CAREER: The Role of Microorganisms in Arsenic Contamination of Groundwater

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ABSTRACT: Arsenic at elevated concentrations in drinking water affects the health of millions of people worldwide from Bangladesh to New Zealand to the USA. Keratosis, skin cancer, lung and bladder cancers, and diseases of the vascular system have all been linked to arsenic exposure through drinking water (Hopenhayn, 2006). To understand the factors that contribute to high arsenic in groundwater, a multidisciplinary and international effort is required. We have found that iron- and arsenate-reducing bacteria contribute to arsenic contamination. Researchers at the University of Manchester have similarly implicated iron reducing bacteria in arsenic contamination of groundwater in West Bengal and Cambodia, and found close relatives of our groundwater isolate, Sulfurospirillum *species NP4, in enrichment cultures using Cambodian sediments (Islam et al., 2004; Lear et al, 2007). Collaboration with Professor Jon Lloyd and others and the Williamson Research Centre at the University of Manchester, UK has allowed us to investigate the effects of iron reducing bacteria at surfaces and probe the ability of* Geobacter uraniumreducens *to cause the release of sorbed arsenic. We have also applied molecular techniques to characterize the microbial communities of groundwater samples of varying arsenic concentrations. The collaboration and techniques learned have enabled UMaine researchers to use techniques employed by geologist and molecular biologists in pursuit of new research directions and have broadened the research networks of both the PI and her graduate student.*

INTRODUCTION

Associate Professor Jean MacRae and her graduate and undergraduate students at the University of Maine have been studying the impacts of microorganisms on groundwater arsenic concentrations, primarily in an aquifer in Northport, Maine. A bacterium, *Sulfurospirillum* NP4, capable of respiring arsenate was isolated from high arsenic well water. This microbe was characterized and found to be capable of respiring a variety of other oxidized inorganic and organic electron acceptors at the expense of hydrogen or a limited number of organic carbon substrates (MacRae et al., 2007). It can also fix nitrogen and carbon through the reverse TCA cycle (manuscript in preparation). By employing molecular techniques, this microbe was found to correlate well with the concentration of As(III), the more toxic and mobile form of arsenic, in the water. The total arsenic concentration was correlated with the *Geobacter* population (Weldon and MacRae, 2006). The genus *Geobacter* contains a variety of iron- and other metal-reducing bacteria, but most of the species characterized so far do not reduce arsenic.

The collaboration established under the IREE program had two primary purposes: to acquire expertise working with iron reducing bacteria by working with one of the leading groups in metal-reducing bacteria and to establish contacts with researchers who have worked on arsenic contamination in several international settings. At environmental conditions, most Fe(III) is insoluble. Thus the culturing system and dynamics of iron reducers are more complex than when the reactions occur in solution. The geomicrobiology group at the University of Manchester, UK has experience combining the tools commonly used in geology to characterize minerals with anaerobic microbiological techniques, and it was anticipated that we could apply these techniques to arsenic-contaminated iron surfaces or substrates to learn more about how they are affected by microbial activity. We also wished to apply molecular techniques to environmental samples from Maine, as had been done by their group with materials from India and Cambodia.

Professor Jonathan Lloyd's Geomicrobiology Laboratory is housed within the Williamson Research Center for Molecular Environmental Science at the University of Manchester. This large, interdisciplinary group has conducted numerous field and laboratory studies involving microbial transformation of inorganic constituents in the subsurface and their effects on water quality. They have done extensive international work on the arsenic problem in West Bengal, Southeast Asia and Australasia. Relevant expertise include the use of advanced molecular tools and microscopy for the characterization of environmental microbial populations, and expertise in X-ray absorption spectrometry, electron microprobe analysis, scanning-tunneling microscopy and atomic force microscopy to study the effects of the microbes on surfaces.

The PI, Jean MacRae, worked at the University of Manchester from January 5-July 18, 2007. Jennifer Weldon, Ph.D., joined the group from June 3-29, 2007.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

The PI primarily worked in the host lab with a relatively newly isolated *Geobacter* species, *G. uraniumreducens*, which was isolated from a uranium-contaminated site. This microorganism was found by members of the Lloyd lab to be capable of arsenate reduction (Gault et al., manuscript in preparation). Since this is a unique trait among the currently characterized *Geobacter* species, it was compared to the better-characterized *G. sulfurreducens*. A series of experiments were conducted to examine the ability of *G. uraniumreducens* to reduce iron (ferrihydrite) and to solubilize arsenic adsorbed to iron.

In the first case, iron was applied to the surface of slides and examined by environmental SEM with EDX capability. The cells were clearly able to solubilize the iron, creating cell-shaped divots in the surface (Figure 1) and releasing Fe(II) into solution, as determined by ferrozine assay. Eventually iron precipitates were observed on the slide surfaces. Since there are several mechanisms of reducing solid phase iron, culture supernatants were filtered and assayed for the ability to reduce ferrihydrite. These experiments did not provide any evidence of the production of chelators or electron shuttles by *G uraniumreducens*.

