. Flood studies have focused on analyses on nonstationarities, mixture distributions, the role of tropical cyclones and orographic precipitation mechanisms.

#### *a) Impact of Tropical Cyclones on Drought*

We have analyzed the contribution of tropical cyclones (TC) to the delayed initiation and early recovery of drought in the eastern US. Given that tropical cyclones can bring up to 30% of total seasonal rainfall in the southeast, we hypothesized that they also contribute significantly to drought recovery. As far as we know, this is the first study to evaluate the impact of Atlantic tropical cyclones on drought in a quantitative manner.

The HURDAT track data (Jarvinen et al. 1984; Neumann et al. 1993) were used to identify TC-related rainfall (1980-2007) from the hourly rainfall dataset of NLDAS2 (Xia et al., 2012). The Atlantic HURDAT data include track information for cyclones from 1985-2011 including the center of TC at 6 hourly timestep, the maximum wind speed, minimum pressure, and the category. Recent studies (Landsea et al, 2010; Kunkel et al., 2010) have used the HURDAT data to analyze the historical trends in the duration of TCs and to examine the contribution of TCs to heavy rainfall. As the NLDAS-2 forcing data have an hourly temporal resolution, the HURDAT tracks locations are linearly interpolated from 6 hourly to hourly following (Villarini et al., 2011). TC-related rainfall is then estimated using a distance threshold value as in previous studies (Shepherd et al, 2007; Jiang and Zipser, 2009; Barlow, 2011). The chosen value of the threshold of 5-degrees (about 500km) was estimated from a sensitivity analysis. Figure 1 shows an example of this for 2005, which experienced 28 tropical cyclone events. The figure shows the tracks from the HURDAT database as well as the accumulated land rainfall associated with the tropical cyclones.

To understand the contribution of TCs to drought recovery (as well as late initiation), we ran the VIC land surface model with and without the contribution of tropical cyclone rainfall. Drought was defined in terms of soil moisture percentiles relative to a set of thresholds and durations to represent various types of drought from short-term severe to long-term moderate droughts.

#### *b) Climatological Analysis of Low Flows over the Eastern US*

Our initial goals were to (1) assess available data for low flow and drought studies in the eastern US, (2) carry out time series analysis of average daily discharge records, and (3) test for non-stationarity in the mean and variance from change point and trend analysis tests. Low flows have been calculated for n-day annual minimum flow values, where n is 1, 7, 30 and 90 days. Statistical tests have been applied for long-term trends (Mann-Kendall non-parametric trend test) and change points (Pettitt test and Rodinov regime shift test) in the low flow values.

To analyze the long-term statistics of low flows over the eastern US, we have collected daily streamflow data from the USGS. Data for 5,475 sites were initially collected for Hydrologic Unit Codes of 01, 02, and 03 and then reduced to 4878 sites after filtering out sites that had no drainage area record or latitude/longitude in the file. Figure 8 shows the location of these sites. Figure 8(a) shows the total number of days of records for each site; Figures 8(b)-(d) show maps of only those sites with at least 30, 50, and 75 years of daily records, respectively. Figure 9 shows that 68% of sites are either still active or have been active until at least 2000.

Initial application of time series analysis has been carried out for a subset of sites from the four river basins identified in the proposal: the Delaware River basin, the Potomac River basin, the Catawba River basin, and the Chattahoochee River basin. These basins have been chosen since they are the major water supply for New York City, Washington D.C., Charlotte in North Carolina, and Atlanta in Georgia, respectively. Initially a total number of 1732 sites from these four basins were retrieved from USGS water data website. A subset of 177 sites with 50 years or more worth of daily streamflow data were identified as shown in Figure 10.

### *c) Flood Studies*

Initial studies of flood climatology have focused on the Potomac River basin (Smith et al. 2012a), the Delaware River basin (Smith et al. 2012b) and Texas (Villarini et al. 2012). We have developed USGS stream gaging data sets and radar rainfall data sets for flood studies in the Catawba and Chattahoochee River basins during Year 1 of the project.

The Potomac River stream gaging stations at Point of Rocks, Maryland has one of the longest records in the stream gaging archive of the USGS. Furthermore, the Potomac River basin is one of the least “regulated” river basins in the eastern US, with a single reservoir (controlling less than 3% of the drainage basin) having been completed in the early 1980s. We examined change points and trends in annual flood peak data and peaks-over-threshold flood data for the Point of Rocks station. In addition to the Point of Rocks station, the Potomac River basin has a relatively large density of stream gaging stations with more than 50 years of record. We have carried out analyses of change points and trends for all of the “long” stream gaging records in the Potomac basin (Smith et al. 2012a)

The East Branch of the Delaware River basin is a critical water supply source for New York City and exhibits large spatial gradients in the runoff and flood magnitudes. There is a dense network of USGS stream gaging stations in the basin linked to assessments of hydrology and water quality of the Pepacton reservoir. The East Branch Delaware River basin has experienced a sequence of major flood events, with Hurricane Irene causing extensive damage and loss of life in August 2011. We have developed high-resolution rainfall analyses for major flood events in the East Branch Delaware and examined spatial variability and heterogeneity in extreme rainfall, runoff and flood magnitudes (Smith et al 2012b).

The Gulf of Mexico region experiences extreme rainfall and flooding from tropical cyclones, warm season thunderstorm systems and winter/spring extratropical systems. We have examined nonstationaries in annual flood peaks for stream gaging stations in Texas with long records. We have also examined the spatially varying role of tropical cyclones in the flood hydroclimatology of Texas (Villarini et al. 2012).

**Principal Findings and Significance** - please provide a progress report if the project is to be continued; if a final report, summarize significant findings. All graphics and tables should be included within the body of the document.

### *a) Impact of Tropical Cyclones on Drought*

Figure 2 shows the total number of landfalling TCs in each year over the contiguous US

(CONUS) for 1979-2007 in terms of cyclone category (based on the Saffir/Simpson hurricane wind scale) and the month of the event. 33 and 27% of landfalling TCs are in the category of tropical storms and Hurricane category 1, respectively. About 30% of landfalling TCs occur in August and September. 2004 had the most landfalling TCs with 9 events of various categories and mostly occurring in August and September (8 events). Figure 3 shows the impact of landfalling TCs in terms of their related rainfall, for (left) number of TCs per state that are within 500km of the center of the TC track, and (right) the accumulated rainfall attributable to these TCs. Four regions were identified for further analysis: Southeastern (SE), South (S), Northeastern (NE), and Midwestern (MW) regions. In general, TCs have large interannual and spatial variability.

Figure 4 (left) shows the interannual variation of the regional contributions of TCs to annual total rainfall and to the TC-season (May-Nov) accumulated rainfall. TCs contribute from 1% (1.5%) to 4% (7%) over the four regions of the annual (TC-season) total rainfall. The maximum regional contribution of TCs are 20% (28%), 17.7% (33%), 11% (17%), and 10% (17%) of annual (TC-season) total rainfall over the SE, S, NE, and MW regions, respectively, mostly in 2004 and 2005. Relative to 1980-2007, the SE, NE, and MW regions have experienced increased contributions of TC-related rainfall in recent years but the South region has experienced a relatively consistent contribution of TC-related rainfall over the whole time period.

While TCs play an important role over the eastern US in terms of heavy rainfall events (Shepherd et al, 2007; Knight and Davis, 2009), our results indicate that their contributions to annual and TC-season total rainfall are quite small compared to non-TC related rainfall (Table 1). Regional average TC-related rainfall varies from 1% to 5% of the annual total. For each region, the standard deviation of TC-related rainfall exceeds the average value, whereas the annual total and non-TC related rainfall far exceeds their standard deviations, which in turn exceed that of the TC-related rainfall. As an example of a year with high TC activity, TCs in 2005 provided about 10% (>15%) of total annual (TC-season) total rainfall in the Midwestern region while its average regional contribution is 1% of annual total rainfall.

Table 1: Mean and standard deviation of annual total rainfall, non-TC related rainfall, and TC-related rainfall and their standard deviations for 1980-2007.

| Region | Area<br>(10 <sup>3</sup> km <sup>2</sup> ) | Annual    |          | Non-TC related |          | TC-related |          |
|--------|--------------------------------------------|-----------|----------|----------------|----------|------------|----------|
|        |                                            | Mean [mm] | Std [mm] | Mean [mm]      | Std [mm] | Mean [mm]  | Std [mm] |
| MW     | 394                                        | 977       | 104      | 968 (99%)      | 105      | 9 (1%)     | 20       |
| NE     | 387                                        | 1148      | 141      | 1122 (98%)     | 134      | 26 (2%)    | 34       |
| S      | 389                                        | 1395      | 174      | 1355 (97%)     | 182      | 40 (3%)    | 53       |
| SE     | 475                                        | 1319      | 152      | 1258 (95%)     | 157      | 61 (5%)    | 59       |

Using the two VIC land surface model simulations (one with and one without TC rainfall) we quantified the impact of TCs on drought. Figure 5 shows an example of the difference between the two simulations for hurricane Katrina, which swept through the central eastern US during late August/early September 2005. The shaded regions represent areas in moderate drought conditions. The impact of the hurricane in recovering from drought conditions is obvious. Figure

6 shows details of how this impact manifests for three selected points along the hurricane path. Point 1 is impacted by one TC during 2005, and the other two points by more than one TC. The TC events cause large increases in soil moisture that in some cases (point 1) delays the initiation of drought by two weeks and the two soil moisture series only converge in the late autumn. For point 2, the TC rainfall keeps the soil moisture out of drought for much of the summer. For point 3, hurricane Katrina breaks the potential long-term drought by about 1 month earlier than in the experiment without TC-related rainfall. For the eastern US as a whole, Figure 7 summarizes the impact of tropical cyclones on drought in terms of the difference in the number of droughts between the simulations without and with TC rainfall, the number of days delay in drought initiation and the number of days earlier in drought recovery. This work has been submitted as a journal paper to J. Climate (Kam et al., 2012).

#### *b) Climatological Analysis of Low Flows over the Eastern US*

Our initial focus has been on the four river basins: Delaware, Potomac, Catawba and Chattahoochee. Figure 10 shows the locations of 117 sites from the Northeast US that are within and near the Delaware and Potomac basins. Step change and trend statistics were calculated on the n-day annual low flow time series and are plotted in Figure 10. The Mann Kendall (MK) test was conducted to find any increasing, decreasing, or no change in the trend of the time series. The nonparametric Pettitt test was used to detect whether there has been a significant change in mean. Figure 10 (mid left through bottom right) shows the result of the MK test. Figure 10 (top right) shows the result of the Pettitt test. The results show that Pettitt test recognizes at least one year when the trend changes but that does not mean that overall the time series will be recognized as an increasing or decreasing time series by MK test.

Figures 11-13 show detailed results from three selected sites out of the 117 northeast USGS sites which display differing results in terms of long-term trends and correlations between different n-day low flow values. Figure 11 (first column) shows an increasing trend for all n-day time series using the MK test, assuming a 95% confidence. The n-day flow series are reasonably correlated, especially for 7-day and 30-day low flows, which may be partly due to the increasing trend in all n-day flows. The attribution of the increasing trends is being investigated. Figure 12 shows a site with a decreasing trend in all n-day low flow series. Again the n-day flow values are well correlated, but only for adjoining n-day values (e.g. 1 and 7 or 30 and 90). The correlation of 1-day and 90-day values diverges greatly for higher values indicating that in drought conditions, the low flows are more tightly correlated than in wet years for this site. Figure 13 shows a step change in behavior around 1955 when low flow values drop dramatically and then steadily increase thereafter, which is likely due to a structural change (gauge change or land use change) rather than a shift in climate.

