The Effect of Speciation and Form on the Bioavailability of Arsenic: Insight into the Behavior of Arsenic in Natural Waters

Elizabeth Ann Diesel

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Geosciences

Madeline E. Schreiber, Chair
Michael F. Hochella, Jr.
J. Donald Rimstidt
Jan R. van der Meer
Peter J. Vikesland

February 4th, 2011
Blacksburg, VA

Keywords: arsenic, organoarsenical, bioavailability, speciation, *E. coli*, *C. fluminea*, colloid, nanoparticle

The Effect of Speciation and Form on the Bioavailability of Arsenic: Insight into the Behavior of Arsenic in Natural Waters

Elizabeth Ann Diesel

ABSTRACT

Drinking water supplies contaminated with arsenic (As), a toxin and carcinogen, adversely impact the health of millions of people worldwide. Previous work has documented that different inorganic and organic As species vary with respect to their toxicities. It is, however, currently not well understood how As speciation affects bioavailability, defined as the capacity of a contaminant to cross an organism's cellular membrane, or how arsenic's form (dissolved vs. non-dissolved) can affect bioavailability. This dissertation addresses the effect of speciation and form on As bioavailability through a combination of field and laboratory studies. In the first project, a poultry litter application experiment was conducted to determine if trace elements (As, Cu, and Zn) are released from litter to underlying soil water, and if so, whether the trace elements are present in dissolved form or complexed to nanoparticles, colloids, or particles. Results showed that Cu and Zn released from the litter were dominantly complexed to organic matter or to iron oxides/clay particles, while As was dominantly dissolved or complexed to organic matter. In the second project, a luminescent *E. coli* bioreporter was created and exposed to different As species, including As(III), As(V), MSMA, and roxarsone. Results showed variable response, with As(III) producing the strongest response, followed by As(V) and MSMA; roxarsone showed no response. The bioreporter was exposed to As solutions with varying cation concentrations to examine the impact of sample geochemistry on performance. Increased monovalent (Na,K) concentrations enhanced luminescent response, while increased divalent (Ca) concentrations inhibited response. These altered responses reflect different As uptake pathways into the cell. The third study addressed bioavailability of As species to *Corbicula fluminea*, a clam commonly used for biomonitoring. Results demonstrate that As(III) is most bioavailable to *Corbicula*, followed by As(V), MSMA, and roxarsone. *Corbicula* also displayed the ability to change As speciation through internal processing and via their shell, demonstrating that *Corbicula* can affect As speciation in solution. Results of these studies enhance the scientific knowledge of how speciation and form affect As bioavailability, and can also inform regulators who use bioavailability to set remediation goals for As-contaminated systems.

ACKNOWLEDGEMENTS

First, I need like to thank my advisor, Dr. Madeline Schreiber. She believed in my prospects as a doctoral candidate when I was only an undergraduate student with little experience. She provided me with numerous opportunities while I was her student, both scientifically and academically, and gave me freedom to pursue research avenues of my own choosing. If it weren't for her support, guidance, and especially patience, I wouldn't have been able to achieve all that I have.

I would also like to thank my committee, Dr. Michael Hochella Jr., Dr. Donald Rimstidt, Dr. Jan van der Meer, and Dr. Peter Vikesland, for all of their guidance and support during my time at Virginia Tech. They challenged me and my research, and through their support in my efforts, they have helped to mold me into the scientist and researcher I am today.

Additionally, I need to thank the many sources of funding for my dissertation research. I was supported on fellowships from the NSF IGERT EIGER program and the Virginia Tech Cunningham doctoral scholar program, and on research assistantships funded by an ICTAS Development grant, a USDA Watershed Processes grant, and a Department of Geosciences D. R. Wones summer fellowship. The EIGER program also provided funding for my internship at the University of Lausanne, Switzerland, to work with Dr. van der Meer. Additional funding for materials, supplies, and analyses was provided by a GSA Hydrogeology research grant, and the AWG Penelope Hanshaw scholarship.

I also need to thank my research collaborators, Matthew Hull and Maya Nadimpalli, who helped make *Corbicula fluminea* research fun and greatly successful. I am grateful to the NSF REU Program for introducing me to Maya and for providing the support for her to work with me during the summer of 2009. Athena Tilley and Bill Henley require thanks as well, as their expert knowledge was key in ensuring the success of my research.

The past and present members of the Hydro research group, as well as the ICTAS ENT research group, need to be thanked. They have been my sounding boards and support groups throughout my dissertation. Other research groups, including Ann Stevens and her microbiology research group, particularly Josh Williams and Revathy Ramachandran, Bobbie Niederlehner and the Stream Team, and the Department of Fundamental Microbiology at the University of Lausanne, especially Edith Coronado, were all instrumental in helping me complete my

dissertation research. I would also like to thank Connie, Ellen, Mary, Linda, and Carolyn, without whom the department could not run, and who helped me every step of the way.

Finally, I would like to thank my husband, Ryan, my parents, and the rest of my family, who have supported and loved me throughout the five and a half years I have been in graduate school.

ATTRIBUTIONS

Several colleagues and coworkers helped in the research and writing of portions of this dissertation. A description of their background and contributions are detailed below.

Dr. Madeline E. Schreiber, *Department of Geosciences, Virginia Tech*, is the primary research advisor and committee chair. She provided scientific guidance and support for the writing of all chapters.

Dr. Jan R. van der Meer, *Department of Fundamental Microbiology, University of Lausanne*, is a committee member and provided scientific guidance, particularly regarding aspects of microbiology. He also aided in the interpretation of results and contributed to the writing of Chapter 2.

Dr. Peter J. Vikesland, *Department of Civil and Environmental Engineering, Virginia Tech*, is a committee member and provided scientific guidance, particularly regarding environmental nanoparticles and *Corbicula fluminea* research.

Dr. J. Donald Rimstidt, *Department of Geosciences, Virginia Tech*, is a committee member and provided scientific guidance, particularly regarding environmental geochemistry and data presentation.

Dr. Michael F. Hochella, Jr., *Department of Geosciences, Virginia Tech*, is a committee member and provided scientific guidance, particularly regarding aspects of environmental nanoparticles.

Matthew S. Hull, *Department of Civil and Environmental Engineering, Virginia Tech*, provided scientific guidance regarding *Corbicula fluminea*, as well as experimental setup. He also contributed to the writing of Chapter 3.

Maya Nadimpalli, *Environmental Sciences and Engineering, University of North Carolina*, assisted with experimental design and set up, and contributed to the interpretations in Chapter 3.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF TABLES

Chapter 1

Chapter 3

INTRODUCTION

Bioavailability is often used as the key indicator of potential risk that chemicals pose to environment and human health, and is regarded as a priority research area for both remediation and risk assessment, as it is an important but poorly quantified regulatory factor (Naidu et al., 2008). It is commonly defined as a measure of the physicochemical access that a toxicant has to the biological processes of an organism (USEPA, 1997). Semple et al. (2004) define a bioavailable compound as "that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time". They introduce another term called bioaccessibility, defined as a compound that "is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical" (Semple et al., 2004). More simply put, a bioaccessible compound is one that has the potential to become bioavailable (i.e., cross an organism's cellular membrane) but is currently not available due to the biogeochemical conditions of the system, while a bioavailable compound is one that actually crosses an organism's cellular membrane.

Both physical and geochemical factors can affect whether a compound is available or accessible to organisms. Some of these factors include the contaminant concentration within the system, dissolution/precipitation and sorption/desorption reactions, speciation of the compound, and complexation of the compound to mobile colloids. For example, sorption/desorption and precipitation/dissolution processes, and the transport of a contaminant both in the system and to a biological membrane are considered bioaccessibility processes, whereas the uptake of a contaminant across a cellular membrane is considered to be bioavailability (Semple et al., 2004). Thus, as physical and geochemical characteristics at different sites are unique, the bioavailability of a contaminant is a site specific quantity.

Several methods have been used to characterize the bioavailability of a contaminant. These methods include chemical extractions of contaminants from soils, toxicity tests on organisms, measuring bioaccumulation in exposed organisms, using soil properties to estimate bioavailability, in vitro and in vivo tests, and microbial biossays (Naidu et al., 2008). Each of these methods measures bioavailability through different mechanisms, which makes comparing the results of one method to another difficult.

As the physical and geochemical characteristics of a system can affect how a contaminant is available, development of a standard method to assess biovailability would remove method disparity and would make field site comparison easier. Using a standard method or set of methods for bioavailability is a necessary step to improving the comparability between various field sites. Using organisms, or bioassays, as a standard method for environmental monitoring would provide information on the wellbeing of an organismal community as well as the general condition of their environmental system (Ravera, 2001).

Another important aspect for assessing bioavailability is the form of the contaminant of interest. For example, many contaminants such as metals or organics, can occur in dissolved form, but can also be non-dissolved (nanoparticle, colloid, particle). This form has implications for bioavailability. For example, Wang and Guo (2000) found that Zn bioavailability to both mussels and clams decreased when bound to colloidal organic carbon (OC), but Cr bioavailability increased when bound to colloidal OC. Chen and Wang (2001) found that Fe bioavailability to diatoms and copepods decreased when it was bound to colloidal OC. Understanding how contaminant association with OC or mineral surfaces affects bioavailability is important, particularly regarding filter feeders, as they are capable of taking up and accumulating contaminants through both water and sediments.

Bioavailability studies can be used to help inform regulators, scientists, and engineers on the amount and type of contaminants to be removed at a field site. For example, if, of the total amount of contaminant in the system, (i.e. all forms/species, dissolved and complexed, both to mobile colloids and matrix), only the dissolved portion of a particular species is bioavailable, then only this portion of the contaminant may need to be remediated, depending on the regulatory requirements. One potential problem of removing only one portion of the contaminant is that changes in the geochemistry in the system (i.e. pH, dissolved oxygen, oxidative/reductive potential, temperature, conductivity) can result in a change in biogeochemical processing that may result in transformation of some of the previously unavailable and/or bioaccessible forms of the contaminant to a bioavailable form.

Although there is a rich literature on the bioavailability of contaminants to a wide variety of organisms, there are some fundamental aspects of bioavailability research that are poorly understood. In particular, there are few data on how the speciation of a contaminant (organic vs. inorganic, oxidized vs. reduced, presence of a functional group) and its form (dissolved vs.

complexed) can impact how it is taken up and accumulated in organisms. The toxicity of a contaminant often varies with its speciation (e.g. inorganic As is more toxic than organic, and As(III) is more toxic than As(V), (Jain and Ali, 2000)), and this speciation information is critical to assess the use of organisms for biomonitoring and the health of an environmental system.

Because speciation and form can both influence toxicity, there is a critical need to determine if organisms used for biomonitoring show differences in uptake of different species and forms, and if so, why. In addition, there have been no studies aimed at integrating bioavailability measurements with the potential geochemical controls. In 2008, the Department of Defense, Department of Energy, and Environmental Protection Agency held a workshop on the research and development needs for understanding and assessing bioavailability of contaminants in soils and sediments. The panel of researchers assembled agreed that the aforementioned areas of bioavailability research are lacking, particularly in the study of arsenic bioavailability (Bridges et al., 2008).

This dissertation is organized into three chapters. The first chapter assesses the extent of arsenic and other trace element association with natural particles, colloids, and nanoparticles in a field experiment using poultry litter as the source of trace elements. The second chapter characterizes the bioavailability of different arsenic species (As(III), As(V), and two organoarsenicals) to a strain of *Escherichia coli* genetically engineered to luminesce in the presence of arsenic. The third chapter characterizes the bioavailability of arsenic species to *Corbicula fluminea*, an invasive species of Asian clam commonly used for bioavailability studies. Results of this work will help regulators to better assess arsenic bioavailability in contaminated systems through an improved understanding of the impact of speciation, complexation, and geochemical controls on bioavailability measurements.

REFERENCES CITED

Bridges, T. Geiger, S., Greenberg, M., Griffin, S., Lanno, R., Leeson, A., Lowney, Y., Luthy, R., Menzie, C., Reible, D., Robert, S., Ruby, M., Speicher, J., Stroo, H., Thompson, T., Unger, M., and von Stackelberg, K., 2008. SERDP and ESTCP expert panel workshop on research and development needs for understanding and assessing the bioavailability of contaminants in soils and sediments. In: SERDP and ESTCP (Editors). DoD, pp. 1-51.

Chen, M., and Wang, W.-X., 2001. Bioavailability of natural colloid-bound iron to marine plankton: Influences of colloidal size and aging. Limnology and Oceanography, 46(8): 1956-1967.

Jain, C.K. and Ali, I., 2000. Arsenic: Occurance, toxicity, and speciation techniques. Water Research, 34(17): 4304-4312.

Naidu, R., Semple, K.T., Megharaj, M., Juhasz, A.L., Bolan, N.S., Gupta, S.K., Clothier B.E., and Schulin, R., 2008. Bioavailability: Definition, assessment and implications for risk assessment. In: R. Naidu (Editor), Chemical Bioavailability in Terrestrial Environments. Linacre House, Oxford.

Ravera, O., 2001. Monitoring of the aquatic environment by species accumulator of pollutants: A reivew. Journal of Limnology, 60(Suppl. 1): 63-78.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., and Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediments is complicated. Environmental Science and Technology, 2: 229A-231A.

USEPA, 1997. Engineering bulletin: Technology alternatives for the remediation of soils contaminated with As, Cd, Cr, Hg, and Pb. In: O.o.E.a.R. Response (Editor), pp. 1-20.

Wang, W.X. and Guo, L., 2000. Influences of natural colloids on metal bioavailability to two marine bivalves. Environmental Science and Technology, 34: 4571-4576.

CHAPTER 1

The Release and Partitioning of Trace Elements from a Poultry Litter Application in an Agricultural Watershed

Elizabeth Diesel and Madeline Schreiber

Department of Geosciences, Virginia Tech, Blacksburg, VA 24061-0420, USA

ABSTRACT

Trace element release to water in watersheds with significant poultry farming is a recent concern, as poultry litter applied to croplands can release organoarsenic compounds, as well as other poultry feed additives (i.e. copper and zinc) to the environment. To examine the mobility of trace elements (As, Cu, and Zn) and nutrients from litter into the soil water system, we conducted a small-scale field application of poultry litter at a site in Rockingham Co. Virginia. Soil water samples were collected from lysimeters before poultry litter application to characterize background hydrogeochemistry, and successive water and sediment samples were collected monthly for a total of four sampling periods. Filtration and ultrafiltration were utilized to aid in the determination of the degree of association of the trace elements with particle (> 1 μm), colloidal (100 nm-1 μm), nanoparticle (1-100 nm) and dissolved (<1 nm) phases within soil water. Results show that P, Cu, and Zn in soil water primarily partition into the particle and nanoparticle/dissolved (nano-dissolved) size ranges, with much less in the colloid size range, whereas most of the As released from the litter into soil water is either as nanoparticles or in dissolved form. The iron and silica, used as proxies for iron oxides and clays, are primarily particles in the soil water samples. Results of sediment digestions before and after litter application demonstrate increases in Cu and Zn concentrations in surface soil after litter application, with subsequent increases at depth (up to 25 cm) during the course of the four month monitoring period. Combined, our results suggest that introduction of poultry litter not only released trace elements into the sediment and soil water system, but also changed trace element partitioning by introducing organic carbon and through mobilization of iron oxides and clays into soil water. Characterization of size fractionation of trace elements is important, as the size can impact both transport and bioavailability of the element within the environment.

INTRODUCTION

Dissolved solutes have conventionally been defined for water quality sampling as anything that passes through a 0.45 micron filter. However, recent research has demonstrated that this is not an accurate assumption (Puls and Barcelona, 1989; Puls et al., 1996), as there are many different possible forms of species in water, including particles, colloids, and nanoparticles, as well as dissolved and macromolecular forms in both natural and engineered systems. Lead et al. (1997) operationally define particles as materials with dimensions larger than 1 μm, colloids as materials with at least one dimension between 1 μm and 100 nm. Nanoparticles are greater than 1 nm and less than 100 nm in size in 2 or 3 dimensions (ASTM Standard E2456, 2006). Within this operational definition, dissolved species are considered to be less than 1 nm in size.

Distinguishing these different forms is important for several reasons. First, dissolved phases are transported differently from their non-dissolved counterparts, and this is of particular importance for trace elements. Trace elements are often associated with solid particles, including clay minerals, iron and aluminum oxides, silica, and natural organic matter (McCarthy and McKay, 2004; Sen et al., 2002), due to adsorption, complexation and co-precipitation. As a result, trace elements are often assumed to have limited mobility within soils due to association with mineral surfaces (Alloway, 1990). However, many studies have indicated that trace element mobility can be enhanced by association with particles, colloids, and nanoparticles in soil water and groundwater. For example, Puls and Powell (1992) found that colloidal associated arsenate has an increased mobility when compared to dissolved arsenate in column experiments. Kent and Fox (2004) found when As(V) was adsorbed to nanometer thick iron oxide films on the surfaces of mobile quartz in an aquifer, arsenic transport was enhanced. Copper and zinc associated with colloids were also found to have an increased mobility as opposed to their dissolved counterparts (Karathanasis, 1999; Karathanasis et al., 2005). In addition to particles, colloids, and nanoparticles, natural organic matter (NOM) can also facilitate trace element transport in soils through the formation of soluble metal-organic complexes (McCarthy and Zachara, 1989). Approximately 10% of dissolved As can be bound to NOM under typical environmental conditions, and considerably more can be complexed if organic carbon concentrations are increased (Buschmann et al., 2006). Cu and Zn also complex with NOM

(Tessier et al., 1979; McBride et al., 1997; Drever, 1997; Ashworth and Alloway, 2004), which can potentially increase mobility.

Another reason for distinguishing size fractionation is that the size of particles can impact reactivity. For example, recent studies have shown that nanoparticles have different reactivities than larger particle sizes of the same materials. Increased reactivity in nanoparticles has been observed for many different natural materials, including galena (Liu et al., 2009), magnetite (Vikesland et al., 2007), hematite (Madden and Hochella, 2005; Madden et al., 2006), as well as a host of others (Hochella and Madden, 2005; Wigginton et al., 2007; Hochella et al., 2008).

Last, the size distribution of trace elements can impact bioavailability. Dissolved trace elements may be more or less bioavailable to organisms than if they are complexed with organic material or adsorbed to particles. Understanding how colloidal complexation affects bioavailability is important, particularly regarding filter feeders, as they are capable of taking up and accumulating contaminants through both water and sediments.

Trace elements in agricultural watersheds

There is a growing concern about trace element mobility in agricultural watersheds where poultry litter is applied as fertilizer, as poultry litter contains Cu, Zn and As, as well as high concentrations of nutrients (N and P) and organic material. Roxarsone, an organoarsenical, is added to poultry feed to improve weight gain, feed efficiency, and pigmentation (Garbarino et al., 2003). Cu and Zn are also added to poultry feed, typically in the sulfate salt or oxide form (Sims and Wolf, 1994; Jackson et al., 2003; Rutherford et al., 2003). These additives aid in growth, pigmentation, and appetite control (O'Dell, 1979) and are typically excreted into the litter (Jackson et al., 2003).

Trace element concentrations in poultry litter can vary significantly. Arsenic concentrations have been reported to vary between < 1 and 77 mg/kg (Vanderwatt et al., 1994; Sims and Wolf, 1994; Moore et al., 1998; Jackson et al., 1999; Jackson et al., 2003). Cu concentrations in poultry litter are reported to range between 97 and 1196 mg/kg (Vanderwatt et al., 1994; Moore et al., 1998; Jackson et al., 1999; Nicholson et al., 1999; Jackson et al., 2003), and Zn concentrations vary between 373 and 718 mg/kg (Vanderwatt et al., 1994; Moore et al., 1998; Jackson et al., 1999; Nicholson et al., 1999; Jackson et al., 2003).

Poultry litter, which is a mixture of manure and bedding material, is rich in nutrients and is often spread on fields as a fertilizer (Jackson and Bertsch, 2001; Bednar et al., 2003; Garbarino et al., 2003; Jackson et al., 2003; Rutherford et al., 2003) or as a means of waste disposal (Hyer et al., 2001). Previous studies have shown that trace elements are easily leached from poultry litter, with up to 72% of As, 49% of Cu and 6% of Zn occurring as water soluble fractions (Jackson and Miller, 1999, Jackson et al., 2003).

Results of the few studies conducted on the impact of poultry litter on trace element mobility in soils and natural waters have documented that despite the strong adsorption potential of trace elements to common aquifer minerals such as iron oxides and clays, As and other trace elements from poultry litter can be found in natural waters in watersheds where litter is applied. For example, Brown et al. (2005) found elevated As (as arsenate), Cu and DOC in soil waters underlying a litter-applied field site, with no As or Cu and lower DOC in soil waters at a reference site on the same property where litter was not applied. Recently, Church et al. (2010) found As and P in drainage ditches adjacent to a poultry production facility with litter amended fields. During storm events, As and P were released from both a litter storage shed (point source) and litter-applied fields (non-point source) to the drainage ditches (Church et al., 2010).

Although the studies above have documented release of trace elements from litter to waters, they have not addressed how the elements are mobilized from the litter. The objectives of this research were to examine trace element release from poultry litter into soil water and to determine the size fractionation of the trace elements. To achieve these objectives, we applied poultry litter in a field experiment and collected soil water samples from lysimeters underlying the field plot for a period of four months following the application. We then used a combination of membrane and ultrafiltration techniques to determine the size fractionation of trace elements.

FIELD SITE

The field site is located within the Muddy Creek subcatchment, which is located 20 km northeast of Harrisonburg in Rockingham County, Virginia (Hyer et al., 2001; Brown et al., 2005). The subcatchment (1.2 km^2) is drained by the Muddy Creek tributary, whose waters flow into the Shenandoah River and the Chesapeake Bay (Hyer et al., 2001; Brown et al., 2005). The primary land use of the area is agriculture, which includes corn, alfalfa, poultry, and cattle production (Hyer et al., 2001).

The underlying bedrock is composed of interbedded limestone, dolostone, and sandstone from the Lower Ordovician-Upper Cambrian Conococheague Limestone (Hyer et al., 2001; Brown et al., 2005). A clay-rich soil has developed from weathering this bedrock, with kaolinite and illite as the dominant clays (Hyer et al., 2001). The vadose zone at the site is highly variable, with thicknesses ranging from 0 to 20 m below surface (Hyer et al., 2001; Brown et al., 2005). The depth to groundwater is also variable, and ranges from 2 to 20 m below the surface (Hyer et al., 2001).

Our sampling site is adjacent to a cornfield to which dried poultry litter is normally applied twice annually as a fertilizer (Hyer et al., 2001; Brown et al., 2005). This site contains three zero tension, or pan, lysimeters with 5 liter capacities (Figure 1) installed at depths of 15, 45, and 90 cm below the surface, which were installed by researchers at the University of Virginia (Kauffman, 1998). A tipping-bucket rain gauge (ISCO 674) was deployed at the site to monitor precipitation during the experiment.

METHODS AND MATERIALS

Field experiment. We conducted a small-scale poultry litter application on a small plot of land (8.4 m^2) (Figure 2). The Virginia Cooperative Extension recommends that litter be applied at a rate of no more than 0.6 kg per square meter (2.5 tons per acre), with up to two applications during the growing season (Collins, 1996). Using those guidelines, our plot should receive a total of 12 kg of litter during the growing season. To improve the success of the experiment, we applied more litter than is recommended; 17 kg of litter were applied to the plot. The litter was tilled into the soil to mimic typical farming practices.

Litter digestion and analysis. A water soluble extraction and a bulk digestion were performed on the litter to determine the amount of As, Cu, and Zn that could be released from the litter application. The water soluble extraction was a modified form of the extraction procedure used by Jackson et al. (2003). Litter samples were placed in a drying oven overnight, cooled in a dessicator for 15 minutes, and then crushed with a mortar and pestle. A 0.5 g subset of the litter was placed in a 100 mL Pyrex bottle with 5 mL of MilliQ Nanopure water and was shaken for 1 hour using a Burrell wrist-action shaker. The extractions were centrifuged for 30 minutes (Fisher Centrific Model 228), and then passed through a 0.2 µm filter and subsequently diluted for analysis.

Litter samples were prepared for bulk digestion through drying overnight at ~30 °C. They were then cooled in a dessicator for 15 minutes and powdered in a SPEX ball mill. The samples were then digested in Aqua Regia and analyzed for trace elements using the Ultratrace1 ICP Analysis (Activation Laboratories, LTD).

Soil core sampling and digestion. Soil cores were collected during each of the four sampling periods. The top 40 cm of the soil was sampled in 10 cm increments. A homogenized portion of these 10 cm increments was placed in a drying oven overnight, allowed to cool in a desiccator, and then crushed with a mortar and pestle. The soils were digested using a modified form of EPA Method 3052 (USEPA, 1996). A 0.5 gram subset of the crushed soil was placed in a 100 mL autoclavable Pyrex bottle, and was reacted with 2 mL of 30% hydrogen peroxide. After approximately 1 hour of reaction time with the hydrogen peroxide, 9 mL of concentrated nitric acid was added, and the soils were autoclaved for 30 minutes in a Napco model 8000-DSE autoclave. The Pyrex bottles were removed from the autoclave, allowed to cool, centrifuged for 30 minutes (Fisher Centrific Model 228), and then diluted for analysis.

