New Jersey Water Resources Research Institute
Annual Technical Report
FY 2016

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, and six grants-in-aid were awarded in FY2016 to graduate students who are beginning their research. 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 FY2016, 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 FY2016, the NJWRRI co-sponsored the seventh annual Passaic River Symposium, held on October 13-14, 2016, which featured and integrated environmental management, watershed science, flood prevention, urban environments, sustainable development, and the Lower Passaic River Restoration Project. More information on the conference can be found at https://www.montclair.edu/csam/passaic-river-institute/conferences/. The NJWRRI also co-sponsored the 2016 Northeast Regional Urban Extension Conference held on November 29-30, 2016. This regional conference convened Extension faculty, government officials, and local stakeholders to highlight research and projects that are helping to build more resilient, sustainable, and healthy urban communities. More information on the conference can be found at http://www.cpe.rutgers.edu/urbanext/.

Research Program Introduction

None.

A molecular tool to measure dechlorinator activity in situ

Basic Information

Title:	A molecular tool to measure dechlorinator activity in situ
Project Number:	2016NJ377B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Biological Sciences
Focus Categories:	Methods, Toxic Substances, Treatment
Descriptors:	None
Principal Investigators:	Valdis Krumins, Donna E. Fennell

Publications

There are no publications.

A molecular tool to measure dechlorinator activity *in situ*

2016-2017 project annual report

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Numbers of Students Supported:

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

Project Summary:

Problem and Research Objectives

Chlorinated organic contaminants accumulate in waterbodies and particularly in sediments because of their relative stability (resistance to physical/chemical and biological transformation). One mechanism for their removal from the environment is reductive dechlorination – the sequential removal of chlorine atoms by bacteria such as *Dehalococcoides mccartyi* that utilize chlorinated organic compounds for respiration. *Dehalococcoides* and other organohalide respiring bacteria are known to be widespread at low numbers, even in uncontaminated environments where they apparently subsist on naturally-occurring organohalides (Krzmarzick et al., 2012). For sites contaminated with chlorinated ethenes such as trichloroethene (TCE) and tetrachloroethene (PCE), bioaugmentation with cultures containing *Dehalococcoides* is a common

and accepted approach (ITRC, 2008). *Dehalococcoides* numbers tend to be elevated at such sites, with high cell titers often correlating with successful transformation (dechlorination) of these contaminants. A rule-of-thumb has emerged that 10^4 – 10^5 *Dehalococcoides* cells per L groundwater is a supporting indicator of successful remediation, although correlations between cell titers and dechlorinating activity across sites is weak (Lebron et al., 2011), indicating that the *Dehalococcoides* are more active (per cell) at some sites than others.

A direct way to monitor *Dehalococcoides* activity and dehalogenation *in situ* would be tracking expression of the reductive dehalogenase (*rdh*) genes. However, this approach is limited to those situations where the specific *rdh* gene responsible for the contaminant of interest is known. To date, the rdh genes have been identified with specificity to the following substrates: PCE (*pceA*, Neumann et al., 1998; *mbrA*, Chow et al., 2010), TCE and cis-dichloroethene (*tceA*, Magnuson et al., 1998, 2000), vinyl chloride (bcvA, Krajmalnik-Brown et al., 2004; *vcrA*, Müller et al., 2004), chlorobenzenes (*cbrA*, Adrian et al., 2007), and 1,2-dichloropropane (*dcpA*, Padilla-Crespo et al., 2014). In previous work, we identified two putative *rdh* genes that increased in abundance in microcosms that also showed increased PCB dechlorination, suggesting that these are involved in PCB dechlorination (Park et al., 2011), and recently, Wang et al. described *Dehalococcoides* strains that can grow by respiring PCE or PCBs, and identified *rdh*s with activity toward PCBs (2014). A major problem with this approach is that *Dehalococcoides* strains studied to date have 15 - 36 putative *rdh* genes in their genomes (Wang et al., 2014), but the substrate targets of most of these have not been identified. There are several compounds that *Dehalococcoides* is known to dechlorinate, such as chlorinated naphthalenes, dibenzodioxins and dibenzofurans (Fennell et al., 2004), for which the responsible *rdh*s have not been determined.

In environments contaminated with organohalides other than those few for which a gene has been identified, or at sites with multiple mixed organohalide contaminants, a more generic tool to assess *Dehalococcoides* activity (growth or contaminant transformation rates) is needed. 16S ribosomal RNA (rRNA) and the 16S rRNA gene (DNA) are the most commonly used gene and gene product used to identify and characterize bacteria in environmental samples. The ribosome is the locus of protein synthesis, and it has long been known that a cell's RNA content, particularly ribosomal RNA, is positively correlated with growth (Caldwell et al., 1950; Schaechter et al., 1958). Several researchers have attempted to show the RNA-growth relationship could be used as a tool for inferring activity or growth in the environment (Kerkhof and Ward, 1993; Kemp et al., 1993; Jeffrey et al., 1996; Campbell et al., 2011). These correlations of cellular RNA content to growth are species-specific (Kerkhof and Ward, 1993; Kemp et al., 1993; Kerkhof and Kemp, 1999; Campbell et al., 2011). But for a given species, ribosomal RNA levels are generally related to activity or potential activity with respect to producing new proteins. In this project we utilize the cellular rRNA content as an overall measure of *Dehalococcoides* activity (growth rate) under controlled conditions, to establish it as a tool for monitoring dehalogenation *in situ* when the specific *rdh* is unknown, or in cases of mixed chlorinated contaminants.

Methodology

Serum bottles (160 mL) were operated as semi-continuous 'chemostats' to establish fixed growth rates at 10, 20, 50, and 100-day hydraulic retention times (HRT). A mixed culture containing *D. mccartyi* strain 195 was grown under anaerobic conditions as described previously (Fennell et al., 2004), with butyric acid as the electron donor and TCE as the electron acceptor. At intervals corresponding to 1/10 of the HRT, 10% of the liquid volume was withdrawn and replaced with media. The TCE concentration in the feedstock was calculated such that overall reactor concentration increased by less than 100% upon feeding (i.e., 50 μM for the 10-day HRT, 3.9 μM for the 100-day HRT). Simultaneous with the TCE cultures, chemostats were operated with the following halogenated pollutants: 1,2,3,4-tetrachlorobenzene and pentachloronitrobenzene, which are suspected of supporting growth of *D. mccartyi*, and 2,3,4,4',5-pentachlorobiphenyl (PCB 114), which is dechlorinated but does not appear to support growth (Zhen et al., 2014). These were operated with a 50-day HRT similar to the TCE reactors, except that sterile Arthur Kill sediment (0.01 g mL^{-1}) was also added to the medium. We have found that adding sterile sediment as a carrier for hydrophobic organohalides yields higher dechlorination activity than coating on glass beads or serum bottles without carrier (Fennell et al., 2004).

The semi-continuous 'chemostats' were operated for a minimum of three HRT before final sampling. During the last 3-4 feeding cycles, chlorinated ethenes (PCE, TCE, cis-DCE, vinyl chloride, and ethene) were analyzed in the headspace of the TCE-fed serum bottles using a gaseous injection on a GS Gaspro column in a gas chromatograph (GC) equipped with a flame ionization detector (FID) (**Figure 1**). For the chlorinated aromatic-fed cultures, endpoint samples (10 mL) were collected and stored (-20 °C) for analysis on a DB-5 column in a GC with an electron capture detector (ECD). At the time of final sampling, 50 mL of culture was filtered through a 47mm diameter 0.1 μm polyethersulfone membrane (SUPOR 100, Pall, Port Washington, NY). The filters were subdivided (cut) using sterile scissors, and total nucleic acids (DNA and RNA) were extracted from one quarter section of each filter using a phenol/chloroform/isoamyl alcohol method (Krumins et al., 2014) (**Figure 2**).

At the time of this annual report, molecular analysis (quantitation of the DNA and RNA) and data analysis are on-going. The nucleic acid extracts will be split, with one aliquot treated with DNAse (RNAse-free DNAse I, ThermoFisher, Grand Island, NY), followed by reverse transcription of the RNA (Superscript VILO cDNA synthesis kit, ThermoFisher, Grand Island, NY). *Dehalococcoides* 16S rRNA gene copies in the genomic DNA and the reverse-transcribed RNA (cDNA) will be quantitated by real-time quantitative PCR (qPCR) using SYBR Green chemistry (PowerUp SYBR, ThermoFisher, Grand Island, NY) with primers Dhc1f - Dhc259r (Duhamel et al. 2004). Transcripts of *tceA* will also be quantitated in the cDNA using primers described by Fung et al. (2007) for comparison with Rowe et al. (2012). The cellular rRNA content (16S copies in the cDNA / copies in the gDNA) will be calculated, and correlated to growth rate (the reciprocal of the 'chemostat' HRT) and substrate utilization rate. This will provide a direct molecular method for estimating *Dehalococcoides* growth and substrate utilization rate in the environment.

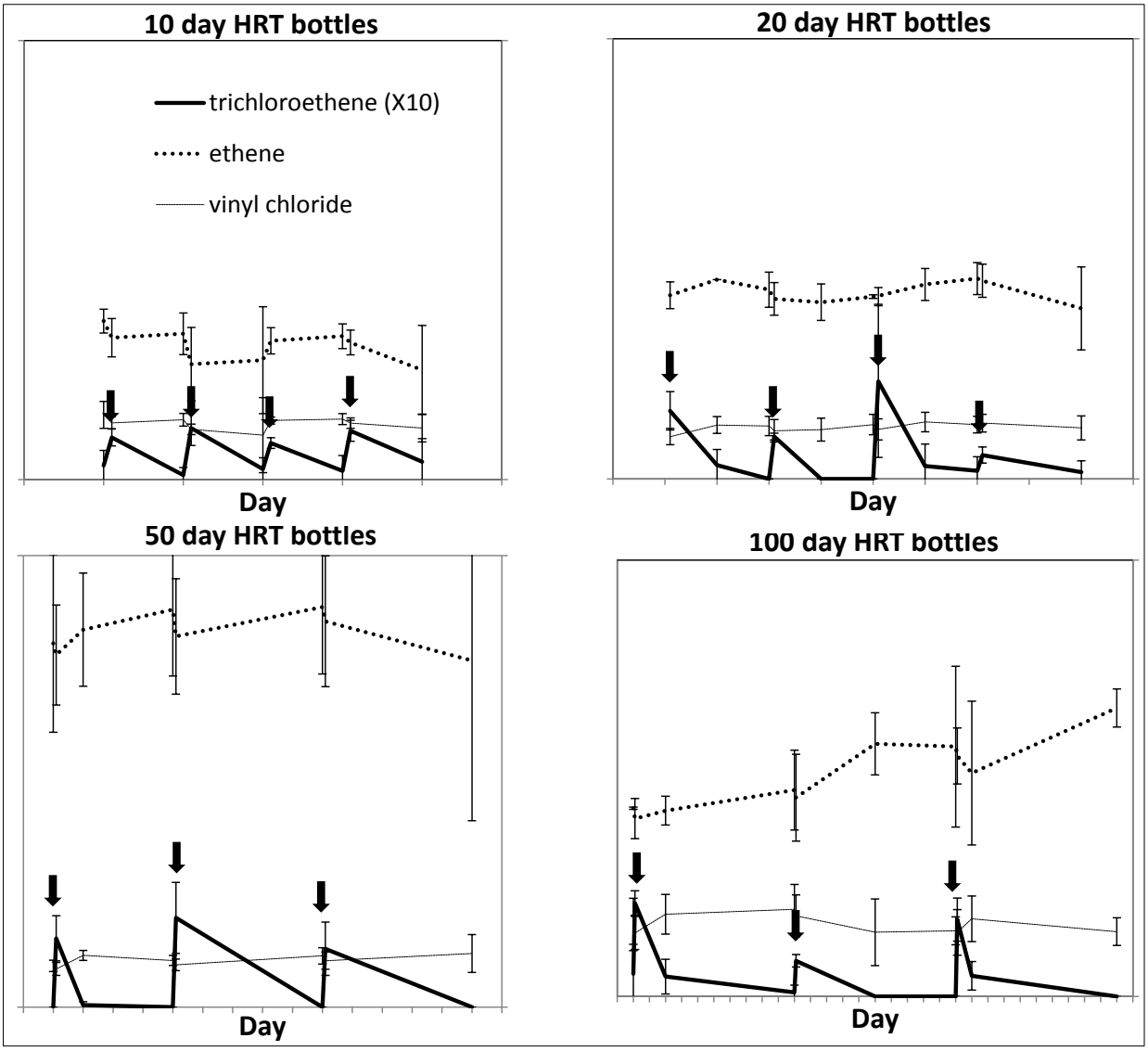

Figure 1. Chloroethenes headspace measurements for trichloroethene (TCE) fed microcosms, during the last 3-4 feeding cycles of the incubations. Black arrows indicate feeding with TCE. Error bars indicate standard deviations of measurements from triplicate microcosms. Note the different x-axis scales: each tick mark indicates one day. The y-axis represents the amount of compound detected in each bottle on a relative scale.

Principal Findings and Significance

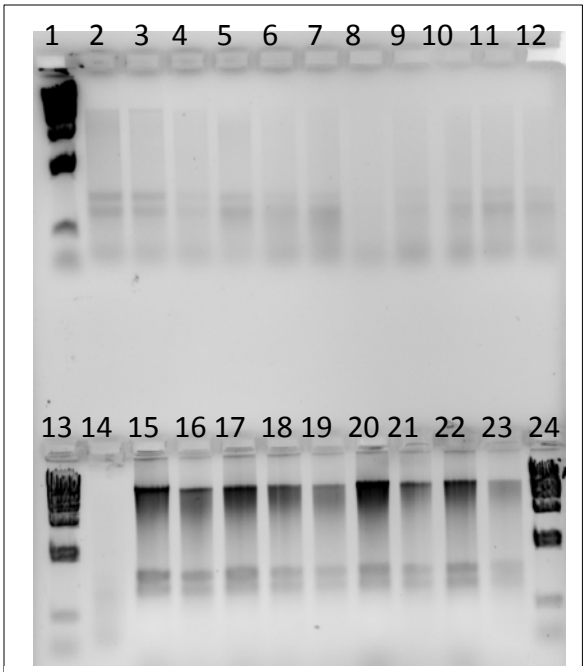

Figure 2. Agarose gel electrophoresis of nucleic acid extracts, showing recovery of both DNA and RNA. Lanes 1, 13 and 24: Lambda Hind III DNA marker. 2-4, 100d HRT; 5-7, 20d HRT; 8-10, 50d HRT; 11,12 and 14, 10d HRT; 15-17, PCB cultures; 18-20, pentachloronitrobenzene cultures; 21-23, tetrachlorobenzene cultures.

To date, we have established that microcosms containing a mixed culture including *Dehalococcoides mccartyi* strain 195 remain metabolically active and retain recoverable amounts of DNA and RNA after incubation on TCE, PCBs, pentachloronitrobenzene, and tetrachlorobenzene. We will determine the functional relationship between cellular rRNA content (ratio of 16S rRNA to the 16S rRNA gene) and growth rate on the various substrates.

This research will lead to a tool to assess growth rates of *Dehalococcoides in situ,* addressing a critical water resource issue in New Jersey: contamination by chlorinated organic (organohalide) pollutants. Over 100 rivers and creeks in New Jersey have water quality impairments due to PCBs and/or other chlorinated contaminants, and dechlorination by organohalide-respiring bacteria is one of the few potential mechanisms to restore water quality in these water bodies. *Dehalococcoides spp*. are capable of reductively dechlorinating a variety of chlorinated organic compounds, including toxic and priority pollutants listed in the Clean Water Act such as polychlorinated biphenyls (PCBs), pesticides, and polychlorinated dibenzo-p-dioxin and -furans ("dioxins"). A molecular tool to indicate the rate (extent) of *Dehalococcoides* activity would facilitate both active bioremediation and monitored natural attenuation approaches for these contaminants.

Publications and Presentations:

None to date. The findings will be summarized in a manuscript to be submitted to *Environmental Science & Technology*.

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Microplastic pollution in New Jersey surface waters

Basic Information

Title:	Microplastic pollution in New Jersey surface waters
Project Number:	2016NJ378B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Categories:	Water Quality, Toxic Substances, Surface Water
Descriptors:	None
Principal Investigators:	Beth Ravit, Keith R Cooper, Brian Buckley

Publications

1. Ravit, Beth, Keith Cooper, Brian Buckley, Sandra Meola, Dayvonn Jones, Molly Greenberg. 2016. Assessing Microplastic Pollution in Urban Watersheds. In: Urban Extension Conference, Rutgers University, Newark, New Jersey. November 29, 2016.
2. Two publications will be submitted for peer review this summer. One will be submitted to AIMS Environmental Science (Dr. Ravit lead author), and will focus on microplastics in the environment. The second article will be submitted to Ecotoxicology (Dr. Cooper lead author), and will focus on the potential toxicological effects of microplastics. We are also collaborating with NOAA to submit a short notes paper describing the various analytical techniques developed for these analyses.

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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: Partial Support - 3 DES Summer Interns Daniel Baron, Dayvonn Jones, Amy Hsieh
Masters' students: 0
Ph. D. students: 1 Partial Support – Cooper PhD student Gina Moreno
Postdoctoral Associates: 0

(3) **Any Notable Achievements:** NY/NJ Baykeeper is using the results of this research in their Plastic Reduction Project Community Outreach efforts. Based on the NJWRRI research, USEPA (Region 2) awarded Baykeeper a $40,000 Urban Water Grant (Rutgers a subcontractor) to add additional water sampling sites on the Passaic River. The Urban Waters grant also supports Baykeeper's community outreach activities. The PIs also received an additional $15,000 NIEHS pilot project grant, which allowed us to increase the number of samples analyzed by EOHSI. NOAA J. J. Howard Sandy Hook Laboratory contributed In-Kind analyses that determined the chemical components of the various microplastic substances, which may be released upon breakdown during environmental exposure. Dr. Ravit has also been awarded a $7,500 Sustainable Raritan River Initiative (SRRI) grant to continue microplastic research during summer 2018, which will add eight new sampling sites on the freshwater portion of the Raritan River.

(3) **Project Summary**:

Problem and Research Objectives:
Plastic "microbeads" (microscopic pieces of plastic smaller than 5 mm) came into widespread use beginning in the 1990s, particularly in personal care products. However, research

documenting the presence of microbeads in freshwaters is more recent, and so the full extent or impacts associated with this environmental pollution are not well understood). Anthropogenic sources of microbead plastic pollution include personal care products that contain microbeads to enhance abrasive qualities – soaps, facial scrubs, toothpastes, cosmetics. Microbeads flushed down the drain are carried through treatment facilities via industrial effluent or wastewaters (thought to be primary upland microbead sources) where they are not removed. Depending on surface water proximity to manufacturing facilities, wastewater treatment plants, or high urban populations, recent studies suggest these particles may be as ubiquitous and present in higher densities in freshwater systems than in the marine environment. NJ waste water treatment plants, built decades ago, were not designed to remove this newly emerging category of pollution during wastewater treatment. When treatment plants legally discharge effluent into NJ surface waters, microbeads in the effluent are released into receiving waters.
There are multiple environmental concerns associated with microplastics in surface waters. In addition to the composition of the plastic itself, there is the potential for persistent organic pollutants (POPs), particularly those that are hydrophobic such as PCBs and PAHs, to attach themselves to the plastic particles. It is estimated that total daily microbead release into aquatic environments may be as high as ***8 trillion microbeads a day***. Microplastics have been documented in fin fish and shellfish tissues, suggesting this pollution has the potential to move into human food chains. Urban rivers receiving effluent discharges may be an important component of microplastic transport, contributing to the global microplastic lifecycle.

NJWRRI funding enabled us to obtain surface ten (10) surface water samples from freshwater reaches of the Raritan and Passaic Rivers. We have calculated microplastic type and density by site, analyzed the chemical composition of recovered microplastics, and are in the process of identifying compounds adsorbed to the recovered microplastic particles that could potentially affect aquatic species. This data will contribute to characterization of microplastic pollution in NJ waters prior to implementation of the 2018-2020 state-wide ban on the manufacture or sale of microbead personal care products. This baseline will allow comparison of microbead pollution before and after the ban, and aid in evaluating the effectiveness of this legislation. We hypothesized that: 1) the concentration of microbeads is greater in Raritan and Passaic River surface waters than in Raritan and Newark Bays, respectively; 2) persistent organic pollutants are sorbed to microbeads present in Raritan and Passaic River surface waters; and 3) uptake of microbeads will result in observable physiological effects in juvenile zebrafish (*Danio rerio*). Given the quantity of daily microbead release, the potential for environmental accumulation, and the unknown effects or the potential for toxicity associated with microbeads, the proposed research is urgently needed.

Methodology:

Sample Collection
We selected five (5) sampling locations on the Raritan River and ten (10) sampling locations on the Passaic River. We modified protocols previously used to obtain Newark and Raritan Bay water samples for microbead analysis. Due to the shallowness of the upriver reaches, it was not possible to sample by pulling the manta trawl net from the Baykeeper boat as originally planned. Therefore, we walked the trawl net into the midsection of the river and positioned the net opening facing upstream. Briefly, water column samples were collected using the manta trawl with a rectangular opening 16 cm high x 61 cm wide, attached to a 333 μm collection net that is 3 m long and 30 x 10 cm^2. The net, equipped with a flow meter, was held in a stationary position

for 15 minutes; the volume of water sampled was calculated using flow meter data at the end of each 15 minute trawl. The distance (flow meter reading x impeller constant) multiplied by the width of the trawl net opening calculated the area sampled, allowing particle density per km^2 to be estimated. Three (3) replicate samples were obtained at each sampling location. One sample was digested and used to determine microplastic density; one sample was separated using a polysucrose gel gradient to obtain microplastics for toxicity studies; one sample was sieved and washed with site water to use of solid phase micro-extraction (SPME) GC/MS/MS analysis of compounds associated with microplastic particles.

Fig. 1. Passaic River sampling (2016) above the Dundee Dam.

Density Analysis-Completed

Fig. 2. Density sample processing: collected sample; sieving, Fenton Reaction digestion; microplastic sorting.

One replicate of each sample was sieved to remove large organic debris prior to a wet peroxide oxidation process in the presence of an iron (Fe II) catalyst to digest organic material (Fenton Reaction). The plastic remains unaltered. After digestion the plastic was separated into three size classes (0.355-0.999 mm, 1.00-4.749 mm, and >4.75 mm) under a dissecting microscope. Plastic particles were then categorized by type (fragment, foam, line, pellet, film) and each type was counted by size.

Chemical Analyses-Sample analysis completed; Compound identification in process

Extractable semi-volatile adsorbed compounds and volatile plastic components of the microbeads, such as plasticizers (e.g. phthalates), were analyzed by solid phase micro-extraction (SPME) GC/MS/MS. Each sample was placed in a SPME vial, and then thermally desorbed from the vial onto the SPME fiber. The analytes were injected onto the GC column using a thermal program, specific for key analytes expected to be present in the plastic pellets and for persistent organic pollutants commonly present in NJ waterways. Key analytes were spiked into the matrix before blowdown to act as internal standards. All major peaks present in the chromatogram are now being identified using the NIST compound library search. Compounds observed will be quantified against the internal standards.

Toxicity Testing – In process

The zebrafish (*Danio rerio*) model is being used to elucidate potential temporal and spatial molecular effects of microplastics on development and/or permanent changes in later stages. The European Union has developed SOPs for conducting toxicant exposures from just post fertilization through reproducing adults (Fish Embryo Acute Toxicity (FET) Test OECD/OCDE 236 Adopted 2013). The AB strain of zebrafish (Zebrafish International Resource Center) was used for all experiments conducted in accordance with the zebrafish husbandry and embryonic exposure protocol (#08-025) approved by the Rutgers University Animal Care and Facilities

Committee. Fertilized embryos were staged based on established criteria, and exposure began at 3hpf (512-cell stage). Preliminary tests indicated development was affected, and we are now beginning experiments to determine if the effects are due to the presence of the plastic or to chemicals associated with the plastic. The exact protocol for exposure will be modified based on whether a particle or extract of the microbead material(s) are being tested. The stage of development will be examined following exposure with different concentrations of particles present on 0.45 filter paper. Chemical extracts supplied by Dr. Brian Buckley and specific pure compounds detected in the microbeads will also be examined.

