New Jersey Water Resources Research Institute
Annual Technical Report
FY 2017

Introduction

The New Jersey Water Resources Research Institute (NJWRRI) supports a diverse program of research projects. 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.

Two faculty initiated projects were awarded in FY2017, and six grants-in-aid were awarded in FY2017 to graduate students who are beginning their research. Two grants-in-aid awarded in FY2016 were carried forward in FY2017. 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 resources issues of importance to the state.

In FY2017, the NJWRRI continued to emphasize the development and upkeep of the website and e-based communications with stakeholder groups. 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. Furthermore, in FY2017, the NJWRRI sponsored the Meadowlands Conference: Super Storm Sandy: Five Years Later, held on October 26-27, 2017. This conference, organized by the Rutgers University Meadowlands Environmental Research Institute in Lyndhurst, New Jersey, brought together a community of scholars, researchers, urban planners, and policy-makers to share ideas and findings around the environmental, social, and design challenges facing East Coast waterways under more frequent extreme weather events. Finally, in FY2017, the NJWRRI worked to form a new partnership with the New Jersey Department of Health (NJDOH) to develop a new program titled “Fostering the Growth of Private Well Researchers at New Jersey’s Universities.” This new program will launch in FY2018.

Research Program Introduction

Two faculty initiated projects and six grants-in-aid were awarded in FY2017. Two grants-in-aid awarded in FY2016 were carried forward in FY2017.

One of the FY2017 projects was completed by 02/28/2018, and the two FY2016 projects were also completed by 02/28/2018. Six of the FY2017 projects have been extended to 08/31/2018.

Comparing the time required for establishment of effective nutrient removal capacity of different stormwater basin designs

Basic Information

Title:	Comparing the time required for establishment of effective nutrient removal capacity of different stormwater basin designs
Project Number:	2016NJ379B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NJ-004
Research Category:	Water Quality
Focus Categories:	Nutrients, Non Point Pollution, Water Quality
Descriptors:	None
Principal Investigators:	Theresa Censoplano, Louise Wootton

Publications

There are no publications.

Comparing the Time Required for Establishment of Effective Nutrient Removal Capacity of Different Stormwater Basin Designs Grant Report

(1) **PI information**: List all PIs, addresses, email addresses, phone numbers

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(2) **Numbers of Students Supported**: list the NUMBER of students in each category that were in any way supported by WRRI funding.

Undergraduates: 2

Masters' students: 1

Ph. D. students: 0

Postdoctoral assocs.:0

(3) **Any Notable Achievements** (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc. – N/A, project not yet completed due to limited rainfall

(4) Project Summary:

A solution to dealing with excessive quantities of phosphorous and nitrogen in the Barnegat Bay lies in limiting the amount of nutrients that make their way into aquatic ecosystems (Dietz and Clausen 2005). One approach is the use of subsurface gravel wetlands (SSGW), which allows a decrease in water flow and filtration as water flows horizontally through a wetland system promoting the conversion of nitrogen fertilizer chemicals to harmless N_2 gas. This study tested the effectiveness of nutrient removal of four different designs of subsurface gravel wetlands, with a focus on nitrogen pollution reduction. The four wetlands that were tested were: a wetland built on the original University of New Hampshire Stormwater Center (UNHSC) subsurface gravel wetland design, two modified UNHSC designs, one of which contains a simplified plumbing design (NJ DEP #2) and the other a deeper gravel layer (NJ DEP #3) and an Advanced Bioretention System (ABS). Water collected in a central well from a large parking lot at Georgian Court University was split into equal flows that fed the four test wetlands. Water samples were collected during major rain events from the central well, as well as from each wetland outlet and tested for ammonia, total Kjeldahl N, nitrate, nitrite, total N, total P and orthophosphate. Nutrient removal efficiency was determined through comparisons of inlet and outlet nutrient concentrations of each test wetland. Based on the results of study thus far, the NJ DEP #3 and the ABS designs were the most effective in terms of nutrient removal, with the ABS design outperforming the other three designs (Tables 1 and 2). The resulting information can be used to inform development of best management practices for stormwater design.

(5) Methodology - give a general summary of procedures and methods actually implemented

A total of fourteen samples were collected from seven storm events over the course of the study, from May 30, 2016 through October 30, 2017 by an automated discrete ISCO Model 6712 sampler per rain event. Samples were collected at each rain event, and then pooled to provide a time-averaged sample from the entire event. All sampling procedures were in compliance with section 5.2.2.2, Automatic Sampling in the NJDEP 2005 Field Sampling Procedures Manual (NJDEP 2005), any applicable USEPA guidance, or any Brick Township Municipal Utilities Authority (BTMUA) standard operating procedure. After collection, samples were placed in proper containers and kept on ice until transport to BTMUA's laboratory for analysis. Once at BTMUA's analytical laboratory, samples were analyzed using EPA certified techniques. In terms of N species, the influent and effluent samples were analyzed for ammonia, total Kjeldahl N, nitrate, nitrite, and total N (TN). For P, the influent and effluent samples were also analyzed for total P and orthophosphate. The water quality data derived from these analyses will be used to calculate the reduction of pollutants in the SSGW and ABS systems. Only storm events that had a minimum of 72 hours elapsed time since the prior event were sampled. Only precipitation events with a minimum of 0.5 inches of rainfall were considered for sampling.

Principal Findings and Significance –

Ammonia

Of the 68 samples collected, 11 were below detectable limits <0.10 mg/L. Ammonia ranged from 0.11 – 0.66 mg/L. 53 of the 68 samples exceeded the surface water quality standard for saline classified water of 0.115 mg/L. Saline classified was water used as the standard for two reasons. The surface water quality standard for ammonia is calculated using pH and temperature. These measurements were not collected during this study. Water on the campus of Georgian Court University eventually leads to the Barnegat Bay Watershed and subsequently the Barnegat Bay.

Orthophosphate

Nearly half of the samples (27 out of 58) collected tested below detectable limits for orthophosphate, <10.0 mcg/L. Of the samples that had detectable limits, these ranged from 10.3 mcg/L to 90.3 mcg/L. Currently, surface and ground water quality standards do not exist for orthophosphate.

Nitrate (NO_3)

For the time frame of the study, several of the samples were below detectable limits (<0.1mg/L) with 15 of the 68 samples falling into this category. Nitrate ranged from 0.11mg/L to 1.38mg/L of the remainder. All of the samples tested for less than the surface water quality standard of 10mg/L.

Nitrite (NO_2)

During the two-year period, of the 68 samples collected, 53 were below detectable limits (<0.025mg/L) for nitrite. The 15 samples that were detectable for nitrite ranging from 0.13mg/L to 0.0946mg/L. None of the samples exceeded the ground water quality standard for nitrite.

Total Phosphorous (TP)

Concentrations for total phosphorous ranged from below detectable limits <10 mcg/L to 218.0 mcg/L. 10 of the 68 samples exceeded the surface water quality standards for total phosphorous of 100 mgc/L. Only 1 of the 68 samples was below detectable limits.

Total Kjeldahl Nitrogen (TKN)

The concentrations for TKN in the samples collected ranged between 0.24mg/L to 18.6mg/L. All samples collected were above detectable limits. Currently, surface and ground water quality standards for TKN do not exist.

The advanced bioretention system showed the most promising results with respect to nutrient removal. With the exception of nitrate, the ABS gravel wetland had positive percent reductions for all other nutrients sampled, with total phosphate percent reduction being the most effective (figs. 1 and 2). In terms of percent reduction for total phosphate, NJ DEP2, NJ DeP3 and the ABS wetlands had positive removal of nutrients. While the UNH design actually had an increase in nutrients for total phosphate.

ABS and NJ DEP 3 had positive reductions in total nitrogen, whereas UNH and NJ DEP2 had an increase in these nutrients. The NJ DEP 2 design had nearly a 30% increase in nutrients for total nitrogen (figs. 3 and 4). All of the SSGWs had positive nutrient reductions for ammonia, with NJ DEP 3 having the most reduction (figs. 5 and 6).

Nitrate reduction was only effective in the NJDEP 3 design, as the other three designs had an increase in nitrates. The UNH and ABS designs had over 100% increase in nitrates (figs. 7 and 8).

Positive nitrite reduction was only effective in the ABS design. Whereas the UNH, NJ DEP 2 and NJ DEP 3 all had an increase in nitrites (figs. 9 and 10).

Ortho-Phosphates were effectively reduced in the NJ DEP 3 and ABS designs. Again, the NJ DEP 2 and UNH designs had an increase in Ortho-P, with the UNH having over 100% increase (figs. 11 and 12). Lastly, in terms of total Kjeldahl N, TKN, the UNH, NJ DEP 3 and the ABS all had a positive reduction in nutrients. The NJ DEP 2 design, however, had an increase in TKN (figs. 13 and 14).

Several factors may have influenced some of the results seen during this study. Not every storm event was captured during the two-year period. Some events occurred unannounced making it impossible to capture samples. The equipment, while very suitable, was inconsistent, as some of the samples were not captured. This was partially due to battery failure. The samplers also failed on multiple occasions and required reprogramming and maintenance. Dry spells prior to storm events yielded an inadequate amount of water flowing through the system and not enough to trigger samplers. Some events also occurred on Thursday, Friday or Saturdays when samples could not be analyzed due to lab unavailability. Therefore, some of the storm systems were missed.

The overall efficacy of each design varied in its ability to reduce nutrients. At conclusion of the preliminary results from the two-year study, the NJ DEP 3 design and the ABS design appear to be the most promising in terms of nutrient removal. The advanced bioretention system outperformed the other 3 designs for overall nutrient removal. Prior studies on the UNH design have demonstrated efficacy in nutrient removal (UNHSC 2010). However, these studies were conducted in New Hampshire with vastly different environmental parameters than in New Jersey. The NJ DEP 2 and NJ DEP 3 design have not been studied in the past, as this is the first study to include these types of designs. Future studies should continue to monitor the efficacy of each of the systems. Some of these studies should also include pH and temperature measurement for the surface quality measurement of ammonia.

Due to plumbing not correctly installed until Fall 2018, only a small portion of the data was collected while the systems were functioning properly. In the early phase of the study, microbial fauna and plant roots had not yet been established; therefore, effecting the creation of organic supplements needed for optimal functioning. Data collected early in the study, is expected to be different than the data collected from the system with greater maturity. Sampling should continue so that a more realistic idea of function of the established system and the performance can be obtained.

Because Georgian Court University is a very well managed property with limited fertilizer usage the influent often lacks pollution. Perhaps the systems should be primed with pollutants prior to rain events to gather a more realistic level of pollution in the influent system.

Figures

Table 1: Mean Values for the stormwater influent and effluent of the gravel wetland designs for the time period between May 2016 and October 2017.

Sample/Gravel Wetland	TP (ug/L)	TN (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	Ortho-P (ug/L)	TKN (mg/L)
INFLUENT	62.86	2.00	0.28	0.188	0.018	17.21	1.79
UNH	89.65	2.06	0.18	0.433	0.025	38.87	1.60
NJDEP2	59.53	2.56	0.21	0.184	0.024	21.84	2.35
NJDEP3	42.25	1.51	0.16	0.154	0.019	11.74	1.34
ABS	32.84	1.50	0.17	0.383	0.015	9.57	1.11

Table 2: Percent Reduction (%R) for the gravel wetland designs for the time period between May 2016 and October 2017. (NOTE – Negative reductions reflect an increase in nutrient)

Gravel Wetland	TP (ug/L)	TN (mg/L)	NH3 (mg/L)	NO3 (mg/L)	NO2 (mg/L)	Ortho-P (ug/L)	TKN (mg/L)
UNH	-42.6%	-2.9%	36.0%	-130.7%	-38.4%	-125.9%	10.9%
NJDEP2	5.3%	-28.0%	26.6%	1.8%	-33.4%	-26.9%	-31.1%
NJDEP3	32.8%	24.6%	43.7%	18.1%	-4.0%	31.8%	25.6%
ABS	47.8%	24.8%	41.2%	-104.0%	16.7%	44.4%	38.3%

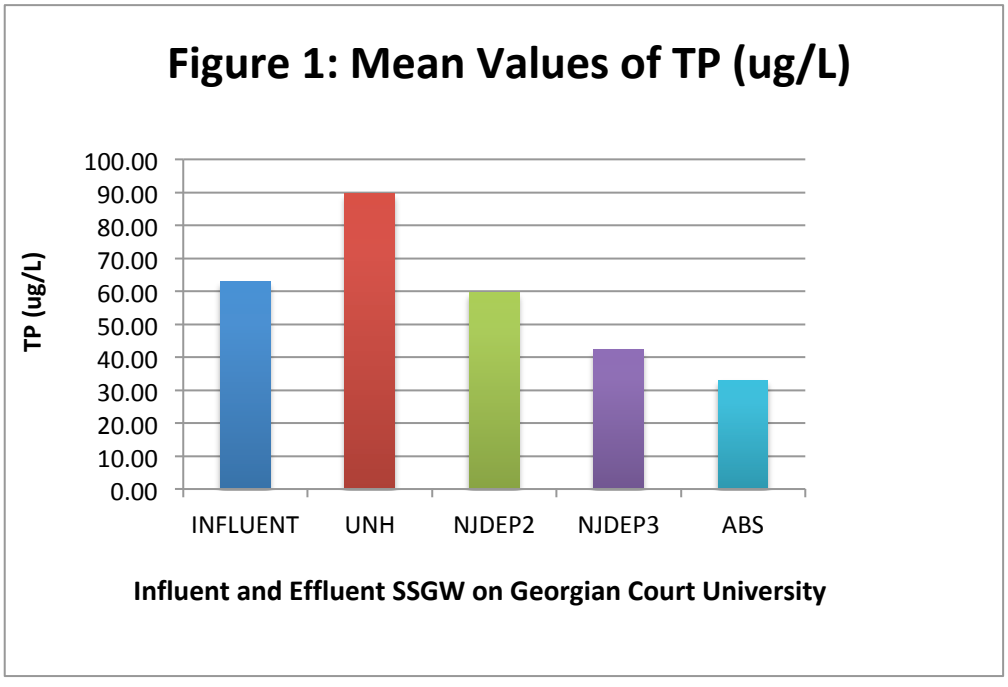


Figure 2: % Reduc. TP (ug/L)

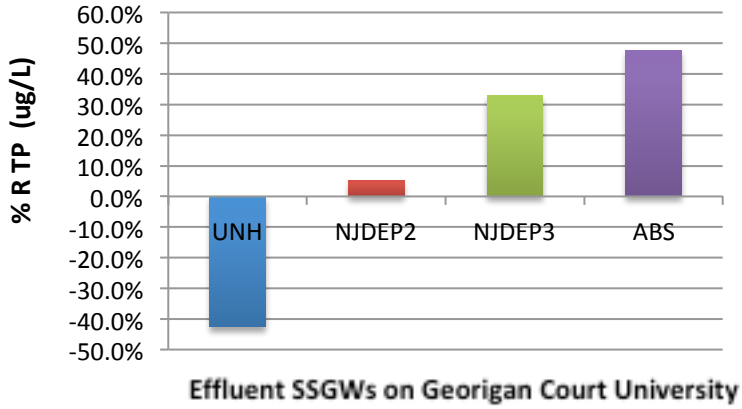


Figure 3: Mean Values of TN (mg/L)

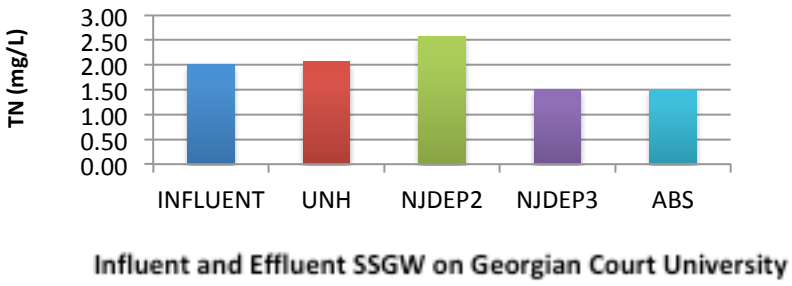


Figure 4: % Reduc. TN (mg/L)

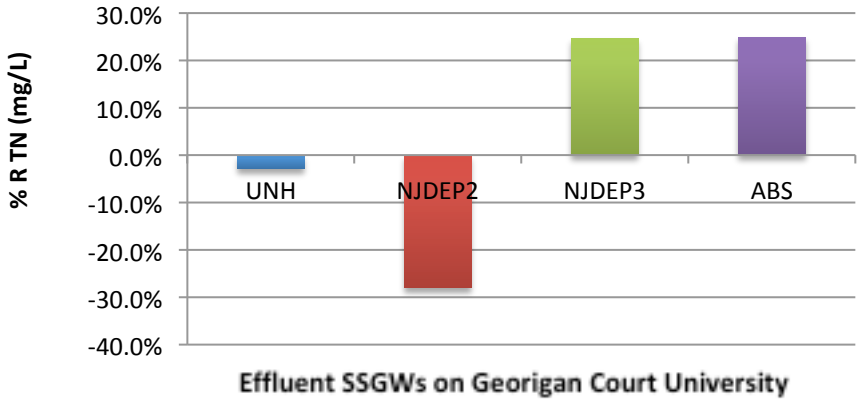
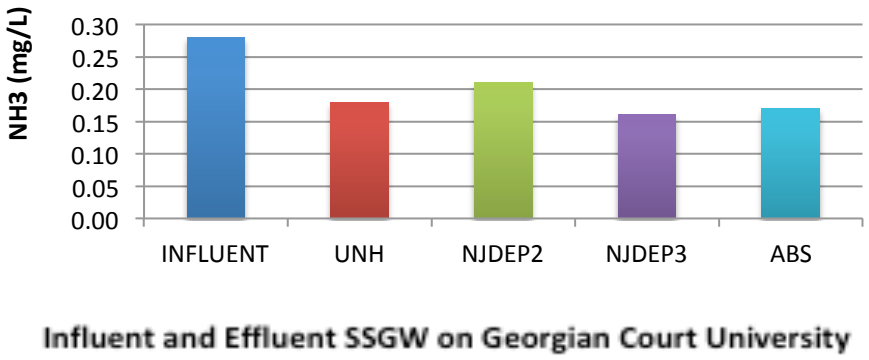
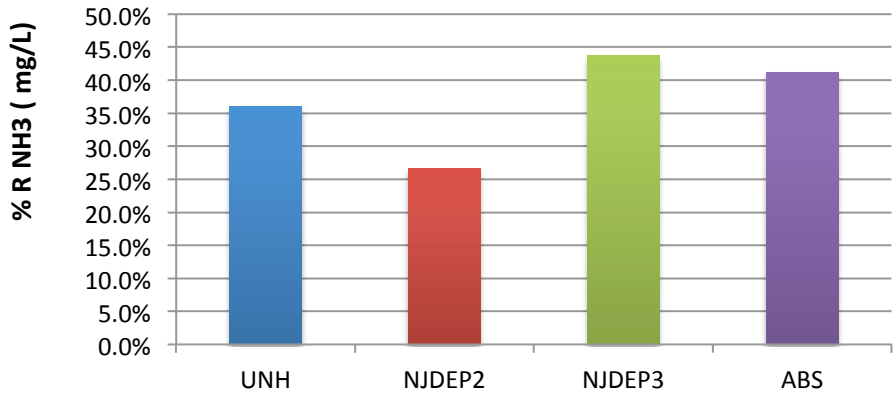


Figure 5: Mean Values of NH3 (mg/L)



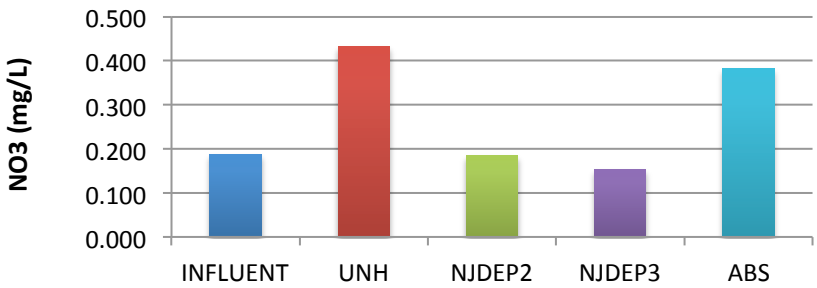
Influent and Effluent SSGW on Georgian Court University

Figure 6: % Reduc. NH3 (mg/L)



Effluent SSGWs on Georigan Court University

Figure 7: Mean Values of NO3 (mg/L)



Influent and Effluent SSGW on Georgian Court University

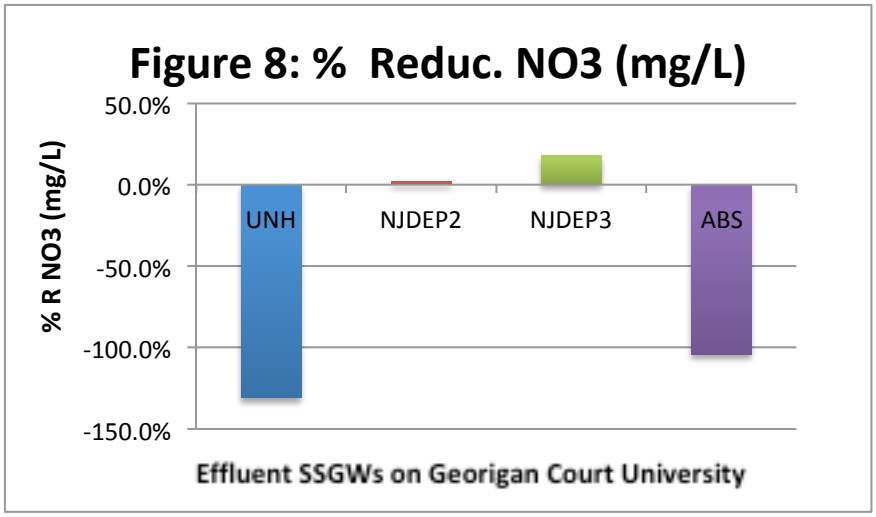


Figure 8: % Reduc. NO3 (mg/L)
% R NO3 (mg/L)
50.0%
0.0%
-50.0%
-100.0%
-150.0%
UNH
NJDEP2
NJDEP3
ABS
Effluent SSGWs on Georigan Court University

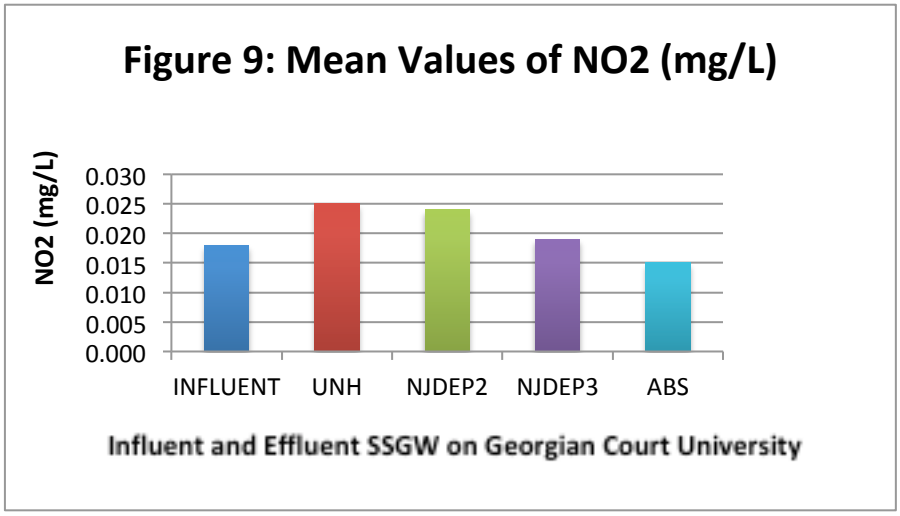


Figure 9: Mean Values of NO2 (mg/L)
NO2 (mg/L)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
INFLUENT
UNH
NJDEP2
NJDEP3
ABS
Influent and Effluent SSGW on Georgian Court University

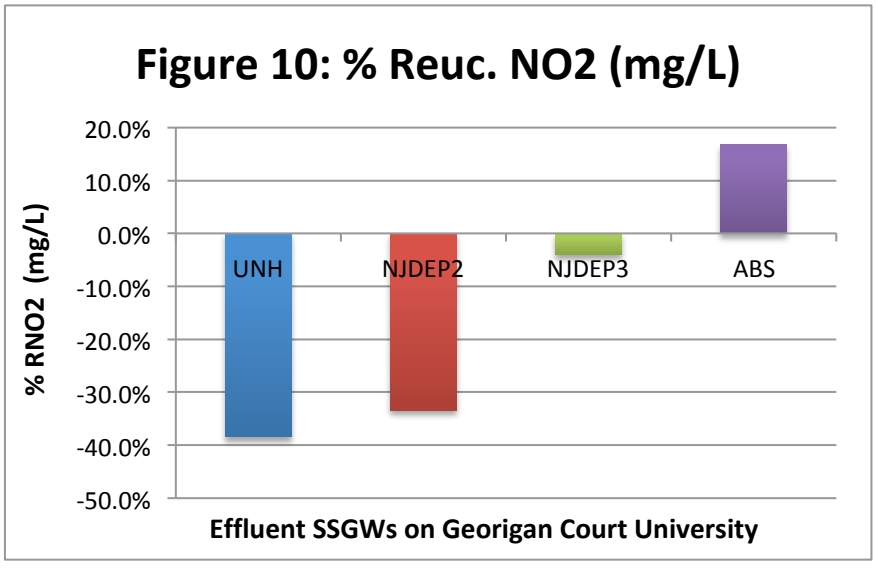


Figure 10: % Reuc. NO2 (mg/L)
% RNO2 (mg/L)
20.0%
10.0%
0.0%
-10.0%
-20.0%
-30.0%
-40.0%
-50.0%
UNH
NJDEP2
NJDEP3
ABS
Effluent SSGWs on Georigan Court University

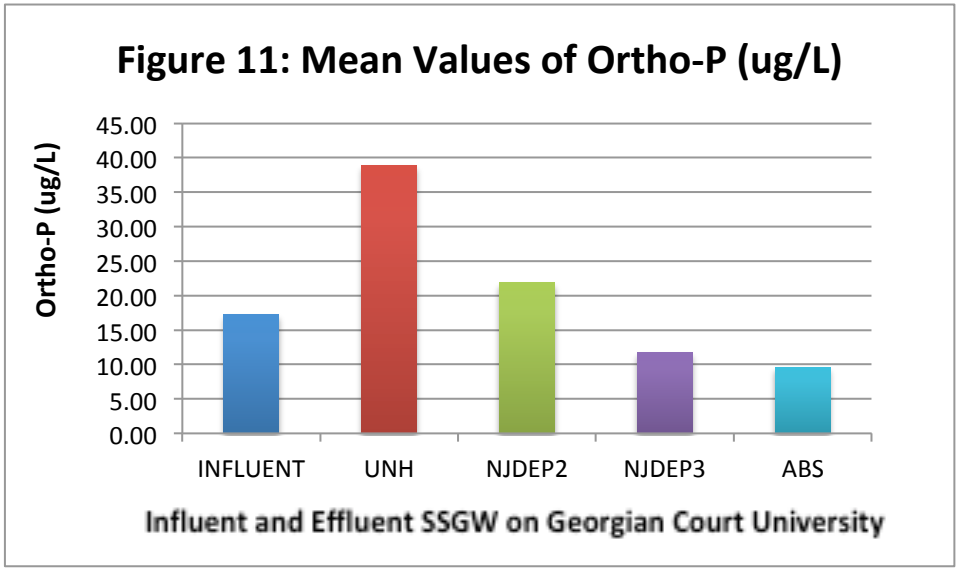


Figure 11: Mean Values of Ortho-P (ug/L)
Ortho-P (ug/L)
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
INFLUENT
UNH
NJDEP2
NJDEP3
ABS
Influent and Effluent SSGW on Georgian Court University

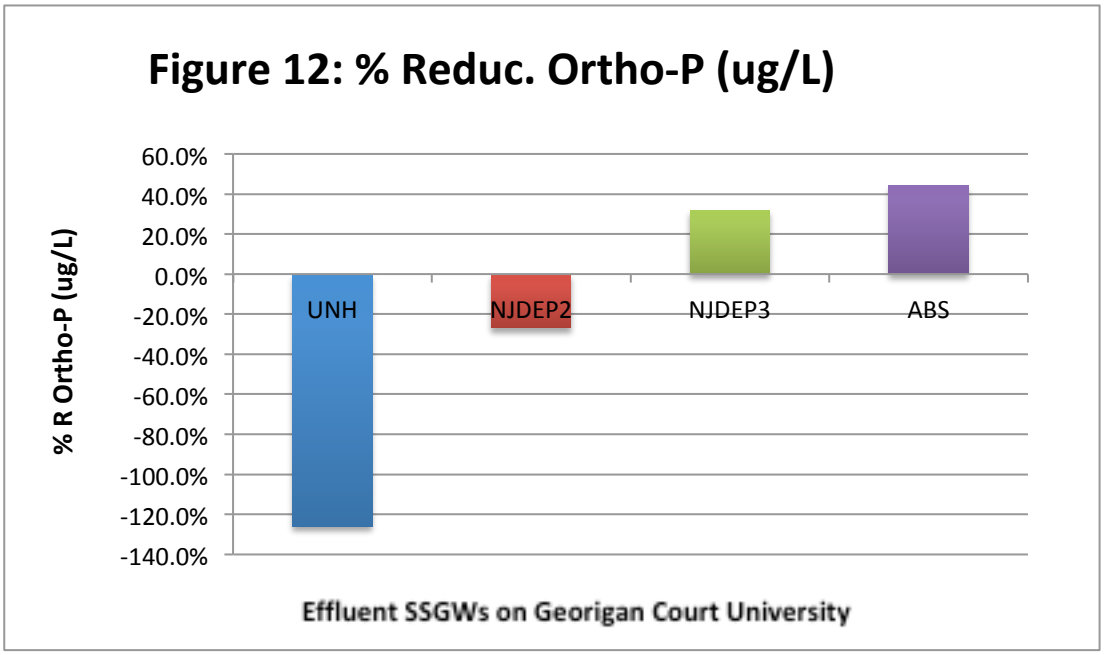


Figure 12: % Reduc. Ortho-P (ug/L)
% R Ortho-P (ug/L)
60.0%
40.0%
20.0%
0.0%
-20.0%
-40.0%
-60.0%
-80.0%
-100.0%
-120.0%
-140.0%
UNH
NJDEP2
NJDEP3
ABS
Effluent SSGWs on Georigan Court University

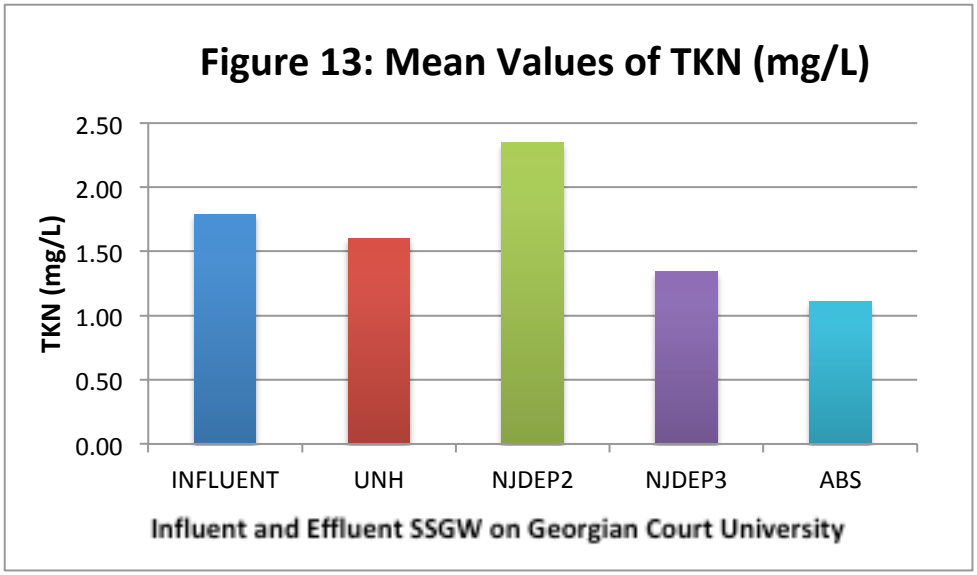


Figure 13: Mean Values of TKN (mg/L)
TKN (mg/L)
2.50
2.00
1.50
1.00
0.50
0.00
INFLUENT
UNH
NJDEP2
NJDEP3
ABS
Influent and Effluent SSGW on Georgian Court University

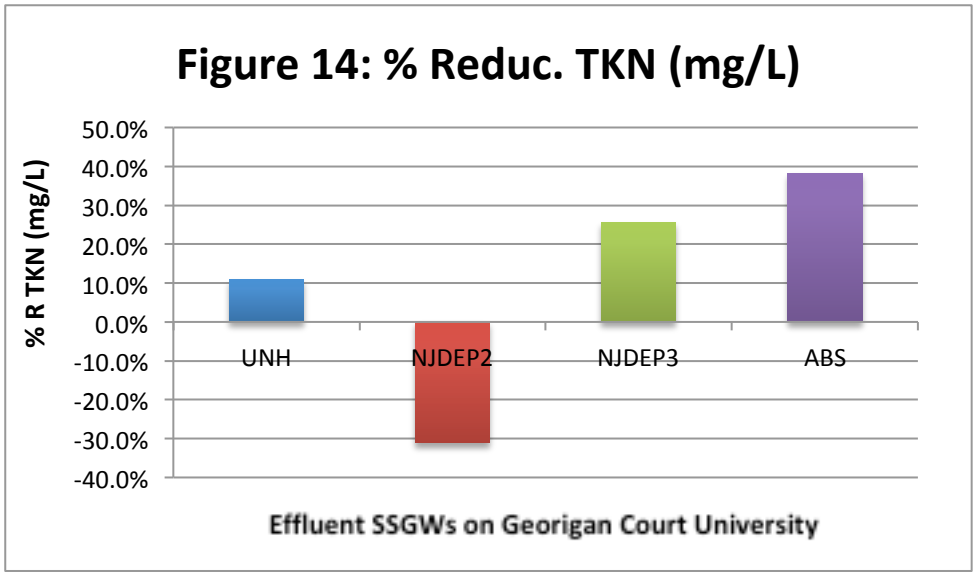


Figure 14: % Reduc. TKN (mg/L)
% R TKN (mg/L)
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
-10.0%
-20.0%
-30.0%
-40.0%
UNH
NJDEP2
NJDEP3
ABS
Effluent SSGWs on Georigan Court University

(4) **Publications or Presentations**:

There are no presentations scheduled as of yet due to timing of data finalization.

