

**Water Resources Research Center
Annual Technical Report
FY 2009**

Introduction

This report covers the period March 1, 2009 to February 28, 2010, the 44th year of the Massachusetts Water Resources Research Center (WRRC). The Center is under the direction of Dr. Paula Rees, who holds a joint appointment as Director of the WRRC and as Director of Education and Outreach of the Engineering Research Center for Collaborative Adaptive Sensing of the Atmosphere at the University of Massachusetts Amherst (UMass).

Several research projects were conducted at the University of Massachusetts Amherst. Dr. David Boutt of the UMass Department of Geosciences evaluated the sustainability of fractured bedrock as a groundwater resource in his project *Characterizing and Quantifying Recharge at the Bedrock Interface*. Dr. Baoshan Xing of the UMass Plant, Soil, and Insect Science Department extended work on a two-year project on the *Environmental Behaviors of Engineered Nanoparticles in Water*. His project was granted a no-cost extension until May 31, 2010. Dr. Xing also worked on a second project, *Bacterial Toxicity of Oxide Nanoparticles and Their Adhesion* with graduate student Wei Jiang. Dr. Chul Park of the Department of Civil and Environmental Engineering worked a second year on his project entitled *Characterization of Wastewater Effluent from Western Massachusetts Publicly Owned Treatment Works using Metaproteomic Analysis*. The *Acid Rain Monitoring Project*, led by WRRC Associate Director Marie-Françoise Walk, was continued for another year in order to document trends in surface water acidification. One Technology Transfer award supported the seventh annual water resources research conference, *Monitoring and Responding to Water Resource Challenges*

Dr. Paul Mathisen and graduate student Suzanne Lepage, Dept. of Civil and Environmental Engineering, Worcester Polytechnic Institute, researched *Characterization of Flow and Water Quality of Stormwater Runoff from a Green Roof*.

Graduate student Deepankar Goyal and Dr. Juliette Rooney-Varga, Department of Biological Sciences, University of Massachusetts Lowell investigated the *Impact of Nanoparticles on the Activated Sludge Process: Effects on Microbial Community Structure and Function*.

Progress results for each project are summarized for the reporting year in the following sections.

Research Program Introduction

Seven research projects were conducted this fiscal year. One research project was funded through the USGS 104G program, and one research project received a no-cost extension for funds received through the previous USGS 104B program. Three new projects and one project continuation were funded through the 104B program and were completed this year. Progress results for the Acid Rain Monitoring Project are also reported.

Bacterial Toxicity of Oxide Nanoparticles and Their Adhesion

Basic Information

Title:	Bacterial Toxicity of Oxide Nanoparticles and Their Adhesion
Project Number:	2009MA177B
Start Date:	4/1/2009
End Date:	3/31/2010
Funding Source:	104B
Congressional District:	First
Research Category:	Water Quality
Focus Category:	Toxic Substances, Water Quality, Geochemical Processes
Descriptors:	None
Principal Investigators:	Baoshan Xing

Publications

1. Jiang, W., H. Mashayekhi and B. Xing. 2009. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environ. Pollut.* 157:1619-1625.
2. Jiang, W., H. Mashayekhi and B. Xing “Bacterial toxicity of oxide nanoparticles and their adhesion to bacteria cell walls”. International Conference on the Environmental Implications and Applications of Nanotechnology, Amherst (MA), June 9-11, 2009, p. 27.
3. Wang, H., R. Wick and B. Xing . “Toxicity of nanoparticulate and bulk ZnO, Al₂O₃ and TiO₂ to the nematode *Caenorhabditis Elegans*”. International Conference on the Environmental Implications and Applications of Nanotechnology, Amherst (MA), June 9-11, 2009, p. 31.
4. Jiang, W. and B. Xing. “Behavior of nanoparticles at the bacteria-water interface” The 7th Annual Massachusetts Water Resources Conference, Amherst, April 8, 2010. Abstract book, p. 25.

Problem and Research Objectives

Oxide nanoparticles (NPs) are widely used, and they are potentially toxic. The goal of this work was to evaluate the toxicity of several engineered oxide NPs to common bacteria species and the adhesion of NPs to the bacteria surface.

Methodology

Batch experiments, FTIR, Characterization of Nanoparticles, Toxicity evaluation, AFM and TEM imaging.

Principal Findings and Significance

Toxicity of nano-scaled aluminum, silicon, titanium and zinc oxides to bacteria (*Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens*) was examined and compared to that of their respective bulk (micro-scaled) counterparts. All nanoparticles but titanium oxide showed higher toxicity than their bulk counterparts. Toxicity of released metal ions was differentiated from that of the oxide particles. ZnO was the most toxic among the three nanoparticles, causing 100% mortality to the three tested bacteria. Al₂O₃ nanoparticles had a mortality rate of 57% to *B. subtilis*, 36% to *E. coli*, and 70% to *P. fluorescens*. SiO₂ nanoparticles killed 40% of *B. subtilis*, 58% of *E. coli*, and 70% of *P. fluorescens*. TEM images showed attachment of nanoparticles to the bacteria, suggesting that the toxicity was affected by bacterial attachment. Bacterial responses to nanoparticles were different from their bulk counterparts; therefore nanoparticle toxicity mechanisms need to be studied thoroughly.

Publications and Conference Presentations

Articles in Refereed Scientific Journals

Jiang, W., H. Mashayekhi and B. Xing, 2009. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environ. Pollut.* 157: 1619-1625.

Conference Proceedings

Jiang, W., H. Mashayekhi and B. Xing "Bacterial toxicity of oxide nanoparticles and their adhesion to bacteria cell walls". International Conference on the Environmental Implications and Applications of Nanotechnology, Amherst (MA), June 9-11, 2009, p. 27.

Wang, H., R. Wick and B. Xing . "Toxicity of nanoparticulate and bulk ZnO, Al₂O₃ and TiO₂ to the nematode *Caenorhabditis Elegans*". International Conference on the Environmental Implications and Applications of Nanotechnology, Amherst (MA), June 9-11, 2009, p. 31.

Jiang, W. and B. Xing. "Behavior of nanoparticles at the bacteria-water interface" The 7th Annual Massachusetts Water Resources Conference, Amherst, April 8, 2010. Abstract book, p. 25.

Student Support

Mr. Hamid Mashayekhi and Miss Wei Jiang, Ph.D. Department of Plant, Soil & Insect Sciences

Notable Achievements and Awards

One graduate student, Wei Jiang, won a first place for her poster presentation at the "Water Dependencies in New England", the 6th Annual Conference:

http://www.umass.edu/psis/news/ne_water_conf.html

Impact of Nanoparticles on the Activated Sludge Process: Effects on Microbial Community Structure and Function

Basic Information

Title:	Impact of Nanoparticles on the Activated Sludge Process: Effects on Microbial Community Structure and Function
Project Number:	2009MA178B
Start Date:	4/1/2009
End Date:	3/31/2010
Funding Source:	104B
Congressional District:	5th
Research Category:	Not Applicable
Focus Category:	Toxic Substances, Wastewater, None
Descriptors:	None
Principal Investigators:	Juliette Rooney-Varga, Deepankar Goyal

Publication

1. Goyal, D., G. Doyle, X. J. Zhang, J. N. Rooney-Varga. Impact of Multi-Walled Carbon Nanotubes on the Structure of Activated Sludge Microbial Communities. Eastern New England Biological Conference, Lowell MA, April 2009.

Problem and Research Objectives

Nanotechnology, or the ability to create and use materials at the scale of 1 to 100 nanometers, is a rapidly expanding sector that is generating materials with unique physical and chemical properties. In particular, carbon nanotubes (CNTs) are known for their unique mechanical, electronic, and biological properties and have far-reaching potential applications as components of personal care products, pharmaceuticals, electronic devices, energy storage devices, stains and coatings, and new environmental clean-up technologies (Masciangioli and Zhang 2003; Boczkowski and Lanone 2007; Chen 2007; Erdem 2007; Rivas et al., 2007; Kislyuk and Dimitriev 2008; Prato et al., 2008; Theron et al., 2008). Massachusetts is poised to be a leader in nanotechnology research and development and this sector is expected to be a major component of the Commonwealth's economy for the foreseeable future. However, while the potential for nanotechnology is vast, relatively little is known about the health and environmental risks posed by nanomaterials (Colvin 2003). Indeed, those watching the industry have commented that concern over unknown risks of nanomaterials is a major determinant of the future success of nanotechnology (Colvin 2003).

Through nanomanufacturing and widespread use of nanomaterials, CNTs and other nanomaterials will inevitably be released into wastewater streams and enter wastewater treatment plants (Wiesner 2006). All publicly owned wastewater treatment facilities rely on the 'activated sludge process,' which relies on controlled microbial degradation of waste materials to remove chemical and biological contaminants (Wagner et al., 2002). However, little is known about how CNTs will affect the complex microbial communities that are responsible for the activated sludge process and whether microorganisms are capable of removing them. Effluent from wastewater treatment plants ultimately is released to the environment, where it can impact aquatic ecosystems and drinking water. Any toxicity to microorganisms exhibited by CNTs has the potential to dramatically reduce the efficacy of the activated sludge process, resulting in the release of untreated sewage, pathogenic microbes, and CNTs into the environment. Shock-loading of other contaminants has shown that treatment performance can be affected for weeks or months, resulting in a reduction in treatment efficiency, environmental release of toxic contaminants, and operation problems that may require months to recover (Boon et al., 2003; Henriques et al., 2007). In addition, the ability of CNTs to strongly adsorb organic matter can reduce the bioavailability and, therefore, microbial degradation of organic pollutants, which would then effectively bypass the treatment process.

The composition and function of activated sludge microbial communities has received considerable attention, although the function of many specific phylogenetic groups and the factors that control are not yet well understood. In broad terms, activated sludge contains microbial eukaryotes ("microeukaryotes"), including protozoa, fungi, and metazoans, as well as a wide diversity of bacteria responsible for varied metabolic functions, including oxidation of organic compounds and removal of nitrogenous pollutants and phosphates (Weber et al., 2007). Within this microbial community, many complex ecological interactions are thought to be necessary for the effective functioning of activated sludge. For example, ciliated protozoa and fungi have been found to form tree-like and filamentous colonies, respectively, that form a back-bone for bacterial colonization, resulting in the production of flocs, which readily settle out of the liquid phase and are collected for effective removal from treated wastewater (Weber et al., 2007). Both bacteria and microeukaryotes are likely to contribute to the formation of extracellular polymeric substances (EPS), which are high molecular weight compounds with adhesive properties that are critical to the formation and integrity of flocs (and biofilms more generally) (Raska et al., 2006; Weber et al., 2007). In addition, specific taxonomic groups of bacteria are

known to carry out key functions in activated sludge. For example, members of the *Planctomycetes* are responsible for anaerobic ammonium oxidation; several lineages within the kingdom *Euryarchaea* produce methane; members of the genus *Nitrospira* oxidize nitrite to nitrate (Juretschko et al., 2002); *Actinomycetes* may contribute to the production of foam and reduce the quality of effluent; and members of the *Chloroflexi* have been associated with bulking events (Kragelund et al., 2007). Thus, analysis of microbial community composition can provide meaningful insight into various activated sludge functions, as well as the factors that control them (Liu et al., 1997; Forney et al., 2001).