Principal Findings and Significance

1. There appears to be wide variation in plastic concentrations and types among the various sampling sites on each river. We are in the process of reviewing all calculations for each sampling location by microplastic type. Density GIS maps for the Raritan and the Passaic River 2016 sampling locations will be finalized by June 30, 2017.
2. Under dry weather sampling conditions, microplastic densities were not always greater in the downstream reaches – our original hypothesis was not supported by this result. Higher densities in the upstream reaches suggest that source tracking is an important aspect of further research to identify where microplastic inputs are originating.
3. Under wet weather sampling conditions (3 sites in the Passaic) the microplastic density appeared to increase at sampling locations downriver. This supports our hypothesis that density would increase with downstream urbanization. However, the number of samples was not significant, and the results may be a function of stormwater washing higher upstream concentrations downstream, rather than be the result of discharges from the more urban sections of the rivers.
4. We have identified the fingerprints of 100 compounds that were most frequently found in the plastic/water samples. We are now using the GC-MS library to determine what these compounds are. This identification is ongoing research during summer 2017.
5. Preliminary toxicity tests indicate there are effects due to the plastic materials. However, we are now conducting further tests to determine if these effects are due to the physical presence of the particles or if they are the result of exposure to chemicals associated with the particles. As actual compounds are identified, we will be exposing zebrafish larvae to the compound (without plastic) and to plastics that are compound free to elucidate a cause-effect relationship.

(4) **Publications or Presentations**:

Conference Presentations

Ravit, Beth, Keith Cooper, Brian Buckley, Sandra Meola, Dayvonn Jones, Molly Greenberg. 2016. Assessing Microplastic Pollution in Urban Watersheds. In: Urban Extension Conference, Rutgers University, Newark, New Jersey. November 29, 2016.

Two publications will be submitted for peer review this summer. One will be submitted to AIMS Environmental Science (Dr. Ravit lead author), and will focus on microplastics in the environment. The second article will be submitted to Ecotoxicology (Dr. Cooper lead author), and will focus on the potential toxicological effects of microplastics. We are also collaborating

with NOAA to submit a short notes paper describing the various analytical techniques developed for these analyses.

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 and the other a deeper gravel layer and an Advanced Bioretention System. 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 will be determined through comparisons of inlet and outlet nutrient concentrations of each test wetland. The resulting information will be used to inform development of best management practices for stormwater design.

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

Run-off water from a large parking lot at Georgian Court University was diverted by a concrete rush way into a collection basin. An outlet from the collection basin was split into four sections so that the collected run-off was diverted equally distributed into three SSGWs and one ABS design (Table 1).

Table 1: Subsurface Gravel Wetlands at Georgian Court University.

Wetland	Design
University of New Hampshire Design (UNH)	Original replicate of wetland designed by UNH
Advanced Bioretention Systems (ABS)	Vertical water flow with mostly sandy soil
Simplified UNH Design #1 (SSGW-3/UNH-1)	UNH design modified by excavated to a depth of 3'and providing an additional foot of gravel
Simplified UNH Design #2 (SSGW-4/UNH-2)	UNH design modified by removal of baffles and simplified plumbing

A total of five samples were collected from each of seven storm events over the course of the study, from May 30, 2016 through April 26, 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 be 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. Additional measurements such as TSS and oxidation/reduction potential were recorded per each rain-sampling event to assess which processes are acting to reduce N and P. 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

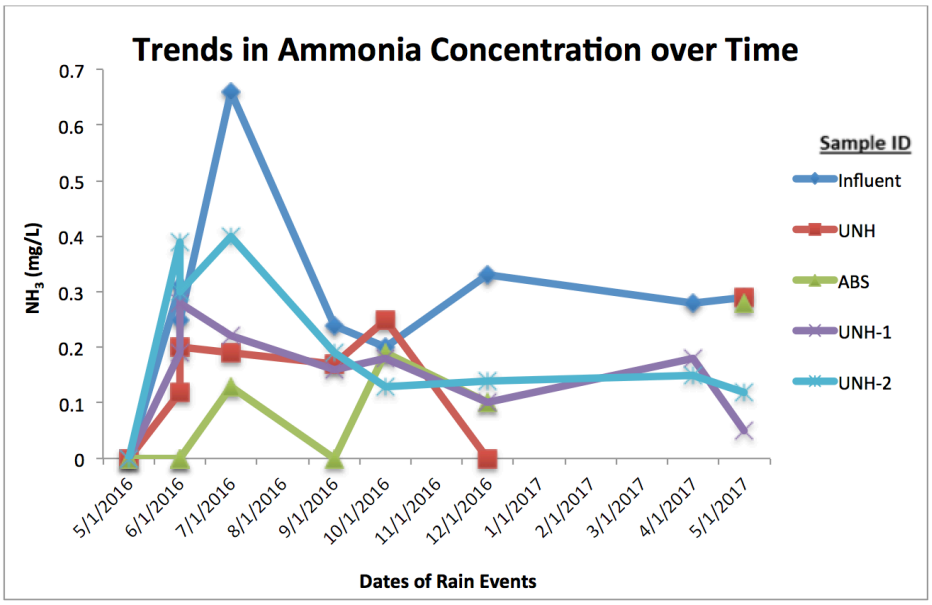

Figure 1: Concentration of Ammonia at GCU's SSGWs for storm events

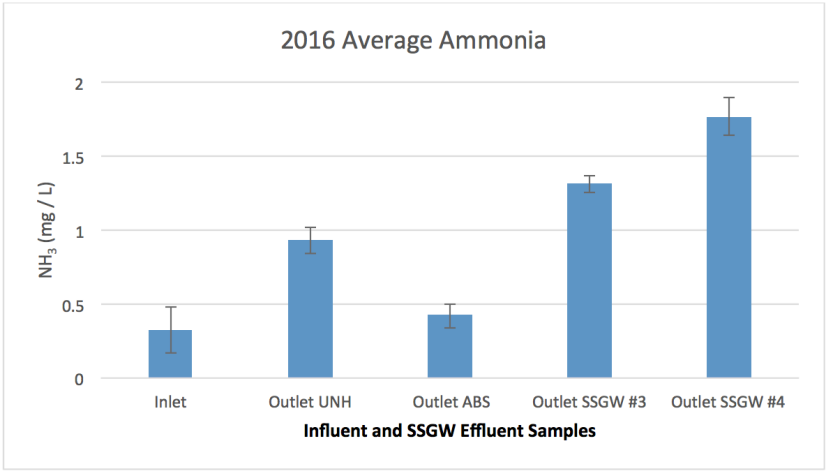

Figure 2: Average Ammonium in 2016 at GCU's SSGWs for storm events

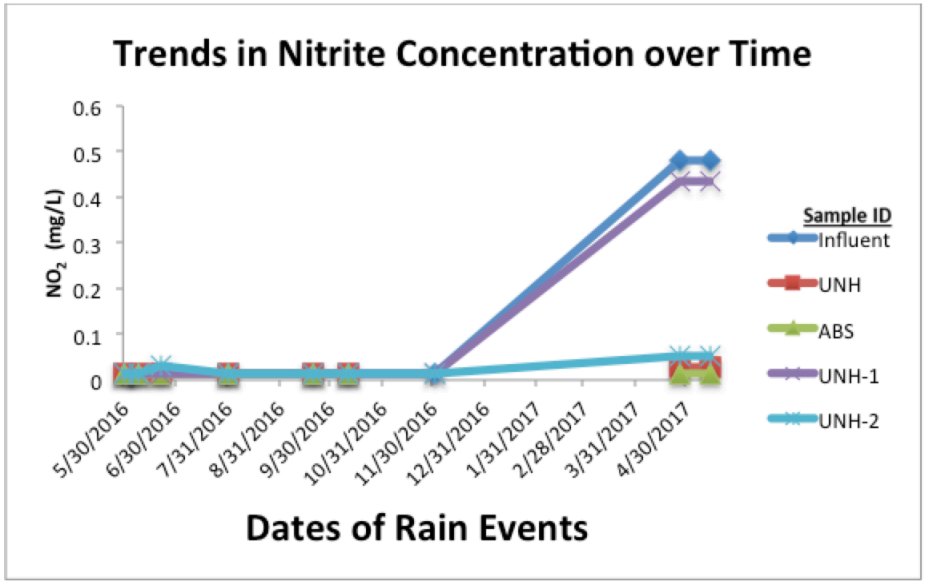

Figure 3: Concentration of Nitrite at GCU's SSGWs for storm events

****Missing Data points reflect no samples collected****

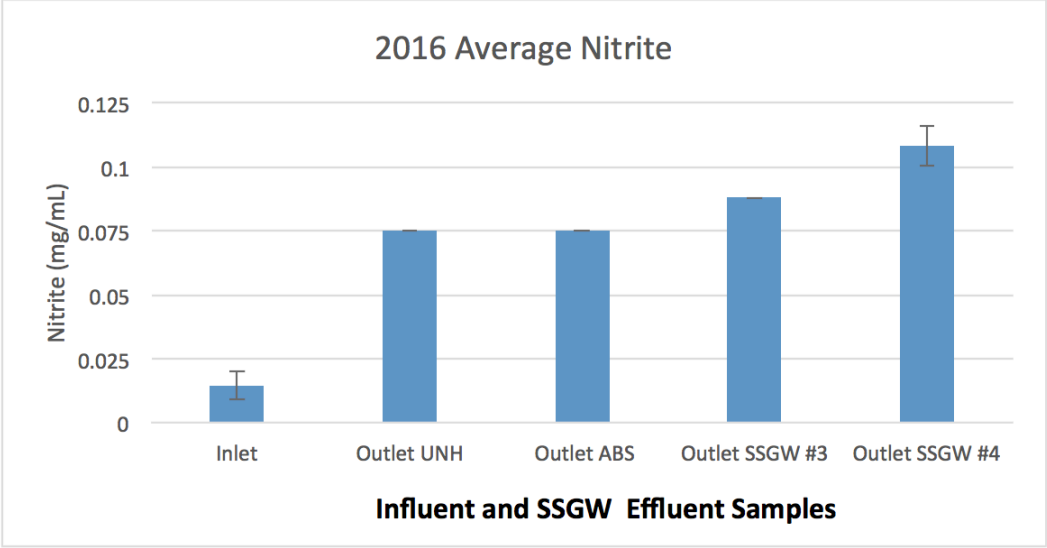

Figure 4: Average Nitrite in 2016 at GCU's SSGWs for storm events

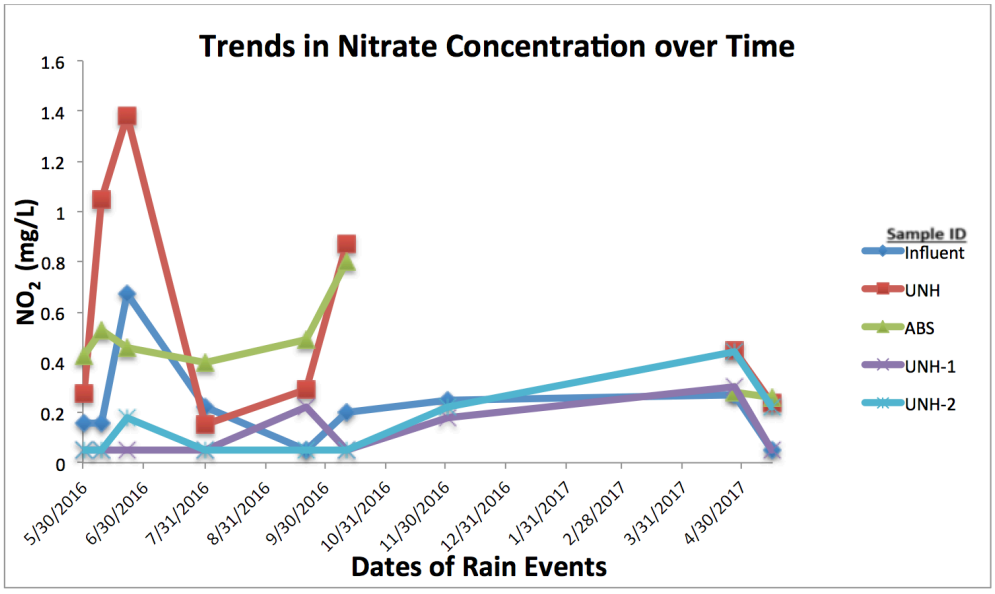

Figure 5: Concentration of Nitrate at GCU's SSGWs for storm events

****Missing Data points reflect no samples collected****

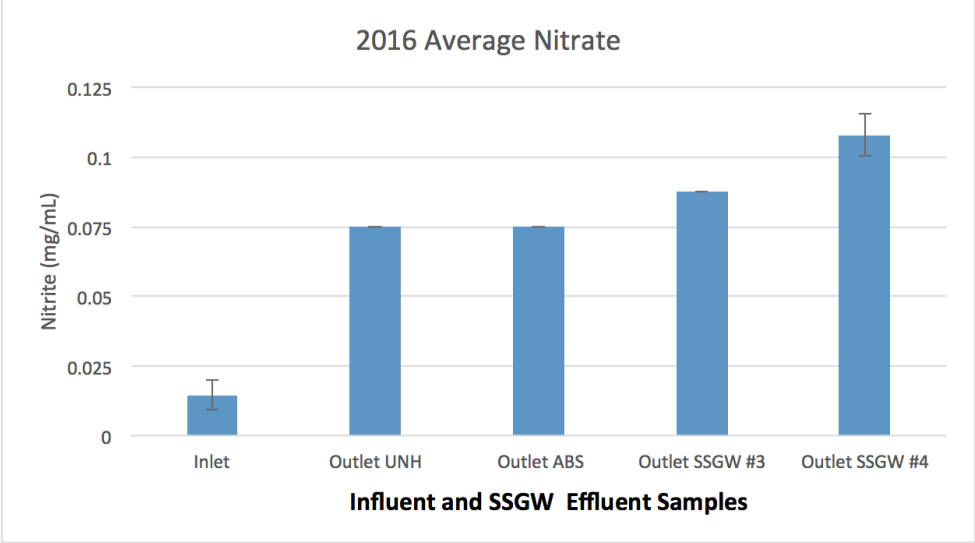

Figure 6: Average Nitrate in 2016 at GCU's SSGWs for storm events

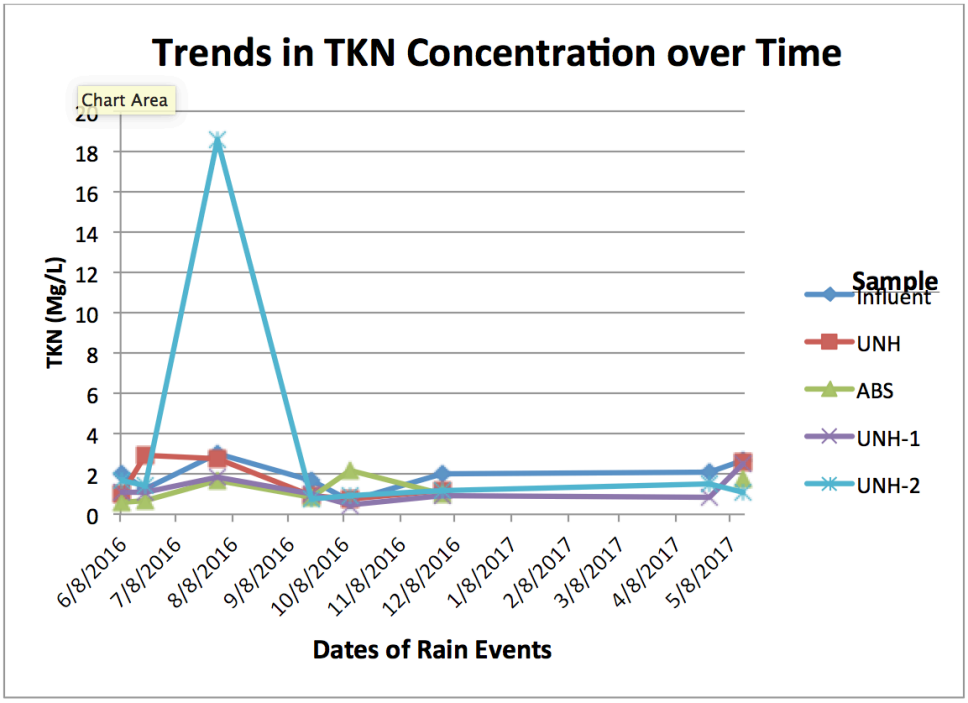

Figure 7: Concentration of Total Kjeldahl Nitrogen at GCU's SSGWs for storm events

****Missing Data points reflect no samples collected****

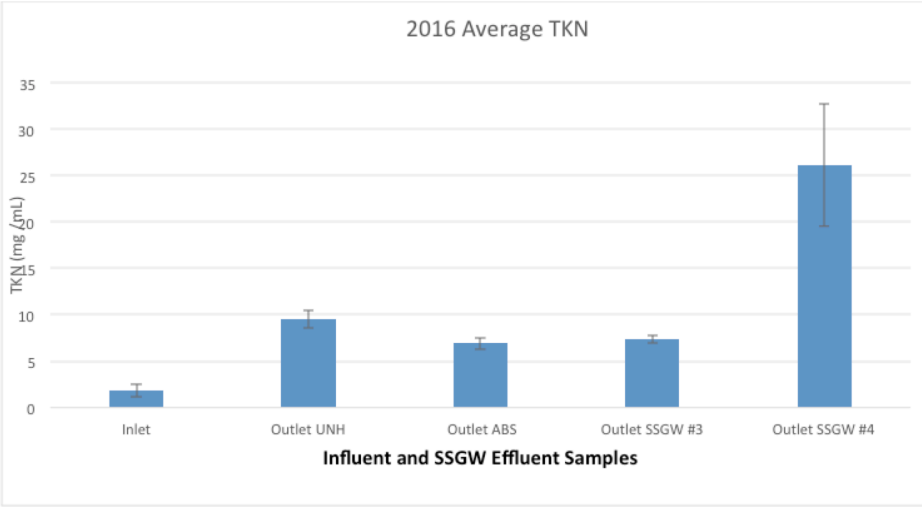

Figure 8: Average Total Kjeldahl Nitrogen in 2016 at GCU's SSGWs for storm events

