ARSENATE UPTAKE, SEQUESTRATION AND REDUCTION BY A FRESHWATER CYANOBACTERIUM: A POTENTIAL BIOLOGIC CONTROL OF ARSENIC IN SOUTH TEXAS

A Thesis

by

CHRISTOPHER THOMAS MARKLEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Geology

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Approved as to style and content by:

Bruce E. Herbert (Chair of Committee)	James W. Golden (Member)
Jennifer T. McGuire (Member)	Rick L. Carlson (Department Head)

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ABSTRACT

Arsenate Uptake, Sequestration and Reduction by a Freshwater Cyanobacterium: A Potential Biologic Control of Arsenic in South Texas.

(May 2004)

Christopher Thomas Markley, B.S., University of Pittsburgh

Chair of Advisory Committee: Dr. Bruce E. Herbert

The toxicity and adverse health effects of arsenic are widely known. It is generally accepted that sorption/desorption reactions with oxy-hydroxide minerals (iron, manganese) control the fate and transport of inorganic arsenic in surface waters through adsorption and precipitation-dissolution processes. In terrestrial environments with limited reactive iron, recent data suggest organoarsenicals are potentially important components of the biogeochemical cycling of arsenic in near-surface environments. Elevated arsenic levels are common in South Texas from geogenic processes (weathering of As-containing rock units) and anthropogenic sources (a byproduct from decades of uranium mining). Sediments collected from South Texas show low reactive iron concentrations, undetectable in many areas, making oxy-hydroxide controls on arsenic unlikely. Studies have shown that eukaryotic algae isolated from arsenic-contaminated waters have increased tolerance to arsenate toxicity and the ability to uptake and biotransform arsenate. In this experiment, net uptake of

arsenic over time by a freshwater cyanobacterium never previously exposed to arsenate was quantified as a function of increasing As concentrations and increasing N:P ratios. Toxic effects were not evident when comparing cyanobacterial growth, though extractions indicate accumulation of intracellular arsenic by the cyanobacterium. Increasing N:P ratios has minimal effect on net arsenate uptake over an 18 day period. However, cyanobacteria were shown to reduce arsenate at rates faster than the system can re-oxidize the arsenic suggesting gross arsenate uptake may be much higher. Widespread arsenate reduction by cyanobacterial blooms would increase arsenic mobility and potential toxicity and may be useful as a biomarker of arsenic exposure in oxic surface water environments.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION: ARSENIC BIOGEOCHEMISTRY: SPECIATION AND SEQUESTRATION IN THE ENVIRONMENT

Introduction

Arsenic is a naturally occurring metalloid that generally exhibits a concentration range of 1-10 μg L^{-1} in freshwaters unimpacted by geogenic or anthropogenic sources of arsenic (Williams, 2001). Estimated background arsenic concentrations in sediments range between 5-10 mg kg^{-1} (Smedley and Kinniburgh, 2002). Arsenic concentrations elevated above unimpacted background values result from the release of arsenic from weathered arsenic containing rocks and minerals and from anthropogenic sources such as the smelting of metal ores, arsenical pesticides and wood preservatives (Jain and Ali, 2000; Smedley and Kinniburgh, 2002). The drinking water standard (as stated by WHO, EPA) for arsenic is 10 μg L^{-1} (Smedley and Kinniburgh, 2002).

Elevated arsenic concentrations can be toxic to humans, causing adverse health effects such as skin lesions, carcinoma, keratosis and blackfoot disease (Lin et al., 1998; Mandal et al., 1998). The U.S. EPA Integrated risk information system (IRIS) indicates chronic oral exposure of arsenic may lead to

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This thesis follows the style of the Soil Science Society of America Journal.

hyperpigmentation, keratosis and possible vascular complications. Arsenic is also listed as a Type A human carcinogen. Research linking arsenic exposure to an increased risk of diabetes has also been reported (Wang et al., 2003).

Arsenic speciation is known to control arsenic mobility and toxicity (Smedley and Kinniburgh, 2002). Historically, the factors controlling arsenic speciation have been assumed to be major inorganic reactions, as controlled by Eh, pH, and the mineralogy of soils and sediments (Daus et al., 1998; La Force et al., 2000). Arsenic tends to co-precipitate or adsorb to metal oxyanion minerals such as iron and manganese oxides, decreasing aqueous concentrations of arsenic (Gebel, 2000; Smedley and Kinniburgh, 2002). Biologic processes and associated organic reactions may also play a role in arsenic speciation and cycling, especially in marine environments (Andreae, 1979; Sanders, 1979). Microbes have been shown to accumulate and release arsenic, thereby influencing aqueous arsenic concentrations and speciation in the environment (Gihring et al., 2001; Nicholas et al., 2003). Inorganic arsenic (arsenate, arsenite) is the dominant form of arsenic, though quantifiable amounts of organic arsenic can be detected in some natural fresh waters (Kuroiwa et al., 1994).

Arsenic Speciation in the Environment

Arsenic in the environment can be divided into two categories: 1) inorganic arsenic and 2) organic arsenic. The more common inorganic arsenic species, arsenate ($H_xAsO_4^{x-3}$) and arsenite ($H_xAsO_3^{x-3}$), are more toxic than the many organic species. Solution Eh and pH affect arsenic speciation (Fig. 1). The anoxic inorganic species, arsenite, is considered the most mobile and biologically toxic species of arsenic found in low Eh surface waters, but this species is generally less common than the oxic form, arsenate, found in high Eh surface waters (Jain and Ali, 2000). The two most common organic species are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Freshwater lake organic arsenic concentrations (MMA, DMA) have been shown to increase during summer, suggesting microbes, with an increased metabolic activity, may methylate inorganic arsenic species (Sohrin et al., 1997).

Controls on the Fate of Arsenic in the Environment

Geochemical Controls

Arsenic speciation and aqueous concentration are a function of pH, Eh and co-precipitation reactions with iron and/or manganese oxyhydroxides (Crecelius, 1975; La Force et al., 2000). Raven et al. (1998) show that arsenic adsorption in controlled settings occurs quickly, stabilizing within 2 hours over a wide range of pH. Reductive dissolution of As-containing iron oxyhydroxides

can release arsenic into solution (Nickson et al., 2000). The geochemistry of inorganic arsenic has been assumed the main control of the speciation and therefore the toxicity of arsenic. Because of this assumption, there have been many studies involving oxidation/reduction reactions (Jain and Loeppert, 2000; Rochette et al., 2000), precipitation/dissolution reactions (Daus et al., 1998; Tournassat et al., 2002) and adsorption/desorption reactions (Goldberg and Johnston, 2001; Klaus et al., 1998; Raven et al., 1998). Fig. 2 describes the

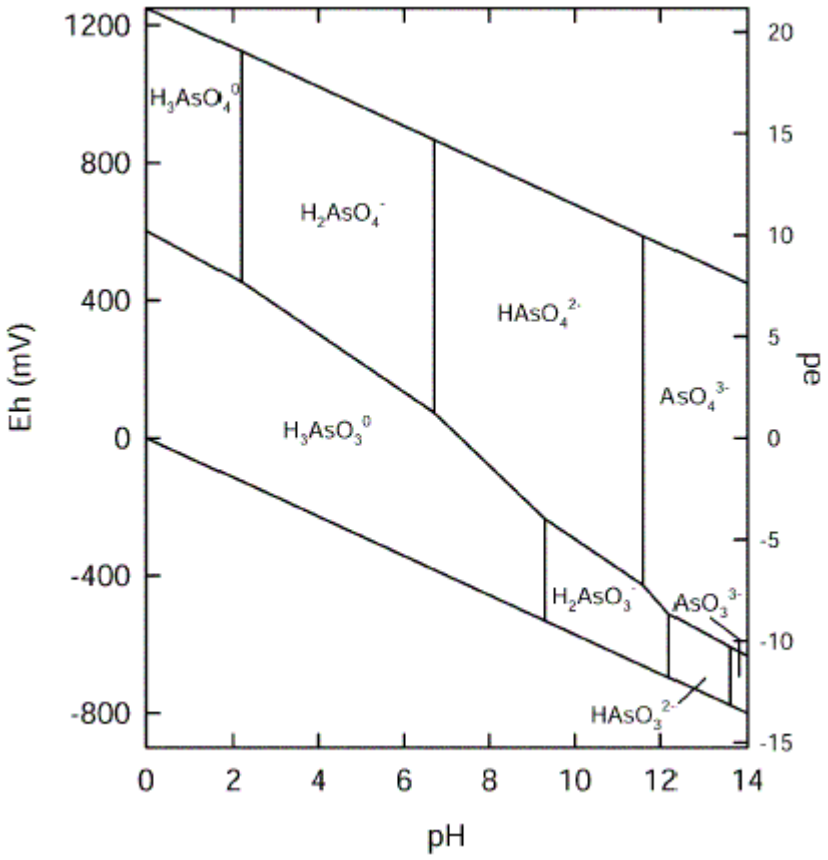


FIGURE 1. Arsenic speciation as a function of Eh and pH. This basic H2O system highlights the effects of Eh and pH on arsenic speciation. Figure from (Smedley and Kinniburgh, 2002).

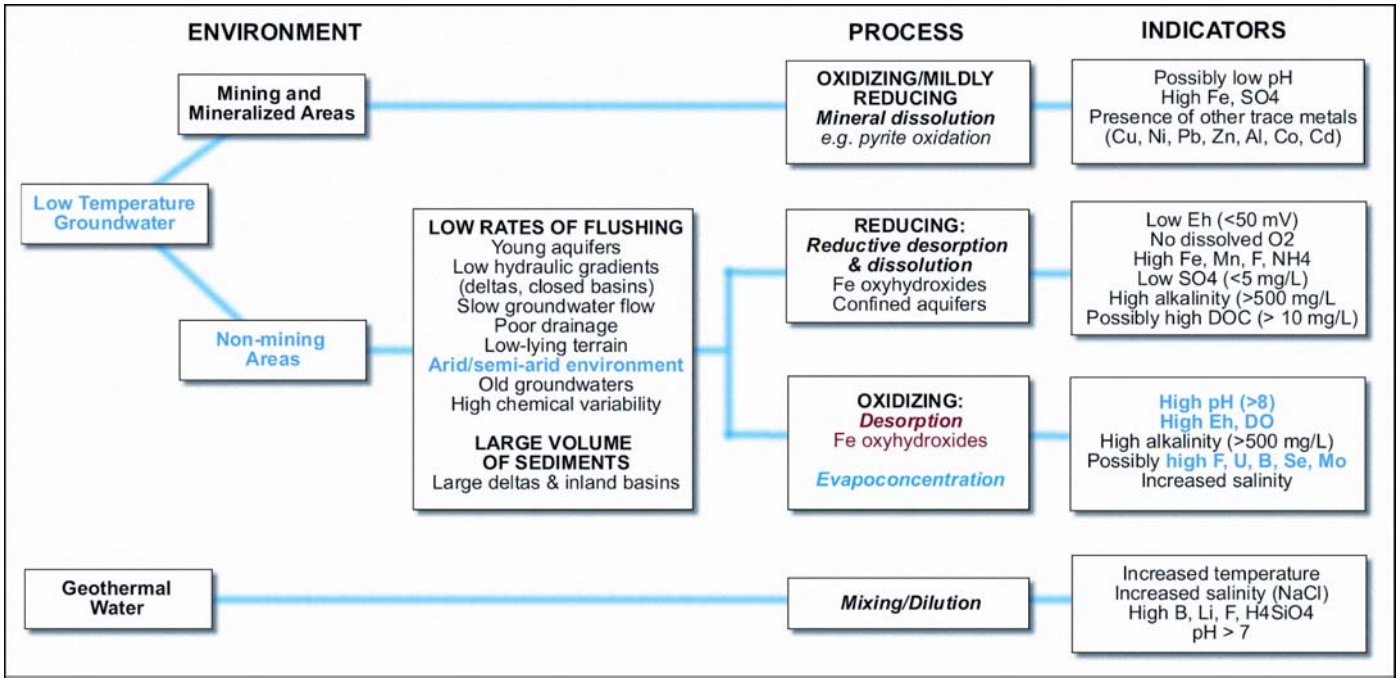


FIGURE 2. Geochemical controls on arsenic fate and transport. Blue text represents conditions present in South Texas. Red text represents hypothesis that these controls do not exist. Figure modified from (Smedley and Kinniburgh, 2002).

geochemical controls of arsenic as related to environmental factors present in South Texas. This illustrates the important role that iron oxyhydroxides play in arsenic sequestration. In oxidizing environments arsenic adsorption/desorption reactions involving iron oxyhydroxides are the dominant fate-controlling mechanism.