Citations

Dietz ME, Clausen JC. 2005. A field evaluation of rain garden flow and pollutant treatment. Water Air Soil Poll. 167:123-38.

University of New Hampshire Stormwater Center (UNHSC) and New England Inerstate Water Pollution Control Commission. 2010. *Investigation of Nutrient Removal Mechanisms of a Constructed Gravel Wetland Used for Stormwater Control in a Northern Climate: Final Report. UNHSC.* Durham, NH.

Composition and diversity of the cutaneous microbiome of amphibians in New Jersey

Basic Information

Title:	Composition and diversity of the cutaneous microbiome of amphibians in New Jersey
Project Number:	2016NJ381B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Biological Sciences
Focus Categories:	Ecology, Conservation, Water Quality
Descriptors:	None
Principal Investigators:	Ariel Kruger, Peter Morin

Publications

1. Kruger, Ariel, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in EcoGSA Seminar, Rutgers University, New Brunswick, New Jersey. November 4, 2016.
2. Kruger, Ariel, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in PPRC Graduate Student Conference, Columbia University, New York, New York. May 6, 2017.
3. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey. February 3, 2017.
4. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit Batrachochytrium dendrobatidis, a deadly fungal pathogen,” in Joint Meeting of Ichthyologists and Herpetologists, Austin, Texas. July 2017 (expected – abstract accepted).
5. Kruger, Ariel, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey. February 2, 2017.
6. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Ecological Society of America, Portland, Oregon. August 2017 (expected – abstract accepted).
7. Kruger, Ariel, 2018 (expected – abstract accepted), “Pine Barrens Treefrog Skin Bacteria Inhibit Growth of the Chytrid Fungus, Batrachochytrium dendrobatidis,” in Joint Meeting of Ichthyologists and Herpetologists, Rochester, New York.
8. Kruger, Ariel, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in PPRC Graduate Student Conference, Columbia University, New York, New York.
9. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey.
10. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit Batrachochytrium dendrobatidis, a deadly fungal pathogen,” in Joint Meeting of Ichthyologists and Herpetologists, Austin, Texas.
11. Kruger, Ariel, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey.

12. Kruger, Ariel; Peter Morin, 2017, “Green frogs harbor microbes that inhibit a deadly fungal pathogen,” in Ecological Society of America, Portland, Oregon.

NJWRRI 2017 Annual Report

Project Title: **Composition and diversity of the cutaneous microbiome of amphibians in New Jersey's aquatic ecosystems**

(1) PI information: List all PIs, addresses, email addresses, phone numbers

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(2) Numbers of Students Supported: list the NUMBER of students in each category that were in any way supported by WRRI funding.
Undergraduates: 0
Masters' students: 0
Ph. D. students: 1
Postdoctoral Associates: 0

(3) Any Notable Achievements (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.

N/A

(4) Project Summary:

Research Objectives:
(1) Measure bacterial diversity in aquatic ecosystems and determine if environmental transmission of microbes to amphibian skin is occurring.
(**2**) Determine if amphibian cutaneous microbial communities are host-specific or site-specific.
(3) Identify potential probiotic bacterial strains that inhibit *Batrachochytrium dendrobatidis (Bd) in vitro*.

Introduction:

Amphibians influence aquatic ecosystems in both stages of their biphasic life cycle. Tadpoles impact primary production and nutrient cycling through consumption of algae and organic matter, while adult amphibians return to aquatic systems to breed, where they deposit nutrient-rich eggs[1]. Recent amphibian declines due to emerging infectious diseases may therefore have reverberating consequences in aquatic ecosystems. Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis (Bd)*, is an emerging pathogen that continues to decimate amphibian populations worldwide[2]. Due to the wave-like spread of the pathogen[3], formulating conservation strategies to prevent declines in New Jersey's amphibian populations is of extreme importance.

Cutaneous microbial communities can alter disease outcome in amphibians[4]. Understanding the composition of the amphibian cutaneous microbiome is increasingly important with the spread of chytridiomycosis. Resistance to *Bd* is correlated with the presence of anti-*Bd* cutaneous symbiotic microbes[3]. Despite overwhelming evidence supporting the role of the amphibian cutaneous microbiome in disease resistance, few comprehensive assessments of microbial symbionts have been performed in wild amphibian populations[5].

While there is evidence that sympatric amphibian species harbor distinct cutaneous microbial communities[5,6], there is also evidence that environmental transmission of bacteria to amphibian skin can occur[7]. To help resolve this discrepancy, previous studies have evaluated the composition of the cutaneous microbiome of tadpoles at different sites[5,8]. However, because there is evidence of a community composition shift in the cutaneous microbiome upon metamorphosis[8], a comprehensive assessment of the symbiotic microbes on adult amphibians is needed to determine if sympatric species have unique cutaneous microbiomes. Furthermore, sites that differ in water quality may support distinct environmental microbes, and thus distinct amphibian cutaneous microbial communities, if environmental transmission is occurring.

By assessing the composition and diversity of the cutaneous microbiome different amphibian species, we will be able to determine if microbiomes tend to be host-specific or site-specific. Once cultured and isolated, we will grow antifungal cutaneous bacterial strains to determine the relative efficacy of *Bd* inhibition. This research will add to a growing body of literature on the amphibian cutaneous microbiome, help identify potential *Bd* probiotic therapy candidates, contribute to our understanding of host-microbe symbioses, and help us determine how these symbioses may affect amphibian susceptibility to *Bd*, a pathogen whose distribution and abundance in New Jersey's aquatic ecosystems has not been recently examined.

Methodology:

I examined the composition and diversity of the cutaneous microbiomes of adult green frogs in three sites that are known to differ in soil and water acidity: Success Pond in Colliers Mills WMA, Jackson, NJ (pH = 4.49), Morin Pond in Somerset, NJ (pH = 9.36), and Imlaystown Bog in Assunpink WMA, Allentown, NJ (pH = 6.13). The skin of 10 tadpole and 10 adult green frogs were sampled using a cotton swab at each site (with the exception of tadpoles at Colliers Mills, where n=8). Each individual was collected with a dipnet and handled using a new pair of nitrile gloves to prevent introduction of human cutaneous microbes. Amphibians were transferred from dipnet to a Whirl-pak, where they were rinsed twice with sterile water to exclude transient matter that is not part of the skin-associated microbiota[5,8]. I collected three swabs from each individual. I also collected three swabs from the pond water at each site to compare environmental microbiota to amphibian cutaneous microbiota. One of the swabs was used immediately (detailed below), and the remaining two swabs from each individual were preserved at -80°C and saved for DNA extraction, PCR, and 16S rRNA sequencing to identify and assess the presence of unculturable microbes (pending analysis).

One of the swabs was plated onto R2A agar in the field. Microbes were allowed to grow for three days at room temperature before bacteria were isolated based on unique colony characteristics. Bacterial isolates were used in *Bd* inhibition assays to detect bacterial strains that inhibit *Bd in vitro*. Briefly, unique colonies were grown in liquid culture to collect cell-free supernatant, which was then plated in a 96-well assay format in triplicate with *Bd*. Wells containing cell-free supernatant were compared to a positive control (*Bd* alone) and a negative control (heat-killed *Bd*) to determine if isolates had an effect on *Bd* growth. Plates were analyzed using a spectrophotometer at 492nm after 7 days of incubation at room temperature based on Bell *et al*.[9]. Isolates that showed greater than 60% inhibition of *Bd* relative to the positive control were identified as inhibitory isolates.

Principal Findings and Significance:

Across sites, there was an average of 8.7 unique isolates per individual (range: 5.9-10.8). The most unique isolates were found at Assunpink WMA, the moderate pH site (Table 1), and the total number of isolates differed significantly among sites (One-way ANOVA, $F_{(2,27)} = 11.59$, $p<0.001$; Figure 1). Shannon's diversity index was highest at Assunpink WMA and lowest at Colliers Mills, the low pH site. Pielou's evenness was relatively consistent across sites (Table 2).

I tested a total of 94 isolates for Bd inhibition (Table 1). Of the isolates tested, 31 showed strong inhibition against Bd, and in some cases, Bd did not grow at all when exposed to isolate CFS (Figure 2). There was no difference in mean inhibition by all isolates across sites (Kruskal-Wallis test: X2 =2.5254 df =2, p=0.28). Assunpink WMA, the moderate pH site, had the greatest percentage of isolates (both skin-associated and environmentally-associated) that were strongly inhibitory against Bd, and the number of inhibitory isolates on each frog was significantly different among sites (One-way ANOVA, $F_{(2,27)} = 8.292$, $p=0.002$; Figure 3). Furthermore, there were significant differences in the frequency of inhibitory isolates on each individual across sites, which takes into account the number of inhibitory isolates on each individual relative to the total number of isolates found on each individual (One-way ANOVA, $F_{(2,27)} = 4.265$, $p=0.025$; Figure 4).

We were able to isolate cutaneous microbes from green frog skin with the ability to inhibit the growth of *Bd*, a deadly fungal pathogen. These bacteria could potentially be used as probiotics applied to the skin of susceptible individuals to help confer disease resistance. We also

showed that sites that vary in pH differ in the percentage of bacterial isolates present on green frog skin that are strongly inhibitory against Bd. However, the presence of anti-*Bd* bacterial species on green frogs at all sites may suggest that green frogs in New Jersey could survive a *Bd* invasion. Understanding how the cutaneous microbiome changes across sites and life stages will inform conservation strategies for protecting amphibians against *Bd*.

Table 1. Green frog adults in New Jersey harbor skin microbes that inhibit *Bd in vitro.* The number of microbes isolated and the percentage of inhibitory isolates vary by site. Isolates exhibiting greater than 65% inhibition of Bd growth in challenge assays were considered strongly inhibitory.

Site	Sampling Date	Unique isolates	Average number of isolates per individual	% Isolates strongly inhibitory*
Morin Pond (high pH)	5/13/16	35	9.4	18.8% (6/32)
Assunpink (mid pH)	7/2/16	45	10.8	45% (18/40)
Colliers Mills (low pH)	7/7/16	23	5.9	31.8% (7/22)

* Denominators are different from "Unique isolates" column because some isolates were not successfully subcultured despite observing microbial growth on initial agar streaking. Therefore, the percentage of isolates that are inhibitory represents the number of inhibitory isolates out of the number isolates that were successfully tested.

Table 2. Shannon's Diversity Index (H') and Pielou's Evenness (J') values for each site, calculated by determining the number of frogs harboring each unique isolate.

Site	H'	J'
Morin Pond (high pH)	0. 3.203	0.9242
Assunpink (mid pH)	3.270	0.9199
Colliers Mills (low pH)	2.871	0.9429

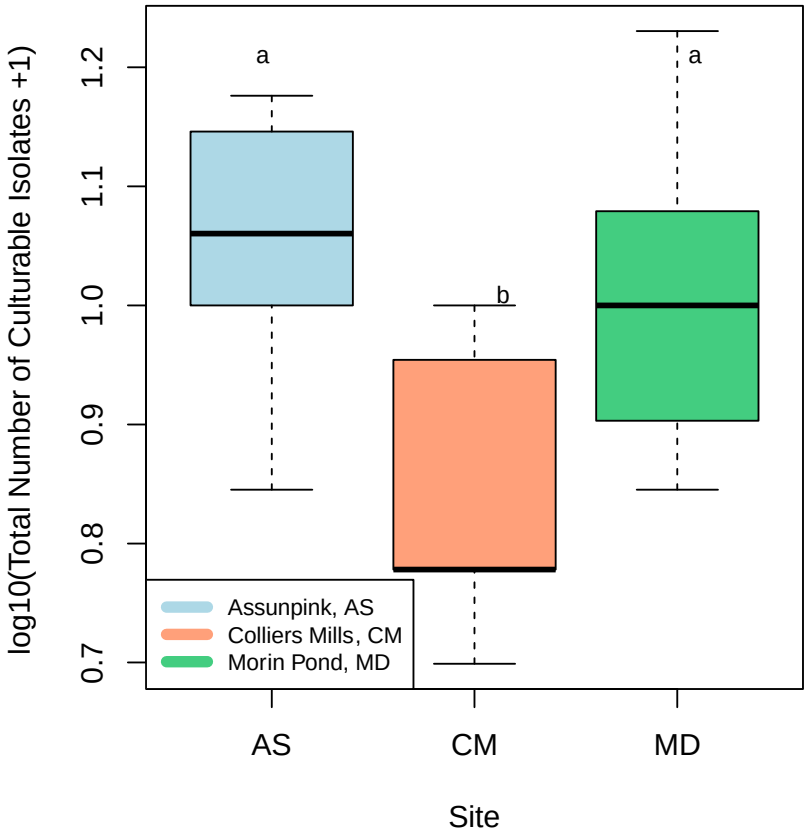

Figure 1. Green frogs at Assunpink WMA, the moderate pH site, had more culturable isolates present on their skin than green frogs at the other two sites. Letters denote differences that are statistically significant ($p<0.05$) using a Tukey's HSD test.

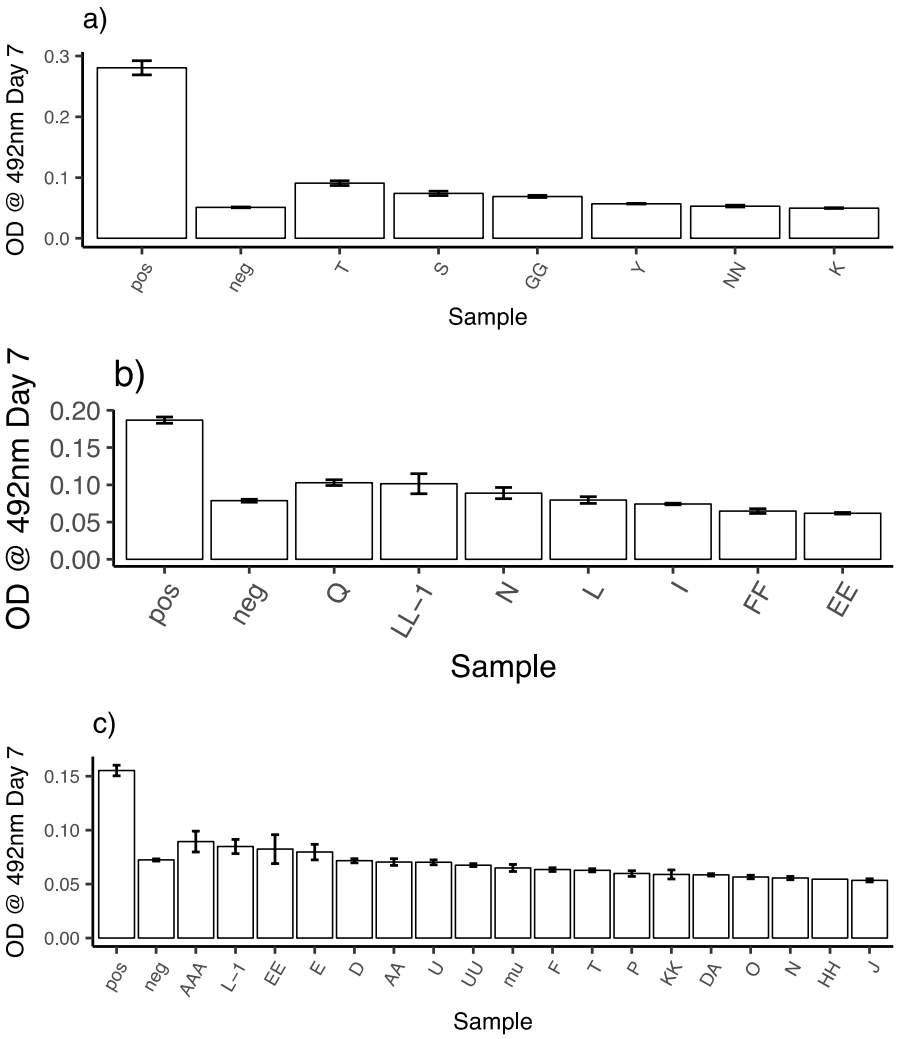

Figure 2. Inhibitory isolates from a) Morin Pond, b) Colliers Mills WMA, and c) Assunpink WMA. Pos = positive control in inhibition assay, or Bd alone, and neg = negative control, or heat-killed Bd. All isolates shown are significantly different from the positive control when means were compared by one-way ANOVA with Tukey's HSD post-hoc test ($p<0.05$). These isolates showed greater than 65% inhibition compared to the positive control on the final day of the assay (Day 7).

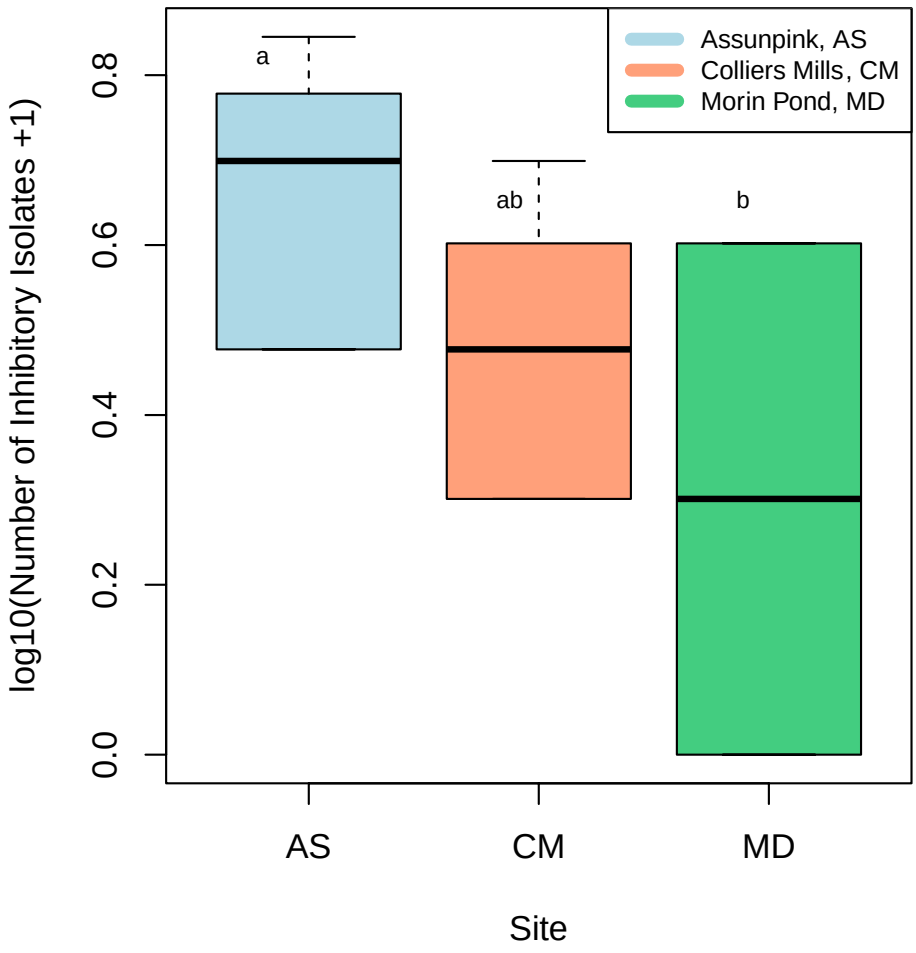

Figure 3. There were more inhibitory isolates present on green frog skin and in the environment at Assunpink WMA compared to the other two sites. Letters denote differences that are statistically significant ($p<0.05$) using a Tukey's HSD test.

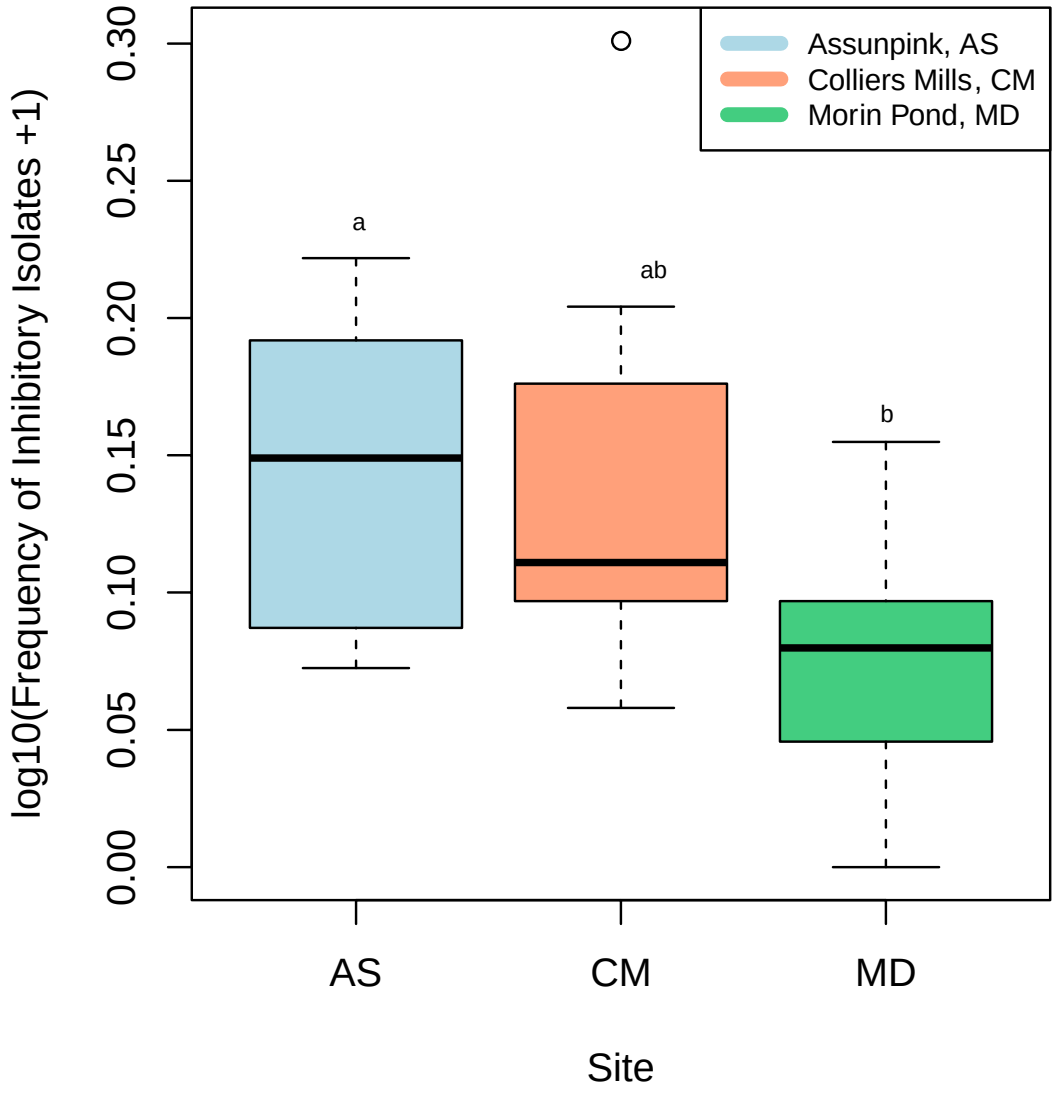

Figure 4. There is a higher frequency of inhibitory isolates (number of inhibitory isolates divided by the total number of isolates on an individual) on green frogs at Assunpink WMA. Letters denote differences that are statistically significant ($p<0.05$) using a Tukey's HSD test.

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(5) Publications or Presentations:

Oral presentations

Kruger, Ariel, 2018 (expected – abstract accepted), "Pine Barrens Treefrog Skin Bacteria Inhibit Growth of the Chytrid Fungus, *Batrachochytrium dendrobatidis*," in Joint Meeting of Ichthyologists and Herpetologists, Rochester, New York.

Kruger, Ariel, 2017, "Green frogs harbor microbes that inhibit a deadly fungal pathogen," in PPRC Graduate Student Conference, Columbia University, New York, New York.

Kruger, Ariel; Peter Morin, 2017, "Green frogs harbor microbes that inhibit a deadly fungal pathogen," in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey.

Kruger, Ariel; Peter Morin, 2017, "Green frogs harbor microbes that inhibit *Batrachochytrium dendrobatidis,* a deadly fungal pathogen," in Joint Meeting of Ichthyologists and Herpetologists, Austin, Texas.

Poster presentations

Kruger, Ariel, 2017, "Green frogs harbor microbes that inhibit a deadly fungal pathogen," in Microbiology Symposium, Rutgers University, New Brunswick, New Jersey.

Kruger, Ariel; Peter Morin, 2017, "Green frogs harbor microbes that inhibit a deadly fungal pathogen," in Ecological Society of America, Portland, Oregon.

Marsh Plants-Derived Biochar for Synergistic Decontamination of Dioxins, PCBs, and Mercury in Passaic River

Basic Information

Title:	Marsh Plants-Derived Biochar for Synergistic Decontamination of Dioxins, PCBs, and Mercury in Passaic River
Project Number:	2017NJ388B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-010
Research Category:	Engineering
Focus Categories:	Water Quality, Non Point Pollution, Treatment
Descriptors:	None
Principal Investigators:	Mengyan Li, Francisco Artigas

Publications

1. Zhang, Yue, Fei Li, Lei Hou, Mengyan Li, 2018, Effective Removal of 1,4-Dioxane using Waste-derived Biochar. (in preparation)
2. Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2018, Biochar for Immobilization of PCBs in Historically Contaminated Sediments. (in preparation)
3. Yue Zhang, 2018, Synthesis and Characterization of Biochar for 1,4-Dioxane Removal, "MS Dissertation," Department of Chemistry and Environmental Science, College of Science and Liberal Arts, New Jersey Institute of Technology, Newark, NJ, 21.
4. Jian Wang, 2018, Biochar as an Effective Capping Material to Stabilize PCBs Contaminations in Water and Sediments, "MS Dissertation," Department of Chemistry and Environmental Science, College of Science and Liberal Arts, New Jersey Institute of Technology, Newark, NJ. (in preparation)
5. Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2019, Biochar for Immobilization of PCBs in Historically Contaminated Sediments, New Jersey Water Environment Association 104th John J. Lagrosa Annual Conference and Exposition, Atlantic City, NJ.
6. Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2019, Biochar for Immobilization of PCBs in Historically Contaminated Sediments, 5th International Symposium on Bioremediation and Sustainable Environmental Technologies, Baltimore, MD.

Marsh Plants-Derived Biochar for Synergistic Decontamination of Dioxins, PCBs, and Mercury in Passaic River

Mengyan Li (NJIT)
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(2) **Numbers of Students Supported**: list the NUMBER of students in each category that were in any way supported by WRRI funding.
Undergraduates: 2 (Matthew Rizzos and Jose Antunes)
Masters' students: 2 (Jian Wang and Yue Zhang)
Ph. D. students: 1 (Fei Li)
Postdoctoral Associates: 0

(3) **Any Notable Achievements** (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.

We have manufactured biochar adsorbents derived from a variety of common biological wastes (e.g., Okara [aka soybean dregs], reed stem, aspen chip, and maize cob) via pyrolysis at three different temperatures (e.g., 300, 500, and 700 °C). These synthesized biochars were further characterized for their physical properties, surface chemistry, and, most importantly, their effectiveness of removing both legacy (e.g., PCBs) and emerging (e.g., 1,4-dioxane) contaminants, which have been widely detected in estuary environments in the U.S. Our results demonstrated biochar as a relatively cost-efficient and green alternative of these manufactured carbonaceous adsorbents for effective stabilization of PCBs in the contaminated sediments and removal of 1,4-dioxane in our drinking water.