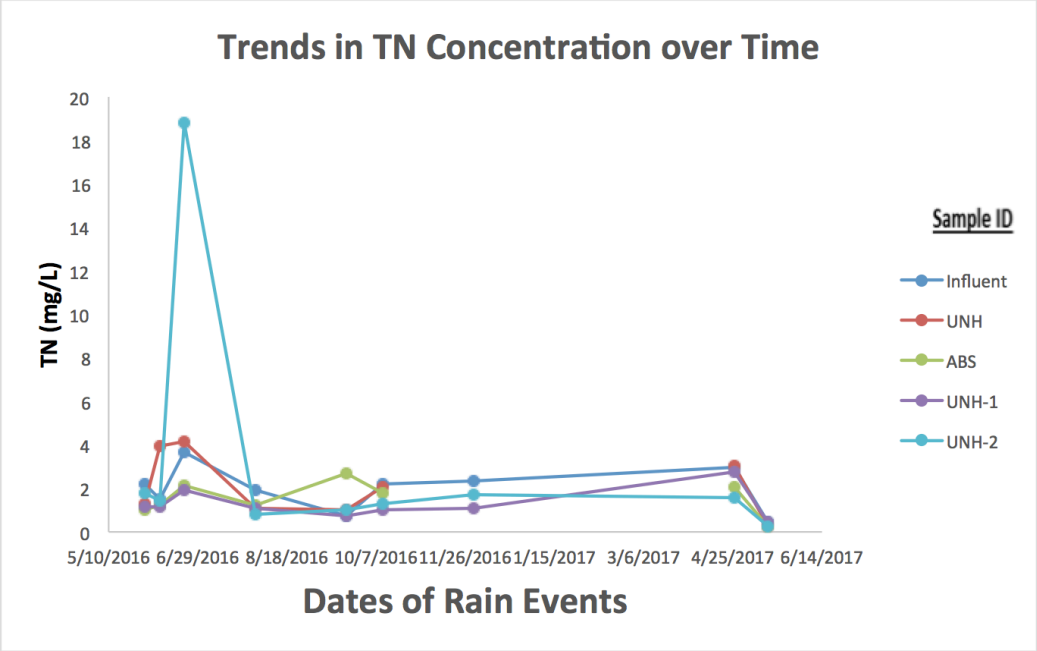

Figure 9: Concentration of Total Nitrogen at GCU's SSGWs for storm events

****Missing Data points reflect no samples collected****

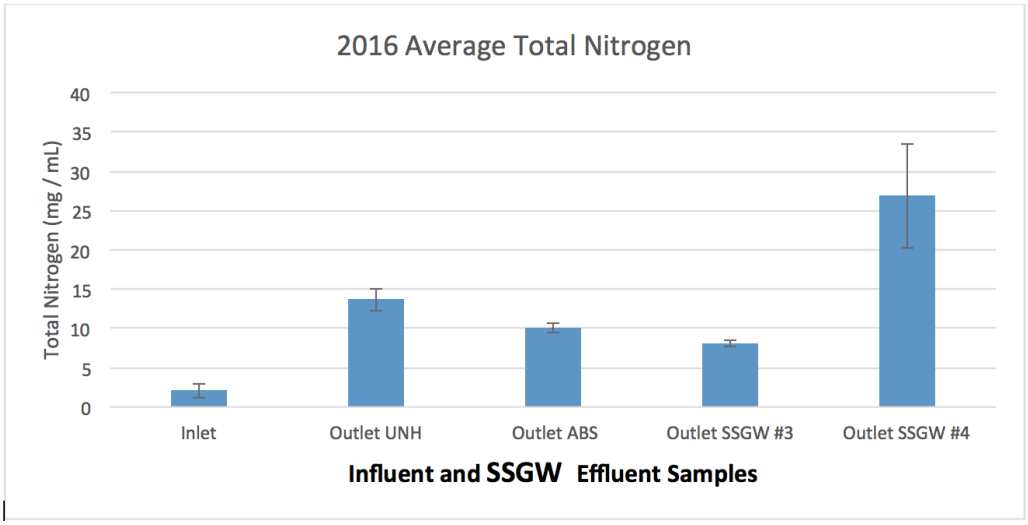

Figure 10: Average Total Nitrogen in 2016 at GCU's SSGWs for storm events

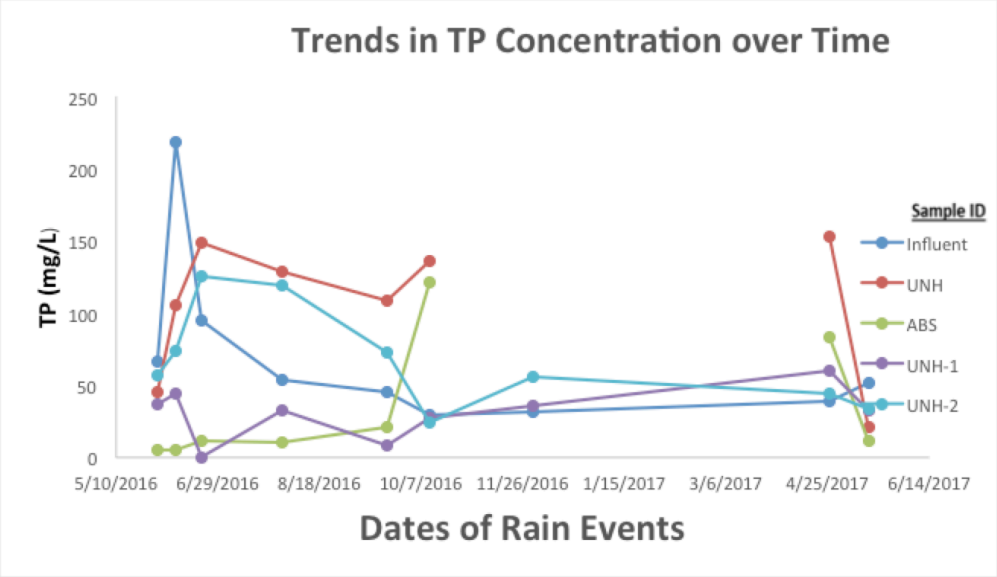

Figure 11: Concentration in Total Phosphate at GCU's SSGWs for storm events

****Missing Data points reflect samples not collected for storm event in 2 SSGW's****

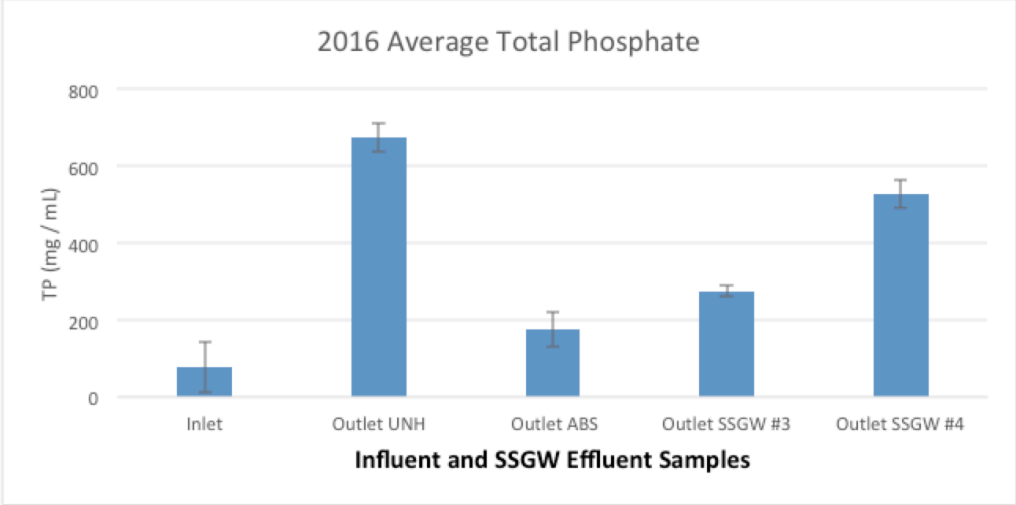

Figure 12: Average Total Phosphate in 2016 at GCU's SSGWs for storm events

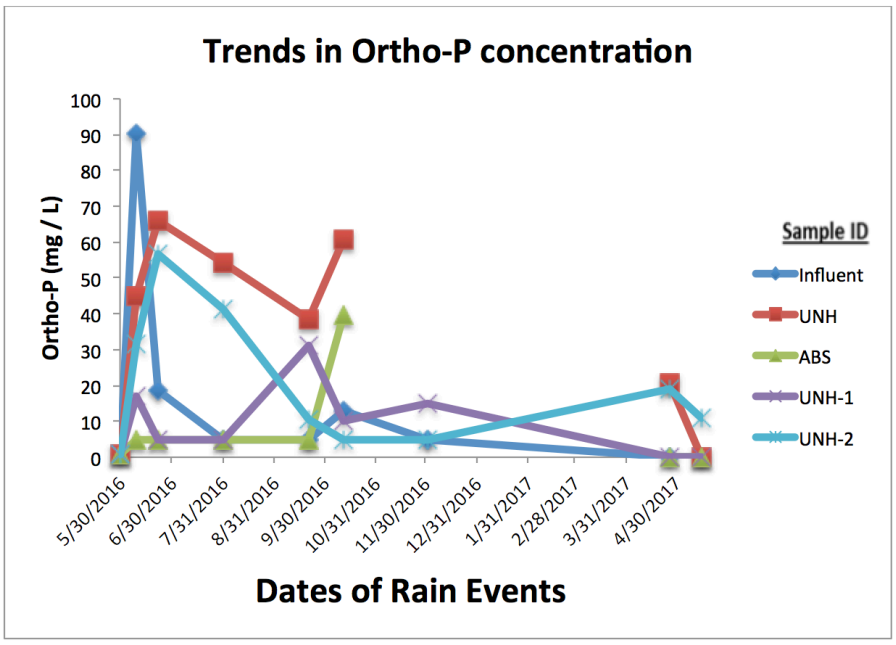

Figure 13: Concentration of Ortho-P at GCU's SSGWs for storm events

****Missing Data points reflect no samples collected in 2 SSGWs****

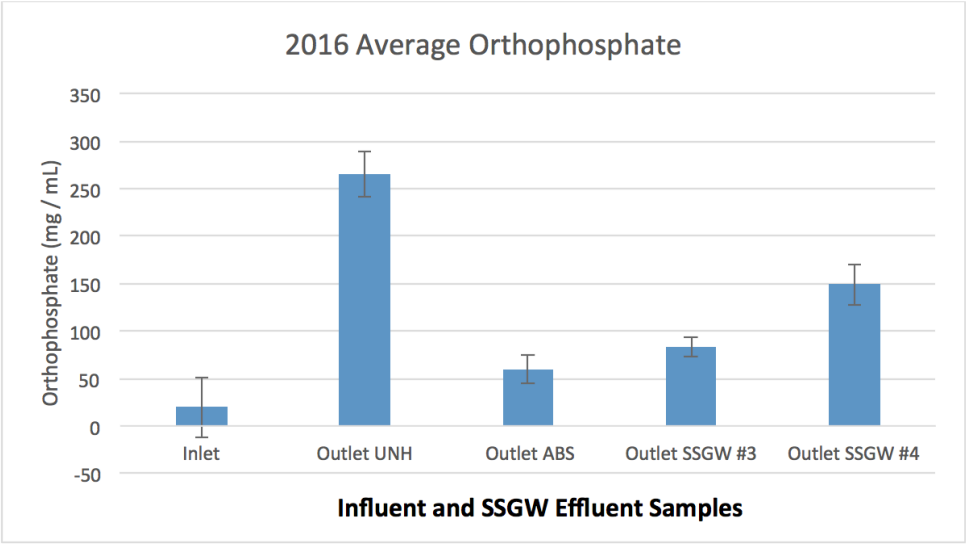

Figure 14: Average Orthophosphate in 2016 at GCU's SSGWs for storm events

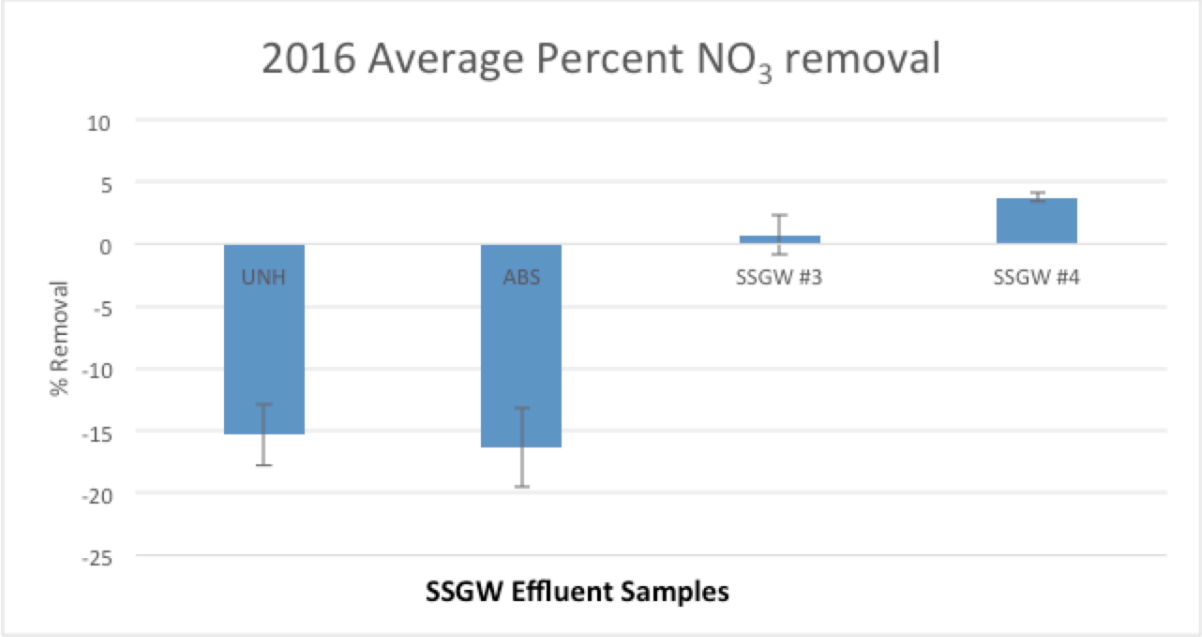

Figure 15: Average Nitrite Removal in 2016 at GCU's SSGWs for storm events

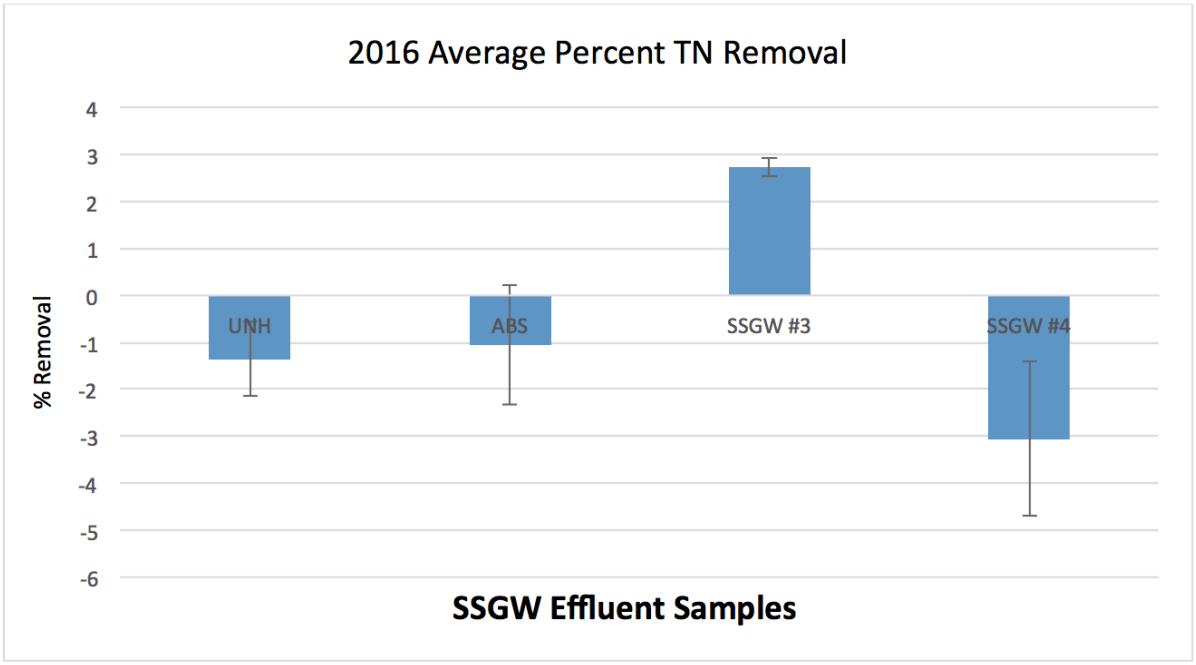

Figure 16: Average Percent Total Nitrogen Removal in 2016 at GCU's SSGWs for storm events

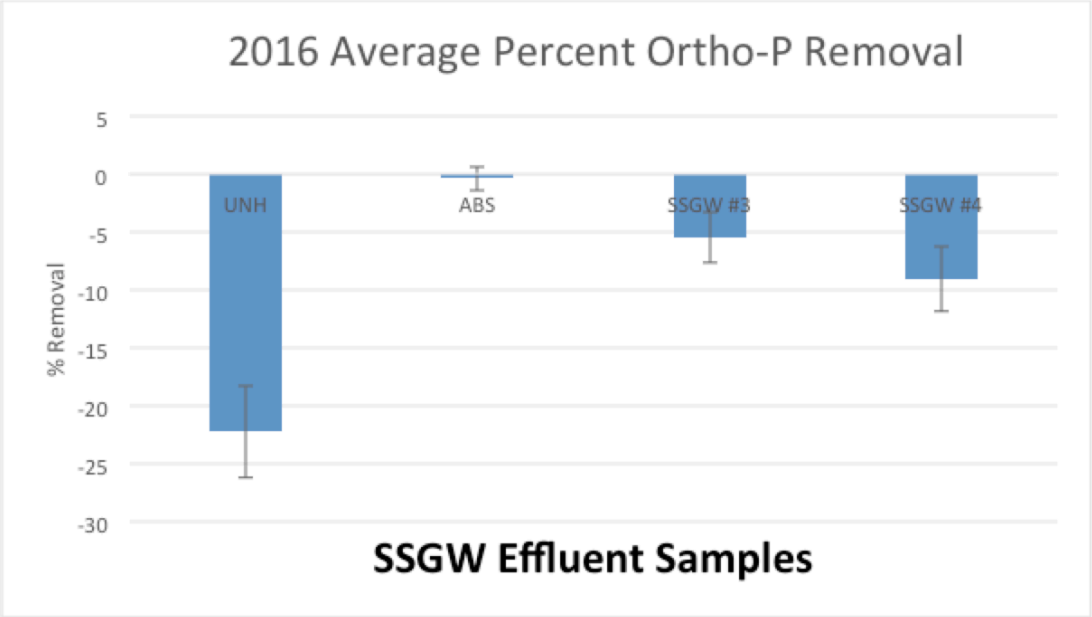

Figure 17: Average Percent Orthophosphate Removal in 2016 at GCU's SSGWs for storm events

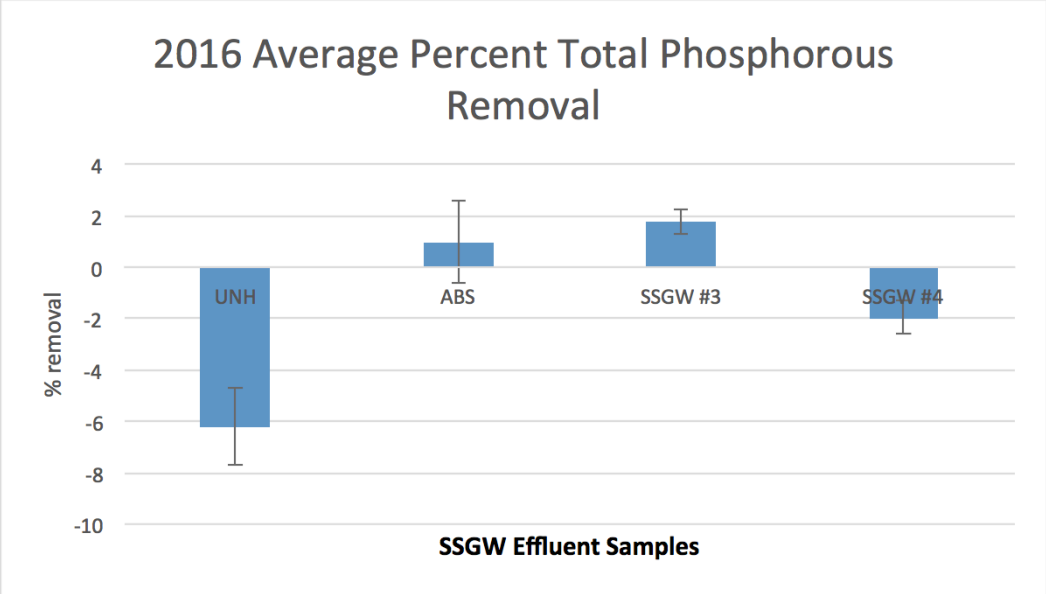

Figure 18: Average Percent Total Phosphorous Removal in 2016 at GCU's SSGWs for storm events

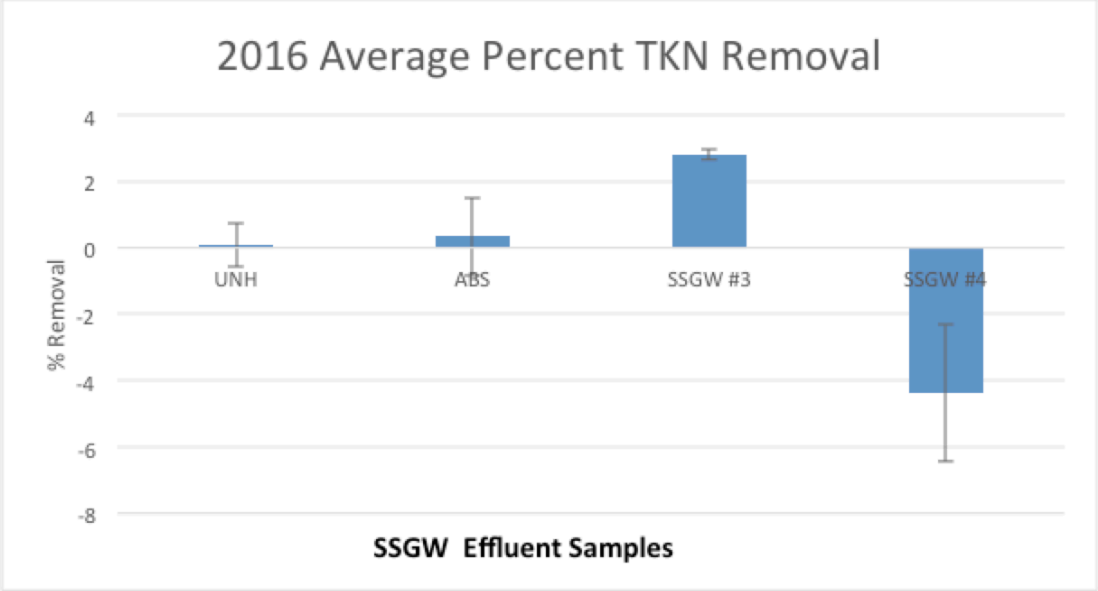

Figure 19: Average Percent Total Kjeladal Nitrogen Removal in 2016 at GCU's SSGWs for storm events

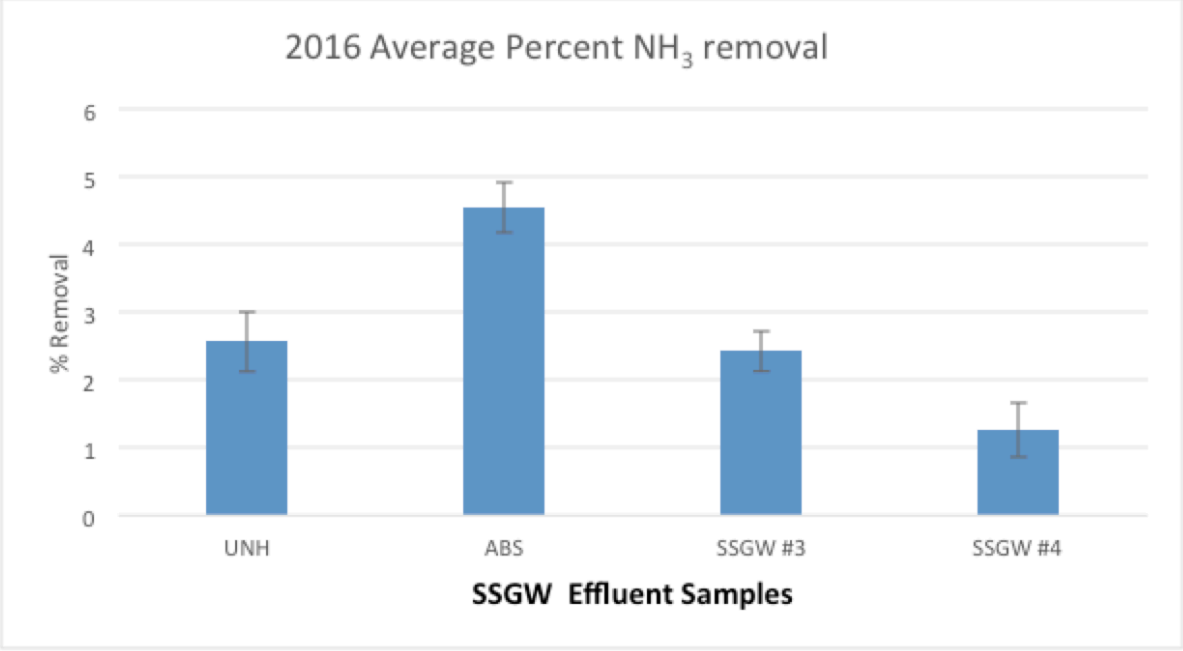

Figure 20: Average Percent Ammonia Removal in 2016 at GCU's SSGWs for storm events

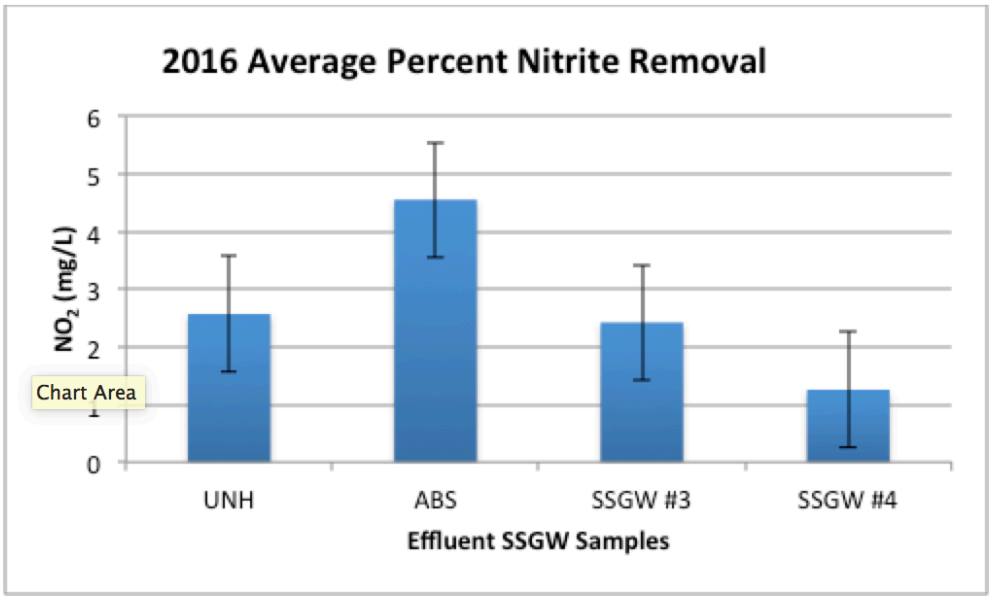

Figure 21: Average Nitrite Percent Removal in 2016 at CGU's SSGWs for Storm Events

Two samplers did not trigger during collection for one rain event, the ABS & the SSGW #3 (UNH-1). Figures 1 and 2 illustrate the ammonia concentration over time and the average concentration for 2016, respectively throughout the study period. While the SSGW #3 (UNH-1) design had a decrease in the ammonia concentration, it contained the second largest average of ammonia for 2016. The amount of nitrite concentration in the original UNH design (Fig. 3), the ABS design and the SSGW #4 (UNH-2) was less than the influent, while the SSGW #3 (UNH-1) had almost no decrease in the nitrite concentration. However, the average for 2016 in nitrite concentration (Fig.4) increased in all of the SSGWs as compared to the inlet concentration. The overall trend in nitrates for SSGW #3 (UNH-1) (Fig. 5) had a decrease in the concentration over the sampling time thus. All of the SSGWs contained more nitrates, on average for 2016 (Fig. 6) than the influent samples. SSGW #4 (UNH-2) had a large increase for both TKN (Fig. 7) and total nitrogen (Fig. 9). Thus far, there is relatively little change in the total concentrations across all samples for both TKN & TN. The average total Kjeldahl nitrogen for 2016 increased in all SSGWs sampled with respect to the inlet concentration (Fig. 8). In comparison to the inlet concentration, all SSGWs had an increase in the total average nitrogen (Fig. 10). The total phosphate concentration over the study period thus far, fluctuated from the initial sampling until present (Fig. 11). While all of the SSGWs contained less total phosphate at initial sampling than the influent sample, the original UNH and the ABS designs output had less total phosphate than the influent, SSGW #3 (UNH-1) and SSGW #4 (UNH-2) samples. The concentration of orthophosphate had trends very similar to that of the total phosphate (Fig. 13), where there was varying fluctuations of concentrations. The concentrations were high for all SSGWs contained a higher output of average total phosphate and orthophosphate in 2016 in comparison to the inlet concentrations (Figures 12 & 14).