A study by Kneebone et al. (2002) indicates a direct correlation ($r^2 = 0.93$) between iron and arsenic concentrations in reservoir sediment pore water. Reservoir sediment analyses show a similar trend. The arsenic in sediment may have precipitated with iron oxyhydroxide minerals. Continued burial may lead to a reducing environment releasing arsenic and the reduced oxy-hydroxide-forming ions, iron and manganese. The released arsenic becomes either bioavailable or may precipitate with various sulfidic minerals (Kneebone et al., 2002; Nicholas et al., 2003).

Biochemical Controls

Biological activity may play a significant role in the fate and transport of arsenic in iron and manganese oxyhydroxide-deficient environments. Microorganisms can indirectly affect arsenic mobility via sulfate reduction, iron-oxide reduction and dissolution of sulfide minerals by oxidation (Gihring et al., 2001). Direct processes include arsenate respiration (Stoltz and Oremland, 1999) and arsenite oxidation (Gihring et al., 2001). Fig. 3 (from (Oremland and Stolz, 2003)) summarizes the oxidation/reduction cycle as it applies to Mono

Lake, CA. Dissimilatory arsenate-reducing prokaryotes (DARP) reduce arsenate, using it as a respiratory oxidant. Arsenite-oxidizing prokaryotes are considered in two classifications: chemolithoautotrophic (CAO) and heterotrophic (HAO) arsenite oxidizers. HAO oxidation is considered a detoxification mechanism. CAO oxidation couples the reaction with the reduction of another molecule for growth.

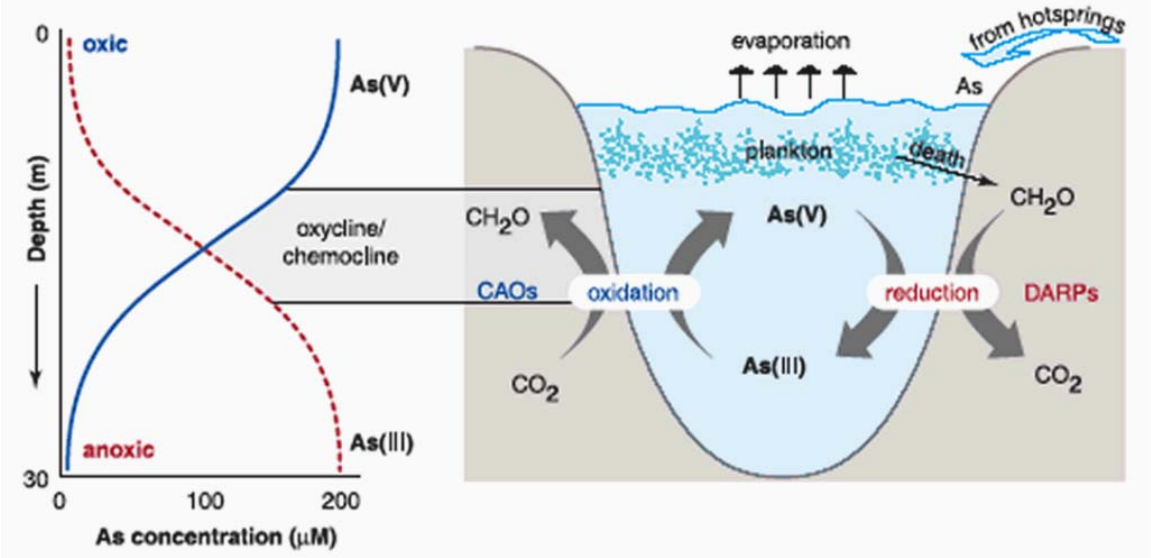


FIGURE 3. Arsenic redox cycling by microbes. Arsenic speciation in the water column of Mono Lake, CA (left). Microbial mediated redox reactions are diagrammed (right). DARPs utilize As(V) as an electron acceptor. CAOs utilize As(III) as an electron donor. Figure from (Oremland and Stolz, 2003).

Although microbes excrete predominantly inorganic arsenic back into the environment, microbes, along with other organisms, can play an important role in converting inorganic arsenic to less toxic organic arsenic species. Many mammals (Vahter and Concha, 2001) and invertebrates have been shown to convert arsenic to organic forms, while microbes can methylate and demethylate arsenic species (Nicholas et al., 2003; Smedley and Kinniburgh, 2002).

It has been shown in a green alga that arsenate is incorporated into the organism due to arsenate's similar chemical characteristics to phosphate (Kuroiwa et al., 1994). Knauer and Hemond (2000) report algae, isolated from arsenic-contaminated habitats, have an increased ability to utilize arsenate in place of phosphate effectively when subjected to phosphate-limited conditions, i.e. enhanced growth and higher cell yields.

A study by Koch et al. (1999) shows the presence of arsenate, arsenite and small amounts of arsenosugars in photosynthetic organisms, suggesting uptake and transformation of these inorganic arsenic species to organic arsenic species. Kuroiwa et al. (1994) report arsenic transformation in marine organisms. There are few studies involving transformations by freshwater organisms. Studying a freshwater environment, Kuroiwa et al. (1994) report a decrease in total arsenic concentrations when following increased trophic levels. The proportion of organoarsenicals also increased, indicating the arsenic is less toxic. Azcue and Nriagu (1995) report seasonal fluctuations of dissolved arsenic

with Increases during summer attributed to low water flow. Organic arsenic ranges from 1.1% in the West Basin 1.2% in the larger East Basin.

Controls on the Bioavailability of Arsenic

Trace metal contaminants are generally considered bioavailable when in the solution phase (Traina and Laperche, 1998). Therefore, the total arsenic concentration in an environment is not indicative of the potential harm to organisms. Arsenic that is adsorbed, precipitated or chelated is difficult for an organism to utilize; it is not bioavailable (Chapman et al., 1998). For example, arsenic can be co-precipitated with iron hydroxides (Daus et al., 1998), removing arsenic from solution. Bioavailability decreases as arsenic is bound with iron hydroxides. It is hypothesized that arsenic contaminated environments will have less bioavailable arsenic as reactive iron concentrations are increased.

The presence of phosphorous can play an important role in arsenic bioavailability. Campos (2002) reports phosphate is physicochemically similar to arsenate, and out-competes arsenate for adsorption sites on sediment particles, especially iron-oxyhydroxides. Aqueous phosphate and silica has been shown to increase arsenate mobility in laboratory simulations of groundwater (Su and Puls, 2003). Phosphate exchange of arsenate sorbed on ferrihydrite increases with increasing pH (Jain and Loeppert, 2000). An influx of phosphate (fertilizers) may release previously non-bioavailable arsenate into the water system through ligand exchange. This is one of the proposed mechanism of arsenic release in

the Bengal basin, a region with elevated arsenic concentrations (Acharyya et al., 2000).

Studies have correlated biologic activity to a change in arsenic concentrations and speciation in marine and estuarine settings. In biologically active surface waters, phosphate depletion is mimicked by arsenate depletion (Smedley and Kinniburgh, 2002). Andreae (1979) correlates an increase in methylated arsenic to an increase of photosynthetic activity in a marine environment suggesting a biotic interaction.

Mechanisms of Arsenic Toxicity and Detoxification

It is reported that arsenite is 60 times more toxic than arsenate and organoarsenicals are 100 times less toxic than inorganic arsenic (Jain and Ali, 2000). The specific mechanism(s) of arsenic toxicity are still not well understood. Vahter and Concha (2001) state arsenate in the cell would be reduced to arsenite and attached to a carrier protein (dithiol) before methylation occurs. Methylated arsenic is less reactive with organic tissues making it less toxic than the inorganic arsenic species (Jain and Ali, 2000) thus methylation of inorganic arsenic is viewed as the major detoxification mechanism of arsenic. Methylated arsenic tends to be more easily excreted from the body compared to inorganic forms (Wildfang et al., 1998). The dominant theory describing arsenite's toxicity is its high affinity for bonding with tissues, specifically enzyme sulfhydryl groups (Jain and Ali, 2000; Wildfang et al., 1998). This interaction would disrupt normal

enzymatic functions.

Role of Photoautotrophs in Arsenic Cycling

Photosynthetic activity has been positively correlated with increased organoarsenical concentrations in marine environments (Andreae, 1979). This suggests photoautotrophic organisms have the ability to biotransform inorganic arsenic to organic forms. Meharg and MacNair (1991) shows arsenate uptake by the grass *H. Lanatus* occurs through phosphate uptake system. The marine alga *Chlorella vulgaris* was shown to uptake arsenic, though minimal biotransformation occurred (Kuroiwa et al., 1994).

Phytoplankton have been shown to increase freshwater sedimentation of arsenic by adding particulate organic matter which sorbs arsenic (Faye and Diamond, 1996). Phytoplankton blooms may play an important role in the cycling of arsenic. The increased biomass may result in increased uptake, biotransformation and sedimentation of arsenic. The importance of phytoplankton increases in areas lacking the oxy-hydroxide controls.

Cyanobacteria Physiology and Habitat

Cyanobacteria (blue-green algae) are a diverse group of photoautotrophic prokaryotes that proliferate in a wide range of freshwater, estuarine and marine environments. Carbon dioxide is utilized as a carbon source in photosynthesis making nitrogen and phosphorous the principle growth-limiting nutrients. Certain

cyanobacteria are capable of atmospheric nitrogen fixation, a process which reduces nitrogen gas to ammonia (Charpy-Roubaud et al., 2001). Phosphorous is the principle growth-limiting nutrient for these species.

Cyanobacteria consist of two basic morphologies, unicellular or filamentous (Paerl et al., 2001). While certain species of each morphology are capable of atmospheric nitrogen fixation, certain filamentous species produce, specialized cells, termed heterocysts, where anaerobic internal conditions allow the fixation of N_2 by the enzyme nitrogenase (Sylvia et al., 1999). Nitrogen fixation utilizes ATP as an energy source and also consumes protons (Stal, 1995). The consumption (reduction) of protons during the reactions may affect solution pH, raising it depending on the amount of nitrogen fixed.