Relatively little is known about the potential toxicity of CNTs to activated sludge microorganisms and studies on pure cultures or defined mixed cultures have yielded conflicting results. There is strong evidence for CNT toxicity to pulmonary cells, as well as potential toxicity to epithelial, brain, and liver cells (Lam et al., 2006; Smart et al., 2006; Warheit 2006). Single-walled CNTs (SWCNTs) have been reported to be highly toxic to *Escherichia coli* str. K12 cells that come in direct contact with them (Kang et al., 2007). (Ghafari et al., 2008) found a moderate impact of SWCNTs on *E. coli*-gfp viability, although they did not differentiate between planktonic cells and those in contact with SWCNT aggregates. Interestingly, they found that *Tetrahymena thermophila*, a ciliated protozoan that is an important member of wastewater treatment microbial communities, ingested CNTs. As a result, the protozoan's ability to ingest and digest bacterial cells was impeded, suggesting a negative impact on an important function of these protozoa, namely bacterivory (Ghafari et al., 2008). Conversely, the CNTs may also have a positive impact on activated sludge processes, as they caused an increase in the production of exudates by ciliates, which may benefit floc formation and, therefore, sludge settleability.

While pure culture studies have shown that nanomaterials can act as antimicrobial agents, the complexity of the activated sludge community make it unlikely to respond to CNTs in the same manner as simple pure culture systems. Our objective was to use state-of-the-art molecular techniques to determine the impact of CNTs on microbial community dynamics in batch reactors that model the activated sludge process.

Methodology

Experimental set-up

Fresh activated sludge was collected from an aeration basin at the Lowell Regional Wastewater Treatment Facility, Lowell. This facility is designed to treat primarily municipal wastewater through conventional primary and secondary treatment processes. Whole unscreened samples were transported to the laboratory and processed within 30 minutes of sample collection. Experimental conditions for batch-scale reactor studies were previously described by Yin et al., (2009). In order to distinguish between effects of CNTs and potential toxic effects of impurities associated with them (such as amorphous carbon and metal catalysts), triplicate CNT-exposed reactors were compared to triplicate reactors exposed to impurities alone. CNTs used in the current study consisted of >90% pure CNTs (Sigma-Aldrich, Inc., St. Louis MO) characterized by Raman spectroscopy (Table 1). Reactors were filled with 2 L of fresh activated sludge, with an initial soluble chemical oxygen demand (sCOD) of 20 mg L⁻¹ from the aeration basin effluent (Yin et al., 2009). The sludge was fed with peptone and aerated prior to and during the experiment as described by Yin et al., (Yin et al., 2009). Sub-samples for microbial community analysis were taken aseptically immediately after adding CNTs or impurities (T₀), at 1.25 hr (T₁) after initial exposure, and at 5 hr (T₄). The samples were placed in cryovials, and stored at -80°C until further processing.

DNA extraction and analysis

Genomic DNA from was extracted and purified from 400 μL sub-samples of sludge using the FastDNA Spin kit for Soil (MP Biomedicals Inc., Solon, OH). ARISA-PCR was performed as previously described (Fisher and Triplett 1999), with minor modifications. Reaction mixtures contained 1 \times AmpliTaq PCR buffer (Applied Biosystems, Inc., Carlsbad, CA), 2.5 mM MgCl_2 , 400 ng μL^{-1} bovine serum albumin (BSA), 200 μM each dNTP, 400 nM each primer, 2.5 U of *Taq* DNA polymerase, and 1, 5, 10, or 20 ng of genomic DNA in a final volume of 50 μL . The primers used were 1392F (5'-G [C/A] ACACACCGCCCGT-3') and 23SR (5'GGGTT[C/G/T] CCCCATTC[A/G]G-3'). The 5' end of primer 1392F was labeled with 6-carboxyfluorescein (6-FAM). The following thermal profile was used for PCR: denaturation at 94°C for 3 min, followed by 30 cycles of amplification at 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, followed by a final extension of 72°C for 7 min. PCR products were analyzed by electrophoresis in 1% agarose gels (Ausubel et al., 1997) and were purified using QiaQuick PCR Purification Kits (Qiagen, Inc., Valencia CA).

20 ng each purified PCR product were lyophilized and subjected to automated capillary electrophoresis (CE) analysis in conjunction with a 50 – 1200 bp size standard labeled with LIZTM (Applied Biosystems, Inc.) at the Center for AIDS Research, UMass Medical School, Worcester MA. ARISA conditions were optimized by comparing profiles generated from multiple DNA template amounts (1, 5, 10, or 20 ng per 50 μL PCR) and PCR product amounts (5, 10, or 20 ng PCR product per well). Comparison of these conditions indicated that the highest diversity (species richness and evenness) and signal to noise ratios were achieved using 1 ng DNA template DNA for PCR and 20 ng PCR product for CE analyses, which were used in subsequent analyses.

ARISA profiles were analyzed using PeakScanner software (Applied Biosystems Inc.) and processed as described by Brown et al., (Brown et al., 2005). The programs Interactive and Automatic Binner were used to bin peaks, with a window size (WS) of 3 bp and a shift value (Sh) of 0.1 (Ramette 2009). Peak areas were normalized to total peak area per sample and peaks representing <1% total peak area for a given sample were considered indistinguishable from background and removed from the analysis. Data visualization and ordination analyses were conducted using the packages Ecodist (Goslee and Urban 2007) and Vegan (<http://vegan.r-forge.r-project.org/>) in the R statistical programming environment (Goslee and Urban 2007). Pairwise Bray-Curtis distances between samples were calculated using the Ecodist package and a hierarchical clustering algorithm with average linkage clustering were used to construct a dendrogram depicting relationships among the samples' ARISA profiles. Correspondence analysis (CA), which assumes a unimodal relationship between relative abundance (i.e., normalized peak area) and ordination axes, was used to analyze relationships between samples. The R package Vegan was used to determine whether CA ordination axes were correlated with environmental variables. The latter included the experiment from which samples were analyzed (E1 for the experiment comparing CNTs to CNT-associated impurities, conducted on June 28, 2007; E2 for the experiment comparing CNT-associated impurities to a control conducted on July 19, 2007); time elapsed from the initiation of the experiment to sampling (0, 1.25, or 5 hours); and treatment (CNTs, associated impurities, or feed alone). "Dummy" variables were assigned for categorical variables and set to 0 or 1 depending on the presence of a given variable. The "envfit" goodness of fit test with 1000 permutations was used to assess the fit of environmental variables to ordination axes.

In order to determine the phylogenetic identity of dominant community members, as detected by ARISA, phylogenetic analysis of 16S rRNA genes contiguous with fragments analyzed in ARISA was used (Brown et al., 2005). DNA amplicons

containing partial 16S rRNA genes and associated intergenic spacer (IGS) regions were generated from selected activated sludge genomic DNA samples using primers 338F and 23SR (5'GGGTT[C/G/T] CCCCATT[C/A/G]G-3') (Amann et al., 1990; Brown et al., 2005). The resulting amplicons were cloned using the TOPO TA Cloning Kit for Sequencing with One Shot® TOP10 Chemically Competent *E. coli*, as described by the manufacturer (Invitrogen Corp., Carlsbad, CA). 90 cloned inserts were analyzed using ARISA, as described above, except that the template DNA for PCR consisted of *E. coli* clone cell lysates (obtained by suspending individual colonies in 0.1 M Tris-Cl, pH 8.0, and incubating them at 99° C for 2 minutes). ARISA peaks from cloned inserts were considered to match OTUs from environmental community ARISA patterns if their peak size was placed within the same 3 bp bin as a given OTU from environmental samples.

At least one cloned insert representative of each ARISA OTU was sequenced in both directions by Beckman Coulter Genomics Inc. (Danvers MA, USA) with M13 primers. Vector and primer sequences were trimmed, trimmed sequences were aligned to the Silva database, and phylogenetic relationships among aligned sequences and their 40 nearest neighbors in the Silva database were analyzed using ARB (Ludwig et al., 2004; Pruesse et al., 2007). Trimmed sequences were deposited in GenBank under accession numbers HM205112 - HM205114.

Principal Findings and Significance

Results

Effects of CNTs and their associated impurities

Analysis of ARISA profiles revealed several differences between bacterial community structure in batch reactors exposed to CNTs for five hours when compared to those exposed to associated impurities alone. For example, the relative peak areas of dominant OTUs represented by peaks 419, 794, and 839 bp were significantly different in communities exposed to CNTs vs. those exposed to CNT-associated impurities (Fig. 1). Similarly, a Chi-square goodness-of-fit test of correspondence analysis (CA) axes revealed that the effect of CNTs on community structure was significant ($p=0.043$), while exposure to impurities alone was not ($p=0.604$). In order to assess the effect of CNTs without interference from the strong effects of time and experiment, CA ordination was repeated with only the time T4 samples from the experiment comparing CNTs to impurities alone (E1). A statistically significant effect of CNTs was observed ($p<0.001$), while a similar analysis of the effects of impurities alone (CA with experiment E2, time T4 samples) revealed no effect ($p=0.316$), as was also evident from direct inspection of ARISA profiles (Fig. 1). Samples taken after only 1.25 hours exposure (time T1) revealed no clear differences in ARISA profiles between either CNT- and impurities-exposed reactors or between reactors exposed to impurities and control reactors), indicating that exposure for 1.25 hours was insufficient for CNT effects to be detected via the approach used here.

Both hierarchical clustering and correspondence analysis (CA) of all samples revealed strong effects of the amount of time elapsed prior to sampling (0, 1.25, or 5 hours) and the date of the experiment (Fig. 2). Baseline (T_0) communities for E1 and E2 were fairly similar. However, these communities diverged substantially over the short experimental time period of five hours, with the resulting communities sharing only 14/29 total OTUs and 4/9 total "dominant" (considered here to be those with average relative peak areas > 5%) OTUs.