#### *c) Flood Studies*

Hydroclimatological analyses of flooding in the Potomac River basin have focused on “mixture” distributions, change points and trends and controls of upper tail behavior of flood peak distributions (Smith et al. 2012a). The dominant flood agents for the Potomac River at Point of Rocks are winter/spring extratropical systems, like the March 1936 flood that produced record flood peaks throughout major drainages of the eastern US (Figure 14). Tropical cyclones play an

important role in determining upper tail properties and contribute to a fall peak in flood frequency (Figure 14). Despite a history of major land use transformation, including deforestation in the late 19<sup>th</sup> and early 20<sup>th</sup> centuries, there is little evidence of nonstationarities in flood occurrences for the Potomac River at Point of Rocks (in Figure 15, we show multidecadal analyses of seasonality in flood occurrence). During year 2 of the project, we will examine spatial properties of flood extremes and couple flood studies with analyses of drought occurrence.

The East Branch Delaware River basin is an important water supply source for the New York City metropolitan region and contains a dense network of USGS stream gages (Figure 16). Hydroclimatological analyses of flooding in the East Branch Delaware have focused on nonstationarities in flood occurrence and spatial heterogeneities in precipitation, runoff and flood peaks (Smith et al 2012b). Orographic precipitation mechanisms play an important role in the flood hydroclimatology of the region. Rainfall analyses (see Figure 17) have been carried out for sequence of major flood events in the Delaware River basin and used to assess precipitation controls of the striking contrasts in runoff and flood peaks between Beaver Kill and the East Branch Delaware above Margaretville (Figure 18). Flooding from Hurricane Irene in August of 2011 produced the record flood peak at Margaretville and devastated portions of the upper Delaware basin. Case study analyses of this event will be completed during year 2 to examine upper tail properties of flood peaks in the region. During year 2, we will also complete coupled studies of drought and flood in the Delaware basin.

Flood studies in Texas have been carried out to characterize the relative role of tropical cyclones as flood hazards. We have focused on spatial analyses of tropical cyclone flood hazards and demonstrated the sharp decrease in tropical cyclone flood frequency with distance from the Gulf Coast in Texas (Figure 19). Analyses of flood hydroclimatology in Texas have also highlighted the role of abrupt changes relative to slowly varying trends (Villarini et al. 2012).

Flood studies have been initiated in the Chattahoochee and Catawba River basins. During Year 2, these studies will be completed and coupled studies of drought and flood occurrence will be performed.

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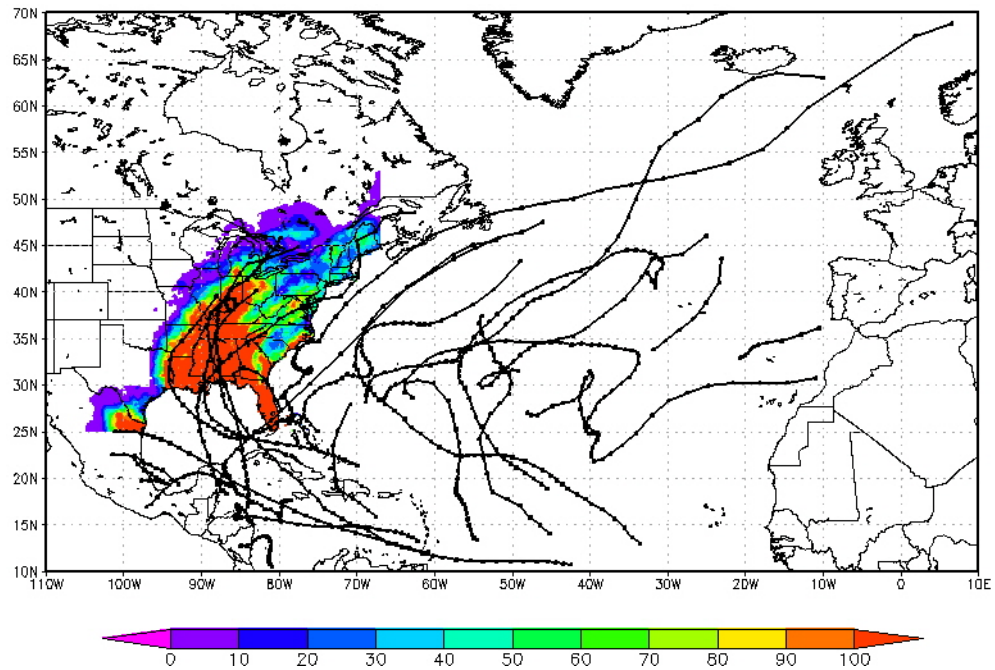


Figure 1: 2005 tropical cyclone tracks from the Atlantic HURDAT database and accumulated rainfall (mm) within 5 degrees (about 500km) from the center of cyclone. Total number of tropical cyclones in 2005 was 28.

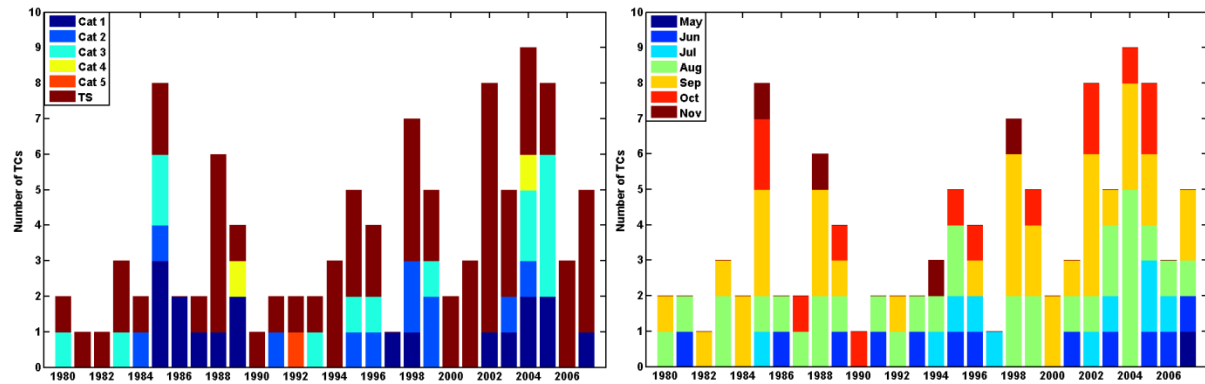


Figure 2: Total number of tropical cyclone landfalls over the United States during 1980-2007 in terms of category (left), and month (right). Storm categories refer to the Saffir/Simpson Hurricane Wind scale number.

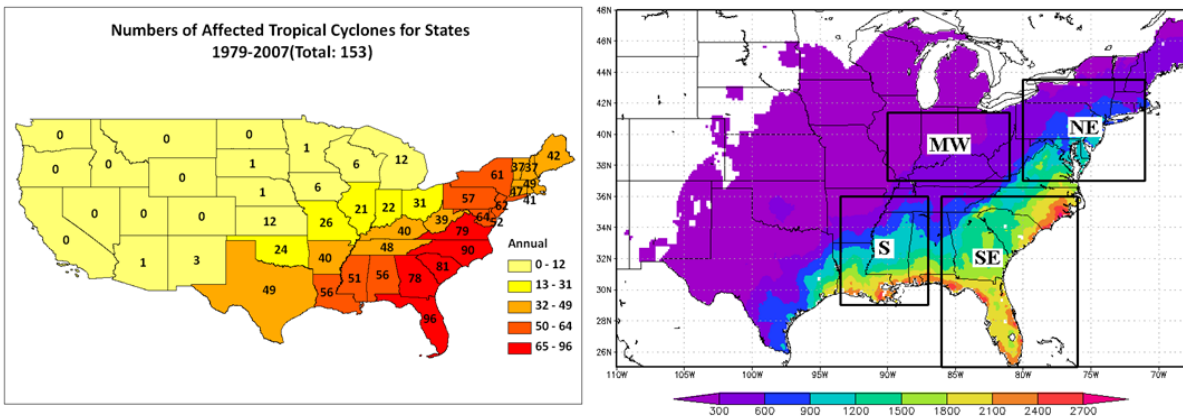


Figure 3: Total number of tropical cyclones by state (left) and accumulated tropical cyclone related rainfall (mm) (right) for 1979-2007. Boxes represent the study regions: Southeastern (SE), the South (S), the Midwestern (MW), and the Northeastern (NE) regions.

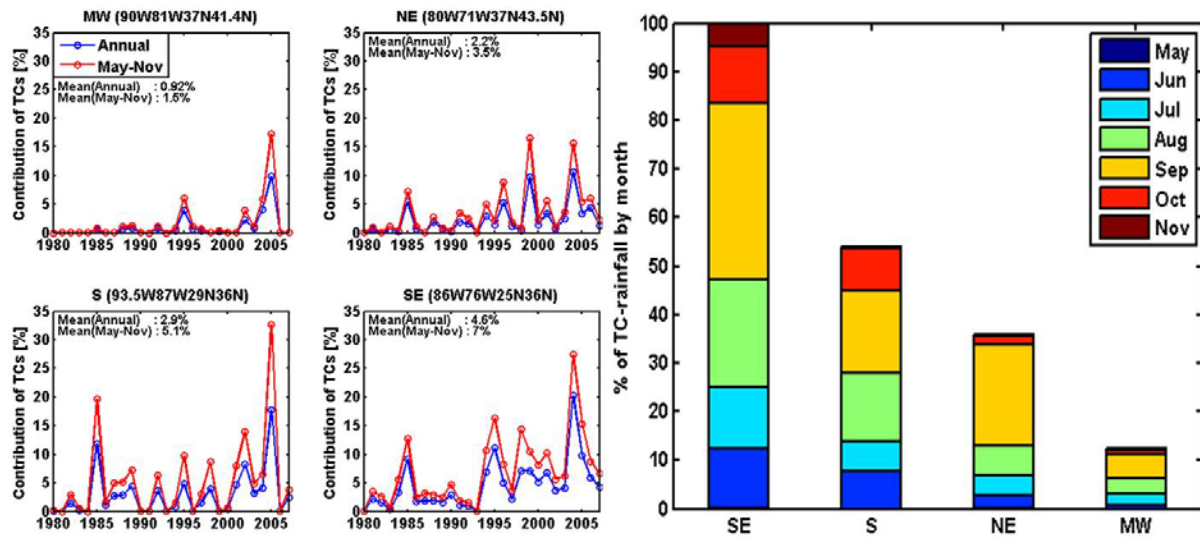


Figure 4: (left) Regional contribution of tropical cyclone-related rainfall to annual (blue) and cyclone-season (red) total rainfall. (right) Average monthly tropical cyclone-related rainfall over each region relative to the total tropical cyclone-rainfall over the Southeastern region (100%).