Cyanobacteria exist as primary producers in many environments, both aqueous and terrestrial. Often they are the base of the ecosystem's food chain (Stal, 1995). The diversity and ubiquity of cyanobacteria make it difficult to describe specific ecosystems in which a cyanobacterium is the dominant species. The ability to compete for resources generally determines the dominant freshwater phytoplankton (Sterner, 1989). Nitrogen-fixing cyanobacteria would tend to be dominant in nitrogen-limited settings. The cyanobacterium, *Anabaena* sp. Strain PCC 7120, used throughout the experiment is capable of nitrogen-fixation (Fig. 4). This cyanobacterium was chosen for 2 reasons. First, the organism is an isolated and documented species. Second, it has not been

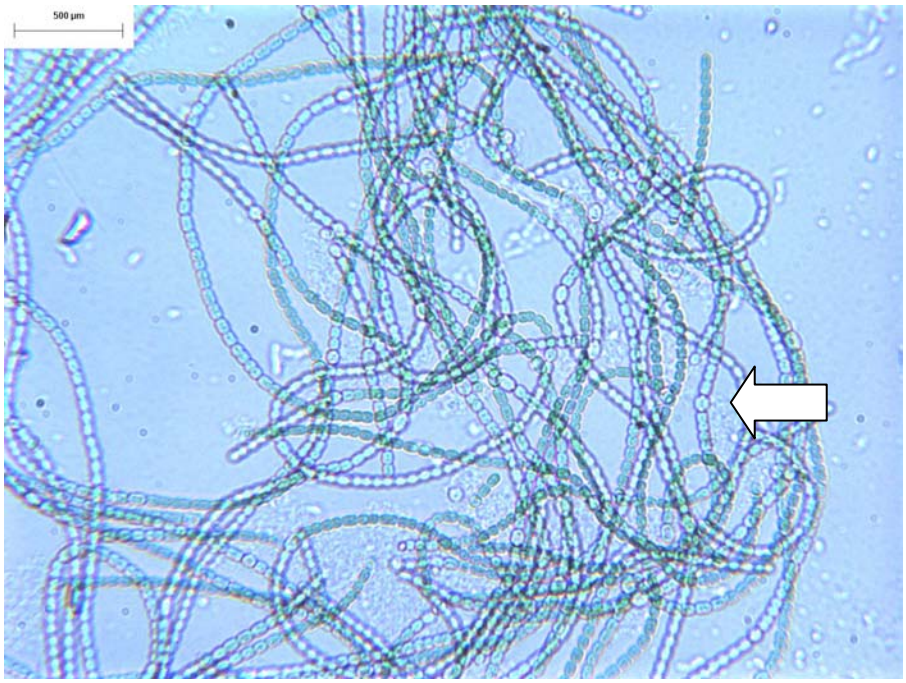


FIGURE 4. Photograph of *Anabaena* sp. Strain PCC 7120. Arrow points to heterocyst.

previously exposed to arsenic. Assuming uptake occurs, it might demonstrate a dormant arsenic uptake mechanism present in other unexposed cyanobacteria.

Anabaena sp. Strain PCC 7120 is a filamentous, freshwater species with the ability to fix atmospheric nitrogen within its heterocysts. Therefore, a selective media lacking combined nitrogen (BG-11) will be used to grow the cyanobacteria in the initial uptake experiments. A final experiment will quantify arsenate uptake and reduction as a function of increasing N:P ratios. The initial experiments have extremely low N:P ratios (N:P << 1). Varying N:P ratios is based on the concept of luxury consumption.

"Luxury consumption refers to increases in organismal nutrients over and above what is immediately required for growth (Sterner and Elser, 2002)."

Low N:P ratios indicate nitrogen limitation. In this instance, nitrogen would limit an organism's growth. Phosphorous consumption (uptake) would continue after growth was reduced or ended, concentrating phosphorous in the cells above what is necessary for growth. It is possible that greater arsenate uptake will occur in lower N:P ratio solutions, being concentrated in the cells similar to phosphorous in nitrogen-limited solutions.

Research Objectives

It is hypothesized that South Texas is deficient in iron-oxyhydroxides, a major geochemical control on arsenic fate and transport. With elevated arsenic concentrations in this region, biological controls may play a significant role in mediating the biogeochemical cycling of arsenic. The ubiquity of cyanobacteria makes it likely cyanobacteria are present in this region. Therefore, there are two objectives of interest:

1. Quantify the reactive iron concentrations in the Nueces River and San Antonio River Watersheds.
2. Identify and quantify arsenate uptake by the freshwater cyanobacterium, A*nabaena* sp. Strain PCC 7120.

3. Identify and quantify arsenate uptake and reduction as a function of increasing N:P ratios.

CHAPTER II

QUANTIFICATION OF REACTIVE IRON IN SEDIMENTS COLLECTED FROM TWO SOUTH TEXAS WATERSHEDS

Introduction

Iron is a common element in the Earth's soil ranging from less than 1% to greater than 20% sediments. Average iron concentration in soil is in the range of 3% (Loeppert and Inskeep, 1996). Primary sources are olivine, pyroxenes, amphiboles and biotite mica. Iron can become mobilized and reactive once these minerals are weathered. Once mobilized, iron may form oxyhydroxides, ferrous sulfides and ferric organic complexes depending on environmental factors such as Eh and pH (Langmuir, 1997).

Arsenic is a known by-product associated with uranium mining. Arsenates, phosphates and sulfates can be concentrated near local ore outcrops (Perel'man and Levin, 1999). The Texas Gulf Coast was the third largest producer of Uranium in the United States during the late 1970s to early 1980s and as such poses arsenic related health risks throughout the region (Parker and Herbert, 2000). Elevated arsenic concentrations in South Texas surface waters have been measured at 44 μg/l; 0.59 μM (United States Department of Energy, 1995). Surface water arsenic concentrations can be seen in Fig. 5.

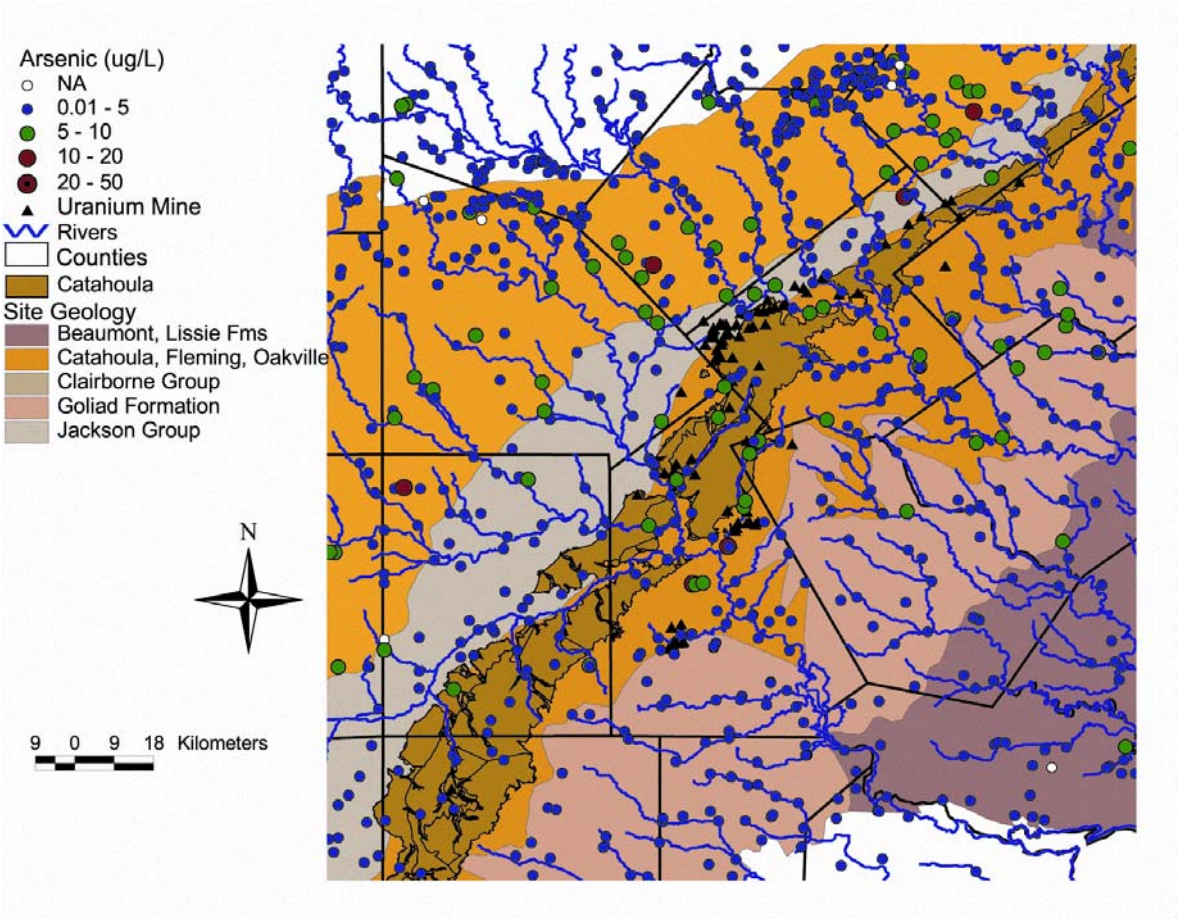


FIGURE 5. Arsenic surface water concentrations (NURE data).

Through prior field visits, it has been visually noted that iron concentrations are highly variable in the soils and sediments of the Nueces and San Antonio River Watersheds. The San Antonio River watershed and the Nueces River watershed have been specifically noted as having a visually distinct sedimentology, with lower levels of iron in the soils and sediments of the