Three of the OTUs found in environmental samples were identified among the 90 cloned inserts analyzed here. These included peaks corresponding to 419, 740, and 812 bp (Fig. 1). Phylogenetic analysis placed these OTUs within the families *Sphingomonadaceae* (419 bp) and *Cytophagaceae* (740 bp), and the genus *Zoogloea*

(812 bp). Two representative of OTU 812 were sequenced and found to be identical. The closest relatives of the sequences representing OTUs 419, 740, and 812 were: an uncultivated *Sphingomonadaceae* bacterium from snow (97.1% similarity); an uncultivated *Cytophagaceae* bacterium from activated sludge (89.5% similarity); and *Zoogloea resiniphila*, a denitrifier isolated from activated sludge (99.8% similarity).

Discussion

While CNTs have the potential to be highly toxic to microbial cells, their impact under the complex abiotic and biological conditions found in environmental microbial communities remains poorly understood. The current study revealed changes in microbial community structure in activated sludge batch reactors exposed to CNTs, while no effects of CNT-associated impurities were detected. Yin et al. (2009) analyzed bulk parameters and performance from the CNT-exposed batch reactors described here and similarly found that CNTs, but not their associated impurities, had several effects on sludge performance. These effects included: increased organic carbon removal primarily through organic carbon adsorption; less negative surface charges of activated sludge flocs; and improved sludge settleability (Yin et al., 2009). Other parameters such as pH, dissolved oxygen, specific resistance to filtration, and relative hydrophobicity were not significantly impacted (Yin et al., 2009). These findings suggest that CNTs impacted community structure through toxicity to some community members, by reducing organic carbon bioavailability, and/or by altering floc properties.

The fact that CNT effects on microbial community structure were detected was especially interesting given that, unlike some previous studies, the experimental conditions used did not maximize CNT-cell interactions. For example, an assay for cytotoxicity developed by Kang et al., (2007) relies on drawing planktonic cells onto a filter that is coated with nanoparticles and observing the resulting effects on cellular membrane integrity over time. Under these conditions, direct cell-nanoparticle contact is artificially induced and CNTs demonstrated high levels of toxicity to Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and, to a lesser extent, Gram-positive (*Staphylococcus epidermis* and *Bacillus subtilis*) cells (Kang et al., 2009). In contrast, here, CNTs were added to activated sludge bioreactors in suspension, making CNT-cell contact much less likely. In addition, the presence of extracellular polymeric substances (EPS) and high concentrations of DOC in the batch reactors used here may have mitigated CNT toxicity to some extent, as CNTs are likely to become embedded in EPS and thereby prevented from coming in direct contact with cell membranes (Neal 2008; Luongo and Zhang 2010). Lastly, the exposure time was kept short in order to avoid confounding effects of starvation and/or accumulation of waste products in closed-system batch reactors. Despite the use of short incubation times, changes in community structure with both CNT exposure and time over the course of the experiment were found (Fig. 1 and 2). Previous studies have shown that cellular inactivation increased with time of exposure (Kang et al., 2009), indicating that use of longer incubation times in continuous reactors may increase effects of CNTs on community structure.

Phylogenetic analysis of cloned inserts that were matched to ARISA peaks revealed the presence of three phylogenetic groups that are responsible for important functions in activated sludge communities, including the members of the families *Sphingomonadaceae* (OTU 419) and *Cytophagaceae* (OTU 740) and the genus *Zoogloea* (OTU 812) (Manz et al., 1996; Neef et al., 1999; Juretschko et al., 2002; Wagner et al., 2002; Li et al., 2008). Of these, the sphingomonad (OTU 419) showed a trend of decreased relative peak intensity with exposure to CNTs (Fig. 1), indicating an adverse impact of CNTs on this group compared to other community members. Within wastewater treatment microbial communities, sphingomonads are

thought to have wide metabolic diversity, are capable of degrading some xenobiotics, and contribute to the formation of flocs (Neef et al., 1999; Wagner et al., 2002). Although directly measuring these parameters was beyond the scope of the current study, the potential for negative impacts on CNTs on these microbial functions deserves further attention.

Differences in the 'baseline' (T_0) community structure from one sampling date to another corroborate results obtained by Wittebolle et al., (2005), who observed that large community shifts occurred over a period as short as a few days in a given wastewater treatment plant and that community structure was related to performance of biological treatment. These findings underscore the need to analyze microbial community structure when assessing the effects of emerging contaminants on environmental systems, as differences in the starting community composition may alter the observed impacts on community performance.

In conclusion, our results indicate that the structure of activated sludge microbial communities is impacted by exposure to CNTs, even when such exposure is limited to a short time period, and that these effects were not due to impurities associated with CNTs. Community shifts found here indicated that CNTs differentially affect microbial species, as has been found under pure culture conditions (Kang et al., 2009). These results raise the concern of CNT impact on biological functions carried out by the activated sludge process.

Table 1. Characteristics of CNTs used in the current study.

Purity	
Carbon nanotubes	>90%
Single-walled nanotubes	>50%
Impurities	
Amorphous carbon	<5%
Co	0.6%
Mg	1.2%
Mo	0.1%
Silicates	0.1%
Average outside diameter	1–2 nm
Density	1.7–2.1
Length	5–15 μm
Specific surface area	>400 m^2/g

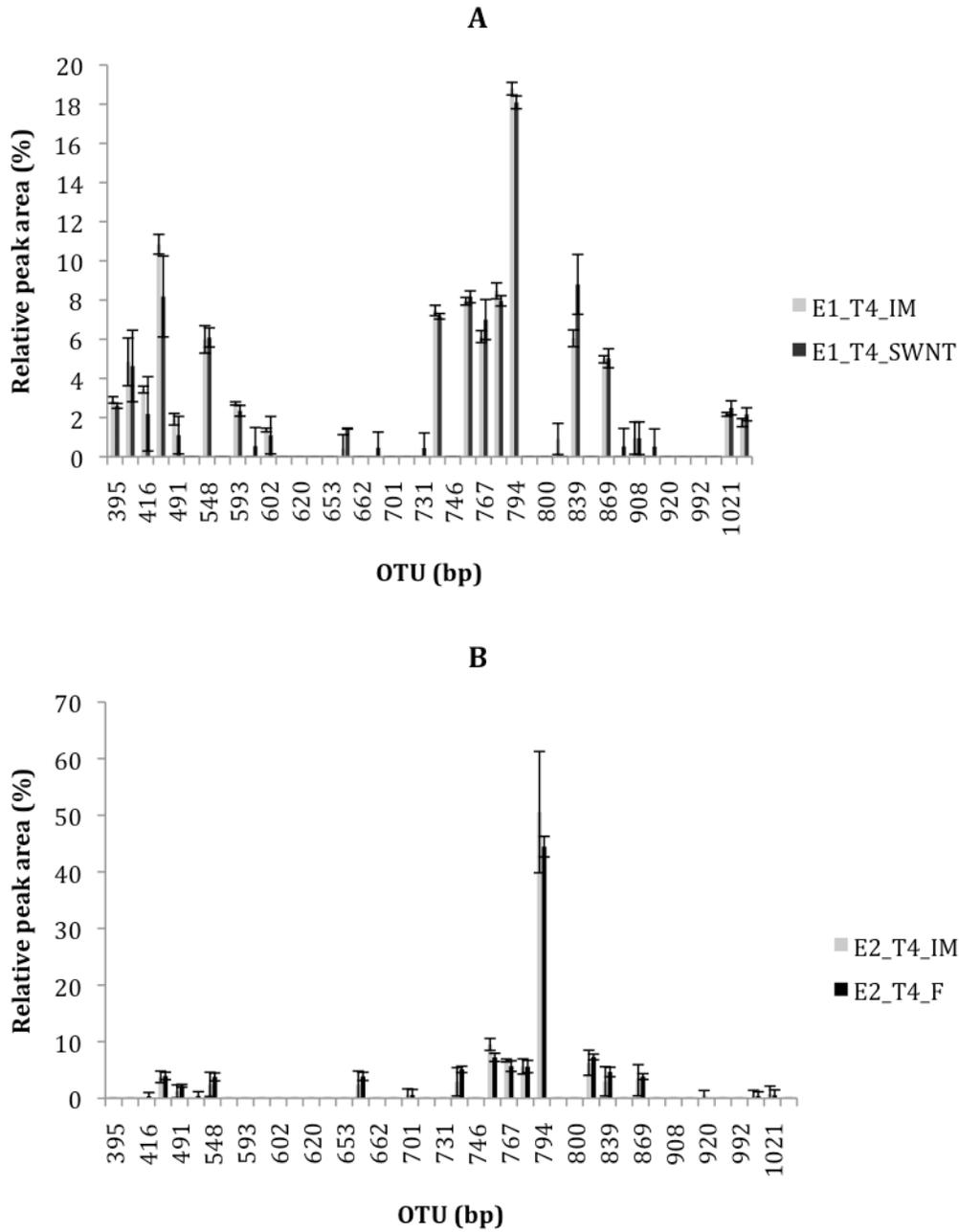


Figure 1. ARISA profiles of activated sludge bacterial communities exposed to CNTs, their associated impurities, or synthetic feed alone at the end of the experiments (T4). Comparisons were made between CNT- and impurities-exposed (IM) reactors during one experiment (designated E1; panel A) and between impurities-exposed and control reactors receiving feed alone (F) in a second experiment (E2; panel B). Means and standard deviations of relative peak areas from triplicate batch reactors are shown.