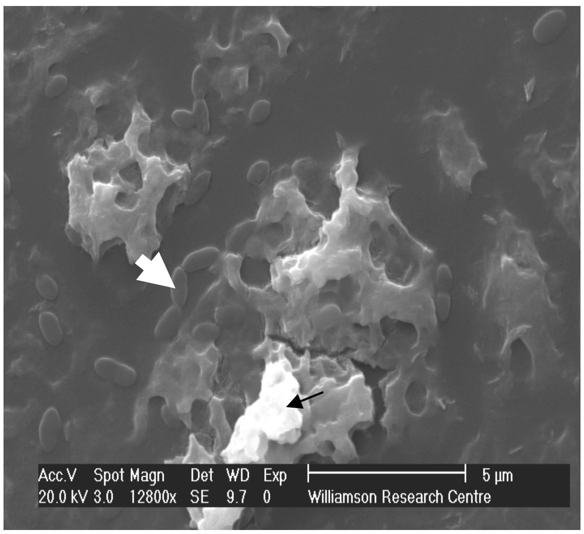

Figure 1, G uraniumreducens on ferrihydrite coated slides after 4 days incubation in phosphate buffered media. The bold white arrow shows cells, and the thin black arrow shows the beginning of a precipitate.

To determine if this organism could cause the release of arsenic bound to ferrihydrite, cells were incubated with iron to which arsenic (As(V) form) was allowed to sorb, and acetate as electron donor. *G. sulfurreducens* had previously been found to produce magnetite, a mixed valence end product of ferrihydrite reduction, which effectively scrubbed the As(V) from solution (Islam et al., 2004). While both microbes reduced ferrihydrite, *G. uraniumreducens* but not *G sulfurreducens* was able to both reduce and solubilize the arsenic in the culture (Figure 2). *G. uraniumreducens* was also able to reduce arsenic sorbed to or incorporated into biogenic magnetite produced by *G. sulfurreducens*.

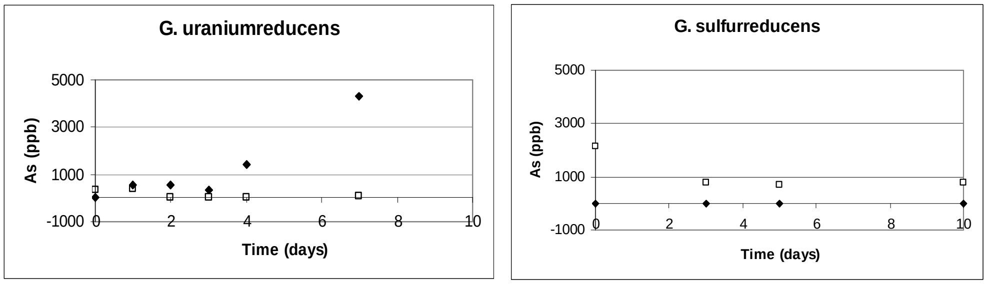

Figure 2 Soluble arsenic speciation (by IC-ICP-MS) in cell suspensions incubated with iron, acetate and 7500 ppb of As(V). As(V) is shown as open squares, and As(III) as filled diamonds.

Samples of groundwater were also brought to the UK from Maine for microbial community analysis. DNA was extracted and clone libraries have been constructed for some of the water samples, and this work continues in our lab.

Both lines of research complement and extend the existing project. The use of ESEM-EDX, XRD and XPS have allowed characterization of the surfaces colonized by iron and arsenic reducing bacteria. A publication describing this work is in preparation. The molecular techniques are now being used to more fully characterize the microbial communities in the aquifer that we have studied in Maine to see if there are any additional links between microorganisms and water chemistry at the site.

Broader Impacts of the International Travel

The international travel allowed the PI and her graduate student to broaden the scope of their work to include chemical and microscopic analysis of surfaces colonized by relevant microbial types, and to expand the analysis of microbes in the arsenic-contaminated Maine aquifer. With this experience, these lines of inquiry may be pursued at the University of Maine by developing collaborations with researchers who have the appropriate research tools on campus but have not yet applied them to the kind of samples we will generate. Prior to this trip we had only worked on solution phase chemistry of both the groundwater and culture media. Experience gained in the UK will allow us to expand our explorations to iron reduction as it is relevant to the cycling of arsenic in the subsurface.

The PI, Jean MacRae, is currently preparing a manuscript based on the results obtained while in the UK, in collaboration with co-author Professor Jonathan Lloyd. Both the PI and her host have expressed an interest in continuing the collaboration. Others in the laboratory continue to correspond with the PI on technical issues. Graduate student participant Jennifer Weldon also corresponds with members of the lab when technical issues relating to molecular biology techniques arise.