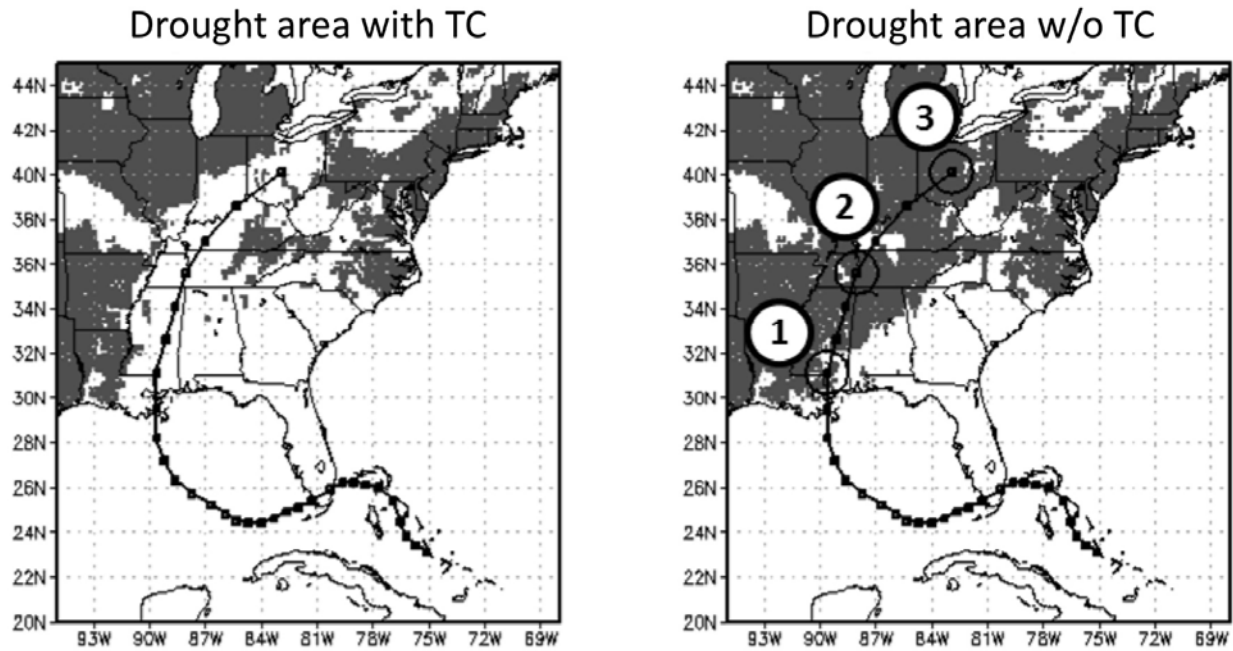


Figure 5: Example of the impact of hurricane Katrina on drought around August 31st 2005 from the control VIC simulation (left) and the VIC simulation without tropical cyclone rainfall (right). Gray area represents relatively dry land surface conditions based on soil moisture less than the 20th percentile. Data for the three grids in the path of the hurricane is shown in detail in Figure 12.

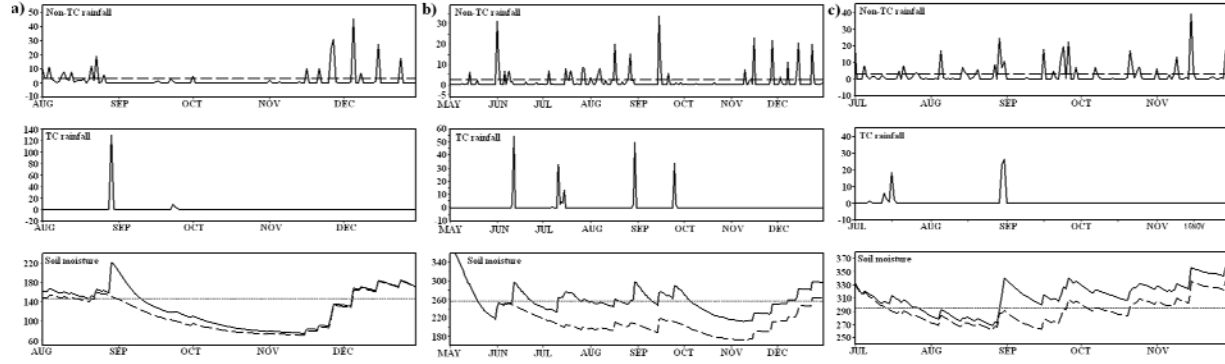


Figure 6: Time series of daily total column soil moisture (third row) from the control simulation (solid line) and the experiment simulation (dotted line) at three grids (first column: Point #1, from August 1st, 2005 to December 31st, 2005; second column: Point #2, from May 1st, 2005 to December 31st, 2005; third column: Point #3, from July 1st, 2005 to November 30th, 2005). The first (second) row represents non-TC related (TC-related) rainfall (mm/day). Long dash lines in the first row represent the mean of daily rainfall from climatology (1980-2007). Solid (long dash) lines in the third row represent daily total column soil moisture from the control (experiment) simulation, and the dot line in the third row represents the corresponding soil moisture to the 20th percentile relative to all monthly simulated soil moisture from the control run.

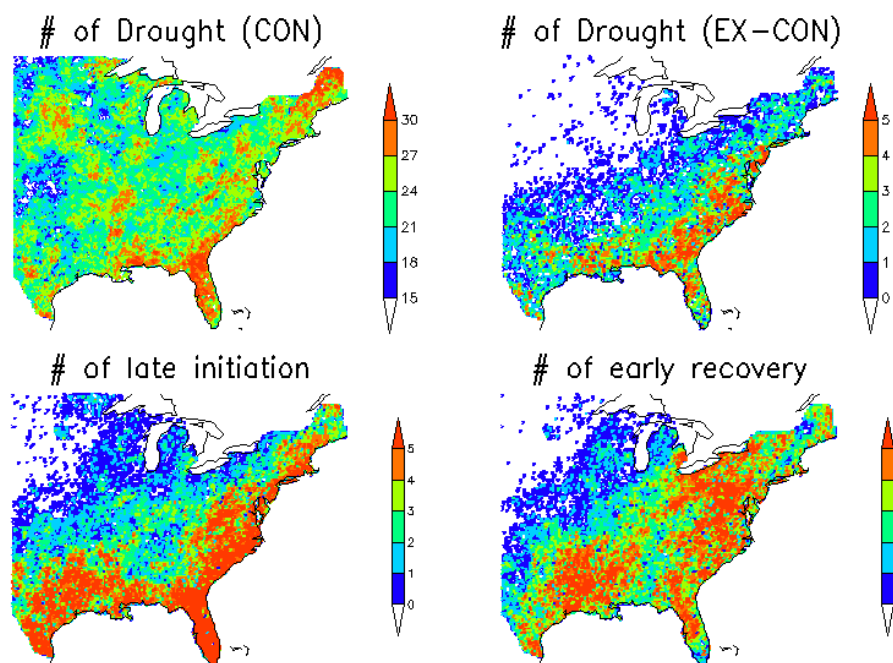


Figure 7: Total number of moderate severe and short-term drought events (top-left), the difference in number of droughts between the control and non-TC simulations (top-right; EX-CON), late drought initiation (bottom-left), and early drought recovery (bottom-right) during 1980-2007.

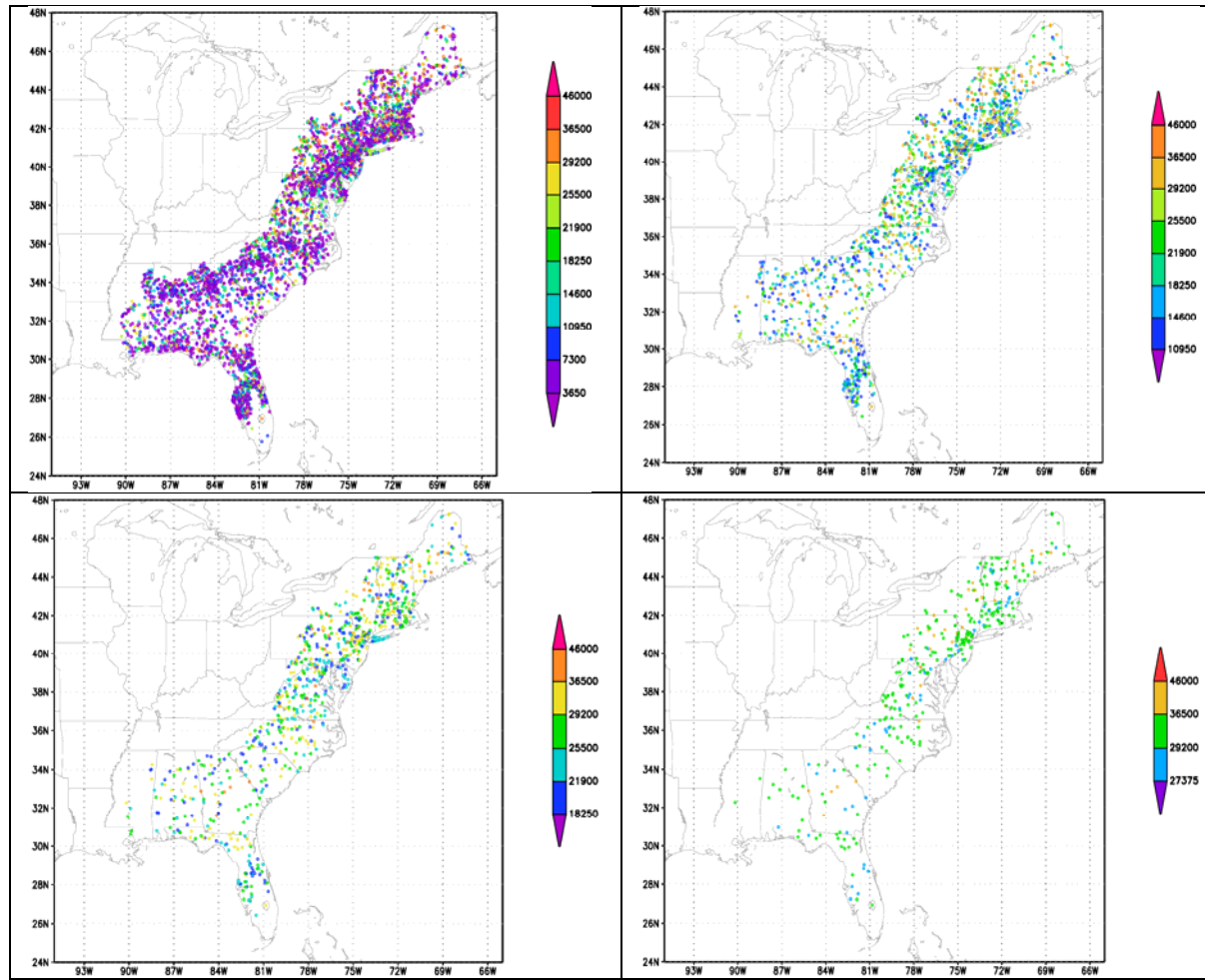


Figure 8. Distribution of the length of streamflow records (days) of 4878 sites (top left); Sites with 30 years or more of streamflow data (1387 sites) (top right); Sites with 50 years or more of streamflow data (807 sites) (bottom left); Sites with 75 years or more of streamflow data (328 sites) (bottom right).