The average percent removal for 2016 was determined for the various components. The percent removal for nitrate (Fig. 15) was negative for the UNH and the ABS designs, which indicated that more nitrates were given off than were introduced. However, SSGW #3 (UNH-1) and SSGW #4 (UNH-2) both had a positive removal of nitrates. The total nitrogen percent removal (Fig. 16) and total Kjeladal Nitrogen (Fig. 19), again, was positive for SSGW #3 (UNH-1). But, all other SSGWs again gave off more TKN than were introduced. All SSGWs gave off more orthophosphate than was introduced (Fig. 17). The average percent total phosphate removal (Fig. 18) was positive for the ABS design and SSGW #3 (UNH-1), but was negative for the UNH design and SSGW #4 (UNH-2). All SSGW#s had a positive removal for ammonia (Fig. 20) and nitrites (Fig. 21).

As sample collection was dependent on rainfall, the minimum number of samples need for definitive analysis has not yet been reached. It is my anticipation to complete the remainder of required samples by late summer, depending on rainfall.

(4) **Publications or Presentations**:

N/A project not yet completed due to minimal rainfall and project still ongoing.

Citations

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

Microplastics as emerging contaminants in surface waters of New Jersey

Basic Information

Title:	Microplastics as emerging contaminants in surface waters of New Jersey
Project Number:	2016NJ380B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Water Quality
Focus Categories:	Surface Water, Water Quality, Toxic Substances
Descriptors:	None
Principal Investigators:	Shirin Estahbanati, Nicole Fahrenfeld

Publications

1. Estahbanati, Shirin. 2016. Influence of Wastewater Treatment Plant Discharges on Microplastic Concentrations in Surface Water. “MS Dissertation”, Civil and Environmental Engineering, Graduate School-New Brunswick, Rutgers, The State University of New Jersey, Piscataway, New Jersey, 39.
2. Estahbanati, Shirin; Nicole, Fahrenfeld, 2016. "Influence of wastewater treatment plant discharges on microplastic concentrations in surface water." Chemosphere 162: 277-284.
3. Parrish, Katie; Shirin Estahbanati: Nicole, Fahrenfeld. Poster. “Putting the Micro in Microplastics” Association of Environmental Engineering and Science Professors (AEESP). Ann Arbor, MI. June 2017.
4. Parrish, Katie; Shirin Estahbanati: Nicole, Fahrenfeld. Poster. “Putting the Micro in Microplastics” 102nd Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 10, 2017.
5. Estahbanati, Shirin; Nicole, Fahrenfeld, 2016, Oral. “Impact of Wastewater Treatment Plants on Microplastics in Freshwater Environment.” 252nd National Meeting of the American Chemical Society. Philadelphia, PA. August 21-25, 2016.
6. Estahbanati, Shirin; Nicole, Fahrenfeld, 2016. Poster. “Microplastics as Emerging Contaminants in NJ Surface Waters.” 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.

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E-mail Address: nfahrenf@soe.rutgers.edu

Phone Number: 848-445-8416.

Numbers of Students Supported:

Undergraduate: Katie Parrish

Masters' Student: Shirin Estahbanati

Notable Achievements:

- New Jersey Water Environment Association(WEA) undergraduate Poster Competition Award May 2017
- Aresty/Honors College Research Fellowship ($700) to continue work
- New Jersey Water Environment Association(WEA) graduate Poster Competition Award May 2016
- New Jersey Water Environment Association (WEA) Louis Fontenelli Award April 2016

Project Summary:

Project Objectives:

The objective of this study was to (1) evaluate effect of wastewater treatment plants (WWTPs) on the number and size classes of microplastic in Raritan River. To achieve this, samples were collected from upstream and downstream of WWTPs to evaluate the effect of each plant on the concentration and distribution of microplastic particles in the River. Samples from each location were prepared for the chemical analysis. The number and size class of microplastics were determined. The second objective (2) was to better understand the role of microplastic as a surface for microbial growth. To do this, microplastic was extracted from personal care products and incubated in Raritan River water or wastewater for 24-48hrs. Then, qPCR was performed to quantify the amount of select target genes in the biofilm. A disinfection study was also performed to understand whether these biofilm were more resistant to disinfection than planktonic cells.

I hypothesized that the number of microplastics would increase downstream of WWTPs effluent discharge. The expectation was the smaller microplastic size category (63-250 µm) will account for most plastic pollution due to its high concentration in personal care products and the potential for it to be a secondary microplastic (i.e., broken off from a larger 'primary' plastic). I also hypothesized that the biofilm microbes would be more resistant to disinfection than planktonic cells.

Methodology

Samples were collected from Raritan River located in central New Jersey, US. Raritan River has two branches that meet and flow to the Raritan Bay. Samples were collected upstream and downstream of four majors, more than 1 million gallon per day, MGD, capacity) municipal WWTPs depicted in Figure 1. Location WWTPc was downstream of a major WWTP and the confluence of the Raritan with the Millstone River. Notably, the Millstone River has several WWTP discharges. In addition, a background site was selected on south branch as a control location to evaluate the impact of other sources of microplastics rather than WWTPs.

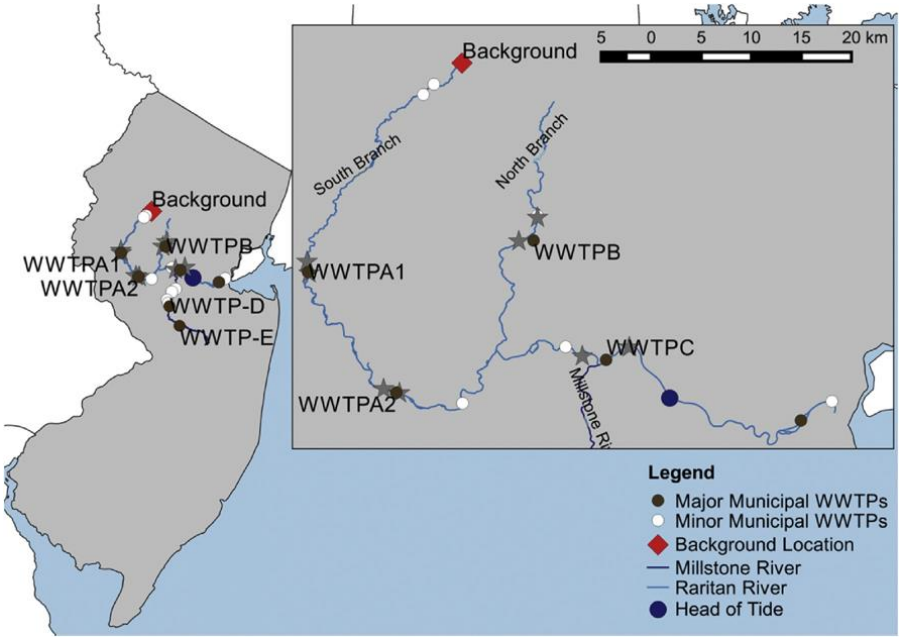

Figure 1-Sampling site map (Estahbanati and Fahrenfeld 2016)

Sampling was performed upstream and downstream of WWTPs for 1 h during the baseflow in duplicate with plankton nets (aperture size 153 μm) paired within 3-72 h of each other. Sample volume was calculated based on the surface water velocity determine by float or pygmy meter and the net cross sectional area. The first analytical step was sieving samples and categorizing the particles into four size classes (63–125 μm, 125–250 μm, 250–500 μm, and 500–2000 μm). Given the net aperture size was larger than 63μm, the results of the 63-125 μm class reported as semi-quantitative. The content of each sieve was rinsed with DI water and transfered into the lab oven. After drying the particles, the organic content of the recovered particles oxidized in a wet peroxide oxidation step. Following the oxidation, the low-density plastic particles were separated from heavier particles such as sand in a density separation step. In the last step, recovered microplastics were classified and counted under a reflected light microscope. Microplastics were categorized into primary and secondary microplastics based on the morphology of the microplastic. Figure 2 depicts the microplastic in four size categories of field's samples versus microplastic of a cosmetic product in the corresponding size class. QA/QC included processing of field blanks and matrix spike duplicates.

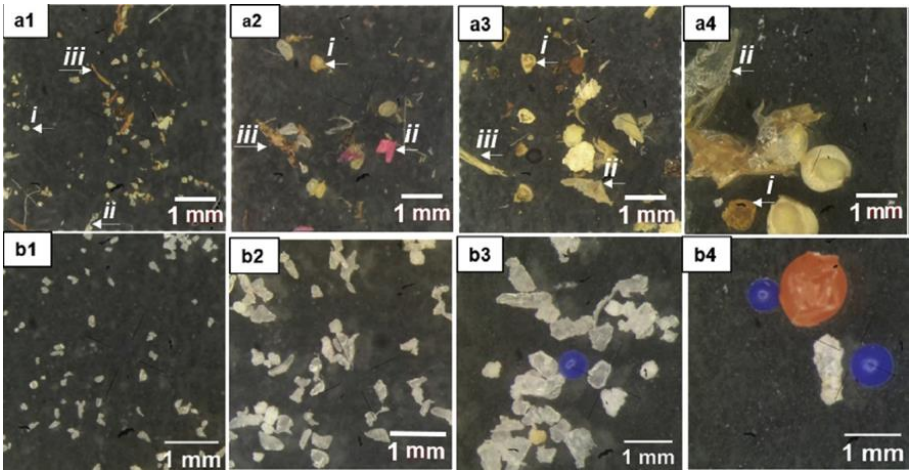

Figure 2- Microplastics in samples 1) 63-125 µm size category, 2) 125-250 µm size category, 3) 250-500 µm size category, and 4) 500-2000 µm counting step. First row (a) presenting the microplastics in field's samples, (b) presenting microplastics in a cosmetic product (Estahbanati and Fahrenfeld 2016)

For the biofilm studies, microplastic was extracted from personal care products and incubated in Raritan River water, untreated wastewater, or DI water (as a control). Microplastics were recovered with sieving, rinsed with sterile DI water, and DNA was extracted using a commercial kit. A second experiment was performed where microplastics were incubated in wastewater then treated with peracetic acid. The microplastics were separated from the wastewater and each sample was split for either total gene copy analysis or treated with propidium monoazide to reduce the signal from non-viable cells. qPCR was performed for BacHum (a human fecal indicator organism marker gene), *sul*1, and/or 16S rRNA genes.

Principal Findings and Significance

This research provided evidence for the spatial distribution of primary and secondary microplastic in the Raritan River. The results indicated that the concentration of primary microplastics increased downstream of several WWTPs (Figure 3). Secondary microplastics were the dominant type in all quantitative microplastic classes (i.e., >125µm, Figure 4). A moderate correlation was observed between microplastic concentration and the distance from Raritan Bay (Figure 5). The decrease from the downstream of a wastewater treatment plant to the upstream of the next WWTP provided insight about the fate and transport of microplastic in the Raritan River. These results indicate that dilution, settling, uptake by biota, or skimming are occurring in the river. Finally, the existence of microplastic at the background location indicated that there are other sources of microplastic pollution other than WWTPs in the River.

With respect to the capacity for microplastic to serve as a surface for biofilm growth, less than one week was sufficient time for a biofilm layer to be completely developed on the surface of microplastics (Fig. 6). Further incubation did not result in the more biofilm growth. Additionally, incubation of microplastic with wastewater resulted in biofilm containing marker genes for fecal indicator organisms and *sul*1 gene carrying organisms. This highlighted the importance of disinfection. Given the potential for microplastic to carry biofilm, viability based molecular methods were applied to microplastic and filtrate from wastewater influent to compare the efficacy of peracetic acid disinfection in the two matrices. Also, the disinfection was more effective on the filtrate samples compared to biofilm (Figure 7).

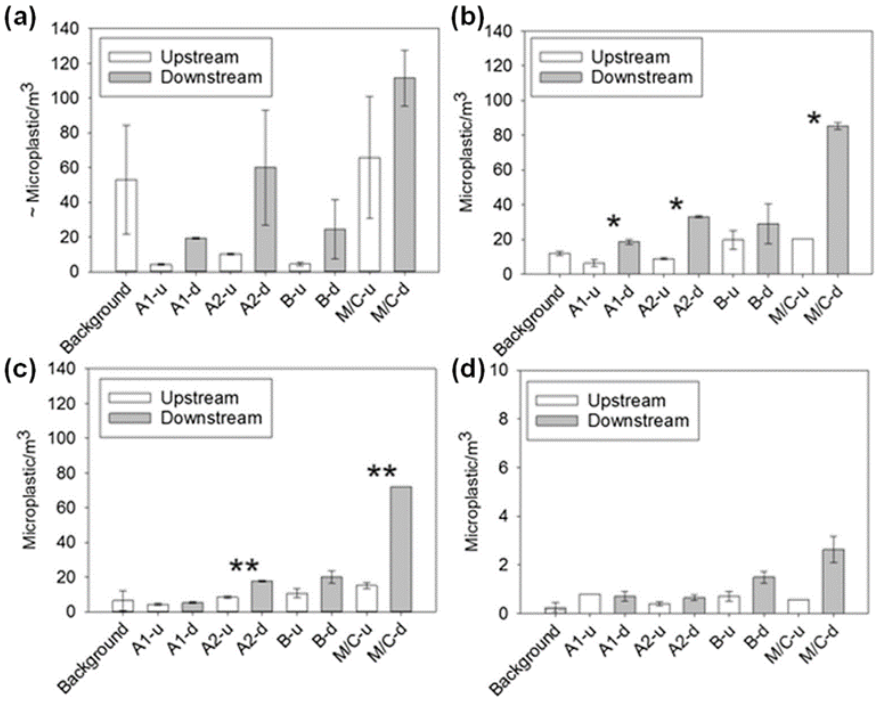

Figure 3-Microplastic concentration at sampling sites (Estahbanati and Fahrenfeld 2016)

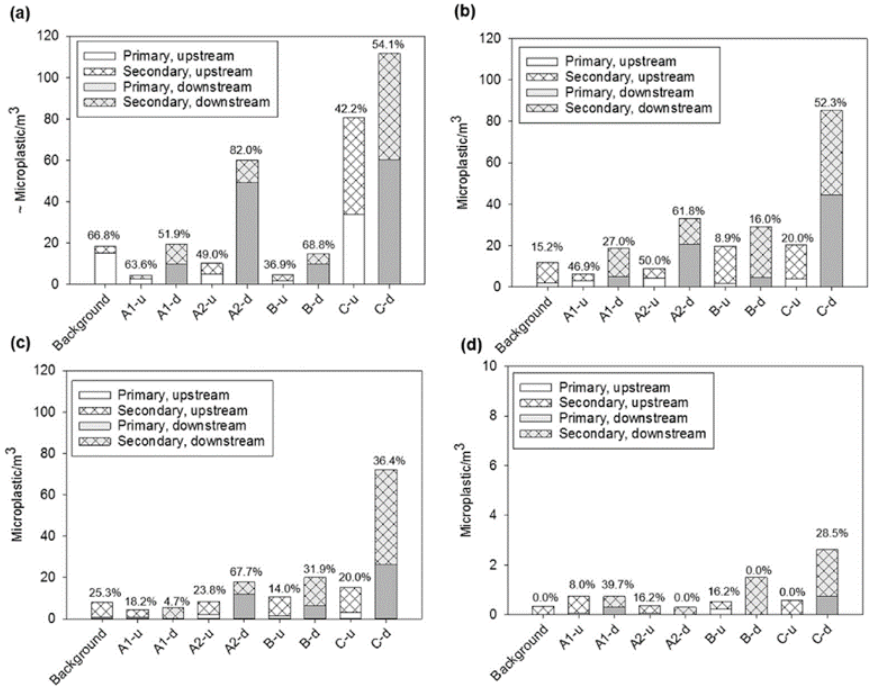

Figure 4-Distribution of microplastic in primary and secondary categories (Estahbanati and Fahrenfeld 2016)

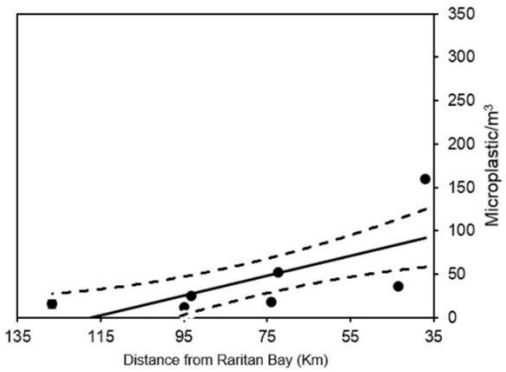

Figure 5-Correlation between quantitative microplastic concentration and distance from Raritan Bay (Estahbanati and Fahrenfeld 2016)

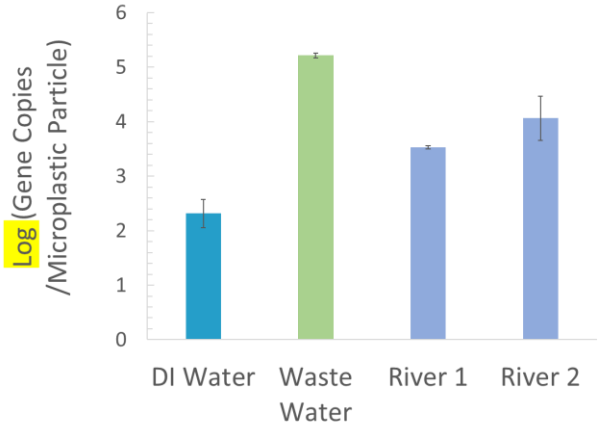

Fig. 6 *sul*1 gene copies per microplastic incubated for one week in wastewater influent, one (River 1) or two weeks in Raritan River water (River 2), and DI water (one week, control).

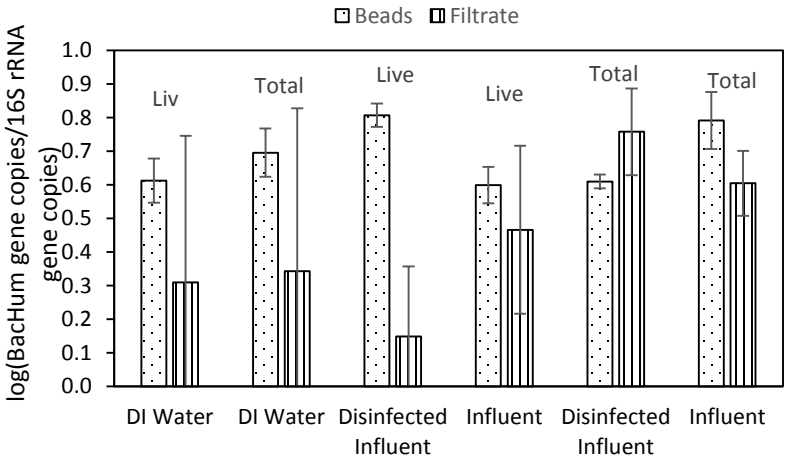

Figure 7-PAA disinfection of microplastic biofilm compared to filtrate.

To my knowledge, this research was the first report of the microplastic in small size classes in freshwater. Given the high specific surface area of small microplastic available to absorb hazardous organic contaminants, the abundance of smaller microplastic may pose a serious risk for the aquatic environment. This research also highlighted the existence and spatial distribution of microplastic in the Raritan River identifying that WWTP were a source of primary microplastic in select size classes. The biofilm studies indicate that microplastic are a surface for microbial growth and that these biofilm are more difficult to disinfect than planktonic cells. This indicates that attachment to microplastic may be a mechanism for fecal microbes to bypass disinfection.

Publications or Presentations

- Estahbanati, Shirin. 2016. Influence of Wastewater Treatment Plant Discharges on Microplastic Concentrations in Surface Water. "MS Dissertation", Civil and Environmental Engineering, Graduate School-New Brunswick, Rutgers, The State University of New Jersey, Piscataway, New Jersey, 39.

- Estahbanati, Shirin; Nicole, Fahrenfeld, 2016. "Influence of wastewater treatment plant discharges on microplastic concentrations in surface water." Chemosphere 162: 277-284.

- Parrish, Katie; Shirin Estahbanati: Nicole, Fahrenfeld. **Poster**. "Putting the Micro in Microplastics" Association of Environmental Engineering and Science Professors (AEESP). Ann Arbor, MI. June 2017.

- Parrish, Katie; Shirin Estahbanati: Nicole, Fahrenfeld. **Poster**. "Putting the Micro in Microplastics" 102nd Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 10, 2017.

- Estahbanati, Shirin; Nicole, Fahrenfeld, 2016, **Oral.** "Impact of Wastewater Treatment Plants on Microplastics in Freshwater Environment." 252nd National Meeting of the American Chemical Society. Philadelphia, PA. August 21-25, 2016.

- Estahbanati, Shirin; Nicole, Fahrenfeld, 2016. **Poster.** "Microplastics as Emerging Contaminants in NJ Surface Waters." 101st Annual New Jersey Water Environment Association Conference and Exhibition. Atlantic City, NJ. May 18, 2016.

Reference

Estahbanati, S. and N. Fahrenfeld (2016). "Influence of wastewater treatment plant discharges on microplastic concentrations in surface water." Chemosphere **162**: 277-284.

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).

NJWRRI 2016 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:
Ph. D. students: 1
Postdoctoral Associates:

(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, life stage-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 both larval and 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 of larvae and adults of different amphibian species, we will be able to determine if microbiomes tend to be host-specific, life stage-specific, or site-specific. Once cultured and isolated, we will grow antifungal cutaneous bacterial strains in both monoculture and polyculture to determine the relative efficacy of *Bd* inhibition in single versus multi-strain assemblages. Because microbial antifungal activity may be the result of interspecific interactions[3], this approach may provide a new perspective on the development of *Bd* probiotic therapies. 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 larval and 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 (not yet completed).

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:

Tadpoles and adult green frogs at all sites harbored cutaneous microbes that completely inhibited *Bd* growth *in vitro* (Figure 1). Green frog adults and tadpoles at Morin Pond, the near-neutral pH site, harbored the largest number of unique isolates (ANOVA, p=0.11; Table 1). At each site, tadpoles harbored a higher frequency of inhibitory isolates than adults, but the differences were not significant across sites (ANOVA, p=0.53; Table 1). There was no difference in the frequency of inhibitory isolates among sites (ANOVA, p=0.11; Table 1). At all sites, greater than 50% of the microbes isolated from the environment were also present on amphibian skin, suggesting that environmental transmission of bacteria to amphibian skin is occurring on some level (Table 2).

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 do not differ in the frequency of inhibitory isolates present on amphibian skin. Green frogs may harbor cutaneous inhibitory isolates regardless of differences in environmental factors such as pH. 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 and tadpoles 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 and life stage of green frog, although the differences are not significant when means compared by one-way ANOVA.