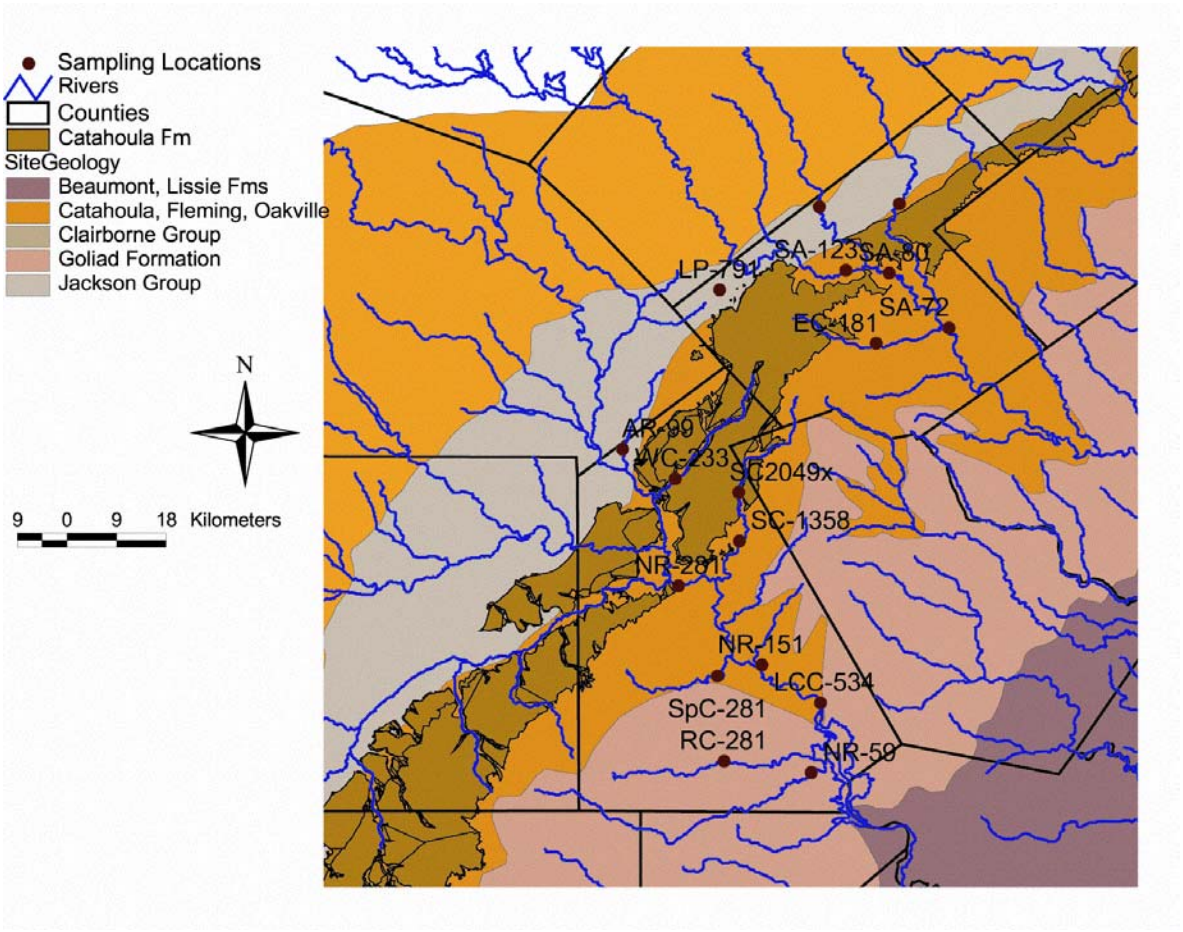


FIGURE 6. Sampling locations related to geology.

Nueces River Watershed. The field sites in relation to local geology can be seen in Fig. 6. Quantifying free iron concentrations are necessary to determine the potential control of iron on arsenic fate and transport in South Texas.

Characterization of South Central Texas Geology

Four major rock unit classifications transect the Karnes and Live Oak County sampling sites trending northeast-southwest. These Tertiary deposits are

the Jackson Group, the Catahoula Formation, the Fleming and Oakville Formations and the Goliad Formation (Galloway et al., 1979).

The Jackson Group is the oldest classification (Upper Eocene) of rock at the sampling sites. The Whitsett Formation is the predominant member consisting of interbedded sand, silt, clay and lignite. Generally, the sand units have produced uranium (Galloway et al., 1979). The Oligocene Catahoula Formation outcrops southeast of the Jackson Group. There are two dominant members: the Fant Tuff which is overlain by the Chusa Tuff. The Chusa has been a productive source of uranium (Galloway et al., 1979). These formations are from Miocene deposits. The Oakville Formation mainly consists of calcareous sandstone with beds of silt and clay (Molina, 2000). It has been a source of uranium (Galloway et al., 1979). These sands were deposited by both major and minor rivers draining the Texas interior (Henry et al., 1982). The Fleming Formation overlays the Oakville Formation and consists of calcareous clay and sand (Galloway et al., 1979). The Goliad Formation consists of Miocene sand deposits and has been a producer of uranium (Galloway et al., 1979). Thick surface deposits of caliche have been identified in this unit (Molina, 2000).

Characterization of South Central Texas Soils

Soil series descriptions below are from the Soil Survey of Karnes County, Texas (Molina, 2000).

The Weigang-Gillett comlex is a mixture of approximately 60% Weigang soils and 30% Gillett soils. The general Weigang sequence consists of 0 to 5 inch surface layer of a neutral, stony fine sandy loam. Five to 19 inches consists of a moderately alkaline stony fine sandy loam. Nineteen to 80 inches is a weakly-consolidated, slightly alkaline sandstone. The Gillett soil has the same surface layer. There is a slightly alkaline clay layer from 5 to 36 inches. Underlying these layers from 36 to 80 inches is a slightly alkaline clay with thin, weakly-consolidated sandstone layers. Due to the degree of mixing, these two soils were mapped as one complex. Low water capacity makes these soils poor for agriculture, but work well for rangelands. The soils also have a low natural fertility. Salinity is measured at 0 - 4 mmhos/cm. Organic matter content ranges from 0.5 - 3%.

The Papalote soil consists of surface and subsurface layers (0-15, 15-19 inches) of neutral loamy coarse sand. The layers from 19 to 80 inches consist of mottled, moderately alkaline sandy clay that changes to a sandy clay loam with increasing depth. This is also a good rangeland soil, though certain crops such as peanuts and watermelon have been grown. This soil has a low natural fertility. Salinity is measured at 0 - 2 mmhos/cm. Organic matter content ranges from 0.5 - 1%.

The Buchel clay consists of moderately alkaline clay throughout. The soil becomes lighter with depth from a very dark gray at the surface to gray at 80 inches. This soil has little use as rangeland or cropland due to the high

frequency of flooding. Salinity is measured at 0 - 8 mmhos/cm. Organic matter content ranges from 2 -5%.

The Rosenbrock clay has a surface layer of very dark gray, slightly alkaline clay. The subsoil layer from 8 to 31 inches is similar to the surface layer. The subsoil layer from 31 to 43 inches is moderately alkaline and grayish brown. A weakly consolidated, alkaline siltstone underlies this material from 43 to 80 inches. The soil is mainly used as rangeland, though crops can be grown. Salinity is measured at 0 - 8 mmhos/cm. Organic matter content ranges from 2 - 5%.

The Sinton sandy clay loam is a very deep floodplain soil. The surface layer (0-25 inches) is a dark grayish brown, moderately alkaline sandy clay loam. The subsoil layer (25-80 inches) is a pale brown, moderately alkaline sandy clay loam. This soil is used for crops and as rangeland. Salinity is measured at less than 2 mmhos/cm. Organic matter content ranges from 1 -3%.

Iron Sources in South Central Texas

Geology plays an important role as parent material in soil formation. Galloway et al. (1977) describe a 3 stage diagenetic history of the Catahoula Formation. In Stage 1, clay (montmorillonite, amorphous aluminosilicates) coat sand grains. As the soil matured, carbonate nodules began to form. Thin section analysis of calcite shows it to be iron-poor (Galloway and Kaiser, 1980). In Stage 2 shallow burial occurs. Meteoric water flow allows the crystallization of

more montmorillonite. Calcite replaces feldspars that are present. Stage 3 describes outcrop weathering. Sands from the outcrop are often mixed with fine-grained crystalline silica, producing the light-colored sediments seen in South Central Texas. There is an apparent lack of iron-bearing minerals in this description.

Henry et al. (1982) describe the effect climate has on soil formation related to the Oakville sandstone. Soils trend from iron rich pedalfers in the northeast to lime enriched pedocals in the southwest. Iron enrichment is attributed to meteoric leaching while lime enrichment is attributed to capillary evaporation. The sampling sites are in this lime-enriched region.

Materials and Methods

Sediment Collection

Sediment collection occurred within a 15-day period in August 2002 at 18 sites (Fig. 7). Eight sites were on riverbanks in the Nueces River Watershed, 10 sites were on riverbanks in the San Antonio River watershed. The sites were distributed through the region along both major rivers and their tributaries. GPS was used to mark site coordinates. Collection site placement was consistently along the water's edge from the top 15 cm of sediment. These sediment grab samples were scooped by a plastic trowel and placed in Ziploc bags. Samples were temporarily stored in an ice chest and taken back to a laboratory refrigerator until analysis. Major geochemical controls (pH, temperature and

dissolved oxygen content) were collected *in situ*. Table 1 shows the specific geologic unit and soil series of each sampling site. Table 2 provides a visual description of each of the sampling sites.

Non-Silicate Bound Iron Extraction

Free iron extraction followed the dithionite-citrate-bicarbonate (DCB) method described by Loeppert and Inskeep (1996) modified to use 50% of the extractant materials. The DCB method extracts non-silicate bound iron including oxides and organically bound iron (Soil Survey Investigation Staff, 1996). The supernatant extractant was diluted with double distilled water (DDW) to reduce iron concentrations to the detection range of the GFAAS. Blanks consisted of iron-free DDW. A 60 μg/L iron standard was measured during the run to ensure accuracy. WC-233 was run twice to ensure correct sampling procedure and quantification repeatability. Flasks used in dilutions were rinsed with DDW and measured for iron to ensure no carryover contamination occurred.

FIGURE 7. Photographs of iron data sampling sites. (top) Olmos Creek (AR-99). (bottom) Cibolo Creek (CBC-887).

FIGURE 7 Cont. (top) Escondido Creek (EC-181). (bottom) Ecleto Creek (EcC-627).

FIGURE 7 Cont. (top) Legarto Creek (LgC-534). (bottom) Nueces River (NR-59).

FIGURE 7 Cont. (top) Nueces River (NR-281). (bottom) Ramirena Creek (RC-281).

FIGURE 7 Cont. (top) San Antonio River (SA-72). (bottom) San Antonio River (SA-80).

FIGURE 7 Cont. (top) San Antonio River (SA-123). (bottom) Sulfur Creek (SC-1358).

FIGURE 7 Cont. (top) Sulphur Creek (SC-2049ext). (bottom) Spring Creek (SpC-281).

TABLE 1. Sediment Sampling Site Geologic Units and Soil Series

Site	Geology [1]	Soil
RC-281	Goliad Fm	Sinton Sandy Clay Loam [2]
LgC-534	Goliad Fm	Sinton Sandy Clay Loam [2]
NR-59	Goliad Fm	Sinton Sandy Clay Loam [2]
SpC-281	Fleming, Oakville Fms	Buchel Clay [2]
NR-281	Catahoula Fm	Buchel Clay [2]
SC-1358	Fleming, Oakville Fms	Sinton Clay Loam [2]
SC2049x	Catahoula Fm	Rosenbrock Clay [2]
AR-99	Jackson Group	Sinton Sandy Clay Loam [2]
LCC-534	Goliad Fm	Sinton Sandy Clay Loam [2]
NR-151	Fleming, Oakville Fms	Buchel Clay [2]
WC-233	Catahoula Fm	Rosenbrock Clay [2]
LP-791	Jackson Group	Weigang-Gillett Complex [3]
EcC-627	Jackson Group	Papalote loamy coarse sand [3]
SA-80	Catahoula Fm	Buchel Clay [3]
SA-72	Fleming, Oakville Fms	Buchel Clay [3]
EC-181	Fleming, Oakville Fms	Buchel Clay [3]
SA-123	Catahoula Fm	Buchel Clay [3]
CBC-887	Jackson Group	Buchel Clay [3]