(4) **Project Summary**:
Problem and Research Objectives

New Jersey has the greatest number of Superfund sites in the US. A number of these sites located along the local rivers (e.g., Passaic and Hudson River) and their tributaries due to significant industrial activity during the 19th and 20th centuries. The Lower Passaic River (LPR) is one of the most contaminated estuary areas in the US. This proposed study is motivated by the recent remediation initiative of US EPA to clean up the lower eight miles of the Passaic River by removing approximate 3.5 million cubic yards of the contaminated sediments and placing a protective cap to sequester the rest of the sediments from bank to bank. The total cost of this project is estimated to be over $1.3 billion, which will be billed to the companies who are responsible for causing the contamination. Conventional capping technique with a two-foot-thick sand layer is initially proposed to cover over the area that is dredged to immobilize the contaminated sediments from resuspension or transport [1]. However, sand may not be optimal to reduce the release of residual contaminants due to its low organic content and poor adsorption capability. Use of reactive capping additives (e.g., activated carbon) with high sorption potential can facilitate chemical separation and settling of underlying sediments. However, the relatively high cost of activated carbon precludes its application for capping in large contaminated areas like the LPR. Thus, an inexpensive and effective capping material is needed to ensure a prompted response to the emerging remediation call for the LPR. Achieving such engineered capping technique innovation will be applicable and effective to decontaminate other heavily polluted rivers and their tributaries in New Jersey and nationwide.

Biochar is a form of charcoal produced by the pyrolysis of carbon-rich biomass. Biochar has been widely used in agriculture to improve soil fertility, stimulate rhizospheric microbial activity, and enhance soil water and nutrient retention. Biochar is also a carbon sequestration strategy with ancillary environmental benefits. Transformation of biomass carbon into stable carbon structures in biochar can be conducive to reduce emissions of CO_2 and other greenhouse gases.

Due to the carbonaceous composition and microporous structure, biochar also serves as an inexpensive sorbent alternative for decontamination of organic and inorganic contaminants in water, soil, and sediments. Previous studies have revealed biochar is applicable for removing dioxins, PCBs, and mercury in aqueous phase and sediments. However, little is known regarding the sorption efficiency for commingled contamination where all three types of pollutants co-exist. Further, removal efficiency of in situ capping materials is linked with the geochemical properties of water and sediments (e.g., pH, redox, and organic content), which urges the need to evaluate the performance of capping materials using real field samples collected from the LPR. Hence, the overarching goal of this study is to investigate the effectiveness of biochar as the capping material to enhance the adsorption of PCBs and dioxins and sequester mercury and other heavy metals primarily via cation exchange, as well as stimulate the intrinsic microbial dehalogenation.

Methodology

Biochar preparation

We selected 4 feedstock materials for biochar synthesis, including the dreg of soybean (which is also called Okara), reed stem, aspen chip, and maize cob. These feedstocks were chosen as they contain varying compositions of cellulose, lignin, lipids, and wax esters, which thus will generate biochar materials with different carbonaceous contents and surface properties.

Okara is the insoluble waste of soybeans that remains after pureeing, which is extensively produced in food industries. The raw soybean was bought from a local grocery store. The raw Okara was made after blended by a blender and placed in the oven at 80 °C until completed dried. Common reeds (*Phragmites australis*) is an invasive species commonly found in the wetlands in Northeast U.S. Reed stem was collected in October 2016 from New Jersey Meadowlands District (Lyndhurst, NJ). The stem was further air-dried and stored in paper bags prior to grinding and use. Aspen chip was purchased from a local pet supply store. Maize cob was obtained from a local grocery store after removing the seeds.

Biochars were prepared by the slow-pyrolysis (5°C/min) of different types of feedstock (50 g) in a muffle furnace (Thermo, Newark, NJ) using different programs according to the experimental requirements. In this experiment, we used three terminal pyrolysis temperatures: 300°C, 500°C, and 700°C (Figure 1). First, approximately 50 g of ground feedstocks were placed in a stainless steel crucible, which was then covered with ceramic fiber to insulate the contact with O_2 and a loose-fitting porcelain lid. The capped crucible was buried in uniformly grained quartz sand inside a large stainless steel, open-top vessel. This vessel reactor will be heated in a muffle furnace at 5 °C min^{-1} and held at a temperature of either 300, 500, or 700 °C for 1 h to make sure all biomass was evenly heated. After the pyrolysis reaction, biochar products in the reactor are cooled to room temperature, crushed into powder, and transferred into the sealed glass bottles for storage. Then, the weight of the products was measured for the estimation of the yield of biochars.

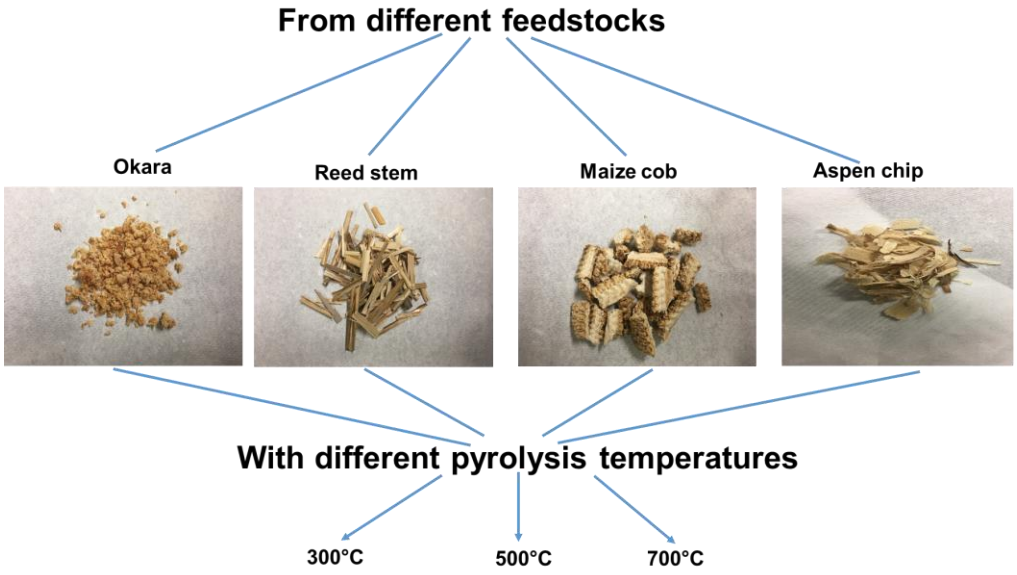

Figure 1. Synthesis of biochars from different feedstocks and at different pyrolysis temperatures.

Adsorption Isotherm by Biochar

To characterize the adsorption performance of the synthesized biochar, we first conducted adsorption isotherm experiment to remove 1,4-dioxane (dioxane), an emerging contaminant detected in the LPR. 20 mg of each type of biochar was mixed with 10 mL water dosed with different concentrations of dioxane (20, 50, 100, 200, and 400 mg/L) in replicate. Then the samples

were placed on an orbital shaker for 24 hours to establish the equilibrium. The supernatant (1 mL) was then collected by passing through 0.45 µm nylon filter. 500 µL of filtered dioxane was transferred to a glass vial and mixed with 500 µL THF (100 mg/L), an internal standard. Dioxane concentration was then determined by gas chromatography (Trace 1300, Thermo Scientific, US) using a TG-BOND Q capillary column (Thermo Scientific, 30 m length×0.25 mm ID×1.4 µm film) equipped with a flame ionization detector (FID) and autosampler (Thermo Scientific, A11310, US). The GC was operated under the following conditions: 1 µL samples were injected with a split ratio of 2:1, purge flow set as 5.0 mL, split flow set as 12 mL min^{-1}; The inlet temperature was 200 °C; the flow rate was constant at 6.0 mL/min with He as the carrier gas; The oven temperature program started at 110 °C for 1 min, then ramped to 180 °C at 15 °C/ min, held at 180 °C for 2 min. The detector temperature was maintained at 250 °C. Dioxane adsorption by biochars was compared with GAC and Ambersorb, which are known for exhibiting relatively high adsorption capacity.

Freundlich Adsorption Isotherm model was used to fit the adsorption data using the following equation:

$$\text{Log } Q_e = \text{Log } K_f + {}^{1}/_{n} \text{ Log } C_e$$

Where K_f = Freundlich isotherm constant (mg/g), is an approximate indicator of adsorption capacity.

n= adsorption intensity, If n = 1 then the partition between the two phases are independent of the concentration. If the value of n is greater than 1, it indicates a favorable adsorption.

C_e= the equilibrium concentration of adsorbate (mg/L).

Q_e= the amount of matter adsorbed per gram of the adsorbent at equilibrium (mg/g).

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of biochars were obtained with the FTIR spectrophotometer (Thermo Scientific, Nicolet iS10) using the diffuse reflectance accessory. The spectra were measured from 4000 to 400 cm^{-1} at the resolution of 4 cm^{-1}. The final spectra were produced by the averaged results of 200 times scans. Assignment of vibrational modes was based on the published spectrum table.

Density and BET Analysis of Biochar Particles

The bulk and skeletal densities of the biochars were measured gravimetrically. For the skeletal density measurement, the biochars were weighed into tared 1.5 mL volumetric flasks, which were then filled with acetone. The volume of acetone required resulted from its mass and density and used to calculate by different the volume occupied by the biochars. The bulk density of the biochars was measured by weighing known volume.

The BET test includes surface areas and total pore volume. They are determined by automated gas sorption analyzer (Quantachrome, Boynton Beach, FL). The surface area was measured with N_2

(0.168 nm^2) while keeping adsorption at liquid nitrogen temperature. The total pore volume was estimated from six N_2 adsorbed point at the pressure of 0.99.

Biochar Application with Mini-Column Test

The mini-column test was conducted using 10 mL plastic syringes of 8.5 cm length and 1.8 cm in internal diameter which was filled with 6.0 g Okara. The biochar was packed in the column between the glass wool, which prevented the wash out of biochar. A rubber stopper was used to keep column sealed. Such prepared column was gravitationally leached from the top flat for 24 h under a constant flow rate of 2.4 mL min^{-1}. 1L water dosed with 10 µg/L of dioxane solution was used for the mini-column test. The apparatus of the experimental setup is delineated in Figure 2.

Leachate was sampled at a series of time points. After preconditioning, the mini-column test was run for 6 h. 0.5 mL of the water samples was mixed with equal volume of dichloromethane (DCM) and vortexed thoroughly for 5 min. Then vials were placed horizontally in -80°C Freezer for 20 min. After removing the ice, 200 µL extract was transferred to a new vial and diluted with equal volume of pristine DCM. GC-MS (Thermo Scientific, Trace 1300-ISQ, US) was used for analyzing the dioxane concentration with the selected ion mode (SIM).

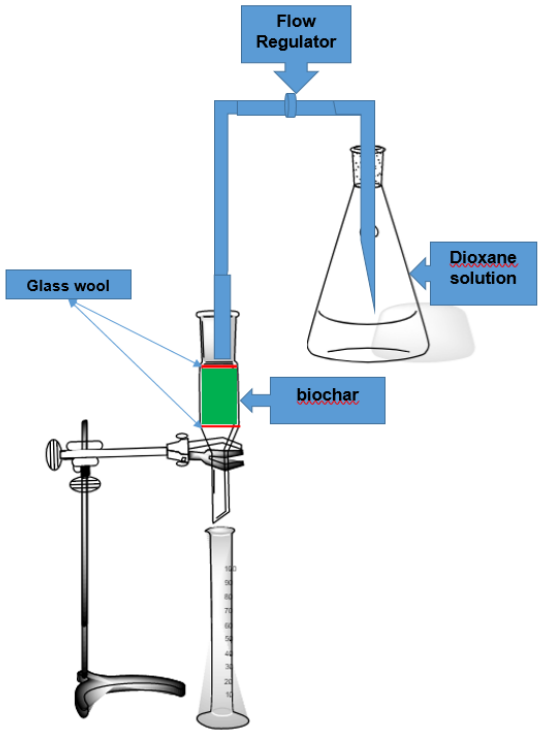

Figure 2. Apparatus of the mini-column setup

Analysis of PCBs using POM Solid Phase Extraction and GCMS

To first assess the PDBs adsorption kinetic by POM, 6 bottles were prepared with an initial concentrations of 10 mg/L PCB-3 (Sigma) and PCB-77 (AccStandard) and same amount of POM strips. Samples were taken at the selected times (e.g., 1, 3, 5, 7, 10, and 14 days). POM strips were

removed from the bottles and dried in the fume hood. Then, the strips were transferred into vials and added with 2mL hexane for desorption for 3 days. Then, the desorption solution was injected directly to GC/MS for the analysis of PCBs. The fingerprint ions include m/z 77, 153, and 188 for the detection of PCB-3.

We further optimize the extraction recovery by testing the absorption of both PCB-3 (100 ppb/10 ppb) and PCB-77 (100 ppb/10 ppb) in water by POM strips. Prior to extraction, PCB-14 (Sigma) was spiked into the solution as a surrogate at a concentration of 100 ppb in order to track extraction recovery. Extracts with lower than 80% surrogate recoveries were discarded. Similar extraction procedures as mentioned above is applied and all compounds are detected by GC/MS.

Sediment Sample Collection and Characterization

Triplicate sediment core samples have been collected at a single location at the fishing deck at the North Arlington along the Lower Passaic River in December 2017. Additional sediment samples were collected at the Hudson River and Hackensack River. Sampling locations were selected given their historical records of contamination and proximity to the "hot spots" at the Superfund site. The sampling efforts were assisted by the Meadowlands Environmental Research Institute (MERI). The collected sediment samples were immediately transferred to sterilized glass jars, placed on ice and transported to our lab at NJIT. A portion of samples was sent to MERI to characterize the concentrations of PCBs, PAHs, organochlorine pesticides (OCPs), and heavy metal contaminants, as well as pH, TOC, N/P content, etc.

Principal Findings and Significance

Adsorption Isotherm of Dioxane by Biochars Derived from Different Feedstocks

The isotherms of different biochars displayed in Figure 3 indicates the biochar derived from Okara and reed stem exhibited better adsorption than the ones synthesized with maize cob and aspen chip. However, Ambersorb and GAC exhibited better adsorption within the tested range of dioxane concentration. In accordance with the previous study, Ambersorb is exceedingly superior in removing dioxane when the dioxane concentration is less than 100 mg/L.

Based on Table 1, we can find that our isotherm data fit the Freundlich Model very well (R^2 > 0.98). Among the tested biochars, Okara and Reed-derived biochar exhibited better dioxane adsorption given the relatively high K_f and n. Thus, they are selected as the potential adsorbents that will be further characterized and verified the suitability to replace manufactured carbonaceous adsorbents like Ambersorb^{TM} 560.

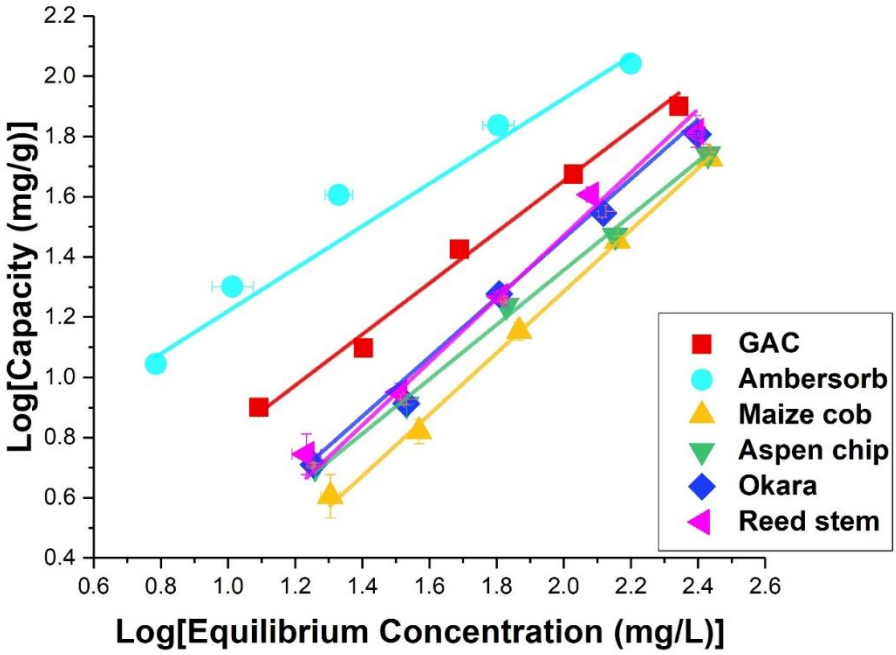

Figure 3. Adsorption isotherms of dioxane by biochars in comparison with Ambersorb and GAC.

Table 1. Freundlich isotherm parameters for dioxane adsorption by biochars

Absorbent	**Freundlich Isotherm parameters**		
	K_f (mg/g)	**n**	**R^2**
Ambersorb™	3.263	1.416	0.984
GAC	0.898	1.177	0.984
Okara	0.344	1.07	0.996
Reed stem	0.311	1.010	0.984
Aspen chip	0.309	1.015	0.987
Maize cob	0.174	0.978	0.999

Effects of Pyrolysis Temperature on Dioxane Adsorption

We further tested the influence of pyrolysis temperature on dioxane adsorption. Figure 4 showed the higher pyrolysis temperature, the higher adsorption capacity exhibited by the biochar derived

from reed stem. Previous studies have revealed that pyrolysis at high temperatures (e.g., 500°C and above) greatly increases the carbon content and thus hydrophobicity of the char materials, which may contribute to the enhanced adsorption of dioxane.

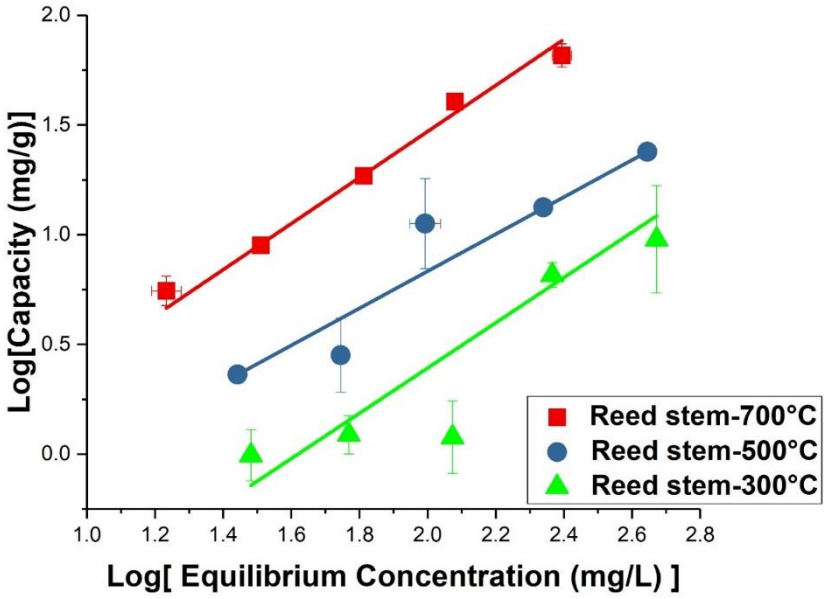

Figure 4. Dioxane adsorption isotherm by reed-derived biochar with different pyrolysis temperatures

Surface Chemistry Characterization by FTIR

Infra-red spectroscopy was employed to uncover the surface chemistry of the synthesized biochar particles and analyze functional moieties formed among C, N, O, and H. First, biochar derived from Okara at three different pyrolysis temperatures were characterized by FTIR. Okara was not sufficiently pyrolyzed under a temperature of 300 °C. Thus, excessive amount of functional moieties remained on its surface (Figure 5), including C-H/O-H (corresponding to the band intensities between 2800 and 3000 cm^{-1}), C≡C/C≡N (corresponding to the band intensities between 2200 and 2400 cm^{-1}), C=C (corresponding to the band intensities between 1500 and 1600 cm^{-1}), and C-O/C-N (corresponding to the band intensities between 1000 and 1200 cm^{-1}). When the pyrolysis temperature increased to 500°C, the abundance of these functional moieties was dramatically reduced. For instance, C-H/O-H and C=C were mostly eliminated on the surface of biochar. The abundance of C-O/C-N was also greatly reduced. However, C≡C/C≡N moieties still persist at a pyrolysis temperature at 500°C. Upon heating at 700°C, an abundance of C≡C/C≡N moieties reduced significantly compared to the Okara-derived biochars synthesized at 300 and 500 °C. No other functional groups were significantly observed based on the FTIR results. The isotherm results showed an increased adsorption performance with an increased pyrolysis temperature. The reduced moieties on the biochar surface at high pyrolysis temperatures may result in less surface electrostatic interactions with dioxane molecules, implying dioxane adsorption may be primarily associated with surface hydrophobicity and pore size/area.

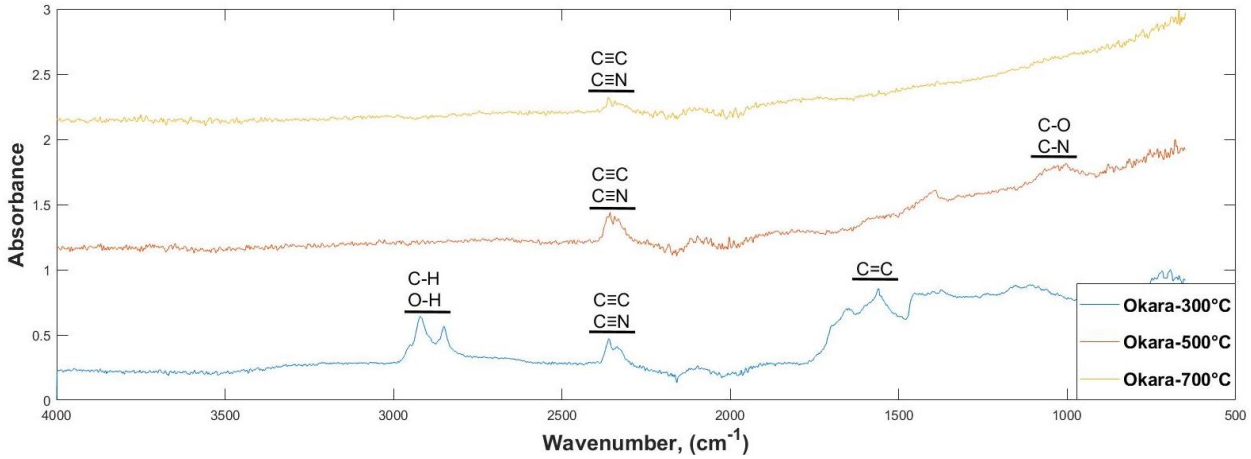

Figure 5. FTIR of Okara-derived biochars pyrolyzed at three different temperatures

Secondly, biochar derived from reed stem at three different pyrolysis temperatures were characterized by FTIR (Figure 6). The dominant functional groups detected on the surface of reed stem-derived biochar at 300°C included C≡C/C≡N (corresponding to the band intensities between 2200 and 2400 cm^{-1}), C=C (corresponding to the band intensities between 1500 and 1600 cm^{-1}), C-O/C-N (corresponding to the band intensities between 1000 and 1200 cm^{-1}) and N-H (corresponding to the band intensities between 500 and 1000 cm^{-1}). With the increase of the pyrolysis temperature, the abundance of these functional moieties was dramatically reduced. This is consistent with the results observed in Okara-derived biochar.

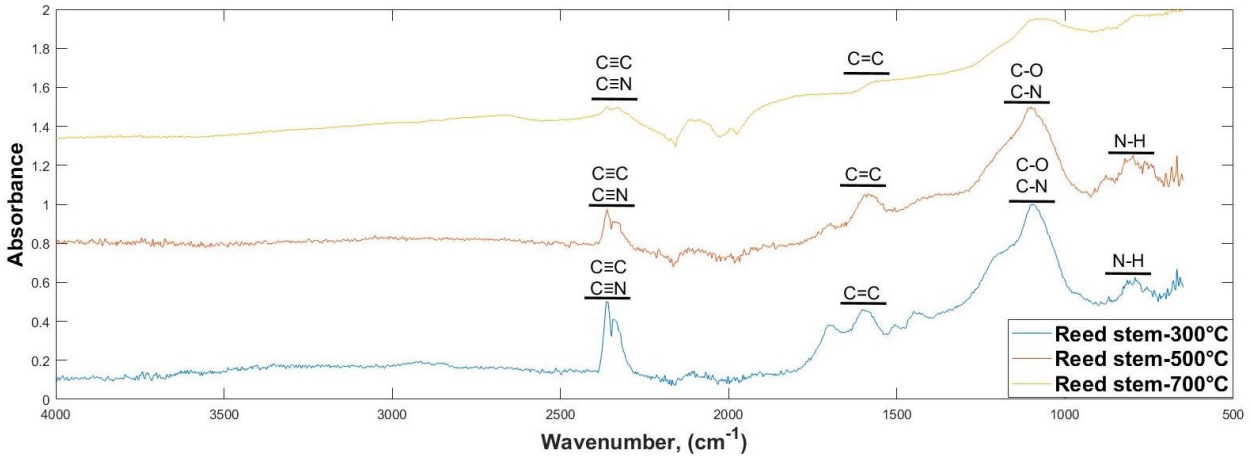

Figure 6. FTIR of reed-derived biochars pyrolyzed at three different temperatures

We further compare the surface chemistries of all biochars pyrolyzed at 700 °C with GAC. Figure 7 showed very few moiety residues were detected when pyrolysis temperature is as high as 700 °C. Relatively small amount of C≡C/C≡N was observed in maize cob-derived biochar and GAC. No clear association was found between any moiety groups and dioxane adsorption.

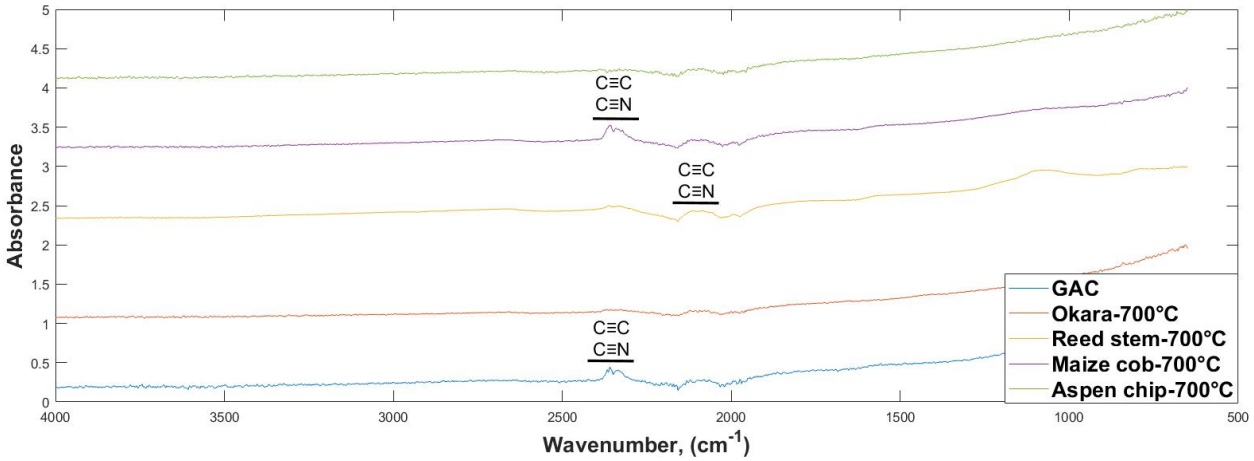

Figure 7. FTIR of different biochars pyrolyzed at 700°C

Density and Specific Surface Area

Based on Table 2, we can find the skeleton density of GAC and Okara-derived biochar are greater than 1 g/cm^3, which facilitates their applications as high-density adsorbents for filter media and *in situ* capping materials. In contrast, reed stem-biochar is lighter than water with a skeleton density of 0.73 g/cm^3. The light biochar is likely disrupted by the water flux and may lead to floating and wash-off issues for field application.

The surface area and total pore volume (TPV) were characterized for selected biochars and GAC. Our results showed that both surface area and TPV of GAC were nearly four times greater than reed stem-derived biochar. As surface area and TPV regulate the interaction with the adsorbate molecule (i.e., dioxane), they may contribute to the adsorption efficiency observed in the isotherm tests.

Table 2. Density and Surface Area Analysis of Biochars and GAC

Item	Bulk Density (g/cm^3)	Skeleton Density (g/cm^3)	Surface Area (m^2•g^{-1})	Total Pore Volume (mL •g^{-1})	Yield (%)
GAC	0.54	1.94	1123.2065	0.4633	N/A
Okara	0.65	1.34	*	*	17
Reed stem	0.38	0.73	331.4197	0.1035	28

***** The data of the surface area and total pore volume for Okara-derived biochar can't be measured. Possibly resulted from the faulted given unfitted analytical mode. We are in the process of seeking for the appropriate analysis method to resolve this issue.

Mini-Column Test

To validate the suitability of Okara-derived biochar as an effective filter media to remove dioxane at the environment-relevant level, the mini-column test was employed. After the preconditioning of the column, monitoring data (Figure 8) showed that the mini-column can effectively remove dioxane from 10 µg/L to approximately 2 µg/L in the effluent. The system will be further improved to increase the removal rate by changing the flow rate or media amount. Breakthrough curves will be established with longer monitoring duration. The effectiveness of the biochar will be compared with manufactured carbonaceous adsorbents, such as GAC and Ambersorb^{TM} 560.

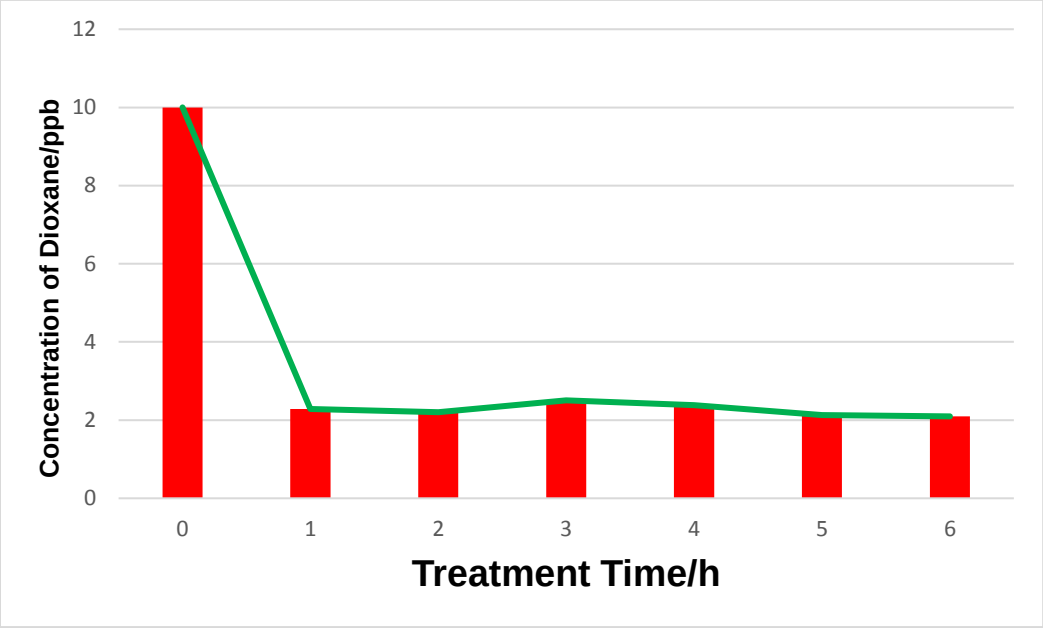

Figure 8. Removal of dioxane in spiked water samples after filtration through the mini-column packed with Okara-derived biochar

Sensitive Detection of PCBs

As shown in Figure 9, all three PCB compounds (i.e., PCB-3, PCB-14, and PCB-77) can all be sensitively detected at ppb levels. The R^2 values for all three calibration curves are all greater than 0.99, indicating a good response of the instrument within the detection range. In comparison with micro-extraction (data not shown), POM solid phase extraction exhibited better recovery and more consistent results, suggesting its suitability for the analysis of PCBs in water solutions in this study.