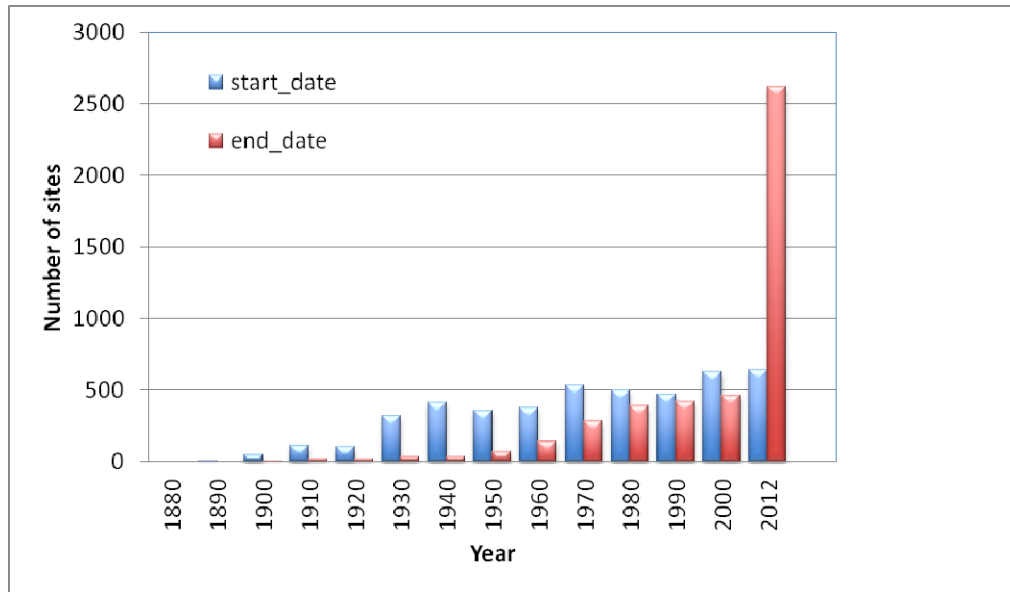


Figure 9. Histogram of starting date and ending date of 4878 USGS sites in the eastern US.

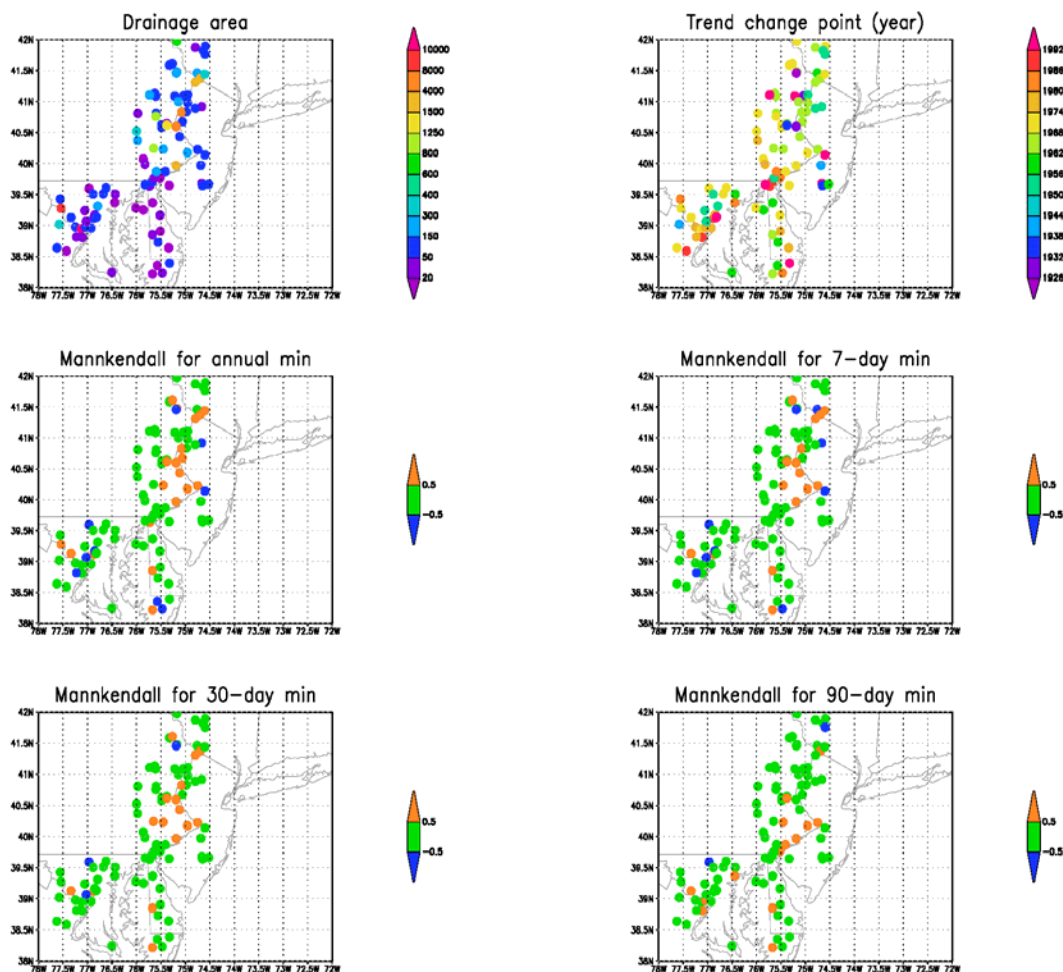


Figure 10. Locations and statistics of 117 sites over the north eastern US showing: drainage areas in km<sup>2</sup> (top left); result of the Pettitt test shows the year that the first step change occurred (top right); Mann Kendall test shows increasing (orange), no trend (green), and decreasing (blue) trends for 1-day minimum low flow (mid left), 7-day low flow (mid right), 30-day low flow (bottom left), and 90-day low flow (bottom right).

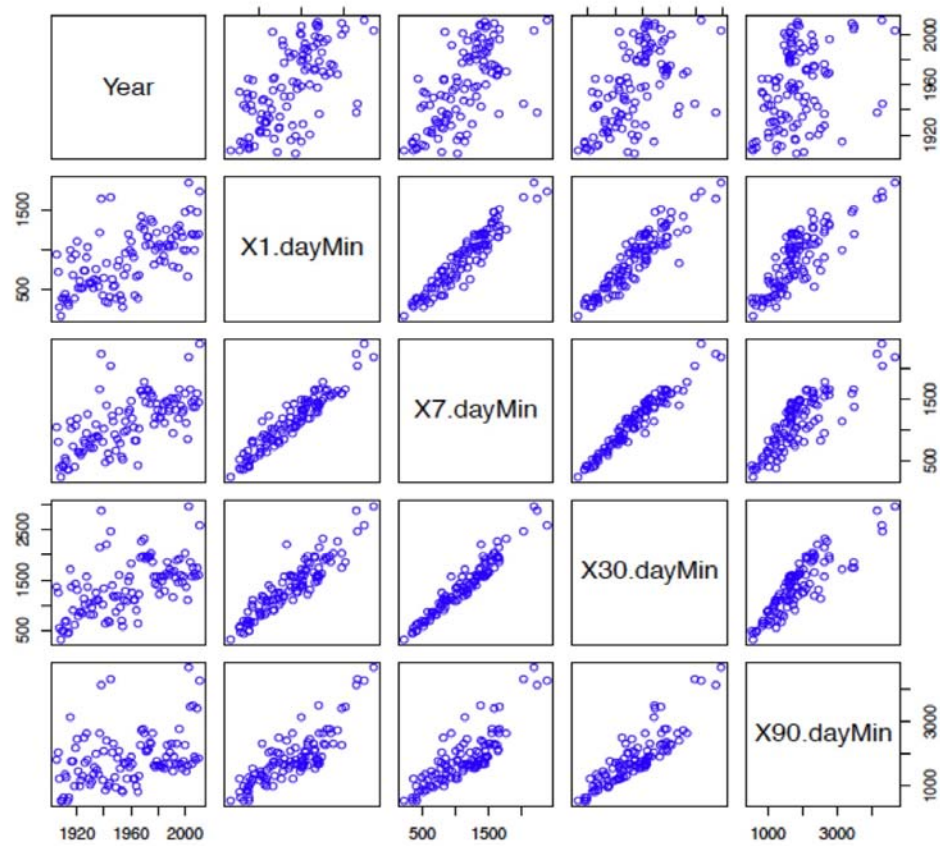


Figure 11. Time series and scatter plots of n-day (1, 7, 30 and 90) annual low flows for USGS gauge 01434008.

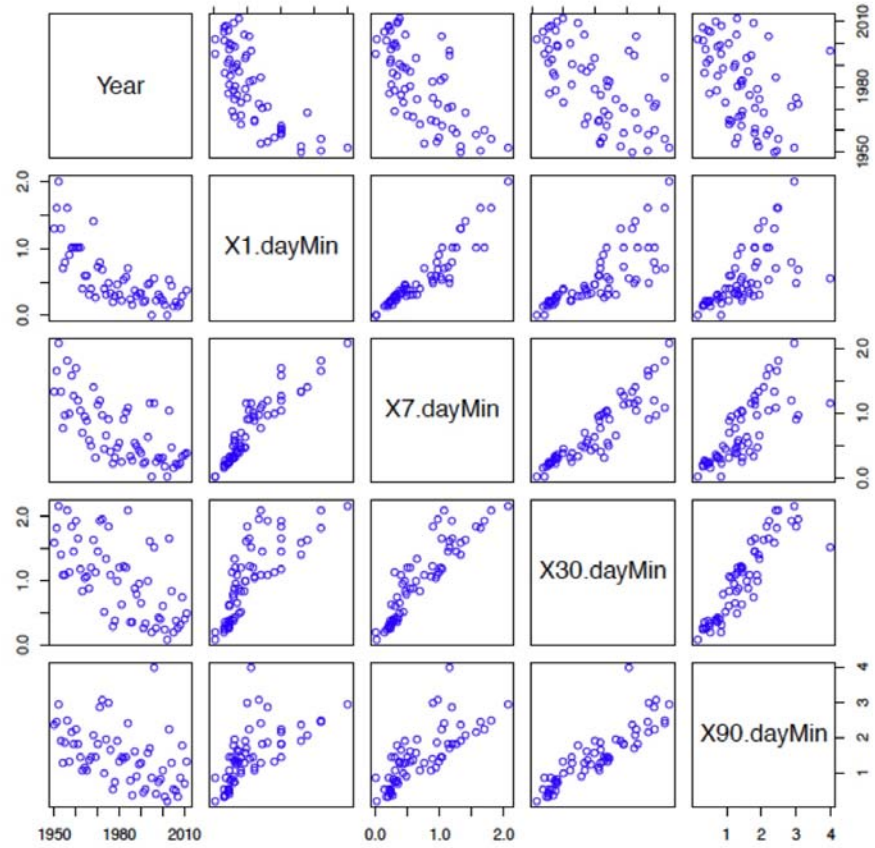


Figure 12. Time series and scatter plots of n-day (1, 7, 30 and 90) annual low flows for USGS gauge 01585500.