[1] Bureau of Economic Geology

[2] SSURGO GIS data

[3] Soil Survey of Karnes County, Texas

TABLE 2: Sampling Site Descriptions

Site	Site Name	Description
Olmos Creek	AR-99	Creek present only as a drying ponds. Many natural dams present. Water has a greenish algal tint.
Cibolo Creek	CBC-887	Water flow is slow upstream and increases speed as the channel narrows downstream. Algae is present on rock in the stream. Algae is present in a drying pond. Clam shells are abundant.
Escondido Creek	EC-181	Creek was small and not flowing. Pooled water was covered with a thick scum layer suggesting cyanobacteria. Vegetation was abundant, specifically grass and wetland vegetation.
Ecleto Creek	EcC-627	The creek was small and slightly-turbid with steep banks on both sides. Water was not flowing.
Legarto Creek	LgC-534	Water was pooled and stagnating. Leaf litter present in the water. A film covering the water suggested high microbe metabolic activity.
Nueces River	NR-59	River was slightly flowing and turbid. The bases of trees were under water. Brown algae were present in water pooled on riverside.
	NR-281	River was turbid with moderate flow. The vegetation was dead 2-3 meters above water level. A green substance (mold, algae, cyanobacteria) was present covering the saturated sediments on the river bank.
Ramirena Creek	RC-281	Sampling occurred upstream of natural dam created by vegetative debris. Water was pooled in the channel leading up to the dam. Water turbidity was high and a green algal tint was present.
San Antonio River	SA-72	Water flow was swift and turbid. Samples were taken downstream of a bridge pier in a low energy depositional environment.
	SA-80	Water flow was swift and turbid. Samples were taken from muddy point bar deposits. Saturated muds showed visible algae.
	SA-123	Water was calm and turbid. River banks on both sides are relatively steep. Vegetation is dead on both shores up to 10m above water line.
Sulphur Creek	SC-1358	Water was pooled and stagnating. Leaf litter was abundant in the water. A film was present on the water surface, as well as an “oil-like” sheen. A green substance (mold, algae, cyanobacteria) was present covering the saturated sediments on the river bank.
	SC-2049 ext.	Sampling occurred just upstream of a culvert that went under FM 2049. Upstream was pooled and displayed a green algal tint. Downstream of the culvert was a small, slow moving stream with a lot of vegetation.
Spring Creek	SpC-281	Creek present only as a drying pond (4 x 10m). Surrounding areas were dry with mud-cracks present.
Lake Corpus Christi	LCC-534	Sediments collected in a protected bay environment. Dense vegetation was present.
Nueces River	NR-151	River flow was very light, slightly turbid.
Weedy Creek	WC-233	Sediment collected upstream of road intersection. Light water flow.
Lyssy Pond	LP-791	Algae was present along the shore.

Iron Analysis

Extractant was analyzed via Graphite Furnace Atomic Absorption Spectrometer (GFAAS) (SpectraAA 200, Varian) using a multi-element lamp (Fe, Co, Ni, Mn, Cu, Cr; Varian). Extracts were diluted with double distilled water (DDW). The default SpectraAA temperature program is used (Table 3). Iron standard solutions were created by adding $FeCl_3$ to an iron-free extract solution and diluted with DDW to appropriate concentrations. The GFAAS-measured iron standard curve had a calculated R^2 of 0.984. Iron concentrations were calculated using the assumptions there was no loss of the 27.5-mL extractant solution used in digesting sediment samples.

TABLE 3. GFAAS Temperature Program for Iron Measurements

Step	Temp (ºC)	Time (s)	Flow (L/min)	Gas Type	Read Signal	Storage
1	85	5	3	Normal	No	No
2	95	40	3	Normal	No	No
3	120	10	3	Normal	No	No
4	700	5	3	Normal	No	No
5	700	1	3	Normal	No	No
6	700	2	0	Normal	No	Yes
7	2300	1.1	0	Normal	Yes	Yes
8	2300	2	0	Normal	Yes	Yes
9	2300	2	3	Normal	No	Yes
10	100	16.2	3	Normal	No	No

Results and Discussion

DCB Iron Extraction Data

The non-silicate bound iron concentrations are variable when looking only at the Nueces River and San Antonio River watersheds. The results can be seen in Figure 8. When compared to the normal range of iron concentrations these values are consistently low throughout the two watersheds. The highest iron concentration observed is 6810 mg/kg (0.681% w/w) while the lowest is below GFAAS detection limits. The average iron concentration for the region is 2770 mg/kg (0.277% w/w). Specific site measurements, including pH, temperature and dissolved oxygen can be seen in Table 4.

Relating Iron Data to Arsenic Fate and Transport

When comparing iron in South Texas soils (0 – 0.681%) to the worldwide average soil concentration (3%) it is determined there is little reactive iron present in this region. This is especially true of the sites where reactive iron is non-existent, as determined by being below GFAAS detection limits. The average iron concentration in South Texas soils (0.277%) is over one order of magnitude lower. These data imply that arsenic adsorption onto iron oxyhydroxides is not a likely mechanism controlling the fate and transport of arsenic in these watersheds. This allows the possibility of another arsenic-

controlling mechanism. The following experiments will examine the role organic cycling may play in South Texas.

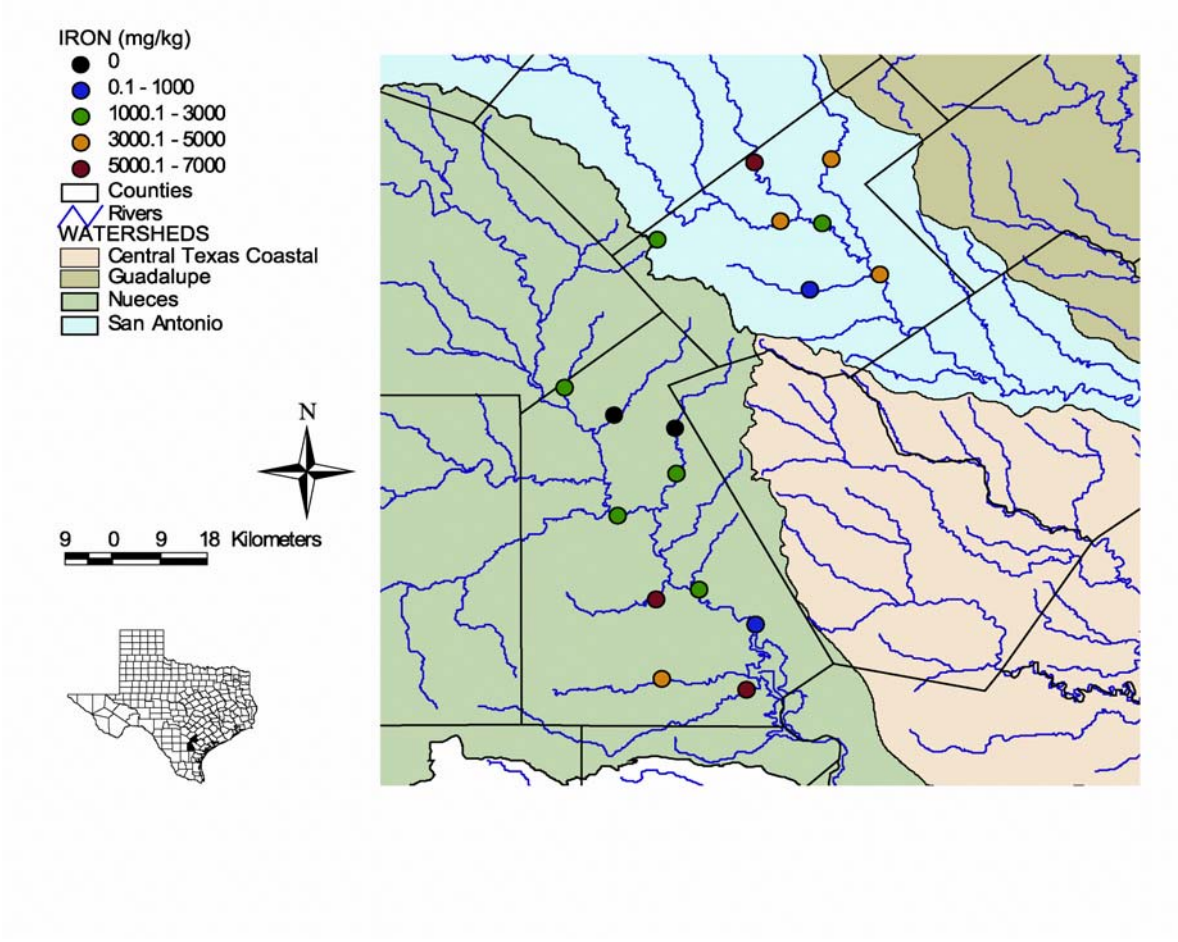


FIGURE 8. Spatial reactive iron oxide data in South Texas.

TABLE 4. Site Data for Iron Oxide Extraction Experiment

Site Name	Latitude	Longitude	pH	T (ºC)	DO (mg/L)	DATE	IRON (mg/kg)
RC-281	28.1420	-98.1040	6.58	30.3	4.54	8/8/2002	3333
LgC-534	28.1240	-97.9620	7.21	31.6	3.79	8/8/2002	3369
NR-59	28.1230	-97.9620	7.45	30.5	3.15	8/8/2002	5608
SpC-281	28.2810	-98.1140	7.61	28.6	0.30	8/8/2002	6363
NR-281	28.4280	-98.1780	7.64	30.4	0.60	8/8/2002	1621
SC-1358	28.5010	-98.0790	7.59	32.5	0.60	8/8/2002	2545
SC2049x	28.5800	-98.0800	7.89	30.6	0.41	8/8/2002	BDL
AR-99	28.6510	-98.2690	7.89	29.9	4.48	8/8/2002	1047
LCC-534	28.2372	-97.9458	6.91	30.6	3.30	8/19/2002	411.7
NR-151	28.2991	-98.0424	7.64	31.8	3.03	8/19/2002	1664
WC-233	28.6029	-98.1829	7.75	32.5	5.03	8/19/2002	BDL
LP-791	28.9106	-98.1113	8.08	37	7.01	8/19/2002	2906
EcC-627	29.0510	-97.8181	6.97	NA	3.62	8/23/2002	3366
SA-80	28.9381	-97.8346	7.17	31.7	5.60	8/23/2002	2988
SA-72	28.8486	-97.7362	7.32	29.9	2.60	8/23/2002	4192
EC-181	28.8232	-97.8553	7.41	29.9	1.92	8/23/2002	704.9
SA-123	28.9424	-97.9042	7.72	29.9	1.53	8/23/2002	3183
CBC-887	29.0461	-97.9484	7.81	30.5	4.13	8/23/2002	6808

CHAPTER III

ARSENATE UPTAKE, SEQUESTRATION AND REDUCTION BY A FRESHWATER CYANOBACTERIUM

Introduction

Results from Chapter II indicate a deficiency in free iron-oxides in the San Antonio and Nueces River Watersheds, South Texas. This increases the potential importance of other mechanisms that control the cycling of arsenic in this region. Microorganisms have been shown to significantly affect arsenic speciation (Oremland and Stolz, 2003). Certain bacteria can reduce (Stoltz and Oremland, 1999) and oxidize (Gihring et al., 2001) arsenic. Various microbes can biotransform arsenic via methylation and demethylation processes (Nicholas et al., 2003; Turpeinen et al., 2002).

Photoautrophic organisms also affect arsenic speciation and bioavailability. Certain grasses (Meharg and MacNair, 1991) and plants (Meharg and Hartley-Whitaker, 2002) have an arsenic resistance that allows arsenate uptake and sequestration. Photosynthetic activity has been positively correlated with increased organoarsenical concentrations in marine environments suggesting biologic cycling of arsenic through photoautotrophs (Andreae, 1979). Biotransformation has been suggested due to the presence of arsenate, arsenite

and arsenosugars in some photosynthetic organisms (Koch et al., 1999). Kuroiwa et al. (1994) report arsenic biotransformation in a freshwater food chain beginning with a green alga. Algae isolated from an arsenic-contaminated freshwater lake have been shown to effectively utilize arsenate under phosphate-limited conditions (Knauer and Hemond, 2000).

