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CAREER: The Role of Microorganisms in Arsenic Contamination of Groundwater

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CAREER: The Role of Microorganisms in Arsenic Contamination of Groundwater

Project Participants

Senior Personnel

Name: MacRae, Jean
Worked for more than 160 Hours: Yes
Contribution to Project:
Directing graduate and undergraduate students in their research projects, contacting and working with area teachers to establish relationships and devise classroom activities for their high school and middle school classes, conducting experiments to determine and keep track of the strains being used in the lab, writing reports and working with members of the public on arsenic in drinking water issues

Post-doc

Graduate Student

Name: Lavine, Ingrid
Worked for more than 160 Hours: Yes
Contribution to Project:
Supported by a fullbright scholarship and then this grant, Ms Lavine conducted experiments to determine the carbon and electron acceptor usage of a strain (NP4) isolated from groundwater that could reduce arsenate. She also made enrichments from groundwater samples to try to isolate additional strains.

Name: Stowe, Jennifer
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms Stowe has developed a 16S rRNA-based FISH method to enumerate the NP4 strain in groundwater. She has used this and a similar assay that enumerated iron-reducing bacteria to determine the prevalence of these organisms in groundwater samples. She was supported during her first two semesters as a TA and has since been funded by this project.

Name: McCormick, Erin
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms McCormick has received supported from a USGS grant and departmental TA/RA positions in addition to summer support from this grant. She has been working on analytical techniques that will be used to measure the release of Arsenic from solids under a variety of conditions to determine which factors affect arsenic release from ground bedrock and model solids.

Undergraduate Student

Name: Tillotson, Jason
Worked for more than 160 Hours: No
Contribution to Project:
Assisted PI and graduate students in the lab over the first summer of the award and during the school year.

Name: Gagnon, Christine
Worked for more than 160 Hours: No
Contribution to Project:

Worked with the PI on educational aspects of the work.

Name: Ricupero, Krista

Worked for more than 160 Hours: Yes

Contribution to Project:

Krista has been working with Jenn Stowe and Jean MacRae on isolating additional arsenate-reducing bacteria from well water and on further characterizing the metabolic capabilities of an existing isolate. She has been supported on the grant as an undergraduate researcher.

Name: Farrington, Lynn

Worked for more than 160 Hours: No

Contribution to Project:

Lynn has worked with Erin McCormick on characterization of an arsenate-reducing bacterium from well water.

Name: Robertson, Seth

Worked for more than 160 Hours: No

Contribution to Project:

Honors student working on thesis project: culturing and characterization of a new arsenate reducing isolate from well water

Name: Livingston, Clarissa

Worked for more than 160 Hours: Yes

Contribution to Project:

Conducted experiments on the chemotaxis of arsenate reducing bacteria in concert with Jenn Stowe, PhD candidate

Name: Erker, Benjamin

Worked for more than 160 Hours: No

Contribution to Project:

Completed carbon source and electron acceptor survey for an arsenate reducing bacterium.

Name: Killip, Kyle

Worked for more than 160 Hours: No

Contribution to Project:

Worked with Graduate Student Jennifer Weldon on microbial community characterization of groundwater samples with different arsenic concentrations.

Name: Cobb, Jacquline

Worked for more than 160 Hours: Yes

Contribution to Project:

Working on arsenic sorption and desorption from iron-coated sand and the effect(s) of microorganisms on desorption rates.

Name: Trainor, Kevin

Worked for more than 160 Hours: No

Contribution to Project:

Studied arsenic adsorption to surfaces

Name: Sturrock, Alexander

Worked for more than 160 Hours: Yes

Contribution to Project:

Studied arsenic adsorption to surfaces and aided graduate student with amplification and cloning of ribosomal RNA genes from DNA isolated from groundwater

Technician, Programmer

Name: Wilson, Tiffany

Worked for more than 160 Hours: Yes

Contribution to Project:

Worked with the PI, undergraduate and graduate students on methods development and operations and maintenance of analytical

equipment. Also supported the project by making media and transferring cultures.

Other Participant

Research Experience for Undergraduates

Organizational Partners

University of Manchester

The PI worked with the Geomicrobiology group at the University of Manchester (with Professor Jonathan Lloyd) on the mobilization of arsenic from iron by Geobacter species. Graduate student Jennifer Weldon received some training in the Lloyd lab on molecular techniques and used their facilities to advance her research.

Other Collaborators or Contacts

Joanne Miller, Bangor High School teacher, worked with PI and a female undergraduate to prepare and deliver classroom activities on arsenic and groundwater quality for her chemistry classes. The PI visited the classes for three years.