Site	Life stage	# Unique Isolates	% Isolates strongly inhibitory
Morin Pond (high pH)	Adult	40	28%
	Tadpole	40	29%
Assunpink (mid pH)	Adult	55	20%
	Tadpole	44	37.5%
Colliers Mills (low pH)	Adult	27	8%
	Tadpole	34	10%

Table 2. Three sites in New Jersey have environmental microbes with the ability to inhibit *Bd in vitro.* Sites were sampled twice during the year at the same time adult and tadpole green frogs were sampled. There is some variation between sampling events that may have been influenced by the microhabitat in which the sample was collected. The majority of environmental isolates were also present on amphibian skin, which suggests environmental transmission of microbes to amphibian skin is occurring at all sites.

Site	Sampling date	# isolates	% isolates inhibitory	% present on amphibian skin
Morin Pond (high pH)	5/13/16	6	33%	100%
	8/9/16	17	35%	64%
Assunpink (mid pH)	7/2/16	15	33%	67%
	8/19/16	16	50%	75%
Colliers Mills (low pH)	7/7/16	4	75%	50%
	10/5/16	10	20%	80%

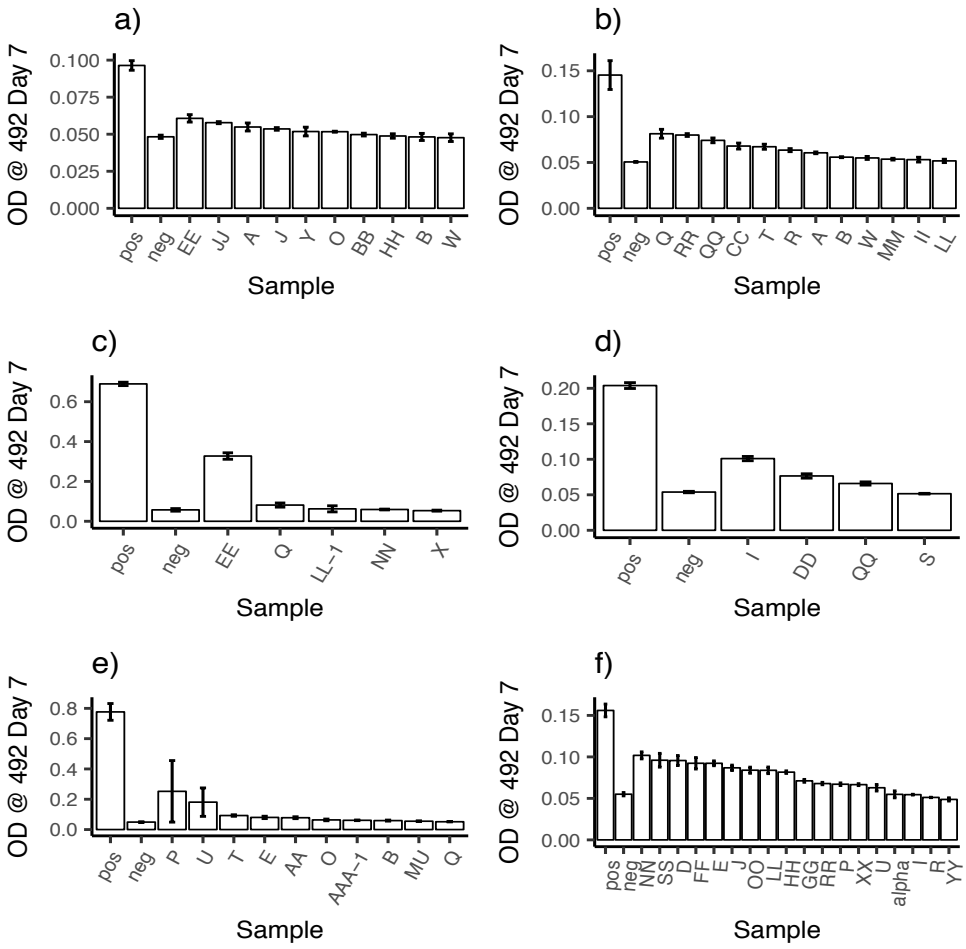

Figure 1. Inhibition assay results for green frog a) adults at Morin Pond, b) tadpoles at Morin Pond, c) adults at Colliers Mills, d) tadpoles at Colliers Mills, e) adults at Assunpink, and f) tadpoles at Assunpink. Letters on the x-axis represent unique isolates within each batch of sampling. Pos = positive control or *Bd* grown alone, neg = negative control or heat-killed *Bd,* and OD @ 492 = Optical density at 492nm, a proxy for *Bd* growth. Error bars denote +/- 1 S.D. 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 60% inhibition compared to the positive control on the final day of the assay (Day 7).

References:

1. Colón-Gaud, C., Whiles, M. & Kilham, S. Assessing ecological responses to catastrophic amphibian declines: Patterns of macroinvertebrate production and food web structure in upland Panamanian streams. *Limnol. Oceanogr.* **54,** 331–343 (2009).
2. Wake, D. B. & Vredenburg, V. T. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 11466–73 (2008).
3. Bletz, M. C. *et al.* Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. *Ecol. Lett.* **16,** 807–20 (2013).
4. Harris, R. N. *et al.* Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME J.* **3,** 818–24 (2009).
5. McKenzie, V. J., Bowers, R. M., Fierer, N., Knight, R. & Lauber, C. L. Co-habiting amphibian species harbor unique skin bacterial communities in wild populations. *ISME J.* **6,** 588–96 (2012).
6. Walke, J. B. *et al.* Amphibian skin may select for rare environmental microbes. *ISME J.* **8,** 2207–17 (2014).
7. Muletz, C. R., Myers, J. M., Domangue, R. J., Herrick, J. B. & Harris, R. N. Soil bioaugmentation with amphibian cutaneous bacteria protects amphibian hosts from infection by Batrachochytrium dendrobatidis. *Biol. Conserv.* **152,** 119–126 (2012).
8. Kueneman, J. G. *et al.* The amphibian skin-associated microbiome across species, space and life history stages. *Mol. Ecol.* **23,** 1238–50 (2014).
9. Bell, S. C., Alford, R. a., Garland, S., Padilla, G. & Thomas, A. D. Screening bacterial metabolites for inhibitory effects against Batrachochytrium dendrobatidis using a spectrophotometric assay. *Dis. Aquat. Organ.* **103,** 77–85 (2013).

(5) Publications or Presentations:

Oral presentations

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.

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.

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.

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).

Poster presentations

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.

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).

Molecular mechanisms by which mercury contamination increases antibiotic resistance in the environment

Basic Information

Title:	Molecular mechanisms by which mercury contamination increases antibiotic resistance in the environment
Project Number:	2016NJ382B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-006
Research Category:	Biological Sciences
Focus Categories:	Toxic Substances, Water Quality, Recreation
Descriptors:	None
Principal Investigators:	Nicole Lloyd, Tamar Barkay

Publications

1. Lloyd, NA, Janssen, SE, Reinfelder, JR, and T. Barkay. 2016. Co-selection of Mercury and Multiple Antibiotic Resistances in Bacteria Exposed to Mercury in the Fundulus heteroclitus Gut Microbiome. Current Microbiology. 73(6) 834-842.
2. Lloyd, Nicole A. and Tamar Barkay. Poster. Horizontal gene transfer of antibiotic resistance genes isolated from the gut of the mummichog fish, Fundulus heteroclitus. Federation of European Microbiological Societies. Valencia, Spain. July 2017.
3. Lloyd, Nicole A. and Tamar Barkay. Poster. Mercury exposure selects for antibiotic resistance in the gut of the mummichog (Fundulus heteroclitus). International Society for Microbial Ecology. Montreal, Canada. August 2016.

NJWRRI FY 2016 Annual Report

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Number of Students Supported

Undergraduates: 0
Masters students: 0
Ph. D. students: 1
Postdoctoral associates: 0

Notable Achievements

None to report.

Problem and Research Objectives

Mercury (Hg) contamination is a serious issue in many of New Jersey's waterways. Research shows that Hg contamination can influence antibiotic resistance in a phenomenon known as co-selection. Co-selection occurs when genes encoding Hg resistance and antibiotic resistance are found together on bacterial mobile genetic elements such as plasmids (1). These elements are freely shared among bacteria in close proximity. Co-selection has been demonstrated in many ecosystems including several types of fish (2), sphagnum bogs (3), and mice guts (4, 5).

With the NJWRRI grant, we studied the co-selection of Hg and antibiotic resistance genes in bacteria isolated from the gut of a benthic, forage feeder fish (Fundulus heteroclitis, aka the mummichog). Samples were obtained from a Hg-contaminated site Berry's Creek and a non-contaminated site, Great Bay, in Tuckerton, NJ. (For further information see (6)). We hypothesized that the mummichog gut may act as a conduit for the emergence of Hg and antibiotic resistant bacteria. To examine the phenomenon of co-selection, we looked at the

phenotypic and genotypic characteristics of three bacterial strains isolated from the gut of the mummichog fish.

Methodology

Isolation

Strains were isolated from mummichog gut ingesta collected from Berry's Creek and Great Bay in Tuckerton, NJ during the summer of 2014. Strains were isolated at room temperature (23ºC) on TSA plates containing either: kanamycin (50 µg/g), ampicillin (100 µg/g) or gentamycin (50 µg/g). Strains were isolated in pure culture and preserved with glycerol and kept at -80ºC.

Antibiograms

Antibiograms were carried out according to (7). Colonies were incubated at 37ºC for 24 h before interpretation. Since resistance profiles are not well established for environmental bacteria, the *Enterobacteriacea* table from (7) and (8) were used to interpret the results. Results are reported in Table 1.

DNA Extraction

Strains were grown overnight in LB liquid media with antibiotics amended. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Plasmids were extracted using the Machery-Nagel Plasmid DNA purification kit (Machery-Nagel).

Sequencing

Plasmids and genomic DNA were sequenced using the HiSeq 2500 system (Illumina).

Sequence Analysis

Raw reads were assembled into contigs using SPAdes (9). The number of contigs for each organism varied and is reported in Table 2. KOALA was used to determine antibiotic resistance proteins in the sequences (10). ANI was calculated for all three of the strains using OrthoANI (11).

MLSA

For strains BC20 and T9, multi-locus sequence analyses (MLSA) were performed to identify the most closely-related species. MLSA was performed on BC20 based on the methods described by (12).The following genes were used for the MLSA: *mreB*, *gapA*, *topA*, *recA*, *gyrB*, *rpoA*, and *atpA*. The genes were extracted from BC20, as well as ten other *Shewanella*

genomes available, concatenated, and aligned. A Neighbor-Joining Tree (Jukes-Cantor method, 1000 bootstrap replicates) was created in Geneious 10.0 (BioMatters). *Aeromonas hydrophilia* ATCC 7966 was used as the outgroup. The tree is reported below in Fig 1.

Principle Findings and Significance

The antibiotic resistance profiles of the three strains are reported in Table 1 below. Previous testing indicates all three strains are also resistant to ampicillin (a beta lactam drug) and trimethoprim (dihydrofolate reductase inhibitor).

Class	Antibiotic	Concentration in disc (µg)	BC20	T9	T21
Aminoglycosides	Tobramycin	10	S	I	S
	Netimicin	30	S	S	S
Carbapenems	Meropenem	10	R	S	S
	Imipenem	10	R	S	S
	Doripenem	10	R	S	S
Penicillins	Ticarcillin	75	R	R	R
	Ticarcillin-clavulanic acid	75 /10	R	R	R
	Piperacillin	75	R	R	R
	Piperacillin-tazobactam	75 /10	R	S	S

Table 1 – Antibiotic resistance profiles of the three strains

Properties of the genomes are reported in Tables 2 and 3. The genome sizes and GC content are typical for the respective genera (Table 2). Table 3 shows the closest available genomes for the three strains, based on the ANI and MLSA results.

Strain	Number of Contigs	Size	GC %
BC20	54	4.7Mb	48.0
T9	70	5.2Mb	45.0
T9 – Plasmid	1	120 kb	44.7
T21	77	4.9Mb	45.4

Table 2 – General properties of the genomes

Strain	Closest available genome	ANI
BC20*	*Shewanella* sp. ANA-3	94.04%
BC20*	*Shewanella* sp. MR-4	95.14%
T9	*Vibrio* sp. EX25	98.30%
T21	*Vibrio parahaemolyticus* FORC 018	98.19%

Table 3 – Genomes with the highest similarity

* Two ANI values are indicated for BC20 due to ambiguity.

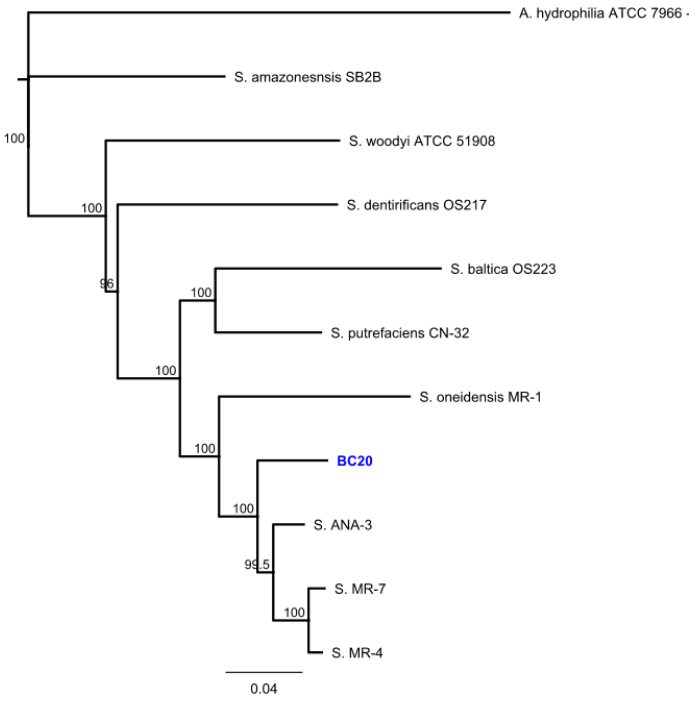

Figure 1 – Neighbor-Joining Consensus Tree of the MLSA of seven genes as described in (12). BC20 shows 94.096% similarity to S. ANA-3 and 92.469% to S. MR-4. Bootstrap values are shown (1000 reps).

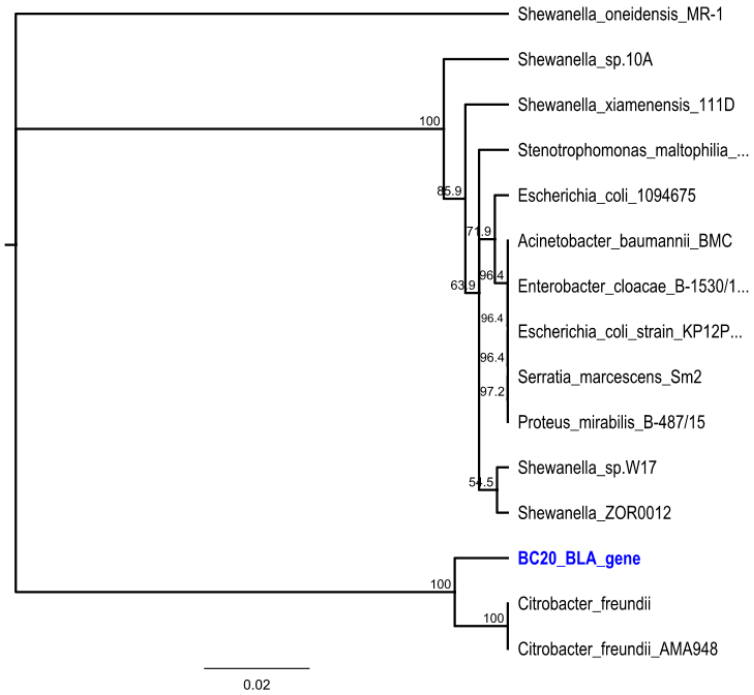

Figure 2 – Phylogeny of BC20 *bla*$_{OXA48}$ gene, compared with *bla*$_{OXA48}$genes from (13). UPGMA tree created in Geneious 10.0 (BioMatters). *bla*$_{OXA48}$ from BC20 shows 97.995% nucleotide similarity to the blaOXA48 genes from *Citrobacter*.

BC20 – Shewanella sp.

Shewanella is an under-studied genus in the Shewanellaceae family, of the gamma proteobacteria group. *Shewanella* are commonly associated with aquatic environments, including marine life, salt water, and fresh water. *Shewanella* have also been isolated in clinical settings and are increasingly being reported as human pathogens (14). A recent study describes the genome of a pneumonia-causing *Sheawnella* strain (15). The genus is considered a reservoir of AB resistance genes (14). There are approximately 59 genomes available in public databases, but only 23 have a species designation (14). As such, the taxonomic tree of *Shewanella* is lacking in specific species information. Some species of *Shewanella*, for example *S. onidiensis*, have been extensively studied for their unique metal metabolisms. However, the majority of Shewanella species are unnamed and hardly characterized. BC20 was isolated from the gut of a mummichog sampled from Berry's Creek.

Multi-locus sequence analysis (MLSA) shows BC20 is closely related to a *Shewanella* strain known as S. sp. ANA-3, a strain studied for its ability to respire arsenate (16). (Fig. 1). ANI analysis shows that BC20 is most closely related to S. sp MR-4. While MLSA analysis is more robust than ANI, the conflicting reports can be attributed to the lack of information available on specific *Shewanella* species.

Several genes of interest were identified in the genome of BC20. Carbapenemases are extended spectrum beta-lactamases (ESBLs), which confer bacterial resistance against the group of last-line therapeutics known as carbapenems. One such gene, bla_{OXA48}, was located in the genome of BC20. BC20 showed phenotypic resistance to various carbapenems (Table 1), which can be explained by the presence of the bla_{OXA48} gene. Bla_{OXA48} genes are thought to have originated in the pathogen *Klebsiella* (13) , but a phylogeny of BC20's bla_{OXA48} gene shows it is most similar to bla_{OXA48} from *Citrobacter* strains (Fig. 2). Interestingly, in the tree, BC20 groups separately from its *Shewanella* neighbors. This provides evidence that *Shewanella* are capable reservoirs of antibiotic resistance genes in the environment. Moreover, many genes for antibiotic and metal resistance were found including *mex* efflux pumps, RND efflux pumps, and *Bcr/CflA* drug resistance transporters. Further, genes involved in resistance to arsenic, a contaminant in the sediment and waters of Berry's Creek, were also located in the genome.

Vibrio group: T9 and T21

Vibrio, like its relative *Shewanella*, are also associated with aquatic environments. The phylogeny of the *Vibrio* genera is more resolved compared to that of *Shewanella*. Vibrio are known to be promiscuous, i.e. share and acquire DNA quite easily, and their genome has high plasticity, which is important in the study of antibiotic resistance genes in the environment (17).

T9 and T21 were both isolated from the gut of mummichogs sampled from Great Bay in Tuckerton, NJ. They were both identified as belonging to the genus *Vibrio*.

T21 is a strain of *V. parahaemolyticus*, showing 98% identity based on Average Nucleotide Identity (ANI) (Table 3). Genes of interest found in the genome of strain T21 include *merA*, the gene for mercury resistance as well as genes for various antibiotic resistances. Like in BC20, we see many genes for multi drug resistance transporters. Perhaps the most interesting genes are *oqxB*, and *qnr* genes, which confer resistance to quinolones, a group of synthetic antibiotics. Quinolones are of public health concern (18). Future studies on this organism include a full metal resistance profile as well as testing for resistance to quinolones.

T9 is most closely related to Vibrio sp. EX25, a strain isolated from deep sea hydrothermal vents (19). The proposed name for this strain is *V. antiquitous* EX25 (19). Interestingly, our strain, T9 contains a 100 kb mega plasmid that is currently being investigated. The plasmid is 63% similar to the megaplasmid pMBL128, isolated from *V. alginolytocus* (20). Antibiotic resistance genes found in the genome of T9 include resistance to chloramphenicol, quinolones, beta lactams, and other multidrug resistance pumps and proteins. T9 also harbors a mercury resistance gene.

In conclusion, we characterized phenotyptically and genomically three strains isolated from mummichogs in New Jersey waterways. The strains were identified as belonging to *Sheawanella* and *Vibrio* genera. Among the three strains, we have found many genes for antibiotic resistance, metal resistance, and mobile genetic elements. Transposases, insertion elements, and integrase genes were found in high numbers in all of the sequences (data not shown). All of the phenotypic antibiotic resistances observed can be corroborated with genes found in the genome. The metal resistances are currently under investigation. We have also observed the presence of antibiotic resistance genes of public concern including resistance genes for carbapenems and quinolones. We also found one megaplasmid in our genome sequences. Since plasmids are easily shared in the environment especially among members of

Vibrio, the plasmid, which may have antibiotic resistance genes, is one way the genes may be propagated in the environment. Our future work on these three strains includes full metal resistance profiles, testing of efflux pump inhibitors, and close examination of the genome annotations. Overall, the research thus far suggests that bacteria living in aquatic environments in New Jersey do act as reservoirs of clinically important antibiotic resistance genes, which raises concern for the health of our waterways.

Publications or Presentations
Articles in Refereed Scientific Journals:

Lloyd, NA, Janssen, SE, Reinfelder, JR, and T. Barkay. 2016. Co-selection of Mercury and Multiple Antibiotic Resistances in Bacteria Exposed to Mercury in the Fundulus heteroclitus Gut Microbiome. Current Microbiology. 73(6) 834-842.

Conference Presentations:

Lloyd, Nicole A. and Tamar Barkay. Poster. *Horizontal gene transfer of antibiotic resistance genes isolated from the gut of the mummichog fish, Fundulus heteroclitus.* Federation of European Microbiological Societies. Valencia, Spain. July 2017.

Lloyd, Nicole A. and Tamar Barkay. Poster. *Mercury exposure selects for antibiotic resistance in the gut of the mummichog (Fundulus heteroclitus).* International Society for Microbial Ecology. Montreal, Canada. August 2016.

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Novel algae based multifunctional biomaterials for the removal of heavy metals from water

Basic Information

Title:	Novel algae based multifunctional biomaterials for the removal of heavy metals from water
Project Number:	2016NJ383B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-010
Research Category:	Water Quality
Focus Categories:	Toxic Substances, Treatment, Methods
Descriptors:	None
Principal Investigators:	Megha Thakkar, Somenath Mitra

Publications

1. Thakkar M and Mitra S*, Removal of Selenium using nanostructured diatom composite. Submitted to Environmental Science: Water Research and Technology.
2. Megha Thakkar, Somenath Mitra, Nanostructures Diatom composites for removal of Water Pollutants like Fluoride, Arsenic and Selenium, New Jersey Water Environment Association Annual Conference, May 2016, 2015, 2014
3. Hua L, Guo L, Thakkar M. et. al (2016) Effects of Anodic Oxidation of a Substoichiometric Titanium Dioxide Reactive Electrochemical Membrane on Algal Cell Destabilization and Lipid Extraction . Bioresource Technology,203:112-7.

Novel Algae based multifunctional biomaterials for the removal of water pollutants.

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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.
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Masters' students: 0
Ph. D. students: 1
Postdoctoral Associates: 0

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(3) **Any Notable Achievements** (Awards, Recognition, etc.), or direct application of the research by Management Agencies, Nonprofits/NGOs, etc.
Second Place for poster presentation at **New Jersey Water Environment Association Annual Conference**, May 2016.