Soil water sampling. Prior to litter application, soil water was sampled from the lysimeters on several occasions, including the day before litter application (8/9/06), to characterize the background geochemical conditions. This is considered the pre-application sampling (A). On 8/10/06, the study site was cleared of vegetation and tilled, litter was applied, and then the site was re-tilled. The lysimeters were sampled three more times after the litter application: on 10/1/06 (S1), 11/12/06 (S2), and 12/16/06 (S3). Water was extracted from the lysimeters using a Masterflex E/S peristaltic pump. After purging ~300 mL of water from the lysimeters (if they were producing), temperature, specific conductance, dissolved oxygen (Orion Model 810A), and pH (Orion Model 290A) were measured and recorded immediately after water extraction. After sampling, lysimeters were fully purged.

After field parameters were measured, water samples were collected for analysis. Samples for trace metal and cation analysis were collected in acid-washed bottles. Subsets of the unfiltered water were set aside and preserved with nitric acid (for cation and trace element analyses), a second subset was set aside and preserved with hydrochloric acid (for TOC analyses), and a third subset was set aside without preservation (for anion analyses).

Filtration. To determine the size fractionation of trace elements in our samples, we used the aforementioned Lead et al (1997) definitions, as well as the ASTM Standard definition for

nanoparticles as guidelines to create operationally defined size categories based upon filtration. Materials that did not pass through a 1 µm filter were considered to be particulate in size, while materials that passed through a 1 µm filter but were retained by a 0.1 µm (100 nm) filter were considered to be colloidal in size, and materials that passed through 0.1 µm (100 nm) filter were considered to be nanoparticles. Materials that passed through a 0.5 KDa (approximately 1 nm pore diameter, (Guo and Santschi, 2007)) ultrafilter were considered to be dissolved. While this size cut off works well for inorganic species, it may not work as well for species such as organic carbon macromolecules that are larger than the pore size of the ultrafilter, but are still considered dissolved. It should be noted that aggregation state is not accounted for in this filtration scheme.

Based on the definitions above, aliquots of the samples were filtered according to the following scheme. Water was filtered through a series of decreasing pore diameter filters: 1, 0.7, 0.45, 0.2 and 0.1 µm. Filtrations using 1 µm and 0.7 µm pore diameter filters were performed in the field, while filtrations using 0.45 µm, 0.2 µm, 0.1 µm pore diameter filters were performed in the laboratory several hours after collection. All samples were stored in a cooler on ice during transport to the lab.

Ultrafiltration was performed on a subset of the 0.1 µm filtered water using 3 kiloDalton, 1 kiloDalton, and 0.5 kiloDalton ultrafilters in stirred cells (Amicon model 8200), although only results from the 0.5 kDa filter are presented here. Ultrafilters were allowed to soak overnight in MilliQ deionized water, were then placed within the UF cells, and were run with 200 mL of MilliQ water in order to flush the membranes. Cells were pressurized with nitrogen gas (55 psi), and stir bar rates were monitored using an Ono Sokki HT-4100 Digital Tachometer and kept at 200 rpm (+/- 10 rpm). Flow rates were monitored and maintained within the manufacturer's clean water flux rates. Two hundred mL of sample were then added to the cells, filtered, and eight 10 mL subsamples were collected to account for membrane concentration according to the scheme outlined by Tadanier (Tadanier et al., 2000; Tadanier et al., 2003). Flow rates were monitored during the filtration to ensure that they were within the manufacturer's sample flux rates.

Chemical analysis of water and digested soil samples. Samples were analyzed on a Varian SpectrAA 220Z graphite furnace atomic adsorption spectrometer with background Zeeman correction for low concentrations (< 25 µg/L) of As and Cu and on a Thermo Elemental ICAP 61E inductively coupled plasma atomic emission spectrometer for higher concentrations

(>25 μg/L) of As and Cu, as well as Zn, sodium, magnesium, calcium, potassium, silica, iron, and phosphorus. Nitrate, sulfate, phosphate, and chloride were analyzed on a Dionex DX-120 ion chromatograph. Total organic carbon (TOC) analyses were conducted using an OI Corporation Model 700 TOC analyzer.

ESEM imaging of filtered sample. A subset of the 0.7 μm filtered water from the 15 cm lysimeter in sampling period S3 was centrifuged for 1 hour (Fisher Centrific Model 228), and the resulting pellet was smeared on a scanning electron microscope sample mount and allowed to dry overnight. The sample was gold coated and then imaged and analyzed for bulk chemical composition in an Environmental Scanning Electron Microscope (ESEM) (FEI Quanta 600 FEG). The chemical composition of the filtrate was determined using Energy Dispersive X-Ray Spectroscopy (EDX).

RESULTS

Litter chemistry. The litter used in our application experiment contained 26 mg/kg acid-extractable As, 404 mg/kg Cu, and 233 mg/kg Zn, with approximately 71% (18 mg/kg) of As, 26% (108 mg/kg) of Cu and 15% (36 mg/kg) of Zn present in the water soluble fraction (Table 1). These percentages are reasonably consistent with water soluble litter extractions (72% of As, 49% of Cu, and 6% of Zn) reported by others (Jackson and Miller, 1999; Jackson et al., 2003).

Soil water chemistry. Between the pre-application sampling (A) and the first post-application sampling (S1), 27 cm of precipitation was measured at our field site (Figure 3). There was 15 cm and 8 cm of precipitation measured between S1 and S2, and S2 and S3, respectively (Figure 3). The 27 cm of rainfall between A and S1 did not result in significant volumes of soil water, as the field experiment followed an extremely dry summer, and the rain likely served to rehydrate the soil. During S1 there was only a small volume (~400 mL total) in the uppermost (15 cm) lysimeter; the other lysimeters (45 and 90 cm) were dry. During the other sampling periods (S2 and S3), the lysimeters were full (5 L) when water was collected.

Field parameters (pH, specific conductance (SC), and temperature) were measured in soil water prior to sample collection (Table 2). Due to the low volume of water collected during sampling period S1, field parameters could not be measured. Soil water temperature was highest at all lysimeter depths before litter application (A), and subsequently decreased during both the S2 and S3 sampling periods, reflecting the change in season from summer to fall. The soil water

pH remained fairly constant between 5 and 7 for all sampling periods and lysimeter depths. SC increased in the 15 cm and 45 cm lysimeters between the A and S2 sampling periods, and then decreased between the S2 and S3 sampling periods. SC in the 90 cm lysimeter increased consistently between the A and S3 sampling periods.

In the 15 cm lysimeter, organic carbon, chloride, sulfate, and nitrate were significantly higher during the S1 event (Figure 4a) then they were at the A, S2 and S3 events ($p = 0.0065$, one way ANOVA), reflecting rapid flushing of these species through the vadose zone. In contrast, As in the soil water did not reach a maximum concentration in the 15 cm lysimeter until S2 (Figure 4b). It should be noted that As was not speciated in samples during this study due to the low concentrations; however, as previous work at the same field site in which speciation of soil water was conducted (Brown et al., 2005) documented that all of the As in soil water was as As(V). Concentrations of the aforementioned constituents, as well as Cu, Zn, P (0.45 µm filtered), and Fe and Si (bulk total) can be found in Table 2.

Soil chemistry. Overall, As concentrations in the soil profile were less than 10 mg/kg, both before and after litter application (Figure 5a). Some small changes in As concentration after litter application are observed (±5 mg/kg), but the patterns did not suggest significant introduction of As to the soil profile.

Prior to this application, P concentrations were highest at the surface (920 mg/kg) and declined with depth. After application, spikes in P concentrations were observed deeper in the soil profile (10-20 cm, 20-30 cm), suggesting some vertical transport of P. There was no evidence of increased P at the deepest soil interval sampled (30-40 cm) during the course of the experiment.

Background Cu and Zn patterns in soils were similar to phosphorous, with highest concentration at the surface (20 mg/kg Cu, 50 mg/kg Zn), declining with depth (Figures 5c and d). Litter application increased the surface concentrations of Cu and Zn, and over time, concentrations of both of these elements exceeded background in deeper intervals (10-20 cm, 20-30 cm). Similar to P, there was no evidence of Cu and Zn at the deepest soil interval sampled (30-40 cm) during the course of the experiment.

Particle, colloid, and nano-dissolved species distribution. When the concentrations of filtered species are divided into the particle, colloid, and nano-dissolved size categories there are clear differences between organic carbon, Si and Fe, and trace elements (Figure 6).

Before litter application, the majority (> 80%) of the organic carbon was nano-dissolved. Despite the increase in OC concentration after litter application (S1), the majority of OC (>80%) remained in the nano-dissolved phase after litter application (Figure 6). It should be noted that while OC falls within the nano-dissolved size range, it is likely that the organic materials released from the poultry litter application are actually macromolecules, rather than a solid phase nanoparticle. Chin et al (1994) found that organic carbon from natural samples was typically 550-2500 Daltons (.55-2.5 kDa) in size, which is within the size range of the fulvic acids they analyzed for reference.

The majority of Si and Fe in soil water was in the particle size range (> 74% of Si, and > 82% of Fe) (Figure 6). ESEM imaging of the filtrate from the 0.7 µm membrane used to filter soil water from the 15 cm lysimeter showed both platy and angular particles less than 1 µm in diameter (Figure 7). Average bulk chemical composition of the suspended particulates using EDX indicates that they are composed primarily of oxygen, silica, and aluminum with lesser concentrations of iron, potassium, magnesium, and carbon (Table 3), giving support to the assumption that particles are composed primarily of clays and iron oxides.

P, Cu, and Zn were found primarily in the particle and nano-dissolved size ranges, with a smaller proportion (< 25%) in the colloid size range (Figure 6). In contrast, the distribution of As was mostly (>50%) in the nano-dissolved range, with lesser presence in the colloid range (Figure 6). Cu and Zn partitioning are comparable, suggesting that these two elements have similar behavior within the subsurface. In contrast, As and P, which are chemical analogues, surprisingly do not exhibit comparable partitioning, indicating different behavior.

Size distribution including dissolved species. For the As and OC data, we were able to separate the nano-dissolved size range into distinct nanoparticle (1 to 100 nm) and dissolved (< 1 nm) size ranges (Figure 8). Results for the 15 cm lysimeter data show that only a small portion (< 31%) of the organic carbon is truly dissolved, while a much larger proportion (> 50%) of the As is dissolved. Additionally, when the OC increases after the litter application (S1), the majority of this increase is seen in the nanoparticle size range, whereas the increase in As after the litter application (between S1 and S2), is seen primarily within the dissolved size range.

DISCUSSION

Release of trace and other elements to the soil and soil water. Arsenic concentrations in the litter were lower (25 mg/kg) than in other studies (> 30 mg/kg; Vanderwatt et al., 1994; Moore et al., 1998; Jackson et al., 1999). As a result, we saw lower As concentrations released into the soil (< 10 mg/kg) and soil water (< 15 µg/L) than was initially expected. Cu and Zn concentrations within the litter were also lower than expected (404 mg/kg and 233 mg/kg, respectively); previous studies have shown that these metal concentrations in the majority of litter samples are typically greater than 400 mg/kg Cu and greater than 300 mg/kg Zn (Vanderwatt et al., 1994; Moore et al., 1998; Jackson et al., 1999; Nicholson et al., 1999; Jackson et al., 2003). Similar to the As concentrations, the Cu and Zn concentrations released into the soil and soil water were lower than initially expected.

Although low producing and dry lysimeters during the S1 sampling period complicate the evaluation of temporal patterns in soil water chemistry following litter application, it is clear that our litter application released nutrients (nitrate, phosphorus, chloride, sulfate), OC, and trace elements (As, Cu, Zn) to soil water. Peak concentrations of nitrate, chloride and sulfate were measured during S1; however, the peak concentration of As occurred in S2, indicating slower vertical transport of this trace element. Bulk (unfiltered) Si and Fe concentrations in soil water increased post litter application (see Table 2), suggesting release of clay and iron oxide particles into the soil water. It is unclear if these elements, occurring as particles in soil water, originated from the litter or whether they were mobilized due to the physical process of tilling litter into the soil, or even from changes in biogeochemical conditions (e.g. increases in OC) induced by the litter.

Although nutrients, OC, and trace elements were present in soil before application due to previous litter use at this site, changes in soil chemistry from our litter application are still apparent. Litter application increased concentrations of Cu, Zn and P within the upper 10 cm of soil. The As signal was much more subtle, and a significant change in As in the soils was not observed. After litter application, increases of Cu, Zn and P were measured in soil to a depth of 30 cm, suggesting that these elements released from the litter were transported vertically and subsequently retained within the soil profile.

Size fractionation of elements in soil water. In soil water, P and the trace elements Cu and Zn primarily partition into the particle and nano-dissolved size ranges, with much less in the

colloid size range. Although the trends for As partitioning are more complicated due to the low concentrations measured in the soil water, most of the As released from the litter into soil water is within the nano-dissolved range. The Fe and Si in the soil water samples are found in the particle size range, with smaller portions in the colloid and nano-dissolved size ranges. Their use as proxies for iron oxides and clay minerals is supported by the EDX results.

Although this study did not use spectroscopic tools that would allow us to directly examine associations of trace elements with OC, clays or iron oxides, our results give us insight into these associations. In soil water, the majority of As is present either as a nanoparticle or in the dissolved phase. Because the organic carbon in soil water is present dominantly in the nanoparticle phase, while the clays and iron oxides were present as particles, our results suggest that most of the As released from the litter into soil water was either dissolved or complexed with organic matter, with less associated with clays or iron oxides. This was surprising, as it is well-documented that inorganic As adsorbs strongly to iron oxides and clays (McBride, 1994; Stollenwerk, 2003, and other references therein) and one study by Harvey (2006) showed similar adsorption patterns between roxarsone and As(V) to goethite and kaolinite. However, recent work has documented As complexation with NOM (Buschmann et al., 2006), supporting the possibility of As complexing with OC released from the litter. It is also possible that the As was outcompeted by organic acids released from poultry litter for sorption sites on mineral surfaces, as has been documented by Grafe et al. (2001; 2002), resulting in lower sorption of As to minerals.

In soil water, Cu and Zn were present mainly associated with particles and nanoparticles. These results suggest that Cu and Zn were either complexed with organic matter, or associated with clays and iron oxides with very little present as a truly dissolved species. Previous studies document that Cu and Zn adsorb to oxides and clays in soils (Harter, 1983; Cavallaro and McBride, 1984; McBride, 1994; Covelo et al., 2007). Cu and Zn will also complex with NOM (Tessier et al., 1979; Drever, 1997; McBride et al., 1997; Ashworth and Alloway, 2004).

Phosphorus did not behave like As (its chemical analogue), but instead it, along with Cu and Zn, associated with clays and iron oxides. Phosphorus was found primarily with particles and nano-dissolved size ranges, which, like Cu and Zn, suggests that the P released from the litter into soil water was either complexed with organic matter, or associated with clays and iron oxides. The association of P, and not As, with clays and iron oxides might indicate that there is

competitive sorption between P and As. Phosphorus was present in the soil in much higher concentrations than As, potentially allowing P to outcompete As for sorption sites on both clays and iron oxides.

Conceptual model of trace element release from poultry litter. In the soil system, transport of trace elements associated with particles, colloids, nanoparticles, organic carbon macromolecules and as dissolved species can be impacted by a host of physical and chemical mechanisms (Figure 9), including complexation, sorption/desorption, aggregation, dispersion, and physical straining within pore spaces (Ryan and Elimelech, 1996; McCarthy and McKay, 2004; DeNovio et al., 2004; Kretzschmar and Schäfer, 2005). Our results suggest that As released from poultry litter was mainly dissolved (Figure 9.1), with a smaller portion complexed with OC (Figure 9.2). Arsenic removal from water via sorption to sediment was not significant. In contrast, Cu and Zn were associated with both particles and nanoparticles in soil water (Figure 9.2), suggesting association with OC, Fe-oxides, and clays. The increasing concentrations of these elements in surface sediment post litter application, with subsequent increases with depth (up to 25 cm), support adsorption to sediment minerals. The increase in bulk Si and Fe in soil water post application demonstrates that the litter application enhanced the release of these elements in the form of particles to soil water. Combined, the results of this study suggest that the introduction of poultry litter not only introduced trace elements to the soil water and sediment systems, but also changed the partitioning of trace elements by introducing OC, and by increasing mobilization of particles and colloids of iron oxides and clays into soil water.

IMPLICATIONS

These results have implications for the use of field geochemical data in speciation solubility models. In our case, if we had used the concentrations from the 0.45 micron filtered samples, we would have greatly overestimated the concentrations of truly dissolved phases. This would impact speciation-solubility calculations used to predict how the trace elements are speciated or complexed, as well as the degree of saturation of minerals of interest.

Another implication of this work is the recognition that different sizes of minerals and organic material exist in natural waters, and that trace elements can be associated with all of these size ranges. The presence of nanoparticles, in particular, is of interest as increased

reactivity with decreasing particle size has been observed for a number of different natural materials. In particular, Liu et al. (2009) showed that the dissolution of galena nanocrystals occurs faster than the dissolution of galena microcrystals, even when aggregated. Vikesland et al. (2007) found that 9 nm magnetite crystals were degraded more quickly in carbon tetrachloride than 80 nm crystals. Studies on nanoscale hematite have shown that manganese oxidizes more quickly on 7.3 nm particles than 37 nm particles (Madden and Hochella, 2005), and that Cu has a higher affinity for sorption onto 7 nm hematite particles when compared to 25 or 88 nm particles (Madden et al., 2006).

Last, the distinction between dissolved and non-dissolved (nanoparticle, colloid, particle) has implications for bioavailability. One notable study found that, depending upon the metal in question, colloidal adsorption can either increase or decrease its bioavailability (Wang and Guo, 2000). In particular, Wang and Guo (2000) found that Zn bioavailability to both mussels and clams decreased when bound to colloidal OC, but Cr bioavailability increased when bound to colloidal OC. Understanding how colloidal complexation affects bioavailability is important, particularly regarding filter feeders, as they are capable of taking up and accumulating contaminants through both water and sediments.

FIGURES

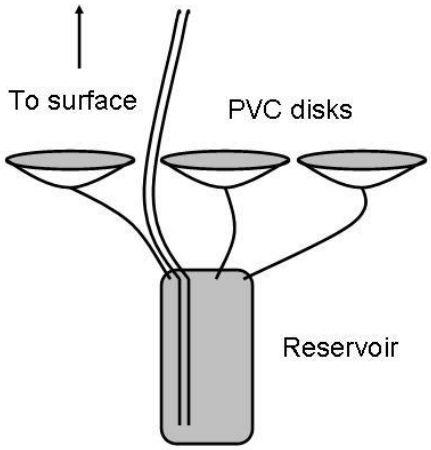

Figure 1: Schematic diagram of pan lysimeters at Muddy Creek site. Lysimeters were installed at 15, 45, and 90 cm below ground surface.

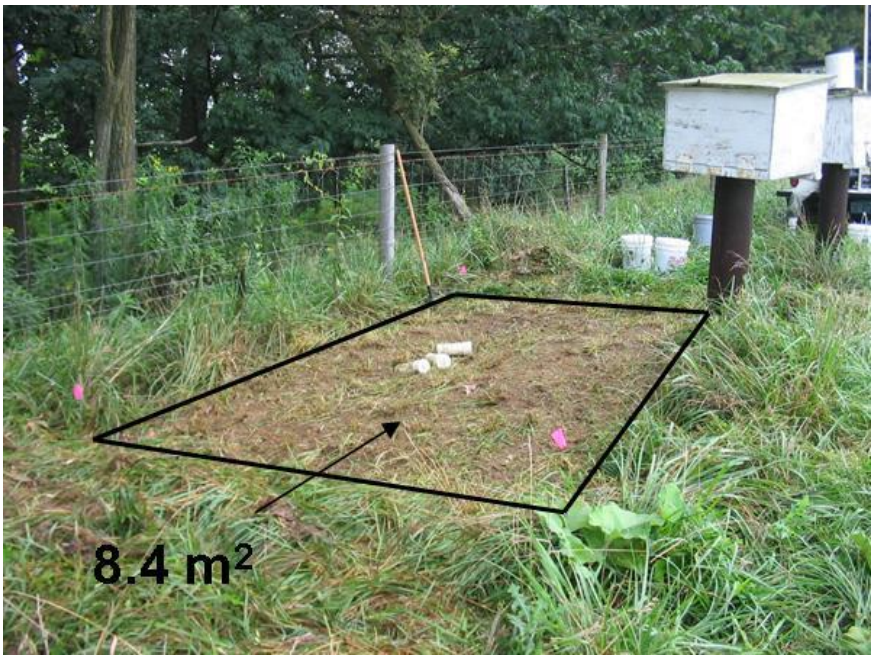

Figure 2: Litter application area at sampling site. The PVC caps indicate the location of the three lysimeters.

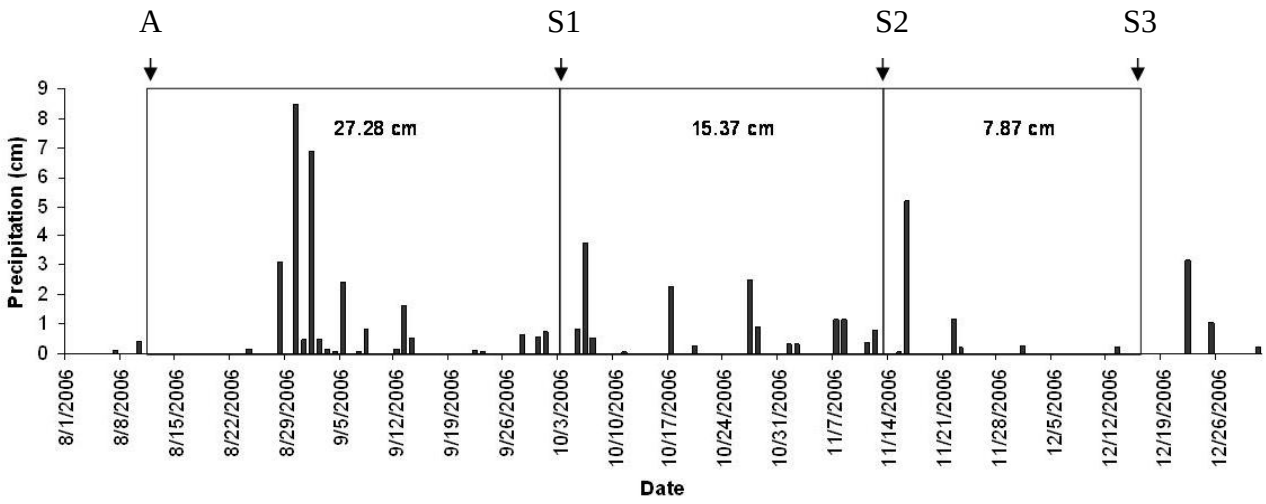

Figure 3: Precipitation at the study site from August 1st, 2006 to January 1st, 2007. Also noted are the four sampling periods: A, S1, S2, and S3.

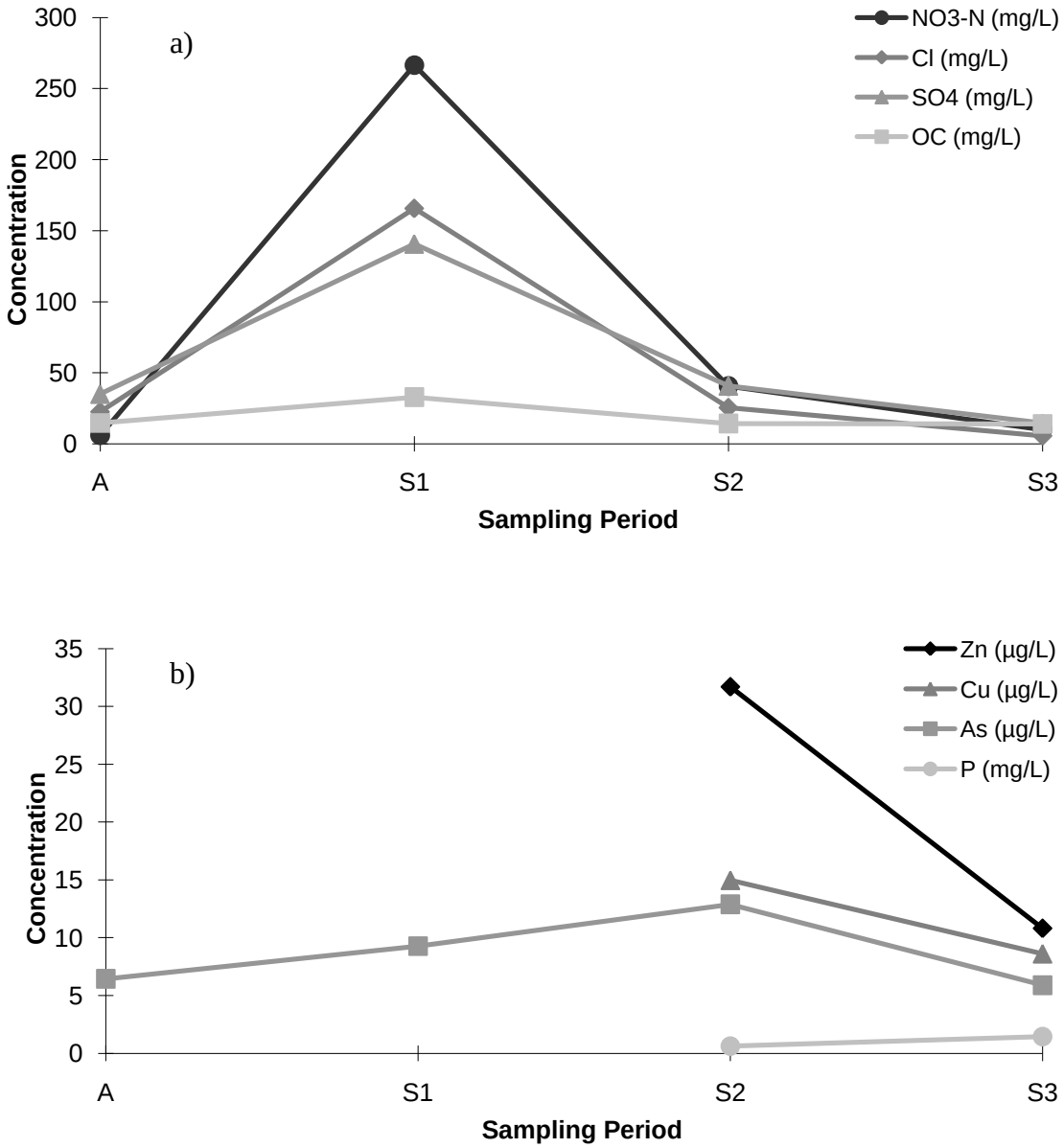

Figure 4: Selected solute concentrations (0.45 µm filtered) in the 15 cm lysimeter: a) NO_3-N, Cl, SO_4, and OC, and b) Zn, Cu, As, and P.