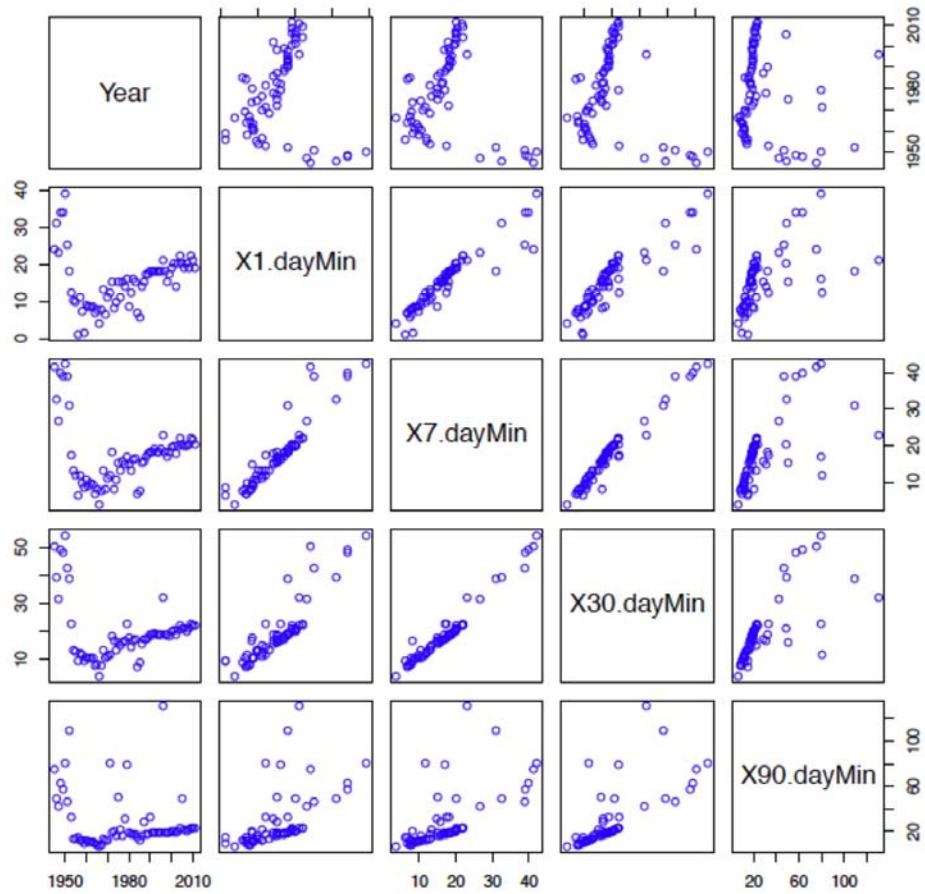


Figure 13. Time series and scatter plots of n-day (1, 7, 30 and 90) annual low flows for USGS gauge 01592500.

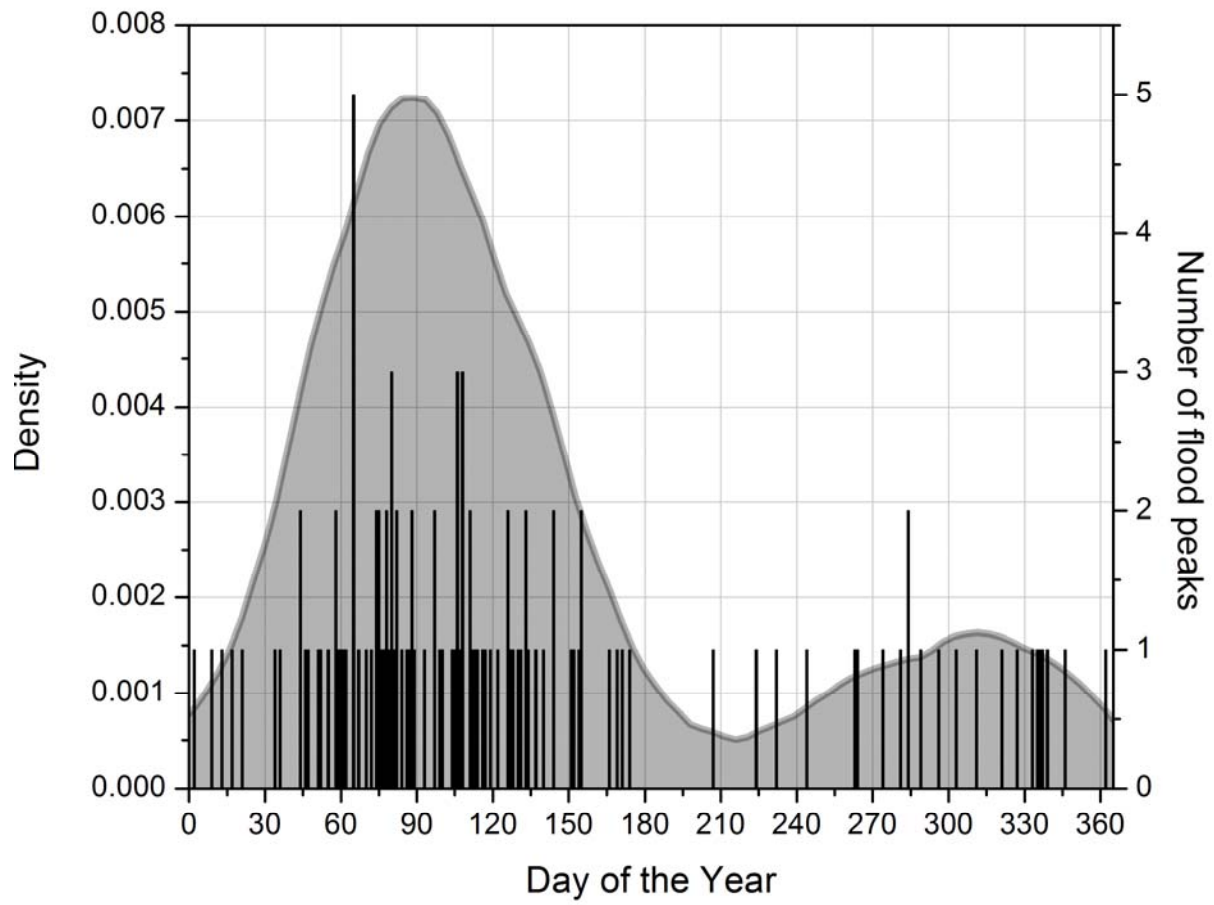


Figure 14. Seasonal distribution of annual flood peaks for the Potomac River at Point of Rocks, MD. Day 1 = January 1.

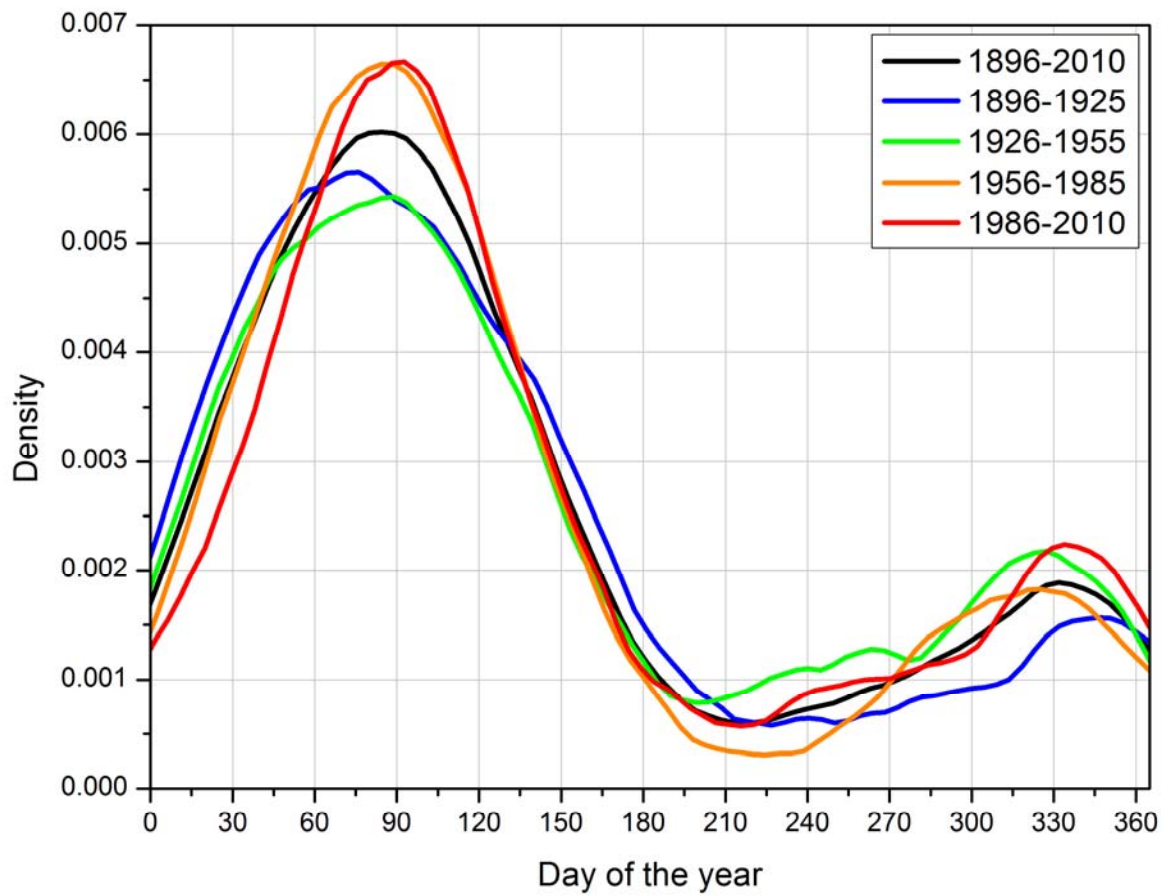


Figure 15. Multidecadal variation in seasonality of flood peak occurrences for the Potomac River at Point of Rocks, MD. Day 1 = January 1.