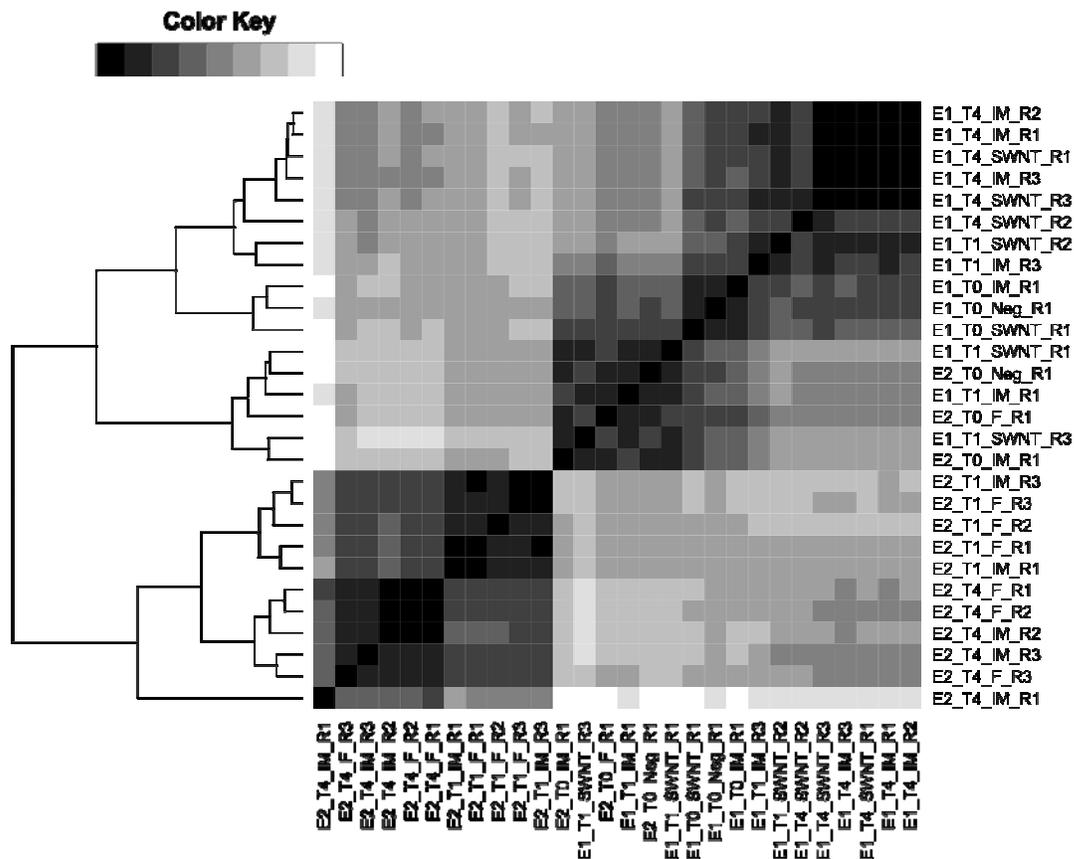


Figure 2. Hierarchical clustering analysis and heatmap of Bray-Curtis distances among samples taken from the first and second experiments (E1 and E2, respectively), at times 0, 1.25 hours, and 5 hours (T0, T1, and T4, respectively), and exposed to CNTs, impurities, or feed alone (SWNT, IM, or F, respectively).

Publications and Conference Presentations

Goyal, D., J. X. Zhang, J. N. Rooney-Varga. 2010. Impacts of single-walled carbon nanotubes on microbial community structure in activated sludge. *Submitted*.

Goyal, D., G. Doyle, X. J. Zhang, J. N. Rooney-Varga, 2009. Impact of Multi-Walled Carbon Nanotubes on the Structure of Activated Sludge Microbial Communities. Eastern New England Biological Conference, Lowell MA, April 2009.

Student Support

Deepankar Goyal, M.S., Biological Sciences
 Gregory Doyle, B.S., Biological Sciences

References

- Amann, R.L., Binder, B., Chisholm, S.W., Olsen, R., Devereux, R. and Stahl, D.A. (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* **56**, 1919-1925.
- Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1997) *Short Protocols in Molecular Biology*. New York: John Wiley & Sons, Inc.
- Boczkowski, J. and Lanone, S. (2007) Potential uses of carbon nanotubes in the medical field: how worried should patients be? *Nanomed* **2**, 407-410.
- Boon, N., Top, E.M., Verstraete, W. and Siciliano, S.D. (2003) Bioaugmentation as a tool to protect the structure and function of an activated-sludge microbial community against a 3-chloroaniline shock load. *Appl Environ Microbiol* **69**, 1511-1520.
- Brown, M.V., Schwalbach, M.S., Hewson, I. and Fuhrman, J.A. (2005) Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. *Environ Microbiol* **7**, 1466-1479.
- Chen, G. (2007) Carbon nanotube and diamond as electrochemical detectors in microchip and conventional capillary electrophoresis. *Talanta* **74**, 326-332.
- Colvin, V.L. (2003) The potential environmental impact of engineered nanomaterials. *Nat Biotechnol* **21**, 1166-1170.
- Erdem, A. (2007) Nanomaterial-based electrochemical DNA sensing strategies. *Talanta* **74**, 318-325.
- Fisher, M.M. and Triplett, E.W. (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol* **65**, 4630-4636.
- Forney, L.J., Liu, W.T., Guckert, J.B., Kumagai, Y., Namkung, E., Nishihara, T. and Larson, R.J. (2001) Structure of microbial communities in activated sludge: potential implications for assessing the biodegradability of chemicals. *Ecotoxicol Environ Saf* **49**, 40-53.
- Ghafari, P., St-Denis, C.H., Power, M.E., Jin, X., Tsou, V., Mandal, H.S., Bols, N.C. and Tang, X.S. (2008) Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. *Nat Nanotechnol* **3**, 347-351.
- Goslee, S.C. and Urban, D.L. (2007) The ecodist package for dissimilarity-based analysis of ecological data. *J Stat Soft* **22**, 1-19.
- Henriques, I.D., Kelly, R.T., 2nd, Dauphinais, J.L. and Love, N.G. (2007) Activated sludge inhibition by chemical stressors - a comprehensive study. *Water Environ Res* **79**, 940-951.
- Juretschko, S., Loy, A., Lehner, A. and Wagner, M. (2002) The microbial community composition of a nitrifying-denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle rRNA approach. *Syst Appl Microbiol* **25**, 84-99.
- Kang, S., Mauter, M.S. and Elimelech, M. (2009) Microbial cytotoxicity of carbon-based nanomaterials: Implications for river water and wastewater effluent. *Environ Sci Technol* **43**, 2648-2653.

- Kang, S., Pinault, M., Pfefferle, L.D. and Elimelech, M. (2007) Single-walled carbon nanotubes exhibit strong antimicrobial activity. *Langmuir* **23**, 8670-8673.
- Kislyuk, V.V. and Dimitriev, O.P. (2008) Nanorods and nanotubes for solar cells. *J Nanosci Nanotechnol* **8**, 131-148.
- Kragelund, C., Levantesi, C., Borger, A., Thelen, K., Eikelboom, D., Tandoi, V., Kong, Y., van der Waarde, J., Krooneman, J., Rossetti, S., Thomsen, T.R. and Nielsen, P.H. (2007) Identity, abundance and ecophysiology of filamentous Chloroflexi species present in activated sludge treatment plants. *FEMS Microbiol Ecol* **59**, 671-682.
- Lam, C.W., James, J.T., McCluskey, R., Arepalli, S. and Hunter, R.L. (2006) A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol* **36**, 189-217.
- Li, A.J., Yang, S.F., Li, X.Y. and Gu, J.D. (2008) Microbial population dynamics during aerobic sludge granulation at different organic loading rates. *Wat Res* **42**, 3552-3560.
- Liu, W.T., Marsh, T.L., Cheng, H. and Forney, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology* **63**, 4516-4522.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A. and Schleifer, K.H. (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363-1371.
- Luongo, L.A. and Zhang, X. (2010) Toxicity of carbon nanotubes to the activated sludge process. *J Haz Mat In Press, Corrected Proof*.
- Manz, W., Amann, R.I., Ludwig, W., Wagner, M. and Schleifer, K.-H. (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiol* **142**, 1097-1106.
- Masciangioli, T. and Zhang, W.X. (2003) Environmental technologies at the nanoscale. *Environ Sci Technol* **37**, 102A-108A.
- Neal, A. (2008) What can be inferred from bacterium–nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? *Ecotoxicol* **17**, 362-371.
- Neef, A., Witzemberger, R. and Kämpfer, P. (1999) Detection of sphingomonads and in situ identification in activated sludge using 16S rRNA-targeted oligonucleotide probes. *Journal of Industrial Microbiology and Biotechnology* **23**, 261-267.
- Prato, M., Kostarelos, K. and Bianco, A. (2008) Functionalized carbon nanotubes in drug design and discovery. *Acc Chem Res* **41**, 60-68.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J. and Glockner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188-7196.

- Ramette, A. (2009) Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. *Appl Environ Microbiol* **75**, 2495-2505.
- Raska, A., Chorvatova, M. and Wanner, J. (2006) The role and significance of extracellular polymers in activated sludge. Part I: Literature review. *Acta Hydrochim Hydrobiol* **34**, 411-424.
- Rivas, G.A., Rubianes, M.D., Rodriguez, M.C., Ferreyra, N.F., Luque, G.L., Pedano, M.L., Miscoria, S.A. and Parrado, C. (2007) Carbon nanotubes for electrochemical biosensing. *Talanta* **74**, 291-307.
- Smart, S.K., Cassady, A.I., Lu, G.Q. and Martin, D.J. (2006) The biocompatibility of carbon nanotubes. *Carbon* **44**, 1034-1047.
- Theron, J., Walker, J.A. and Cloete, T.E. (2008) Nanotechnology and water treatment: applications and emerging opportunities. *Crit Rev Microbiol* **34**, 43-69.
- Wagner, M., Loy, A., Nogueira, R., Purkhold, U., Lee, N. and Daims, H. (2002) Microbial community composition and function in wastewater treatment plants. *Antonie Van Leeuwenhoek* **81**, 665-680.
- Warheit, D.B. (2006) What is currently known about the health risks related to carbon nanotube exposures? *Carbon* **44**, 1064-1069.
- Weber, S.D., Ludwig, W., Schleifer, K.H. and Fried, J. (2007) Microbial composition and structure of aerobic granular sewage biofilms. *Appl Environ Microbiol* **73**, 6233-6240.
- Wiesner, M.R. (2006) Responsible development of nanotechnologies for water and wastewater treatment. *Water Sci Technol* **53**, 45-51.
- Yin, Y., Zhang, X., Graham, J. and Luongo, L. (2009) Examination of purified single-walled carbon nanotubes on activated sludge process using batch reactors. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **44**, 661-665.

Assessing the Transport and Fate of Effluent Organic Nitrogen in the Connecticut River and Long Island Sound Using Mass-Mapping Proteomics Technology

Basic Information

Title:	Assessing the Transport and Fate of Effluent Organic Nitrogen in the Connecticut River and Long Island Sound Using Mass-Mapping Proteomics Technology
Project Number:	2009MA186B
Start Date:	4/1/2009
End Date:	5/31/2010
Funding Source:	104B
Congressional District:	MA-001
Research Category:	Water Quality
Focus Category:	Water Quality, Acid Deposition, Nutrients
Descriptors:	
Principal Investigators:	Chul Park

Publications

1. – Westgate, P. and Park, C. (In revision) Evaluation of proteins and organic nitrogen in wastewater treatment effluents. Environmental Science and Technology.
2. – MS Thesis: Characterization of Proteins in Effluents from Three Wastewater Treatment Plants that Discharge to the Connecticut River, MS Environmental Engineering, Aug 2009
3. – Westgate, P. and Park, C. (2009) Evaluation of Effluent Proteins: Towards Characterization of Effluent Organic Nitrogen, Water Environment Federation 82nd Annual Technical Exhibition and Conference (WEFTEC 2009), October 2009, Orlando, FL.