PI has worked on programming with teachers in Old Town, Belfast and Ellsworth, ME but did not work in the classrooms herself at these schools.

Graduate student Jennifer Weldon worked with three area schools (one primary, one middle and one high school) on water related programming and treatment to remove arsenic.

PI and graduate students worked with Expanding Your Horizons organizers at UMaine - primarily through the Women's Resource Center to provide workshops at the Annual conferences.

Activities and Findings

Research and Education Activities:

The major objective of this research was to determine how microorganisms affect arsenic mobility in groundwater, and to identify factors that contribute to increased or decreased mobility. The educational objectives were to increase awareness of arsenic and groundwater drinking water issues more generally, by working with area schools, undergraduate and graduate level classes and making the research findings available to the public.

A combination of laboratory studies and field work has been employed while studying the dynamics of arsenic mobility in the groundwater environment. The primary field site was located in Northport, ME in an aquifer that has a gradient of arsenic concentrations from low in the upland recharge area to very high (upwards of 1300 ppb) in the low-lying discharge region. The maximum contaminant level for this toxic element is 10 ppb.

Enrichment of groundwater from one of the discharge wells with arsenic and lactate yielded an isolate labeled NP4 capable of respiration using arsenate (As(V)) as an electron acceptor. Phylogenetic analysis indicates this bacterium is from the Sulfurospirillum genus. Laboratory experiments showed it to be capable of using several water-soluble and some insoluble electron acceptors and a limited number of organic electron donors. It is also capable of growth autotrophically using H2 as electron donor and can fix nitrogen, which gives it the flexibility to live in various anaerobic and nutrient-limited conditions. These findings have been reported in one journal article and at four conferences.

A study of groundwater chemistry and microbiology using the fluorescence in situ hybridization (FISH) method revealed correlations between Sulfurospirillum NP4 and the As(III) concentrations, and Geobacter/delta proteobacteria and the total arsenic and total iron concentrations. These findings support a model of arsenic mobilization through the reduction of ferric iron-based binding sites on aquifer surfaces causing the release of arsenic adsorbed to the surface. Sulfurospirillum NP4 may be important in reducing As(V) to As(III) in the groundwater environment. These results have been reported in one journal paper and at four conferences.

Laboratory experiments on the effects of arsenate reduction on mobilization of arsenic from amorphous iron minerals have shown that

reduction of arsenate to arsenite by Geobacter uraniireducens results in an increase in the aqueous phase arsenic concentration. The form of arsenic recovered from the liquid phase was primarily As(III) while the arsenic was supplied to the solid phase as As(V). Results from these experiments are being included in a book chapter and comparative work using NP4 is being completed for inclusion with these results in a paper. The G. uraniireducens work has been presented at a conference.

A broader community analysis that then FISH analysis described above has also been completed to explore the relationships between groundwater chemistry and microbiology. In this study, two broad phylogenetic methods, rRNA intergenic space analysis (RISA) and cloning and sequencing of the 16S rRNA gene, were employed to characterize the microbial communities in four wells: two in the recharge area and two in the discharge area. These results confirmed the increase in delta-proteobacteria in the discharge area, which came largely at the expense of alpha- and gamma-proteobacteria and with a loss of overall diversity. These results have been presented at two conferences and are being prepared for submission for publication.

A field experiment in which sand, iron-coated sand and arsenic and iron-coated sand were placed in the Northport wells for several months, and then retrieved for community analysis has also been conducted. The communities associated with the surfaces differed from the groundwater communities, and also differed from each other. Delta proteobacteria were present on the surfaces retrieved from the high-arsenic discharge wells regardless of surface coating, however when arsenic was added, there was a shift toward the beta proteobacteria. The results of this experiment are being prepared for presentation and publication.

Two additional lines of investigation were initiated with this funding and are currently being pursued: the respiratory arsenate reductase gene of Sulfurospirillum NP4 is being characterized, and an arsenite oxidizing bacterium that was isolated from the Northport aquifer is being studied to investigate the potential effects of this activity in groundwater.

The PI has incorporated the results and approaches used in this research into both undergraduate and graduate classes. The problem of arsenic in groundwater has formed the basis of two honors thesis projects where undergraduate students undertook independent research projects and were advised by the PI. Another such project is currently underway. Several undergraduate research assistants worked on various aspects of the project, mentored by the PI and in some cases also by the graduate students involved, and their work has been included in conference presentations and publications. Graduate students involved in the project have presented their work at regional, national and international conferences.