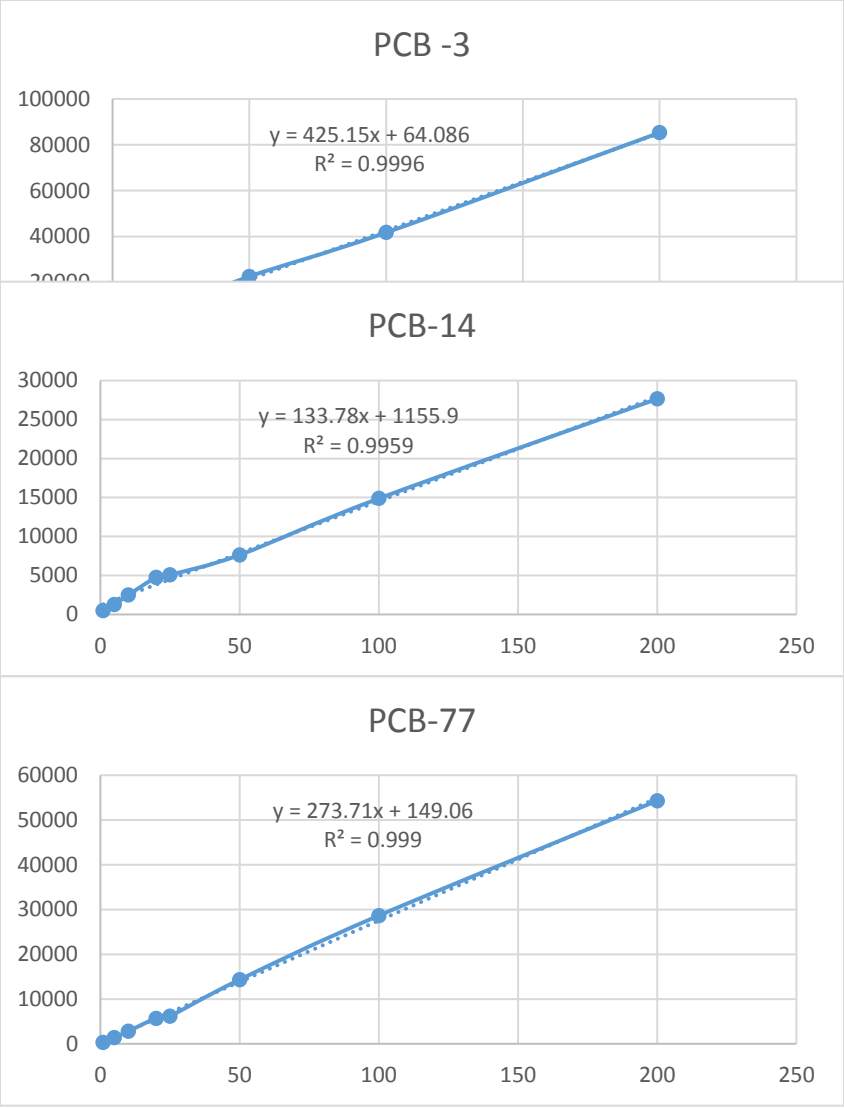

Figure 9. Calibration curves for the analysis of three selected PCBs

Sediment Sample Analysis

To mimic field remediation, sediment samples were collected at selected hot spots along three local rivers that are known for PCBs contamination. Table 3 presents the total PCBs and organochlorine pesticides (OCPs) in replicated or triplicated field samples. As highlighted in yellow, sediments collected at the Berry's Creek showed the highest contamination, which will thus be used for microcosm assays to evaluate the effectiveness of biochars for immobilization of PCBs in sediments.

Table 3. Concentrations of PCBs in collected sediment samples

SAMPLE #	90965	90966	90967	90968	90969	90970	90971	90972	90973	90991	LAB BLANK
LOCATION	Berrys	Berrys	Berrys	Passaic	Passaic	Passaic	Hudson	Hudson	Hudson	Hudson	LAB BLANK
SAMPLE DATE	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17	12/15/17
TOTAL CONCENTRATION *(ug/Kg)*											
PCB	1289.25	493.36	6644.70	109.65	129.80	166.74	72.27	84.89	97.25	26.77	7.96
OCP	51.47	39.82	231.85	14.45	15.96	11.99	4.08	4.34	2.81	2.58	3.25
SURROGATE RECOVERY (%)											
PCB 14	39%	27%	63%	70%	79%	56%	35%	43%	36%	16%	13%
PCB 65	31%	21%	85%	91%	101%	99%	51%	49%	45%	29%	15%
PCB 166	10%	7%	54%	29%	27%	48%	27%	26%	25%	7%	2%
Dibutylchlorendate	75%	96%	113%	125%	88%	127%	44%	36%	26%	36%	31%

(5) **Publications or Presentations**:
List any publications, presentations, published abstracts reporting the WRRI-supported work. Oral or poster presentations at conferences should be specified as such. Even if there are no conference proceedings, you need to report any poster or oral presentations you give. Please include the name, date and place of the conference.

1. Articles in Refereed Scientific Journals:
Zhang, Yue, Fei Li, Lei Hou, Mengyan Li, 2018, Effective Removal of 1,4-Dioxane using Waste-derived Biochar. *(in preparation)*

Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2018, Biochar for Immobilization of PCBs in Historically Contaminated Sediments. *(in preparation)*

2. Dissertations:

Yue Zhang, 2018, Synthesis and Characterization of Biochar for 1,4-Dioxane Removal, "MS Dissertation," Department of Chemistry and Environmental Science, College of Science and Liberal Arts, New Jersey Institute of Technology, Newark, NJ, 21.

Jian Wang, 2018, Biochar as an Effective Capping Material to Stabilize PCBs Contaminations in Water and Sediments, "MS Dissertation," Department of Chemistry and Environmental Science, College of Science and Liberal Arts, New Jersey Institute of Technology, Newark, NJ. *(in preparation)*

3. Conference Presentations
Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2019, Biochar for Immobilization of PCBs in Historically Contaminated Sediments, New Jersey Water Environment Association 104th John J. Lagrosa Annual Conference and Exposition, Atlantic City, NJ.

Wang, Jian, Fei Li, Yue Zhang, Mengyan Li, 2019, Biochar for Immobilization of PCBs in Historically Contaminated Sediments, 5th International Symposium on Bioremediation and Sustainable Environmental Technologies, Baltimore, MD.

Using geophysical technologies to delineate and monitor saltwater intrusion within New Jersey coastal aquifers

Basic Information

Title:	Using geophysical technologies to delineate and monitor saltwater intrusion within New Jersey coastal aquifers
Project Number:	2017NJ389B
Start Date:	3/1/2017
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NJ-002
Research Category:	Ground-water Flow and Transport
Focus Categories:	Groundwater, Hydrology, Water Quality
Descriptors:	None
Principal Investigators:	Judy Robinson, Lee Slater

Publications

There are no publications.

NJWRRI Final Report: May 2018

Using geophysical technologies to delineate and monitor saltwater intrusion within New Jersey coastal aquifers

Rutgers University, Newark
Department of Earth & Environmental Sciences

1. **PI information**

Judy Robinson	Previous: Assistant Research Professor, RU-N Current position (as of 3/5/2018): Computational Scientist at Pacific Northwest National Laboratory
Lee Slater	Henry-Rutgers Professor, RU-N

2. **Number of Students Supported**
   Undergraduates: 2
3. **Notable Achievements: N/A**
4. **Project Summary**

Two geophysical surveys were conducted in Cape May County, New Jersey (NJ) along the Delaware Bay to characterize and delineate saltwater intrusion. Marine electrical imaging was used to collect continuous vertical electrical soundings (CVES) adjacent to the Bay; and self-potential (SP) measurements were collected within a deep monitoring well and compared with fluid specific conductance. Electrical measurements as CVES were collected from two creeks and the raw data provided general trends in resistivity but lacked depth information. The results from 1D laterally constrained inversions of the CVES data provided data at depth and the freshwater/saltwater interface was inferred by assuming the location of the freshwater/saltwater transition at depth. This inferred boundary was parallel to the shoreline at a distance of approximately 500 m inland. Measurements of fluid specific conductance within a deep monitoring well, 800 Obs, remained constant over the timespan of this experiment; as such SP measurements within the well were not indicative of saltwater intrusion but of electrokinetic mechanisms from tidal pressure gradients. This was verified by performing a continuous wavelet transform (CWT) and comparing scalograms of SP data and tidal gage heights from two monitoring stations within 10 km of the site. Future work to study saltwater intrusion in Cape May County, NJ could focus on repeating marine electrical surveys using larger electrode spacings during varying seasons and weather events. In addition, SP measurements could be collected in wells where variations in salt concentration and/or fluid specific conductance reflect groundwater-saltwater exchange.

Table of Contents

I. Problem and Research Objectives

Saltwater intrusion of coastal aquifers in Cape May County, New Jersey (NJ) is one of the most critical water resource issues in the State (Lacombe & Carleton, 2002; Lacombe, Carleton, Pope, & Rice, 2009). Regional groundwater investigations to map saltwater intrusion most often rely on chemical analysis and potentiometric measurements from monitoring wells at discrete intervals and locations. While point-scale measurements provide highly accurate local information, this data must be interpolated for up-scaling which leads to uncertainty.

Electrical geophysical methods, namely direct-current electrical resistivity and self-potential (SP), have vast untapped potential to improve regional understanding of groundwater quality issues. Specifically, these geophysical methods are sensitive to conductivity contrasts and pressure and concentration gradients associated with salt-water intrusion and can provide information at a scale consistent with the operation of subsurface processes that resource professionals need to understand in order to make informed decisions regarding water management.

This study used two geophysical methods, electrical resistivity (ER) imaging and self-potential (SP) to look at saltwater intrusion in Cape May County, NJ. Field campaigns included: ER continuous vertical electrical soundings (CVES) within the Delaware Bay to collect electrical measurements; SP measurements within a USGS monitoring well at the Rutgers Cape Shore Laboratory. Electrical measurements were collected to map the freshwater/saltwater interface along the Delaware Bay in two creeks. SP measurements were collected to capture changes in electrical conductivity (as a proxy for salinity) ahead of the occurrence of a salt water intrusion event, marked by increases in conductivity.

It was found that the CVES data and inverse modeling results show increasing resistivities as the distance inland from the shoreline increased. Using resistivity, the inverse of conductivity, as

a proxy for salinity, a location of the freshwater/saltwater interface was inferred, however additional work needs to be performed to determine the location of the transition zone at depth. The borehole SP signals were dominated by streaming potentials (i.e. the movement of fluid) caused by pressure gradients from tidal fluctuations. Fluid specific conductance changes in the borehole were minimal, therefore increases and/or decreases in conductivity ahead of any saline intrusion front could not be captured using SP deployed from the available wells. The signal was compared to tidal gage data to determine that signal sources were electrokinetic from tidal pressure variations. This study provides a first step in using geophysical tools to delineate freshwater/saltwater interfaces in Cape May County, NJ.

a. Study Site

The study site was along the Delaware Bay in Cape May County, NJ at the Rutgers Cape Shore Laboratory (Figure 1). The Rutgers Cape Shore Laboratory is located in Green Creek approximately 16 km from Cape May, NJ. The area surrounding the Laboratory is a part of the Dennis Creek Wildlife Management Area. The Bay is an estuary outlet for the Delaware River and fresh bay water mixes with salt water of the Atlantic Ocean. The shore is composed of salt marshes and mudflats such that only seasonal or small communities can inhabit the area. Dead standing tree trunks along the edges are evidence of saltwater intrusion creeping inland at the surface during high tides. Tidal fluctuations within

Figure 1: Cape May County, NJ with the study area designated and locations of tide gages at South Dennis Creek and Cape May Harbor (credit: Google maps).

10 km of the site are recorded at two USGS stations, Sluice Creek at South Dennis and Cape May Harbor, Cape May (Figure 1).

II. Methodology

a. Marine continuous vertical electrical soundings (CVES)

Continuous vertical electrical soundings (CVES) were collected on December 22, 2017 spanning two creeks inland from the Bay: Green Creek and another creek designated here as Creek 2 (Figure 2). An electrode cable consisting of 13 floating electrodes was towed from a Go-Devil boat rented from the Meadowlands Environmental Research Institute (MERI) (Figure 3). An IRIS Syscal 48 Pro was used to collect the resistance measurements and a Garmin GPSmap 420s was used for GPS locations and depths to the sediment layer. The fluid specific conductance was 33 mS/cm, both in the Delaware Bay, Green Creek and Creek 2.

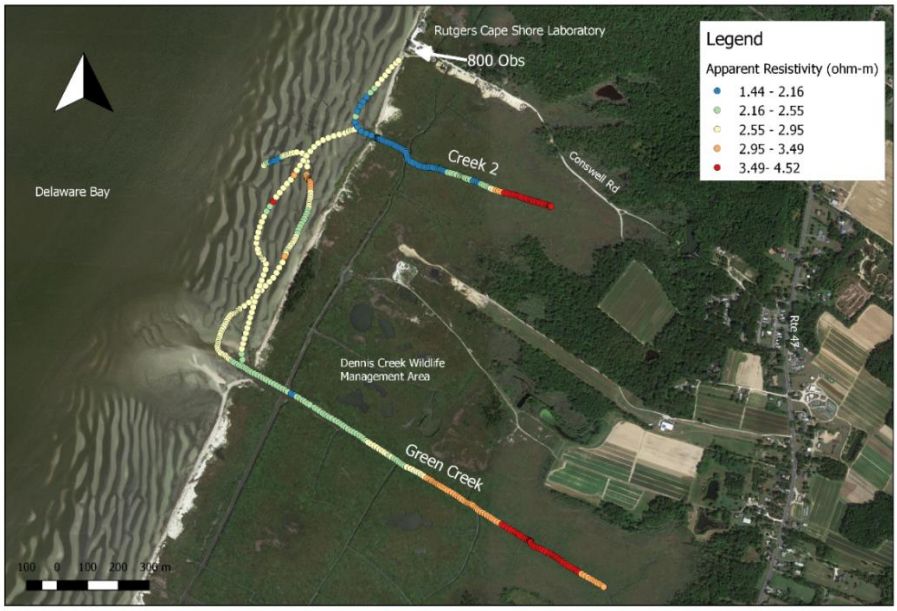

Figure 2: Location of CVES towed survey within Green Creek and Creek 2, south of the Rutgers Cape Shore Laboratory. Averaged apparent resistivities from the data of each sounding are shown for the survey.

The marine survey configuration proposed by Mansoor & Slater (2007) was used which is for a 13-electrode, 10-channel instrument. A single current injection allows for a combination of 10 measurements from the electrode array to be collected at each location. Within the survey, the electrode spacing varies which provides information at different depths. Thus, measurements at each location can be considered as a 1D sounding. Aarhus workbench software (streamed ERT package) was used to invert the data as a series of 1D layered soundings (Auken et al., 2005).

Figure 3: Marine CVES survey. An electrode cable, shown behind the boat, was dragged through Green Creek and Creek 2 by boat pilot and MERI director, Francisco Artigas.

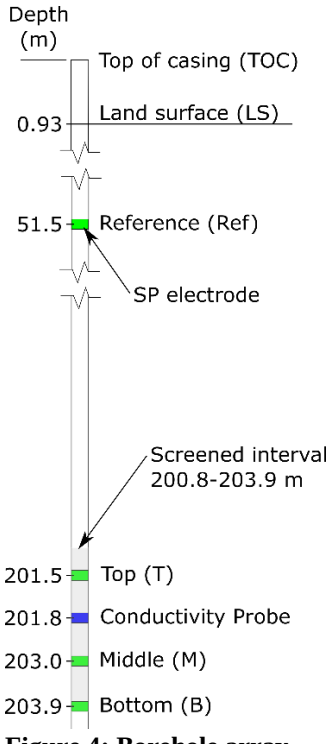

Figure 4: Borehole array schematic for the array used in Oyster 800 Obs.

b. Self potential (SP)

A USGS monitoring well adjacent to the Rutgers Cape Shore Laboratory, Cape May 800 Obs (090302) was selected for this study (Figure 1). Monitoring for this study occurred between November 1, 2017 to December 29, 2017. Water depths and temperature were recorded by USGS every 15 minutes and downloaded monthly. The well is constructed of PVC piping and the screened interval is between 200.8-203.9 m from the top of casing (Figure 4). The open interval is within the Atlantic City sands confined aquifer. Historical borehole logs including gamma, electromagnetic induction (EM) and self potential (SP) show that there is no significant geologic

variability within the screened interval (Figure 5A) which would impact measurements collected in this study.

A SP borehole array (Figure 4) was constructed and installed in 800 Obs using Ag/AgCl Silvion WE300 self potential (SP) electrodes. In this electrode, the Ag/AgCl rod is housed inside a plastic casing containing KCl gel. The casing is separated from its surroundings by a low permeability ceramic disk. The WE300 electrodes have a 0.05M internal KCl concentration. Stranded copper wire (20 gauge or lower) was connected to the lead from each electrode.

Differential voltage measurements were logged at a 15 min time interval with a Campbell Scientific CR10X data logger. This device has a 20 GΩ internal impedance, a resolution of 3.33 μV, and an accuracy of 0.2%. A HOBO conductivity U24 logger was placed in line with the SP electrodes to monitor fluid specific conductance changes over time (Figure 4). Conductivity and temperature were logged every 10 minutes.

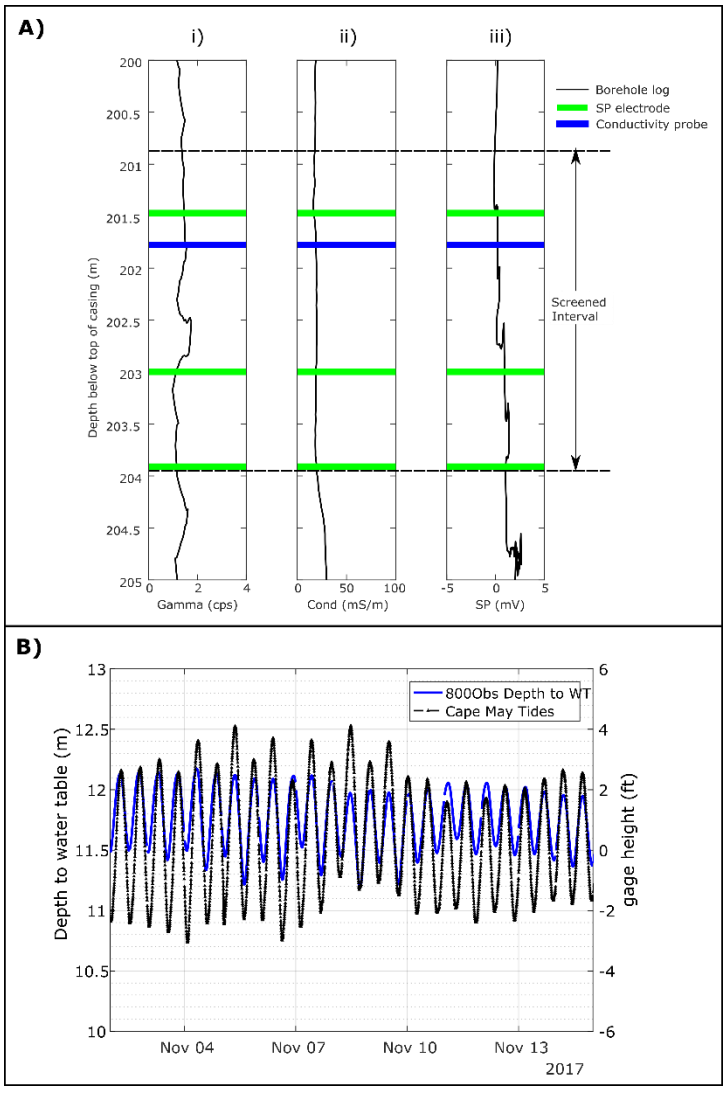

Figure 5: Background information in 800Obs A) Historical Oyster 800 Obs borehole logs obtained from USGS. Gamma log a) electromagnetic induction (EM) log b) and SP log c) are truncated to show the screened interval and locations of self potential electrodes and conductivity probe used in this study. Gamma and SP logs were collected in 1989; EM induction log collected in 2014. B) The depths to the water table (WT) compared to tidal gage height in Cape May.

The array contained four SP electrodes (Figure 4), with the reference electrode near the top of the borehole below the water table. Water level changes due to tidal influence impacted the surrounding landscape; this did not permit the reference electrode to be placed outside of the borehole.

SP measurements are sensitive to concentration gradients and pressure gradients resulting from tidal stage fluctuations. To better understand the tidal influences on water elevations within 800 Obs which would have an impact on the SP data, gage heights and water table elevations were reviewed from Sluice Creek at South Dennis and Cape May Harbor, Cape May (Figure 1). It was found that the Cape May Harbor tides directly correspond to the depth to water table in 800 Obs. As evident in Figure 5B, increases in gage height at Cape May Harbor coincide with increases in the water elevations, suggesting tidal fluctuations immediately have an impact on 800 Obs.

III. Principal Findings and Significance

a. Electrical resistivity: Continuous vertical electrical soundings (CVES)

The CVES raw data is plotted as average sounding apparent resistivity (Ω-m) in Figure 2. The general trend of values reflects an increase in apparent resistivity as the distance from the Bay increases. The entire dataset is shown for completeness, however higher averaged apparent resistivities observed in the Bay are counterintuitive given the observed increased water depth. We presume lack of electrode contact with the water surface (due to the more rapid speed of the boat) caused these lower values. While this data plot gives insight into general trends and values, it lacks insight into the depth of subsurface features to determine a freshwater/saltwater interface. For example, the actual depth to the first sediment layer is not taken into account and

spatially averaging over this very conductive layer can produce a freshwater/saltwater boundary either closer or farther away from the shoreline.

The 1D laterally constrained inversions for Green Creek and Creek 2 are shown in Figure 6. The results are shown as a profile which represents a series of 1D inversions; in the profile there is no image smoothing between each 1D inverted sounding. Missing vertical sections reflect data that was filtered. In most cases, filtering was due to highly unlikely variations in apparent resistivity within a single profile. Sounding depths were used to constrained the inversion and this water layer is shown in as blue within the colorscale in Figures 6b and 6c. A depth of investigation (DOI) is shown as a solid gray line and reflects the boundary below which the inverted model is not sensitive to the data and therefore should not be interpreted. The DOI in Green Creek is shallower than in Creek 2. Injected current tends to follow the path of least resistance and this results in less penetration into a formation below a higher conductive top layer. Therefore, the shallower DOI in Green Creek is likely due to the deeper water depth in Green compared to Creek 2.

The inversions show a progression of higher resistivity within the layering as the distance from the Bay increases. The classic Ghybern-Herberg relation (Ghyben, 1888; Herzberg, 1901) depicts saltwater undercutting freshwater inland due to the higher density of saltwater. The position of the interface is related to the differences in densities of the two fluids. This relation makes several assumptions, one of which is the definition of this interface as a sharp boundary. In reality, this is not the case. More recent work by Barlow & Reichard, (2010) represent this interface as a transition zone where there is a zone of diffusion or dispersion between the freshwater and seawater. There is a wide variation reported (30 m to 670 m) in the transition zone between freshwater and saltwater along the Northern Atlantic Coastal Plain in part due to

global sea level fluctuations (Barlow & Reichard, 2010). For these profiles, a boundary was defined where the 2nd layer was ~3 Ω-m (3.3 mS/cm) and the 3rd layer was ~6 Ω-m (1.7 mS/cm). These values represent two orders of magnitude difference from the Delaware Bay water fluid specific conductance (33 mS/cm). Following from the 2nd and 3rd layers, a bold oval is shown in Figures 6b and 6c to represent the inferred transition zone at depth, which is below the DOI and can only be indirectly estimated from these images. Using this as a guideline places the freshwater/saltwater interface for Green Creek and Creek 2 about 500 m from the Delaware Bay shoreline (Figure 6) at the surface. Viewing Figure 6a, this boundary is approximately parallel to the shoreline. We recognize the assumptions presented in this figure and view this image as a first step towards identifying this boundary.

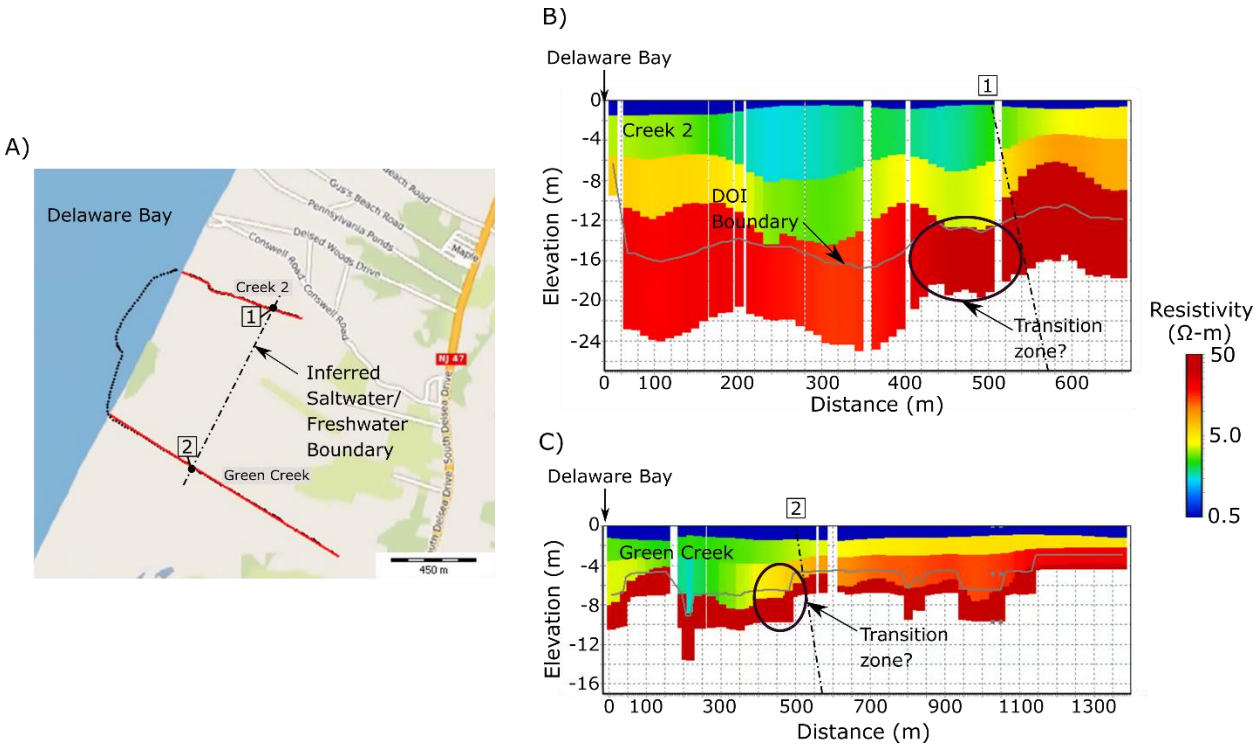

Figure 6: 1D Laterally constrained inversions. Site map shown in A) with profiles Creek 2 B) and Green Creek C). The uppermost layer in the profiles (shown in blue) is the water surface above the first sediment layer.

b. Self potential (SP)

The entire timespan of the SP dataset collected from the borehole array is shown in Figure 7, which consisted of potential measurements between the reference electrode to the top (blue) and bottom (black) electrode and the middle and bottom electrodes (red) (refer to Figure 4 for depths). In order to download the HOBO conductivity probe data, the array was pulled out of 800Obs on Nov 15th and this resulted in a data gap at this time. The measurement between the middle and bottom electrode (Figure 7), is representative of any noise or variations within the data within the open interval of the borehole. Interestingly this measurement, about 5 mV, is approximately the difference between the reference-bottom and reference-top measurements. The SP signal between the reference-bottom and reference-top electrodes exhibits an overall decreasing trend in magnitude, diurnal rises and falls, and a variety of fluctuations. Of primary concern to this study is a comparison of the SP measurements to the groundwater fluid specific conductance in the 800Obs and the ability of changes in the SP measurements to precede any changes in these values. A representative subset of this dataset in Figure 8 shows a constant fluid specific conductance where there was a decreasing trend in the reference-top SP measurements. Given there were no changes in fluid specific conductance, the

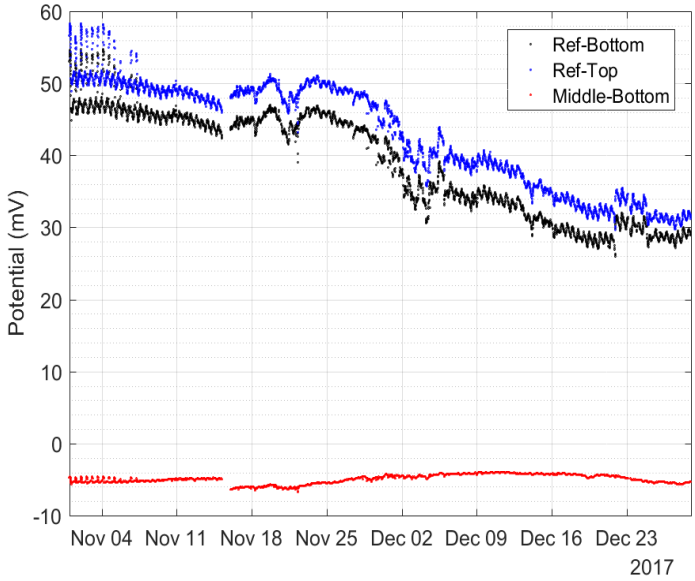

Figure 7: Self potential (SP) measurements within 800Obs for the timespan of this study.

changes in SP cannot be attributed to exclusion-diffusion potential mechanisms since there was no concentration gradient which can lead to differences in ion mobility. Rather, the SP signal must be dominated by electrokinetic mechanisms arising from flow due to the presence of an external pressure gradient, such as the tidal fluctuations.

In order to decipher the time domain signal, a frequency transform was performed using a continuous wavelet transform (CWT) on temporal subsets of SP data and the gage heights at Sluice Creek at South Dennis and Cape May Harbor, Cape May (locations shown in Figure 1). The advantage of using the CWT over a fast-fourier transform (FFT) is that the CWT can represent abrupt changes that are localized in time and frequency. Although the SP signal contains abrupt changes which is ideal for CWT analysis, gage heights are cyclical. It was chosen to perform the CWT analysis on both datasets so that results of the analysis could be more easily compared.