Problem and Research Objectives

Significant efforts have been made to reduce the nitrogen released from wastewater treatment plants (WWTPs) and this has been mainly achieved by upgrading the facility for enhanced nitrogen removal through nitrification and denitrification. Though these processes are effective for removing inorganic nitrogen (ammonia and nitrate) organic nitrogen remains little changed, presumably due to its recalcitrant nature, which leads to organic-N becoming a substantial fraction of the N in the final effluent. Thus, one major issue with effluent organic-N is whether it degrades and becomes bioavailable in receiving waters.

Our research group proposed a unique research plan that bases on proteomics analysis to characterize effluent proteins and to assess their fate in receiving waters. Better characterization of effluent proteins and better understanding of their fate in receiving water are critical as proteins comprise a major fraction of effluent organic nitrogen. Furthermore, as proteins can be characterized at a molecular level, profiling of effluent proteins and tracking them in a well defined laboratory bioassay (that mimics receiving waters) will further enable us to determine the fate of proteins, thus a significant fraction of organic-N, in receiving waters. The specific objectives of this project are as follows:

- Determine and characterize proteins in wastewater effluents from major dischargers to the Connecticut River, thus to Long Island Sound.
- Perform a laboratory bioassay and apply proteomics analysis before and after the bioassay to evaluate the bioavailability of effluent proteins in receiving waters.

Methodology

This research has been conducted in two phases: 1) collecting effluents samples and characterizing effluent proteins from various wastewater treatment plants and 2) performing a laboratory bioassay to investigate the fate of effluent proteins and organic nitrogen in receiving waters.

Collection of samples. Primary and secondary effluents were collected from three wastewater treatment facilities that discharge to the Connecticut River in Western Massachusetts. The Northampton and Amherst facilities use conventional activated sludge while the Springfield Regional Wastewater Treatment Facility uses the Ludtzac Ettinger process to treat their

wastewater. Samples were collected in plastic containers kept on ice until processed later the same day. Total suspended solids (TSS), volatile suspended solids (VSS) and chemical oxygen demand (COD) measurements were taken the day of collection, while samples were frozen for later measurement of protein, total nitrogen, ammonium, nitrate and nitrite. The secondary effluent from each sample was also separated through a 0.45 μm filter.

Quantification of Proteins in Effluent. The Frølund adaptation of the Lowry method (1995) was mainly used to quantify proteins in wastewater effluents. This method can account for the interference of humic compounds in protein measurement. This method, however, often produced falsely negative protein concentrations for unconcentrated effluent samples. Thus, in this research the quantity of proteins in unconcentrated effluent was measured using the original Lowry method, while the protein concentration in ammonium sulfate concentrated samples were measured using the Frølund adaptation of the Lowry method. Light absorbance was measured with the Thermo Spectronic Genesys 10 UV Spectrophotometer (Thermo Spectronic, Madison, WI, USA) and concentrations calculated from a standard curve created from 0, 10, 25, and 50 mg/L BSA standards.

Ammonium Sulfate Precipitation. In order to visualize proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), secondary effluent and secondary filtered effluent were concentrated with 50% ammonium sulfate. The appropriate mass of ammonium sulfate was combined with 150 ml of primary effluent, 1.2 L of secondary effluent, and 2.2 L of 0.45 μm filtered secondary effluent, in 500 ml centrifuge bottles and one glass pyrex bottle (for 1 L of the 0.45 μm filtered secondary effluent). Precipitation procedures were conducted on ice for more than 12 hours, followed by centrifuging at 11,730 g for 45 minutes. The precipitate was re-suspended in a known volume in phosphate buffered saline (PBS: 10mM NaCl, 1.2mM KH_2PO_4 , 6.0mM Na_2HPO_4), then dialyzed extensively in the same buffer with multiple changes at 4°C using a 6-8 kDa cellulose membrane.

Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis. The SDS-PAGE was performed according to the method of Laemmli (1970). Ammonium sulfate concentrated samples were prepared for size separation on polyacrylamide gels by incubating at approximately 95°C for at least 10 minutes with a 3.3x sample buffer consisting of XT Mops sample buffer and a reducing agent (Bio-Rad, Hercules, CA, USA). Some samples were heat concentrated for up to one hour. Following heat concentration, samples were centrifuged at

12,000 rpm for 3 minutes and the supernatant was used for SDS-PAGE. Prepared samples were loaded onto pre-cast Criterion XT 4-12% gradient gels (Bio-Rad, Hercules, CA, USA) and separated on the gels by a current of 80V for 20 minutes, followed by 100V for two hours. After electrophoresis, gels were stained with silver nitrate or coomassie brilliant blue using Bio-Rad's Silver Stain Kit or Bio-Safe stain (Bio-Rad, Hercules, CA, USA). Gel images were digitally recorded using a CanoScan 8800F desktop scanner (Canon, Tokyo, Japan).

Zymogram analysis. Samples were subjected to zymogram analysis to determine if they contained active proteolytic enzymes. Enzyme activity was determined by separating proteins using electrophoresis in a casein infused gel (Bio-Rad, Hercules, CA, USA). Before electrophoresis the samples were combined with zymogram buffer (Bio-Rad, Hercules, CA, USA) and centrifuged at 12,000 rpm for 3 minutes; the supernatant was used for the zymogram analysis. Gel images were digitally recorded using a CanoScan desktop scanner (Canon, Tokyo, Japan).

Chemical Analysis. Total protein in each of the effluents, both raw and concentrated with ammonium sulfate, was measured using the Lowry method (1951) and determined with a calibration curve generated with bovine serum albumin (Fisherbrand Scientific, Pittsburg, PA, USA). On the day of sample collection, COD, TSS and VSS were measured for primary and secondary effluents according to Standard Methods (2005). COD was measured for secondary effluent filtered through a 0.45 μm filter, as well. Light absorbances for COD tests were determined using the ThermoSpectronic Genesys 10 UV Spectrophotometer (Thermo Spectronic, Madison, WI, USA) and concentrations calculated from a standard curve using 0, 10, 75, and 150 mg/L KHP standards.

Nitrogen species. Total nitrogen concentrations in primary and secondary effluent, and 0.45 μm filtered secondary effluent were determined using the persulfate method (Hach, Loveland, CO, USA) and confirmed using a Shimadzu TN analyzer (Shimadzu TOC-VCPH with TNM-1, Shimadzu North America, SSI Inc., Columbia, MD, USA). Ammonium, nitrate and nitrite ions in the solution phase (<0.45 μm) of primary and secondary effluents were measured using a Metrohm ion chromatograph (Metrohm, Herisau, Sz). Organic nitrogen was estimated by subtracting the sum of the nitrogen ions from the total nitrogen.

Laboratory bioassay. Several incubation conditions have been tested in an effort to establish the final protocol of laboratory bioassay for this research. Some earlier incubation

conditions included: 1) no mixing, 2) intermittent mixing, and 3) continuous mixing of the bioassay bottles. Each set of bioassay included a killed control set to make sure that changes in proteins and organic nitrogen during the bioassay were caused by biological activity. The earlier experiments also tested different dilution sets between effluents and the Connecticut River water at 1:9 and 5:5. For this laboratory bioassay, effluents from Springfield Regional Wastewater Treatment Facility were mainly used. The final bioassay protocol includes following conditions:

- 1) Filter river water using 100 μm filter.
- 2) Use 5:5 ratio for river water and secondary effluent for incubation.
- 3) Perform a separate bioassay on dissolved and whole fraction of secondary effluents.
- 4) Provide continuous mixing during the incubation.
- 5) Place the incubation bottles under natural sunlight conditions.

During this laboratory bioassay we also performed Tangential Flow Filtration (TFF) to effectively concentrate the sample before conducting all protein related analysis. Following the concentrating stage, proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Some effluent concentrate samples were sent to another laboratory for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis to identify the proteins. In addition, various effluent parameters such as TSS, total organic carbon (TOC), cations, anions, and inorganic nitrogen species, as described earlier, were also measured.

Principal Findings and Significance

The current project has revealed several important and new findings regarding effluent organic nitrogen and effluent proteins. The most important finding of this research is that facilities with more advanced N removal processes contain a greater amount of organic nitrogen with a higher diversity of proteins and active enzymes in their final effluents. This indicates that effluent organic-N in advanced wastewater treatment plants differs significantly than that found in conventional wastewater treatment systems. The full effects of released enzymes and proteins in the receiving ecosystem are unknown, but are thought to increase the bioavailability of natural organic matter and to modulate nutrient cycling in the receiving water. As advanced removal of N becomes mandatory in wastewater treatment, it is imperative that this process and potential unintended consequences be fully understood.

The consistent differences between effluent protein profiles from each of the treatment plants investigated further suggest that effluent proteins and enzymes could serve as “fingerprints” of distinct wastewater treatment works and provide a means to track their fate in receiving waters. This fingerprinting concept was employed in the bioassay during the later part of this research and partially used to track the fate of preselected proteins during the incubation. Other major findings and significance of this research can be further summarized as below.

- The research revealed that proteins are significantly correlated with organic nitrogen in effluent from each of the wastewater treatment facilities, demonstrating the significance of protein molecules in effluent organic nitrogen. We believe that there has been no precedent study that has found this relationship or addressed the issue of proteins being an indication, or representative, of effluent organic nitrogen.
- We believe that this is also the first study showing changes in protein profiles, at a molecular level, across processes in a wastewater treatment plant. The results from this approach allowed direct evidence that some influent wastewater proteins persist through the wastewater treatment process and some of these proteins are actually active proteolytic enzymes.
- The research also showed that some bacterial proteins and enzymes that are generated during a biological treatment do indeed end up in the secondary effluent, as so-called soluble microbial products (SMP).
- The finding of active enzymes and proteins in filtered effluent samples is also important to note since the addition of a filtration process to a facility, such as microfiltration, is not likely to improve the capture of these potentially biological compounds.
- Because of these protein results, we gained new knowledge that different treatment works release different sets of proteins (thus, organic nitrogen) and proteolytic enzymes: this information could not be achieved by simple quantitative data or conventional size fractionation techniques. These results are important in that we do not know how these proteins and enzymes behave in the receiving water and what ecological and environmental impacts they may have. The study has provided us a chance to better characterize and identify effluent proteins and enzymes, which can be tracked thoroughly in well defined laboratory bioassays or even directly in the receiving water.