Outreach has included work on programming and experimental activities with area high school teachers. In the case of Bangor High School, the PI was invited to work with several classes on groundwater chemistry and did chemical analyses on samples brought in by the students or their teacher. Some samples were also brought to the University for further analysis and the results were sent back to the teacher for additional tie in to their activities. The PI and graduate students also participate in the Expanding Your Horizons conferences regularly to provide middle school girls with a chance to learn about career options in STEM fields and introduce them to water quality and pollution issues in environmental engineering.

More recently, and faculty of Engineers Without Borders, the PI has worked with undergraduate (primarily) students on many aspects of water pollution, including arsenic contamination of drinking water. While this work involves less formal instruction than in the venues described above, the arsenic issue has been a useful example in discussions since it is a water quality issue that faces people here in Maine as well as in Southeast Asia, South America, China and elsewhere.

Findings:

Sulfurospirillum NP4 is an arsenate-respiring bacterium that was isolated from groundwater taken from a high-arsenic well located in Northport, ME. This organism not only respires arsenate, but can also reduce nitrate, selenate, Mn(IV), sulfite, thiosulfate, sulfur and TCE. It can fix nitrogen and can grow autotrophically, using CO2/HCO3- as a carbon source using H2 as the electron donor, or grow at the expense of lactate, pyruvate, formate or fumarate. It can grow fermentatively at the expense of fumarate or lactate. When cysteine is present in the media, Sulfurospirillum NP4 can also reduce Fe(III). Since the cysteine was present at a low concentration, it is likely that it uses the cysteine as an electron shuttle. This organism can also reduce AQDS, an electron acceptor which has also been shown to shuttle electrons to solid phase electron acceptors in other microorganisms.

Partial gene sequences have been obtained for the nitrogenase, arsenate reductase and citrate lyase enzymes of Sulfurospirillum NP4. The lack of RuBisCO gene and the presence of citrate reductase in NP4 support the conclusion that this organism uses a reverse TCA pathway for carbon fixation. The arsenate reductase gene is being further characterized.

Groundwater samples taken from wells in the Northport aquifer with a range of arsenic concentrations were tested for water chemistry and

microbial populations. Using a fluorescence in-situ hybridization technique, we discovered that the total arsenic and total iron concentrations in groundwater correlated with the Geobacter/delta-proteobacteria population. Sulfurospirillum NP4 was strongly correlated with the As(III) concentration. These results indicate that conditions that favor the delta-proteobacteria also favor reduction of Fe and increased mobility of As. Given that Geobacter and many other members of the delta proteobacteria are capable of iron reduction, they could be responsible for the mobilization of arsenic. Sulfurospirillum NP4 could be responsible for a shift in arsenic species toward the more mobile and toxic As(III) form.

Groundwater and surface-associated populations were compared for high and low arsenic wells in the Northport aquifer. In general, the diversity of microorganisms in the water phase was higher than on the surfaces that were incubated in wells for several months. Higher diversity was also observed in the lower-arsenic wells. The higher arsenic wells also showed a shift away from gamma proteobacteria toward the delta proteobacteria The type of surface affected the community composition. In the high arsenic wells, the community bound to the surfaces coated with iron were enriched with delta-proteobacteria, and when arsenic was co-precipitated with iron there was a greater shift toward the beta proteobacteria. It is not clear whether the groundwater populations represent an integration of the populations attached to various surface minerals available in the aquifer for colonization, or if they are shaped more by the tendencies of different microbial populations to spend more time in suspended vs attached form.

Arsenic mobilization is intertwined with iron dynamics. Comparison of arsenic behavior in pure culture laboratory experiments using organisms that can reduce iron but not arsenic (Geobacter sulfurreducens), iron and arsenic (Geobacter uraniireducens), or arsenic but not iron (Sulfurospirillum NP4), demonstrated that the form of arsenic is very important in its mobility. When iron(III) oxyhydroxide and A(V) are incubated together with the microorganisms, the arsenic concentration in the aqueous phase is significantly increased as it is reduced to the As(III) form. As long as the iron is present in the system in excess, even as Fe(III) is reduced, arsenic remains immobilized in the As(V) state but is released into the aqueous phase as it is reduced to As(III).

An arsenite oxidizing microorganism has been isolated from Northport aquifer groundwater. It can fix carbon can grow under aerobic or nitrate reducing conditions. Characterization of this microorganism, which could also impact arsenic mobility, continues.