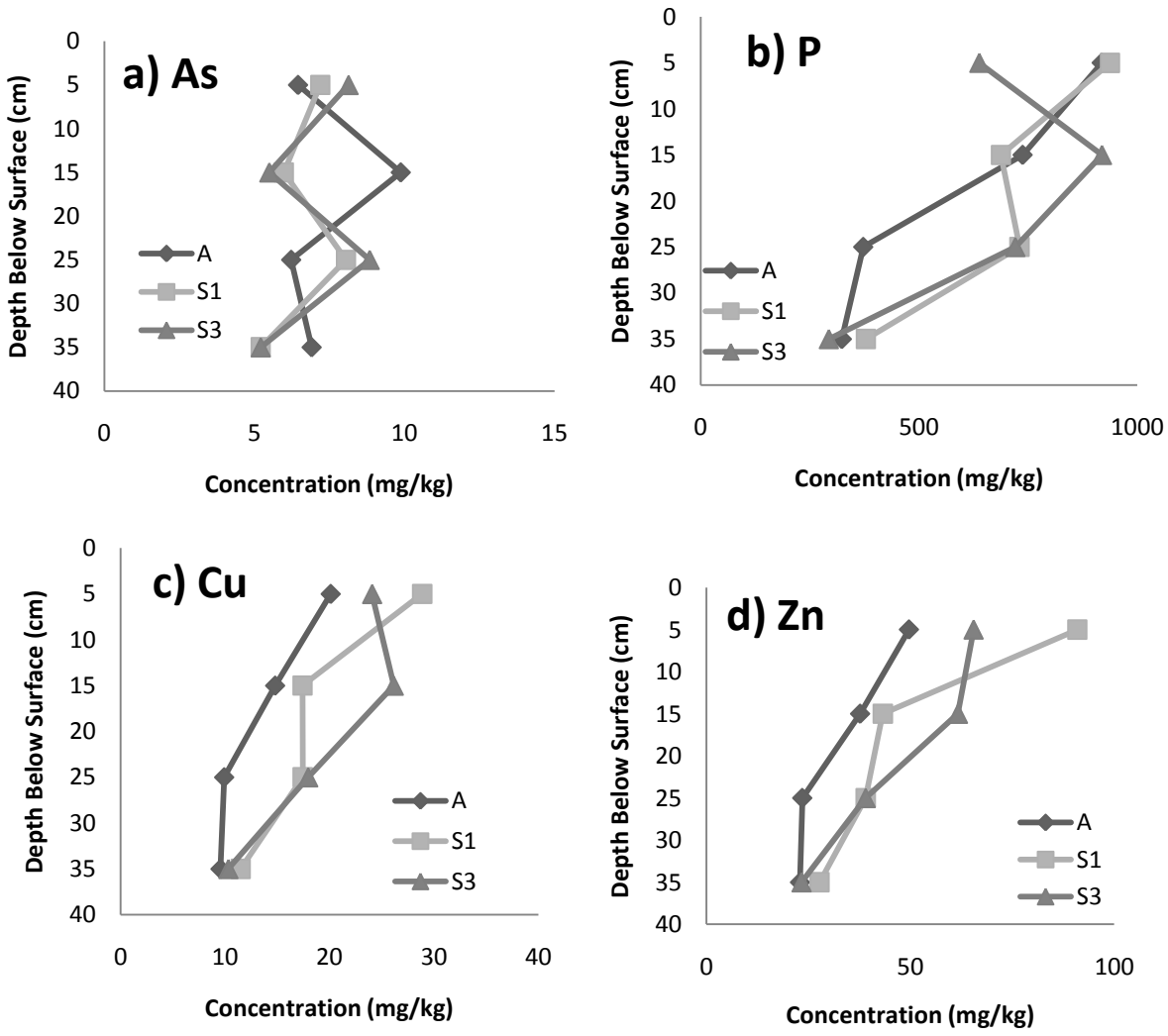

Figure 5: Concentration of a) As, b) P, c) Cu, and d) Zn in the upper 40 cm of soil during sampling periods A, S1, and S3. During S2 the soil was saturated and could not be sampled below 10 cm. Samples were depth integrated over the sampling interval, and reported depths on axes are at the midpoint of the sample core.

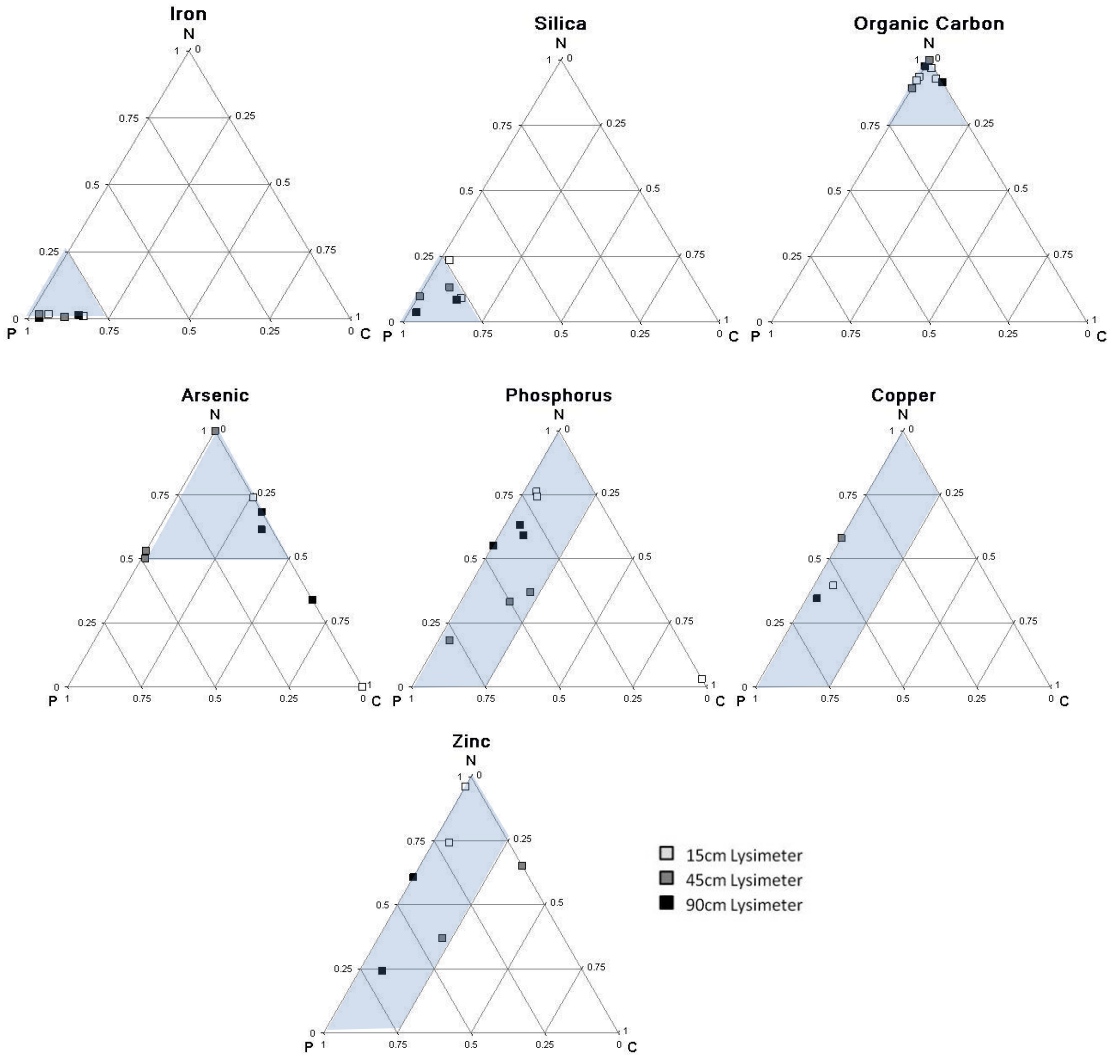

Figure 6: Proportions of As, P, Zn, Cu, organic carbon, iron, and silica present in the particle (P), colloid (C), and nano-dissolved (N) size ranges from the A, S2, and S3 sampling periods in all lysimeters; 15cm (white squares), 45cm (gray squares), and 90cm (black squares). The shaded areas highlight regions in the ternary diagram where the majority of samples are located.

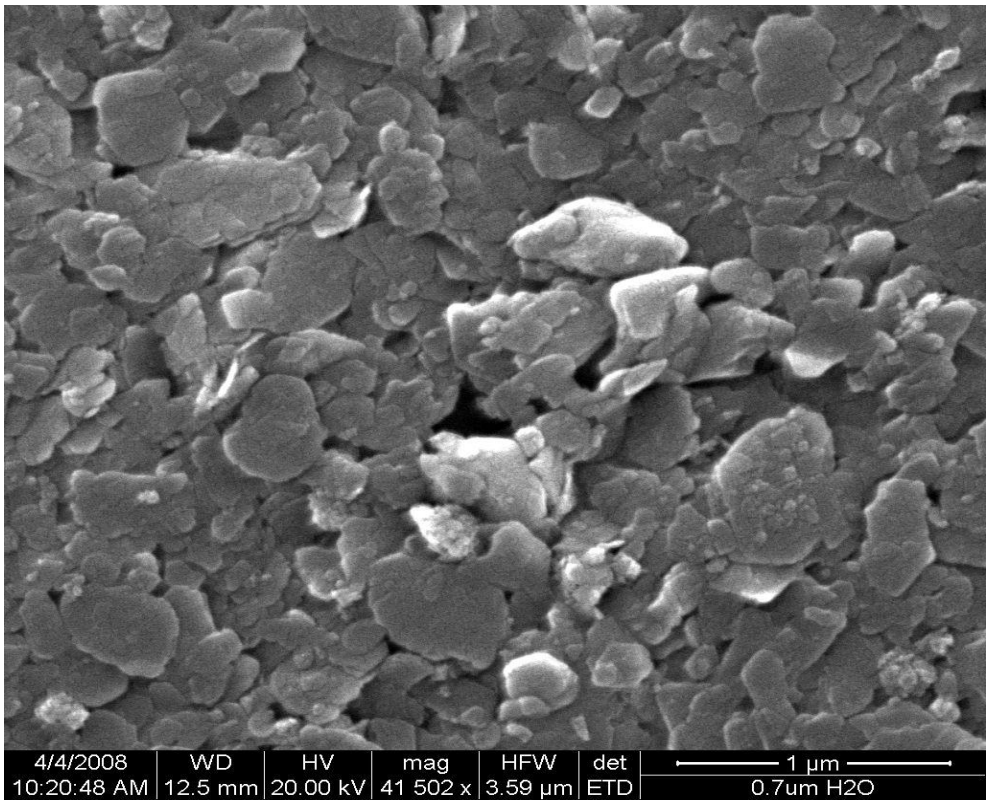

Figure 7: ESEM image of particles from 0.7um filtered water sample from 15cm lysimeter. Note 1 micron scale bar at the bottom. EDX results shown in Table 3.

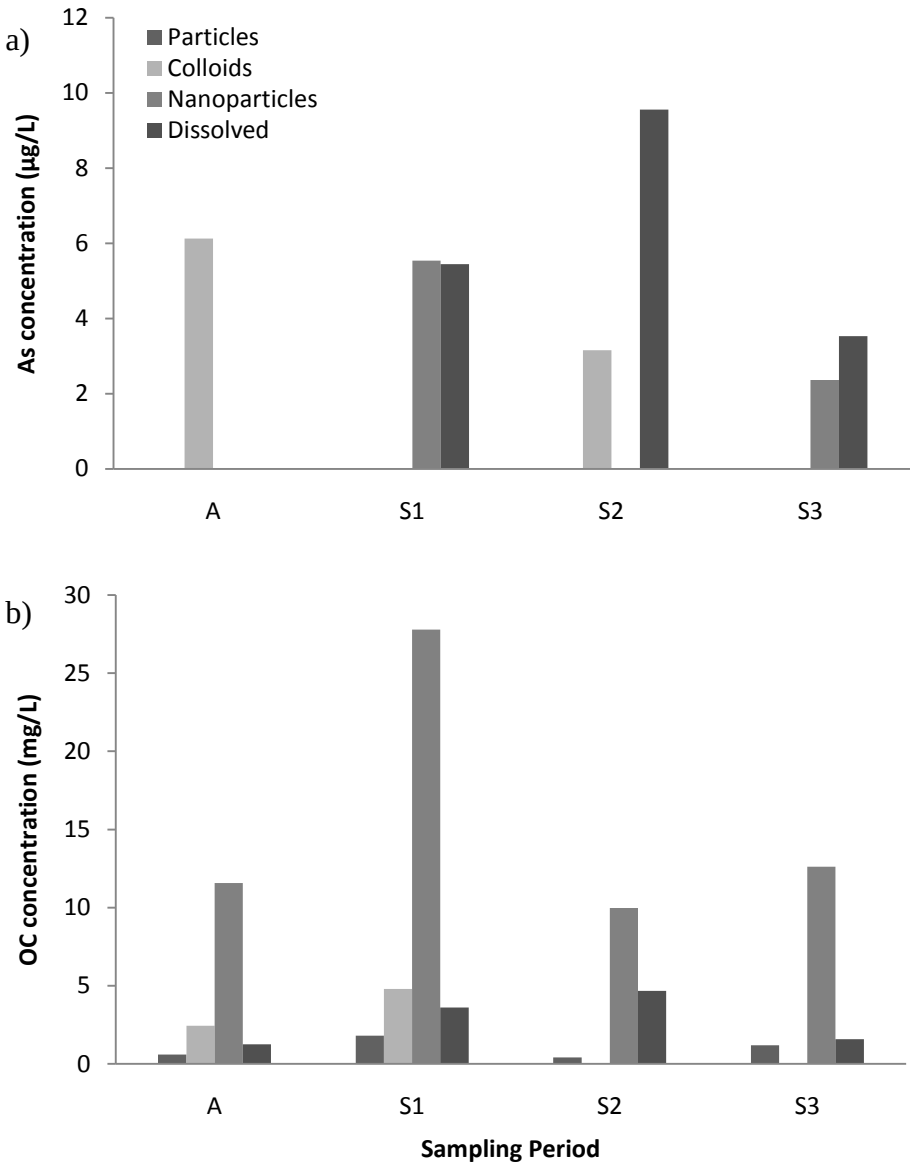

Figure 8: Concentrations of a) As and b) organic carbon present within the particle, colloid, nanoparticle, and dissolved size ranges from all sampling periods within the 15cm lysimeter. Note that the organic carbon falling into the nanoparticles range most likely reflects macromolecules.

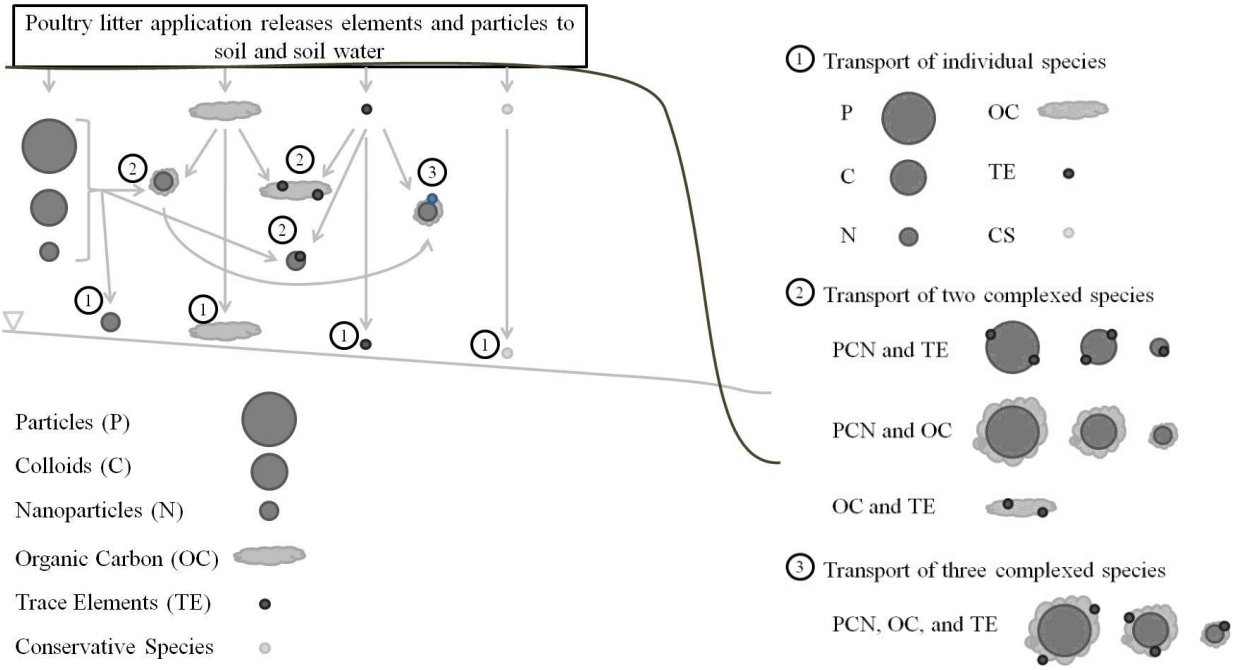

Figure 9: Conceptual model of particle (P), colloid (C), nanoparticle (N), organic carbon (OC), and trace element (TE) interaction and transport within the subsurface. Results suggest that As released from poultry litter was present either in dissolved form (Mechanism 1) or complexed with organic matter (Mechanism 2), while Cu, Zn and P were complexed with clay or iron oxide particles or OC (Mechanism 2). Although not shown in the diagram, aggregation of particles is likely occurring.

TABLES

Table 1: Acid-extractable and water soluble concentrations of As, Cu, and Zn within the poultry litter.

	Acid extractable (mg/kg)	Water Soluble (mg/kg)	Percent Water Soluble
As	26	18	71%
Cu	404	108	27%
Zn	233	35	15%

Table 2: Soil water chemical parameters (temperature (Temp), pH, specific conductance (SC)), and 0.45 µm filtered concentrations for all analytes during all sampling periods, except for Si and Fe, which are bulk total concentrations. Soil water chemical analysis during S1 was limited by low volume or dry lysimeters. All concentrations in mg/L unless otherwise specified.

		Temp (°C)	pH	SC (µS/cm)	As (µg/L)	Cu (µg/L)	Zn (µg/L)	P	OC	Bulk Si	Bulk Fe	NO3-N	SO4	Cl	Ca	K	Mg	Na
15cm	**A**	20.5	6.1	210	6.4	---	---	0.72	14.7	2.6	0.9	6.0	35.1	22.7	35.9	48.2	8.5	1.1
	S1	---	---	---	9.3	---	---	---	32.9	---	---	266.5	140.7	165.7	---	---	---	---
	S2	10.5	5.4	714	12.9	9.2	31.7	0.63	14.4	9.4	3.0	40.7	40.9	25.6	67.7	28.3	15.2	10.3
	S3	9.6	6.7	332	5.9	8.6	10.8	1.44	14.0	17.3	5.9	10.0	14.6	5.8	31.1	17.8	6.7	5.1
45cm	**A**	19.0	5.7	140	9.2	---	---	0.93	15.9	6.0	1.0	2.9	17.1	0.9	14.6	14.3	5.5	2.3
	S2	11.0	5.4	252	9.4	15.5	18.6	0.99	15.8	20.8	7.0	6.4	14.7	4.6	17.9	16.6	6.4	3.3
	S3	8.7	5.8	197	8.7	10.4	11.4	1.00	16.0	42.1	15.1	1.2	14.4	3.1	14.0	13.9	5.0	2.8
90cm	**A**	21.5	6.2	178	4.4	---	---	0.70	9.2	3.6	3.0	1.7	35.9	0.6	9.7	7.4	3.4	3.9
	S2	12	5.9	188	11.0	9.2	66.1	0.19	8.2	19.1	6.5	4.4	26.2	4.3	13.7	11.8	4.9	5.4
	S3	9.5	5.1	196	2.0	5.9	10.8	0.12	6.3	12.6	4.3	3.8	31.5	5.0	13.7	11.4	4.8	5.0

Table 3: Average percent of bulk chemical composition of suspended particulates from 15 cm lysimeter during S3.

Chemical Species	% of Total Composition
O	54.23
Si	20.07
Al	14.82
Fe	5.96
K	2.20
Mg	1.45
C	1.30

REFERENCES CITED

Alloway, B.J., 1990. Heavy metals in soils. Blackie; Halsted Press, Glasgow; New York.

Anderson, B.K. and Chamblee, T.N., 2001. The effect of dietary 3-nitro-4-hydroxyphenylarsonic acid (roxarsone) on the total arsenic level in broiler excreta and broiler litter. Journal of Applied Poultry Research, 10(4): 323-328.

Ashworth, D.J. and Alloway, B.J., 2004. Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. Environmental Pollution, 127(1): 137-144.

ASTM Standard E2456, 2006. "Standard Terminology Relating to Nanotechnology," ASTM International, West Conshohocken, PA, 2006, DOI: 10.1520/E2456-06, www.astm.org

Bednar, A.J. et al., 2003. Photodegradation of roxarsone in poultry litter leachates. Science of The Total Environment, 302(1-3): 237-245.

Brown, B.L., Slaughter, A.D. and Schreiber, M.E., 2005. Controls on roxarsone transport in agricultural watersheds. Applied Geochemistry, 20(1): 123-133.

Buschmann, J. et al., 2006. Arsenite and arsenate binding to dissolved humic acids: Influence of pH, type of humic acid, and aluminum. Environmental Science and Technology, 40(19): 6015-6020.

Cavallaro, N. and McBride, M.B., 1984. Zinc and copper sorption and fixation by an acid soil clay: Effect of selective dissolutions. Soil Science Society of America Journal, 48(5): 1050-1054.

Chin, Y.P., Alken, G., and O'Loughlin, E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environmental Science and Technology, 28(11):1853-1858.

Collins, E., 1996. Fact sheet no. 10: Poultry litter management and carcass disposal. Virginia Farmstead Assessment System(Publication No. 442): 910.

Covelo, E.F., Vega, F.A. and Andrade, M.L., 2007. Simultaneous sorption and desorption of Cd, Cr, Cu, Ni, Pb, and Zn in acid soils I. selectivity sequences. Journal of Hazardous Materials, 147(3): 852-861.

DeNovio, N.M., Saiers, J.E., and Ryan, J.N. 2004. Colloid movement in unsaturated porous media: Recent advances and future directions. Vadose Zone Journal, 3: 338-351.

Drever, J.I., 1997. The geochemistry of natural waters: surface and groundwater environments. Prentice Hall, Upper Saddle River, N.J.

Garbarino, J.R., Bednar, A.J., Rutherford, D.W., Beyer, R.S. and Wershaw, R.L., 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environmental Science and Technology, 37(8): 1509-1514.

Grafe, M., Eick, M.J., and Grossl, P.R., 2001. Adsorption of arsenate and arsenite on goethite in the presence and absence of dissolved organic acid. Soil Science Society of America Journal, 65(6): 1680-1687.

Grafe, M., Eick, M.J., Grossl, P.R., and Saunders, A.M., 2002. Adsorption of arsenate and arsenite on ferrihydrite in the presence and absence of dissolved organic carbon. Journal of Environmental Quality, 31(4): 1115-1123.

Guo, L. and Santschi, P., 2007. Ultrafiltration technique and its application to sampling aquatic colloids. in J.R. Lead and K.J. Wilkinson, editors. Environmental colloids and particles: behaviour, separation and characterisation. Hoboken: John Wiley & Sons Ltd, p.

Gustafsson, J.P., 2001. Modeling the acid-base properties and metal complexation of humic substances with the Stockholm Humic Model. Journal of Colloid and Interface Science, 244(1): 102-112.