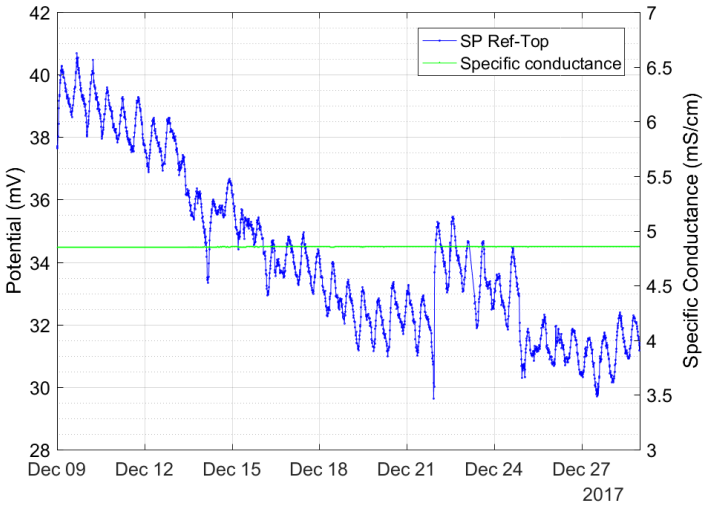

Figure 8: SP measurements alongside fluid specific conductance within 800Obs.

Given the variability in the SP signals, a CWT analysis was performed for two different time periods. The data and results of the CWT analysis for the period from November 2-15, 2017 are shown in Figure 9. The data in Figure 9a shows a wide range of variation in the SP signal, particularly from November 2-7 whereas gage data appear cyclical. CWT results are displayed as scalograms and the y-axis is on a $\log_2$ scale. Since the applied filters in the CWT perform moving averages against the signal which is of finite duration, edge effects manifest at the

beginning and end of a dataset. The dashed white line within each scalogram is the cone of influence, which shows where edge effects of the CWT become significant. The SP CWT is bimodal with two dominate periods at 750 and 375 minutes (Figure 9b). Other vertical features are shown at depth. The sluice gate (Figure 9c) has two dominate periods at the same values as the SP CWT and also has similar vertical features. The dominate period for the Cape May Harbor gage height (Figure 9c) is 750 minutes. The dominant period of 750 minutes can be directly correlated to tidal periods, which is 745 minutes between successive high and/or low

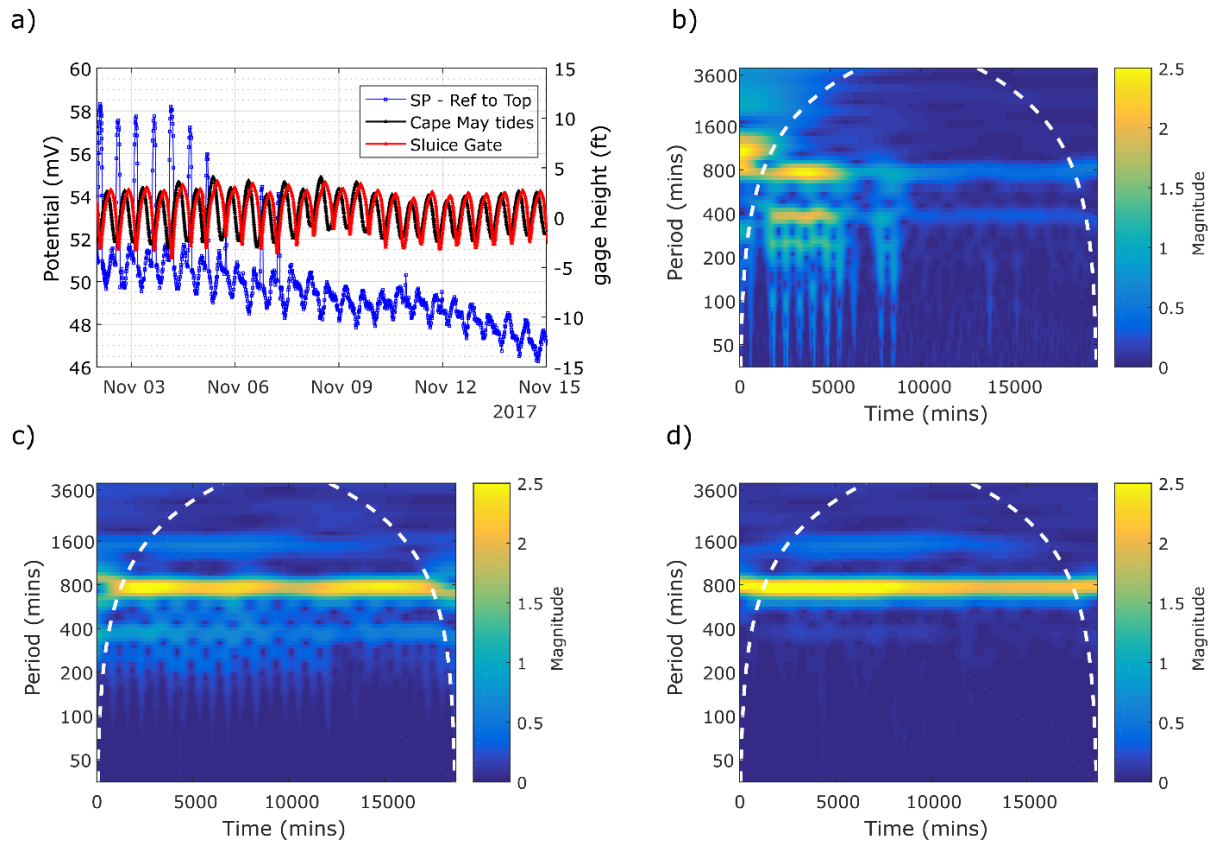

Figure 9: CWT for signal generated between November 2-15, 2017 a) for SP electrodes b) gage height at South Dennis Sluice Gate c) and Cape May Harbor d).

tides. The 375 minutes is half of the tidal period; this is only observed at the sluice gate location and not Cape May Harbor. In Figure 9, it is observed that the SP signal is caused from tidal fluctuations as measured at the two locations.

The results of CWT analysis from Dec 9-15 is shown in Figure 10. The SP data shows a decreasing trend with a large drop in Dec 14th. All signals appear cyclical in nature and the SP signal did not exhibit widely varying values at any point within this timespan compared to the November 2-15th dataset. Again, there are two dominant periods in the SP data at approximately 750 and 375 minutes (Figure 10b). This is observed within the sluice gate CWT (Figure 10c); the Cape May Harbor CWT (Figure 10d) shows a dominant period equal to 750 minutes. Again, the SP signal appears to originate from the gage heights recorded at the two stations.

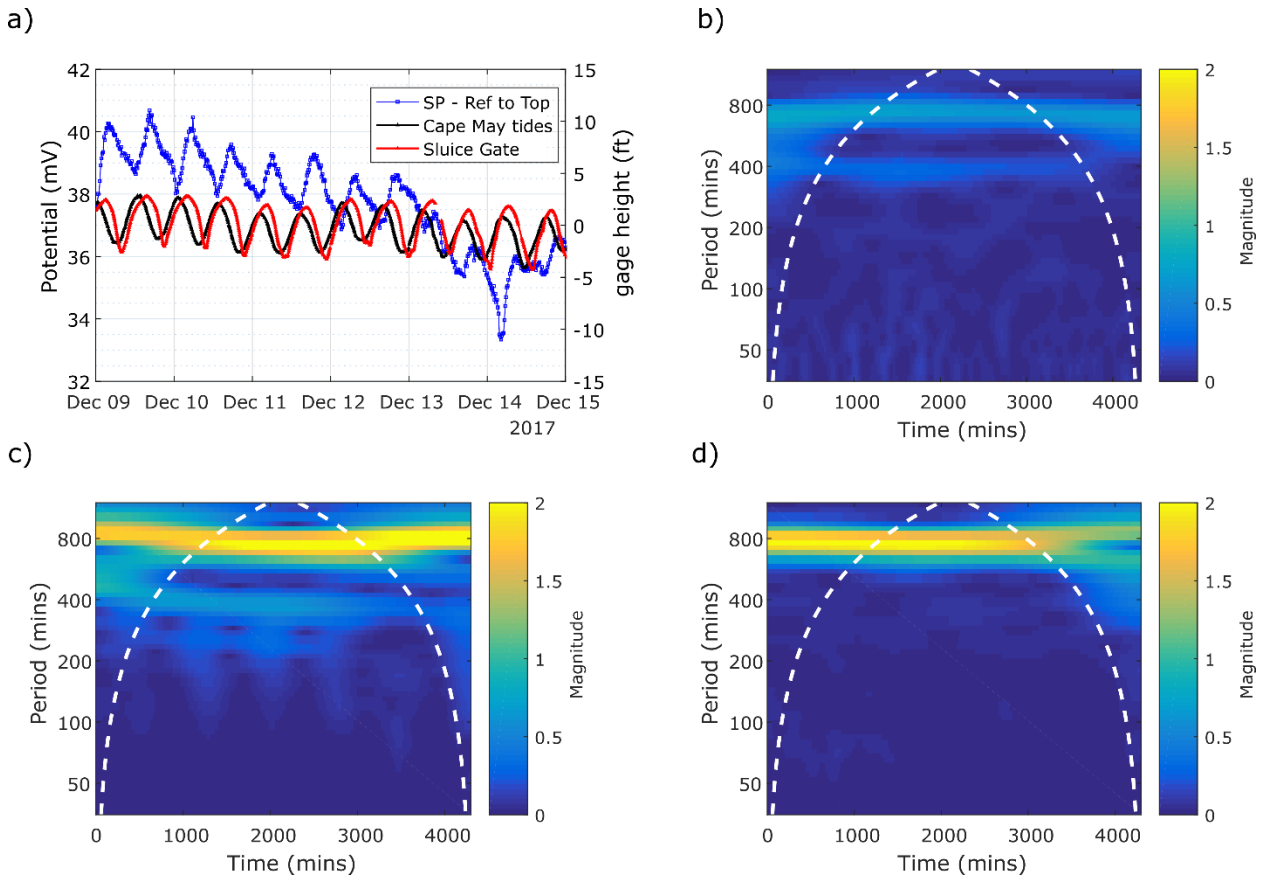

Figure 10: CWT for signal generated between December 9-15, 2017 a) for SP electrodes b) gage height at South Dennis Sluice Gate c) and Cape May Harbor d).

IV. Discussion

Continuous vertical electrical soundings (CVES) and self-potential (SP) measurements within a deep borehole were used to characterize and detect salt water intrusion within Cape May

County, NJ. The CVES survey was used to infer a freshwater/saltwater boundary, however additional surveys need to be performed to refine this model. Similar surveys could be conducted increasing electrode spacings to more reliably image deeper into the subsurface. Additionally, surveys could be collected over different seasons and weather events to map future changes of this boundary from the shoreline.

Unfortunately, SP monitoring within the Cape May 800Obs well did not experience changes in fluid specific conductance in the duration of this study. Therefore, detecting concentration gradients ahead of any conductive front with SP was not possible. An CWT analysis was performed to verify that the SP signal recorded was in fact due to electrokinetic mechanisms due to changes in pressure gradients from tidal variations.

The original proposal planned for SP monitoring in two wells. However, it was learned shortly before deployment that one borehole had a steel casing which would produce erratic SP signals. Efforts to find another well to monitor in Cape May County were not fruitful. The proposal also intended to collect time lapse surface electrical resistivity data. Upon arriving at the site, it was determined infeasible to collect measurement in the adjacent wetlands areas and the tidal range was such that electrical cables could not be left unattended on the Bay shoreline.

V. Outreach/Education

Two undergraduate students were directly supported through this NJWRRI project. Jalise Wright is a senior at Rutgers-Newark majoring in geoscience and is president of the Association of Environmental and Engineering Geologists (AEG) student chapter. She performed extensive laboratory measurements to ensure the SP electrodes were performing as expected before field deployment. She also programmed the Campbell data logger to collect differential measurements and tested the logger for consistent measurements that matched with our

laboratory high impedance voltmeter. She tested the operation of the conductivity probe in the lab. Jalise helped prepare all wire and materials for field deployment and helped build the SP borehole array in the field.

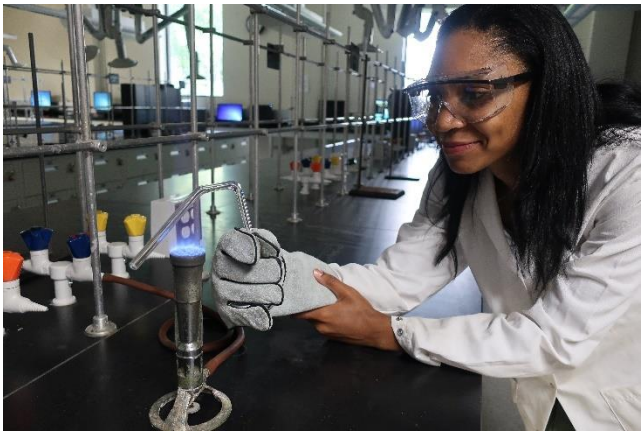

Figure 11: Undergraduate student Jalise Wright making a glass salt bridge to test SP electrodes in the lab using different conductivity fluids.

Undergraduate Daniel Silva, a first-generation college student, recently graduated with a BS degree in January 2018. Participation in this project and a write-up of field activities was one part of his three-part research requirement to graduate. Under my direction, Daniel assisted Jalise in building and deploying the SP borehole array in the field and scouted out a nearby field for a possible surface resistivity campaign. As one of his only field experiences in his undergraduate studies, Daniel gained experience with borehole measurements and learned how to operate the data logger. His write-up earned high marks and showed an understanding of the issues with saltwater intrusion and the theory behind using SP measurements to monitor this phenomenon. A USGS hydrologic technician, Aric Vanselous, talked with Jalise and Daniel about careers and his tasks on a daily basis.

Figure 12: Daniel Silva, now graduated, assisting Jalise Wright in building the SP borehole array in the field.

VI. Conclusion

Using electrical resistivity imaging, the freshwater/saltwater interface was inferred along the Delaware Bay in Cape May County, NJ. This model, while in need of refinement, represents a first step in delineating this boundary. A much greater time and instrumentation effort would have been needed to produce this image if traditional point sampling techniques were used. This boundary is dependent on several variables including tidal gage fluctuations and global sea level changes, periodic CVES mapping of this boundary can better determine the mechanisms and responsible for the movement of this transition zone. Further, larger electrode spacing can potentially look deeper within the aquifer system. While the deep well used in this study did not have conductivity changes, the CWT analysis confirmed the changes within the borehole were due to electrokinetic mechanisms. Future work could focus on using other boreholes in Cape May County.

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Removal of polyfluoroalkyl substances (PFASs) and other micropollutants using spiky sweetgum seeds as renewable bioadsorbents to support "waste control by waste" and point-of-use (POU) water treatment devices

Basic Information

Title:	Removal of polyfluoroalkyl substances (PFASs) and other micropollutants using spiky sweetgum seeds as renewable bioadsorbents to support "waste control by waste" and point-of-use (POU) water treatment devices
Project Number:	2017NJ390B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-010
Research Category:	Water Quality
Focus Categories:	Sediments, Surface Water, Toxic Substances
Descriptors:	None
Principal Investigators:	Likun Hua, Wen Zhang

Publications

1. Shi, X.; Hua, L.; Zhang, W., Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants. 2018 (Under preparation)
2. Column Filtration Using Spikey Balls, Student Filter Competition of AWWA, New Jersey, 2018
3. Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants, Tech Quest Innovation/Prototype Awards of New Jersey Innovation Institution, New Jersey, 2018
4. Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants, Undergraduate Research and Innovation Phase-2 of New Jersey Innovation Institution, 2018
5. Can we use spiky sweet gum seeds as bio adsorbents for the removal of water contaminants? Undergraduate Research and Innovation Phase-1 of New Jersey Innovation Institution, 2017

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2. Numbers of Students Supported:

Undergraduates: 4
Masters' students: 2
Ph. D. students: 1
Postdoctoral Associates: 0

3. Any Notable Achievements

2018 Third Place Award, Student Filter Competition of AWWA NJ
2018 Tech Quest Innovation/Prototype Awards of New Jersey Innovation Institution
2018 Undergraduate Research and Innovation Phase-2 of New Jersey Innovation Institution
2017 Undergraduate Research and Innovation Phase-1 of New Jersey Innovation Institution

4. Project Summary:

4.1 Problem and Research Objectives

This proposal addressed **Research Priority II** - ***Novel approaches to water resource problems and water science*** as it would perform a holistic evaluation of removal of multiple micropollutants in surface waters by sorption. We would use our fully renewable, scalable, developed sweetgum seed bioadsorbent, which results in low carbon and low energy footprint. The U.S. Environmental Protection Agency (EPA) launched its drinking water guidelines for PFOA and PFOS in response to rising concerns of these chemicals toward drinking water security. Recent research has focused on the environmental occurrence, human exposure, and potential health effects of PFASs in the environment. This project delivered new insight into the development

of novel and sustainable water treatment technologies to support waste-control-by-waste concepts and POU applications in small community applications.

Based on the above-mentioned knowledge gaps, research need, and our preliminary work, we hypothesized that the sweetgum seed capsule materials could be a good candidate of renewable biomass as a starting point to develop novel, cost effective, simple, and user friendly adsorption devices. They are particularly suitable for small scale communities and point-of-use (POU) water treatment facilities to remove trace level micropollutants. The results provided new insight into the sustainable water pollution prevention, waste conversion, and waste-control-by-waste concepts. To test the hypothesis, the following **specific objectives** were pursued:
Objective I: Characterization of seed materials for better understanding the physicochemical properties and the influences of thermal treatment.
Objective II: Batch tests for adsorption kinetics and adsorption capacities of different water micropollutants on bioadsorbent surfaces will be determined.
Objective III: Adsorption column tests for longevity, reusability and cost effectiveness.

4.2 Methodology
(1). Materials
Adsorbent. Sweetgum tree seed capsules as shown are collected from local areas of New Jersey and undergo proper treatment (cleaning, drying, and thermal activation). Thermal treatment is conducted by placing capsule materials in the oven under 100-300 ^{o}C for different times to carbonize the structure. Then treated seed capsules will be appropriately characterized with different analytical techniques to determine changes in functional groups, morphology, specific surface areas and mechanical properties.

Model micropollutants. PFOA (98%) and Methylene blue (MB) purchased from Sigma Aldrich and Fisher Scientific was proposed to be used as options of model micropollutants for the adsorption studies. These were selected owing to their environmental relevance and recalcitrant properties in traditional water and wastewater treatment. During this project, MB was first chosen and thoroughly tested while other pollutant will still be tested afterwards.
(2). Experimental design

Characterization. Surface morphology and chemistry of biomass materials before and after thermal treatment will be examined by scanning electron microscopy (SEM)-Energy Dispersive Spectroscopy (EDS) (Hitachi S-3400N), Nicolet ThermoElectron FTIR spectrometer, WITEC ALPHA300 Confocal Raman microscope. BET surface areas analysis is performed on Micromeritics® AutoChem II 2920.

Batch tests to determine adsorption kinetics and adsorption capacity. Polluted water is simulated by blending pure water with the above mentioned pollutants at desirable concentrations. Batch adsorption tests will be carried out in flasks with 50 ml in a shaker (See **Fig. 1**). The reaction temperature is controlled at room temperature ~22^{o}C. Adsorption isotherm will be studied by mixing micropollutants and capsule adsorbents at different appropriate mass ratios as was previously performed. At different adsorption time, the liquid samples are withdrawn to determine the residual concentrations of different pollutants. The concentration of heavy metals in the supernatant is determined by inductive coupled plasma-mass spectrometry (ICP-MS). The amount of adsorbed metal is calculated by the difference between the initial and final solution concentrations. Control experiments without adding adsorbent are also included. The PFOA concentration is measured by an Acquity UPLC (XEVO G2 QTOF, Waters Co., Ltd, USA) equipped with a C18 column. The mobile phase consists of acetonitrile and 0.1% formic in water. The adsorption isotherm data may be fitted with Langmuir or Freundlich equations.

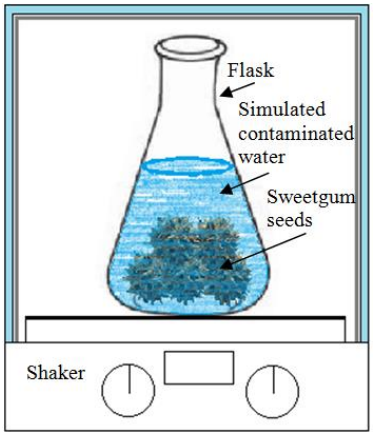

Fig. 1 Schematic of the laboratory batch tests set up.

4.3 Principal Findings and Significance

The thermal treated spiky ball showed a lower organic composition than original spiky ball, as shown in the FTIR spectrum in **Fig. 2**. The experiments conducted on MB shows effective result (**Fig. 3**). During the experiments, we found thermally treated spiky balls have a better absorption performance than raw spiky balls, which was possibly caused by a higher porosity of the absorbent since the structure was carbonized by heat (**Fig. 3a**). Additionally, we also found the treated spiky balls have a higher absorption capacity for the MB tested with different concentrations of MB (**Fig. 3a**). Additionally, we have also prepared the PFOA and PFOA experiment precedures, which will be tested next. The calibration curve will follow **Fig. 4** for HPLC measurement.

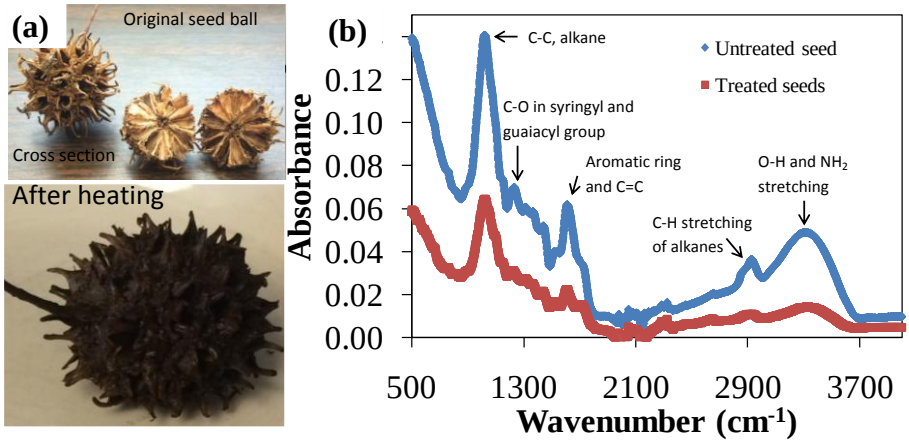

Fig. 2 (a) Original spiky ball and thermally treated spiky ball; (**b)** FTIR spectral changes before and after thermal treatment.

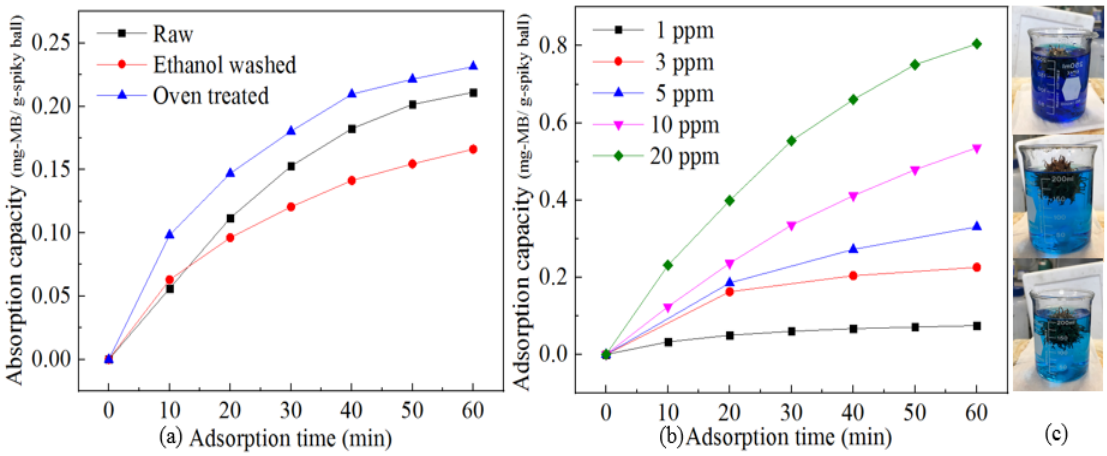

Fig. 3 Adsorption capacity of **(a)** three types of spiky balls for 3 ppm MB, **(b)** raw spiky ball for MB with different initial concentration, **(c)** the change in color of MB solution with time

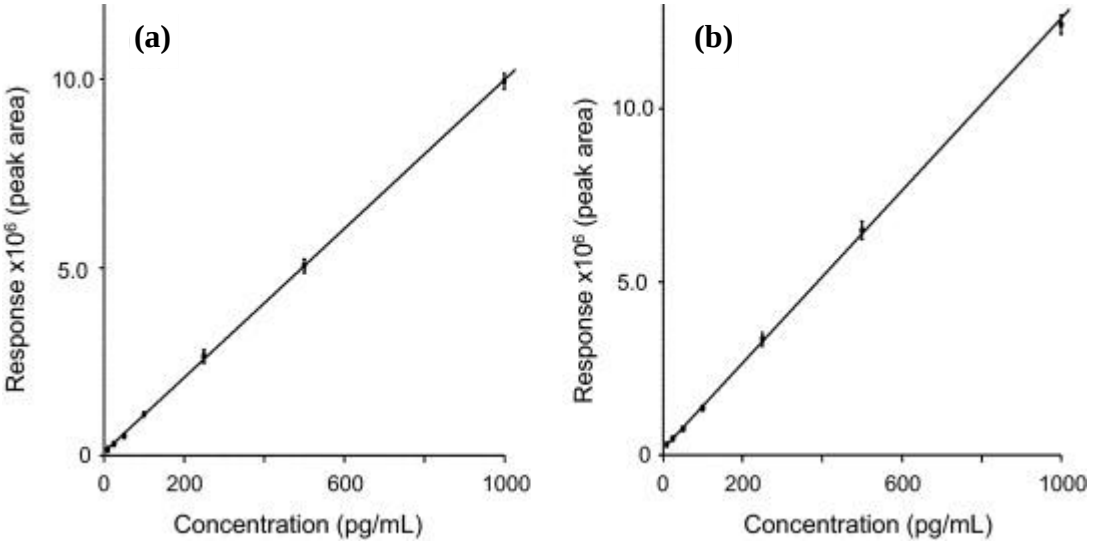

Fig. 4 HPLC Calibration curves for the triplicate injections of PFOA **(a)** and PFOS **(b)** in the concentration range from 10 pg/mL to 1000 pg/mL. (Dolman et al, 2011)

The outcome of this study provides the scientific basis for a synthetic chemical-free method employing sweet gum tree seed to enable sustainable drinking water treatment in the developing world. Sweet gum tree seed capsule is a local biomass found in New Jersey that often ends up in solid waste. Due to the unique porous structures, these spiky balls have been converted by us into cost effective adsorbents for point-of-use (POU) water treatment devices. We have demonstrated this bio adsorbent can be fully renewable, scalable and involve low carbon and energy footprint in the preparation. To fully implement the proposed filter in the field, we propose to further validate the effectiveness against organic dye and other pollutant. Filter regeneration should also be considered for further work. For the treatment of highly concentrated source waters, we have designed and tested a hybrid column with a regular sand layer on top of the seed layer (**Fig. 5**). In addition, testing in the field is required before comprehensive environmental implications can be assessed.

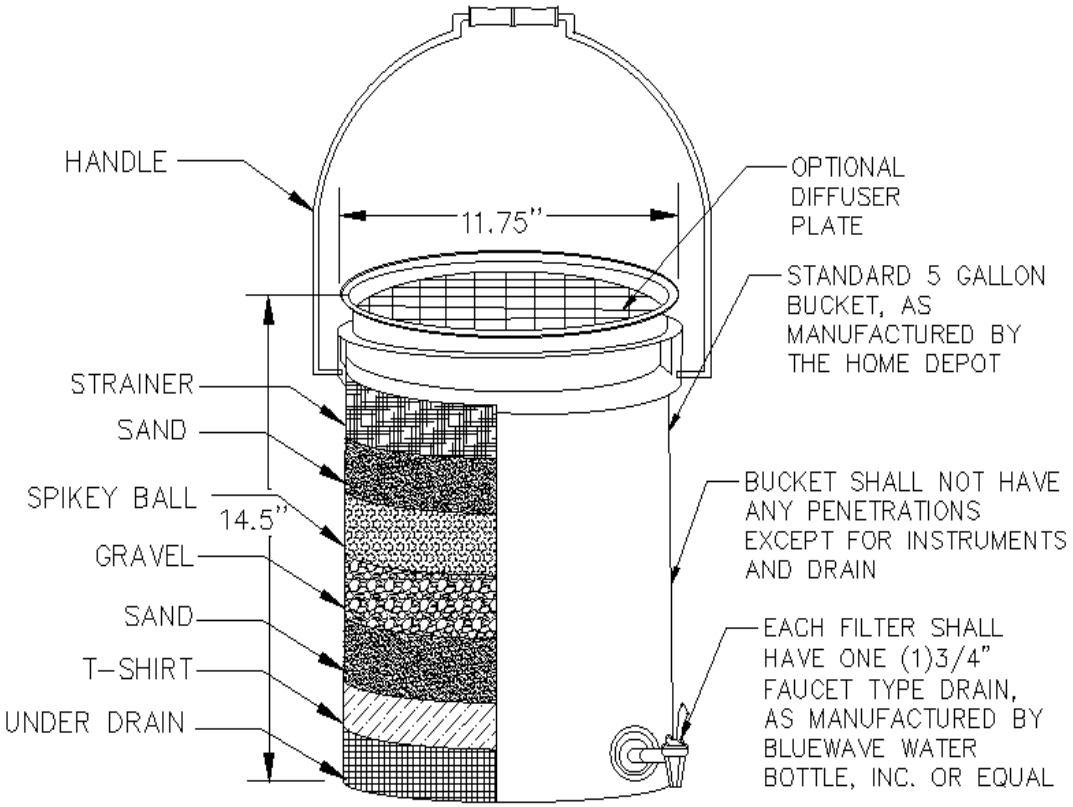

Fig. 5 Hybrid column filtration system with different layers addition to the spiky ball

5. Publications or Presentations:

5.1 Publications

Shi, X.; Hua, L.; Zhang, W., Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants. **2018** (Under preparation)

5.2 Poster Presentations

1. Column Filtration Using Spikey Balls, ***Student Filter Competition of AWWA***, New Jersey, 2018
2. Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants, ***Tech Quest Innovation/Prototype Awards of New Jersey Innovation Institution***, New Jersey, 2018

5.3 Oral Presentations

1. Development of Sustainable Bio-Adsorbent using spiky sweet gum for the removal of water contaminants, ***Undergraduate Research and Innovation Phase-2 of New Jersey Innovation Institution***, 2018
2. Can we use spiky sweet gum seeds as bio adsorbents for the removal of water contaminants? ***Undergraduate Research and Innovation Phase-1 of New Jersey Innovation Institution***, 2017

Designer Bacteria for Restoring the Passaic River

Basic Information

Title:	Designer Bacteria for Restoring the Passaic River
Project Number:	2017NJ391B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Biological Sciences
Focus Categories:	Groundwater, Toxic Substances, Treatment
Descriptors:	None
Principal Investigators:	Rachel Dean, Donna E. Fennell

Publications

1. Dean, Rachel; Cassidy, Schneider; Haider, Almnehlawi; Fennell, Donna; 2018, Dechlorination of Chlorinated Dioxins in the Passaic River, New Jersey.(In progress)
2. NEMPET 2018: Northeast Microbiologists: Physiology, Ecology and Taxonomy June 22nd - 24th. Minnowbrook Lodge, Blue Mountain Lake, New York Oral presentation titled “Benzene be unseen: apparent benzene loss in iron-reducing cultures”

Rachel Dean
FY 2017 NJWRRI Project Report Summary

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(2) **Numbers of Students Supported**:
Undergraduates: 0
Masters' students: 0
Ph. D. students: 2
Postdoctoral Associates: 0

(3) **Any Notable Achievements**
None.