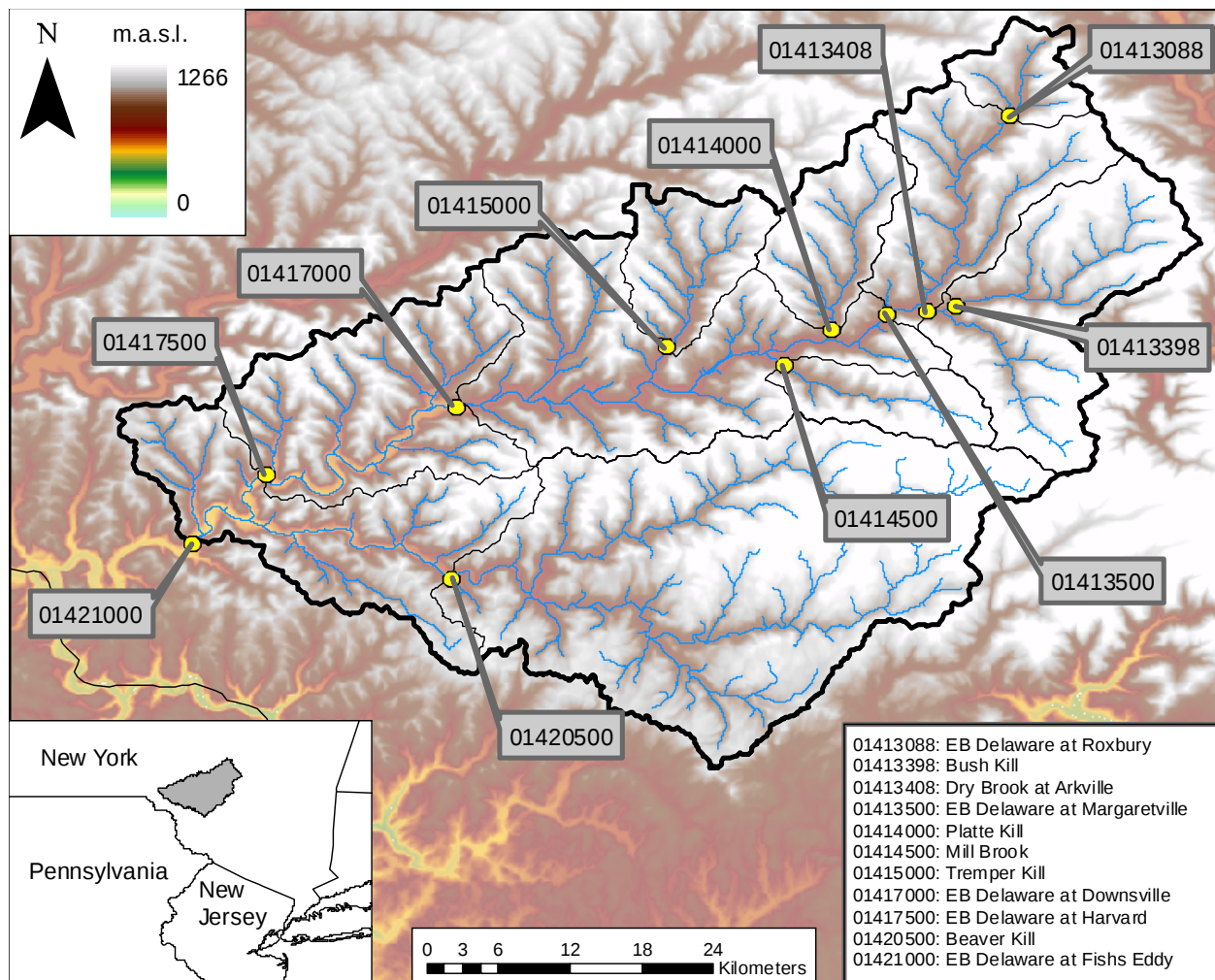


Figure 16. Location map for East Branch Delaware study watersheds, with USGS stream gaging locations.

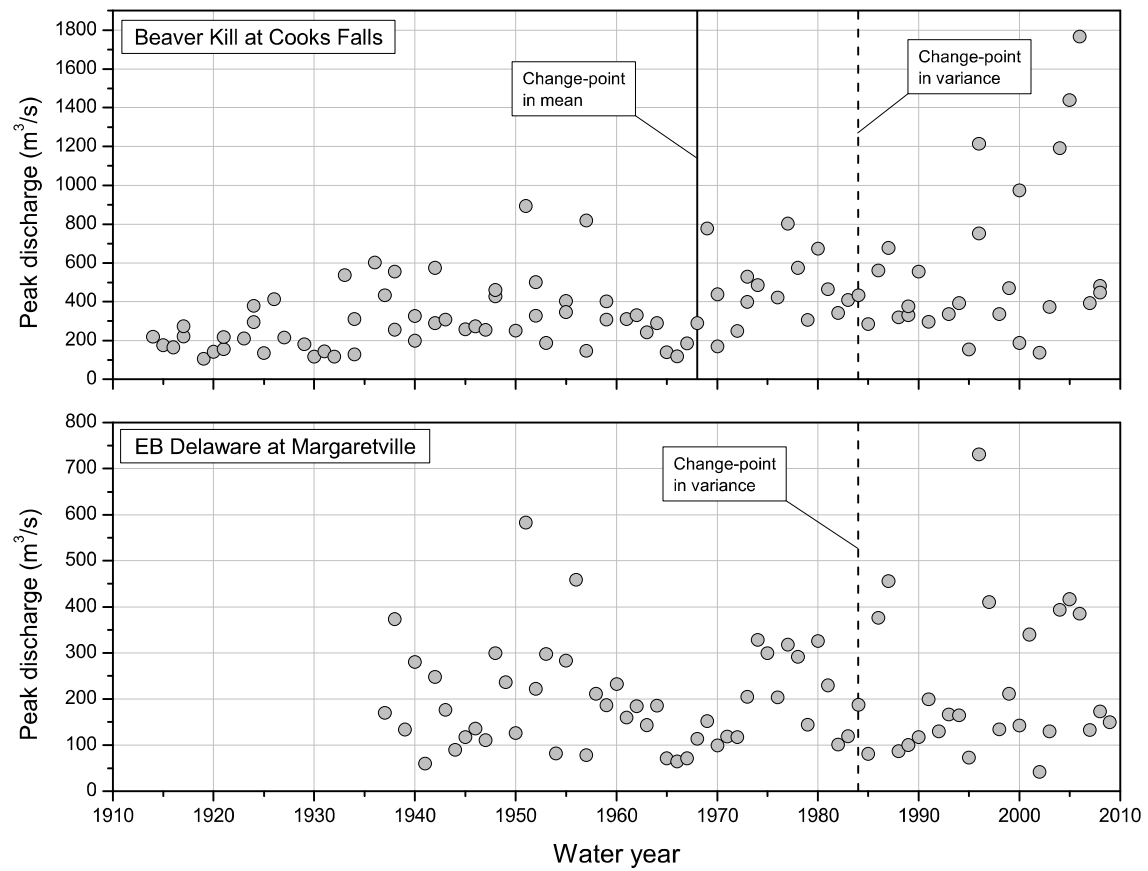


Figure 17. Annual flood peaks for Beaver Kill (top) and East Branch Delaware River at Margaretville (bottom). Change point times are shown.

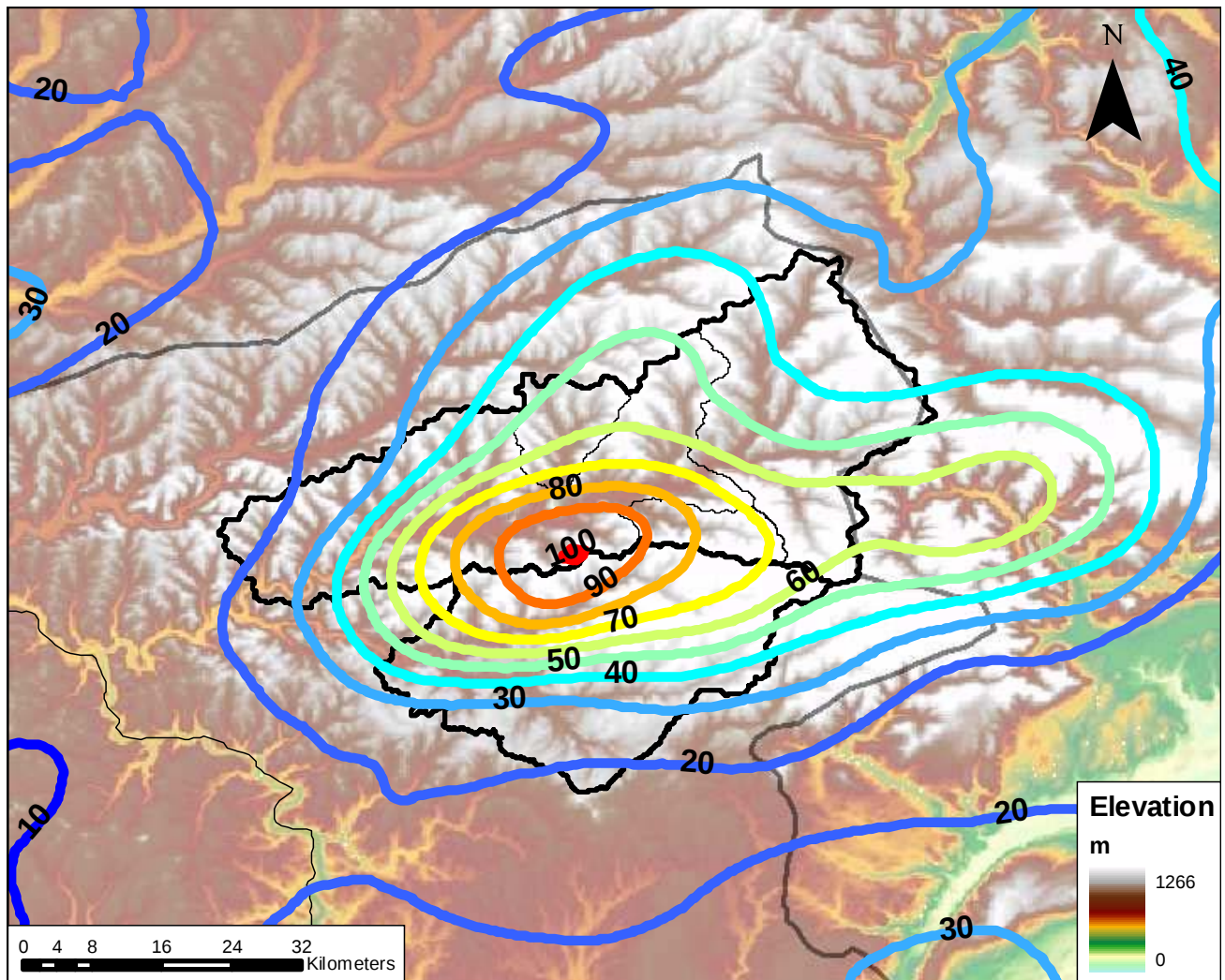


Figure 18. Storm total rainfall accumulation (mm) for 19 June 2007 storm. Basin boundaries and terrain are included.