Gustafsson, J.P., van Schaik, J. W. J., 2003. Cation binding in a mor layer: batch experiments and modelling. European Journal of Soil Science, 54(2): 295-310.

Harvey, M.C., 2006. Adsorption properties of roxarsone and arsenate on goethite and kaolinite. M.S. Thesis, Virginia Tech

Hach, 2006. Hydrolab DS5X, DS5, and MS5 Water Quality Multiprobes USER MANUAL.

Harter, R.D., 1983. Effect of soil pH on adsorption of lead, copper, zinc, and nickel. Soil Science Society of America Journal, 47(1): 47-51.

Hochella, M.F. Jr. and Madden, A.S. 2005. Earth's nano-compartment for toxic metals. Elements, 1(4): 199-203.

Hochella, M.F. Jr., Lower, S.K., Maurice, P.A., Penn, R.L., Sahai, N., Sparks, D.L., and Twining, B.S., 2008. Nanominerals, mineral nanoparticles, and earth systems. Science, 319(5870): 1631-1635.

Hyer, K.E., Hornberger, G.M. and Herman, J.S., 2001. Processes controlling the episodic streamwater transport of atrazine and other agrichemicals in an agricultural watershed. Journal of Hydrology, 254(1-4): 47-66.

Gustafsson, J.P., and van Shaik, J.W.J., 2003. Cation binding in a mor layer: batch experiments and modelling. European Journal of Soil Science, 54(2): 295-310.

Jackson, B.P. and Bertsch, P.M., 2001. Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environmental Science and Technology, 35(24): 4868-4873.

Jackson, B.P. et al., 2003. Trace element speciation in poultry litter. Journal of Environmental Quality, 32(2): 535-540.

Jackson, B.P. and Miller, W.P., 1999. Soluble arsenic and selenium species in fly ash/organic waste-amended soils using ion chromatography-inductively coupled plasma mass spectrometry. Environmental Science and Technology, 33(2): 270-275.

Jackson, B.P., Miller, W.P., Schumann, A.W. and Sumner, M.E., 1999. Trace element solubility from land application of fly ash/organic waste mixtures. Journal of Environmental Quality, 28(2): 639-647.

Karathanasis, A.D., 1999. Subsurface migration of copper and zinc mediated by soil colloids. Soil Science Society of America Journal, 63(4): 830-838.

Karathanasis, A.D., Johnson, D.M.C. and Matocha, C.J., 2005. Biosolid colloid-mediated transport of copper, zinc, and lead in waste-amended soils. Journal of Environmental Quality, 34(4): 1153-1164.

Kauffman, S.J., 1998. Colloid and contaminant transport through an unsaturated soil. PhD Dissertation, University of Virginia.

Kent, D.B, and Fox, P.M., 2004. The influence of groundwater chemistry on arsenic concentrations and speciation in a quartz sand and gravel aquifer. Geochemical Transactions, 5(1): 1-12.

Kretzschmar, R. and Schäfer, T., 2005. Metal retention and transport on colloidal particles in the environment. Elements, 1: 205-210.

Lead, J.R., Davison, W., Hamilton-Taylor, J. and Buffle, J., 1997. Characterizing colloidal material in natural waters. Aquatic Geochemistry, 3(3): 13-232.

Lead, J.R., and Wilkinson, K.J., 2006. Aquatic colloids and nanoparticles: Current knowledge and future trends, Environmental Chemistry, 3(3): 159-171.

Liu, J., Aruguete, D.M., Murayama, M., and Hochella, M.F., Jr. 2009. Influence of size and aggregation on the reactivity of an environmentally and industrially relevant nanomaterial (PbS). Environmental Science and Technology, 43(21): 8178-8183.

Madden, A.S. and Hochella, M.F., Jr. 2005. A test of geochemical reactivity as a function of mineral size: Manganese oxidation promoted by hematite nanoparticles. Geochimica et Cosmochimica Acta, 69(2): 389-398.

Madden, A.S., Hochella, M.F., Jr., and Luxton, T.P., 2006. Insights for size-dependent reactivity of hematite nanomineral surfaces through Cu2+ sorption. Geochimica et Cosmochimica Acta, 70: 4095-4104.

McBride, M., Sauve, S. and Hendershot, W., 1997. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. European Journal of Soil Science, 48(2): 337-346.

McBride, M.B., 1994. Environmental chemistry of soils. Oxford University Press, New York.

McCarthy, J.F. and McKay, L.D., 2004. Colloid transport in the subsurface: past, present, and future challenges. Vadose Zone Journal, 3(2): 326-337.

McCarthy, J.F. and Zachara, J.M., 1989. Subsurface transport of contaminants. Environmental Science and Technology, 23(5): 496-502.

Moore, P.A. et al., 1998. Decreasing metal runoff from poultry litter with aluminum sulfate. Journal of Environmental Quality, 27(1): 92-99.

Morrison, J.L., 1969. Distribution of arsenic from poultry litter in broiler chickens, soil, and crops. Journal of Agricultural Food Chemistry, 17(6): 1288-1290.

Nicholson, F.A., Chambers, B.J., Williams, J.R. and Unwin, R.J., 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. Bioresource Technology, 70: 23-31.

O'Dell, B. 1979. Copper and zinc in poultry nutrition. in NFIA literature review on copper and zinc in poultry, swine and ruminant nutrition. West Des Moines: National Feed Ingredients Association. p. 1-62.

Puls, R.W. and Barcelona, M.J., 1989. Filtration of ground-water samples for metals analysis. Hazardous Waste & Hazardous Materials, 6(4): 385-393.

Puls, R.W., Eychaner, J.H., and Powell, R.M. 1996. Colloid-facilitated transport of inorganic contaminants in ground water: Part 1. Sampling considerations. in J.R. Boulding, editor. EPA Environmental Assessment Sourcebook. Chelsea: Ann Arbor Press Inc. p 345-358.

Puls, R.W. and Powell, R.M., 1992. Transport of inorganic colloids through natural aquifer material: implications for contaminant transport. Environmental Science and Technology, 26(3): 614-621.

Robinson, J.S. and Sharpley, A.N., 1995. Release of nitrogen and phosphorus from poultry litter. Journal of Environmental Quality, 24(1): 62-67.

Rutherford, D.W. et al., 2003. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. Environmental Science and Technology, 37(8): 1515-1520.

Ryan, J.N. and Elimelech, M., 1996. Colloid mobilization and transport in groundwater. Colloids and Surfaces A: Physiochemical and Engineering Aspects, 107(20): 1-56.

Sen, T.K., Mahajan, S.P. and Khilar, K.C., 2002. Colloid-associated contaminant transport in porous media: 1. Experimental studies. Aiche Journal, 48(10): 2366-2374.

Sims, J.T., 1987. Agronomic evaluation of poultry manure as a nitrogen source for conventional and no-tillage corn. Agronomy Journal, 79(3): 563-570.

Sims, J.T., and Wolf, D.C. 1994. Poultry waste management: Agricultural and environmental issues. in Sparks, D.L., editor. Advances in Agronomy 52:1-83.

Stollenwerk, K.G. 2003. Geochemical processes controlling transport of arsenic in groundwater: A review of adsorption. in Welch, A.H., and Stollenwerk, K.G., editors. Arsenic in groundwater: Geochemistry and occurrence. Boston: Kluwer Academic Publishers. p 67-100.

Tadanier, C.J., Berry, D.F. and Knocke, W.R., 2000. Dissolved organic matter apparent molecular weight distribution and number-average apparent molecular weight by batch ultrafiltration. Environmental Science and Technology, 34(11): 2348-2353.

Tadanier, C.J., Berry, D.F. and Knocke, W.R., 2003. Modeling uncoupled solute transport in natural organic matter size fractionation by ultrafiltration. Journal of Environmental Engineering-Asce, 129(1): 33-42.

Tessier, A., Campbell, P.G.C. and Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51(7): 844-851.

USEPA, 1996. Microwave assisted acid digestion of siliceous and organically based matrices. Method 3052.

Vanderwatt, H.V., Sumner, M.E. and Cabrera, M.L., 1994. Bioavailability of copper, manganese, and zinc in poultry litter. Journal of Environmental Quality, 23(1): 43-49.

Vikesland, P.J., Heathcock, A.M., Rebodos, R.L., and Makus, K.E., 2007. Particle size and aggregation effects on magnetite reactivity toward carbon tetrachloride. Environmental Science and Technology, 41(15): 5277-5283.

Wang, W.X., and Guo, L., 2000. Influences of natural colloids on metal bioavailability to two marine bivalves. Environmental Science and Technology, 34: 4571-4576.

Wigginton, N.S., Haus, K.L., and Hochella, M.F., Jr. 2007. Aquatic environmental nanoparticles. Journal of Environmental Monitoring, 9: 1306-1316.

CHAPTER 2

Effect of pH, Ionic Strength, and Speciation on the Performance of an *E. coli* Bioreporter for Detection of Arsenic in Natural Waters

Elizabeth Diesel (1), Jan Roelof van der Meer (2), Madeline Schreiber (1)

(1) Department of Geosciences, Virginia Tech, Blacksburg, VA 24061-0420, USA

(2) Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland

ABSTRACT

A new luminescent *E. coli* bioreporter for arsenic was created and tested using laboratory arsenic (As) solutions and a natural sample from a former As mine. Solutions for four different species of As (As(III), As(V), and two organoarsenicals, monosodium methyl arsenate (MSMA) and roxarsone) across a range of concentrations were utilized. Exposure of the bioreporter to these different arsenic species resulted in variable luminescent response, with the strongest response from As(III), followed by As(V) and MSMA. Roxarsone produced no response above background luminescence. Field samples were analyzed to determine the response from a solution containing both As(III) and As(V); this showed that solutions containing more than one As species in solution necessitates sample speciation or a standard curve slope analysis to account for all forms of As present. Arsenic solutions with altered pH and cation concentrations were utilized to determine the effect of sample geochemistry on bioreporter performance. Cation concentration was found to have an impact on luminescent response; it increases *E. coli* growth and also effects arsenic transport to the cell interior. In particular, increased NaCl and KCl in solution increased both growth and luminescent response, while $CaCl_2$ in solution increased growth but decreased luminescent response. In contrast to cation concentration, pH values ranging from 4 to 8 were found to have no effect upon *E. coli* luminescent response, regardless of speciation. These results not only have implications for using bioreporters to monitor As in natural systems, but also for applying bioreporters to assess bioavailabiliy.

INTRODUCTION

Arsenic (As) is a ubiquitous metalloid in the environment. It is present in natural waters worldwide due to both natural and anthropogenic sources (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002). Arsenic is both a toxin and a carcinogen, implicated in cardiovascular, pulmonary, immunological, neurological, and endocrine disorders, as well as skin, lung, bladder, and kidney cancers (NRC, 1999; NRC, 2001). In areas such as Taiwan, Bangladesh, Vietnam, India, and Hungary, among other countries, residents rely on As-contaminated groundwater for their drinking water supplies, and as a result, many people in these countries have succumbed to arsenicosis (arsenic poisoning), cancer, and other illnesses (Smith et al., 2002; Mandal and Suzuki, 2002).

Arsenic in natural waters can occur as different species and forms, such as arsenite (As(III)) or arsenate (As(V)) oxyanions, as complexes with colloidal particles or natural organic matter (NOM), or as organoarsenicals, depending on the source of contamination and the environmental conditions of the natural system (Jain and Ali, 2000). In addition to many naturally-occurring mineral sources of As, there are also human sources. Organoarsenicals are used as animal feed additives, such as 3-nitro 4-hydroxyphenolarsonic acid (also known as roxarsone), used to improve weight gain, feed efficiency, and pigmentation in poultry (Garbarino et al., 2003). Organoarsenicals are also used to control insects and weeds. For example, monosodium methyl arsonate (MSMA) is a herbicide commonly used on golf courses (Cai et al., 2002; Feng et al., 2005).

Understanding the speciation and form of As is critical, as the toxicity differs: arsenite (As(III)) is more toxic than arsenate (As(V)), which in turn is more toxic than organic forms, such as mono-methylarsonate (MMA), and dimethylarsinate (DMA) (Jain and Ali, 2000). Redox potential and pH are the two most important factors controlling inorganic As speciation, although microbially mediated reactions also play an important role in As biotransformations (Oremland and Stolz, 2003).

Methods for arsenic analysis

Analytical techniques such as atomic absorption and atomic fluorescence spectrometry, inductively coupled plasma techniques, and high-pressure liquid chromatography, while highly effective (Hung et al., 2004; Melamed, 2005), can be costly and time consuming. Other methods

of As detection include abiotic sensors, such as colorimetric tests, electrochemical sensors, and anodic stripping voltammetric probes (Melamed, 2005). However, analysis of the most common colorimetric test, which uses mercury bromide, showed that it is not accurate in the concentration range below 50 µg/L, and, consequently, gives rise to a large number of false-positive and false-negative results (Rahman et al., 2002). On the basis of this poor performance, the method has been optimized using an instrumental readout of the colorimetric signal, which resulted in significantly better As concentration prediction (Melamed, 2005). Two new colorimetric kits (Quick Arsenic and Hach EZ) were recently developed and tested in the field (Steinmaus et al., 2006). Despite this improvement, there have been major concerns regarding the use of toxic chemicals (i.e., mercury bromide, zinc, release of arsine gas) in the colorimetric tests (Melamed et al., 2005), which merits the development of alternative protocols and methods.

Whole-cell bacterial biosensors are an inexpensive and toxicant-sensitive method of monitoring environmental contamination. Due to these attributes, numerous strains of bacterial bioreporter assays capable of detecting organic compounds, xenobiotics, metals, radiation, and changes in pH have been developed (Gu et al., 2004). Additionally, these bioreporters are an effective method of monitoring the bioavailable fraction of a contaminant in environmental systems (Belkin, 2003). *Escherichia coli* biosensors have been used for the determination of bioavailability for a variety of contaminants, including mercury (Rasmussen et al., 2000), antimony (Tauriainen et al., 1997), phosphate (Dollard and Billard, 2003), and alkanes (Sticher et al., 1997). Additionally, there have been many bacterial bioreporter assays created for As detection, the majority of which are *E. coli* (Diesel et al., 2009). The reporter systems used in these bioassays include luciferase, β-Galactosidase, green fluorescent protein, cytochrome *c* peroxidase, all with varying concentration ranges of As detection (Diesel et al., 2009).

Study Objective

Most bioreporter assays have used arsenite as specific inducer for the bioreporter cells, but relatively few studies have addressed bioreporter response to different As species and forms (see, for example, Baumann and van der Meer, 2007). The practicality of As bioreporters in the field depends on their efficacy to distinguish different As species. Thus, the main objective of this study was to examine the response of the *E. coli* bioreporter to different As species. A second goal of the study was to examine the effect of geochemical conditions (e.g. cation

concentration, pH) on the response of the *E. coli* reporter to As species, which has not been previously examined. The luminescent response of the reporter is a measure of arsenic bioavailability, and to better assess bioavailability in contaminated systems, an improved understanding of the impact of speciation and geochemical controls on bioavailability measurements is needed. In particular, changes in system geochemistry may transform some of the contaminant to a bioavailable form.

In this study, we used an *E. coli* luciferase bioreporter for As. In contrast to previously developed luciferase reporter strains (Stocker et al., 2003), this strain generates the substrate for the luciferase reaction. This enables real-time kinetic analysis, as there is no need to add a separate substrate for the luciferase. Real-time analysis can be useful to measure the relative increase of the luminescence signal in response to different As concentrations. On the other hand, the system can also be used in a more classical sense by recording the signal output after a predefined assay incubation time and comparing this to a well-defined standard curve.

METHODS AND MATERIALS

Bacterial bioreporter construction

The bioluminescent *E. coli* used in this study was engineered to synthesize bacterial luciferase and regenerate the luciferase substrate in the presence of arsenite. The reporter strain (*E. coli* DH5α-2697) carries plasmid pSB403-arsR, in which a one kilobase *Eco*RI fragment with the *arsR* gene and its cognate promoter from plasmid pPROBE-arsR (Stocker et al., 2003) was placed upstream of the *luxCDABE* genes in plasmid pSB403 (Winson et al., 1998). The plasmid confers tetracycline resistance to the strain, which was added at 10 µg per ml in reporter cell cultures order to maintain the presence of the reporter plasmid.

Essentially, a low level ArsR protein is produced by the reporter strain, which is used by the bacteria to "sense" arsenite that has entered the cell. Arsenite-binding to ArsR diminishes affinity of the protein to repress its promoter, the result of which is increased expression of ArsR itself and of the LuxCDABE genes (Xu et al., 1998; Stocker et al., 2003). Arsenite binding to ArsR is quite specific, and apart from arsenite only antimonite binds as efficiently (Xu et al., 1998). Therefore, when the reporter cells are brought into contact with an arsenite-containing sample, this causes luciferase synthesis and spontaneous bioluminescence because of the substrate generation (i.e., LuxC, LuxD and luxE). In contrast, in the absence of As, leakage of

the ArsR control results in a low luciferase signal only. Because of the presence of a chromosomally located *arsRBC* operon in *E. coli* DH5a, reporter cells also produce an arsenite efflux pump (ArsB) and an arsenate (As(V) reductase (ArsC). This second *arsR* gene is not identical with the plasmid-borne *arsR* gene. As a result of the reductase the *E. coli* reporter may also respond to other forms of As than arsenite (the direct effector of ArsR), such as arsenate or organoarsenicals, which are converted intracellularly to arsenite.

Arsenite reporter bioassay

To prepare the bioluminescent assay, a small amount of *E. coli* reporter cells were transferred from a -80 °C glycerol stock to fresh medium (Luria Broth (LB) with tetracycline) and cultured for ~16 hours at 30-37°C. The culture was then diluted into fresh medium to an optical density (OD_{600}) of ~ 0.2, and regrown to exponential phase (OD_{600} of ~0.6) in order to have active cells. 100 µL culture aliquots were then pipetted into a 96 well plate and mixed with 100 µL sample aliquots in triplicate. The plate was covered and incubated at 30 °C for 1 hour, after which bioluminescence and culture turbidity were analyzed using a Tecan SpectraFluor Plus bench-top luminometer with one second integrated measurements taken on each well. Assay plates were removed and further incubated for an additional hour, after which the luciferase signal and culture turbidity were re-read on the luminometer. Where indicated, luciferase signals were normalized for culture turbidity to account for increased growth.

Arsenical stock solutions

Arsenic solutions were created in the laboratory from As salts of sodium arsenite (Fisher Scientific), sodium arsenate (J.T. Baker), monosodium methyl arsonate (Supelco Analytical), and roxarone (ICN Biomedicals Inc.). Fresh solutions were prepared before each experiment to ensure that As speciation was maintained.

For As solutions with increased cation concentrations, solutions were created using salts of NaCl, KCl, and $CaCl_2$. pH was adjusted to 4, 6, or 8 using either 0.01 M HCl or NaOH.

Field sample collection

Spring water was collected at the Brinton Arsenopyrite Mine site (BAMs) in Floyd County, Virginia, on November 6, 2009. This water contains both arsenate and arsenite, which is

derived from arsenopyrite oxidation. This site has been extensively investigated by previous researchers (Rocovich and West, 1975; Dove and Rimstidt, 1985; Walker et al., 2005; Chaffin et al., 2005; Valenti et al., 2005; Harvey et al., 2006; Lottig et al., 2007; Brown et al., 2007). This is an ideal field site to collect water for exposure experiments, as As is the dominant trace element leaching from the ore and waste piles. Ni, Co, Mo, Cu, Zn are found at only trace concentrations in water at the site (Chaffin et al., 2005; Lottig et al., 2007). Because antimonite also binds to ArsR which would induce luminiscient response of the bioreporter and thus interfere with the analysis, it is important to note that previous analysis of samples from the field site show antimony concentrations below detection (< 5 mg/kg Sb in sediments, < 8 µg/L in water).

Water samples were collected from a spring channel approximately 100 m downstream of the mine tailings piles. Field parameters (i.e. temperature, pH, dissolved oxygen, and conductivity) of the water were recorded during sampling. Arsenic speciation (As(III) and As(V)) was conducted in the field using pre-conditioned SPE SAX columns (see Wilde et al., 2004 for description of procedure). The aliquots of speciated samples were preserved with a few drops of concentrated nitric acid for chemical analysis. Additionally, 1 liter of water was collected and preserved with 10 mL 0.125 M EDTA, which has been shown to preserve the distribution of As species through chelating metal cations, buffering pH, and decreasing microbial activity (Gallagher et al., 2001; Bednar et al., 2002). Previous work by Trang et al. (2005) showed that EDTA does not affect the luminescent response of the bioreporter at concentrations < 0.2 mM. A serial dilution of the EDTA-amended water samples was used in the *E. coli* reporter assay, and EDTA concentrations in exposures were between 0.08 and 0.31 mM.

Chemical analysis of arsenic solutions

Laboratory and field samples were analyzed for As on a Varian SpectrAA 220Z graphite furnace atomic adsorption spectrometer with background Zeeman correction for low concentrations (< 25 µg/L) of As; this method has a detection limit of ~3 µg/L. Higher concentrations of As (>25 µg/L) were analyzed on a Thermo Elemental ICAP 61E inductively coupled plasma atomic emission spectrometer; the detection limit for this method is ~ 25 µg/L. For quality control, check and internal addition standards were utilized. Duplicate samples from the field and experiments were regularly analyzed to check sampling procedures. Replicate analyses were conducted every 20 samples to check instrument accuracy.

RESULTS AND DISCUSSION

Effect of speciation on luminescent response

The *E. coli* 2697 reporter strain displayed a linear proportional response of luciferase signal as a function of arsenite concentration between 10 and 200 µg/L and arsenate between 100 µg/L and 2 mg/L (Figure 1, a and b). For monosodium methyl arsonate (MSMA), the response was roughly linear between 100 µg/L and 5 mg/L (Figure 1c). The reporter strain did not measurably react to roxarsone (Figure 1d). The standard curves created from luciferase activity measurements after 1 and 2 hour incubation times were similar, with comparable R^2 values and linear equations, whereas the overall light emission from the 2 hour incubation was higher for all forms of As (Figure 1). The increase in luminescent output between 1 and 2 hours incubation is due to increased growth of cells in the assay. The two time steps analyzed were from the same batch of exposed cells, enabling us to see changes in luminescence over time from the same cells. This is an improvement for most microbial bioassays for As, as no substrate needs to be added at each time step, and multiple time steps can be analyzed with the same set of cells.

Response of the *E. coli* 2697 luciferase reporter was strongest after exposure to arsenite, followed by arsenate and MSMA, which indicates that As species are sensed differentially by the reporter strain. This makes sense, as the ArsR protein reacts directly with arsenite, and both arsenate and MSMA have to be converted into arsenite before they can derepress the reporter circuit. The luminescence resulting of exposure to As(V) is one-tenth that of As(III) at any given concentration (Figure 1, a and b), which is similar to results reported by Baumann and van der Meer (2007) for another *E. coli* As reporter strain.

Reporter cells produced very little signal increase after 1 hour exposure to MSMA, but after 2 hours incubation the luciferase activity had clearly increased above background (Figure 1c). This result is in contrast with what was seen by Baumann and van der Meer (2007) who exposed the bioreporter *E. coli* DH5α (pJAMA-arsR) to monomethylarsonic acid (MMAA or MMA). In their study, strain *E. coli* DH5α (pJAMA-arsR) produced no luminescent response beyond background in the presence of 100 µg/L MMAA in solution. MMAA is almost identical in structure to MSMA; the only difference is that MSMA has a Na atom rather than a H atom bonded to an O on the structure. It is possible that the increase in luminescence above background could be from the dissociation of Na from the MSMA structure. The Na could

increase *E. coli* growth (see discussion below), and subsequently luminescence. It is also possible that higher concentrations of MMAA, comparable to those used here for MSMA, could produce a luminescent response above background.

The low responses elicited for MSMA and roxarsone, which are both As(V) organoarsenicals, may be due to poor transport of the compounds into the reporter cell's interior, or a lowered ability of the biochemical Ars-system to produce arsenite from MSMA and roxarsone.

Effect of solution cation concentration on luminescent response

Increases in monovalent cation concentration (0.01 and 0.1 M NaCl and KCl) increased the growth of the bioreporter, as shown by higher optical densities compared with control solutions (Figure 2, a and b). However, even when normalized for optical density (Figure 3), there is still an increase in the luminescent response of the bioreporter to As in the presence of higher cation concentration solutions. This effect was seen for As(III) (Figure 3, a and d) and As(V) (Figure 3, b and e), but was not as strong for MSMA (Figure 3, c and f), and was stronger for KCl (Figure 3, a-c) than for NaCl in the assay (Figure 3, d-f).

Increases in concentration of divalent cations (0.01 and 0.1 M $CaCl_2$) also resulted in an increase in growth of the bioreporter (Figure 2c). However, the increase in $CaCl_2$ concentration resulted in a decrease of the bioluminescence, normalized to optical density (Figure 4). This effect was observed for As(III) (Figure 4a) and As(V) (Figure 4b), but not for MSMA (Figure 4c).