Training and Development:

Three MS students worked on various aspects of this project. Each learned analytical and culturing techniques, reporting and analysis of results as well as experimental design. Two had their findings published and all had the opportunity to present their results at conferences.

A fourth MS student completed her degree, published the results and has continued to work on her PhD dissertation research on the project. As part of her training she spent a year on a fellowship that allowed her to spend a significant amount of time teaching in area classrooms and learning about effective instructional techniques. This enhanced her experience in training for a faculty position and created an excellent educational experience for the classes she regularly worked with. She has presented her work at seven local, national and international conferences, and spent several weeks working in the laboratory of our collaborators at the University of Manchester, UK on molecular techniques. While working on this degree she had her second child and so took some time off and started back at somewhat reduced time (the reason for the extension of this grant). She anticipates completing her laboratory and field work within six months, submitting her work for publication and completing her dissertation within the year.

Eleven undergraduate students participated in this project. They also developed laboratory skills, record keeping and reporting skills, and experimental design to a level far beyond what they had attained in classes. One worked primarily on the educational aspects of the project, and she carried on in a science education program to obtain teaching certification. Three have gone on to grad school, two honors theses have been written with another underway by a student who had worked on the project as a summer job. One student did an independent study to develop an activity that can be done with high school students on arsenic removal from well water.

Work with area High School teachers has increased awareness of drinking water and groundwater issues among school aged children and young adults. It has also been used as a context within which to teach basic science principles. Working with teachers has proved to be a very efficient way to transfer science content, since teachers are in the best position to know how to pitch materials so that their students will understand, and to adapt materials so that they satisfy Maine Learning Results.

The web site developed at the beginning of the project still generates inquiries about arsenic chemistry, mobility and microbiology. It also serves as a venue for groups or individuals to find me and seek advice on what to do about the arsenic in their drinking water, and for graduate students to enquire about the program.

Outreach Activities:

Participation in the Expanding your Horizons programs over the years provided hands on experience to approximately 150 middle school girls and their chaperones. In the workshops, girls participated in a non-point source water pollution simulation and did a number of short, related experiments demonstrating how pollutants move in surface and groundwater environments. These activities introduced participants to arsenic as a pollutant and some of the basics of the field of environmental engineering. They also provided the girls with role models (female grad students also participated in these activities) to encourage them to consider a career in engineering.

The Consider Engineering program was hosted in my lab in one summer during which groups of four high school students did experiments each afternoon for a week. We studied effectiveness of water treatment options to clean up a turbid, colored water sample from a local pond. Arsenic treatment was included in their discussion and report.

The web site created at the beginning of the project has provided the PI with several opportunities to work with individuals on groundwater quality issues and related treatment options.

The PI, an undergraduate assistant and the PhD student have developed classroom activities related to groundwater quality, chemistry and treatment. Instructions for these activities and materials have been made available to teachers.

The PhD student worked with the Branch Lake Association while doing her MS degree project. The PI and she provided information to the Lake Association on arsenic in drinking water and treatment options, and tested a number of samples for arsenic and microorganisms. These data were included in the student's analyses along with results from her primary field site in Northport.

This research brought together several researchers from the University of Maine System. The PI has submitted proposals with a researcher at the University of Southern Maine, and convened meetings with other researchers at UMaine working on different aspects of arsenic research. These for a provided opportunities for graduate students to talk about their work with an audience knowledgeable about arsenic, but not necessarily in their primary area of expertise (economists, toxicologists, cell biologists, geologists, environmental engineers).

Journal Publications

MacRae, J.D., Lavine, I.N., McCaffery, K.A., Ricupero, K., "Isolation and characterization of NP4, an arsenate-reducing Sulfurospirillum from groundwater in Northport, Maine.", Journal of Environmental Engineering, p. 81, vol. 131, (2007). Published,

Weldon, J., MacRae, J.D., "Correlations between arsenic in Maine groundwater and microbial populations as determined by fluorescence in situ hybridization.", Chemosphere, p. 440, vol. 63, (2006). Published,

Books or Other One-time Publications

Weldon, J.M., and J.D. MacRae., "Do Microbes Affect Groundwater Arsenic Concentrations? A Study On An Arsenate-Reducing Bacterium.", (2005). Book, Published
Editor(s): William Lyon, et al.
Collection: Proceedings of the International Conference on Environmental Science and Technology
Bibliography: American Science Press, New Orleans, LA. pp510-515.