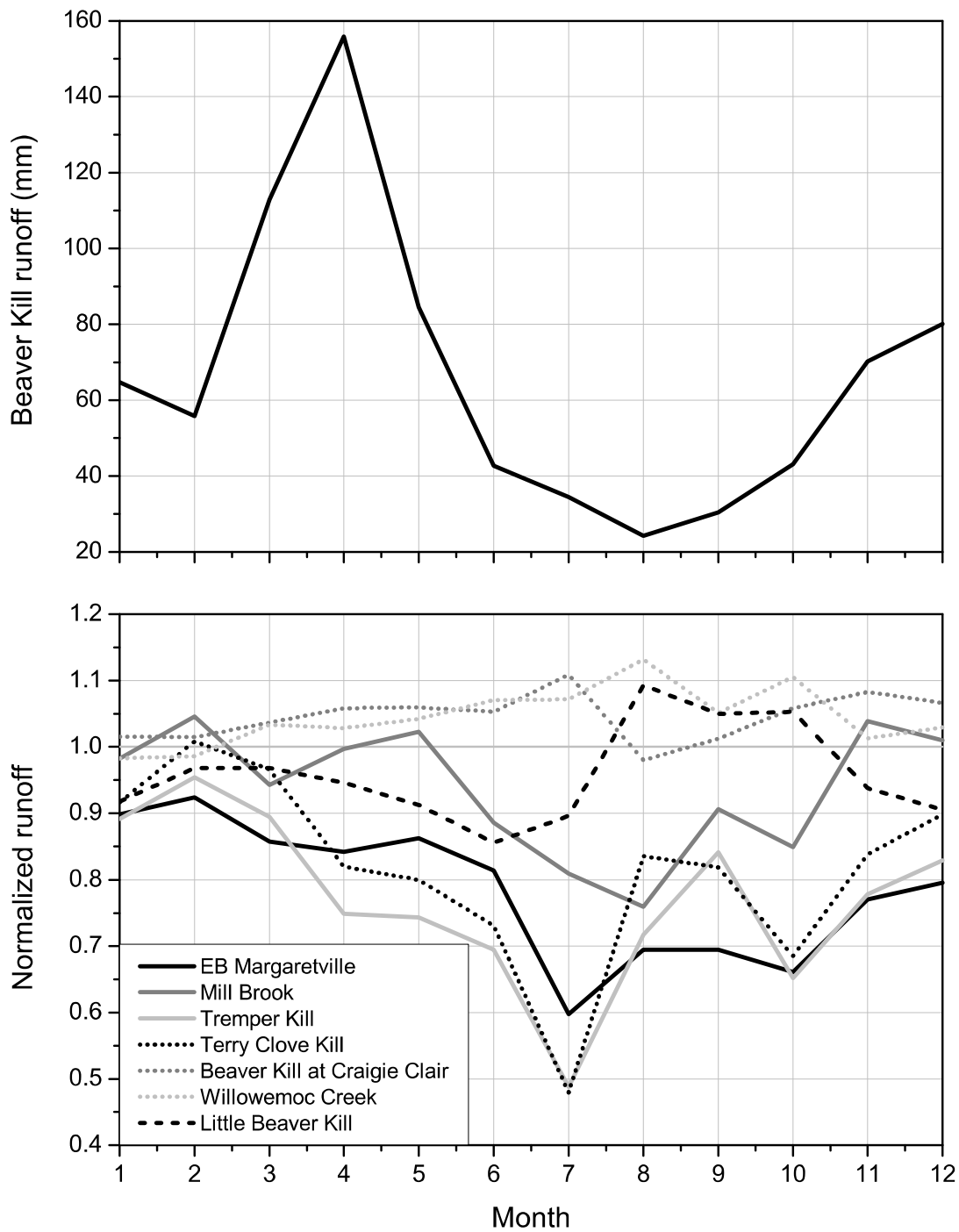


Figure 19. Mean month runoff (mm) in Beaver Kill (top). Normalized monthly runoff values for other stream gaging stations in the East Branch Delaware are computed by dividing by Beaver Kill values. Results are shown in bottom figure.

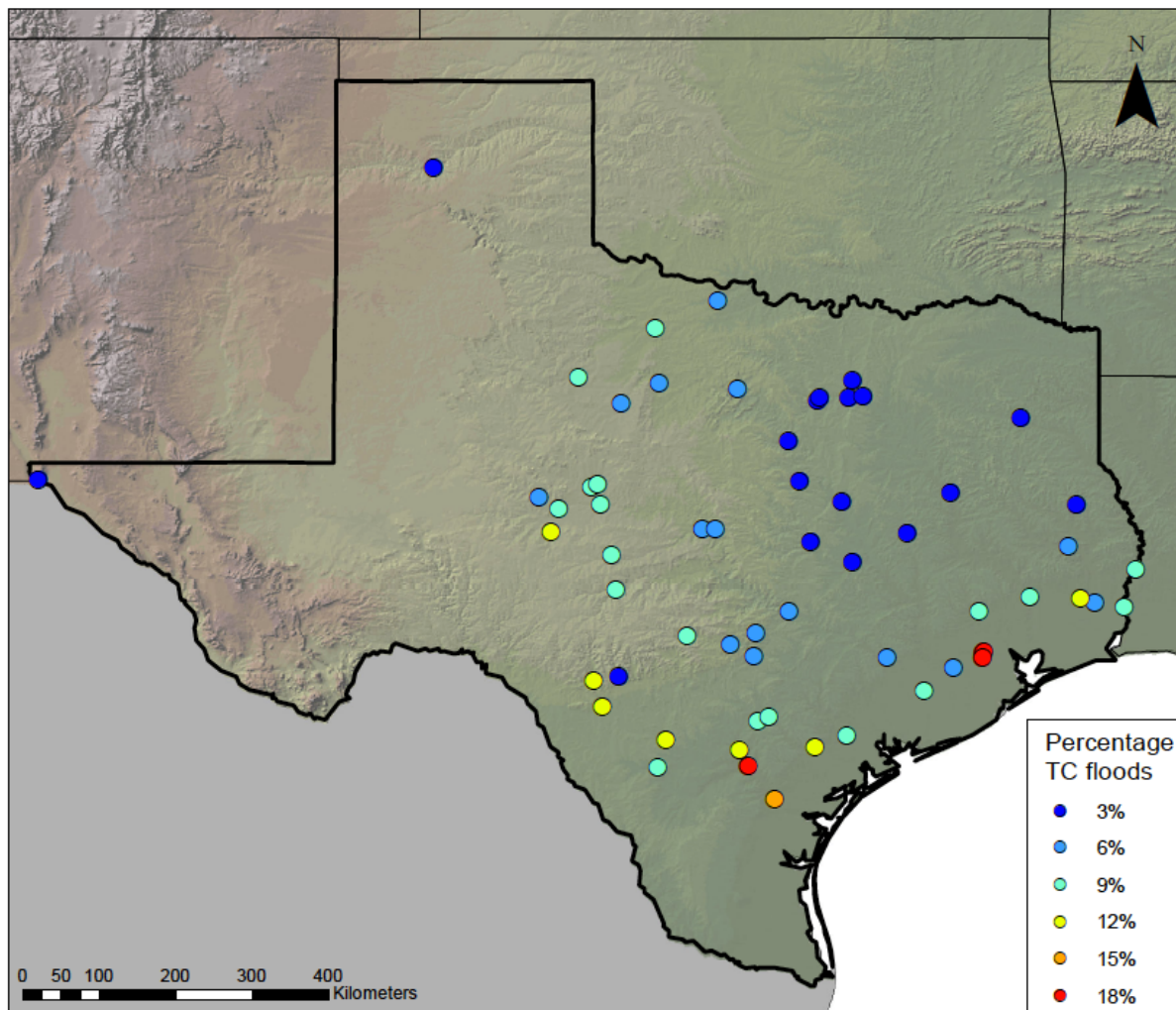


Figure 20. Percentage of annual flood peaks from tropical cyclones for USGS stream gaging stations in Texas with long records.

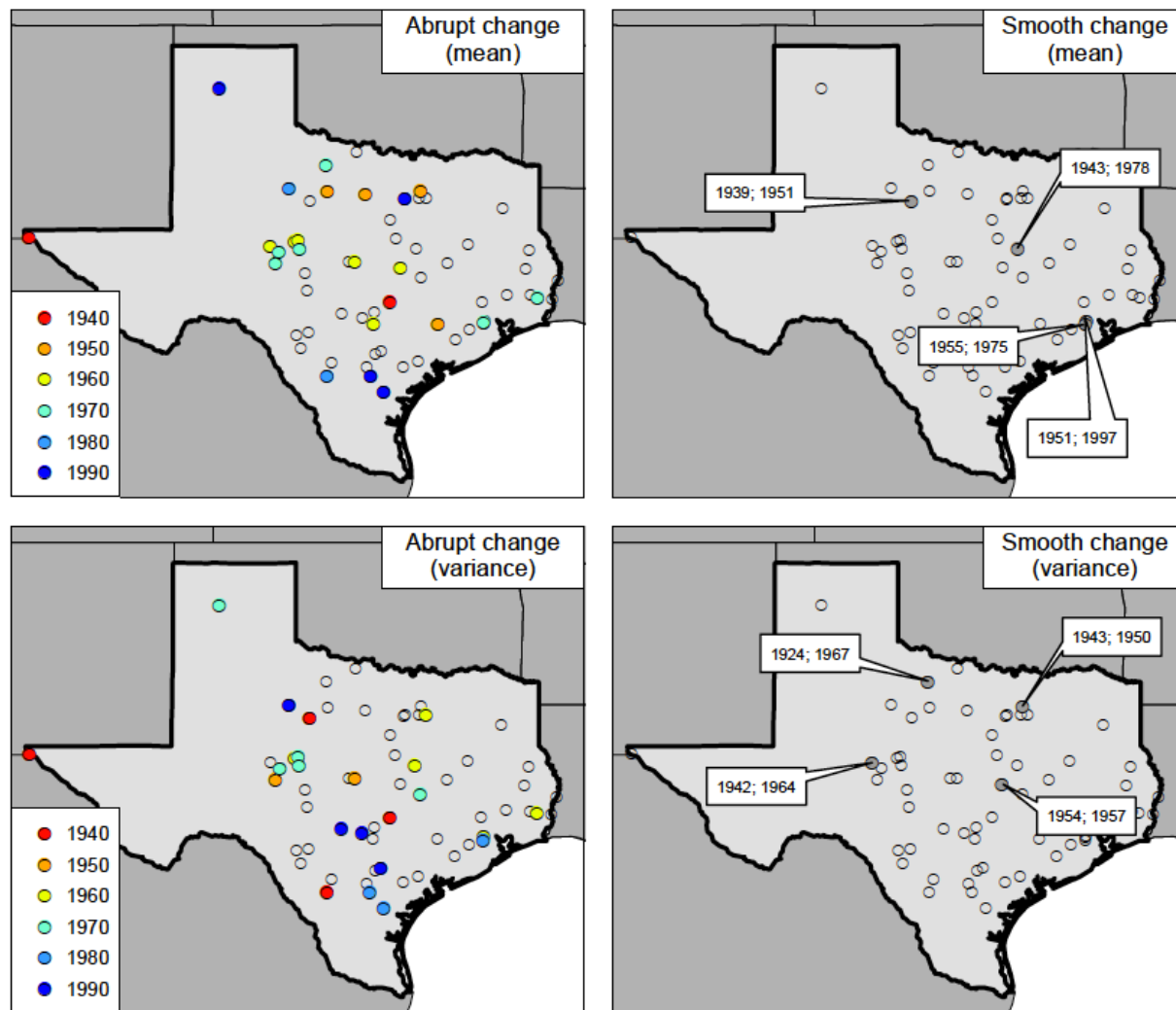


Figure 21. Analyses of change points and trends for annual flood peak observations from Texas.

## **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](http://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

|                                 |                                   |
|---------------------------------|-----------------------------------|
| <b>Title:</b>                   | Information Transfer Program      |
| <b>Project Number:</b>          | 2011NJ282B                        |
| <b>Start Date:</b>              | 3/1/2011                          |
| <b>End Date:</b>                | 2/29/2012                         |
| <b>Funding Source:</b>          | 104B                              |
| <b>Congressional District:</b>  | NJ-006                            |
| <b>Research Category:</b>       | Not Applicable                    |
| <b>Focus Category:</b>          | None, None, None                  |
| <b>Descriptors:</b>             | None                              |
| <b>Principal Investigators:</b> | Christopher Obropta, Diana Morgan |

## Publications

There are no publications.

## Information Transfer Program

The information transfer program has emphasized development of the website and e-based communications with stakeholder groups. It has also focused on the production of substantive newsletters addressing specific water resource issues as an effective way to communicate information to the public.

Although no issues of the newsletter were produced during FY2011, one issue of the newsletter is about to be released and a second is in the planning process. Our annual research update issue is currently in production. It is intended to showcase NJWRRI-funded research and also to illustrate the importance of research in solving water-related problems. A second issue will focus on research efforts currently underway in the Barnegat Bay. 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, as paper copies to all members of the New Jersey legislature and Congressional delegation, and it is posted on our website.