Our results show two effects of increasing cation concentrations. First, the increase results in an increase in *E coli* growth, observed for both monovalent and divalent cations. Na has been shown to increase *E. coli* growth in solutions where glutamate is the sole carbon source (Frank and Hopkins, 1969). In those same experiments, K was shown to also aid growth, but not to the extent of Na (Frank and Hopkins, 1969). The concentrations of Na and K used by Frank and Hopkins (1969) were lower (0.003 and 0.01M, respectively) than the concentrations of Na and K used in our study. We chose concentrations (0-0.1M solutions) that are within the typical ranges for natural waters (Langmuir, 1997). It is important to note that cells were cultured in LB, and the LB broth used in these assays contains 0.098 M Na, 0.007 M K, 0.0002 M Ca, and 0.103 M Cl. The 0.01M and 0.1M salt solutions add further Na, K, and Ca to the assay (0.108

and 0.198 M total Na, 0.017 and 0.107 M total K, 0.0102 and 0.1002 M total Ca).

Second, the increase in cation concentration impacted the luminescent response of the *E coli*, even when luminescence is normalized to the optical density. With monovalent cations (Na, K), increased concentrations increased response, whereas divalent cations (Ca) decreased response. These results suggest that monovalent and divalent cations play different roles in transporting As into the cell interior.

Arsenite is likely to enter the *E. coli* cell via a glycerol channel, GlpF (Sanders et al., 1997), whereas arsenate is likely to enter via phosphate specific channels, Pit and Pst (Rosenberg et al., 1977; Willsky and Malamy, 1980). Phosphate, however, has been shown to enter the cell through both phosphate and glycerol channels (Hayashi et al., 1964; Willsky et al., 1973). Complexation of phosphate with divalent metals (e.g. Mg, Ca, Co, Mn) has been shown to aid in phosphate transport across the *E. coli* cell membrane (van Veen et al., 1994), and K has been shown to enhance phosphate transport into the cell, via symport (Russell and Rosenberg, 1979; Russell and Rosenberg, 1980). This suggests that increasing either monovalent or divalent cation concentrations would enhance the transport of arsenate, a chemical analogue to phosphate, across the cell membrane. This effect is seen with increasing Na and K in solution, but our results suggest that increasing Ca concentrations inhibits, rather than enhances, response. We are currently investigating if the concentrations of Ca and As are high enough to approach saturation with respect to calcium arsenate and calcium arsenite minerals, for which solubility products have been estimated (Bothe and Brown, 1999; Moon et al., 2004; Zhu et al., 2006).

Additionally, it is likely that phosphate (PO_4) in the LB broth (51.4 mg/L) complexes with Ca in solution, thereby increasing the amount of PO_4 that is entering the *E. coli* cell. The Ca-PO_4 complexes are likely stronger than Ca-AsO_4 complexes, which could allow Ca-PO_4 complexes to outcompete the arsenate counterparts for transport into the cell. This would result in lowered transport of arsenate into the cell interior, subsequently decreasing the luminescence produced. The effect of increasing $CaCl_2$ on luminescient response is less with arsenite (see Figure 4 a and b). A possible explanation for this phenomenon is that arsenite transport into the cell is different than for arsenate, as it enters through a glycerol channel (GlpF), rather than a PO_4 specific channel. Additionally, at pH 6.5, arsenite is mainly present as H_3AsO_3 (Smedley and Kinniburgh, 2002), which is neutrally charged, and only weakly complexes with Ca (Marini and Accornero, 2007).

It should be noted that the ionic strength of the divalent solution is higher than the monovalent solutions and thus the experiments are not directly comparable. However, a difference in ionic strength, although it may affect response, cannot be the sole reason for the difference in response of the bioreporter to the different cation solutions, as the response increased with increasing monovalent cation concentration, but decreased with increasing divalent cation concentration.

Effect of sample pH

Sample pH had little to no effect on the luminescence produced by the *E. coli* reporter culture (Figure 5). The optical density normalized standard curves produced at sample pH 4 (Figure 5a), 6 (Figure 5b), and 8 (Figure 5c) are essentially identical, regardless of As speciation. Additionally, the optical densities of these cell solutions are essentially identical, indicating that sample pHs of 4, 6, or 8 do not affect *E. coli* growth (data not shown). These pHs are environmentally relevant, as they are well within the range seen in natural aqueous systems. The main reason for the negligible effect of sample pH is that LB acts as an excellent buffer (pH ~6.5), and was able to buffer the sample pH from all solutions to ~6.5, regardless of starting pH.

Analysis of field samples using the *E. coli* arsenic bioreporter

The field sample collected at BAMs contains both As(III) (0.11 mg/L) and As(V) (4.79 mg/L), as determined by the SPE SAX speciation followed by GFAA analysis, for a total arsenic concentration of 4.9 mg/L. A separate volume preserved with EDTA was serially diluted (1/4, 1/8, 1/16) for exposure to the *E. coli* biosensor (Figure 6a).

Because the sample contains both As(III) and As(V), the luminescent response observed is a combination of the response from both As(III) and As(V) entering the cell of the *E. coli.* If the luminescent response from this mixed species field sample is assumed to be from only one species or the other, total As concentration will be either over- or under-estimated. For example, if the luminescent response from the field sample (e.g. 2 hour response from 1/8 diluted sample at 457 RLU, Figure 6a) is plotted on a standard curve for only As(V), this results in an overestimation of the total As concentration at 1180 µg/L (solid line in Figure 6b). In contrast, if the sample is assumed to be 100% As(III), there is an underestimation of the total As concentration at 110 µg/L (solid line in Figure 6c).

When the sample is speciated for As(III) and As(V), and the luminescent contribution from each of these species is considered by determining the luminescent response from both standard curves, the total As response can be better replicated. Using the two hour 1/8 diluted field sample and knowing the speciation of the sample, the contribution from 13 µg/L As(III) (1/8 dilution of 0.11 mg/L) is 198 RLU (dashed line in Figure 6c) and the contribution from 599 µg/L As(V) (1/8 dilution of 4.79 mg/L) is 317 RLU (dashed line in Figure 6b), summing for a total luminescent response of 515 RLU. Applying this value (515 RLU) to the standard curve for the field sample in Figure 6a yields a total As concentration of 810 µg/L, which is much closer to the measured total As concentration of 613 µg/L (1/8 dilution of 4.90 mg/L). This procedure does not completely replicate the original data (457 RLU, 600 µg/L) due to the imperfect fit of the linear standard curve to the data. For example, when the luminescent response of 457 RLU is plugged into the standard curve, an As concentration of 660 µg/L is calculated, instead of the 613 µg/L As measured in the original sample.

It is, however, possible to remove these analytical complications by recognizing that the slope of the standard curve produced by the natural sample reflects the combined response of As(V) and As(III). For example, if some proportion of the As(V) and As(III) standard curve slopes (0.24 and 2.60, respectively) are assumed to make up the whole of the standard curve slope for the natural sample (0.39), we can write:

$$0.39 = 0.24x + 2.60y$$

with the proportions of the As(V) and As(III) slopes (x and y, respectively) summing to 1, or 100% of the slope of the natural sample.

Using these two equations, we can determine the slope of the standard curve from the natural sample (0.39) is comprised of 93.6% of the slope of the standard curve for arsenate (0.24) and 6.4% of the slope for the standard curve for arsenite (2.60). These proportions are very close to the proportions of As(III) and As(V) in the natural sample, with 0.11 mg/L As(III) comprising 2.2% of the total As in solution, and 4.79 mg/L As(V) comprising 97.8% of the total As.

CONCLUSIONS

This new luminescent *E. coli* bioreporter developed for this study improves microbial bioassays for As, as no substrate needs to be added at the desired time step, and multiple time steps can be analyzed with the same assay. The ability to analyze at multiple time steps enabled

the creation of a standard curve for MSMA at the two hour time step, even though increased luminescent response was not evident after 1 hour incubation. These results are promising for extending the application of the bioreporter to other more complex organoarsenicals.

Exposure of the *E coli* bioreporter to different As species results in variable luminescent response, with As(III) having the strongest response, followed by As(V) and MSMA, while roxarsone did not derepress the reporter system measurably. These results demonstrate that the use of this bioreporter for analyzing As in natural water samples must be done with caution. Analysis of the field sample from BAMs has shown that samples containing more than one species of As in solution may necessitate sample speciation to account for all forms of As present. Assuming the luminescent response will be from only one form of As in the sample will generate an over- or under-estimation of the total As concentration in the sample. However, if the slopes of the As(III) and As(V) standard curves are considered to comprise the natural sample slope, the proportion of As(III) and As(V) contributing to the luminescent response can be more effectively considered, without requiring speciation information.

This study also examines the impact of geochemical conditions on the response of the *E coli* biosensor to As. Cation concentration has an impact upon luminescent response; it can potentially increase or decrease As transport to the cell interior, as well as increase *E. coli* growth. In particular, increased monovalent cations (Na and K) in solution have increased *E. coli* growth as well as luminescent response to As, while increased divalent cations (Ca) increased growth but decreased luminescent response. This change in luminescence, when not accounted for through optical density normalization and a standard curve adjusted with added cations, could be misinterpreted as an overestimation or underestimation of the total As concentration.

In contrast to increased cation concentrations, pH values ranging from 4 to 8 were found to have no effect upon *E. coli* luminescent response, regardless of speciation, as LB was able to sufficiently buffer the pH to ~6.5 for all samples. In the range of 4-8, pH does not need to be accounted for when analyzing natural samples.

When using a bioreporter to study the concentrations of As in a natural aqueous system, it is important to understand the geochemistry of that system. Factors such as As speciation and cation concentration may have an effect upon the luminescent response of the *E. coli*, which in turn may affect the results that are reported. Our results demonstrate that speciation is the most

important factor to consider when analyzing a natural sample for As. If As speciation is not determined for natural samples, then an inaccurate measurement of concentration and in turn, bioavailability, is likely.

FIGURES

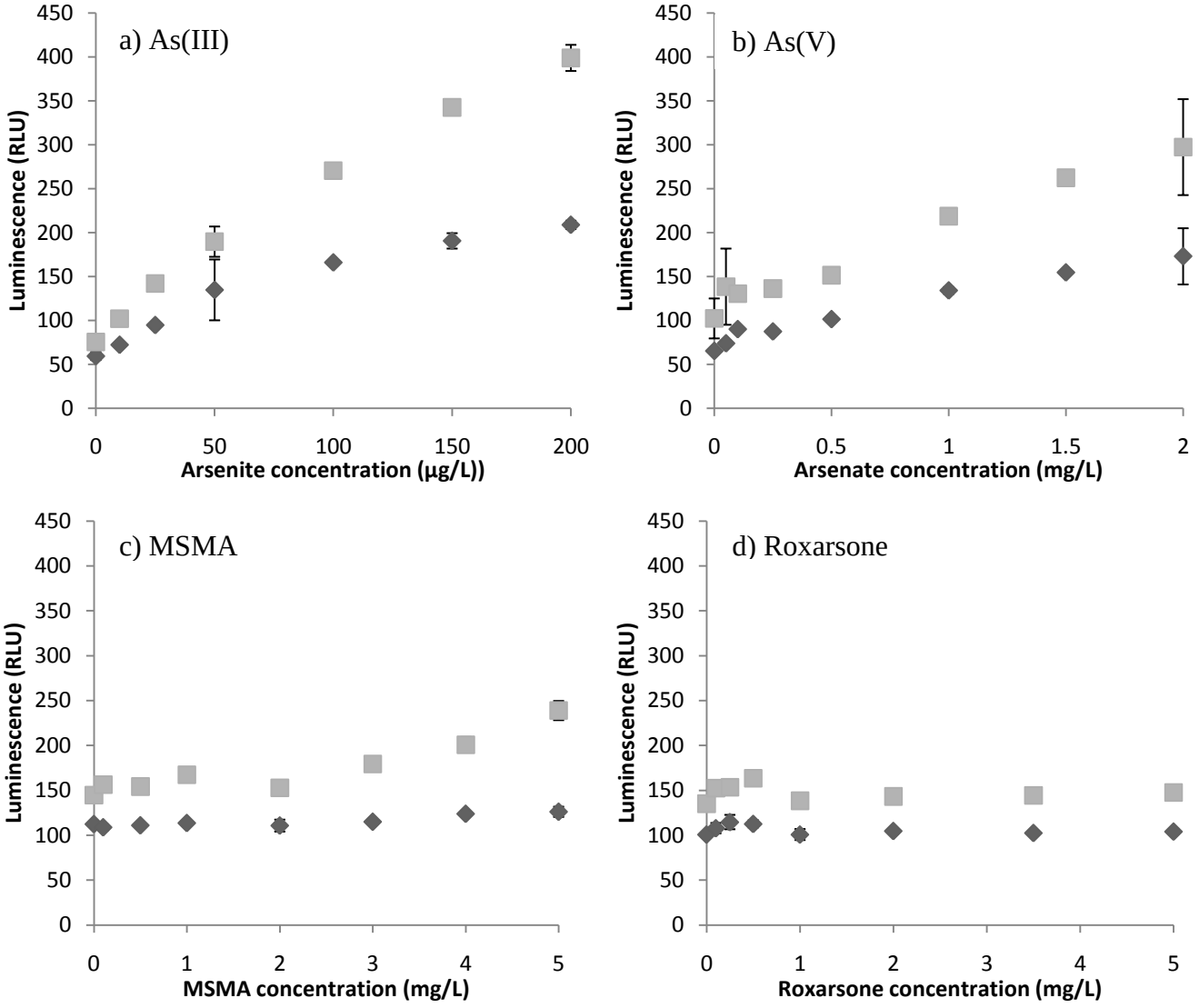

Figure 1: Light emitted in relative light units from induced bioluminescent *E. coli* over a range of a) arsenite, b) arsenate, c) MSMA and d) roxarsone concentrations after 1 hour incubation (black diamonds) and 2 hours incubation (gray squares). Error bars represent the standard deviation of the triplicates. Note the differences in concentrations used for the different As species.

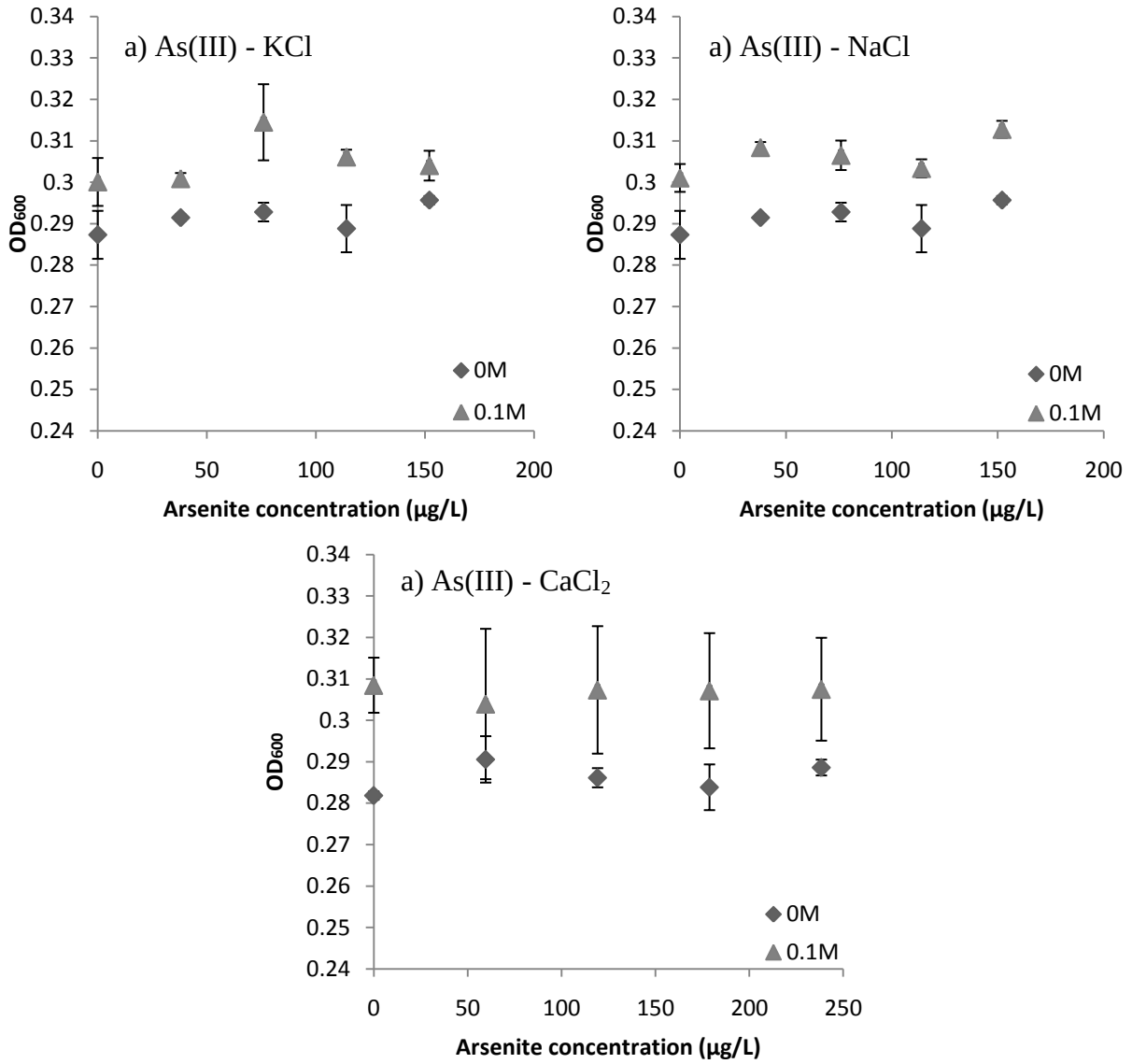

Figure 2: Optical density (growth) of induced bioluminescent *E. coli* over a range of arsenite concentrations after 2 hours incubation in solutions of 0M and 0.1M a) KCl, b) NaCl, and c) $CaCl_2$ solutions. Error bars represent the standard deviation of the triplicates.

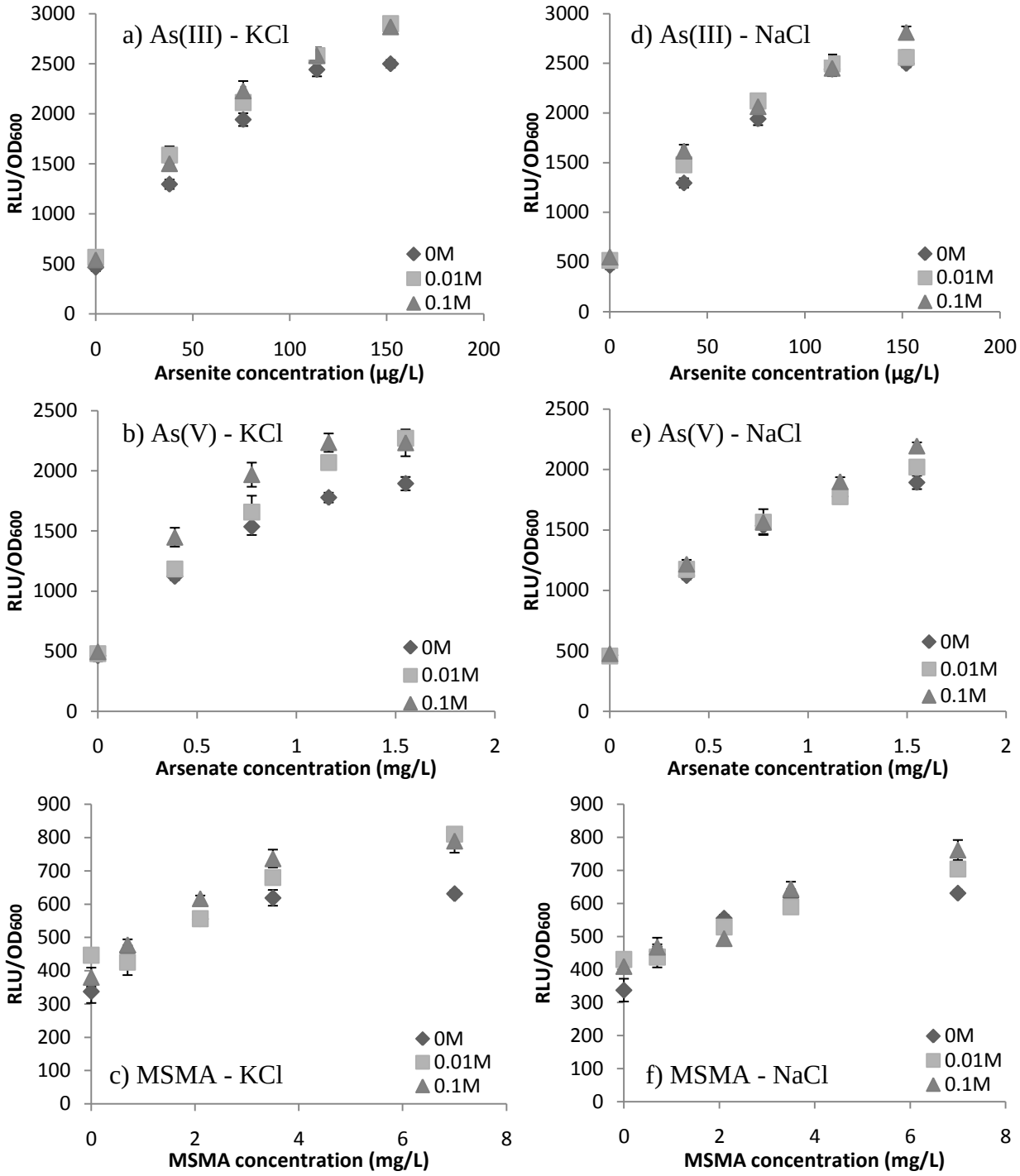

Figure 3: Light emitted in relative light units from induced bioluminescent *E. coli* over a range of arsenite, arsenate, and MSMA concentrations after 2 hours incubation in solutions of 0, 0.01, and 0.1M a-c) KCl and d-f) NaCl solutions. Error bars represent the standard deviation of the triplicates. Luminescent response has been normalized against assay optical density. Note the differences in concentrations used for the different As species.

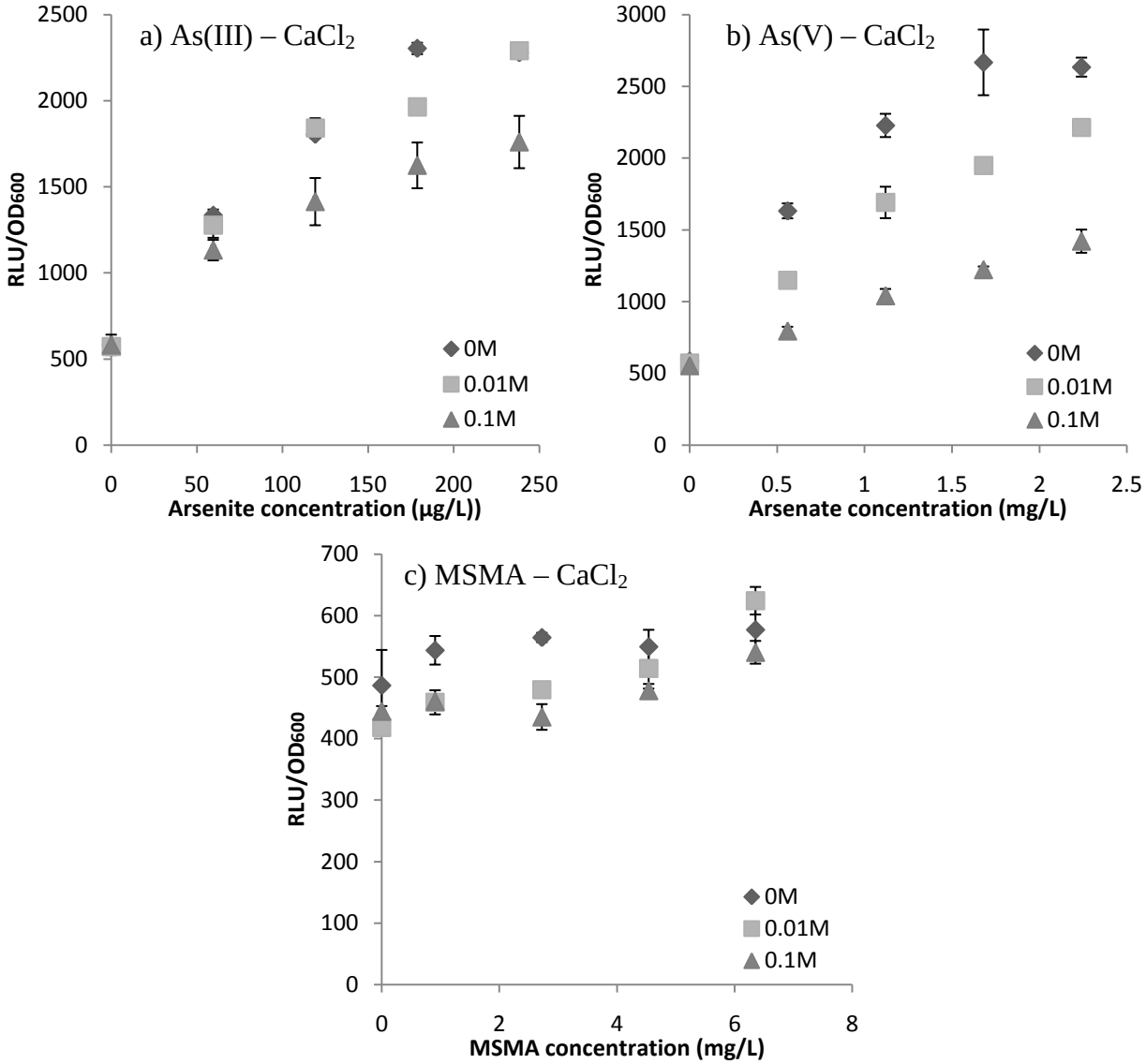

Figure 4: Light emitted in relative light units from induced bioluminescent *E. coli* over a range of a) arsenite, b) arsenate, and c) MSMA concentrations after 2 hours incubation in solutions of 0, 0.01, and 0.1M $CaCl_2$ solutions. Error bars represent the standard deviation of the triplicates. Luminescent response has been normalized against assay optical density. Note the differences in concentrations used for the different As species.