(3) **Project Summary**:

Problem and Research Objectives:

The sediments of the Passaic River harbor dioxins, in particular 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD), which are dangerous to fish, wildlife, and human health (1). The lower Passaic River will soon undergo dredging to remove dioxin-contaminated sediments and this effort is expected to last for six years (2,3). Dredging is disruptive in terms of noise and smell and limits the recreational use of the river. For instance, swimming was discouraged in the Hudson River during dredging due to operational traffic and the potential danger from disturbed PCBs (4). Because dredging will be used to remove only the most highly contaminated, accessible sediments, dioxins will still persist in sediments below the dredging depths. Therefore, it is important to design a more permanent remediation solution which is not limited to superficial layers of contamination. Eliminating TCDD through stimulation of naturally occurring microorganisms or amendment of biological materials, in contrast to mechanical removal of dioxins, may be the least invasive way to restore the Passaic River to a safe and functional state.

Since dioxins are fat soluble and accumulate in the food chain, the main route of dioxin exposure for humans is through the ingestion of contaminated meat and fish (5). NJ residents in surrounding areas are still advised not to eat any fish coming from the lower Passaic River due to PCBs and dioxin contamination (6). In other words, the River remains an unfit source of fish for residents who may rely on it for sustenance or recreation. To be specific, levels of TCDD as high as 29,000 ppt have been found in the Passaic River (7). Above 50 ppt TCDD, the state of New Jersey deems that an area should be screened (8). Many NJ residents live near the Passaic River,

where TCDD concentrations far exceed the screening threshold of 50 ppt. Furthermore, despite warnings and requests not to, many residents eat the fish and shellfish from the polluted river (9). TCDD is the most harmful potential dioxin tainting the Passaic River. Identifying active bacteria which could detoxify TCDD and producing large amounts of their detoxifying enzymes would be beneficial for New Jersey residents living near the Passaic River.

Under oxygen-free conditions such as the sediment bed where dioxins are present, organohalide respiring bacteria (OHRB) are the only known microorganisms involved in dioxin transformation (10). OHRB are bacteria that can utilize chlorinated organic compounds (organohalides) for energy to support growth (11). The OHRB produce reductive dehalogenase (rdh) enzymes to take electrons from an electron donor, often hydrogen, and use them to reduce organohalides such as chlorinated dioxins. In doing so, the bond between the halogen and the carbon compound it is attached to is cleaved (12) and the chlorine is released as a harmless chloride ion. Depending upon the position of the chlorine substituents, de-chlorination can substantially detoxify the compound (13). OHRB genera such as *Dehalococcoides* and *Desulfitobacterium* often have multiple rdh's, can use a number of electron donor compounds, and use a wide range of organohalides as electron acceptors (11). Very few dioxin-dehalogenating bacteria have been identified (14,15) and enriching cultures with TCDD could be expensive and slow. Therefore, in this project we will take advantage of OHRB's wide substrate range and first enrich for OHRB on more soluble and available organohalides. We will then enrich for OHRB which can degrade TCDD. After obtaining TCDD-active cultures, we will analyze metagenomic data of these cultures to identify specific rdh's involved in TCDD degradation and express those enzymes in an external microbial host.

Development of dioxin-active microbial cultures would supply valuable data applicable to the Passaic River. Moreover, only three rdh's have been successfully produced outside of OHRB (11,16,17), all within the past two years. Production of dioxin-active enzymes from these dechlorinating cultures would provide cutting-edge, raw biological product applicable to the remediation of the Passaic River and beyond.

Methodology - a general summary of procedures and methods actually implemented:

Microcosm studies of dioxins degradation:

Because the organohalides trichloroethene (TCE) and 1,2-dichlorobenzene (1,2-DCB) are more lightly chlorinated and are also more soluble, they were expected to allow faster growth of desired OHRB than direct incubation with dioxin. Therefore, TCDD-amended microcosms were created using previously established dichlorobenzene (DCB)- and trichloroethylene (TCE)-degrading Passaic river sediment microcosms as the inocula. Similar microcosms were also set up using fresh sediment from the Lower Passaic River at the mouth of Newark Bay (the same inoculum as the DCB and TCE enrichment cultures). The use of the original sediment in the microcosms supported the ideology that direct enrichment of dioxin-dechlorinating microorganisms may immediately turn on transcription of relevant reductive dehalogenase genes. **Table 1** outlines the setup of microcosms, which were created in triplicate. The TCE co-substrate in Sets 9-11 and the DCB co-substrate in Set 15 were added in the case that these

original chemicals were needed to stimulate dechlorinating activity in the cultures. Sediment samples were taken approximately every two months and frozen for later analysis.

Table 1: Description of established microcosms amended with various dioxin congeners. Original sediment = un-enriched sediments from the original sampling location in the Passaic River, DCB enrichments = microcosms established with and actively dechlorinating dichlorobenzene, TCE 2nd Transfers = 2nd transfer microcosms established with and actively dechlorinating trichloroethene.

	Source	Congener	Concentration (µM)	Note
1	Original Sediment	All	--	killed control
2	TCE 2nd Transfers	All	--	killed control
3	Original Sediment	2, 3, 7, 8	2	
4		1, 2, 3, 4	21	
5		2,7	20	
6	TCE 2nd Transfers	2, 3, 7, 8	2	
7	TCE 2nd Transfers	1, 2, 3, 4	21	
8	TCE 2nd Transfers	2,7	20	
9	TCE 2nd Transfers	2, 3, 7, 8	2	+ TCE co-substrate
10	TCE 2nd Transfers	1, 2, 3, 4	21	+ TCE co-substrate
11	TCE 2nd Transfers	2,7	20	+ TCE co-substrate
12	DCB Enrichments	2, 3, 7, 8	2	
13		1, 2, 3, 4	21	
14		2,7	20	
15		2, 3, 7, 8	2	+ DCB co-substrate

Gas chromatography-mass spectrometry

Dioxin analysis methods are being developed using gas chromatography-mass spectrometry (GC-MS) with a HP-5MS 60.0 m capillary column. Standards containing all amended dioxin congeners and potential daughter products were developed. Samples are extracted into toluene:acetone and cleaned up on a Florisil column prior to analysis.

Genetic screening

Because the methods for genetic analysis mentioned in the proposal (DNA stable isotope probing (SIP) and metagenomic analysis) first require GC-MS confirmation of activity against one or more of the amended dioxin congeners, these analyses have not yet been conducted. However, a collaborator in a dioxin degradation - focused research group at Michigan state University has analyzed DNA from the TCE and DCB enrichment cultures. These cultures served as the inoculum for the dioxin-amended microcosms which are the focus of this study. Therefore, further analysis of this data can help us predict which dehalogenase genes may be active under exposure to dioxin.

Protein expression

The process of strain construction has begun. In preparation for identification of a dehalogenase gene of interest, we have begun streamlining the process of introduction of a gene into the appropriate vector DNA and host bacterium. As the gene to introduce, we are currently using the reductive dehalogenase gene PceA from *Desulfitobacterium hafniense* DCB-2, which

dechlorinates the organohalide perchloroethene. This is the gene that Mac Nelly et al. introduced into a host bacterium which led to successful production of its active protein. To first confirm their methods as well as to validate our own amendments, we are attempting to reproduce their results. A particular change we are making is to create a single DNA construct which includes both functional protein (RdhA) and chaperone protein (RdhT). This is in contrast to MacNelly's group which created DNA constructs with single genes and therefore had to introduce two constructs into the receiving microbial host instead of one when testing the effect of the added chaperone protein on functional protein viability. Introducing two constructs into a bacterium was expected to be less efficient than introducing just one, and also raised the possibility of the host bacterium losing one construct over time. Therefore, the strains we create with this amended method should be more stable over time. Additionally, instead of the pASK vector used by their group which contains a tetraycline-inducible gene promoter, we will be utilizing a pMAL vector, which contains a hybrid tryptophan and lactose (tac) operon. This promoter allows for much finer control of protein expression levels than other promoters, which is important if the protein is burdensome or toxic to the host bacterium producing it. A schematic of the planned DNA construct is depicted in **Figure 1**. Another change I will implement, as mentioned in the original proposal, will be to include the needed VitaminB12 into the media during growth although the microbial host organism is capable of producing it on its own, to ensure the host bacteria have plenty of this vitamin required for proper production of the protein.

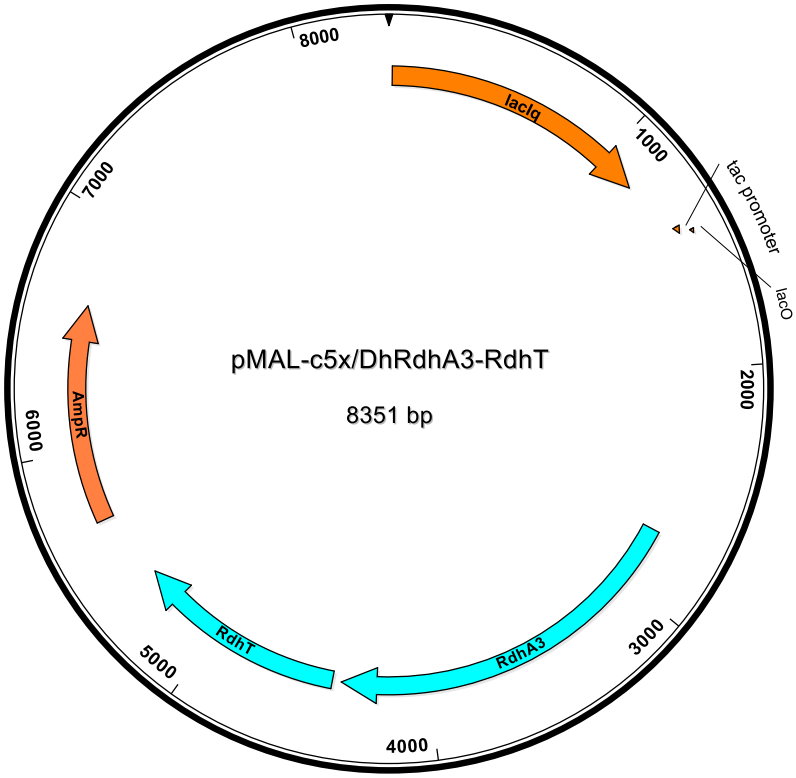

Figure 1. Schematic of the DNA construct including functional gene RdhA3 and chaperone gene RdhT of *D. hafniense* strain DCB-2.

Principal Findings and Significance - a summary of significant findings:

Microcosm studies of dioxins degradation:
As can be seen in **Figure 2**, early results showed dechlorination of one of the amended dioxin congeners in microcosms created with TCE enrichment cultures. The microcosms created with DCB enrichment cultures are expected to show de-chlorination as well, yet with a potentially different de-chlorination pattern or substrate range. As DCB is more hydrophobic than TCE and is also aromatic, we expect that the DCB cultures may be more likely to de-chlorinate the very hydrophobic compound, TCDD.

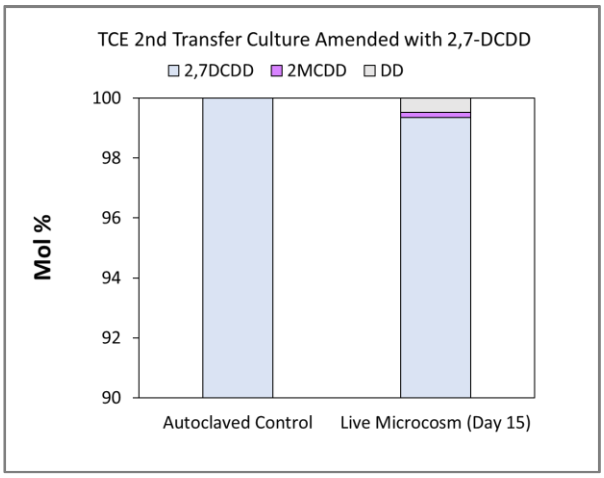

Figure 2: Early results showing biologically- mediated partial de-chlorination of the lightly chlorinated dioxin 2,7-dibenzo-p-dioxin (2,7DCDD) to 2-monochlorodibenzo-p-dioxin (2MCDD) and complete de-chlorination to dibenzo-p-dioxin (DD).

Preliminary genetic analysis:
DNA from the DCB- and TCE- enrichment cultures were subjected to analysis on a WaferGen SmartChip by a collaborator. To do the analysis, DNA sequences from five strains of *Dehalococcoides mccartyi* and three species of *Dehalogenimonas* were pooled into a database for primer design. These primers were then used on the chip for simultaneous qPCR reactions. Four reductive dehalogenase genes from the DCB enrichment culture and twenty-three from the TCE enrichment culture were shown to have a significant DNA copy number, indicating that these genes are active under exposure to the respective chemicals. The low identification of reductive dehalogenases in the DCB enrichment culture is likely due to the low overall DNA concentration in the sample which was analyzed. Another sample from this culture lineage with higher DNA concentration will be re-analyzed.

Protein expression:
As Mac Nelly's group did, we will test functional protein alone (RdhA) and functional protein along with chaperone protein (RdhA + RdhT) for PceA from *D. hafniense*. We will follow this same process when we have identified one or more dehalogenases of interest from our own study. The expression vector containing RdhA3 of *D. hafniense* was confirmed with appropriate primers (**Fig. 3a**). The expression vector containing both RdhA3 and RdhT of *D. hafniense* has not yet been successfully created, but a vector containing RdhT was confirmed (**Fig. 3b**). These vectors can be further cleaved and re-ligated to produce the desired vector containing both RdhA3 and RdhT genes.

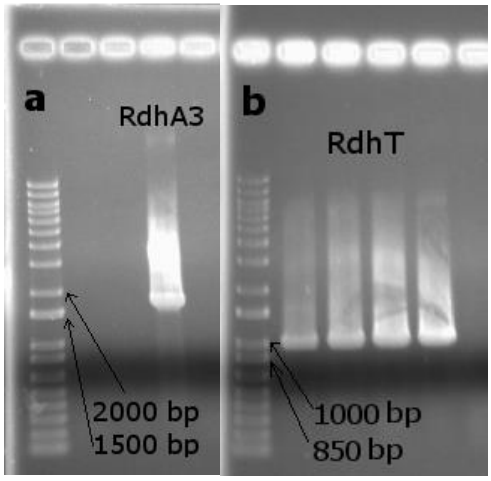

Figure 3: Agarose gels showing amplified DNA of the expected size correlating to RdhA3 (a), and RdhT (b), from the constructed pMAL-c5x expression vectors.

Significance:
The results from the early timepoint showing dechlorination of 2,7-dichlorodibenzo-p-dioxin after just 15 days are promising. Now that the microcosms have incubated with the various dioxin congeners for over one year, we expect to see activity on the more heavily chlorinated congeners, 1,2,3,4- and 2,3,7,8-tetrachlorodibenzo-p-dioxin. The SmartChip data from the cultures which were used as inocula for the current study indicates upregulation of multiple reductive dehalogenase genes. This same analysis can be performed on any cultures active toward one of the dioxin congeners, which may implicate a reductive dehalogenase involved in dioxin dechlorination. While analytical methods are being optimized for GC-MS analysis of dioxins, DNA construct creation and protein expression methods are also being optimized. After confirmation of further activity toward one or more dioxin congeners in the dioxin-amended cultures, further genetic analysis and heterologous protein production can be performed as previously proposed. The results from genetic analysis will give valuable knowledge on which genes are most active during dioxin dechlorination. Functionally active protein of any of the reductive dehalogenase genes will be useful for the field of anaerobic remediation of chlorinated compounds, and production of a dioxin-active protein will be particularly important in this research field as well as for the potential remediation of the heavily contaminated Passaic River.

(4) **Publications or Presentations**:

1. Articles in Refereed Scientific Journals:
In progress-
Dean, Rachel; Cassidy, Schneider; Haider, Almnehlawi; Fennell, Donna; 2018, Dechlorination of Chlorinated Dioxins in the Passaic River, New Jersey.

2. Book Chapter:
None.

3. Dissertations:

None.

4. Conference Proceedings
NEMPET 2018: Northeast Microbiologists: Physiology, Ecology and Taxonomy
June 22nd - 24th. Minnowbrook Lodge, Blue Mountain Lake, New York
Oral presentation titled "Benzene be unseen: apparent benzene loss in iron-reducing cultures"

5. Other Publications
None.

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Developing a Multitasking "Green" Technology for Removal of Wastewater Contaminants and Bioethanol Production

Basic Information

Title:	Developing a Multitasking "Green" Technology for Removal of Wastewater Contaminants and Bioethanol Production
Project Number:	2017NJ392B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-008
Research Category:	Biological Sciences
Focus Categories:	Management and Planning, Wastewater, Water Quality
Descriptors:	None
Principal Investigators:	Saumik Panja, Dibyendu Sarkar

Publications

1. Panja, Saumik. & Dibyendu, Sarkar. (2018). Developing A Multitasking Green Phytotechnology to Remove Pharmaceuticals and Nutrient from Secondary Wastewater Effluent. Innovation Expo, Stevens Institute of Technology, Hoboken, NJ, May 2018.
2. Panja, Saumik. & Dibyendu, Sarkar., & Rupali, Datta. (2017). Removal of Ciprofloxacin and Tetracycline by Vetiver Grass from Nutrient Amended Secondary Wastewater Matrix. Association for Environmental Health and Sciences Foundation, Amherst, MA, October 2018.

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Numbers of Students Supported: 0

Any Notable Achievements

- Panja, Saumik. & Dibyendu, Sarkar. (2018). Developing A Multitasking Green Phytotechnology to Remove Pharmaceuticals and Nutrient from Secondary Wastewater Effluent. Innovation Expo, Stevens Institute of Technology, Hoboken, NJ, May 2018. (2nd place in **Doctoral Student Poster Presentation Competition**).

- Panja, Saumik. & Dibyendu, Sarkar., & Rupali, Datta. (2017). Removal of Ciprofloxacin and Tetracycline by Vetiver Grass from Nutrient Amended Secondary Wastewater Matrix. Association for Environmental Health and Sciences Foundation, Amherst, MA, October 2018. (2nd place in **Doctoral Student Poster Presentation Competition**).

Project Summary

Problem and Research Objectives

Pharmaceutical compounds are one of the major emerging contaminants that pose a severe threat to water quality as well as to public health all over the world (Kolpin et al. 2002; Marx et al. 2015). The major sources of pharmaceutical compounds include domestic sewage and wastewater generated from public health sectors (Pereira et al. 2015). The majority of the pharmaceutical compounds, after consumption is weakly absorbed and poorly metabolized by our physiological system and excreted in intact form (McArdell et al. 2003; Rossmann et al. 2014). Following excretion, they are released to our sewage system, which eventually ends up in wastewater treatment plants (WWTPs). As of now, there is no regulation enforced by the US Environment Protection Agency (EPA) for these compounds (He et al. 2015), hence, the WWTPs do not have any mandate, nor an infrastructure to screen pharmaceutical compounds from wastewater. Antibiotics, whether it is present within a living system or in the environment, tend to put biological pressure on microorganisms, and enable them to develop new metabolic pathways to

acquire immunity against them. High concentration of COD in urban wastewater and the presence of pharmaceutically active compounds generate a favorable condition for diverse microorganisms which are already present in the wastewater to develop antibiotic resistance genes (ARGs) through the process of anaerobic treatment, an integral part of all WWTPs (Aydin et al. 2015). Through horizontal gene transfer (HGT) mechanism, the developed ARGs are spread across different strains and species of microorganisms. Thus, the antibiotic(s) become ineffective against its target pathogenic species and can no longer be used for treatment purposes. The major concern is that the development of new antibiotics or modification of old ones are not as fast as the microorganisms acquire resistance against them. According to the report of Center for Disease Control and Prevention (CDC), more than 2 million people in the US are affected by antibiotic-resistant infections every year, of whom 23,000 people die (CDC report 2013). Benotti et al. (2009) reported the presence of 51 wastewater-derived pharmaceutical endocrine disrupting compounds in the reclaimed water of 19 drinking water facilities that serves more than 28 million people in the United States.

Antibiotics have been the pillar of public health welfare since its discovery in the early 20th century. Apart from human health, antibiotics are also used in veterinary and agricultural sectors. The diverse application of antibiotics in various sectors has increased its production tremendously in recent years. According to a Food and Drug Administration (FDA) report, sale of antibiotics in the United States was approximately 3.3 million kg in 2011, which accounts for only human consumption, not taking into account the veterinary sector (Wang et al. 2015).

We performed several preliminary experiments in a hydroponic setup to test if vetiver is capable of removing pharmaceutical compounds from secondary wastewater effluents. Within first 7 days, 100% CIP (at an initial concentration of 0.05 mg/L), 87% CIP (at an initial concentration of 0.1 mg/L), 78% CIP (at an initial concentration of 1 mg/L) and 63% CIP (at an initial concentration of 10 mg/L) was removed by vetiver. Within 15 days, more than 90% of CIP was removed from all systems except for 10 mg/L initial concentration, where 70% of removal was achieved.

Objective:
Investigate the molecular mechanisms of uptake and potential detoxification of antibiotics in the vetiver system.

Methodology

Chlorophyll extraction

Chlorophyll was extracted from vetiver leaf according to Liu et al. (2013). 0.1 g of leaf tissue was crushed using liquid nitrogen in a mortar pestle. The crushed tissue was incubated in 80% (v/v) aqueous acetone for 24 hours in dark. The solution was filtered, and absorbance of the solution was measured using Citation 3, Biotek® microplate reader at 663 and 645 nm for chlorophyll a and b respectively.

Crude protein extraction

Plant protein was extracted according to Farkas et al. (2007). 70mg of crushed plant material was transferred to a 2 mL microcentrifuge tube containing 0.5 mL of protein extraction buffer (50mM Tris-HCL (pH 7.2), 1 mM ethylenediaminetetraacetic acid, 10 mM β-mercaptoethanol, and 1μL of 20 mg mL^{-1} PMSF). The reaction mixture was subjected to cold bath sonication for 30 minutes and centrifuged (10,000g for 15 minutes) thereafter. The supernatant containing crude plant protein was collected in microcentrifuge tube and stored at -80°C freezer. The protein quantification was performed using Pierce™ bicinchoinic acid (BCA) protein assay kit (Thermo Scientific).

Stress Enzyme Analysis

Glutathione-s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activity assay were performed using enzyme assay kits (Cayman Chemicals). Crude plant protein extract was used for performing GST and CAT assay. A Citation 3, Biotek® microplate reader was used for all spectrophotometric analysis.

Sample preparation for ESI-MS analysis and instrumental parameters

ESI-MS analysis was performed to detect CIP degradation products present in vetiver root and shoot. The plant material was obtained for this analysis after the experimental period (30 days). The plant materials were crushed with liquid nitrogen and the crushed biomass was eluted in deionized water. The mixture was vortexed and then filtered using 0.45μM syringe filter. The filtrate was used as ESI-MS samples. Unit mass resolution Collision Induced Dissociation (CID) spectra were recorded on a Waters Quattro Ultima Triple Quadrupole Mass Spectrometer equipped with an Electrospray Ionization (ESI) source using nitrogen as the desolvation gas. The samples were introduced to the source at a flowrate of 20μL/min. The source and the desolvation gas temperature were held at 100 and 250°C, respectively. The collision energy was varied between 4-30 eV. The capillary and cone voltage were set at 3 kV and 15 kV, respectively. The desolvation gas flow was maintained at 222 L/hr.

Principal Findings and Significance

Chlorophyll content

With the increasing CIP concentration in aqueous media, the depletion of chlorophyll increased (fig.1). A significant ($p<0.05$) drop in the chlorophyll content was observed for 1 and 10 mg/L CIP treatment. For 1 and 10 mg/L CIP treatment, the chlorophyll drop was 33% and 46% respectively. For lower CIP concentrations (0.05 and 0.1 mg/L), the drop was 9% and 14% respectively. There were no such changes observed in the chlorophyll content for positive control where no CIP was present.

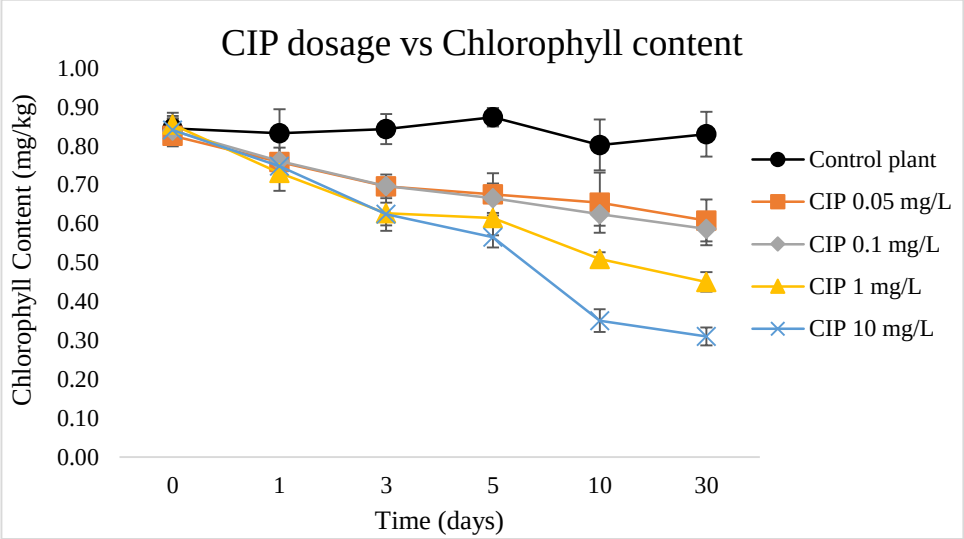


Fig. 1: Chlorophyll content in vetiver grass exposed to different CIP concentrations.

Protein content:

A significant ($p<0.05$) decrease in the protein content was observed in vetiver root exposed to different concentrations of CIP (Fig. 2). For the first 5 days, the decrease of root protein content was rapid in 0.05 and 0.1 mg/L CIP treatment. On the other hand, the depletion continued for 10 days in the vetiver root exposed to higher concentrations of CIP (1 and 10 mg/L). The protein content in vetiver shoot exposed to CIP treatments didn't exhibit rapid decrease. Vetiver grass exposed to the higher CIP concentrations (1 and 10 mg/L) showed initial decrease followed by a short recovery on 5th day. But at the end of the experimental period, the protein content was reduced by 52% and 60% in vetiver shoot exposed to 1 and 10 mg/L CIP (Fig. 3).

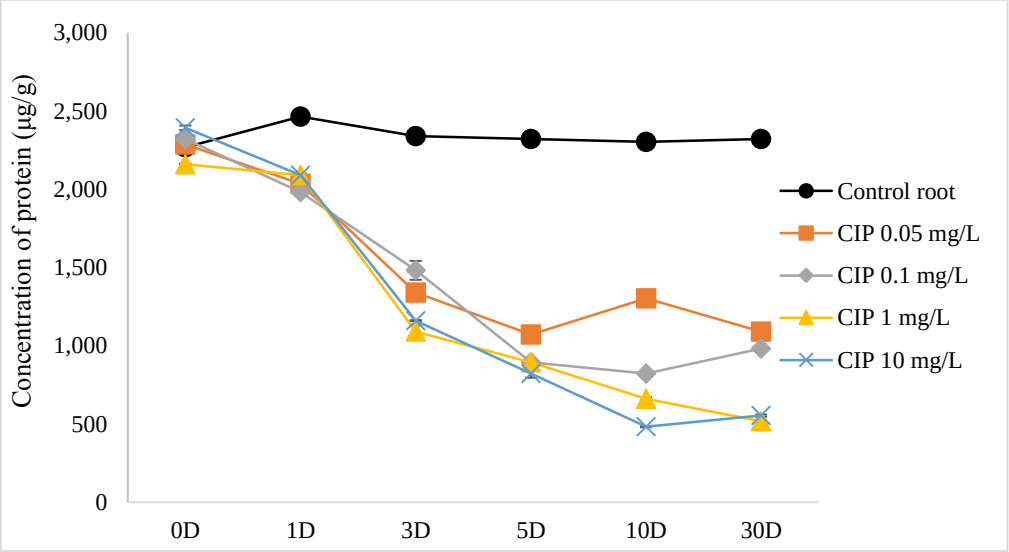


Fig. 2: Total Protein content in Root

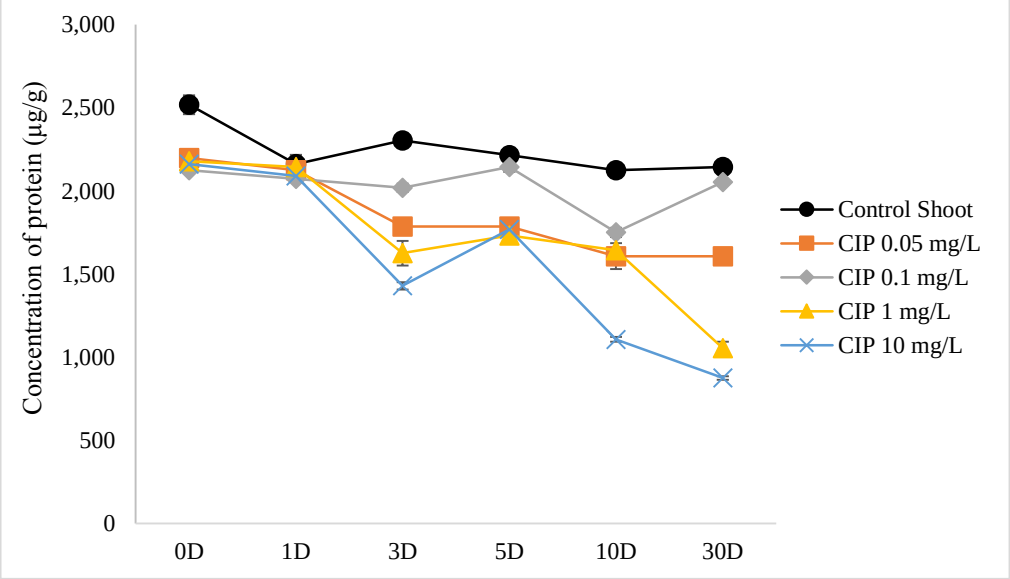


Fig. 3: Total Protein content in Shoot

GST activity

The activity of GST increased significantly ($p<0.05$) in vetiver root and shoot exposed to different concentrations of CIP (except 0.05 mg/L). Maximum GST activity in plant exposed to 1 and 10 mg/L was observed during the 5th day of the experiment. Vetiver shoot exposed to 0.05 and 0.1 mg/L didn't exhibit any remarkable change in the GST activity compared to control plant. However, the plants exposed to higher CIP concentrations (1 and 10 mg/L) exhibit a stress recovery (both root and shoot) at the end of the experimental period, and low GST activity was observed. One of the reasons of increased GST activity might be the presence of reactive oxygen species (ROS) in plant physiological system. The generation of ROS might be linked to the exposure to CIP.