NJWRRI co-sponsored a series of design workshops in support of the New Jersey Environmental Infrastructure Financing Program's Barnegat Bay Initiative (<http://www.njeit.org/barnegatbayinitiative.html>). The workshops offered instruction on recent innovations in two areas: Subsurface Gravel Wetland Design and Porous Pavement Design. Workshops were held March 14, 2012 in Morristown, March 15, 2012 in New Brunswick and March 16, 2012 in Toms River. More information can be found at the following website: <http://www.njwrri.rutgers.edu/pdfs/subsurface-gravel-wetland-and-porous-pavement-design-workshops.htm>

Our website ([www.njwrri.rutgers.edu/](http://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. The home page and 'events' pages are regularly updated to highlight upcoming events, publications and other water-related news. This year is the first time that traffic to the website has been analyzed, and a comprehensive report has been compiled. Overall, there were about 16,000 visits to the website last year. The pages receiving the most visits, outside of the home page, were the watershed organizations, new book releases and research pages. This is important information for us in terms of developing and maintaining the website. 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 the benefit of this new information.

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. As a member of the council, we co-sponsored the 2011 New Jersey Water Monitoring

Summit, held December 1-2, 2011 in West Trenton, NJ.

[http://www.state.nj.us/dep/wms/2011\\_summit.htm](http://www.state.nj.us/dep/wms/2011_summit.htm)

NJWRRI will be a co-sponsor of the 5th Passaic River Symposium to be held October 19, 2012 at Montclair State University. We intend to provide scholarships for students to attend the symposium, and NJWRRI-funded research projects will be presented.

# **USGS Summer Intern Program**

None.

| <b>Student Support</b> |                               |                               |                             |                            |              |
|------------------------|-------------------------------|-------------------------------|-----------------------------|----------------------------|--------------|
| <b>Category</b>        | <b>Section 104 Base Grant</b> | <b>Section 104 NCGP Award</b> | <b>NIWR-USGS Internship</b> | <b>Supplemental Awards</b> | <b>Total</b> |
| <b>Undergraduate</b>   | 3                             | 2                             | 0                           | 0                          | 5            |
| <b>Masters</b>         | 2                             | 1                             | 0                           | 0                          | 3            |
| <b>Ph.D.</b>           | 6                             | 1                             | 0                           | 0                          | 7            |
| <b>Post-Doc.</b>       | 1                             | 2                             | 0                           | 0                          | 3            |
| <b>Total</b>           | 12                            | 6                             | 0                           | 0                          | 18           |

## Notable Awards and Achievements

For project 2011NJ280B, "Development of a Push-Pull Technique to Simultaneously Characterize Volatilization and Biodegradation Rates of VOCs in Shallow Wetland Sediments," the following is reported: Research funded by this grant produced preliminary results which were incorporated in a larger proposal to the National Science Foundation, which was funded with a \$310,000 grant (CBET-1133281). The purpose of the NSF grant is to expand and scale up the promising early results generated from NJWRRI-funded experiments.

For project 2011NJ281B, "Release of Hazardous Metals into Surface and Groundwater by Microbial Oxidation of Sulfide Minerals," the following award is reported: Rutgers SAS Award for Distinguished Contributions to Undergraduate Education.

Project 2011NJ277B, "Assessment of the water-borne pathogen, *Batrachochytrium dendrobatidis*, in New Jersey amphibians and their habitat," reported the first case of Ranavirus in New Jersey amphibians. Results from this discovery are being shared with the NJ Division of Fish and Wildlife to implement wide-scale sampling for Ranavirus throughout the state and to create educational materials for wildlife managers in order to help document outbreaks and contain the spread of the disease. In addition, more stringent sterilization protocols were recommended for the consulting firm, Herpetological Associates, Inc., when moving from site to site to contain the disease.

This project also reports an invited seminar: Monsen-Collar, Kirsten; Lisa Hazard; Paola Dolcemascolo. 2012. Molecular Detection of Pathogens in New Jersey Amphibians. University of Pennsylvania, Eco Lunch Seminar Series, February 24, 2012.

For project 2010NJ231B, "Green Remediation of Tetracycline in Soil-Water Systems," the following award is reported: 1st prize in the graduate student oral presentation competition for the presentation "Drinking water treatment residuals as a "green" sorbent for veterinary antibiotics: Results from an incubation study." 2012 Annual Meeting of the Hudson-Delaware Chapter of the Society of Environmental Chemistry and Toxicology, April 26-27, 2012 in Montclair, NJ.

For project 2010NJ230B, "Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersey Pinelands," the following award is reported: a "Best Student Presentation" award for the oral presentation "Subsurface hydrologic controls on sensitive wetland ecosystems in the New Jersey Pinelands" was given at the Mid-Atlantic Chapter of the Ecological Society of America meeting on April 9, 2011 in Montclair, NJ.

## Publications from Prior Years

1. 2007NJ144B ("Using assimilated C-DNA to fingerprint active microorganisms in methylmercury demethylation by stable-isotope probing") - Dissertations - Yu, Riqing. May 2011. Microbial Mercury Methylation and Demethylation: Biogeochemical Mechanisms and Metagenomic Perspectives in Freshwater Ecosystems. Ph.D. dissertation. Department of Environmental Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 206 pages.
2. 2008NJ161B ("Microbial Mobilization of Arsenic and Selenium Oxyanions in Subsurface Aquifers") - Articles in Refereed Scientific Journals - Rauschenbach I, Narasingaro P, Haggblom MM. 2011. Desulfurispirillum indicum sp. nov., a selenate and selenite respiring bacterium isolated from an estuarine canal in Southern India. Int. J. System. Evol. Microbiol. 61: 654-658. (doi:10.1099/ijs.0.022392-0)
3. 2008NJ161B ("Microbial Mobilization of Arsenic and Selenium Oxyanions in Subsurface Aquifers") - Articles in Refereed Scientific Journals - Rauschenbach I, Yee N, Haggblom MM, Bini E. 2011. Energy metabolism and multiple respiratory pathways revealed by genome sequencing of Desulfurispirillum indicum strain S5. Environmental Microbiology 13:1611-1621. (doi:10.1111/j.1462-2920.2011.02473.x)
4. 2008NJ154B ("Volatilization of PCBs from the Tappan Zee region of the Hudson River") - Articles in Refereed Scientific Journals - Sandy, Andy L.; Jia Guo; Robert J. Miskewitz; Wade R. McGillis; Lisa A. Rodenburg. 2012. Fluxes of polychlorinated biphenyls volatilizing from the Hudson River, New York measured using micrometeorological approaches. Environmental Science and Technology, 46, 885-891.
5. 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") - Dissertations - Bugel, S.M. 2011. Contaminant effects on vitellogenesis and oogenesis in zebrafish (Danio rerio) and killifish (Fundulus heteroclitus) from the chemically impacted Newark Bay, NJ. Ph.D. dissertation. Department of Environmental Sciences, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 205 pages.
6. 2009NJ188B ("Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities") - Dissertations - Honarbakhsh, Maryam. 2011. Impact of wastewater effluents on the structure and antibiotic resistance of native microbial communities. MS dissertation. Department of Biochemistry and Microbiology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 58 pages.
7. 2010NJ216B ("Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues") - Other Publications - Hazard, L.C., K. Kwasek, E. Koelmel, M. Gonzalez-Abreu, and S. Gerges. 2011. Variation in salinity aversion of northeastern amphibian species may influence response to anthropogenic salinization. Partners in Amphibian and Reptile Conservation, Northeast Chapter Annual Meeting. Millersville, MD. (Poster presentation)
8. 2010NJ216B ("Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues") - Other Publications - Kwasek, K. and L. Hazard. 2011. Behavioral responses of the eastern newt to road deicers. Partners in Amphibian and Reptile Conservation, Northeast Chapter Annual Meeting. Millersville, MD. (Poster presentation)
9. 2010NJ216B ("Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues") - Dissertations - Gonzalez-Abreu, Madelyn. 2011. Behavioral response of American Bullfrogs and Northern Leopard Frogs to increased environmental salinity. Master's thesis. Department of Biology and Molecular Biology, Montclair State University, Montclair, NJ. 28 pages.
10. 2010NJ216B ("Impact of salinization on New Jersey amphibian species: A physiological approach to water quality issues") - Dissertations - Koelmel, Erika. 2011. Behavioral responses of two Ranid frogs

- to road deicers: Does terrestriality influence sensitivity? Master's thesis. Department of Biology and Molecular Biology, Montclair State University, Montclair, NJ. 26 pages.
11. 2010NJ221B ("Comparative toxicological assessment of gasoline oxygenates MTBE, ETBE and TAME, and their metabolites, in the zebrafish cardiovascular system") - Articles in Refereed Scientific Journals - Bonventre, Josephine A., Lori A. White, Keith R. Cooper. 2012. Craniofacial abnormalities and altered wnt and mmp mRNA expression in zebrafish embryos exposed to gasoline oxygenates ETBE and TAME. *Aquatic Toxicology* 120-121: 45-53. Epub: <http://dx.doi.org/10.1016/j.aquatox.2012.04.008>.
  12. 2010NJ222B ("Investigation into the Role of Arsenic Reducing Bacteria in the Mobilization of Arsenic into Ground Water in New Jersey") - Articles in Refereed Scientific Journals - Mumford, A.C., Barringer, J.L., Benzel, W.M., Reilly, P.A., and Young, L.Y. 2012. Microbial transformations of arsenic: mobilization from glauconitic sediments to water. *Water Research* 46: 2859-2868.
  13. 2010NJ231B ("Green Remediation of Tetracycline in Soil-Water Systems") - Conference Proceedings - Punamiya, P., D. Sarkar and R. Datta. 2011. Evaluating the potential of Al-based drinking water treatment residuals as a "green" sorbent for tetracycline: A comprehensive batch sorption study. Proceedings of the Joint Annual Conference of the Hudson Delaware and Chesapeake Potomac Regional Chapters of the Society of Environmental Toxicology and Chemistry, Wilmington, DE
  14. 2010NJ231B ("Green Remediation of Tetracycline in Soil-Water Systems") - Conference Proceedings - Punamiya, P., D. Sarkar, and R. Datta. 2011. Aluminum-Based Drinking-Water Treatment Residuals: A "Green" Sorbent for Tetracyclines. 2011 Geological Society of America Annual Meeting, Geological Society of America Abstracts with Programs, Boulder, CO, Vol 43, No 5, pp 456.
  15. 2010NJ230B ("Hydrogeophysical investigation of subsurface controls on persistent canopy gaps in the New Jersey Pinelands") - Dissertations - Parsekian, Andrew, 2011, Geophysical estimation of free-phase gas content and distribution in peatlands, "Ph.D. Dissertation." Department of Earth and Environmental Sciences, Graduate School Newark, Rutgers, the State University of New Jersey, Newark, New Jersey, 116 p.
  16. 2008NJ156B ("Process-based modeling of nitrogen removal dynamics in brownfield and intact remnant wetland systems") - Dissertations - Palta, Monica M. 2012. Denitrification in Urban Brownfield Wetlands. Ph.D. Dissertation. Department of Ecology & Evolution, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ. 153 pp.