Cyanobacteria are photoautotrophic prokaryotes that proliferate in a wide range of freshwater, estuarine and marine environments (Stal, 1995). An *Anabaena* strain of cyanobacteria (Fig. 9) was identified in the watersheds analyzed for iron. The following experiments test a freshwater cyanobacterium's ability to uptake, sequester and reduce arsenate in a limited-iron setting. The cyanobacterium used is *Anabaena* sp. Strain PCC 7120 and has no previous exposure to arsenate.

The following experiments quantify arsenate net uptake using 3 arsenate concentrations (0, 1.0 μM As, 10 μM As) in a fixed nutrient solution. Subsequent experiments quantify net arsenate uptake and reduction as a function of N:P ratios, thereby testing the concept of luxury uptake. Luxury uptake occurs when one nutrient limits growth, yet the organism continues to uptake some other nutrient (Sterner and Elser, 2002). In this scenario, P uptake should continue when subjected to N-limited conditions. Arsenate uptake should also continue based on the similarity between arsenate and phosphate (Campos, 2002).

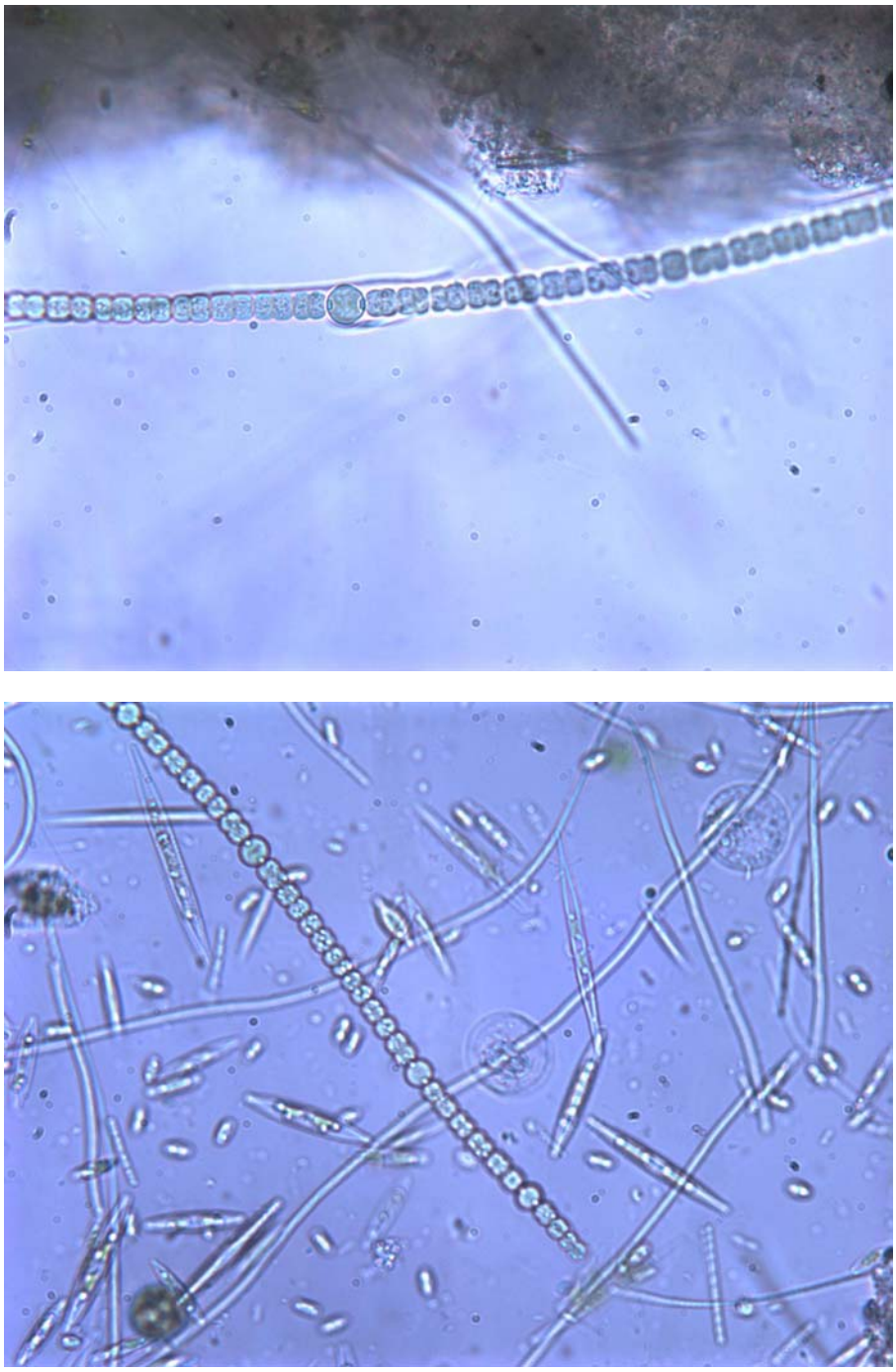


FIGURE 9. *Anabaena* sp. in water samples collected from Weedy Creek, Texas.

Materials and Methods

Laboratory Practices

All chemicals used throughout the following experiment were ACS certified reagent grade and used without any further purification. All solutions were made with NanoPure water (NPW) 18.2 MΩ. Glassware was washed before each use by rinsing 3 times with 1% ultrapure nitric acid and 3 times with NPW. All glassware was allowed to air dry. All microbiological work was done aseptically. All growth media and containers were autoclaved before use. All inoculations occurred in a 70% ethyl alcohol washed hood.

Cyanobacteria Growth and Enumeration

A large number of cyanobacteria-inoculated samples were needed throughout the course of the experiments. These stock cultures were contained in 2 L Pyrex Erlenmeyer flasks containing 1 L BG-11 growth media (Golden, 2002). The stock cultures were inoculated with the cyanobacterium, *Anabaena* sp. Strain Pasteur Culture Collection (PCC) 7120, generously donated by Dr. Golden. Stock cultures were maintained at room temperature under natural lighting conditions.

Cell count is related to the optical density (OD) of a homogeneous cell suspension (Theil, 1988). Optical density was determined by measuring the cyanobacteria suspension absorbance at 700 nm (2 nm slit) on a U-3010

Spectrophotometer (Hitachi). Five suspensions were measured, including one blank solution.

A cell counting chamber (borrowed from Dr. Zuberer) was used to count the cells. The cell counting chamber, filled with the cyanobacteria suspension of known optical density, was placed on a microscope for enumeration. Due to the sheer number of cells, direct counts were deemed impractical. Photos were taken of 20 fields of view to achieve a statistically significant average (Fig. 10).

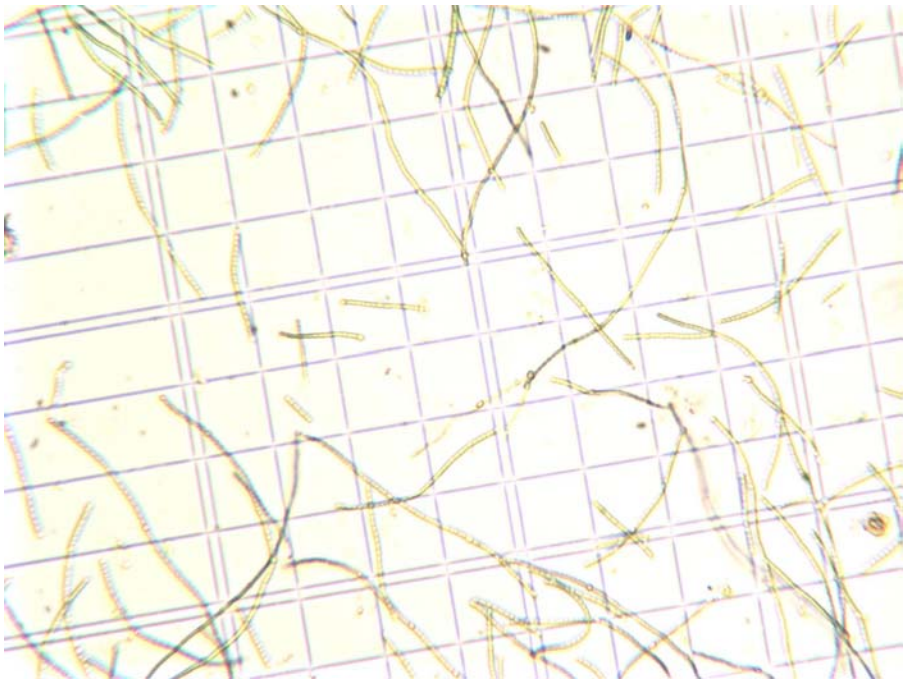


FIGURE 10. *Anabaena* sp. Strain PCC 7120 in the cell-counting chamber.

Photos were opened in ArcView 3.3 and an average cell length was determined using the ArcView 3.3 length tool. The average cell length was determined to be 10.6 units (arbitrary) by counting 236 individual cells and

determining a total length of these cells to be 2499 units. After determining the number of cells in each increasing OD sample, the following equation was used to determine the final cell count in cells / mL suspenion:

$$(\text{Cells / mL}) = (\#\ \text{cells}) * (1000\text{mm}^3) / (\text{Volume}) / (1\ \text{mL}),$$

where (# cells) is the number of cells counted for each sample and (volume) is the calculated volume of the area counted. The cell counts calculated from the equation above were then correlated to optical density.

Arsenic Analysis

Arsenate was measured throughout the experiments using 10 mL aliqouts from each sample. Aliquots were filtered through 0.45 *μ*m polycarbonate filters (Poretics Corp.) and measured using GFAAS (SpectraAA 200, Varian) with a nickel modifier and arsenic specific lamp (Lake, 2002). The temperature program used can be seen in Table 5. Arsenite was measured using continuous flow Hydride Generation Atomic Absorption Spectroscopy (Perkin Elmer). A borosilicate/sodium hydroxide solution was used to volatilize arsenite in a method developed by (Loeppert, 2003).

TABLE 5. GFAAS Temperature Program for Arsenic Measurements

Step	Temp (ºC)	Time (s)	Flow (L/min)	Gas Type	Read Signal	Storage
1	95	5	3	Normal	No	No
2	100	60	3	Normal	No	No
3	125	10	3	Normal	No	No
4	1200	5	3	Normal	No	No
5	1200	10	3	Normal	No	No
6	1200	2	0	Normal	No	Yes
7	2600	0.7	0	Normal	Yes	Yes
8	2600	2	0	Normal	Yes	Yes
9	2600	2	3	Normal	No	Yes
10	100	30	3	Normal	No	No

Preliminary Arsenate Uptake Experiments

Six experimental conditions were tested in triplicate (Table 6). The flasks were filled with a phosphate-deficient BG-11 growth media (Golden, 2002) and spiked with the appropriate arsenate concentrations using sodium arsenate. Phosphate was excluded to maximize any potential arsenate uptake. Uptake was operationally defined as arsenate loss from solution. Solutions were adjusted to pH 7.1 using a 1% nitric acid solution and a 0.25 M NaOH solution. Flasks were inoculated with 5 mL of a cyanobacteria suspension with an OD of 0.49 at 700 nm. The suspension was adjusted to pH 7.1 before inoculation. All samples were placed on an orbital shaker (Forma Scientific) set at 110 rpm to ensure thorough mixing and aeration. All flasks were maintained in natural light and room temperature conditions. Samples were allowed 28 days of growth before pH, OD and arsenic measurements were taken.