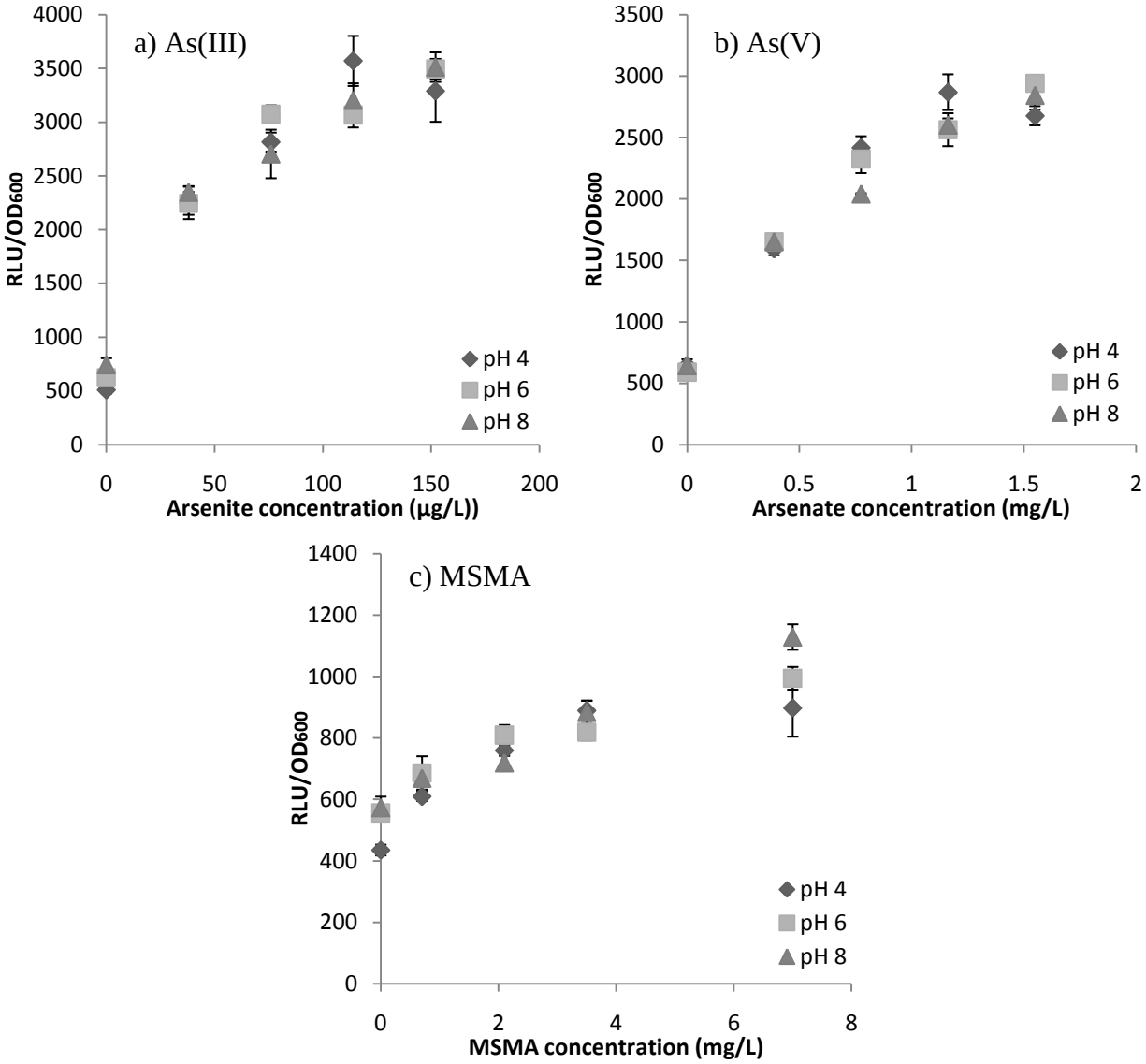

Figure 5: Light emitted in relative light units from induced bioluminescent *E. coli* over a range of a) arsenite, b) arsenate, and c) MSMA concentrations after 2 hours incubation in solutions of pH 4, 6, and 8. Error bars represent the standard deviation of the triplicates. Luminescent response has been normalized against assay optical density. Note the differences in concentrations used for the different As species.

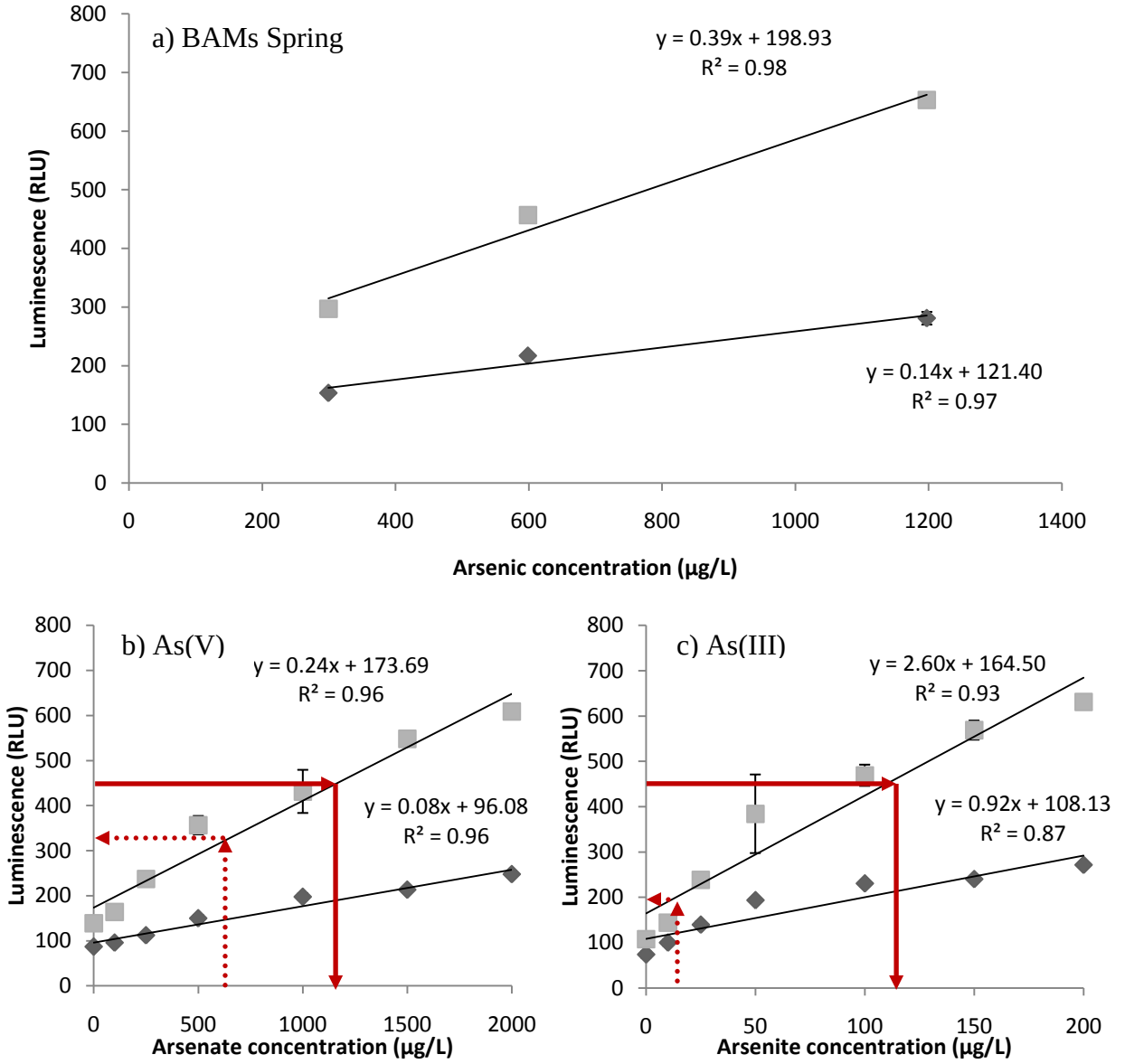

Figure 6: Light emitted in relative light units from induced bioluminescent *E. coli* over a range of a) serial dilutions of the BAMs sample (1/4, 1/8, 1/16 dilutions of original sample); total As concentrations measured using GFAA on SPE-SAX speciated samples, b) arsenate concentrations, and c) arsenite concentrations plotted against luminescent response. Black diamonds represent 1 hour incubation; gray squares represent two hours incubation. Error bars represent the standard deviation of the triplicates. Thick solid line represents overestimation (b) and under-estimation (c) of As concentration if incorrect speciation is assumed using the 457 RLU response of the two hour 1/8 dilution of the BAMs sample. Dashed line represents the RLU response if speciation is known and used to estimate concentration.

REFERENCES CITED

Alloway, B.J., 1990. Heavy metals in soils. Blackie ; Halsted Press, Glasgow; New York.

Bednar, A.J., Garbarino, J.R., Ranville, J.F., and Wildeman, T.R., 2002. Preserving the distribution of inorganic arsenic species in groundwater and acid mine drainage samples. Environmental Science and Technology 36(10): 2213-2218.

Bednar, A.J. et al., 2003. Photodegradation of roxarsone in poultry litter leachates. Science of the Total Environment, 302(1-3): 237-245.

Belkin, S., 2003. Microbial whole-cell sensing systems of environmental pollutants. Current Opinion in Microbiology, 6(3): 206-212.

Bothe, J.V., and Brown, P.W., 1999. Arsenic immobilization by calcium arsenate formation. Environmental Science and Technology, 33: 3806-3811.

Brown, B.L., Slaughter, A.D. and Schreiber, M.E., 2005. Controls on roxarsone transport in agricultural watersheds. Applied Geochemistry, 20(1): 123-133.

Brown, B.V., Valett, H.M. and Schreiber, M.E., 2007. Arsenic transport in groundwater, surface water, and the hyporheic zone of a mine-influenced stream-aquifer system. Water Resources Research, 43(11): 1-14.

Cai, Y., Cabrera, J.C., Georgiadis, M., and Jayachandran, K., 2002. Assessment of arsenic mobility in the soils of some golf courses in South Florida. Science of the Total Environment, 291: 123-134.

Chaffin, J.L., Valett, H.M., Webster, J.R. and Schreiber, M.E., 2005. Influence of elevated As on leaf breakdown in an Appalachian headwater stream. Journal of the North American Benthological Society, 24(3): 553-568.

Diesel, E., Schreiber, M., and van der Meer, J.R., 2009. Development of bacteria-based bioassays for arsenic detection in natural waters. Analytical and Bioanalytical Chemistry, 394(3): 687-693.

Dietrich, R.V., 1959. Geology and mineral resources of Floyd County of the Blue Ridge Upland, Southwestern Virginia, Engineering Experiment Station Series no. 134. Virginia Polytechnic Institute.

Dollard, M.A. and Billard, P., 2003. Whole-cell bacterial sensors for the monitoring of phosphate bioavailability. Journal of Microbiological Methods, 55(1): 221-229.

Dove, P.M. and Rimstidt, J.D., 1985. Solubility and stability of scorodite, $FeAsO_4.2H_2O$. The American Mineralogist, 70(7-8): 838-844.

Feng, M., Schrlau, J.E., Snyder, R., Snyder, G.H., Chen, M., Cisar, J.L., and Cai, Y., 2005. Arsenic transport and transformation associated with MSMA application on a golf course green. Journal of Agricultural and Food Chemistry, 53(9): 3556-3562.

Frank, L., and Hopkins, I., 1969. Sodium-stimulated transport of glutamate in Escherichia coli. Journal of Bacteriology, 100(1): 329-336.

Gallagher, P.A., Schwegel, C.A., Wei, X., and Creed, J.T., 2001. Speciation and preservation of inorganic arsenic in drinking water sources using EDTA with IC separation and ICP-MS detection. Journal of Environmental Monitoring, 3(4): 371-376.

Garbarino, J.R., Bednar, A.J., Rutherford, D.W., Beyer, R.S. and Wershaw, R.L., 2003. Environmental Fate of Roxarsone in Poultry Litter. I. Degradation of Roxarsone during Composting. Environ. Sci. Technol., 37(8): 1509-1514.

Gu, M.B., Mitchell, R.J., and Kim, B.C., 2004. Whole-cell-based biosensors for environmental biomonitoring and application. Advances in Biochemical Engineering/Biotechnology, 87: 269-305.

Harvey, M.C., 2006. Adsorption properties of roxarsone and arsenate on goethite and kaolinite. MS Thesis, Virginia Tech.

Harvey, M.C., Schreiber, M.E., Rimstidt, J.D. and Griffith, M.M., 2006. Scorodite Dissolution Kinetics: Implications for Arsenic Release. Environmental Science & Technology, 40(21): 6709-6714.

Hayashi, S.I., Koch, J.P., and Lin, E.C.C., 1964. Active transport of L-α-Glycerophosphate in Escherichia coli. The Journal of Biological Chemistry, 239(9): 3098-3105.

Hung, D.Q., Nekrassova, O., and Compton, R.G., 2004. Analytical methods for inorganic arsenic in water: A review. Talanta, 64(2): 269-277.

Jain, C.K. and Ali, I., 2000. Arsenic: Occurrence, toxicity, and speciation techniques. Water Research, 34(17): 4304-4312.

Langmuir, D., 1997. Aqueous environmental geochemistry. Prentice Hall, Upper Saddle River, N.J.

Lottig, N.R., Valett, H.M., Schreiber, M.E. and Webster, J.R., 2007. Flooding and arsenic contamination: Influences on ecosystem structure and function in an Appalachian headwater stream. Limnology and oceanography, 52(5): 1991.

Mandal, B., K. and Suzuki, K.T., 2002. Arsenic round the world: A review. Talanta, 58(1): 201-235.

Marini, L., and Accornero, M., 2007. Prediction of the thermodynamic properties of metal-arsenate and metal-arsenite aqueous complexes at high temperatures and pressures and some geological consequences. Environmental Geology, 52(7): 1343-1363.

Meighen, E.A., 1991. Molecular Biology of Bacterial Bioluminescence. Microbiological Reviews, 55(1): 123-142.

Melamed, D., 2005. Monitoring arsenic in the environment: A review of science and technologies with the potential for field measurements. Analytica Chimica Acta 532(1): 1–13.

Moon, D.H., Dermatas, D., and Menounou, N., 2004. Arsenic immobilization by calcium-arsenic precipitates in lime treated soils. Science of the Total Environment, 330: 171-185.

Morrison, J.L., 1969. Distribution of arsenic from poultry litter in broiler chickens, soil, and crops. J. Agric. Food Chem., 17(6): 1288-1290.

National Research Council (NRC), 1999. Arsenic in Drinking Water. National Academy Press, Washington DC.

National Research Council (NRC), 2001. Arsenic in Drinking Water Update. National Academy Press, Washington DC.

Oremland, R.S. and Stolz, J.F., 2003. The ecology of arsenic. Science, 300(5621): 939-944.

Rahman, M.M., Mukherjee, D., Sengupta, M.K., Chowdhury, U.K., Lodh, D., Chanda, C.R., Roy, S., Selim, M., Quamrussaman, Q., Milton, A.H., Shahidullah, S.M., Rahman, M.T., and Chakraborti, D., 2002. Effectiveness and reliability of arsenic field testing kits: Are the million dollar screening projects effective or not?, Environmental Science and Technology, 36(24): 5385–5394.

Rasmussen, L.D., Sorensen, S.J., Turner, R.R., and Barkay, T., 2000. Application of a mer-lux biosensor for estimating bioavailable mercury in soil. Soil Biology & Biochemistry, 32(5): 639-646.

Rocovich, S.E. and West, D.A., 1975. Arsenic Tolerance in a Population of the Grass Andropogon scoparius Michx. Science, 188(4185): 263-264.

Rosenberg, H., Gerdes, R.G., and Chegwidden, K., 1977. Two systems for the uptake of phosphate in Escherichia coli. Journal of Bacteriology, 131(2): 505-511.

Russell, L.M., and Rosenberg, H., 1979. Linked transport of phosphate, potassium ions and protons in Escherichia coli. Journal of Biochemistry, 184: 13-21.

Russell, L.M., and Rosenberg, H., 1980. The nature of the link between potassium transport and phosphate transport in Escherichia coli. Journal of Biochemistry, 188: 715-723.

Sanders, O.I., Rensing, C., Kuroda, M., Mitra, B., and Rosen, B.P., 1997. Antimonite is accumulated by the glycerol facilitator GlpF in Escherichia coli. Journal of Bacteriology, 179(10): 3365-3367.

Smedley, P.L. and Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Applied Geochemistry, 17(5): 517-568.

Smith, A.H., Lopipero, P.A., Bates, M.N., and Steinmaus, C.M., 2002. Public health - Arsenic epidemiology and drinking water standards. Science, 296(5576): 2145-2146.

Steinmaus, C.M., George, C.M., Kalman, D.A., and Smith, A.H., 2006. Evaluation of two new arsenic field test kits capable of detecting arsenic water concentrations close to 10 µg/L. Environmental Science and Technology, 40(10): 3362–3366.

Sticher, P., Jaspers, M.C., Stemmler, K., Zehnder, A.J., and van der Meer, J.R., 1997. Development and characterization of a whole-cell bioluminescent sensor for bioavailable middle-chain alkanes in contaminated groundwater samples. Applied Environmental Microbiology, 63(10): 4053-4060.

Stocker, J. et al., 2003. Development of a Set of Simple Biosensors for Quantitative and Rapid Measurements of Arsenite and Arsenate in Potable Water. Environmental Science & Technology, 37(20): 4743-4750.

Tauriainen, S., Karp, M., Chang, W., and Virto, M., 1997. Recombinant luminescent bacteria for measuring bioavailable arsenite and antimonite. Applied Environmental Microbiology, 63(11): 4456-4461.

Trang, P.T.K., Berg, M., Viet, P.H., Mui, N.V., and van der Meer, J.R., 2005. Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples. Environmental Science & Technology, 39(19): 7625-7630.

Valenti, T.W. et al., 2005. Bioassessment of an Appalachian Headwater Stream Influenced by an Abandoned Arsenic Mine. Archives of Environmental Contamination and Toxicology, 49(4): 488-496.

van Veen, H.W., Abee, T., Kortstee, G.J.J., Konings, W.N., and Zehnder, J.B., 1994. Translocation of metal phosphate via the phosphate inorganic transport system of Escherichia coli. Biochemistry, 33: 1766-1770.

Walker, F.P., Schreiber, M.E., and Rimstidt, J.D., 2005. Kinetics of arsenopyrite oxidative dissolution by oxygen. Geochimica et Cosmochimica Acta, 70(7): 1668-1676.

Wilde, F.D., Radtke, D.B., Gibs, J., and Iwatsubo, R.T., eds., 2004. Processing of water samples (version 2.1): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5, April, accessed 11/23/09 at http://pubs.water.usgs.gov/twri9A5/

Willsky, G.R., Bennett, R.L., and Malamy, M.H., 1973. Inorganic phosphate transport in Escherichia coli: Involvement of two genes which play a role in alkaline phosphatase reduction. Journal of Bacteriology, 113(2): 529-539.

Willsky, G.R., and Malamy, M.H., 1980. Effect of arsenate on inorganic phosphate transport in Escherichia coli. Journal of Bacteriology, 144(1): 366-374.

Winson, M.K., Swift, S., Fish, L., Throup, J.P., Jorgensen, F., Chhabra, S.R., Bycroft, B.W., Williams, P., and Stewart, G.S., 1998. Construction and analysis of luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorom sensing. FEMS Microbiology Letters, 163(1): 185-192.

Xu, C., Zhou, T., Kuroda, M. and Rosen, B.P., 1998. Metalloid Resistance Mechanisms in Prokaryotes. Journal of Biochemistry, 123(1): 16-23.

Zhu, Y.N., Zhang, X.H., Xie, Q.L., Wang, D.Q., and Cheng, G.W., 2006. Solubility and stability of calcium arsenates at 25°C. Water, Air, & Soil Pollution, 169(1-4): 221-238.

CHAPTER 3

Effect of Speciation on the Bioaccumulation of Arsenic in *Corbicula fluminea*

Elizabeth Diesel (1), Matthew Hull (2), Maya Nadimpalli (3), Peter Vikesland (2), and Madeline Schreiber (1)

(1) Department of Geosciences, Virginia Tech, Blacksburg, VA 24061, USA
(2) Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061, USA
(3) Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, 27599, USA

ABSTRACT

Bivalves have been used extensively as monitors for environmental pollution because they are excellent indicator organisms, particularly as bioassays for determining the bioavailable concentrations of environmental contaminants. Specifically, *Corbicula fluminea* has been used successfully for biomonitoring, both because of its abundance and its biological similarity to rarer bivalve species. This research addresses the effects of speciation on the bioavailability and bioaccumulation of arsenic (As) to *C. fluminea*. Arsenic bioavailability to *Corbicula fluminea* was found to be affected by As speciation at exposure concentrations >5 mg/L. Under these conditions, arsenite was more bioavailable than arsenate, monosodium methyl arsonate, and roxarsone. At lower As concentrations (< 5 mg/L), speciation did not affect bioavailability; all As species tested accumulated similarly. The shell of *C. fluminea* was found to be an efficient As(III) oxidizer, with As(III) oxidation at lower concentrations controlled solely by the shell. At higher As concentrations, a portion of As(III) oxidation occurred within the clam. The oxidation of As(III) to As(V) indicates that *C. fluminea* is able to alter arsenic's bioavailability, as As(V) is less bioavailable than As(III). The ability to oxidize As indicates that *C. fluminea* can alter the geochemistry of their surrounding environment.

INTRODUCTION

Bioavailability

Both physical and geochemical factors can affect the bioavailability of aquatic contaminants. Examples of factors include the concentration of the compound within the system, dissolution/precipitation and sorption/desorption reactions, speciation of the compound, and complexation of the compound to mobile colloids and nanoparticles. These factors can affect both the contaminant's bioaccessibility (the potential for transport across a biological membrane) and its bioavailability (the uptake of the contaminant by the organism) (Semple et al., 2004).

Several methods have been used to characterize contaminant bioavailability, and each has its own unique set of advantages and disadvantages. These methods include chemical extractions of contaminants from soils, *in vitro* as well as *in vivo* measures of toxicity, contaminant bioaccumulation in bioassays, and estimates of bioavailability based on physicochemical properties of soils, sediments, and surface waters (Naidu et al., 2008). Each of these methods measure bioavailability through different mechanisms (i.e. toxicity measures lethal response, whereas bioaccumulation measures contaminant uptake), which makes comparing the results of one method to another difficult. Using organisms, or bioassays, as a standard method for environmental monitoring can be extremely useful as they can provide information on the well-being of an organismal community as well as the general condition of their environmental system (Ravera, 2001).

C. fluminea as a biomonitor

Bivalves have been used for many years to monitor environmental pollution as they are excellent indicator organisms, especially as a bioassay for bioavailable concentrations of environmental contaminants (see Farris and Van Hassel, 2007 and references therein). Biomonitoring with mussels and clams can be more informative than collecting grab samples of sediments or water, as they are capable of integrating contaminant exposure over time, as well as providing a measure of bioavailability (Farris and Van Hassel, 2007). *Corbicula fluminea* in particular have been used extensively for biomonitoring, both because of their abundance and biological similarity to rarer unionid bivalve species (Milam and Farris, 1998; Hull et al., 2006). *Corbicula* have been found to concentrate organic pollutants from both water and sediment and

heavy metals from water, as well as exhibit a high tolerance for exposure to toxic substances (Doherty, 1990), making them ideal organisms for monitoring contamination.

Corbicula fluminea has been utilized to examine the bioaccumulation of trace elements, such as copper (Cu), zinc (Zn), and cadmium (Cd) (Andre`s et al., 1999; Graney et al., 1984). Results of these studies indicate that *C. fluminea* will bioaccumulate Cu, and to a lesser extent, Cd, in proportion to the exposure concentration, while bioaccumulation of Zn is not correlated to concentration (Andre`s et al., 1999; Graney et al., 1984). Other trace elements that *C. fluminea* has been shown to bioaccumulate include selenium (Lee et al., 2006) and uranium (Simon and Garnier-Laplace, 2004).

While the application of *C. fluminea* for biomonitoring an array of metal contaminants in aquatic ecosystems is extensive, the suitability of *C. fluminea* for biomonitoring aquatic arsenic (As) has been investigated only recently. Previous studies have shown that *Corbicula* can survive in solutions with high arsenite concentrations where it is capable of bioaccumulating As in proportion to exposure concentrations (Sebesvari et al., 2005; Santos et al., 2007; Liao et al., 2008a). Chen et al. (2010) described a linkage between the daily valve activity (i.e., opening and closing of shell) of *C. fluminea* and As bioavailability and bioaccumulation, and suggested that this linkage could be used to predict As binding at the gills of *C. fluminea* as well as As bioaccumulation. Mason et al. (2000) examined bioaccumulation of As in crayfish and fish; results suggested that sorption of As to the exoskeletons may be an important mechanism of bioaccumulation.