- The bioassay that was designed to mimic the reaction of wastewater effluents in natural receiving waters requires natural sunlight and continuous and uniform mixing during the incubation.
- Concentrations of both inorganic nitrogen (ammonia and nitrate) and organic nitrogen changed greatly during the bioassay, indicating a degradation of organic nitrogen and release of newly generated organic nitrogen.
- After the incubation, new soluble protein bands were detected along with a substantial increase in algal biomass. This observation suggests that receiving waters utilized available effluent nitrogen, including organic nitrogen, and release of proteins from grown algal biomass contributed to a new set of dissolved organic nitrogen remaining in the bioassay.

Characterization of Flow and Water Quality of Stormwater Runoff from a Green Roof

Basic Information

Title:	Characterization of Flow and Water Quality of Stormwater Runoff from a Green Roof
Project Number:	2009MA199B
Start Date:	4/1/2008
End Date:	3/30/2009
Funding Source:	104B
Congressional District:	3
Research Category:	Water Quality
Focus Category:	Water Quality, Hydrology, Water Quantity
Descriptors:	None
Principal Investigators:	Paul Mathisen, Suzanne LePage

Publications

1. LePage, Suzanne “An investigation of the hydrologic and geochemical processes contributing to green roof performance”, MS Thesis, Worcester Polytechnic Institute, completed in May 2010
2. LePage, Suzanne and Paul Mathisen, “An investigation of the hydrologic and geochemical processes contributing to green roof performance.” presentation at the American Society of Civil Engineers (ASCE) World Environmental and Water Resources Congress 2010. Providence, Rhode Island. May 20, 2010.
3. LePage, Suzanne, “An Investigation into the Water Quality Impacts of a Green Roof”, poster presentation at WPI Graduate Achievement Day, Worcester Polytechnic Institute, Worcester, MA on March 31, 2010.
4. LePage, Suzanne, “An Investigation into the Water Quality Impacts of a Green Roof”, poster presentation at the 7th Annual Water Resources Research Conference, Amherst, MA on April 8, 2010

Problem and Research Objectives

Low Impact Development (LID) techniques for site design are increasingly being utilized to mitigate the negative impacts associated with stormwater runoff, and green roofs are one such application. The ability of green roofs to reduce the total and peak volumes of stormwater runoff has been fairly well documented, but performance varies in different climate zones, and there is limited information available regarding green roof effectiveness in New England, a region whose weather patterns are notoriously variable from season to season and often even day-to-day. Additionally, there are questions regarding the impact that green roofs have on water quality. While there seems to be a general consensus that green roofs will leach phosphorus, and sometimes other contaminants, into stormwater runoff within the first few years after installation, it is assumed that this phenomenon will not continue after the green roof vegetation has been established. However, it is still unclear whether or not this assumption is valid, and very few research projects have attempted to provide the necessary insight into the hydrologic and chemical processes that are contributing to this question.

Accordingly, the goals of this research were to provide insight into the hydrologic and geochemical processes that contribute to green roof performance. The specific objectives included the following:

- Determine the effectiveness of a green roof in attenuating stormwater flow
- Document a green roof's impact on water quality, specifically regarding phosphorus
- Identify the key components of the processes that are likely leading to the highest variability in observed water quality parameters – hence, the highest potential that a change in design could lead to significant improvements

In addition to providing insight into green roof performance, these objectives are intended to provide a foundation for future research efforts to explore the behavior of phosphorus in soil solutions and its implications for stormwater treatment.

Methodology

The methodology for achieving the project objectives combined field monitoring and laboratory testing and analyses to characterize the quality of runoff associated with the Nitsch/Magliozzi Green Roof, an extensive green roof located on top of a new residence hall at WPI. This roof, which was donated to enhance the sustainability of the building and foster continued research and education, provided the context for this project. The research tasks included field monitoring of the roof drainage, laboratory testing of green roof panels under simulated rainfall conditions, bench-scale testing of phosphorus desorption from the growing medium, and laboratory analyses of water quality, soil characteristics, and plant phosphorus content. The methodology provided a basis for gaining a better understanding of the relationship between rainfall and runoff volumes, phosphorus sorption/desorption in the growing medium, and plant uptake processes.

The field monitoring program focused on the seasonal variations of water quality throughout a complete growing season. Two flow meters and sampling ports have been installed within the storm drain system of the residence hall: one to measure drainage from the green roof; and the other to measure drainage from the “non-green” portion of the roof. Using these sampling ports, a total of 25 grab samples from each roof were collected and analyzed between June 2009 and April 2010.

The laboratory testing and analysis program was developed to characterize both the stormwater retention performance and water quality characteristics of the green roof. For this program, two (2) of the green roof panels were brought into a

greenhouse maintained at WPI by WPI's Biology Department. A stand was constructed which allowed for the application of simulated rainfall and collection and measurement of runoff for each panel. For water quality monitoring, runoff from each panel was detoured through a flow-through device attached to a water quality monitoring sonde (Hach MS5 Hydrolab unit), and grab samples were collected at key points during the simulated storms. The Hach MS5 Hydrolab units, one of which was acquired using support from this grant, were important components of this system. Soil and plant samples were also collected, and additional bench-scale tests were completed to characterize the nature of the phosphorus desorption from the media. All samples of water, plant, and soil were analyzed in the water quality laboratory in WPI's Department of Civil and Environmental Engineering.

Principal Findings and Significance

In regards to storm-water flow attenuation, results from the greenhouse experiments showed that green roof performance was more effective for smaller storms, and was influenced by the soil properties (including field capacity and moisture content). Overall, these results are consistent with the published literature. For example, the reduced retention capacity observed during higher flow conditions is a common trend that has been reported for extensive green roof performance. At high rainfall intensities, the field capacity of the green roof panels is quickly exceeded, and the thin layer of the extensive green roof design does not provide much storage capacity. However, while the growing medium did not provide much storage during the heavier simulated rain event, the green roof vegetation's ability to rapidly uptake water when it becomes available did provide a stormwater retention benefit. The improved performance during the lower flow conditions was found to be more heavily influenced by the soil than by the plants. The highest retention rates in the simulated rain events were observed when the antecedent moisture content was low (9-11%). In contrast, for a light rain event, the moisture content of the soil at the beginning was the highest of all tests (26%), and the green roof panels retained only 38% of the influent volume, despite the fact this simulated storm used the smallest volume of water of all simulated events. Clearly the growing medium's field capacity is a critical design factor that is indicative of green roof performance.

In regards to water quality, phosphorus concentrations observed in runoff during greenhouse tests, were similar in magnitude to the concentrations in samples collected from the green and white roofs, which were relatively high. These high concentrations were found to be primarily influenced by phosphorus in the growing medium, which quickly desorbs in response to flushing due to storm events. For all greenhouse tests, the phosphorus concentrations (and other constituents as well) showed up in the "first flush" runoff samples and continued to increase throughout the duration of the storm and after the simulated rainfall had stopped. This trend was consistently observed in all storms, regardless of their size or intensity. These results indicate that the desorption of phosphorus from the growing medium happens quickly, and the soil is not rapidly depleted of its phosphorus content. Also, the green roof panel whose soil was higher in phosphorus concentration (Stand B) also produced runoff with higher phosphorus concentrations than the other panel tested in the greenhouse (Stand A). Meanwhile, the growth of green roof plant material and its associated nutrient uptake processes did not appear to reduce the amount of phosphorus that ended up in the runoff. These results confirm that the growing medium is the source of phosphorus in runoff. However, while a bench-scale laboratory experiment indicated that phosphorus levels in runoff may decrease over time, the rate of desorption is not constant and cannot be easily predicted. Additional investigations will be needed in order to predict the long-term impact of a green roof on phosphorus loading.

With consideration to the design of green roofs, a number of key processes/factors were defined. First, this research showed that soil storage and soil moisture content are particularly important considerations with respect to green roof performance. Soil storage is heavily influenced by antecedent moisture content, and soil moisture content is a function of both weather, which cannot be controlled, and plant variety, which generally can be controlled. These results should help future designers determine whether the weather patterns in a particular location where a green roof is being considered will be hindrance to the effectiveness of a green roof. Areas experiencing significant amounts of rainfall that may keep the soil at field capacity would not be a good choice. However, selecting plant varieties that quickly uptake water, such as sedum and delosperma, will provide the ability to regenerate the holding capacity of the growing medium and will improve the performance of green roofs. Also, efforts should be taken to engineer new soil media that will maximize the field capacity of green roof designs. Second, the research showed that the leaching of phosphorus from the growing medium must be taken into consideration when designing a green roof. Previous studies have made assumptions that the leaching of phosphorus will decrease over time and many have predicted that the phenomenon will only occur for a few years after installation. However, the results of this study indicate that this assumption may not be valid. The long-term phosphorus loading resulting from a green roof may continue longer than previously assumed. Until additional investigations are conducted to develop a prediction model, the impacts of a green roof must be given careful consideration if being installed where phosphorus levels in stormwater are a concern. Further, it is recommended that phosphorus use be minimized in the growing medium. The typical green roof plant varieties, such as those studied here, do not appear to uptake very much of this nutrient, even in their first few establishment years.

In general, these results provide a basis for developing improved predictions of storm-water retention performance, gaining deeper insight into the transformations of phosphorus in the green roof panels, and developing a process by which continued, in-depth study could be performed under controlled laboratory and field conditions.

Publications and Conference Presentations:

The results summarized in this summary report are also described in more detail in a Master of Science thesis prepared by Suzanne LePage in partial fulfillment of the requirements for her Master of Science degree at Worcester Polytechnic Institute. The results were also disseminated via a presentation at the EWRI Congress of the American Society of Civil Engineers (ASCE), and via a poster presentation at the Annual Water Resources Conference in Amherst, MA. The details of these items are included in the following listing:

Publications and Conference Presentations

Dissertations/MS Theses

LePage, Suzanne, 2010. An investigation of the hydrologic and geochemical processes contributing to green roof performance. MS Thesis, Worcester Polytechnic Institute, completed in May 2010

Other Publications and presentations

LePage, Suzanne and Paul Mathisen, 2010. *An investigation of the hydrologic and geochemical processes contributing to green roof performance.* Presentation at the American Society of Civil Engineers (ASCE) World Environmental and Water Resources Congress 2010. Providence, Rhode Island. May 20, 2010.