(4) **Project Summary**:
Include the following sections:

Problem and Research Objectives - Arsenic affects millions of people across the globe and is a priority for the World Health Organization (WHO) [1-4]. The sources of arsenic in ground water are primarily associated with oxidative weathering, but anthropogenic activities such as industrial effluents from metal processing and pesticides manufacturing also contribute to arsenic contamination [5]. The WHO guideline calls for a maximum drinking water arsenic concentration

of 10 ppb and the US-EPA plans to lower the standards to 5 ppb [6]. Arsenic can exist as arsenite (As III) and in oxidized conditions as arsenate (As V). Several studies have concluded that the former is more toxic [7] and is more mobile because it is less strongly adsorbed on most mineral surfaces [8]. Selenium is an important micronutrient for animals and humans but is toxic in excess [9] . Higher Se concentration can lower reproduction rates and increase birth defects [10]. In water, Selenium exists predominantly in inorganic forms selenite (SeO_3^{2-}, where Se is present as the Se^{4+} and selenate (SeO_4^{2-}) where selenium is present as the Se^{6+}[11]. The toxicity of selenium depends on its oxidation state and Se (IV) is considerably more toxic than Se (VI) [12]. Drinking water is a primary source for selenium exposure and the U.S. Environmental Protection Agency has set the maximum contaminant level in drinking water to be 0.05 mg/L. The objective of this project is to develop a Novel Algae based multifunctional biomaterials for the removal of contaminants from water.

Biomaterials were developed by immobilizing metal oxides on diatom surface and in pores of diatom frustules. It is well known that mixed metal oxides usually exhibit better sorption abilities than individual oxides in terms of higher capacities, pH tolerance and faster kinetics. Hydrous zirconium oxide is known to retain various oxo metal anions, especially those that can form weak conjugate acids. It is also known to be chemically stable, nontoxic and does not dissolve in water even at a wide range of pH (49). At the same time the iron oxides/hydroxides and have been gaining popularity in water treatment and are known to adsorb heavy metals. Therefore specific objective is to develop a bimetallic oxide composite by immobilizing iron and zirconium oxides on diatom frustules for effective removal of water pollutants.

Preparation of Diaton Bimetallic Composite (BMDC) :

Diatom Phaeodactylum tricornutum was cultured and maintained in artificial sea water Aquil using a diurnal Chamber with 12 hour day/night cycles at 19°C±1°C. 6L of diatom culture was flocculated with 6 g of $ZrOCl_2.8H_2O$ and 6 g of $FeCL_36H_2O$ purchased from Sigma Aldrich) at pH 9. One molar NaOH was used to adjust to pH 9.

After flocculation, the culture was conditioned overnight with shaking (150 rpm, 2880 VWR orbital shaker). The resulting Bimetallic diatom mixture was separated by gravitational settling and membrane filtration (< 5 psi, 0.2 µm PTFE filter), and was washed with 500 mL Milli-Q water. The resulting slurry was transferred to 50-mL centrifuge tube

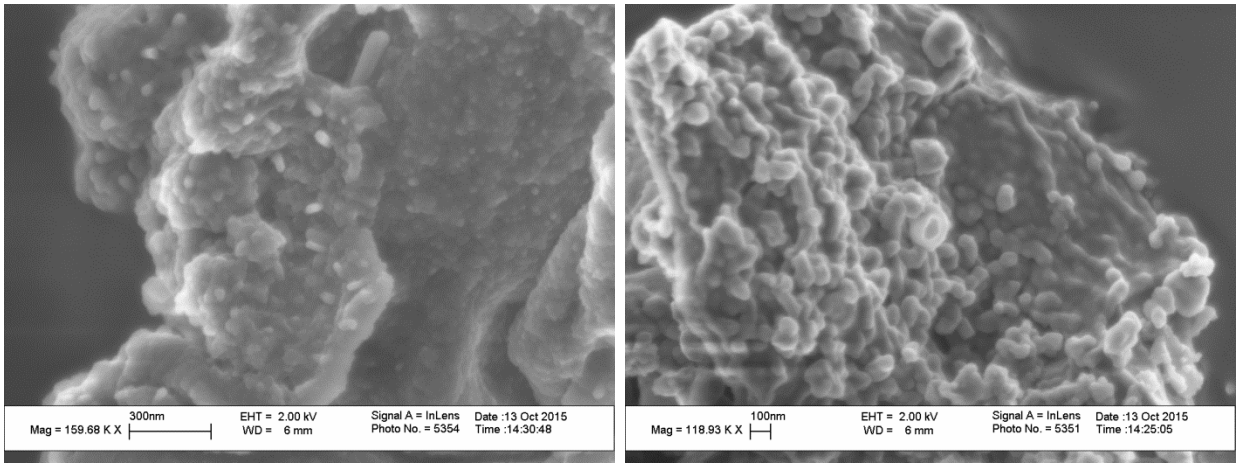
and thermally treated at 70°C in an oven for 6 h. Then, it was treated with 10 mL of concentrated H_2SO_4 and heated for 2 hrs at 200°C, vacuum filtered using 0.2µm filter, washed with Milli-Q water to neutral pH and then dried at 200°C in a vacuum oven.

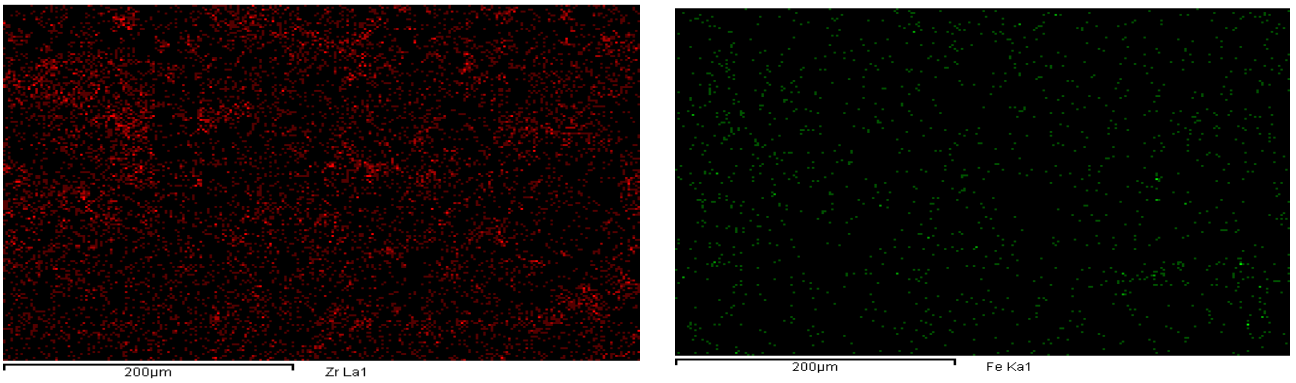
The kinetics of adsorption was performed as follows. For Arsenic, 100 ml of different concentrations of arsenite (61, 89,133, 193 and 299 ppb) and arsenate (68, 103, 151, 213 and 334 ppb) were contacted with 0.014 g of BMDC and placed in the shaker at 250 rpm. 5 mL aliquot was withdrawn at different time intervals, filtered using 0.2 µm membrane filter and residual arsenic in the media was quantified using Agilent 7500 ICP-MS. All standards were prepared from multi-element solution 2A, 10mg/L (Spex Certiprep) with addition of internal standard mix (Li6, Ge, Y, In, Tb, Bi). Multi-element instrument calibration standard 1 and 20 mg/L (Spex Certiprep) was used for the verification of calibration. For Selenium, a 50 millilitre of 5 mg/L of Se (IV) and (VI) solutions were contacted with 0.010 g and 0.025 g of BMDC in polycarbonate bottles and the samples were collected at 5, 15 and 30 minutes followed by 1, 3, 6 and 24 hours. 5 mL aliquot was withdrawn at different time intervals, filtered using 0.2 µm membrane filter and residual Se in the media was quantified using Agilent 7500 ICP-MS.

Principal Findings and Significance –

The bimetallic oxide-diatom composite (BMDC) was characterized using transmission electron (HRTEM), scanning electron microscope equipped with Energy dispersive X-ray Spectrometer (SEM, LEO 1530 VP)

The presence of Zr and Fe particles on diatom surface was studied at using SEM. Fig. 1a shows SEM of the original diatom, Fig. 1b is bimetallic composite and Figs. 1c and 1d are mapping of Zr and Fe from BMDC. Similarly, Fig. 1e and Fig. 1f show TEM image of the diatom and BMDC. SEM and TEM images showed that the original diatom was reduced to porous nano biosilica. EDX analysis using SEM confirmed the presence of Zr (89.87%), Fe (8.75%) in BMDC.





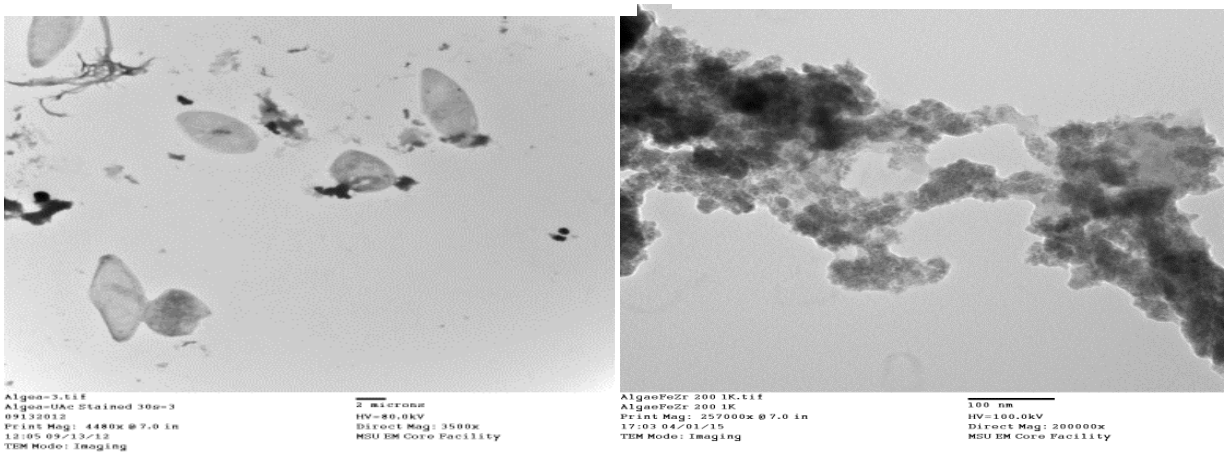


Figure 1: (a) SEM of diatom, (b) diatom bimetallic oxide composite; (c, d) represents mapping for Zr and Fe immobilized on diatom surface. TEM of (e) diatom and (f) diatom bimetallic oxide composite

Arsenic was not adsorbed on the pure diatom, but BMDC was effective in removing arsenic from water. 0.014 g of adsorbent was contacted with 100 ml arsenic containing water. Arsenite concentrations of 61, 89, 133, 193, 299 ppb and arsenate concentrations of 64, 103, 150, 213, 334 ppb were studied. Adsorption increased with contact time. Highest adsorption was seen in the first 45 min of exposure (Figure 2). The slower adsorption was due to the decrease in concentration gradient between the bulk solution and the sorbent surface, which decelerated the transport of the arsenic species to the BMDC.

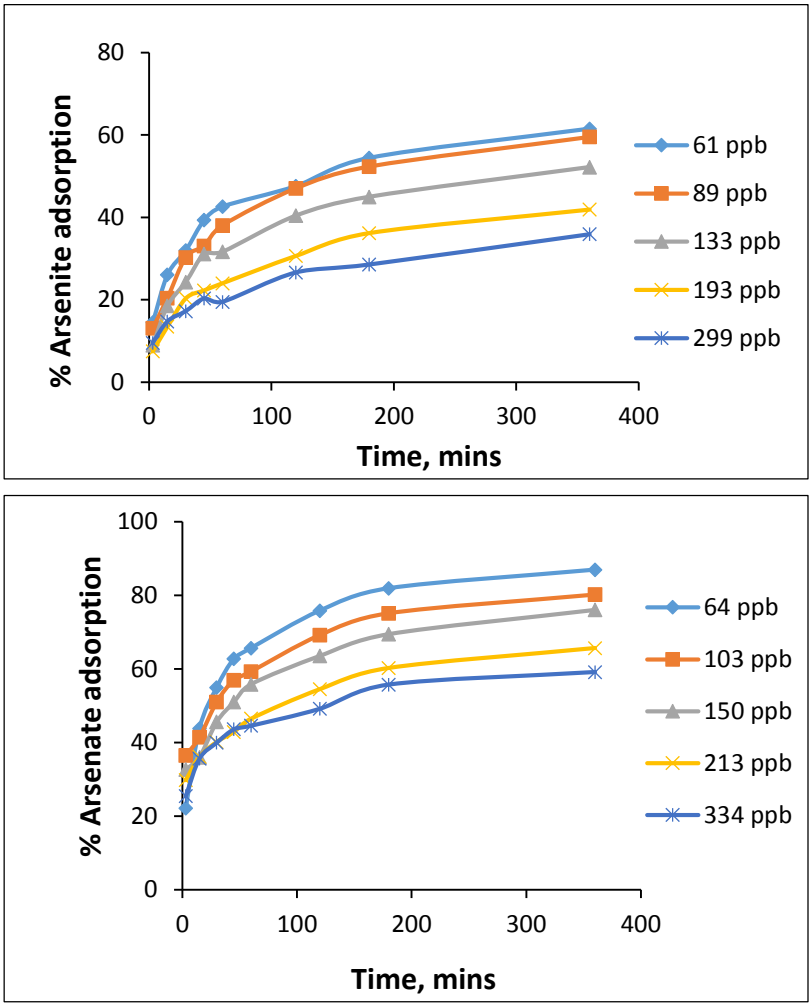


Figure 2: Percent adsorption of arsenite (a) and arsenate (b) as a function of time, adsorbent dosage 0.014 g in 50 mL of solution.

It was observed that no selenium was adsorbed on the pure diatom, but the BMDC was effective in removing selenium from water. Selenite and Selenate uptake was studied as a function of time and is presented in Fig. 3. Selenium sorption increased as a function of contact time. Selenium sorption increased as a function of contact time. 0.010 g of bimetallic composite showed Se (IV) uptake of 50 and 95% at 15 mins and 3 hrs respectively, whereas 0.025 g of bimetallic composite showed removal only 40% Se (VI) after 24hrs of contact. This shows that bimetallic diatom composite shows better Se (IV) removal efficiency compared with Se (VI).

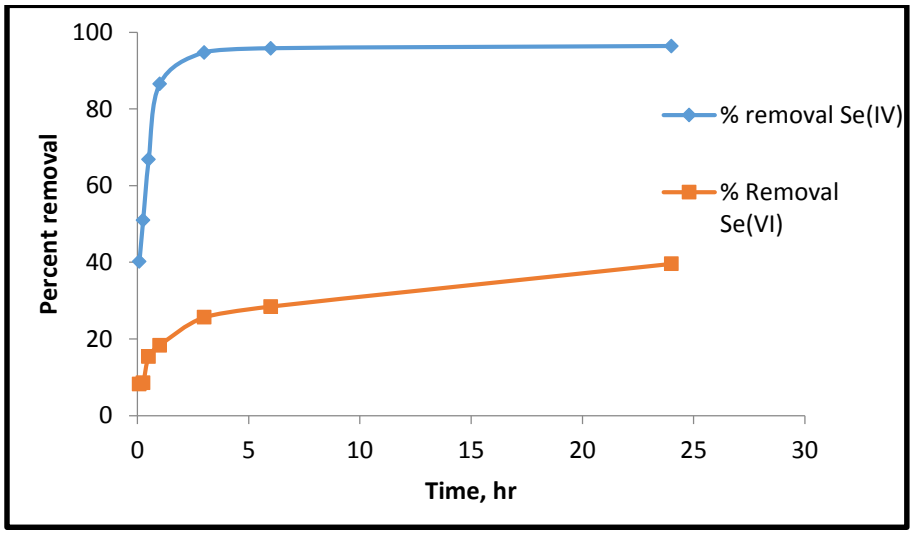


Figure 3: Percent removal of Se (IV) and Se (VI) as a function of time.

Diatom offers unique architecture with excellent mechanical strength. This highlights the potential of diatom as the host for immobilizing nanomaterials to form composite. A bimetallic composite was successfully synthesized for removal of As and Se from water.

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(5) **Publications or Presentations**:

1. **Thakkar M** and Mitra S*, Removal of Selenium using nanostructured diatom composite. Submitted to Environmental Science: Water Research and Technology.

Presentations

Megha Thakkar, Somenath Mitra, Nanostructures Diatom composites for removal of Water Pollutants like Fluoride, Arsenic and Selenium, **New Jersey Water Environment Association Annual Conference**, May 2016, 2015, 2014

Other Publications

1. Hua L, Guo L, **Thakkar M.** et. al (2016) Effects of Anodic Oxidation of a Substoichiometric Titanium Dioxide Reactive Electrochemical Membrane on Algal Cell Destabilization and Lipid Extraction . *Bioresource Technology,203:112-7.*
2. **Thakkar M**, Wu Z, Wei L, Mitra S* (2015) Defluoridation using Diatom-ZrO_2 composite from Algal Biomass. *Journal of Colloidal and Interface Science,450: 239-245*.
3. Randhawa V, **Thakkar M**, Wei L*. (2014). Effect of algal biomass on its inactivation by hydrogen peroxide: culture study and empirical modeling. *Journal of Applied Phycology*, 26:349-355.
4. Randhawa V, **Thakkar M**, Wei L* (*2013*). Effect of algal growth phase on *Aureococcus anophagefferens* susceptibility to hydrogen peroxide. *Aquatic Toxicology 142-143: 230-238*
5. **Thakkar M,** Randhawa V, Wei L* (2013) Comparative responses of two species of marine phytoplankton to metolachlor exposure. *Aquatic Toxicology 126:196-206*
6. Randhawa V, **Thakkar M**, Wei L* (*2012*) Applicability of hydrogen peroxide in brown tide control - culture and microcosm studies. *Plos One* 7(10): e47844
7. Wei L*, **Thakkar M**, Chen Y, Ntim S.A, Mitra S, Zhang X (2010) Cytotoxicity effects of water dispersible oxidized multiwalled carbon nanotubes to marine alga, Dunaliella tertiolecta. *Aquatic toxicology 100: 194-20.*

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Toward a greener water reuse technology with ferrate(VI) - phosphorus removal and recovery

Basic Information

Title:	Toward a greener water reuse technology with ferrate(VI) - phosphorus removal and recovery
Project Number:	2016NJ384B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NJ-011
Research Category:	Engineering
Focus Categories:	Nutrients, Wastewater, Water Supply
Descriptors:	None
Principal Investigators:	Lei Zheng, Yang Deng

Publications

1. A manuscript entitled “Performance of Ferrate(VI) for the Removal of Phosphorus from Secondary Effluent” will be submitted to Journal of Hazardous Materials in the June of 2017 (by invitation)
2. A manuscript entitled “ Ferrate(VI) for Chemically Enhanced Primary Treatment of Municipal Wastewater” will be submitted to Water Research (in preparation).
3. Zheng, L., N. Li, Y. Deng (2017) Ferrate(VI) for Chemically Enhanced Primary Treatment of Municipal Wastewater in Northeast Graduate Student Water Symposium (University of Massachusetts-Amherst, during September 8-10, 2017.
4. The project was primarily used to support the dissertation research of Lei Zheng (the Student Awardee). Part of data was integrated into Nanzhu Li’s dissertation. Li obtained her PhD in Environmental Management in 2017. Li, Nanzhu, 2017, Ferrate as a New Treatment Chemical for Removal of Effluent Organic Matter (EfOM) and Emerging Micro-pollutants in Treated Municipal Wastewater for Water Reuse, Ph.D. Dissertation, Department of Earth and Environmental Studies, Montclair State University, Montclair, New Jersey.

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(2) Numbers of Students Supported:

Undergraduates: 0

Masters' students: 0

Ph. D. student: 2

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

4.1 Problem

Phosphorus (P) pollution in NJ & P removal and recovery. Municipal wastewater typically contains 5.0-20.0 mg/L total phosphorus (TP), of which a majority (4.0-15.0 mg/L) is inorganic (orthophosphate and polyphosphate) and the rest in its organic form[1]. Unfortunately, secondary treatment

in traditional wastewater treatment plants only removes 1.0 – 2.0 mg/L TP. Consequently, a large excess of P remains in secondary effluent subsequently discharged into natural receiving water bodies. As a primary nutrient causing noxious harmful algal blooming in surface water, P is recognized as a major wastewater-derived contaminant. Algal-induced eutrophication represents a ubiquitous water quality issue across this country. Specifically, in the mid-Atlantic region, four coastal waters (i.e., Hudson River/Raritan Bay, Barnegat Bay, NJ Inland Bays, and Delaware Bays) are under a moderate – high eutrophic state[2]. For NJ fresh waters, NJ Department of Environmental Protection (NJDEP) set 0.05 and 0.10 mg/L as the TP numerical criteria for lakes and streams, separately. NJDEP has been fighting with the TP present in the effluent of WWTPs, an essential source causing NJ water algal blooming. For instance, NJDEP required 42 WWTPs within the Passaic River Basin to reduce TP discharges by 83% [3]. The current core strategy to remove P from wastewater involves the incorporation of P into total suspended solids (TSS) through chemical precipitation or enhanced biological phosphorus removal (EBPR). Despite of a water pollutant, P is a non-renewable resource itself in the world, particularly important for global food production. In 2000, 19.7 Mt of P was mined as phosphate rock, of which 15.3 Mt was used to produce fertilizers[4]. However, the P production and use remain inefficient, while the P demand is linearly increased, with very few recycling routes. Therefore, recovery of P from secondary sources gains special attention. Municipal wastewater is such a secondary source, given that ca. 1.3 Mt of P is globally treated in WWTPs[4]. Different P recovery options from wastewater are developed, but have limitations. Agricultural application of biosolids is restricted by a high transport cost and a low P purity. The techniques for struvite production require the use of EBPR that is not widely employed in practices, apart from its limited P recovery potential. And the recovery of P from biosolid ash relies heavily upon expensive incineration infrastructure. Consequently, the development of new wastewater P recovery technologies is highly demanded. Ferrate (VI) for water reuse and P removal. Water reuse is a strategically sound approach to meet the current and future water demand, particularly for arid, water-stressed, or highly urbanized areas. In NJ, drought and population density constantly put a significant stress on the state's water resource. In the Water Supply Action Plan 2003-04, NJDEP acknowledges that "the availability of fresh water is a limiting factor in the potential development and redevelopment of the State." WWTP effluent represents a stable non-seasonal reclaimed water source, generally meeting 87 of the 93 numerical primary and secondary drinking water standards without further treatment[5]. Every day, the U.S. population generates 121 million m3 municipal wastewater, of which one-third could be reused. However, only 7 - 8% of the water is now reused, leaving a tremendous potential for expanding the use of reclaimed water in the future.

Ferrate (VI), i.e. the oxyanion FeO_4^{2-} containing iron in + 6 oxidation state, is an emerging, green treatment agent[6-8]. It has been preferentially studied for water reuse because of simultaneous removal of a broad range of pollutants (e.g. emerging micro-pollutants and TP) through multiple reaction mechanisms (i.e., chemical oxidation, coagulation, precipitation and disinfection), little production of unwanted disinfection byproducts, and the decreased costs due to advances in ferrate(VI) manufacture. Recently, it has been selected at a New Orleans WWTP to produce reclaimed water for wetland restoration, due to its outperformance of chlorine, ozone and UV irradiation based on a 7-yr study [9]. Although the ability of ferrate (VI) for water treatment was early demonstrated, ferrate (VI) chemistry remains undeveloped. It is traditionally believed that Fe (III) from Fe (VI) reduction can precipitate P in a similar fashion with which ferric salts work. However, Lee et al.[10] found that ferrate (VI) performed better than Fe (III) or Fe (II) in terms of the P removal from secondary effluent, suggesting that various iron sources remove P with different mechanisms. The limited knowledge in ferrate (VI) reaction with P disables optimal ferrate (VI) application for water reuse.