Arsenic in the environment

Arsenic is a ubiquitous metalloid in the environment. It can be found in natural waters worldwide due to natural and anthropogenic sources (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002). Arsenic can be both toxic and carcinogenic, the cause of cardiovascular, pulmonary, immunological, neurological, and endocrine disorders, as well as skin, lung, bladder, and kidney cancers (NRC, 1999; NRC, 2001). In areas such as Taiwan, Bangladesh, Vietnam, India, and Hungary, residents depend upon As-contaminated groundwater for their drinking water supplies, and many people in these countries have succumbed to arsenicosis (arsenic poisoning), cancer, and other illnesses (Smith et al., 2002; Mandal and Suzuki, 2002).

Arsenic in natural waters can occur as different arsenic species and forms, such as As(III) or As(V) as an oxyanion in solution, as complexes with colloidal particles or NOM, or as organoarsenicals, depending on the source of contamination and the environmental conditions of the natural system (Jain and Ali, 2000). Understanding the speciation and form of As is critical, as the toxicity differs. Arsenite (As(III)) is more toxic than arsenate (As(V)), which in turn is more toxic than organic forms, such as mono-methylarsonate (MMA), and dimethylarsinate (DMA) (Jain and Ali, 2000). In natural waters, As is typically found as inorganic As(III) and As(V) species. Redox potential and pH are the two most important factors controlling inorganic As protonation and speciation. Thermodynamics predicts that As(V) is the dominant inorganic species under oxidizing conditions, while As(III) dominates under reducing conditions. However, it is well known that these species may be in disequilibrium due to slow oxidation and reduction kinetics, the presence of oxidizing mineral surfaces, and the presence of microorganisms that mediate many As biotransformation processes (Oremland and Stolz, 2003).

Organoarsenicals, including 3-nitro 4-hydroxyphenolarsonic acid (roxarsone) and monosodium methyl arsonate (MSMA), can also be found in environmental systems due to human activity. Roxarsone is added to poultry feed to improve weight gain, feed efficiency, and pigmentation (Garbarino et al., 2003). Roxarsone is not retained in the tissues of the animal, but is excreted into the litter (Morrison, 1969; Alloway, 1990; Bednar et al., 2003; Garbarino et al., 2003). This litter is then applied as fertilizer, which releases roxarsone, and subsequently As(V) into the environment. MSMA is a herbicide that is used mostly on golf courses (Cai et al., 2002; Feng et al., 2005). After application, the portion of MSMA not intercepted by plants is biotransformed in the soil to arsenate, with lesser amounts of transformation to DMA, MMA, and arsenite, respectively (Feng et al., 2005).

Study Objective

The purpose of this study is to determine the effect of speciation on As bioaccumulation in *Corbicula fluminea* in both laboratory prepared As solutions and field samples of As contaminated water. We conducted a series of As exposures using four species of arsenic (As(III), As(V), MSMA, and roxarsone) at a range of concentrations. To investigate As bioaccumulation under environmentally-relevant conditions, we exposed *C. fluminea* to As contaminated water collected from a field site. The results of this study will enhance our

understanding of how speciation affects accumulation of As by *C. fluminea*, and will also provide practical information about the importance of speciation to risk assessment. .

METHODS AND MATERIALS

Corbicula fluminea collection and acclimation

Clams were collected from the New River in Radford, VA, under permit from the Virginia Department of Game and Inland Fisheries. Clams were manually removed from the sediment and placed in a plastic bag filled with site-collected water. Clams measuring between approximately 9 and 12 mm in length, umbo to ventral margin, were collected; clams measuring larger or smaller than this range were excluded to minimize potentially confounding effects of age and body size on bioaccumulation or mortality.

Upon returning to the lab, clams were rinsed with deionized water to remove any sediment or other particles that cling to their shells and placed in a 10 liter aquarium filled with EPA Moderately Hard Synthetic (EPA MHS) freshwater (USEPA, 2002). The ionic composition of EPA MHS utilized for these experiments is shown in Table 1.

To prevent the introduction of fecal matter into the exposure assay that might alter arsenic bioavailability, clams were allowed to depurate in the aquariums for about 3 days prior to use in all exposures. While depuration periods of greater length are preferred, the time between collection and exposure was minimized to eliminate the need to feed the *C. fluminea* test organisms during an exposure. Sorption of metals to algal cells, which are a preferred laboratory food-source for clams such as *C. fluminea*, has been reported in the literature, and such sorption processes could potentially confound results either by enhancing or retarding the mass of metal available for biological uptake (Mehta and Gaur, 2005).

Arsenic stock solutions

Arsenic solutions were created from reagent-grade arsenic salts of sodium arsenite (Fisher Scientific), sodium arsenate (J.T. Baker), monosodium methyl arsonate (Supelco Analytical), and roxarone (ICN Biomedicals Inc.). Salts were massed with an analytical balance, added to volumetric flasks, and then diluted to the desired concentration with deionized water. Fresh solutions were prepared before each experiment to ensure that As speciation was maintained.

Laboratory exposures

For each exposure, three clams were placed in a 150 mL beaker containing As stock solution diluted with EPA MHS as appropriate to achieve the desired range of exposure concentrations (10 µg/L to 100 mg/L). Three beakers of three clams each were used for all experimental concentrations and conditions. No additional dissolved oxygen was added during the course of the experiments. Any clams that died during the course of an exposure were removed from their beakers shortly after death to ensure that they did not affect the filtration of the other clams in the beaker. Controls consisting of clams exposed only to EPA MHS were used in all experiments to determine the base-line concentration of As in *C. fluminea* tissue prior to exposures; other controls were added as needed. Water samples were taken throughout the exposures and preserved with nitric acid for analysis of total As.

Effect of speciation on bioaccumulation

To determine the effect of speciation on As bioaccumulation, an initial exposure at concentrations from 10 µg/L to 100 mg/L of arsenite (As(III)), arsenate (As(V)), and roxarsone was performed. A second exposure exploring the effect of As speciation on bioaccumulation was conducted over a narrower concentration range (500 µg/L to 50 mg/L) of As(III), As(V), and monosodium methyl arsonate (MSMA).

Change in arsenic speciation during exposure

To evaluate changes in As speciation during exposure, an experiment tracking the oxidation of As(III) by *Corbicula* was conducted. Clams were exposed to As(III) concentrations of 0.5, 1, and 10 mg/L for 3 days. Controls consisting of empty shells fastened shut with plastic cable ties and controls consisting only of the plastic cable ties exposed to these same solutions were used to determine As oxidation due to interaction with the shell, plastic cable ties, glassware, and the atmosphere. Another set of controls was utilized; beakers with As solutions and cable ties were utilized to determine As(III) oxidation due to interaction with just ties, glassware, and the atmosphere. Water samples were taken before and after each exposure.

Samples were speciated for As (As(III)) and As(V)) using solid phase extraction strong anion exchanger (SPE SAX) columns (see Wilde et al., 2004 for description of procedure).

Effect of natural samples on arsenic bioaccumulation

Clams were also exposed to field-collected samples of water from the Brinton Arsenopyrite Mine site (BAMs). These samples were serially diluted to allow for a range of exposure concentrations (~1 to 5 ppm total As).

Clam dissection and digestion

Upon termination of each exposure, the soft tissue of *C. fluminea* was separated from the shell using a surgical steel blade. Wet tissue weights were determined on an analytical balance. Arsenic body burdens were determined after a nitric acid microwave digestion of the total wet body tissue amalgamated from 3 clams in each exposure beaker (CEM MARS method #375). These digestate samples were diluted to < 10% acid for subsequent analysis.

Field sample collection

Field samples were collected from spring water from the Brinton Arsenopyrite Mine site (BAMs) in Floyd County, Virginia, on November 6, 2009. The As at this field location is derived from arsenopyrite oxidation, and water samples collected from this site contain both arsenate (As(V)) and arsenite (As(III)).

This site has been extensively investigated by previous researchers (Rocovich and West, 1975; Dove and Rimstidt, 1985; Walker et al., 2005; Chaffin et al., 2005; Valenti et al., 2005; Harvey et al., 2006; Lottig et al., 2007; Brown et al., 2007). In the early 1900's, arsenopyrite was mined and roasted at this site, and multiple tailing piles were placed next to the stream channel (Dietrich, 1959). The tailings consist principally of unconsolidated sediments containing arsenopyrite, scorodite, and As-rich iron oxides (Dove and Rimstidt, 1985; Harvey et al., 2006), as well as part of the host rock, a quartz sericite schist (Dietrich 1959). This is an ideal collection site for water for exposure experiments, as As is the dominant trace element leaching from the waste piles and ore; other toxic elements often found in sulfide deposits (e.g. Ni, Co, Mo, Cu, Zn, Sb) are only found at trace concentrations in water at the site (Chaffin et al., 2005; Lottig et al., 2007).

Water samples were collected from a spring channel approximately 100 m downstream of the mine tailings piles. Field parameters (i.e. temperature, pH, dissolved oxygen, and conductivity) of the water were recorded during sampling. Arsenic speciation (As(III)) and As(V)) was conducted in the field using SPE SAX columns (see Wilde et al., 2004 for description of procedure). The aliquots of speciated samples were preserved with a few drops of concentrated nitric acid for chemical analysis. Samples were placed in a cooler with ice during transport from the field site.

Additionally, 1 L of water was collected and preserved with 10 mL 0.125 M EDTA, which has been shown to preserve the distribution of As species through chelating metal cations, buffering pH, and decreasing microbial activity (Gallagher et al., 2001; Bednar et al., 2002). A serial dilution of the EDTA-amended water samples was used in the *C. fluminea* experiment.

Arsenic analysis

Laboratory and field samples, as well as diluted tissue digestates, were analyzed for As on a Varian SpectrAA 220Z graphite furnace atomic adsorption spectrometer with background Zeeman correction for low concentrations (< 25 µg/L) of As. The detection limit of this method is ~ 3 µg/L. Higher As concentrations (>25 µg/L) were analyzed on a Thermo Elemental ICAP 61E inductively coupled plasma atomic emission spectrometer. The detection limit for this method is ~ 25 µg/L. For quality control, check and internal addition standards were utilized. Additionally, duplicate samples from the field and experiments were regularly analyzed to check sampling procedures. Replicate analyses were conducted periodically to ensure instrument accuracy.

RESULTS

Effect of speciation on arsenic accumulation

Exposures of *C. fluminea* to dissolved As(III), As(V), roxarsone, and MSMA at concentrations ranging from 10 µg/L to 100 mg/L were used to examine As bioaccumulation. After 5-6 days of exposure to dissolved As solutions, all clams exposed to As at concentrations <50 mg/L were actively filtering. At As concentrations of 50 to 100 mg/L of As(III) and As(V), however, clams avoided filtering and had up to 66% mortality prior to termination of the exposure.

Concentrations of As measured in the soft-tissue of clams were plotted as a function of the As concentration in water to which clams were exposed (Figure 1 a and b). We assumed that all As in the tissue was bioaccumulated, as tissues were thoroughly rinsed prior to digestion. Arsenic accumulated in tissue was positively related to, but not linearly correlated with, the concentration of As measured in the water column. Background As levels were below detection limits, as measured in the soft tissue of *C. fluminea* controls (exposed to EPA MHS only, without As). For all As species, As levels measured in clams exposed to 10 and 100 μg/L As were at or below instrument detection limits. Clams exposed to 1 and 10 mg/L As had increased concentrations of As, but there was no difference in accumulation between As(III), As(V), and roxarsone. However, exposures of 100 mg/L As showed elevated concentrations of As in the digestate. Additionally, there was a much higher accumulation of As(III) than As(V) and roxarsone, respectively.

For the second exposure, which compared accumulation of As(III), As(V), and MSMA, As body burden for *C. fluminea* was again plotted against the water column As concentration (Figure 2a and b). Again, As body burdens were positively related to, but not linearly correlated with, water column As concentration with the greatest accumulation evident in exposures performed at the highest water column As concentration. The amount of As bioaccumulation in the body tissue of the *Corbicula* was below the detection limit for the control clams, and was near background concentrations for the 500 μg/L exposed clams. The clams exposed to 1, 5, and 10 mg/L As showed elevated concentrations of As in the digestate, but there was no difference in accumulation between As(III), As(V), and roxarsone. Exposures performed at 50 mg/L As showed higher accumulation of As(III) than As(V) and MSMA, respectively.

The relationship between As bioaccumulation and exposure concentration is non-linear between 1 and 100 mg/L (Figures 1 and 2). When plotted on log-log axes, a power law best describes the relationship (Figure 3).

<u>*Corbicula fluminea* oxidation of arsenic</u>

C. fluminea demonstrated an ability to oxidize As(III) to As(V) (Figure 4). At lower As concentrations (0.5 and 1 mg/L), the majority of As(III) oxidation is controlled by the shell (Figure 4, a and b; compare "end – shell controls" to "end – live clams"). At higher concentrations (10 mg/L), a portion of As(III) is oxidized within the tissue of organism, 1.7 mg/L

of the total As(III) (Figure 4c). Oxidation due to zip ties and/or the beaker (Figure 4; compare "end, no clams" to "start") is inconsequential compared with the oxidation that occurs due to interaction with the shell and within the tissue, indicating that the oxidation of As(III) to As(V) is mediated by the organism, and not as a result of the experimental setup.

C. fluminea exposure to field samples

The spring sample collected at BAMs contains both As(III) (0.07 mg/L) and As(V) (5.11 mg/L) and was serially diluted for the *C. fluminea* exposure. This sample had a specific conductance of 139 µS/cm and pH of 5.4. When exposed to these field samples, clams accumulated less As than those exposed to comparable concentrations of stock As solutions (Figure 5, a and b), with significant differences observed at the highest concentrations. Temperature, pH, and water hardness can affect clam filtration (Graney et al., 1984; Vidal et al., 2002; Loayza-Muro and Elias-Letts, 2007), and thus can impact As accumulation. These characteristics for our sample were not outside the normal ranges for *C. fluminea*, and only minimal avoidance behavior (i.e. lowered filtration) was observed.

DISCUSSION

Effect of speciation on arsenic accumulation

Roxarsone showed very little accumulation within the tissue of *C. fluminea*, which may be due in part to the possibility that it is not sequestered by metallothioneins in the gills, mantle, or digestive glands, which is potentially how As(III), As(V), and MSMA are accumulated, as other trace elements are accumulated this way (Gosling, 2003).

At high As concentrations (> 10 mg/L), As(III) is accumulated much more readily, and is thus more bioavailable than As(V), MSMA, and roxarsone. This may be due to different detoxification pathways, which could lead to longer residence times for As(III) within the clam. At lower concentrations (< 10 mg/L), however, speciation does not seem to affect As bioavailability.

Although As speciation has never been studied in clams before, Spehar et al. (1980) reported that in laboratory bioassays with several species of invertebrates and one fish species, As(III) was the most toxic of several different arsenic compounds (As(III), As(V), DMA, and disodium methyl arsenate), inducing a significant reduction in survivorship of amphipods after 7

days exposure at an As concentration of 1 mg/L. In our experiments, we observed fatality of clams at the high (50 to 100 mg/L) concentrations of As(III) and As(V), but not MSMA or roxarsone at similar concentrations, which is consistent with the toxicity observations of Spehar et al. (1980).

Corbicula fluminea oxidation of arsenic

The shell of *C. fluminea* is an efficient As(III) oxidizer, as most oxidation at lower As concentrations seems to be controlled solely by the shell. At higher concentrations (> 10 mg/L), however, a portion of the As(III) oxidation occurs within the clam tissue itself. This internal oxidation of As may partially explain the increased bioaccumulation of As(III) at exposure concentrations > 10 mg/L; a longer internal As residence time may be necessary to oxidize arsenite to arsenate, allowing for more As to accumulate before depuration.

Although not studied previously in *C. fluminea*, As biotransformation has been documented for a number of different species. For example, Kuroiwa et al. (1994) demonstrated bioaccumulation, biotransformation, and excretion of As compounds by several freshwater species. Maeda et al. (1990) reported the accumulation and biomethylation of As(V) from water by a three-step freshwater food-chain consisting of an autotroph (algae), grazer (daphnid), and carnivore (fish). Vahter (1981) reported that in mice As(III) was methylated to a greater extent than As(V), which led to increased whole-body retention of As(III) relative to As(V). Similar to our results, Vahter (1981) demonstrated that for both As(III) and As(V), whole-body retention increased with increasing dose.

C. fluminea exposure to field samples

Lowered bioaccumulation of As from the BAMs field sample compared with the laboratory solutions could be caused by lowered filtering due to avoidance of high water hardness or increased nitrate. Nitrate was higher in the water sampled at BAMs (Table 2) when compared to that of the EPA synthetic freshwater (Table 1). Increased aqueous nutrient concentrations, particularly increased nitrate, can lower the viability of *C. fluminea* in natural stream systems (Sousa et al., 2008). The nitrate in BAMs water (12.5 mg/L) is much higher than the highest (2.7 mg/L) total nitrogen concentration (nitrate, nitrite, and ammonia) in solution during the monitoring of Sousa et al. (2008). Additionally, increased ammonia in solution has

been shown to affect *C. fluminea*, and can contribute to die offs, particularly when dissolved oxygen is low (Cherry et al., 2005).

It is also possible that the BAMs water hardness could have affected As accumulation. Water hardness has been shown to affect contaminant bioavailability; lower water hardness tends to result in higher bioaccumulation in *C. fluminea* for a number of trace metals (Cd, Cu, mercury (Hg), lead (Pb), and Zn) (Shoults-Wilson et al., 2010). Water hardness has been shown to affect bioaccumulation in other species (*Daphnia*) as well (Yim et al., 2006). According to Markich and Jeffree (1994), the greater abundance of Ca present in the field-collected water relative to EPA MHS (11.6 versus 5.3 mg/L, respectively, Tables 1 and 2) may be sufficient to explain at least some of the differences in As body-burden. These researchers suggest that the Ca water concentration is the primary variable for determining the maximum exposure concentrations of certain trace metals tolerable by freshwater biota (Markich and Jeffree, 1994). While total water hardness in general and Ca in particular have been shown to reduce the bioavailability and subsequent toxicity of certain metals, it is important to note that the ameliorative effects of these parameters on As species are unclear.

Additionally, it is possible that As could be adsorbed to natural particles suspended in solution, which might render the As less bioavailable to the *Corbicula*. One study found that Zn bioavailability to both mussels and clams decreased when bound to colloidal organic carbon (OC) (Wang and Guo, 2000). Similarly, Chen and Wang (2001) found that Fe bioavailability decreased to diatoms and copepods when it was bound to colloidal OC.

CONCLUSIONS

Arsenic bioavailability to *C. fluminea* is affected by As speciation, especially at higher exposure concentrations (> 10 mg/L), with As(III) more bioavailable than As(V), MSMA, and roxarsone, respectively. At lower As concentrations (< 10 mg/L), however, speciation does not appear to affect accumulation and hence, bioavailability. The shell of *C. fluminea* was found to be an efficient As(III) oxidizer, with most oxidation at low As concentrations controlled by interaction with the shell. However, at higher As concentrations (> 10 mg/L), a portion of the As(III) oxidation appears to occur as As passes through the clam. The bioavailability of As in a field sample was lower than what would have been predicted from laboratory exposures,

potentially due to As adsorption to suspended particles, colloids, and/or nanoparticles, or decreased filtration due to other water chemistry (i.e. high nitrate concentrations, high water hardness).

FIGURES

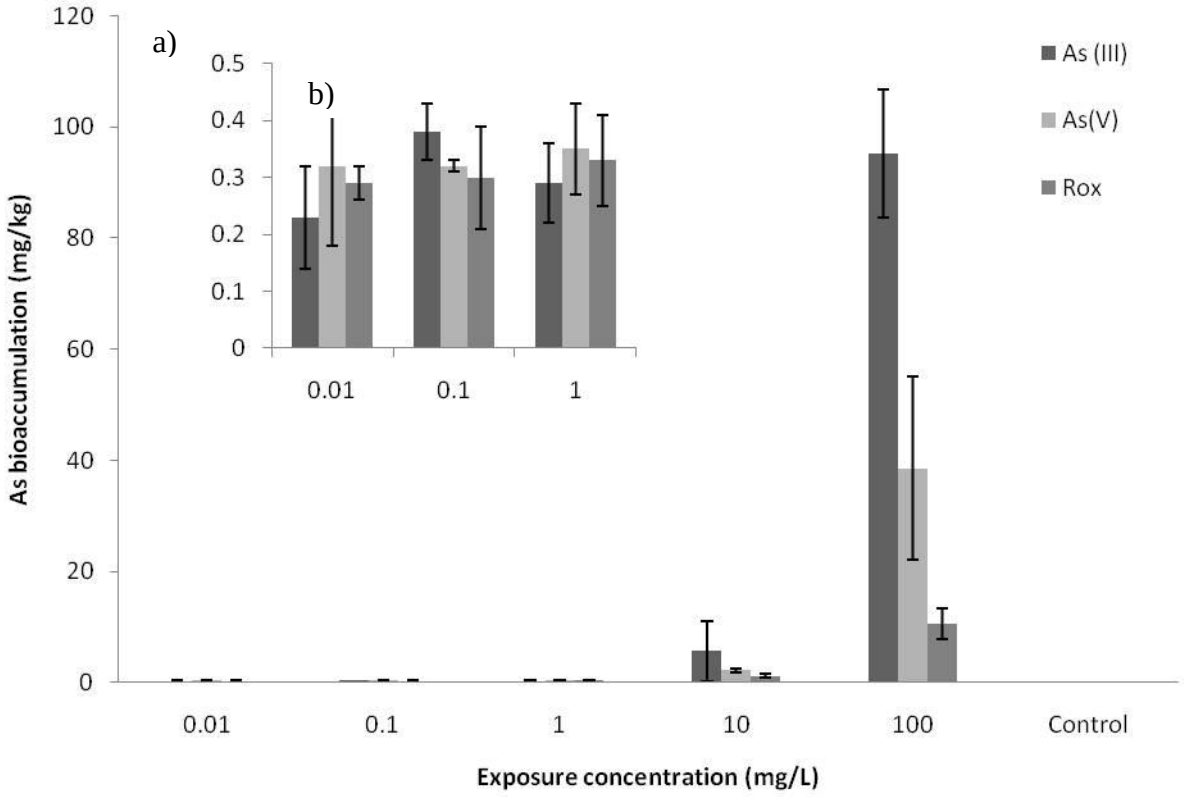

Figure 1: Arsenic accumulated in clam tissue (mg/kg wet weight) from 10 µg/L to 100 mg/L exposure solutions of arsenite, arsenate and roxarsone at a) all concentrations and b) low concentrations. Results presented are the averages of triplicate measurements, and error bars represent the standard deviation of the triplicates. Arsenic accumulation in control clams is below detection limit (< 3 µg/L).

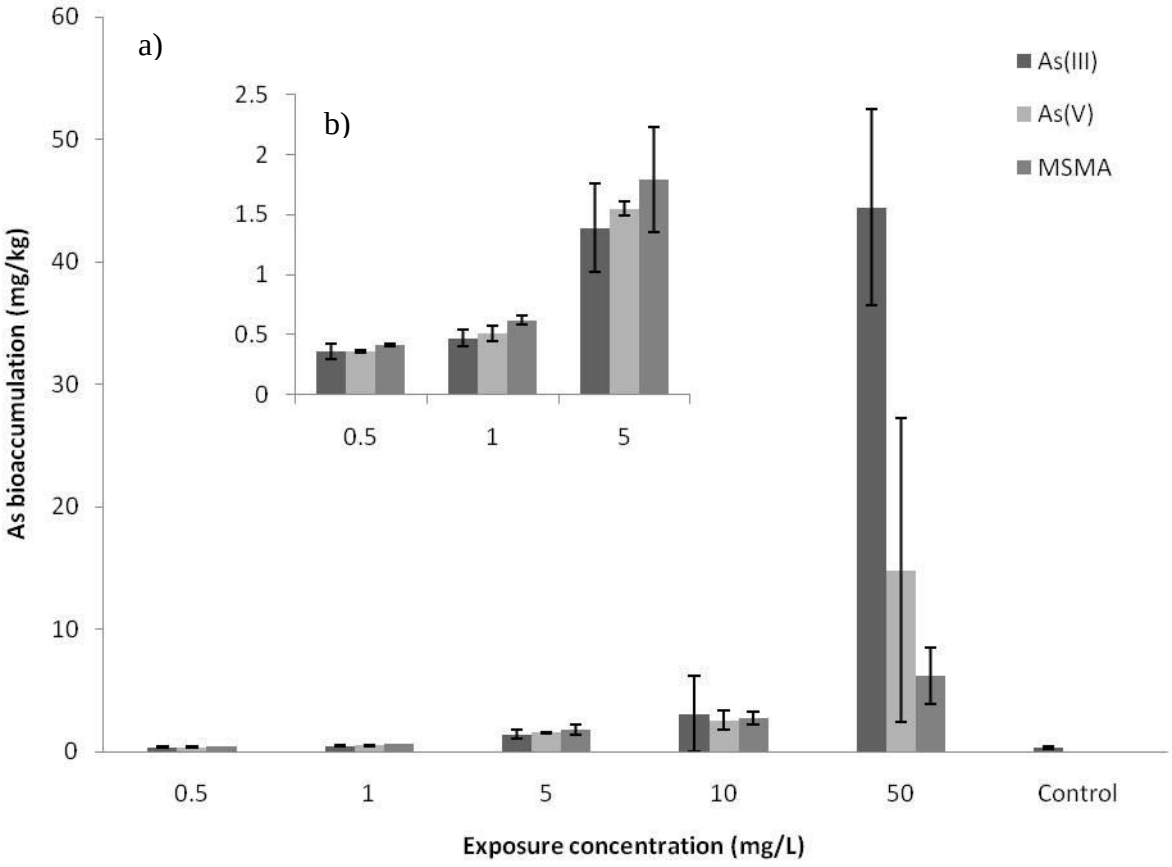

Figure 2: Arsenic accumulated in clam tissue (mg/kg wet weight) from 500 µg/L to 50 mg/L exposure solutions of arsenite, arsenate and MSMA at a) all concentrations and b) low concentrations. Results presented are the averages of triplicate measurements, and error bars represent the standard deviation of the triplicates. Arsenic accumulation in control clams was near the detection limit (3 µg/L detection, 0.2 mg/kg in tissue).