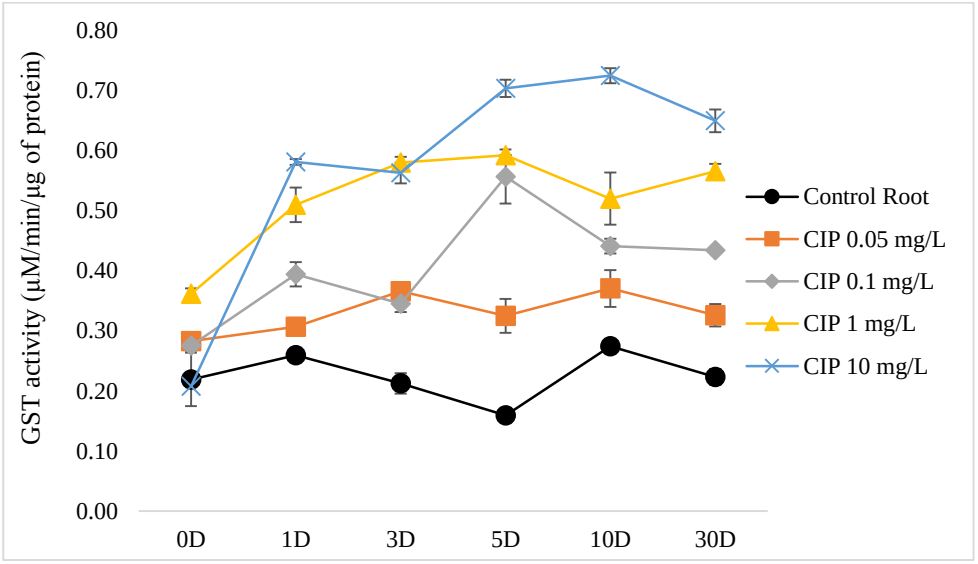


Fig. 4: GST activity in vetiver root

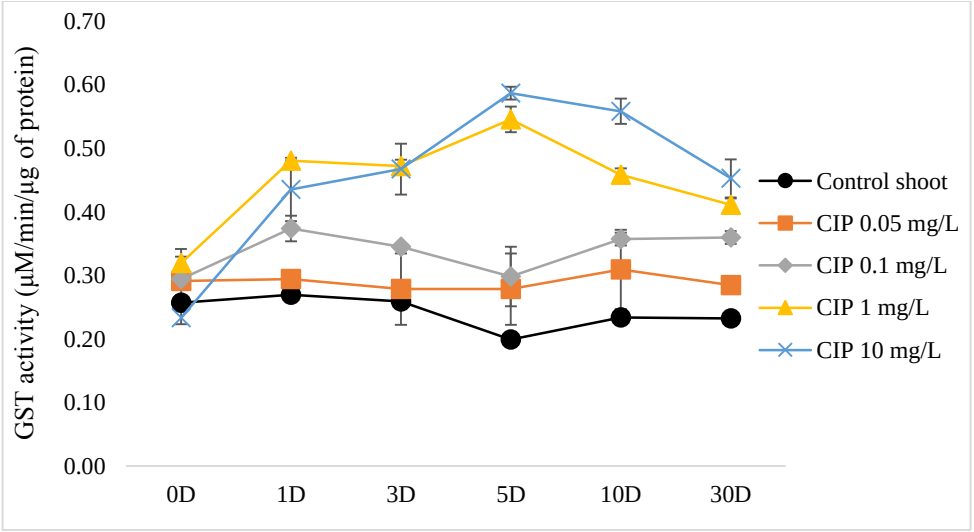


Fig. 5: GST activity in vetiver shoot

SOD activity:

Initially, an increased SOD activity was observed in vetiver root exposed to all CIP treatments (except 0.05 mg/L) (fig. 6). However, a sharp drop of SOD activity was noticed in roots after 5th day. The drop of SOD units was significant ($p<0.05$) in roots exposed to 1 and 10 mg/L CIP treatment. There was a 45% drop in the SOD activity observed in vetiver roots exposed to 10 mg/L CIP. No remarkable change was observed in SOD concentration was observed in vetiver leaves compared to positive control (fig. 7).

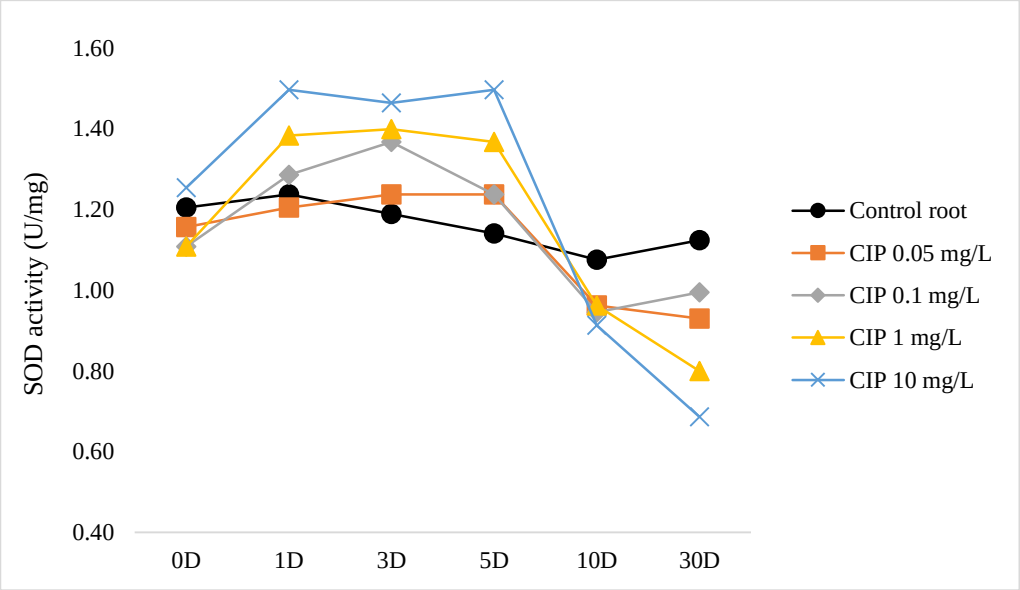


Fig. 6: SOD activity in vetiver root

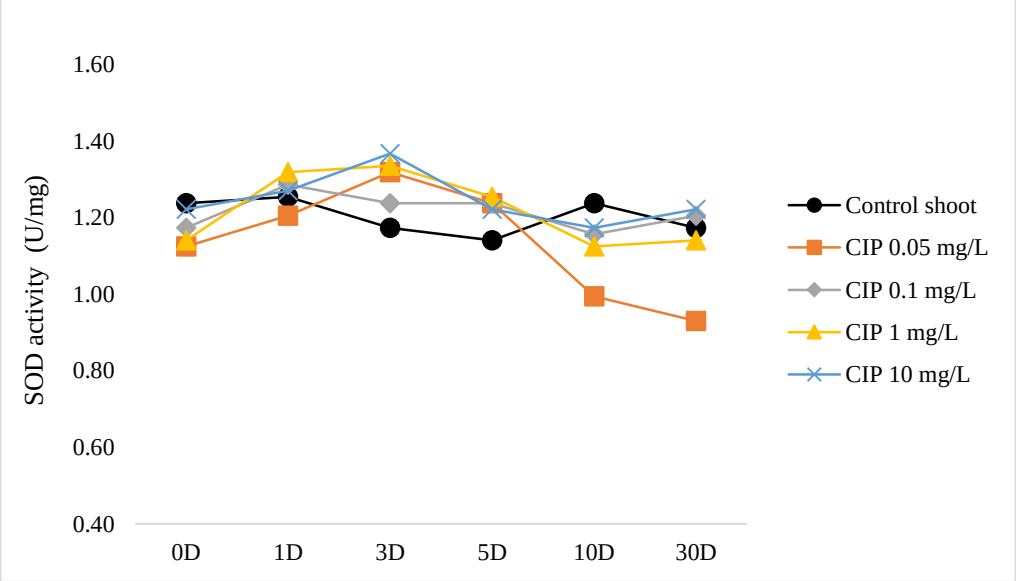


Fig. 7: SOD activity in vetiver shoot

CAT activity:

Initially, a significant ($p<0.05$) increase in the catalase activity was observed in vetiver root and shoot. Vetiver roots exposed to the CIP treatment exhibited continuous increase in the CAT activity (except 0.1 mg/L) at the end of the experimental period (fig. 8). However, vetiver shoots exhibited initial increase in CAT activity (until 10th day) and then followed by a gradual decrease which indicates a stress recovery phase (fig. 9). There were no significant changes observed in the CAT activity in the root and shoot of positive control. Increased CAT activity indicates that the vetiver grass was under ROS induced stress.

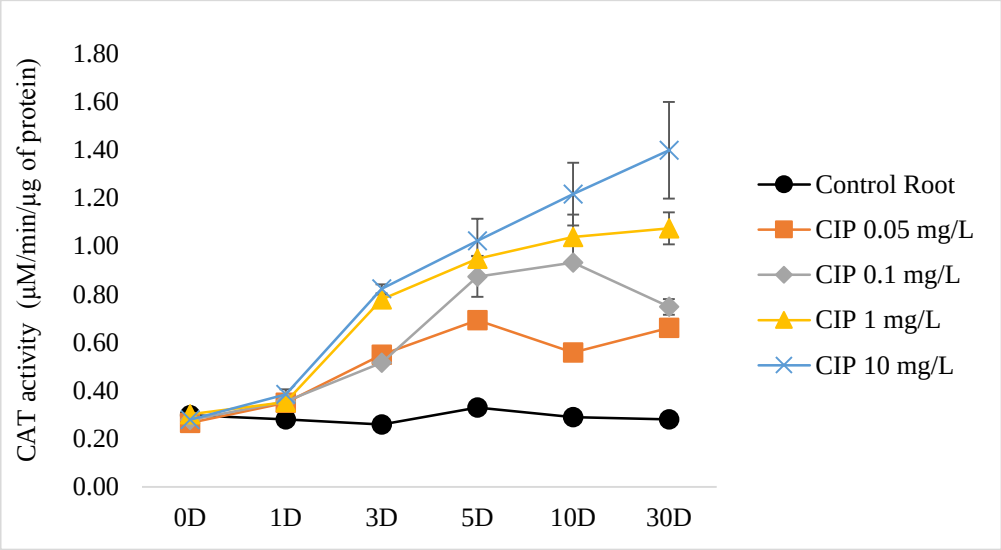


Fig. 8: Catalase activity in vetiver root

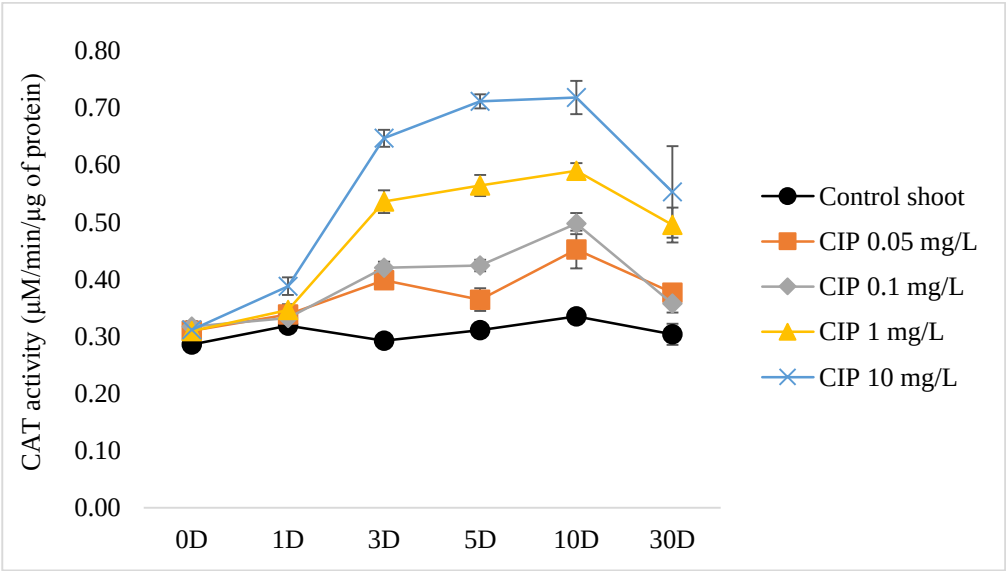
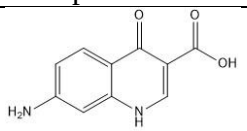
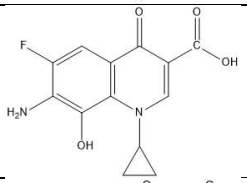
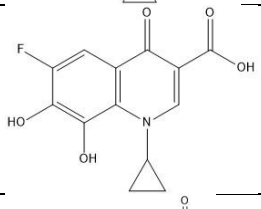
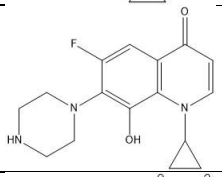
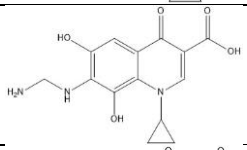
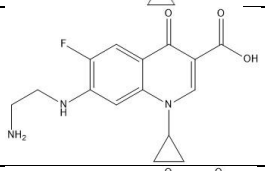
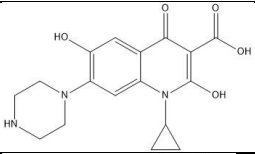
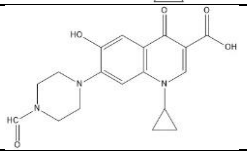


Fig. 9: Catalase activity in vetiver shoot

CIP degradation products:

ESI-MS analysis revealed eleven CIP degradation products present in vetiver root and shoot. Although root to shoot CIP translocation factor was low (20-30%), more CIP degradation products were found in vetiver shoot (Table 1). It implies that the primary detoxification of CIP takes place in the vetiver shoot. A few degradation mechanisms involve the cleaving of fluorine from the main structures (m/z 206, 304, 346a and b, and 362a). In most cases, the fluorine was replaced by a hydroxyl group. It is still unknown whether these degradation products had any toxic influence on the physiological activities of plant. Literature review revealed that the majority of the degradants are formed due to fungal biodegradation.

Location	m/z	Proposed structure	IUPAC name	Reference
Root	206		7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Liao et al. 2016)
Root and shoot	262		7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Prieto et al. 2011)
Shoot	280		1-cyclopropyl-6-fluoro-7,8-dihydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Wetzstein et al. 1999)
Shoot	288		1-cyclopropyl-6-fluoro-8-hydroxy-7-(piperazin-1-yl)quinoline-4(1H)-one	(Vasconcelos et al. 2009)
Shoot	304		7-((2-aminomethyl)amino)-1-cyclopropyl-6,8-dihydroxy-4-oxo-1,4- dihydroquinoline-3-carboxylic acid	(Ji et al. 2014)
Shoot	306		7-((2-aminomethyl)amino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihyroquinoline-3-carboxylic acid	(Prieto et al. 2011)
Shoot	346a		1-cyclopropyl-2,6-dihydroxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid	(Ji et al. 2014)
Shoot	346b		1-cyclopropyl-7-(4-formylpiperazin1-yl)-6-hydroxy-4-oxo-1,4- dihydroquinoline-3-carboxylic acid	(Ji et al. 2014)

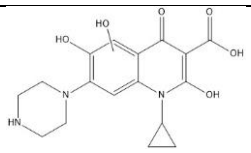
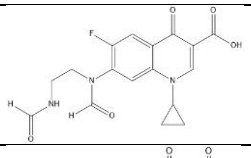
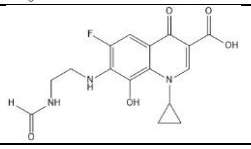
Shoot	362a		1-cyclopropyl-2,6-dihydroxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid compound with methanol (1:1)	(Ji et al. 2014)
Shoot	362b		1-cyclopropyl-6-fluoro-7-(N-(2-formamidoethyl)formamido)-4-oxo-1,4- dihydroquinoline-3-carboxylic acid	(Ji et al. 2014)
Shoot	362c		7-((2-Acetamidoethyl)amino)-1-cyclopropyl-6-fluoro-8-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	(Prieto et al. 2011)

Table 1: CIP degradation product detected in ESI-MS analysis

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- Panja, Saumik. & Dibyendu, Sarkar. (2018). Developing A Multitasking Green Phytotechnology to Remove Pharmaceuticals and Nutrient from Secondary Wastewater Effluent. Innovation Expo, Stevens Institute of Technology, Hoboken, NJ, May 2018.
- Panja, Saumik. & Dibyendu, Sarkar., & Rupali, Datta. (2017). Removal of Ciprofloxacin and Tetracycline by Vetiver Grass from Nutrient Amended Secondary Wastewater Matrix. Association for Environmental Health and Sciences Foundation, Amherst, MA, October 2018.

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He K, Soares AD, Adejumo H, McDiarmid M, Squibb K, Blaney L. Detection of a wide variety of human and veterinary fluoroquinolone antibiotics in municipal wastewater and wastewater-impacted surface water. Journal of Pharmaceutical and Biomedical Analysis. 2015;106:136-43. doi:http://dx.doi.org/10.1016/j.jpba.2014.11.020.

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Prieto A, Möder M, Rodil R, Adrian L, Marco-Urrea E. Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. Bioresource technology. 2011;102(23):10987-95.

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Finding the source: towards quantitative methods for microbial sourcing tracking

Basic Information

Title:	Finding the source: towards quantitative methods for microbial sourcing tracking
Project Number:	2017NJ393B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Categories:	Wastewater, Water Quality, Non Point Pollution
Descriptors:	None
Principal Investigators:	Sarah Phelan, Nicole Fahrenfeld

Publications

1. Phelan, Sarah, Disha Soni, William R. Morales Medina, Kris Parker, Nicole Fahrenfeld Ph.D; 2018, Finding the source: towards quantitative methods for microbial sourcing tracking, Poster presentation in NJWEA 103rd John J. Lagrosa Conference & Exposition, Atlantic City, NJ
2. Phelan, Sarah 2018, Finding the source: towards quantitative methods for microbial sourcing tracking, Apr 01 '18, Amplicon sequencing data submitted to NCBI SRA Database:, Accessible at https://www.ncbi.nlm.nih.gov/sra/SRP139593, after publication or 10/31/18, SRP139593

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Undergraduates: 2 *(Disha Soni, Kris Parker)*
Masters' students: 1 *(Sarah Phelan)*
Ph. D. students: 1 (*William R. Morales-Medina*)
Postdoctoral Associates: 0

(3) **Any Notable Achievements**:

1st place – graduate (Master's) research project poster- NJWEA 103rd Annual Conference and Exposition

(3) **Project Summary**:

Problem and Research Objectives

Understanding the source of fecal contamination is critically important to managing fecal contamination in surface waters and protecting human health. Existing regulations on this contamination are based on coliform counts while the method for identifying and quantifying total coliforms is labor intensive, and does not provide information on the source of the waste. There are multiple alternative methods that are available for identifying and understanding the source of fecal contamination, however, many of the current methods have unique benefits and drawbacks that limit their usefulness as analytical tools. One emerging method that has only recently become a viable method for source tracking is amplicon sequencing. Amplicon sequencing is a fingerprinting method that involves sequencing a selected gene (often the 16S rRNA gene for describing the microbial community structure) of tens of thousands of microbes in a single sample. Historically, amplicon sequencing was prohibitively expensive when compared to other available methods such as qPCR or other finger printing methods like tRFLP, but recently the cost has dropped considerably and made the method a comparable alternative to other analyses. A major benefit of amplicon sequencing when compared to other methods is that it will provide a large amount of information the microbial community in the sample and is not limited to a prior understanding of the source, compared to qPCR which uses assays to quantify specific source concentrations. This method has the potential to be used to identify a broad range of information on contaminant sources and will expand the understanding of microbial communities beyond 'indicator' microbes such as *E. coli* and *Enterococci.*

The objectives of the study are twofold: (1) to determine if amplicon sequencing distinguishes clearly between samples of wastewater and fecal material from different animal sources in lab controlled spiked samples (2) to compare the results of amplicon sequencing to qPCR (a demonstrated technology) to determine if they are consistent in field samples from a fecal contaminated waterway in New Jersey.

The hypothesis is that amplicon sequencing will allow for the identification of distinct microbial signatures that can be used to identify the source of fecal contamination in water sampling. To test this hypothesis, correlations are being tested for sources identified using amplicon sequencing with qPCR analysis of paired samples.

Methodology

To create a representative 'library' of samples from mixed sources, fecal samples were collected from multiple locations in NJ (Table 1 and Figure 1). Wastewater influent (2L) was collected from a wastewater treatment plant located in northern NJ, as representative of human feces and sewage. Surface water samples (11L total volume) were collected from two sites on the Navesink River, which based on previous data (NJDEP Sampling data) had little to no fecal coliform present. The sites selected were NJDEP Site 32 and NJDEP Site 58 (Figure 1).

Table 1: *Library fecal sample collection sites*

Sample type	Sample Location	Description
Horse	Horse Farm in Middlesex County	Fresh Manure from 2 horses from two stables
Horse	Rutgers Horse Farm	Fresh Manure Samples from multiple horses in farm
Goose	Park in Red Bank, New Jersey	Multiple fresh samples collected adjacent to park pond
Goose	Park in Somerset, New Jersey	Fresh samples collected from park
Dog	4 Domestic dogs from 3 owners – mixed breeds	Fresh samples provided by owners

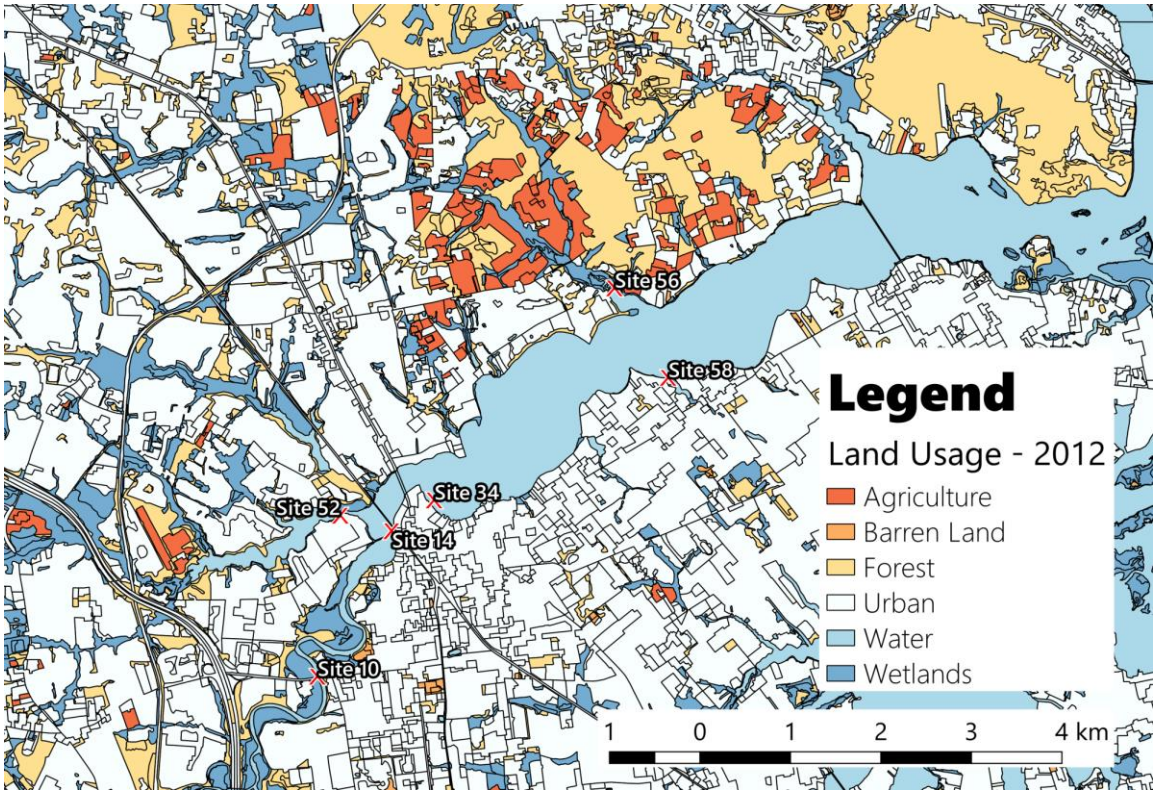

Figure 1: *Sampling locations for library samples (Site 32 and Site 58) and field samples*

The fecal library consisted of 0.9L of surface water with varying volumes of WW (0.1L) and/or masses of homogenized feces (1g) (Table 2). Volumes were chosen based on data supporting the average human coliform results, and the highest concentration results observed in the Navesink River from fecal coliform sampling completed in the summer of 2016 and by membrane filtration using a simultaneous detection technique. Reported coliform counts per gram of feces were $2.5x10^3$ for cow manure, 10^8 coliform for geese, and $13x10^6$ for human, and concentrations in wastewater ranging from $3.6x10^3$ to $7.9x10^5$ FCU/100 mL. The surface water used for the library creation was a 1:1 (v:v) ratio of samples from Sites 34 and 58. The library mixtures were homogenized with a 10 speed blender (Black and Decker BL2010BG). Each library mixture was prepared in duplicate and preserved for coliform or biomolecular (DNA) analysis, as described below.

Table 2: *Library sample mixtures*

Sample name	Contents	Surface Water	Wastewater	Goose	Horse	Dog
Sa, Sb	Surface Water	900 mL	0 mL	0 g	0 g	0 g
SWa, SWb	Surface Water, Wastewater	900 mL	100 mL	0 g	0 g	0 g
SWGa, SWGb	Surface Water, Wastewater, Goose	900 mL	100 mL	1 g	0 g	0 g
SWHa, SWHb	Surface Water, Wastewater, Horse	900 mL	100 mL	0 g	1 g	0 g
SWDa, SWDb	Surface Water, Wastewater, Dog	900 mL	100 mL	0 g	0 g	1 g
SWGHa, SWGHb	Surface Water, Wastewater, Goose, Horse	900 mL	100 mL	1 g	1 g	0 g
SWGDa, SWGDb	Surface Water, Wastewater, Goose, Dog	900 mL	100 mL	1 g	0 g	1 g
SWDHa, SWDHb	Surface Water, Wastewater, Dog, Horse	900 mL	100 mL	0 g	1 g	1 g
SWGHDa, SWGHDb	Surface Water, Wastewater, Goose, Horse, Dog	900 mL	100 mL	1 g	1 g	1 g
SDGHa, SDGHb	Surface Water, Dog, Goose, Horse	900 mL	0 mL	1 g	1 g	1 g
B	De-Ionized Water	0 mL	0 mL	0 g	0 g	0 g

Two field sampling campaigns were performed. Wet weather samples were collected during a storm event with a total rainfall accumulation of 0.15" during the storm event from surface water in various locations along the Navesink River. Sites sampled during the wet weather event were: NJDEP Site 10, Site 14, Site 34, Site 52, and Site 56 (Figure 1). Historical rainfall data was collected from USGS data at the nearby USGS station 402156073582901. Dry weather samples were collected on 10/15/17 8AM-12PM from the same surface water locations along the Navesink River as the wet weather samples. Additionally, one downstream sample was collected from NJDEP Site 58. Duplicate (1L) samples were collected from each location.

Both the library and field samples from the wet weather event were analyzed at a certified laboratory (NJ Analytical Lab, Ewing, New Jersey) for total coliform analysis using analytical method SM9222B as described in standard methods (APHA, 2015[i]). Samples collected during the dry weather sampling event were analyzed at Rutgers using analytical method EPA SM1604 (EPA, 2002 [ii]).

Field and library samples were filter concentrated and DNA was extracted from filter concentrated samples, wet weather, dry weather, and library samples using a commercial kit (FastDNA Spin Kit for Soil, MP Biomedicals, Hurcules, CA) following the manufacturer's directions. DNA extracts were stored at -20 C until analysis. qPCR was performed on all samples using HF183 as a human fecal indicator (Seurinck et al., 2005[iii]) and HOF597 as a horse fecal indicator (Dick et al., 2004[iv]). qPCR was also performed for the 16S rRNA gene as an estimate of total bacterial population (Suzuki et al. 2000[v]). A summary of the qPCR primers is below.

Table 3: Summary of primers used in qPCR analysis

Target Fecal Source	Primer/Probe name	Primer/Probe Sequence 5' to 3'	Ta	Reference Paper
Horse	Bac708R	CAATCGGAGTTCTTCGTG	53° C	Dick et al., 2004
	HoF597F	CCAGCCGTAAAATAGTCGG		
Human	HF183F	ATCATGAGTTCACATGTCCG	53° C	Seurinck et al., 2005
	Bac242R	TACCCCGCCTACTATCTAATG		

To determine the microbial community present in the fecal library and surface samples, amplicon sequencing (Illumina MiSeq, 300 bp, paired end) was performed targeting the V3-4 region of the 16S rRNA gene at a commercial lab (MR DNA, Shallowater, TX, United States). Sequences were processed using the QIIME v. 1.9.1 (Accessed 2/2018)(Kuczynski et al, 2012[vi]). Briefly, sequences were trimmed using Trimmomatic 0.36[vii] prior to joining the paired ends using Pandaseq.[viii] After demultiplexing (split_libraries.py), chimeras were removed (identify_chimeric_seq.py with UCHIME61) the reads parsed into operational taxonomic units (OTUs) within a 97% sequence identity cutoff (pick_de_novo_otus.py). To allow for comparison between samples with a different number of sequences, the samples will be rarified to the lowest sequencing depth within the samples (N=32404 sequences).

A Bray–Curtis similarity matrix was calculated on log-normalized subsampled operational taxonomic unit data at the class level followed by cluster analysis with a SIMPROF test and non-metric multidimensional scaling (nMDS) in PRIMER 7. To determine which OTUs were preferentially associated with a given sample type from the library samples (human, horse, canine, or goose) biomarker analysis was performed on class-level relative abundance data for the library samples. The linear discriminant analysis effect size (LEfSe) tool (Segata et al., 2011[ix]) was used to identify biomarkers using relaxed parameters (comparisons performed between subclasses with different names, and one-against-all comparisons).