Currently, chemical precipitation using Fe or Al slats is the most popular method to remove P from wastewater. Compared with Al salts, Fe salts are usually preferred due to a lower cost. In future, Fe would play an more essential role in the next-generation WWTPs that often apply A-B processes, i.e. a high loaded biological treatment (A stage) followed by a bio-oxidation or B stage for N recovery[11]. In the

A stage, Fe addition is the cheapest option for reduction of colloidal and particulate COD and elimination of P. Most of the treatment techniques in the future WWTPs are being used or tested at the pilot scale, but the ONLY missing process is economically feasible P recovery from iron-phosphate (FePs) sludge. Traditionally, the presence of Fe is perceived as negative for P recovery. However, P is commonly mobilized from various FePs in nature. This disparity indicates our poor understanding of the Fe and P chemistry. In a very recent critical review in *Environmental Science & Technology*, Wilfert et al. [11] pointed out that advances in Fe-P chemistry would provide basis for efficient recovery P from FePs for future energy and resource recovery WWTPs, and also suggested future research approaches.

4.2 Research Objectives:

The primary **purpose** of this proposed project is 1) to advance fundamental understanding of ferrate (VI) removal of P from wastewater, and 2) to evaluate the technical feasibility of P recovery from Fe- sludge produced from ferrate (VI) oxidation, with an emphasis for water reuse. The **central hypothesis** is 1) that ferrate (VI) can remove inorganic P in wastewater, in addition to organic P subsequent to Fe (VI) oxidative destruction of P-containing organic compounds, through chemical precipitation and complexation, thus being advantageous over Fe (III) salt- induced precipitation only for removal of inorganic P; and 2) that P in certain FeP precipitates produced from ferrate (VI) treatment is extractable, thereby providing a potential approach for P recovery. To test the hypothesis, three specific objectives will be pursued:

- To determine stoichiometric and kinetic information on ferrate(VI) removal of P in distilled water and secondary effluent under different conditions;
- To characterize Fe(VI)-induced FeP precipitates and determinate their speciation;
- To evaluate P release from Fe(VI)- induced FeP precipitates under different conditions.

4.3 Methodology

4.3.1 P removal from biologically treated municipal wastewater (secondary effluent)

Secondary effluent sample was collected from local wastewater treatment plant located in Verona New Jersey. Water sample was stored in a cold room at the Montclair State University Environmental Remediation Lab at 4 ˚C until use. The experiments were completed within three days after the sample arrival in order to minimize any significant change in water quality.

The experiments were carried out using a regular jar-test procedure in duplicates. Water samples were moved out from the cold room. Experiments began after the water temperature increased to the room one. The treatment tests were performed in 500 ml beakers containing 400 ml secondary effluent. Initial pH was adjusted to a desired level (6.5 or 7.5). The treatment was initiated after the addition of an appropriate mass of solid potassium ferrate (VI) (Sigma, >98% purity). Ferrate (VI) concentrations varied at 1, 3, 5, 7 and 9 mg/l as Fe. The treatment process included a 1-min rapid mixing (150 rpm), a 1.5-hour slow mixing (30 rpm) and a 2-hour settlement (no mixing). Upon completion, water samples were collected at the center of the supernatant for analysis of phosphorus concentration in the treated wastewater. The identical experiments were performed with ferric chloride as a control group.

4.3.2 P removal from raw municipal wastewater

Raw municipal wastewater was collected from two local wastewater treatment plants located in Verona NJ, and Elizabeth NJ respectively. Water sample was stored in MSU's cold room at 4 ˚C until use. The experiments were performed using the aforementioned procedure with a few modifications. Firstly, 1000 ml beakers were used as the reactors with 800 ml raw wastewater. Secondly, more water

quality parameters were analyzed, including Total Suspended Solid (TSS), Biological Oxygen Demand (BOD_5), Total Nitrogen (TN), Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), Organic Phosphorus (OP), Poly Phosphorus (PP) and Reactive Phosphorus (RP). Concentrations of different P species were analyzed in particulate and dissolved forms. Here particulates are defined as the matters that cannot pass through 0.45 μm-pore size microfiltration fitters. Control tests were carried out with ferric chloride under identical conditions.

4.3.3 Characterization of Fe-P products and evaluation of P release

The produced Fe(VI)-induced particles were analyzed with different analytical techniques, including TEM analysis, size distribution (Malvern Nano Zetasizer), and zeta potential (Malvern Nano Zetasizer). Amorphous and crystalline Fe were also determined using the citrate-ascorbate extraction method for the iron precipitates from Fe(VI) treatment of phosphate in distilled water.

5. Results and Findings

5.1 Water Sample Quality Information

Water quality of secondary effluent and raw wastewater were summarized in **Table 1**.

Table 1. Water Quality Parameters of Secondary Effluent and Raw Municipal Wastewater

Parameters	Secondary Effluent (Verona, NJ)	Raw Wastewater (Verona, NJ)	Raw Wastewater (Elizabeth, NJ)
pH	7.28	7.49	7.38
UV_{254} (cm^{-1})	0.120	0.361	0.566
TOC (mg/l)	9.49	23.28	56.46
BOD_5 (mg/l)	N/A	117	255
TP (mg/l as PO_4^{3-})	10.3	11.1	13.7

5.2 Fe(VI) Removal of P from Secondary Effluent

Fe(VI) and Fe(III) removals of TP from the secondary effluent at different doses and pH are shown in **Figure 1** and **2**, respectively. As seen from **Figure 1**, for either pH, the TP removal exhibited a linear correlation with the increasing Fe(VI) dose. At any specific Fe(VI) dose, the TP removal at pH 7.5 was slightly better than that at pH 6.5. The finding was likely associated with the different Fe(III) formation rates and the formation of different Fe hydroxides/Fe-P minerals at different pH. Any significant difference was not observed between Fe(VI) and Fe(III) treatments. This is different from the result from other literature that reported a better TP removal with Fe(VI). Moreover, the two different pH levels did not affect the TP removals at the different Fe(III) doses in this study.

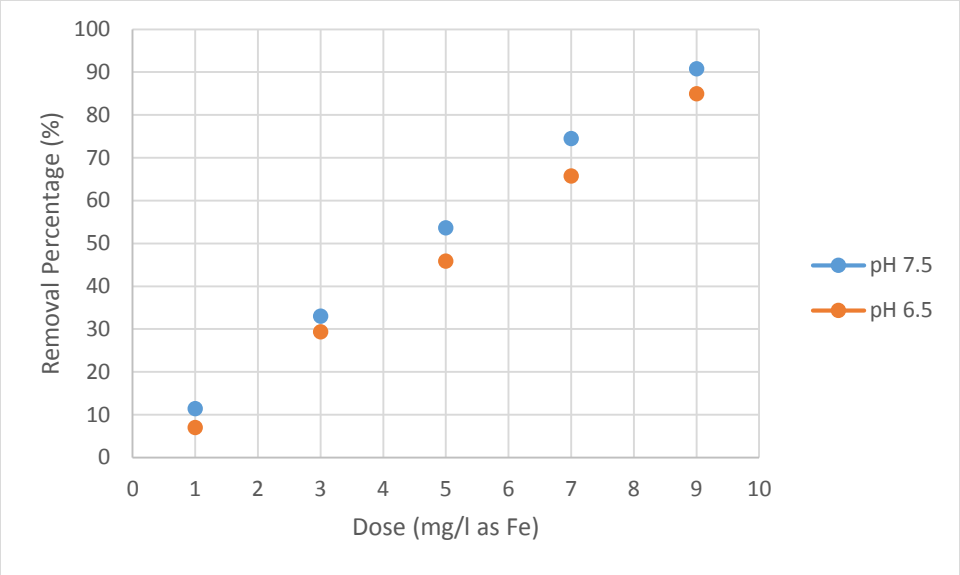


Figure 1. Fe(VI) Removal of TP from Secondary Effluent at Different Doses and pH

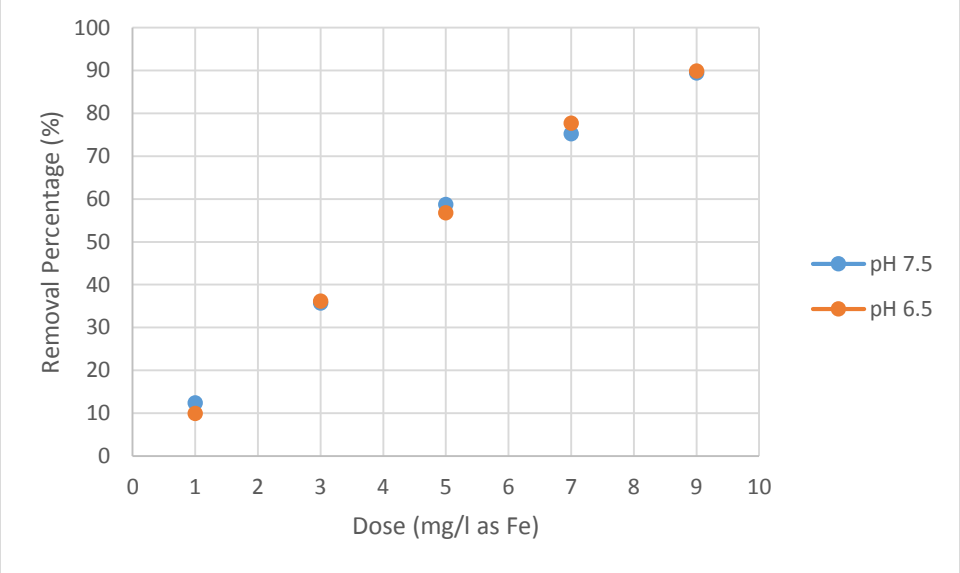


Figure 2. Fe(III) Removal of TP from Secondary Effluent at Different Doses and pH

5.3 Performance of Fe(VI) Treatment of Raw Wastewater

Fe(VI) or Fe(III) removals of TSS from Verona and Elizabeth WWTP wastewaters are shown in **Figure 3** and **4**, respectively. For the Verona raw wastewater at pH 6, TSS was increased with the increasing Fe(VI) from 0 to 9 mg/l, but gradually deceased with the increase in the Fe(III) dose. This finding suggested a poor settling property of Fe(VI)-induced particles at a weakly acidic condition. However, when the pH was increased to 7.5, TSS decreased with the increasing iron dose, regardless of the iron source. However, at any specific dose, a better TSS was achieved with Fe(III).

BOD_5 after Fe(VI) or Fe(III) treatment of Verona and Elizabeth raw wastewaters are shown in **Figure 5** and **6**, respectively. And COD after Fe(VI) or Fe(III) treatment of Verona and Elizabeth raw wastewaters are shown in **Figure 7** and **8**, respectively. Of interest, Fe(III) provided a better removal in both BOD_5 and COD for the two wastewater samples at identical experimental conditions. It should be noted that total BOD_5 and COD, rather than dissolved BOD_5 and COD, were measured in this study. Therefore, the findings are likely ascribed to the poor removal of particulate BOD_5 and COD in the Fe(VI) treatment than that in the Fe(III) treatment.

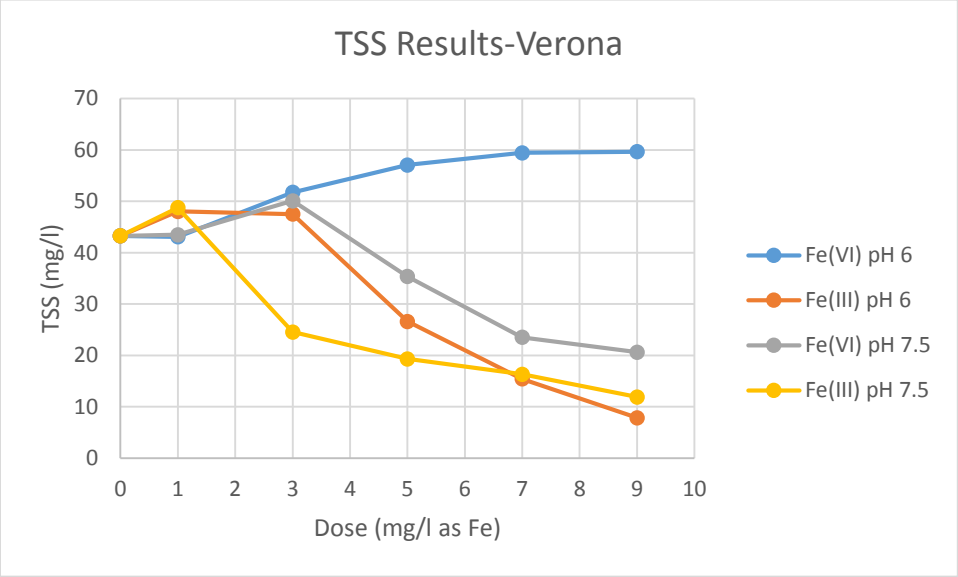


Figure 3. Fe(VI) or Fe(III) Removals of TSS from Raw Wastewater (Verona, NJ)

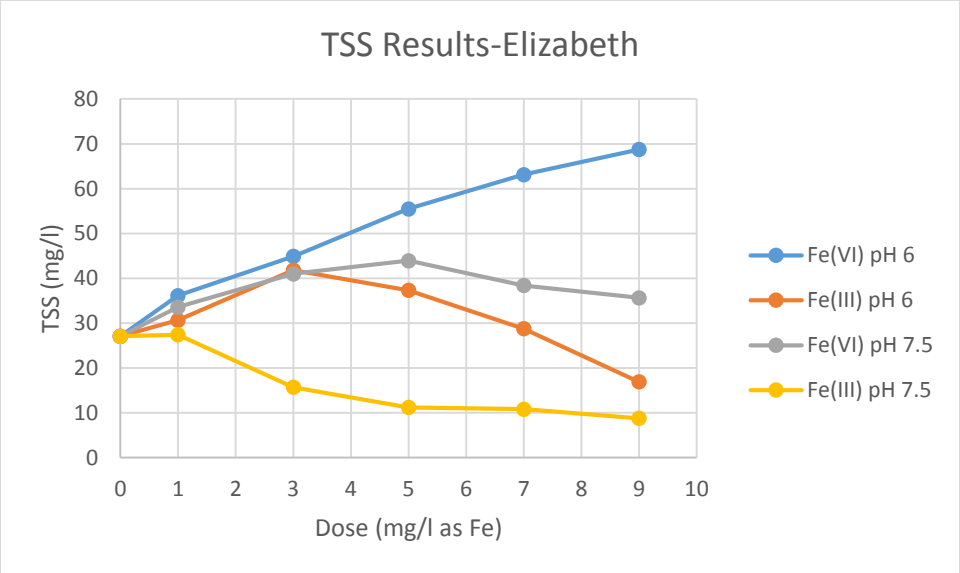


Figure 4. Fe(VI) or Fe(III) Removals of TSS from Raw Wastewater (Elizabeth, NJ)

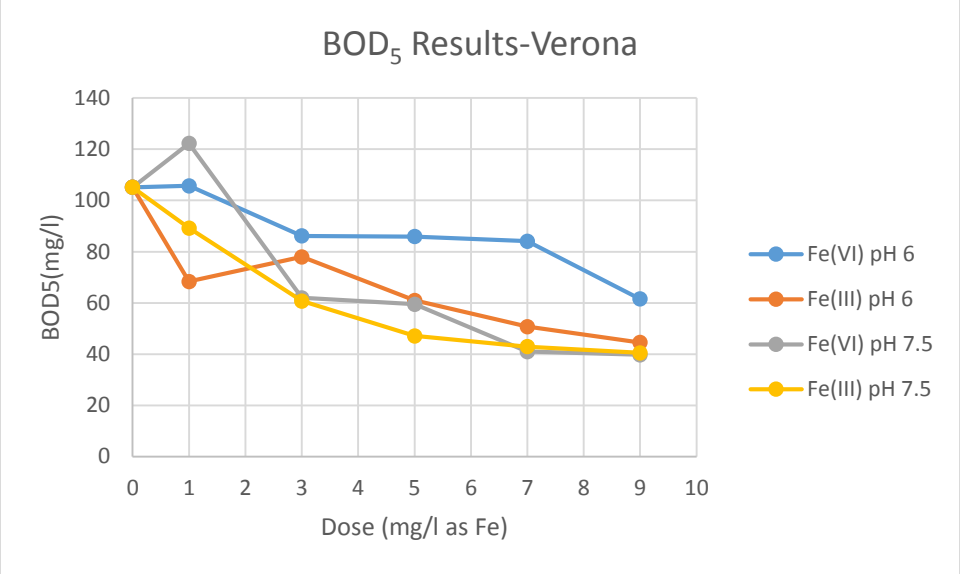


Figure 5. BOD_5 after Fe(VI) or Fe(III) Treatment of Raw Wastewater (Verona, NJ)

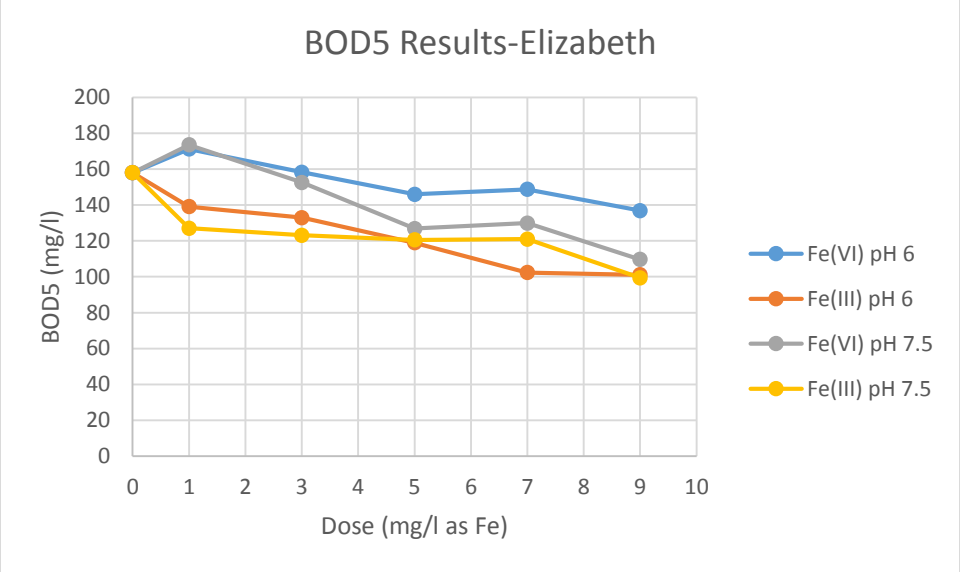


Figure 6. BOD_5 after Fe(VI) or Fe(III) Treatment of Raw Wastewater (Elizabeth, NJ)

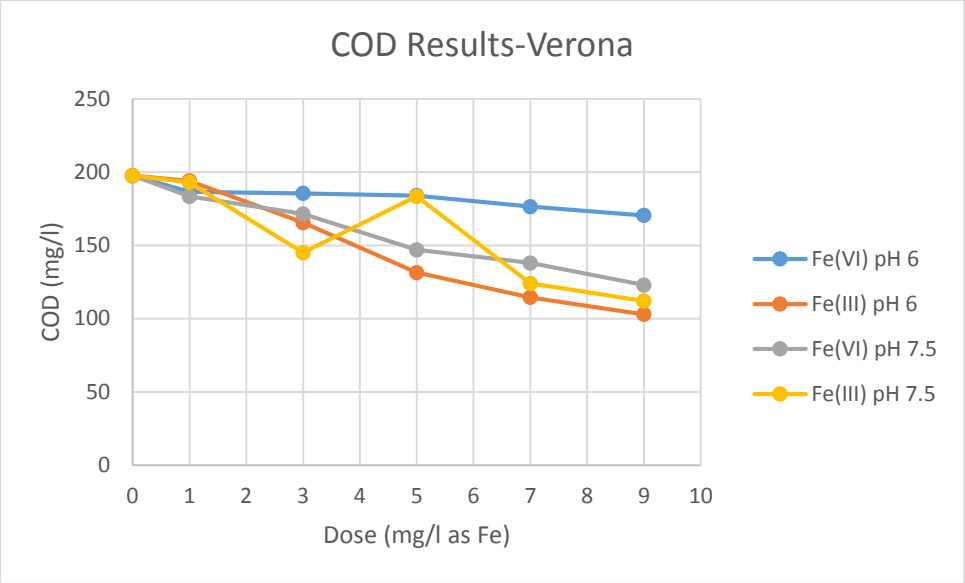


Figure 7. COD after Fe(VI) or Fe(III) Treatment of Raw Wastewater (Verona, NJ)

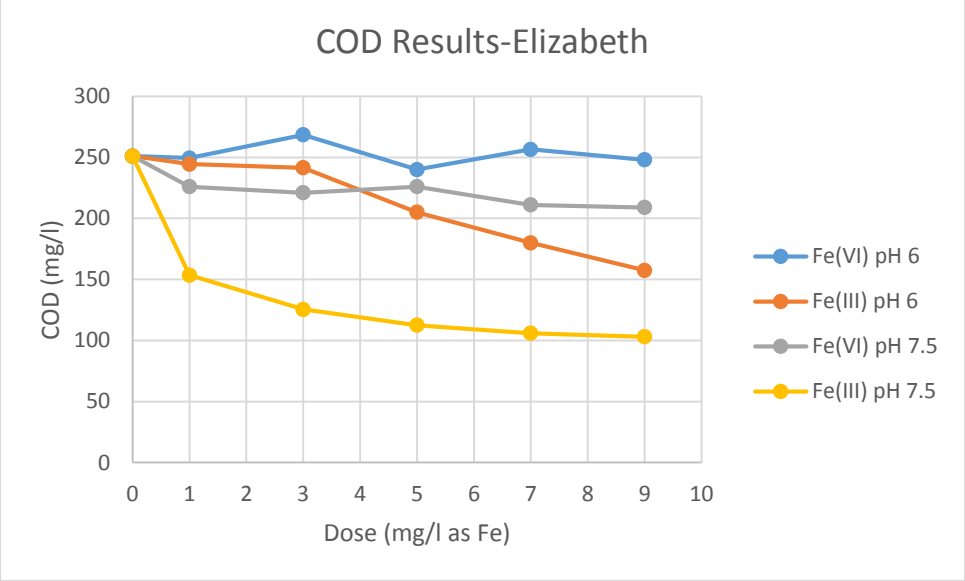


Figure 8. COD after Fe(VI) or Fe(III) Treatment of Raw Wastewater (Elizabeth, NJ)

Residual TP and dissolved P (DP) in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Verona, NJ) are shown in **Figure 9** and **10**, respectively. And residual TP and dissolved P (DP) in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Elizabeth, NJ) are shown in **Figure 11** and **12**, respectively. Important findings could be observed from these results. Firstly, P in untreated raw water primarily existed in a dissolved state. Secondly, Fe(III) achieved a slightly better removal of TP than Fe(VI), because Fe(III) better removed TSS, thus reducing the particulate P in the treated wastewater. Thirdly, Fe(VI) better removed DP than Fe(III) for the Verona wastewater, while Fe(III) was better in the DP removal than Fe(VI) for the Elizabeth wastewater.