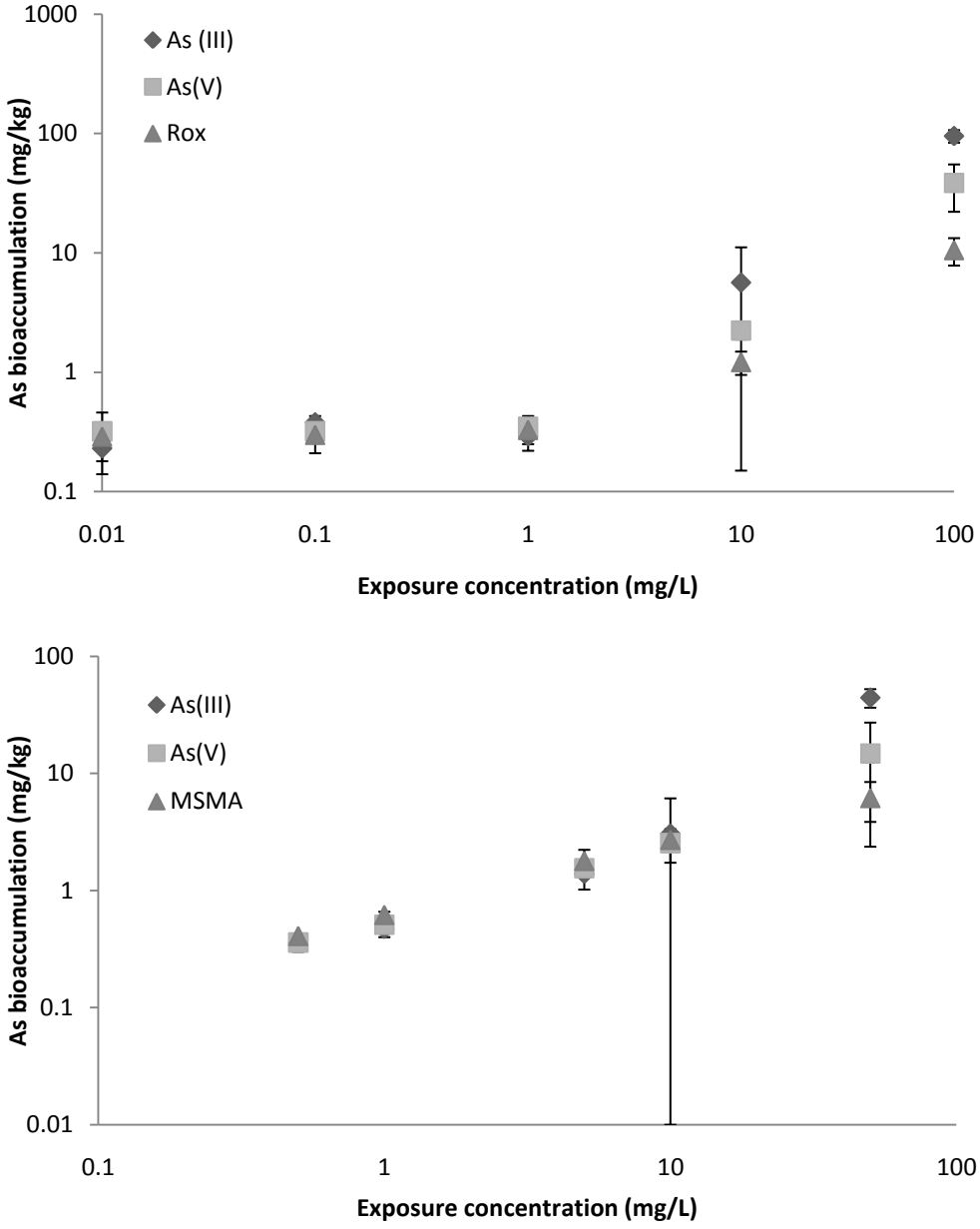

Figure 3: Log-log plot of arsenic bioaccumulation versus exposure concentration for a) 0.01 to 100 mg/L As(III), As(V), and roxarsone, and b) 0.5 to 50 mg/L As(III), As(V), and MSMA. Results presented are the averages of triplicate measurements, and error bars represent the standard deviation of the triplicates.

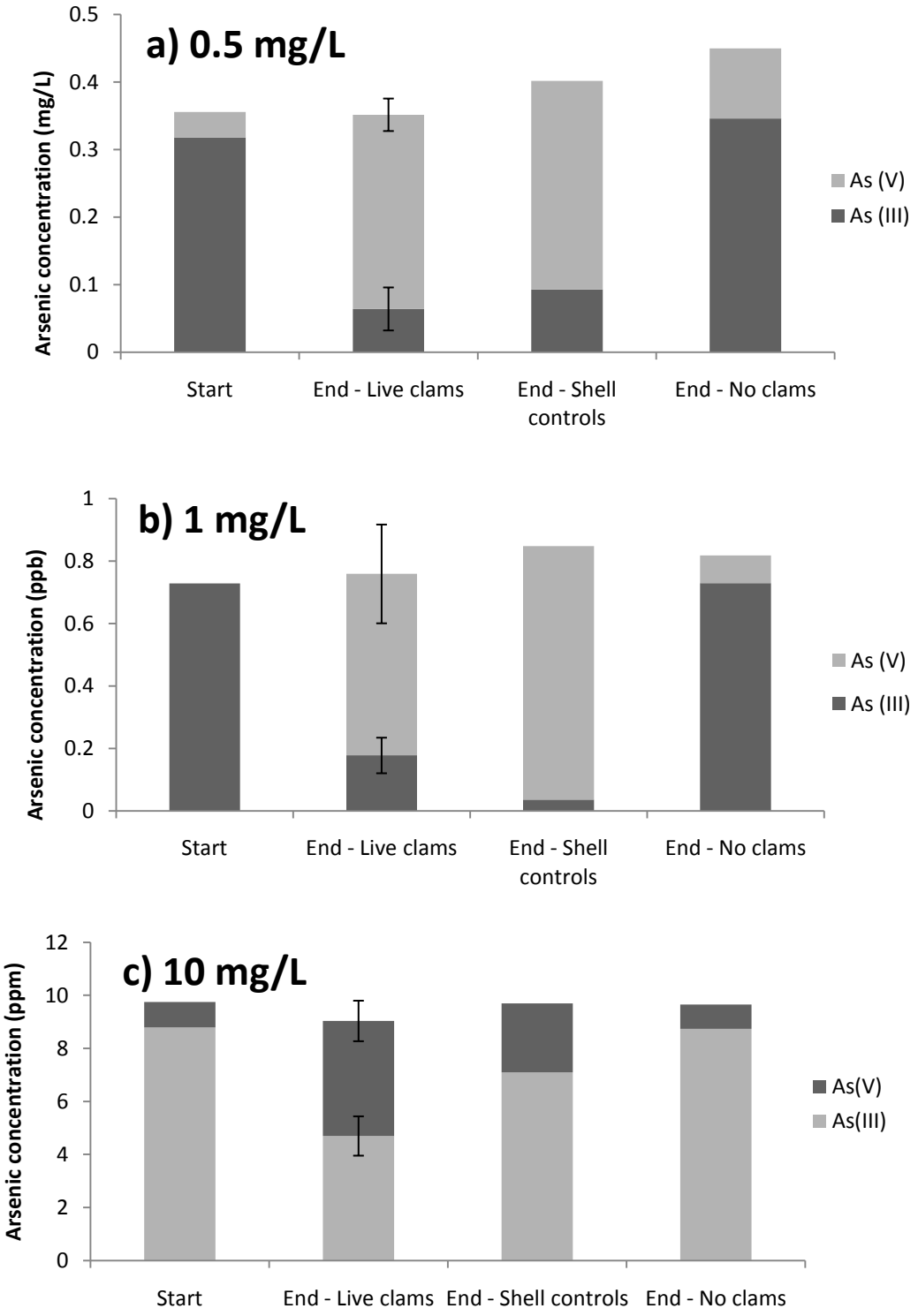

Figure 4: Arsenite exposure solution concentrations from start solutions of arsenite at a) 0.5 mg/L, b) 1 mg/L, and c) 10 mg/L, before exposure and after 3 days exposure. Results presented are the averages of triplicate measurements, and error bars represent the standard deviation of the triplicates.

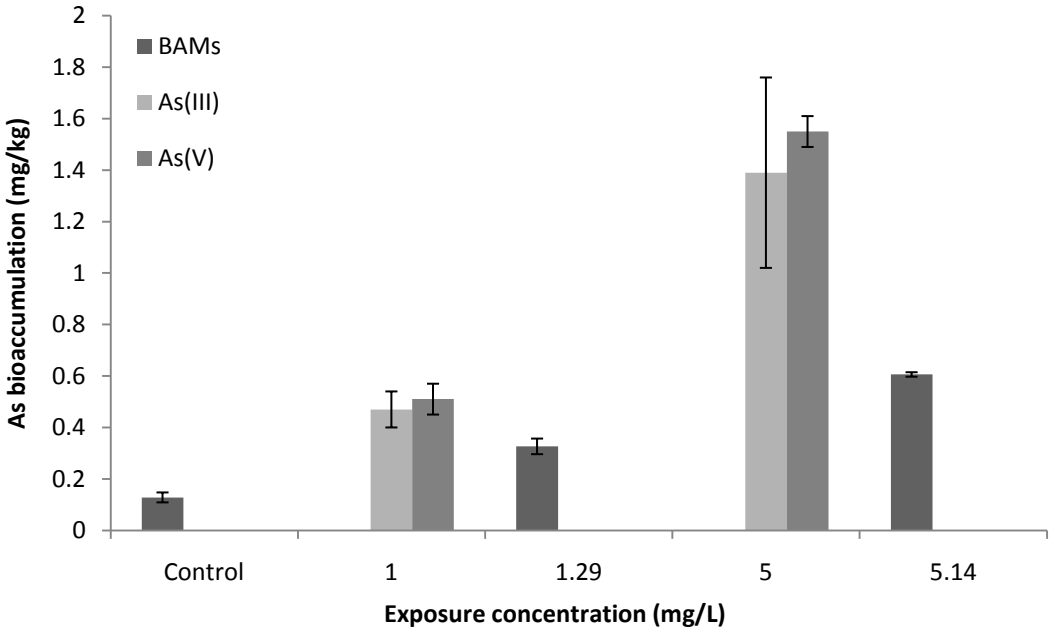

Figure 5: Arsenic accumulated in clam tissue from serially diluted BAMs spring water and 1 and 5 mg/L arsenite and arsenate solutions. Results presented are the averages of triplicate measurements, and error bars represent the standard deviation of the triplicates. Note that BAMs water contains both As(III) and As(V).

TABLES

Table 1: The actual composition of EPA synthetic freshwater used for the exposure assays, and the expected composition based upon the method.

	Analyzed concentration (mg/L)	**Expected concentration (mg/L)**
Ca	9.5	14.0
K	2.0	2.1
Mg	11.6	12.1
Na	26.9	26.3
Cl	1.2	1.9
SO_4	75.3	81.4

Table 2: Composition of BAMS field samples from November 2009, which were utilized for the exposure. BDL = below detection limit.

	Concentration (mg/L)
Ag	BDL (< 0.009)
As	4.95
Ca	11.6
Cu	0.07
Fe	0.95
K	2.6
Mg	4.02
Mn	0.04
Na	52.1
Pb	BDL (<0.016)
Zn	0.01
F	0.46
Cl	1.52
NO_3-N	12.5
SO_4	25.5

REFERENCES CITED

Allen, H.J., Waller, W.T., Acevedo, M.F., Morgan, E.L., Dickson, K.L., and Kennedy, J.H., 1996. A minimally invasive technique to monitor valve-movement behavior in bivalves. Environmental Technology, 17(5): 501-507.

Alloway, B.J., 1990. Heavy metals in soils. Blackie ; Halsted Press, Glasgow; New York.

Andre`s, S., Baudrimont, M., Lapaquellerie, Y., Ribeyre, F., Maillet, N., Latouche, C., and Boudou, A., 1999. Field transplantation of the freshwater bivalve Corbicula fluminea along a polymetallic contamination gradient (River Lot, France): I. Geochemical characteristics of the sampling sites and cadmium and zinc bioaccumulation kinetics. Environmental Toxicology and Chemistry, 18(11): 2462–2471.

Bednar, A.J., Garbarino, J.R., Ferrer, I., Rutherford, D.W., Wershaw, R.L., Ranville, J.F., and Wildeman, T.R., 2003. Photodegradation of roxarsone in poultry litter leachates. Science of The Total Environment, 302(1-3): 237-245.

Brown, B.V., Valett, H.M. and Schreiber, M.E., 2007. Arsenic transport in groundwater, surface water, and the hyporheic zone of a mine-influenced stream-aquifer system. Water Resources Research, 43(11): 1-14.

Cai, Y., Cabrera, J.C., Georgiadis, M., and Jayachandran, K., 2002. Assessment of arsenic mobility in the soils of some golf courses in South Florida. Science of the Total Environment, 291: 123-134.

Chaffin, J.L., Valett, H.M., Webster, J.R. and Schreiber, M.E., 2005. Influence of elevated As on leaf breakdown in an Appalachian headwater stream. Journal of the North American Benthological Society, 24(3): 553-568.

Chen, M., and Wang, W.-X., 2001. Bioavailability of natural colloid-bound iron to marine plankton: Influences of colloidal size and aging. Limnology and Oceanography, 46(8): 1956-1967.

Chen, W.-Y., Liao, C.-M., Jou, L.-J., and Jau, S.-F., 2010. Predicting bioavailability and bioaccumulation of arsenic by freshwater clam Corbicula fluminea using valve daily activity. Environmental Monitoring and Assessment, 169: 647-659.

Cherry, D.S., Scheller, J.L., Cooper, N.L., and Bidwell, J.R., 2005. Potential effects of Asian clam (Corbicula fluminea) die-offs on native freshwater mussels (Unionidae) I: water-column ammonia levels and ammonia toxicity. Journal of the North American Benthological Society, 24(2): 369-380.

Dietrich, R.V., 1959. Geology and mineral resources of Floyd County of the Blue Ridge Upland, Southwestern Virginia, Engineering Experiment Station Series no. 134. Virginia Polytechnic Institute.

Doherty, F.G., 1990. The asiatic clam, Corbicula spp., as a biological monitor in freshwater environments. Environmental Monitoring and Assessment, 15(2): 143-181.

Dove, P.M. and Rimstidt, J.D., 1985. Solubility and stability of scorodite, $FeAsO_4.2H_2O$. The American Mineralogist, 70(7-8): 838-844.

Ehlers, L.J. and Luthy, R.G., 2003. Contaminant bioavailability in soil and sediment: Improving risk assessment and remediation rests on better understanding bioavailability. Environmental Science and Technology, 37(15): 295A–302A.

Farris, J.L., and Van Hassel, J.H., 2007. Freshwater bivalve ecotoxicology. Chapter 2: A review of the use of unionid mussels as biological indicators of ecosystem health. Society of Environmental Toxicology and Chemistry, Pensacola, FL.

Feng, M., Schrlau, J.E., Snyder, R., Snyder, G.H., Chen, M., Cisar, J.L., and Cai, Y., 2005. Arsenic transport and transformation associated with MSMA application on a golf course green. Journal of Agricultural and Food Chemistry, 53(9): 3556-3562.

Garbarino, J.R., Bednar, A.J., Rutherford, D.W., Beyer, R.S. and Wershaw, R.L., 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environmental Science and Technology, 37(8): 1509-1514.

Gosling, E., 2003. Bivalve molluscs: Biology, ecology, and culture. Chapter 2: Morphology of bivalves. Fishing News Books, Blackwell Science.

Graney, R.L., Cherry, D.S. and Cairns, J., 1984. Heavy metal indicator potential of the Asiatic clam (Corbicula fluminea) in artificial stream systems. Hydrobiologia, 102(2): 81-88.

Harvey, M.C., Schreiber, M.E., Rimstidt, J.D. and Griffith, M.M., 2006. Scorodite dissolution kinetics: Implications for arsenic release. Environmental Science and Technology, 40(21): 6709-6714.

Hull, M.S., Cherry, D.S., and Neves, R.J., 2006, Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, Virginia. Hydrobiologia 556: 341-355.

Jain, C.K. and Ali, I., 2000. Arsenic: Occurance, toxicity, and speciation techniques. Water Research, 34(17): 4304-4312.

Kuroiwa, T., Ohki, A., Naka, K., and Maeda, S., 1994. Biomethylation and biotransformation of arsenic in a freshwater food chain: Green alga (Chlorella vulgaris), shrimp (Neocaridina denticulate), killifish (Oryzias iatipes). Applied Organometallic Chemistry, 8(4): 325-333.

Lee, B.G., Lee, J.S., and Luoma, S.N., 2006. Comparison of selenium bioaccumulation in the clams Corbicula fluminea and Potamocorbula amurensis: A bioenergetic modeling approach. Environmental Toxicology and Chemistry, 25(7): 1933-1940.

Liao, C.-M., Jau, S.-F., Chen, W.-Y., Lin, C.-M., Jou, L.-J., Liu, C.-W., Liao, V.H.-C., and Chang, F.-J., 2008a. Acute toxicity and bioaccumulation of arsenic in freshwater clam Corbicula fluminea. Environmental Toxicology, 23(6): 702-711.

Liao, C.-M., Lin, C.-M., Jou, L.-J. and Chen, W.-Y., 2008b. Sodium gill potential as a tool to monitor valve closure behavior in freshwater clam Corbicula fluminea in response to copper. Sensors, 8: 5250-5269.

Loayza-Muro, R., and Elias-Letts, R., 2007. Responses of the mussel Anodontites trapesialis (Unionidae) to environmental stressors: Effect of pH, temperature and metals on filtration rate. Environmental Pollution, 149(2): 209-215.

Lottig, N.R., Valett, H.M., Schreiber, M.E. and Webster, J.R., 2007. Flooding and arsenic contamination: Influences on ecosystem structure and function in an Appalachian headwater stream. Limnology and oceanography, 52(5): 1991.

Maeda, S., Inoue, R., Kozono, T., Tokuda, T., Ohki, A., and Takeshita, T., 1990. Arsenic metabolism in a freshwater food chain. Chemosphere, 20(1-2): 101-108.

Markich, S.J., and Jeffree, R.A., 1994. Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves: An explanation of how water hardness reduces metal toxicity. Aquatic Toxicology, 29(3-4): 257-290.

Mandal, B., K. and Suzuki, K.T., 2002. Arsenic round the world: A review. Talanta, 58(1): 201-235.

Mason, R.P., Laporte, J.-M., and Andres, S., 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contaminant Toxicology, 38: 283-297.

Mehta, S.K., and Gaur, J.P., 2005. Use of algae for removing heavy metal ions from wastewater: progress and prospects. Critical Reviews of Biotechnology, 25(3): 113-152.

Milam, C.D., and Farris, J.L., 1998. Risk identification associated with iron-dominated mine discharges and their effect upon freshwater bivalves. Environmental Toxicology and Chemistry, 17(8): 1611-1619.

Morrison, J.L., 1969. Distribution of arsenic from poultry litter in broiler chickens, soil, and crops. Journal of Agricultural Food Chemistry, 17(6): 1288-1290.

Naidu, R. Semple, K.T., Megharaj, M., Juhasz, A.L., Bolan, N.S., Gupta, S.K., Clothier, B.E., and Schulin, R., 2008. Bioavailability: Definition, assessment and implications for risk assessment. In: R. Naidu (Editor), Chemical Bioavailability in Terrestrial Environments. Linacre House, Oxford.

National Research Council (NRC), 1999. Arsenic in Drinking Water. National Academy Press, Washington DC.

National Research Council (NRC), 2001. Arsenic in Drinking Water Update. National Academy Press, Washington DC.

Oremland, R.S., and Stolz, J.F., 2003. The ecology of arsenic. Science, 300: 939-944.

Ravera, O., 2001. Monitoring of the aquatic environment by species accumulator of pollutants: A reivew. Journal of Limnology, 60(Suppl. 1): 63-78.

Rocovich, S.E. and West, D.A., 1975. Arsenic tolerance in a population of the grass Andropogon scoparius Michx. Science, 188(4185): 263-264.

Santos, H.M. Diniz, M.S., Costa, P.M., Peres, I., Costa, M.H., Alves, S., and Capelo, J.L., 2007. Toxicological effects and bioaccumulation in the freshwater clam (Corbicula fluminea) following exposure to trivalent arsenic. Environmental Toxicology, 22(5): 502-509.

Sebesvari, Z., Ettwig, K.F., and Emons, H., 2005. Biomonitoring of tin and arsenic in different compartments of a limnic ecosystem with emphasis on Corbicula fluminea and Dikerogammarus villosus. Journal of Environmental Monitoring, 7: 203-207.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., and Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediments is complicated. Environ. Sci. Technol., 2: 229A-231A.

Shoults-Wilson, W.A., Unrine, J.M., Rickard, J., and Black, M.C., 2010. Comparison of metal concentrations in Corbicula fluminea and Elliptio hopetonensis in the Altamaha river system, Georgia, USA. Environmental Toxicology and Chemistry, 29(9): 2026-2033.

Simon, O. and Garnier-Laplace, J., 2004. Kinetic analysis of uranium accumulation in the bivalve Corbicula fluminea: effect of pH and direct exposure levels. Aquatic Toxicology, 68(2): 95-108.

Smedley, P.L. and Kinniburgh, D.G., 2002. A review of the source, behavior and distribution of arsenic in natural waters. Applied Geochemistry, 17: 517-568.

Smith, A.H., Lopipero, P.A., Bates, M.N., and Steinmaus, C.M., 2002. Public health - Arsenic epidemiology and drinking water standards. Science, 296(5576): 2145-2146.

Sousa, R., Rufino, M., Gaspar, M., Antunes, C., and Guilhermino, L., 2008. Abiotic impacts on spatial and temporal distribution of Corbicula fluminea (Müller, 1774), in the River Minho Estuary, Portugal. Aquatic Conservation: Marine and Freshwater Ecosystems, 18: 98-110.

Spehar, R.L., Fiandt, J.T., Anderson, R.L., and DeFoe, D.L., 1980. Comparative toxicity of arsenic compounds and their accumulation in invertebrates and fish. Archives of Environmental Contamination and Toxicology 9:53-63.

USEPA, 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition, Washington, D.C.

USEPA, 1997. Engineering bulletin: Technology alternatives for the remediation of soils contaminated with As, Cd, Cr, Hg, and Pb. In: O.o.E.a.R. Response (Editor), pp. 1-20.

Vahter, M., 1981. Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. Environmental Research, 25(2): 286-293.

Valenti, T.W., Chaffin, J.L., Cherry, D.S., Schreiber, M.E., Valett, H.M., and Charles, M., 2005. Bioassessment of an Appalachian headwater stream influenced by an abandoned arsenic mine. Archives of Environmental Contamination and Toxicology, 49(4): 488-496.

Vidal, M.-L., Basseres, A., and Narbonnea, J.-F., 2002. Influence of temperature, pH, oxygenation, water-type and substrate on biomarker responses in the freshwater clam *Corbicula fluminea* (Müller). Comparative Biochemistry and Physiology Part C, 133: 93-104.

Walker, F.P., Schreiber, M.E., and Rimstidt, J.D., 2005. Kinetics of arsenopyrite oxidative dissolution by oxygen. Geochimica et Cosmochimica Acta, 70(7): 1668-1676.

Wang, W.X., and Guo, L., 2000. Influences of natural colloids on metal bioavailability to two marine bivalves. Environmental Science and Technology, 34: 4571-4576.

Wilde, F.D., Radtke, D.B., Gibs, J., and Iwatsubo, R.T., eds., 2004. Processing of water samples (version 2.1): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5, April, accessed 11/23/09 at http://pubs.water.usgs.gov/twri9A5/

Yim, J.H., Kim, K.W., and Kim, S.D., 2006. Effect of hardness on acute toxicity of metal mixtures using Daphnia magna: Prediction of acid mine drainage toxicity. Journal of Hazardous Materials, B138: 16-21.