Additional statistical analysis is ongoing but will include cross comparison of amplicon sequencing, qPCR, and coliform results utilizing a Wilcoxon rank sum test, a paired Student's t-test (normality of data confirmed by a Shapiro test) and a Kruskal–Wallis rank sum test followed by a post hoc pairwise t-test as appropriate.

Principal Findings and Significance

Coliform results for wet and dry weather sampling are shown in Figure 2. Coliform results were elevated at site S-10 during wet and dry weather sampling, S-56 during wet and dry weather sampling, and S-14 during dry weather sampling (results too numerous to count or greater than 950 CFU/100 mL). The

lowest field sampling result was 85 CFU/100 mL, at S-58 during dry weather sampling. For context, when classifying surface waters for shellfish harvesting, the geometric mean of samples must be below 88 CFU/100 mL, and the 90th percentile must be below 163 CFU/100 mL for the water to be classified as "special restricted", which is the lowest tier that still allows shell fish harvesting before it is classified as "prohibited" (NJDEP, 2015).

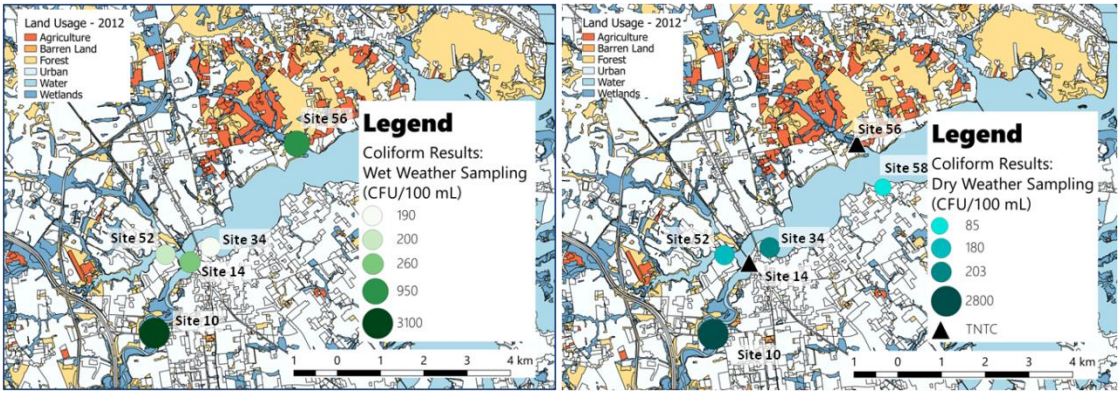

Figure 2: *Coliform results for wet and dry weather field sampling events*

qPCR results are in progress, for the human fecal indicator gene HF183 and the horse fecal indicator gene HoF 597F, however preliminary results with HF183F show good correspondence with expected detections in library samples, with elevated results in library samples spiked with wastewater relative to samples that were missing that matrix. Interestingly, the field samples with elevated total coliform (Site 10 wet, Site 10 dry, Site 14 dry, and Site 56 dry) did match those sites with elevated qPCR results. In the field samples, all qPCR results were low (at or below the trip blank results) except for S-34 Wet, which was approximately 5 log gene copies/mL (2 log gene copies/mL higher than the other samples). It is possible that other fecal bacteria (not a result of the presence of human fecal material) have resulted in elevated coliform results in S-10, S-14, and S-56. Testing for the presence horse fecal contamination (using the HoF597F primer) is in progress and it is anticipated that results will correspond well in the library samples. Figure 3 shows current qPCR results for library and field samples, and field samples are compared against total coliform results.

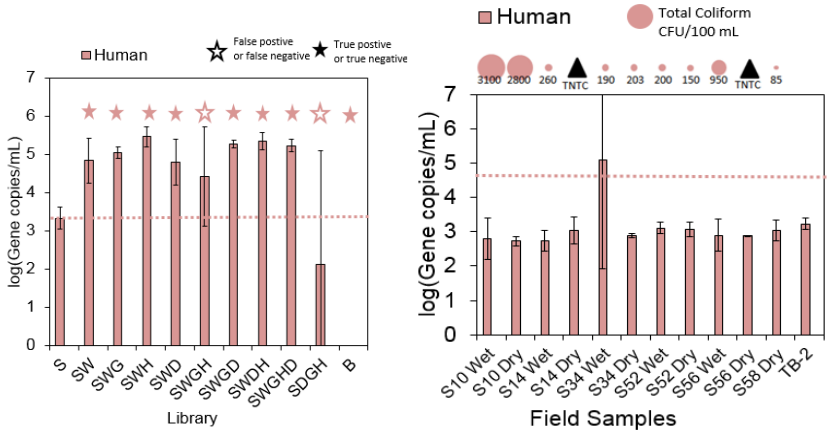

Figure 3: *qPCR results for the Bac183 indicator gene for human fecal contamination in library and field samples. Field samples total coliform also shown.*

Amplicon sequencing was performed on library samples, and on field samples from both wet and dry weather events. Results were compared for relative abundance at the class level, and a Brays-Curtis similarity matrix was calculated on the log normalized sub sampled Operational Taxonomic Units (OTUs). Results for the Library samples, excluding the surface water library samples which are shown with the field samples, are shown in Figure 4. Samples spiked with wastewater (SW), wastewater and horse (SWH), and wastewater and dog (SWD) fecal material clustered with approximately 75% similarity. All other library samples including samples spiked with wastewater and goose, and all library samples containing more than two fecal spikes, clustered at approximately 70% similarity, and had higher relative abundance of Firmicutes, a bacterial phylum typically associated with wastewater. All of the library samples except for SW-d contained Firmicutes (*Bacilli*, *Clostridia*, or both) ranging from 5.6% to 54.6% relative abundance within the library samples. Not all of the library replicates clustered as similar, and in fact three of nine duplicates were significantly different from one another, as shown in figure 4. All of the library samples were within 70% similarity of their library replicates.

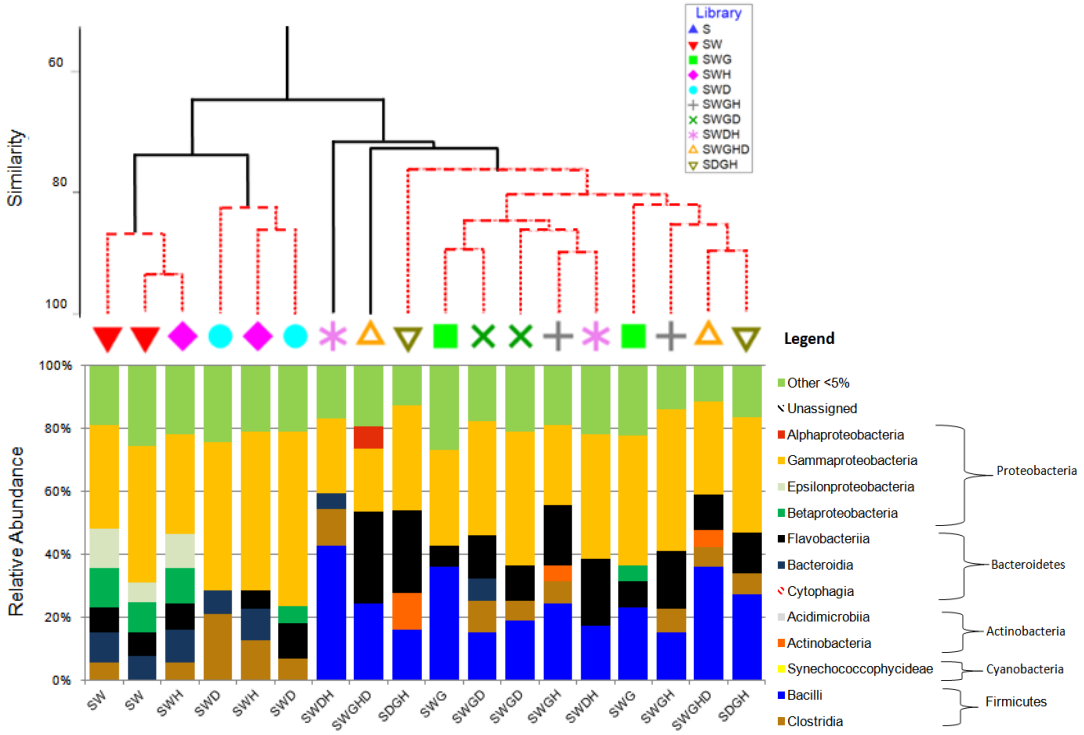

Figure 4: *OTU relative abundance data and cluster analysis of Brays Curtis similarity results for library samples. Red cluster links represent samples without significant differences.*

Field samples collected during wet and dry weather events were compared for relative abundance the class level and using cluster analysis of the Brays Curtis Similarity Index, as described above. Replicate field samples clustered together with greater than 80% similarity between field samples at each location. Samples collected during wet and dry weather sampling events did not necessarily show a high level of similarity- a dendogram was produced using the Bray Curtis similarity measure, and S-10 wet and dry weather samples were 80% similar, but S-14 wet and S-14 dry, and S-52 wet and S-52 dry were less than 40% similar between sampling events. Results with elevated coliform (too numerous to count or >1000 CFU/100 mL) varied in terms of relative bacterial abundance: site S-10 wet and dry weather samples clustered together, and site S-14 and S-56 dry were similar. Figure 5 shows OTU relative

abundance tables and Brays Curtis similarity results. Red arrows indicate samples with elevated total coliform results.

The field samples did not contain Firmicutes at >5% relative abundance. While library samples were dominated by gammaproteobacteria, many field samples had higher relative abundances of Betaproteobacteria or Alphaproteobacteria. Library and field samples were clustered at approximately 30% similarity.

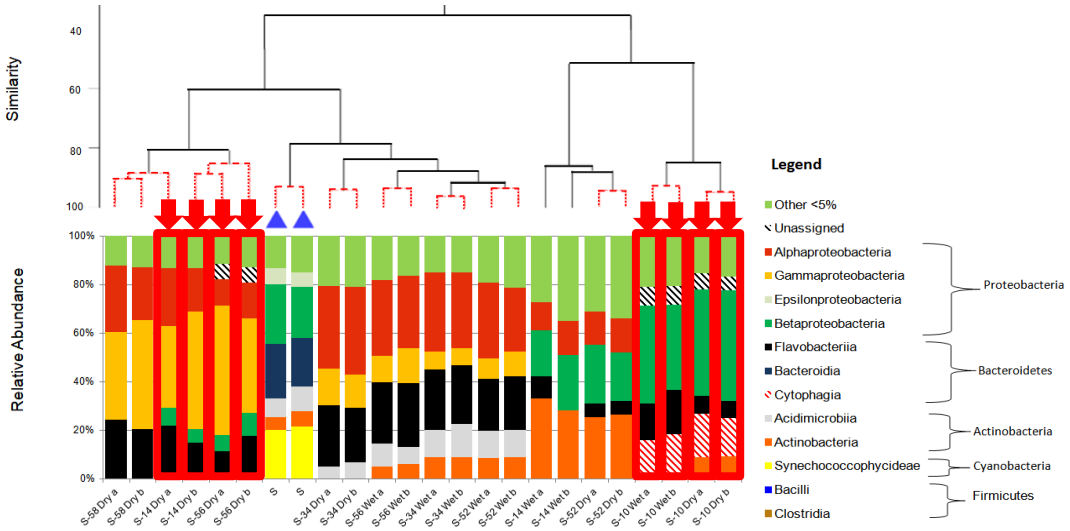

Figure 5: *OTU relative abundance data and cluster analysis results for field samples. Red arrows indicate samples with elevated fecal coliform counts. Red links on the cluster analysis indicate samples without significantly different microbial community structures.*

Microbial community structures for library and field samples were compared using non-metric multidimensional scaling (nMDS). Results show the relative similarity of library and field samples from the sampling. As shown above, samples clustered separately, with surface waters differentiating from spiked library samples by greater than 40%. Using the relative abundance data only, it was not possible to identify fecal contaminant sources by clustering and comparison of relative similarity.

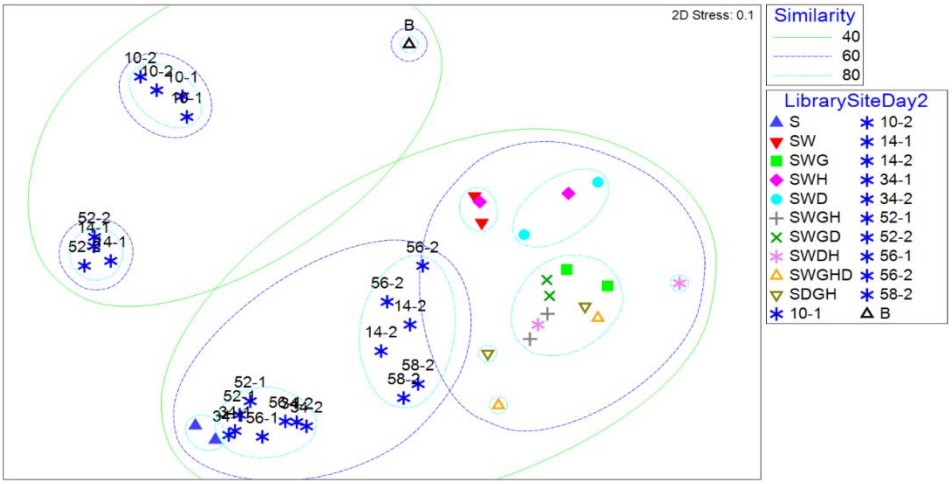

Figure 6: *nDMS plot showing relative similarity of library and field samples. Overlays represent results of the cluster analysis. Field samples are represented by blue stars with the site number labeled followed by -1 or -2 for wet and dry weather sampling, respectively.*

A review of results and data comparison is ongoing. In addition to statistical comparisons, the linear discriminant analysis effect size (LEfSe) tool (Segata et al., 2011[x]) was used to identify biomarkers using relaxed parameters (comparisons performed between subclasses with different names, and one-against-all comparisons) on the library samples. Relaxed parameters were selected because more stringent parameters yielded few significantly different/discriminate features within the library. Initial comparison of the relative abundance of indicator organisms in the library and field samples did not visually appear to correlate with the matrix/coliform levels in the samples, however these results are being re-run and checked for statistically significant features.

(4) **Publications or Presentations**:

Phelan, Sarah, Disha Soni, William R. Morales Medina, Kris Parker, Nicole Fahrenfeld Ph.D; 2018, Finding the source: towards quantitative methods for microbial sourcing tracking, Poster presentation in NJWEA 103rd John J. Lagrosa Conference & Exposition, Atlantic City, NJ

Phelan, Sarah 2018, Finding the source: towards quantitative methods for microbial sourcing tracking, Apr 01 '18, Amplicon sequencing data submitted to NCBI SRA Database:, Accessible at https://www.ncbi.nlm.nih.gov/sra/SRP139593, after publication or 10/31/18, SRP139593

References

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[x] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.*12:R60. doi: 10.1186/gb-2011-12-6-r60

Mercury (Hg) Flux at the Sediment-Water Interface: The Effect of Microbial Hg(0) Oxidation

Basic Information

Title:	Mercury (Hg) Flux at the Sediment-Water Interface: The Effect of Microbial Hg(0) Oxidation
Project Number:	2017NJ394B
Start Date:	3/1/2017
End Date:	8/31/2018
Funding Source:	104B
Congressional District:	NJ-012
Research Category:	Water Quality
Focus Categories:	Sediments, Solute Transport, Toxic Substances
Descriptors:	None
Principal Investigators:	Yuwei Wang, Nathan Yee

Publications

1. Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., Adsorption of methylmercury onto Geobacter bemidijensis Bem, submitted to ES&T.
2. Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., 2017, Adsorption of methylmercury onto Geobacter bemidijensis Bem, in the 13th International Conference on Mercury as a Global Pollutant, Providence, Rhode Island [Poster]
3. Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., 2018, Adsorption of methylmercury onto Geobacter bemidijensis Bem, in Goldschmid Conference, Boston, MA [Talk]
4. Wang, Yuwei; Sarah, Janssen; Jeffra, Schaefer; John Reinfelder; Nathan, Yee., 2018, Cellular uptake of mercury in anaerobic bacteria: Application of mercury stable isotopes to elucidate physiological controls, in NorthEastern Microbiologists: Physiology, Ecology and Taxonomy conference, Blue Mountain Lake, NY [Talk]

Mercury and anaerobic bacteria interactions in aquatic system

(1) PI information:

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Co-PI: Nathan Yee

Address: 14 College Farm Road, New Brunswick, NJ

Email address: nyee@envsci.rutgers.edu

Phone numbers: 848-932-5714

(2) Numbers of Students Supported:

Undergraduates: 1

Masters' students: 0

Ph. D. students: 1

Postdoctoral Associates: 0

(3) Any Notable Achievements:

Submitted an application for NEMPET (NorthEastern Microbiologists: Physiology, Ecology and Taxonomy) scholarship, status pending.

(4) Project Summary:

Problem and Research Objectives:

In New Jersey (NJ), there are a number of mercury contaminated sites on the Superfund National Priority list that have significantly affected water quality. The fate and transport of mercury in contaminated sediments is strongly influenced by microbe-mercury interactions. Dissolved elemental mercury [Hg(0)] is mobile in pore waters and can be oxidized by anaerobic microorganisms. The conversion of Hg(0) to Hg(II) affects the bioavailability of mercury for bacterial uptake and production of neurotoxic methylmercury (MeHg). MeHg adsorption onto bacterial cells can affect the release of MeHg into aquatic environments as well as limiting the uptake of MeHg for enzymatic demethylation processes.

These critical mercury transformation processes alter the mobility and fate of mercury contamination in sediments.

In this study, we conducted experiments to understand the interactions between mercury and sediment microbial communities. The objectives of this study are (1) to identify the microbial community that is resistant to Hg(0) in sediments collected from Newark Bay, NJ; (2) to investigate the uptake of Hg(II) into Hg methylating bacteria; (3) to quantify the adsorption of MeHg onto bacterial cells .

Methodology:

Collection and incubation of sediments: Sediments were collected in Newark Bay. Aliquots of samples were taken and analyzed for total mercury (THg). To investigate Hg(0) resistant microbial populations in the sediments, an aliquot of sediment suspension was exposed to continuous source of Hg(0) gas. To examine the microbial community structure and activity in the sediments exposed to Hg(0), both RNA and DNA will be extracted using RNA PowerSoil kits with DNA elution (Qiagen) for 16S rDNA sequencing and RNA to convert to cDNA for 16S rRNA sequencing. The genes and transcripts encoding *hgcA* will be quantified using RT-qPCR.

Hg(II) uptake experiments: Hg(II) uptake experiments were performed using strain *Geobacter sulfurreducens* PCA. Cells were incubated in reactors amended with of 50 nM mercuric nitrate that was equilibrated with 10 μM cysteine. Periodically, reactors were sacrificed and aliquots of samples were taken for THg, cell associated Hg (Hg_{cell}), aqueous Hg (Hg_{aq}), and intracellular Hg (Hg_{in}) measurements. Mercury stable isotope composition of the intracellular Hg was also analyzed. To goal of this experiment was to use mercury stable isotopes to examine the mechanisms of Hg uptake by Hg methylating bacteria.

MeHg adsorption experiments: MeHg experiments were conducted using *Geobacter bemidjiensis* Bem. Cells were exposed to various amounts of MeHg sealed reactors. To examine the adsorption kinetics, individual reactors were sacrificed at specific time points for total Hg (THg), cell associated Hg (Hg_{cell}) and aqueous Hg (Hg_{aq}) analysis. Adsorption isotherm experiments were performed to determine MeHg binding constants.

Principal Findings and Significance:

1) Microorganisms in NJ sediments are highly resistant to Hg(0): Sediments incubated with different electron donors (glucose, pyruvate) and acceptors (sulfate and iron citrate) exhibited robust growth. In all growth conditions tested, bacteria grew to a high cell density within 48 h in (Figure 1A-D). Furthermore, cultures grown with glucose, pyruvate and sulfate were highly resist Hg(0) (Figure 1A-C). The only

growth condition that did not show Hg(0) resistance was the medium amended with iron citrate. The microbial communities resistant to Hg(0) will be further examined by DNA sequencing.

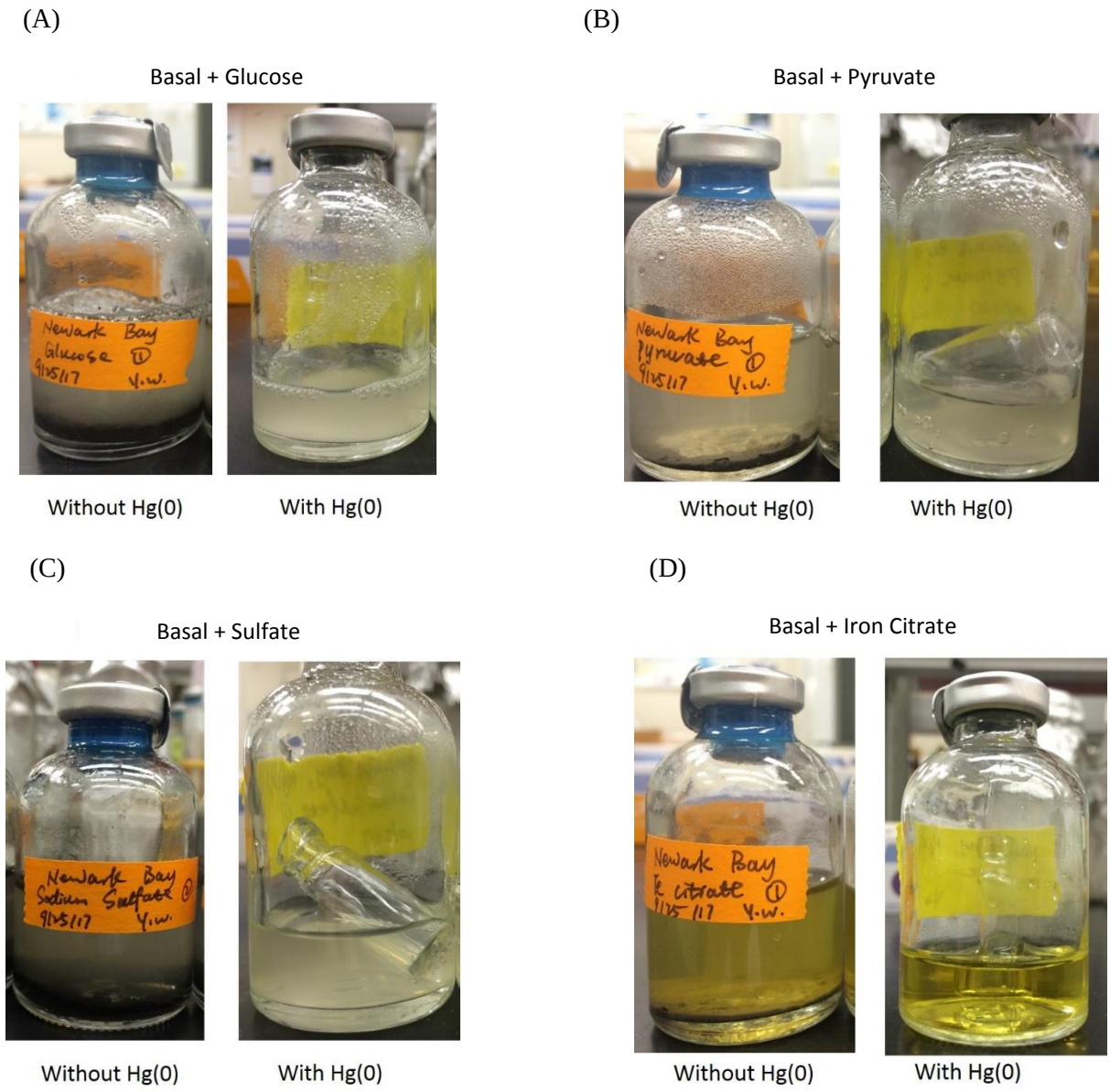
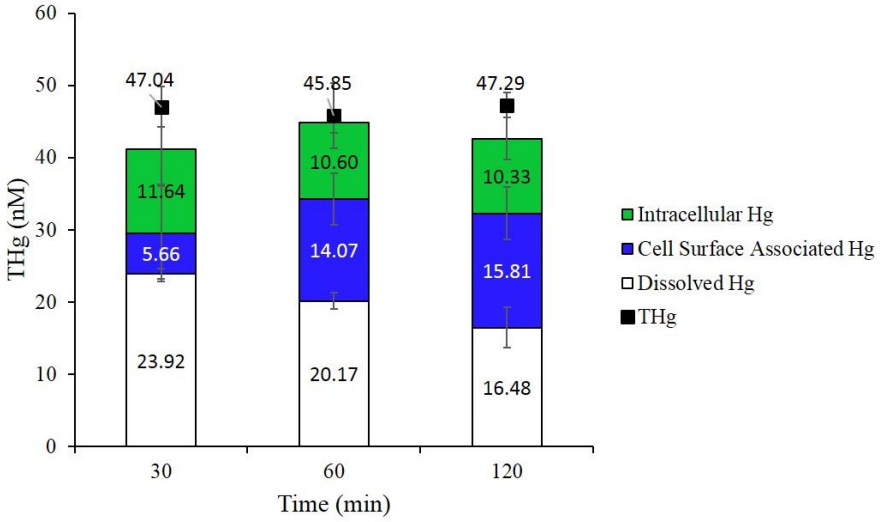


Figure 1: Growth of bacteria incubated from Newark Bay sediments with and without Hg(0) source in Basal medium amended with (A) glucose, (B) pyruvate, (C) sulfate, (D) iron citrate

2) Hg(II) uptake by *G. sulfurreducens* PCA: The uptake of Hg(II) by *G. sulfurreducens* PCA resulted in fractionation of Hg stable isotopes. The intracellular Hg is isotopically enriched with the heavier isotopes compared to Hg(II) outside the cells. This effect corresponded to isotope depletion in dissolved Hg

(Figure 2B). The results indicated that strain *G. sulfurreducens* PCA uptakes isotopically heavier Hg(II) pool and methylates it to MeHg. The use of Hg(II) isotope ratios allows us to further probe the physiology of Hg uptake and methylation to better understand the dynamics of Hg bioavailability in microorganisms.

(A)



(B)

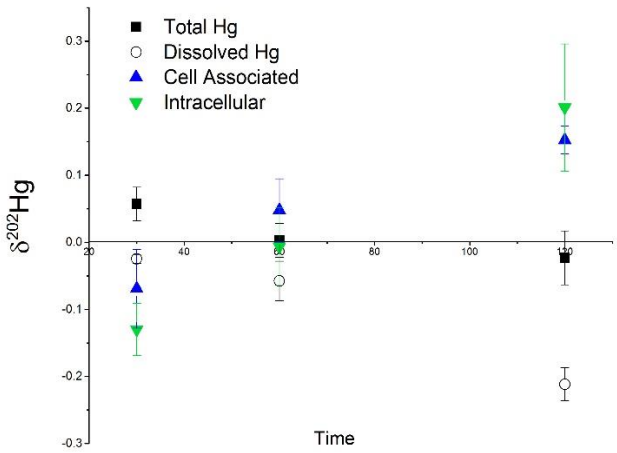


Figure 2: Hg(II) uptake by *G. sulfurreducens* PCA. (A) Mass balance of Hg(II) accumulated in different fractions of bacterial cells in the process of uptake; (B) Fractionation of ^{202}Hg in Hg(II) taken up by bacterial cells.

3) MeHg adsorption by *G. bemidijensis* Bem: The results indicated that *G. bemidijensis* strongly adsorbs MeHg via complexation to thiol functional groups. The log K binding constant of MeHg onto the thiol sites was determined to be 10.2 ± 0.2. These findings provide a quantitative framework to describe

MeHg binding onto bacterial cell surfaces and elucidate the importance of bacterial cells as possible carriers of adsorbed MeHg in natural aquatic systems.

(4) Publications or Presentations:

Publications:

- Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., Adsorption of methylmercury onto *Geobacter bemidijensis* Bem, submitted to ES&T.

Presentations:

- Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., 2017, Adsorption of methylmercury onto *Geobacter bemidijensis Bem*, in the 13th International Conference on Mercury as a Global Pollutant, Providence, Rhode Island [Poster]
- Wang, Yuwei; Qiang, Yu; Bhoopesh, Mishra; Jeffra, Schaefer; Jeremy, Fein; Nathan, Yee., 2018, Adsorption of methylmercury onto *Geobacter bemidijensis* Bem, in Goldschmid Conference, Boston, MA [Talk]
- Wang, Yuwei; Sarah, Janssen; Jeffra, Schaefer; John Reinfelder; Nathan, Yee., 2018, Cellular uptake of mercury in anaerobic bacteria: Application of mercury stable isotopes to elucidate physiological controls, in NorthEastern Microbiologists: Physiology, Ecology and Taxonomy conference, Blue Mountain Lake, NY [Talk]

Information Transfer Program Introduction

None.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	13	0	0	0	13
Masters	6	0	0	0	6
Ph.D.	8	0	0	0	8
Post-Doc.	0	0	0	0	0
Total	27	0	0	0	27

Notable Awards and Achievements

For 2017NJ388B: We have manufactured biochar adsorbents derived from a variety of common biological wastes (e.g., Okara [aka soybean dregs], reed stem, aspen chip, and maize cob) via pyrolysis at three different temperatures (e.g., 300, 500, and 700 °C). These synthesized biochars were further characterized for their physical properties, surface chemistry, and, most importantly, their effectiveness of removing both legacy (e.g., PCBs) and emerging (e.g., 1,4-dioxane) contaminants, which have been widely detected in estuary environments in the U.S. Our results demonstrated biochar as a relatively cost-efficient and green alternative of these manufactured carbonaceous adsorbents for effective stabilization of PCBs in the contaminated sediments and removal of 1,4-dioxane in our drinking water.

For 2017NJ390B: 2018 Third Place Award, Student Filter Competition of AWWA NJ; 2018 Tech Quest Innovation/Prototype Awards of New Jersey Innovation Institution; 2018 Undergraduate Research and Innovation Phase-2 of New Jersey Innovation Institution; 2017 Undergraduate Research and Innovation Phase-1 of New Jersey Innovation Institution

For 2017NJ392B: Panja, Saumik. & Dibyendu, Sarkar. (2018). Developing A Multitasking Green Phytotechnology to Remove Pharmaceuticals and Nutrient from Secondary Wastewater Effluent. Innovation Expo, Stevens Institute of Technology, Hoboken, NJ, May 2018. (2nd place in Doctoral Student Poster Presentation Competition).

For 2017NJ392B: Panja, Saumik. & Dibyendu, Sarkar., & Rupali, Datta. (2017). Removal of Ciprofloxacin and Tetracycline by Vetiver Grass from Nutrient Amended Secondary Wastewater Matrix. Association for Environmental Health and Sciences Foundation, Amherst, MA, October 2018. (2nd place in Doctoral Student Poster Presentation Competition).

For 2017NJ393B: 1st place – graduate (Master’s) research project poster- NJWEA 103rd Annual Conference and Exposition

For 2017NJ394B: Submitted an application for NEMPET (NorthEastern Microbiologists: Physiology, Ecology and Taxonomy) scholarship, status pending.