LePage, Suzanne, 2010. *An Investigation into the Water Quality Impacts of a Green Roof*. Poster presentation at WPI Graduate Achievement Day, Worcester Polytechnic Institute, Worcester, MA on March 31, 2010.

LePage, Suzanne, 2010. *An Investigation into the Water Quality Impacts of a Green Roof*. Poster presentation at the 7th Annual Water Resources Research Conference, Amherst, MA on April 8, 2010 (see note below on award).

Student Support

This project provided equipment that assisted the research program of 1 graduate student, Suzanne LePage, at Worcester Polytechnic Institute. The matching funds designated in this grant included the student time and effort as part of an independent study project (ISP) completed in the fall of 2009.

Notable Achievements and Awards

2nd Place award for Poster entitled "*An Investigation into the Water Quality Impacts of a Green Roof*", which was presented at the Seventh Annual Water Resources Research Conference Poster Contest on April 8, 2010

Acid Rain Monitoring Project (ARM)

Basic Information

Title:	Acid Rain Monitoring Project (ARM)
Project Number:	2009MA211B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	1st
Research Category:	Water Quality
Focus Category:	Acid Deposition, Surface Water, Water Quality
Descriptors:	None
Principal Investigators:	Marie-Francoise Walk, Paula Sturdevant Rees

Publications

There are no publications.

The Acid Rain Monitoring project continued for the 8th consecutive year following an 8 year hiatus which was preceded by 10 years of consecutive sampling. Approximately 150 sites (mostly streams) were sampled by volunteer collectors and tested for pH and alkalinity by volunteer labs.

Of those, 26 long-term sites were analyzed for color, SO₄, NO_{3-N}, Cl, Ca, Mg, K, Mn, Fe, Cu, Si, and Al. New data on lakes and streams collected over the past 8 years will aid in evaluating whether or not changes result from passage of state and federal Clean Air Act revisions. These analyses are in process and should provide important evidence in the ongoing debate about clean air standards.

The more than 43,000 records of water chemistry for Massachusetts' lakes and streams, now covering 1983-2010, are posted on a web site in a searchable and downloadable form so that additional data analyses specific to the user may be conducted (<http://umatei.resuo.ads.umass.edu/armproject1/>).

Students Supported

- 1 BS student in Economics at UMass Amherst
- 1 BS student in Mathematics at UMass Amherst
- 1 BS student in Chemical Engineering at UMass Amherst
- 1 PhD student in Chemistry at UMass Amherst.

Characterizing and Quantifying Recharge at the Bedrock Interface

Basic Information

Title:	Characterizing and Quantifying Recharge at the Bedrock Interface
Project Number:	2009MA213G
Start Date:	9/1/2009
End Date:	8/31/2012
Funding Source:	104G
Congressional District:	1st
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Water Supply, Water Quantity
Descriptors:	None
Principal Investigators:	David Boutt, Stephen B. Mabee

Publication

1. Characterizing Groundwater Recharge Across the Surficial/Bedrock Interface. Bevan, L.B., D.F. Boutt, S.B. Mabee. Massachusetts Water Research Resource Center Annual Conference. April 8, 2010. (Poster session).

Problem and Research Objectives

Evaluating the sustainability of fractured bedrock as a groundwater resource and understanding the environmental impacts of water withdrawals from the bedrock on nearby streams, wetlands, ecosystems and unconsolidated aquifer systems requires an estimate of the recharge and an understanding of the advective flux across the bedrock –overburden boundary. Few published studies address this issue with direct measurement (Rodhe and Bockgard, 2006, White and Burbey, 2007) while others use tracers (e.g. Rugh and Burbey, 2008) and numerical models to study the distribution of groundwater flow in the soil and bedrock (eg., Harte and Winter, 1995; Tiedeman et al., 1998). However, quantifying the flux of water between the overburden and bedrock remains one of the major sources of uncertainty in numerical models (Lyford et al., 2003). The only long term monitoring well that is screened in bedrock in Massachusetts (Figure 1) shows an interesting downward trend in hydraulic head over a period of almost 20 years while the hydraulic head from a nested piezometer in the surficial material above the bedrock piezometer does not show a similar trend. Understanding the dynamics of how systems like these interact is fundamental to improving our ability to manage and regulate these important resources.

The objectives of this project are to evaluate water flux across the overburden-bedrock interface under ambient and stressed conditions and to estimate its hydraulic conductivity in three typical hydrogeologic settings in the glaciated terrain of eastern Massachusetts. The hydrogeological conditions that will be examined include thick till overburden, thin till-shallow bedrock and coarse-grained stratified deposits. The work will be conducted in the Assabet River watershed because this watershed has previously been modeled by the USGS (DeSimone, 2004). The project complements past and ongoing work by the USGS in the New England region that evaluates water availability and the impacts of pumping on shallow aquifers and riparian systems (eg., DeSimone et al., 2002, DeSimone, 2004; Carlson et al., 2008). The proposed project is also designed to complement a project underway by the USGS Water Science Center in Northborough, MA that is assessing the factors affecting bedrock well yields in the Nashoba terrane. Many of these projects benefited from several years of cooperation between the Office of the Massachusetts State Geologist, University of Massachusetts, USGS and the MA Department of Environmental Protection.

The expected outcome of this work is a clearer understanding of the groundwater flux across the overburden-bedrock boundary and how the coupled systems respond to seasonal changes, individual recharge events and potential stresses due to pumping. Acquisition of these data will provide a basis for calibrating numerical models that investigate the effects of groundwater withdrawals (both surficial and bedrock) on stream baseflows and ecosystems.

Methodology

Site Selection

Well sites will be chosen in the context of the existing groundwater flow model by the USGS that meet the following characteristics: 1) lie in areas where the Nashoba formation outcrops or subcrops, 2) are covered by till or in an area of exposed bedrock, 3) are relatively close to outcrop data collection stations and bedrock wells studied previously, and 4) are in a model predicted recharge area.

At each location we will use (where applicable) 2D seismic refraction and resistivity surveys to determine the depth to bedrock along a number of cross sections. General topographic surveys will be performed and maps created for each well site.

Site Instrumentation

Each well site will be instrumented with the following: 1) a precipitation gage including air temperature, 2) soil moisture probes at 0.1, 0.2, 0.3 m depth, 3) a multilevel well screened at 2 locations in the surficial materials and 2 screened locations in the bedrock, 4) pressure transducers and thermocouples at each of the borehole screened locations. Precipitation will be measured with Texas Electronics Rain Gage (TE525-L) with air temperature being measured with a Campbell Scientific 107-L Temperature Sensor. The precipitation gage has a 0.254 mm tip resolution and a 15 cm orifice. Soil moisture will be measured at specified depths using the CS616-L water content reflectometer by Campbell Scientific. The probe uses time-domain measurement methods to measure the volumetric water content from 0% to saturation at a resolution of 0.1% volumetric water content. Rods having lengths of 30 cm will be buried to the specified depths.

Six-inch boreholes will be drilled through the overburden and at least 10 m into the bedrock. Split spoon samples will be taken at 2 m intervals in the surficial materials and the bedrock will be cored using a NX size core barrel. Wells will be furnished with the Solinst CMT multi-level sampling system that allows a maximum of 7 discrete measurement locations within a single 6 cm pipe. The exact depth of the borehole screened area (we envision 0.25 m screened regions) will be determined based on site characteristics, such as depth to water table and thickness of till. The Solinst system requires the isolation of individual screened regions using bentonite clay. Care will be taken to make sure that regions are hydraulically isolated. Hydraulic head and water temperature will be measured at each screened region with a 35/D Druck pressure transducer (designed to fit in the 3/8" diameter port of the Solinst CMT) and a Campbell Scientific 107-L Temperature Sensor, respectively.

Data Collection

Data collection will take place over at least an 18 month time period starting in the late summer of 2010 and ending in the summer of 2011. At each site 13 separate pieces of data (precipitation, air temperature, 3 soil moisture contents, 4 hydraulic heads, and 4 water temperatures) will be recorded at 15 minute intervals. At each site all thirteen channels will be connected to a Campbell Scientific CR-1000 data logger equipped with an additional compactflash storage card with a capacity of 1GB. At 15 minute intervals this consists of 1248 measurements over the period of 1 day at each site. Bi-weekly visits to the field sites will be made to ensure proper functioning of the data collection efforts and to retrieve data. The hydraulic response of both the surficial and bedrock system to recharge events will be measured using the data specified above. Historically water levels in these materials reach their maximum during the late spring and minimum during early fall periods. In summary, field data collected during spring and fall hydrologic regimes will consist of time-synced hydraulic head measurements at multiple vertical locations at each of the study sites.

Data Reduction, Interpretation, Modeling

Data collected from the deployed instruments will be time-synced to ensure proper data analysis. Self consistency in the data sets will be determined based on an assumed relationship between precipitation and soil moisture. Precipitation and air temperature data will be compared with climate data from the nearby Bedford and West Medway, MA, weather stations (about 5 and 15 mi, respectively, from the basin). These stations were found to be representative of conditions in the study area by DeSimone (2004). Ground water level fluctuation and soil moisture will be analyzed to examine relationships and to determine the significance of vertical versus horizontal movement of water at the chosen sites. Hydraulic head distributions in the till and the bedrock will be examined for evidence of vertical

transmission of water. Time series analysis (Eltahir and Yeh, 1999) will examine corresponding lag times between precipitation, soil moisture, and ground water level at various depths. Summary data sets will be developed for each site.

The hydraulic data together with the temperature data will be used to build 1-dimensional coupled saturated-unsaturated water flow and temperature models using the general finite-element method solver COMSOL multiphysics (Fleming, 2009). Hydraulic and heat boundary conditions will be provided by the field measurements. The integration of both head and temperature data into a model such as this reduces the degrees of freedom and will allow an estimation of the vertical flux across various boundaries (water table, till-bedrock interface) present in the model (Anderson, 2005) that is constrained by observations of head and temperature. A split data approach will be used to calibrate the model reserving the other half of the data to predict water flux under different hydraulic conditions throughout the data set. The hydraulic properties of the bedrock-till interface will be the main calibration parameter. For each a site a detailed quantitative model will be developed to understand the movement of water from the surface through to the bedrock. Recharge rates to the till (where present) will be estimated and the amount of leakage (recharge) to the bedrock will also be determined using the data collected. Results from this analysis will yield a detailed set of water fluxes for the hydrologic periods during data collection. The hydraulic properties of the bedrock-till interface will be an important calibration and model result.