TABLE 6. Preliminary Arsenate Uptake Experiment Test Conditions

Condition Name	Description
BG-11	BG-11 Growth Media
INOC	Inoculated BG-11 Growth Media
AsL	1.0 μM Arsenate spiked Bg-11 Growth Media
AsH	1.0 mM Arsenate spiked Bg-11 Growth Media
INOC-AsL	1.0 μM Arsenate spiked Bg-11 Growth Media, inoculated
INOC-AsH	1.0 mM Arsenate spiked Bg-11 Growth Media, inoculated

The polycarbonate filters used in obtaining the arsenate aliquot were placed into 50 mL centrifuge tubes containing 25 mL 1.0 mM PO^{4-} solution and placed on an orbital shaker at 120 rpm for 2 hours to free any arsenate sorbed to cell surfaces. Ten mL aliquots of the phosphate solution were filtered and measured for arsenate using GFAAS.

A brown precipitate formed randomly during the experiment in certain flasks. The precipitate formed a ring at the liquid-air interface. The precipitate was identified as a possible iron-oxide. Arsenate is known to co-precipitate with iron oxides (La Force et al., 2000) making this brown precipitate a possible arsenate sink and source of error in these experiments.

Flasks were emptied and allowed to air dry for 24 hours. One hundred and fifty mL of 0.2 M Nitric acid were added to dissolve the precipitate. Flasks were allowed to stand for 3 hours before being measured for arsenate and iron via GFAAS.

Four modified BG-11 conditions (Table 7) were tested to prevent formation of the brown precipitate. Triplicates of each condition were inoculated to determine effects on cyanobacteria growth. Triplicates of each were spiked with arsenate to determine arsenate loss from solution.

TABLE 7. Modifications of BG-11 Growth Media

Condition Name	Inoculated	Arsenate Spiked	Modification
N-0.1 BG-11		X	10% BG-11 components added
I-0.1 BG-11	X		10% BG-11 components added
N-0.01 BG-11		X	1% BG-11 components added
I-0.01 BG-11	X		1% BG-11 components added
N-0.1 TMS, Fe		X	10% Trace Metal Solution, Fe added
I-0.1 TMS, Fe	X		10% Trace Metal Solution, Fe added
N-0.01 Fe		X	1% Fe added
I-0.01 Fe	X		1% Fe added

The samples were adjusted to pH 8±0.1 using a 1% nitric acid solution and a 0.25 M NaOH solution. Flasks were inoculated with a 5 mL cyanobacteria suspension. All samples were placed on an orbital shaker set at 110 rpm to ensure thorough mixing and aeration. All flasks were maintained in natural light and room temperature conditions. All samples were measured and adjusted to pH 8±0.1 on days 6 and 13. Samples were allowed 20 days of growth before pH, OD and arsenate measurements were taken. Original and modified BG-11 components can be seen in Table 8.

TABLE 8. BG-11 Component Concentrations

Chemical	BG-11 Solution Molarity	Modified BG-11 Solution Molarity
$NaNO_3$	1.765E-03	0.000E+00
$CaCl_2*2H_2O$	2.449E-04	2.449E-04
$Fe(NH_4)_2(SO_4)2*6H_2O$	3.060E-05	3.060E-07
$C_{10}H_{14}N_2O_8Na_2$	2.974E-06	2.974E-06
K_2HPO_4	2.297E-04	1.148E-04
$MgSO_4*7H_2O$	3.043E-04	3.043E-04
Na_2CO_3	1.887E-04	1.887E-04
H_3BO_3	4.618E-05	4.618E-05
$MnCl_2*4H_2O$	9.146E-06	9.146E-06
$ZnSO_4*7H_2O$	7.720E-07	7.720E-07
$Na_2MoO_4*2H_2O$	1.612E-06	1.612E-06
$CuSO_4*5H_2O$	3.164E-07	3.164E-07
$Co(NO_3)_2*6H_2O$	1.684E-07	1.684E-07

Arsenate Uptake and Sequestration by a Cyanobacterium

Six experimental conditions were tested in triplicate. The conditions were the same as the initial uptake experiment except that the high arsenate concentration was reduced to 10 μM. Samples were contained in 70 mL polystyrene tissue culture flasks (Fisher Scientific) with vented caps to prevent contamination. The flasks were filled with the modified BG-11 growth media (1% iron, 50% phosphate) and spiked with the appropriate arsenate concentrations using sodium arsenate. Solutions were adjusted to pH 7.0 using a 1% nitric acid solution and a 0.25 M NaOH solution. Flasks were inoculated with a 5 mL

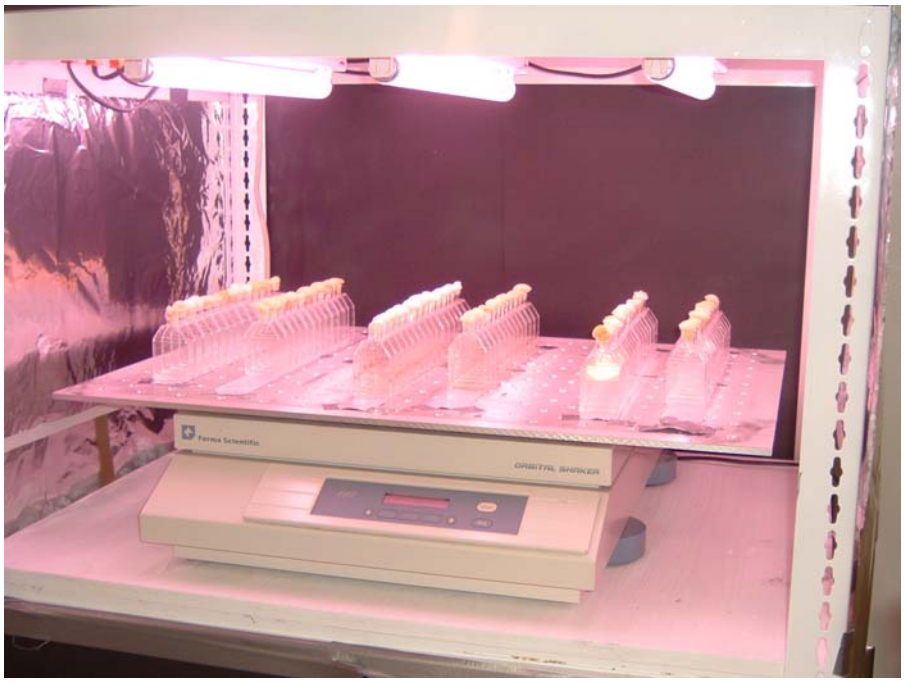


FIGURE 11. Cyanobacteria growth chamber. Another orbital shaker is located below.

cyanobacteria suspension (OD of 1.028 at 700 nm). All samples were placed on 2 orbital shakers set at 110 rpm to ensure thorough mixing and aeration. The orbital shaker was housed in a growth chamber (Fig. 11) where light could be controlled. The light schedule was 13 hours on, 11 hours off using 3 timed lights (Sylvania, 20W GROLUX) for each orbital shaker to simulate summer lighting conditions in South Texas. Temperature ranged from 23ºC (day) to 22ºC (night). Optical density, pH and arsenate uptake, defined as loss from solution, were measured at 9 time steps over 43 days. Arsenic speciation was qualitatively analyzed using High Performance Liquid Chromatography (HPLC) (DX600 Model, Varian), with an IonPac AS-14 anion exchange column and appropriate

guard column. Table 9 details the HPLC method (Jackson, 2001). Arsenate-spiked BG-11 will be compared to inoculated, arsenate-spiked BG-11.

TABLE 9. HPLC Method for Arsenic Speciation. Table modified from (Jackson, 2001)

Time (min)	NanoPure Water	10 mM PO_4 pH 7.2	flow rate (mL / min)
0.00	80%	20%	1
3.00	80%	20%	1
3.03	0	100%	2
10.00	0	100%	2
10.01	80%	20%	2

Cellular Arsenic Partitioning

An arsenic extraction was done to differentiate extracellular arsenic, intracellular arsenic and arsenic sorbed to the cell surface. Cells were cultured for 18 days in a 5.3 μM (400 μg/L) arsenate solution. This is the lowest concentration to allow reliable arsenic measurements after dilution. Cell suspensions were filtered to determine arsenic in solution. NanoPure water (15 mL) was used to rinse any residual arsenic in the filtration apparatus. The cells were rinsed with 15 mL 3 mM EDTA to determine arsenic sorbed to cells. The cells were then digested using 15.5 mL 1 M ultra-pure nitric acid diluted in NPW. This extraction was based on the methods of Mirimanoff and Wilkinson (2000). Arsenic was quantified via GFAAS.

Arsenate Reduction by a Cyanobacterium

Six experimental conditions testing 3 N:P ratios were tested. Cells were cultured for 18 days in a 1.0 *μ*M (74.9 ppb) arsenate solution. Samples were contained in 70 mL polystyrene tissue culture flasks (Fisher Scientific) with foam plugs to prevent contamination. The flasks were filled with the reduced iron BG-11 growth media (1% iron), spiked with the appropriate arsenate and inoculated. The 3 N:P ratios can be seen in Table 10. Total arsenic was measured with GFAAS. Net arsenate uptake is defined as total arsenic loss from solution. Arsenite was measured with HGAAS within 6 hours of collection. Minimal amounts of arsenite oxidize if samples are analyzed within 25 hours of collection (Bednar et al., 2002). Optical density, pH and Eh were also measured.

Results and Discussion

Analysis of Preliminary Experiments

The cyanobacteria suspension optical density correlated to cell number has an R^2 value of 0.9928 when measuring absorbance up to 0.36 (Fig. 12). A suspension with an absorbance of 1.6 was diluted to a value of less than 0.36. A cell count was back calculated using the dilution factor back to 1.6. This extrapolated optical density correlates to cell count with an R^2 of 0.999.