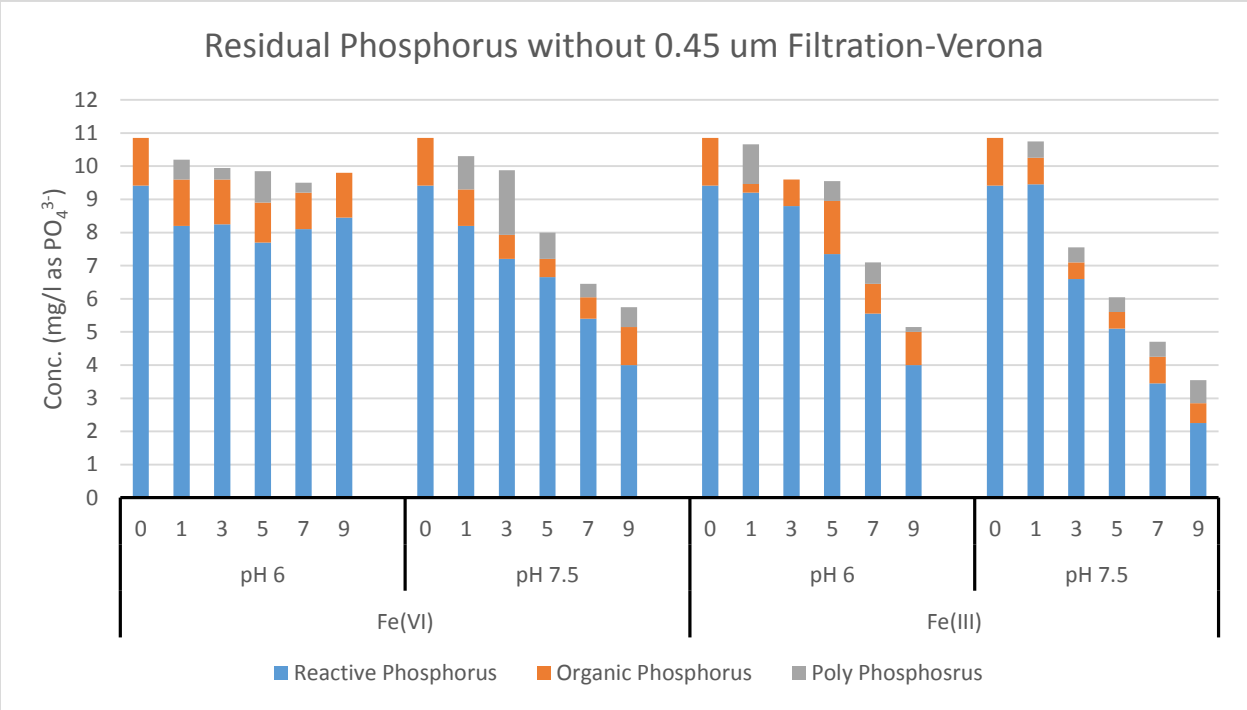


Figure 9. Residual TP in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Verona, NJ)

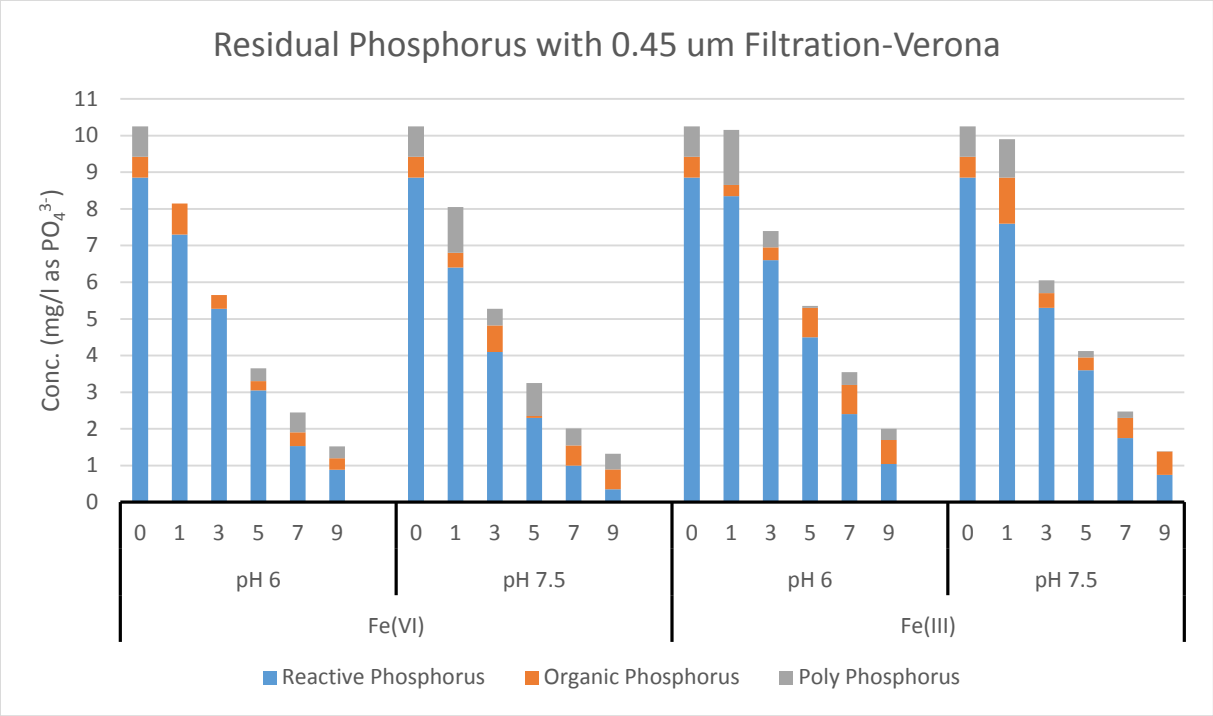


Figure 10. Residual DP in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Verona, NJ)

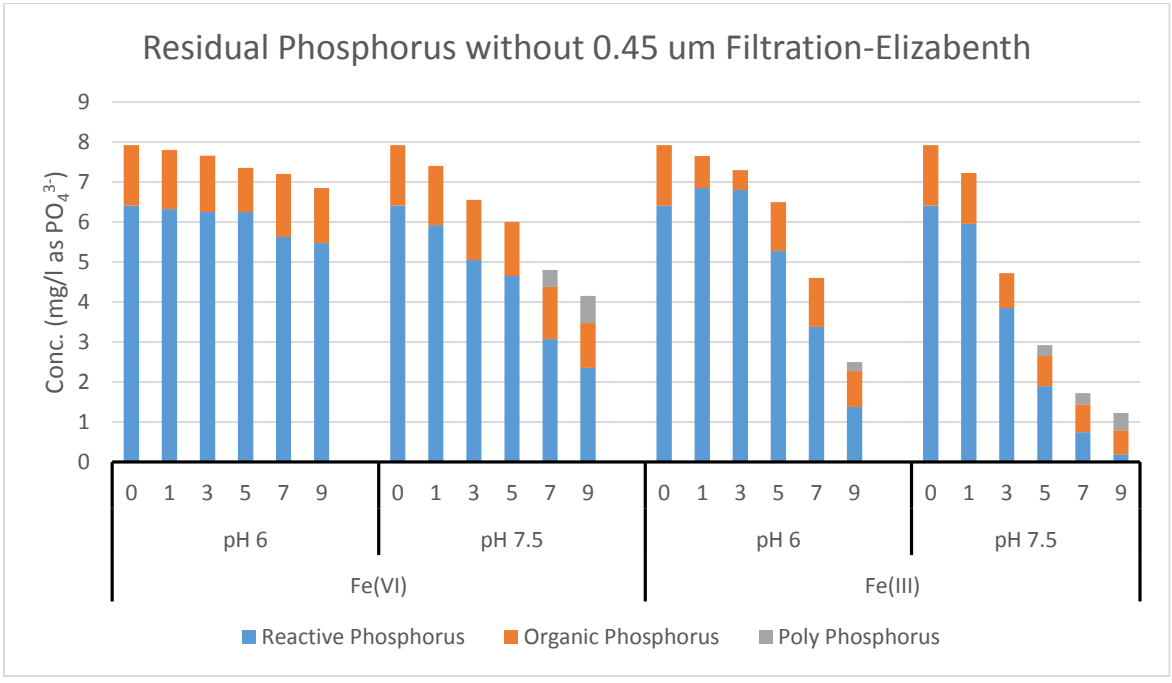


Figure 11. Residual TP in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Elizabeth, NJ)

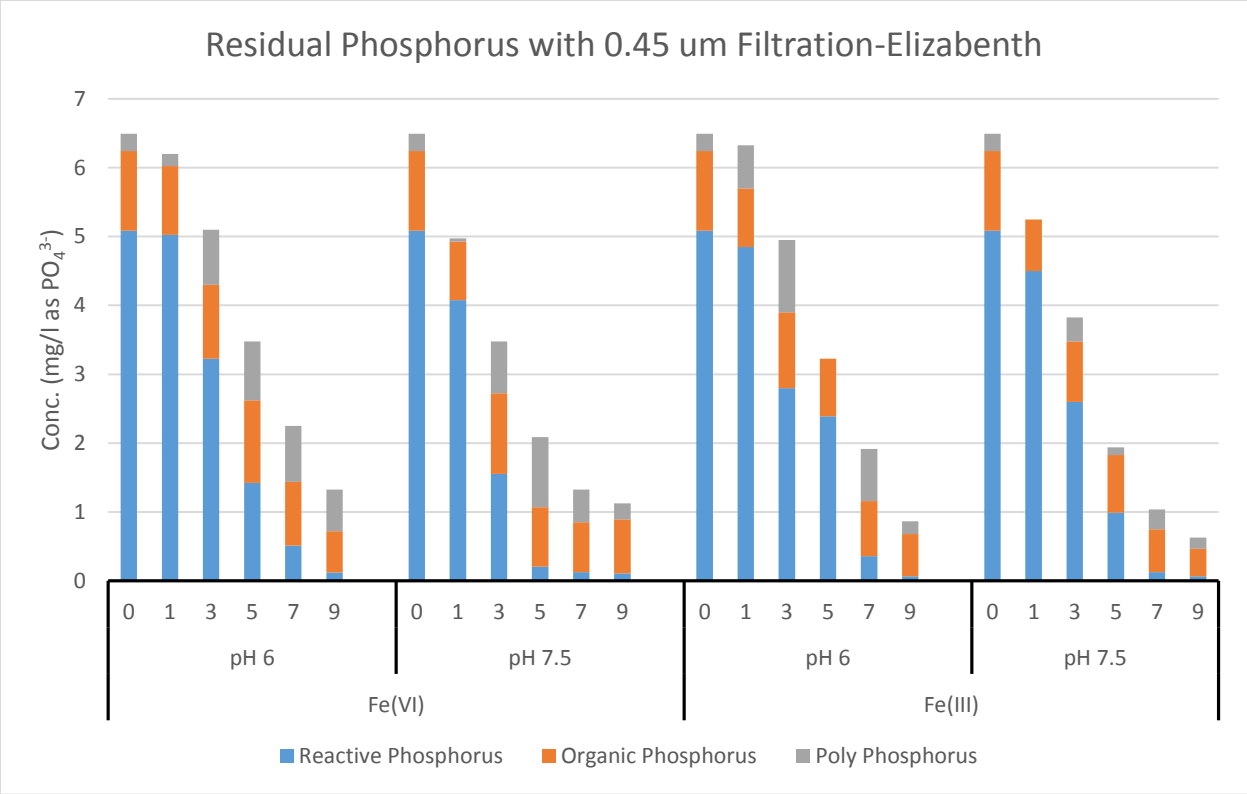
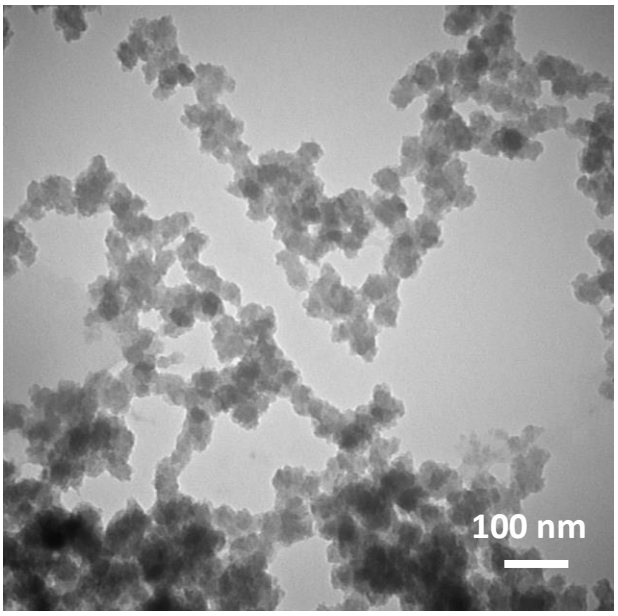


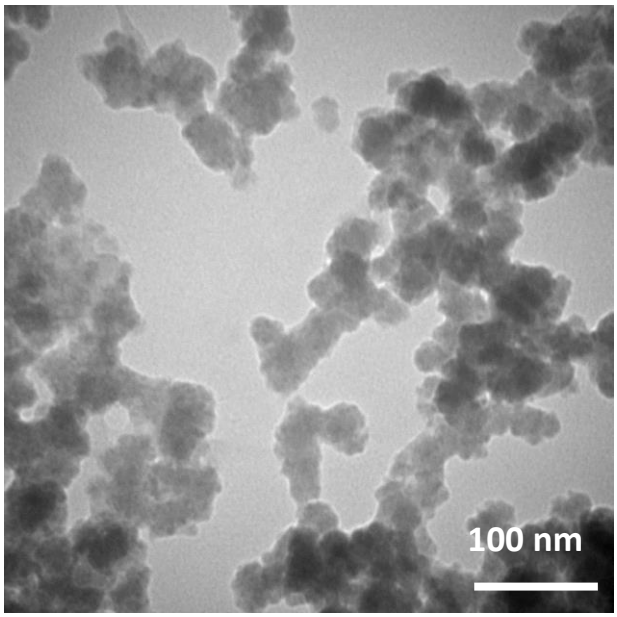
Figure 12. Residual DP in different P species after Fe(VI) or Fe(III) treatment of raw municipal wastewater (Elizabeth, NJ)

5.4 Characterization of Iron Precipitates

TEM images of Fe(VI)-induced particles from Fe(VI) treatment of secondary effluent are shown in **Figure 13**. Numerous nanoparticles were observed with spherical shapes and uniform sizes approximately ranging within 30-70 nm. These nanoscale particles tended to aggregate and produce a few micrometer flocs. As demonstrated before, these aggregates had a poor settling velocity reduce TSS. Zeta potentials of these suspended Fe(VI)-induced iron nanoparticles were measured under different experimental conditions (**Figure 14**). The zeta potentials were slightly increased from the initial -9.37 mV to -8.23, -8.45 and -7.88 mV at 2.50, 5.00, and 7.50 mg/L Fe(VI) at 120 min, respectively. The zeta potentials allowed these particles under a relatively state.



(a)



(b)

Figure 13 TEM images of suspended Fe(VI)-induced particles after Fe(VI) treatment of secondary effluent (initial pH = 7.23, Fe(VI) = 5.00 mg/L, and contact time = 120 min)

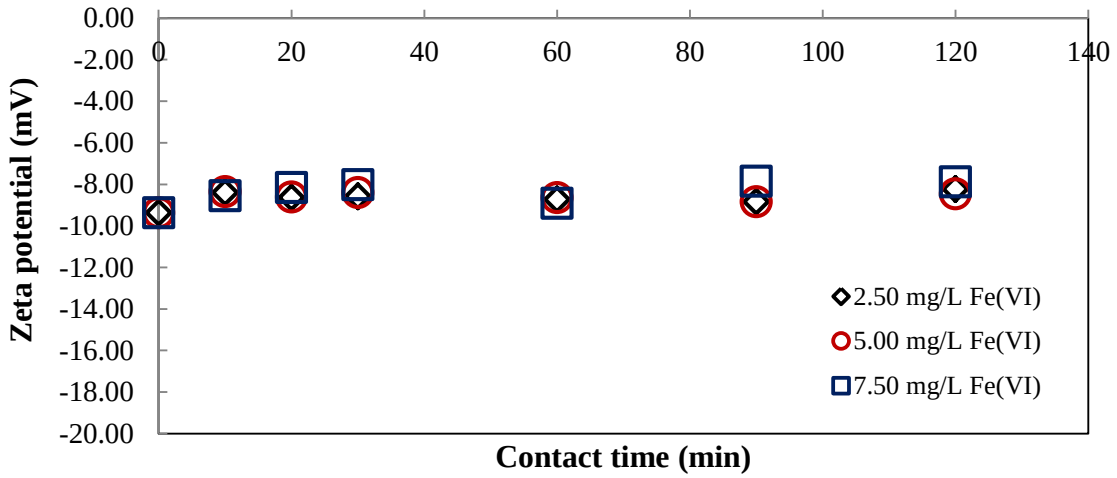


Figure 14 Zeta potentials of suspended particles during Fe(VI) treatment of secondary effluent (initial pH = 7.23; relative standard deviations are below 5%, not shown in the figure)

Size distributions of suspended particles in secondary effluent during Fe(VI) treatment are shown in **Figure 15**. At any specific settling time, the particle size was almost normally distributed. Although the particle numbers varied with contact time, the size distribution curves were very similar: the peaks were observed around 0.5 μm; and the most of particles had a size below 5.0 μm. These findings suggested that the size distribution and turbidity were almost consistent within 120 min and were not influenced by the varied particle number. Finally, the amorphous and crystalline iron fractions were determined for Fe(VI)-induced particles from Fe(VI) treatment of phosphate-containing solution (Fe(VI) = 8.12 mg/L; P = 3.00 mg/L) at pH 7.5 (**Figure 15**). Both amorphous and crystalline iron were observed in the produced iron precipitates. It should be noted that a majority of iron was present in the amorphous iron precipitates (71%), greater than crystalline iron (29%).

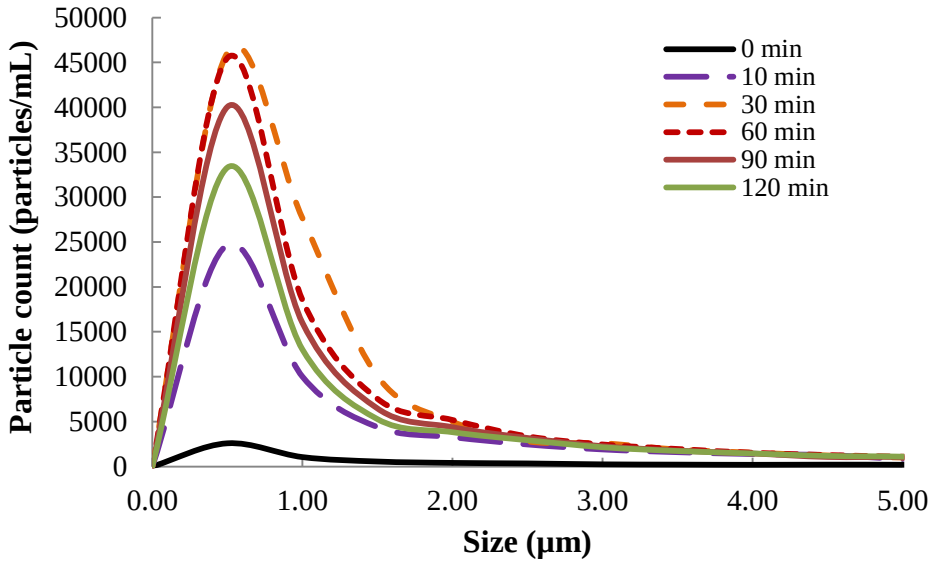


Figure 15 Size distributions of suspended Fe(VI) particles after Fe(VI) treatment of secondary effluent (initial pH = 7.23; 5.00 mg/L Fe(VI))

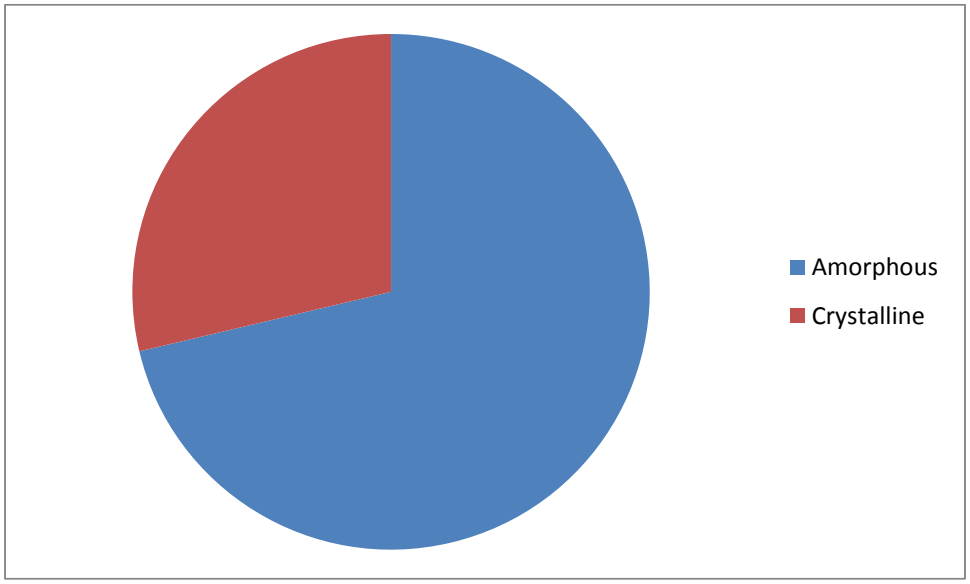


Figure 16 Distributions of amorphous and crystalline Fe in Fe(VI)-induced particles after Fe(VI) treatment of phosphate-containing solution (initial pH = 7.23; 5.00 mg/L Fe(VI); pH 7.5)

(5) Publications or Presentations:

5.1. Articles in Refereed Scientific Journals:

1) A manuscript entitled “Performance of Ferrate(VI) for the Removal of Phosphorus from Secondary Effluent” will be submitted to Journal of Hazardous Materials in the June of 2017 (by invitation)

2) A manuscript entitled " Ferrate(VI) for Chemically Enhanced Primary Treatment of Municipal Wastewater" will be submitted to *Water Research* (in preparation).

5.2 Oral presentation:

Zheng, L., N. Li, Y. Deng (2017) Ferrate(VI) for Chemically Enhanced Primary Treatment of Municipal Wastewater in Northeast Graduate Student Water Symposium (University of Massachusetts-Amherst, during September 8-10, 2017.

5.3 Dissertation:

The project was primarily used to support the dissertation research of Lei Zheng (the Student Awardee). Part of data was integrated into Nanzhu Li's dissertation. Li obtained her PhD in Environmental Management in 2017.

Li, Nanzhu, 2017, Ferrate as a New Treatment Chemical for Removal of Effluent Organic Matter (EfOM) and Emerging Micro-pollutants in Treated Municipal Wastewater for Water Reuse, Ph.D. Dissertation, Department of Earth and Environmental Studies, Montclair State University, Montclair, New Jersey.

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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	7	0	0	0	7
Masters	2	0	0	0	2
Ph.D.	6	0	0	0	6
Post-Doc.	0	0	0	0	0
Total	15	0	0	0	15

Notable Awards and Achievements

Notable Achievements -

For 2016NJ378B: NY/NJ Baykeeper is using the results of this research in their Plastic Reduction Project Community Outreach efforts. Based on the NJWRRI research, USEPA (Region 2) awarded Baykeeper a $40,000 Urban Water Grant (Rutgers a subcontractor) to add additional water sampling sites on the Passaic River. The Urban Waters grant also supports Baykeeper's community outreach activities. The PIs also received an additional $15,000 NIEHS pilot project grant, which allowed us to increase the number of samples analyzed by EOHSI. NOAA J. J. Howard Sandy Hook Laboratory contributed In-Kind analyses that determined the chemical components of the various microplastic substances, which may be released upon breakdown during environmental exposure. Dr. Ravit has also been awarded a $7,500 Sustainable Raritan River Initiative (SRRI) grant to continue microplastic research during summer 2018, which will add eight new sampling sites on the freshwater portion of the Raritan River.

For 2016NJ380B: New Jersey Water Environment Association (WEA) undergraduate Poster Competition Award, May 2017 Aresty/Honors College Research Fellowship ($700) to continue work New Jersey Water Environment Association (WEA) graduate Poster Competition Award, May 2016 New Jersey Water Environment Association (WEA) Louis Fontenelli Award, April 2016

For 2016NJ383B: Second Place for poster presentation at New Jersey Water Environment Association Annual Conference, May 2016