MacRae, J.D., "Microorganisms and arsenic contamination of groundwater in Maine: is there a link?", (2006). Conference Proceedings, Published
Collection: Contaminated Soils, Sediments and Water Vol 11
Bibliography: Conference Proceedings of the 21st Annual International Conference on Soils, Sediments and Water, UMass Amherst, October 18, 2005.

Web/Internet Site

URL(s):
http://www.civil.umaine.edu/MacRae/Arsenic%20Main.htm

Description:
This web site was created to provide general information about arsenic to the public, and as support material for work with middle and high school classes.

Other Specific Products

Contributions

Contributions within Discipline:
NP4 was isolated from groundwater taken from a field site in Northport, ME and was the first organism, to our knowledge, that respires arsenate to be isolated from groundwater that is not anthropogenically contaminated with solvents. It may contribute to increased mobility and toxicity of arsenic in groundwater by altering the redox state of arsenic. We have found that it is present in the area where it was isolated as a significant fraction of the population (up to 16%) and correlates with the concentration of As(III) in the groundwater. Total arsenic in the groundwater at the site was better correlated with the abundance of delta-Proteobacteria, which includes many iron-reducing bacteria. This finding indicates that indirect release mechanisms such as solubilization of iron-based arsenic sorption sites may be of greater importance in producing high arsenic ground water than direct transformations of arsenic.

Experiments on iron reducing bacteria in pure culture have shown that reduction of ferric oxyhydroxide alone is insufficient to release arsenic bound to the iron surface. If arsenate is reduced to arsenite, a large percentage of the arsenic in the system may be released. It appears both the species of arsenic and the availability of binding sites is important in determining the solubility of arsenic in a mixed system.

The molecular characterization of the microbial populations in Northport groundwater samples with varying concentrations of arsenic has demonstrated that the low arsenic sites have greater microbial diversity than the higher arsenic sites. Delta Proteobacteria are more abundant in the high arsenic water. Molecular analysis of microbial populations that colonized surfaces (sand, iron-coated sand, and arsenic and iron-coated sand) incubated in wells for several months revealed that the surface type significantly affected the microbial population on the surface, and that bound populations differed from the groundwater population.

These studies contribute to a better understanding of the factors, biotic and abiotic, that contribute to arsenic mobility, and will contribute to the development of better models and tools for watershed management to protect groundwater supplies.

Contributions to Other Disciplines:
Arsenic concentrations in groundwater are imperfectly correlated with geology. Our findings indicate that microbial populations correlate with groundwater chemistry and that the activities of anaerobic bacteria can contribute to arsenic mobility. Anaerobic conditions, indicated by low dissolved oxygen, a sulfur smell or high iron, could act as a risk factor along with geological factors, to be used to identify those communities at greatest risk of arsenic exposure and thus in need of public education.

Including the additional factors required to cause arsenic mobility will help modelers, geologists, regulators, educators and drinking water professionals create better tools to identify potential areas of contamination. This information will be useful for targeting communities in need of public education (on well testing and treatment options) and identifying geographical areas where the geology may dictate more careful watershed management to prevent contamination of the water.

Contributions to Human Resource Development:
Engineering is a field in which women are chronically under-represented. Three graduate students and five of the undergraduates who have been involved in the project are women. The PI is committed to preparing women and minorities for life in the world of engineering through mentoring graduate and undergraduate students, as well as through programs like Expanding your Horizons. Working with high school classes also gives young women the message that they can pursue careers in STEM fields. The undergraduate student who worked with the high school and middle school students served as a role model to encourage young people, and young women in particular, to consider careers in engineering, and is now pursuing a career in science education.

Contributions to Resources for Research and Education:
A hydride generator was purchased for an existing AAS in the department, which allows researchers and students in lab courses to use this instrument for As and Hg analysis. This expands the PI's ability to involve classes in research.
HPLC columns and additional molecular biology equipment were purchased for use in this and other projects.
Additional components were purchased for an existing protein gel apparatus to allow us to run denaturant gradient gels for community analysis.

This has expanded our capacity to do community molecular analyses
A pump was purchased for well sampling.
A new water treatment activity has been developed that can be used at education and outreach events.

Contributions Beyond Science and Engineering:

The results should provide data required for risk analysis. To this end, data have been shared with the state toxicologist, Andy Smith. Findings were presented as they were made at the Maine Water Conference, which is attended by water professionals, state employees who work on water, policy makers and academic researchers, as well as national and international conferences that reach practitioners. One of the recommendations from our research is careful management of organic matter in watersheds in geological high risk areas to avoid arsenic contamination of drinking water and costly remediation.

Conference Proceedings

Categories for which nothing is reported:

Any Product

Any Conference