Synthesis and Modeling

Observations of water and energy transport (i.e. temperature) will be summarized and compared to site characteristics such as till thickness, till composition, topographic setting, hydraulic heads of till and bedrock, bedrock hydraulic properties, distance to mapped structural features, and characteristics of fracture networks mapped in nearby outcrops. Recharge rates will be calculated and summarized for each site at monthly intervals. Relationships between the hydraulic conductivities determined at the bedrock-till interface and field observations will be made. Using the calibrated 1-dimensional models, we will perform some preliminary estimates of the impact of bedrock water withdrawals on the flux of water across the boundaries at the different sites. Fluid withdrawal will be simulated by modeling a pumping well placed into the bedrock at a specified depth. Results from the models will be used to infer the impact of further use of bedrock water supplies on surficial water supplies and recharge rates into the bedrock.

Principal Findings and Significance

Work on this project began on September 1, 2009. No findings have been developed yet as most of the effort to date has focused on site selection and preparing for field work in the summer of 2010. Here is an update on what has been completed to date.

1. A GIS based map was developed for the Assabet River watershed. The map includes spatial distribution of soil types, surficial geology, bedrock geology, land use types and existing bedrock monitoring well locations. This map was used to assess possible study site locations, data collection station locations, as well as developing a soil sampling program.
2. Study site selection criteria were developed and locations within the Assabet River catchment were evaluated. Based upon selection criteria, Gates Pond Reservoir, Berlin, MA was selected.
3. Access agreements between the Geosciences Department at the University of Massachusetts, Amherst and the Town of Hudson, Department of Public Works (the controlling entity of Gates Pond) were negotiated and approved.

4. Access agreements with the lessee of farmland located adjacent to Gates Pond Reservoir (231 Sawyer Hill Rd., Berlin, MA) are still under negotiation. Should negotiations prove fruitless, adequate contingency plans have been developed with the Town of Hudson, MA Department of Public Works.
5. Instrumentation and data collection methods for measuring aquifer response to precipitation inputs were researched and selected. Instrumentation is currently being ordered and will be installed in Summer 2010. The instrumentation will communicate with the Primary Investigators via cellular telemetry.
6. Preliminary soil samples were taken for a baseline grain size analysis. The analysis is currently in progress.
7. Geophysical methods for effectively evaluating the soil profile at Gates Pond Reservoir were evaluated and selected. Resistivity profiles will be taken using the University of Massachusetts, Amherst, Department of Geosciences' AGI Sting© R-1. Soil resistivity profiles will be performed in summer 2010.
8. A soil sampling regime and methods for characterizing the hydraulic properties of the site's soil were evaluated and selected. Soil sampling and hydraulic properties will be evaluated beginning summer 2010.
9. Statistical time-series analysis methods for establishing a hydraulic conductivity gradient across a surficial/bedrock interface were researched and tested for viability using MATLAB©. The data used for the statistical analysis was collected from a nested/bedrock piezometer located in Pelham, MA. The results of the statistical analysis helped determine the required sampling frequency of instrumentation that will be deployed in Summer 2010.
10. Bedrock wells at Gates Pond Reservoir were modified to accommodate down-hole instrumentation. Currently, two wells are equipped with Solinst LevelLoggers© and one BaroLogger© to gather baseline water level data.
11. Designs for down-hole fiber optic distributed temperature probes are currently being developed. The investigators are hopeful that all bedrock wells at the Gates Pond Reservoir will be equipped with fiber optic probes and will be functional by the end of Summer 2010.

Study area selection is complete and study design is on track and will continue through early Summer 2010. Liam B. Bevan, an M.S. student who will be performing the majority of the field and laboratory work for this project, began work in earnest beginning in the fall of 2009. His initial efforts will be in characterizing the study area catchment using in-situ and laboratory techniques and installing and maintaining instrumentation.

Publications and Conference Presentations

Bevan, L.B., Boutt, D.F., Mabee, S.B. 2010. *Characterizing Groundwater Recharge Across the Surficial/Bedrock Interface*. Massachusetts Water Research Resource Center Annual Conference. April 8, 2010. (Poster session).

No other publications at this time.

Student Support

Liam B. Bevan is fully supported by this project. He is pursuing an M.S. degree in geology in the Department of Geosciences at the University of Massachusetts, Amherst.

References

Anderson, M., 2005: Heat as a ground water tracer. *Ground Water*, 43(6), 951–968.

- Carlson, C.S., DeSimone, L.A., Weiskel, P.K., 2008, Simulated effects of year 2030 water-use and land-use changes on streamflow near the Interstate-495 corridor, Assabet and Upper Charles River Basins, eastern Massachusetts: U.S. Geological Survey Scientific Investigations Report 2008-5132, 108 p.
- DeSimone, L.A., 2004, Simulation of ground-water flow and evaluation of water-management alternatives in the Assabet River Basin, eastern Massachusetts: U.S. Geological Survey Scientific Investigations Report 2004-5114, 133 p.
- DeSimone, L.A., Walter, D.A., Eggleston, J.R., and Nimiroski, M.T., 2002, Simulation of ground-water flow and evaluation of water-management alternatives in the upper Charles River basin, eastern Massachusetts: U.S. Geological Survey Water-Resources Investigations Report 02-4234, 94 p.
- Eltahir, E.A.B., and Yeh, P.J.-F., 1999, On the Asymmetric response of aquifer water level to floods and droughts in Illinois, *Water Resources Research*, Vol. 35, No. 4, pp. 1199-1217.
- Fleming, B.J., 2009, Ground water-surface water interactions in the Deerfield River watershed, Charlemont, MA. M.S. Thesis, University of Massachusetts-Amherst.
- Harte, P.T. and Winter, T.C. 1995. Simulations of flow in crystalline rock and recharge from overlying glacial deposits in a hypothetical New England setting. *Ground Water*, v.33, no.6, pp.953-964.
- Lyford, F.P., Carlson, C.S. and Hansen, B.P., 2003, Delineation of Water Sources for Public-Supply Wells in Three Fractured-Bedrock Aquifer Systems in Massachusetts: U.S. Geological Survey Water-Resources Investigations Report 02-4290, 114 p.
- Rodhe, A. and Bockgard, N. 2006. Groundwater recharge in a hard rock aquifer: A conceptual model including surface-loading effects. *Journal of Hydrology*, v.330, pp.389-401.
- Rugh, D.F. and Burbey, T.J. 2008. Using saline tracers to evaluate preferential recharge in fractured rocks, Floyd County, Virginia, USA, *Hydrogeology Journal*, 16: 251-262.
- Tiedeman, C.R., Goode, D.J. and P.A. Hsieh. 1998. Characterizing a ground water basin in a New England mountain and valley terrain. *Ground Water*, v.36, no.4, pp.611-620.
- White, B.A. and Burbey, T.J. 2007. Evidence for structurally controlled recharge in the Blue Ridge Province, Virginia, USA. *Hydrogeology Journal*, v.15, pp.929-943.

Information Transfer Program Introduction

A significant portion of 104B funds retained at the Center supports the information transfer objective of 104B. Our main information transfer tool is the Annual Water Center Conference, initiated in 2003 by then Director David Reckhow. The conference provides an interdisciplinary forum for scientists, practitioners, and policy makers to discuss current critical water research, foster greater collaboration among scientists and practitioners, and strengthen the connection between research, education, and policy. Participants include researchers, stakeholders, and managers of water resources from academia, government, non-profits, and the private sector. The 7th Annual Water Resources Research Center Conference is described in the subsequent section. The Center publishes programs from all of our conferences on our website (<http://www.umass.edu/tei/wrrc/WRRC2004/WRRCconferences.html>).

The Center relies heavily upon the Internet for information transfer. Several of the Center's projects have significant Internet information transfer elements that are still in existence and utilized today. One of these funded through 104B is the Acid Rain Monitoring Project (ARM) (<http://umatei.tei.umass.edu/ColdFusionProjects/AcidRainMonitoring/>).

2009 Water Resources Conference

Basic Information

Title:	2009 Water Resources Conference
Project Number:	2009MA206B
Start Date:	3/1/2009
End Date:	2/28/2010
Funding Source:	104B
Congressional District:	1st
Research Category:	Not Applicable
Focus Category:	Water Quality, Water Quantity, Water Use
Descriptors:	None
Principal Investigators:	Paula Sturdevant Rees, Marie-Francoise Walk

Publications

There are no publications.

The Water Resources Research Center organized the seventh annual Water Resources Research Conference: Monitoring and Responding to Water Resource Challenges. While the conference took place in April, most of the work for this conference was accomplished in the reporting period. The Cooperative State Research, Education, and Extension Service New England Regional Program again cooperated in planning the conference. Seven co-sponsors helped underwrite the cost of the conference.

Thirty posters were presented and there were 32 platform presentations as well as a panel presentation in three concurrent sessions. The presentations were grouped into the following 12 sessions:

- Climate Change and Precipitation
- Climate Change and Water Resources Planning
- Climate Change and Habitat Vulnerability
- Coastal Issues
- Pathogens in Water
- Sustainable Water Resources
- Environmental Monitoring
- Harmful Algae Blooms
- Streamflow
- Stormwater Management
- Hydrology and Ecosystem Services
- Water/Energy Nexus

The Keynote Address was given by Cameron J. Brooks, PhD, Director, Solutions and Business Development, Big Green Innovations, IBM Corporation, on Smarter Water Management: Whether Too Much or Not Enough, the World Needs a Smarter Way to Think About Water.

152 people registered for the event, representing 13 colleges and universities, 16 companies, 14 governmental agencies, 10 non-profit organizations, and 2 municipalities.

Students supported by project

- 1 BS student in Mathematics at UMass Amherst
- 1 BS student in Chemical Engineering at UMass Amherst

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	6	0	0	0	6
Masters	3	1	0	0	4
Ph.D.	8	0	0	0	8
Post-Doc.	0	0	0	0	0
Total	17	1	0	0	18

Notable Awards and Achievements

Under the grant for "Bacterial Toxicity of Oxide Nanoparticles and Their Adhesion at Bacteria-Water Interface (2009MA177B)", graduate student Wei Jiang won a first place for her poster presentation at the 6th Annual Conference titled "Water Dependencies in New England"
http://www.umass.edu/psis/news/ne_water_conf.html

Under the grant for "Characterization of flow and water quality of stormwater runoff from a green roof (2009MA199B), MS student Suzanne LePage received a 2nd Place award for Poster entitled "An Investigation into the Water Quality Impacts of a Green Roof", which was presented at the Seventh Annual Water Resources Research Conference Poster Contest on April 8, 2010.

Publications from Prior Years