The PI also had several very enlightening and useful conversations with Dr. David Polya, a member of the Williamson Research Centre on the challenges and rewards of international field work and collaborations. One of the aims of establishing this collaboration was to obtain additional ties with researchers who have worked with the Geomicrobiology group on international projects. The PI is currently the faculty advisor for the University of Maine's new Engineers Without Borders (EWB) chapter. Since she has little international development experience, she used the opportunity of meeting with Dr. Polya to gain some insights into the factors that are important in achieving success. He indicated that having a very involved and enthusiastic collaborator in country, and learning about potential health and other risks in advance and preparing well for these were essential. He indicated his willingness to provide contacts if we decide to initiate links with communities where he is working. This could open the door to links between the PI's research and an EWB project in the future, since arsenic issues are important in these locations.

The PI also had the opportunity to meet with K.M. Ahmad from Dhaka University who has worked extensively on the arsenic issue in Bangladesh. They discussed some of the difficulties not only in assessing the health risks of As in water in Bangladesh, but also in communicating risks to the population and monitoring water treatment.

While language is not generally an issue in the UK, there are, of course, cultural differences. The structure and funding at the University are quite different from ours, although the group there seemed to be moving more toward the American model of external research funding. The host lab is quite an international one, however, with students and post-docs from Portugal, France, Germany, Hong Kong, China, Mongolia, India and Pakistan included in the group, and several had international components of their work. Since some of these researchers will eventually form their own research groups, this could expand the PI and graduate student's potential international collaborators.

The graduate participant, Jennifer Weldon, had the opportunity to interact with a large interdisciplinary research group and to experience a different academic setting. She also met students from a variety of European and Asian countries. She has been very upbeat about the value of this experience to her, and has significantly expanded her international contacts. The training and experience she gained in Manchester is guiding her now as she completes her dissertation research.

DISCUSSION AND SUMMARY

The problem of arsenic in groundwater is a complex one involving not just arsenic redox cycling, but also iron cycling and probably carbon cycling as well. While the intense interest in the problem generated by the magnitude of the health impacts in the Bengal basin and elsewhere has fostered a lot of research, there are still many unanswered questions. Among them, how do iron reducers affect arsenic mobility? Some research, such as our own (Weldon and MacRae, 2006), has linked Fe reducers with increased As mobility, yet others have also found that iron reducers may reduce the mobility of arsenic (e.g. Islam et al, 2004) or that iron is not released into solution concurrently with As (e.g. Horneman et al., 2004; van Geen et al., 2004). More process level understanding is needed to really resolve the mechanism(s) of As release.

The work done in the context of the collaboration between the PI and her host at Manchester University demonstrated that the outcome of iron reduction on mobility may be dependent on the iron reducer. The activity of *G uraniumreducens*, but not *G. sulfurreducens* resulted in As mobilization from ferrihydrite. Whether this is a function of the rates of reaction at a micro scale influencing end products, the mechanism of iron reduction, the order of reduction (concurrent, As or Fe first), or the form of arsenic involved remains to be determined. These are new lines of research that the PI intends to pursue moving forward, in collaboration with the Manchester group, with other researchers in Maine and independently.

The Williamson Research Centre houses faculty and researchers with disciplinary roots in the earth sciences, biological sciences, and chemistry (organic and inorganic), including numerical analysis and modeling as well as atmospheric sciences and hydrology. The formal links and central support for activities like joint facilities and weekly seminars have accelerated and facilitated successful interdisciplinary research and contributed to the success of its participants. This success has attracted an array of excellent international researchers who in turn add to the scope and breadth of the research done at the Centre. Having this opportunity to work within a different system was valuable on several levels. It became clear that much can be achieved when disciplinary boundaries are ignored, at least where research is concerned, and that having researchers who come from different (international) research traditions results in more different approaches being tried. This ultimately leads to the kind of lively research community that can create significant advances in knowledge and understanding, and encourage students to pursue careers in science and engineering.

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BRIEF BIOGRAPHIES OF RESEARCHERS

Jean MacRae received a B.S. degree in Life Science from Queen's University in Kingston, Ontario, Canada in 1988. She received her M.S. in Microbiology in 1991 and Ph.D. in Environmental Engineering in 1997 from the University of British Columbia. She worked for Environment Canada and then Health Canada prior to taking her position in the Department of Civil and Environmental Engineering at the University of Maine in 1999. She was promoted to Associate professor in 2006. Her research interests include anaerobic microbial processes and element cycling, biodegradation and bioavailability of organic contaminants.

Jennifer Weldon attended the State University of New York Canton College of Technology where she was the Earl W. MacArthur Honor Scholar. She graduated with an associate degree in Liberal Arts and Sciences with highest honors. She obtained her B.S. degree in Chemical Engineerin in 2002 from Clarkson University and her M.S. in Civil Engineering from the University of Maine in 2005. She is now a Ph.D. candidate at the University of Maine.