TABLE 10. N:P Ratios in Arsenate Reduction Experiment

South Texas Inorganic N	**N:P (ratio)**	**N (mg/L)**	**P (mg/L)**
Maximum	98	11	1.3
Minimum	0.31	< .05	<0.01
Average	8.2	1.4	0.28
Standard Deviation	12	2.4	0.37

South Texas Total N	**N:P (ratio)**	**N (mg/L)**	**P (mg/L)**
Maximum	128	11.5	1.3
Minimum	0.5	<0.05	<0.01
Average	11.4	1.73	0.28
Standard Deviation	15.0	2.59	0.37

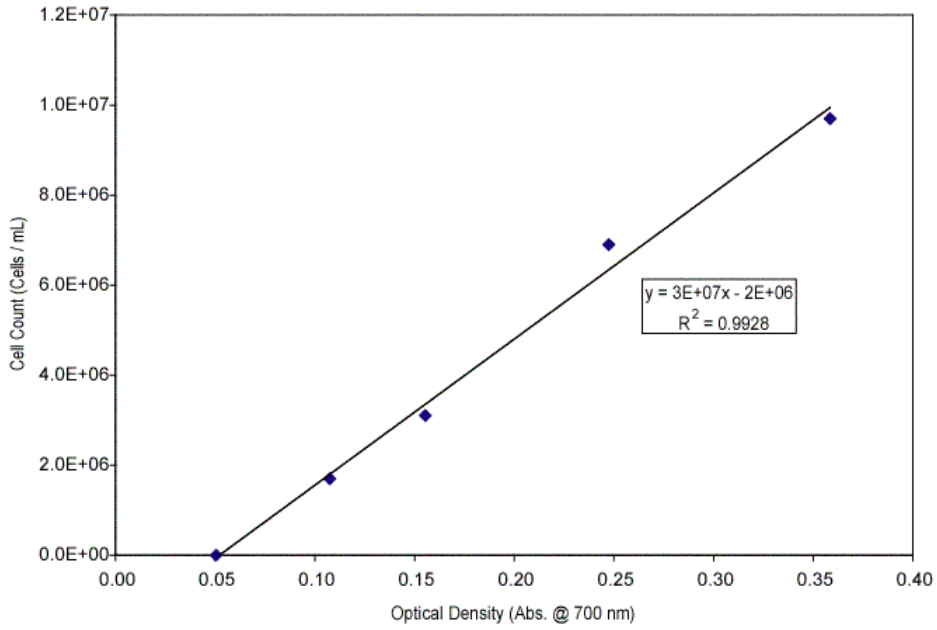
BG-11 Total N[1]	**N:P (ratio)**	**N (mg/L)**	**P (mg/L)**
High N:P	295	52.5	0.178
Mid N:P	69.5	12.4	0.178
Low N:P	17.4	3.09	0.178

[1] Organic N in BG-11 solution is negligible (3 orders of magnitude lower)

Arsenate uptake is evident in the initial experiment for both arsenate spike concentrations; 1.0 μM, 1.0 mM (Fig. 13). Uptake amounts appear similar though it is more visible in the 1.0 μM As solution (with similar uptake masses, uptake would represent a higher percentage in the 1.0 μM As solution).

Cyanobacteria growth is not adversely affected by arsenate in the solution. Growth in the 1.0 mM arsenate solution is higher than the lower and non-arsenate spiked solutions (data not shown). Arsenate may be filling the role of phosphate (Knauer and Hemond, 2000). Total distribution for arsenic in the three phases (solution, brown precipitate, cell wall) can be seen in Table 11. Arsenate sorbed to the cell walls is negligible (Fig. 14). Arsenic in this phase is at least 3 orders of magnitude less than the total arsenate spike.

The brown precipitate contained up to 35.8% of the arsenic in the 1.0 μM arsenate samples. As a result, the BG-11 solution was modified. Results from the BG-11 modification experiment are listed in Table 12. It was determined that iron be reduced to 1% of the normal BG-11 solution. Phosphate was reduced to 50%.



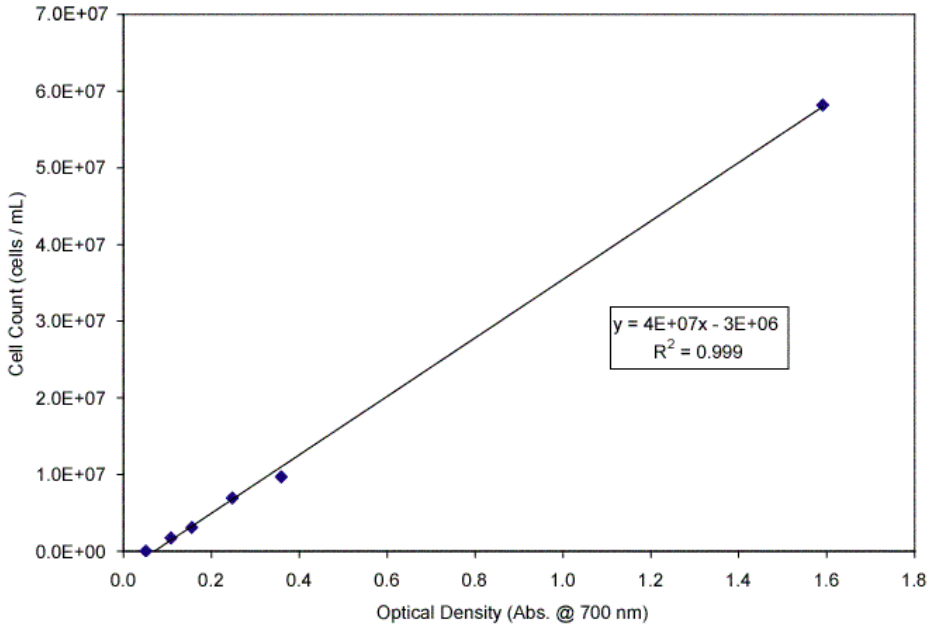


FIGURE 12. Correlating cell count and optical density (top). Extrapolated data obtained by diluting high OD sample to lower analytical range and back calculating for cell count (bottom).

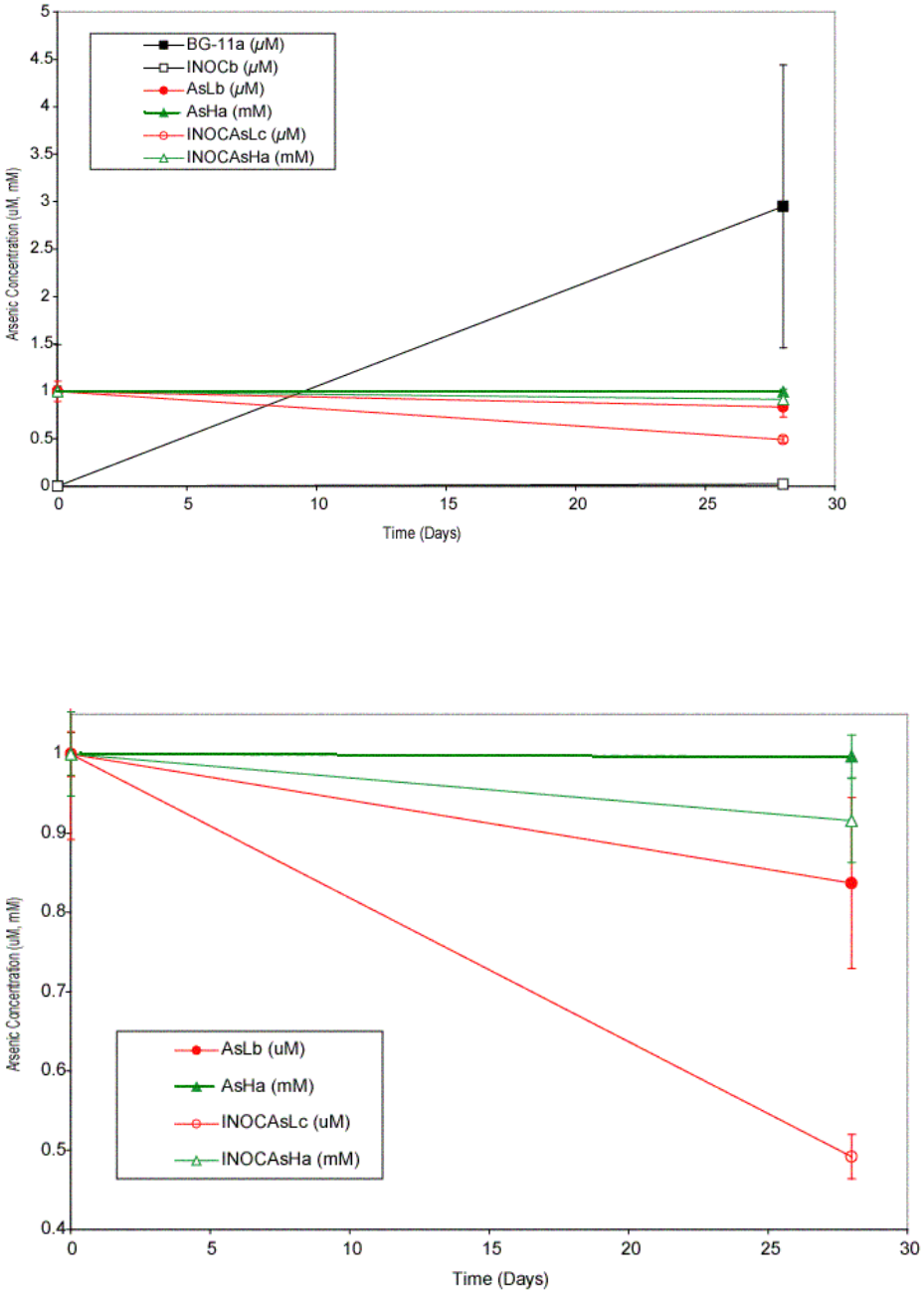


FIGURE 13. Arsenate uptake in initial experiment. Arsenic in BG-11a was traced back a single contaminated sample (top). Arsenate uptake in initial experiment with enlarged Y-scale (bottom).

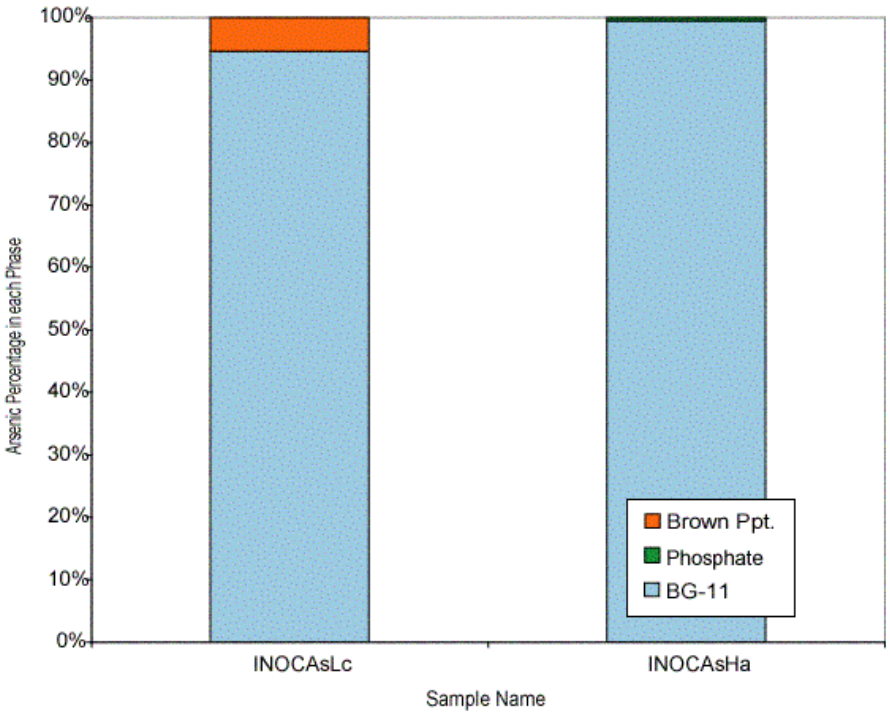


FIGURE 14. Arsenic distribution in the initial uptake experiment. Low arsenate-spiked solutions show a higher percentage of arsenic sequestered in the brown precipitate.

TABLE 11. Arsenic Distribution in Initial Uptake Experiment

Sample Name[1]	Total Arsenic (mM) initial	final	BG-11 As (mM)	Phosphate Rinse As (mM)	Brown Precipitate As (μM)
BG-11_a	NA	2.93E+00	1.39E-05	3.89E-05	0.0240
BG-11_b	NA	5.86E-01	7.44E-06	7.72E-06	
BG-11_c	NA	1.47E-01	5.74E-06	1.89E-06	
INOC_a	NA	7.15E-03	1.19E-05	-6.42E-08	
INOC_b	NA	1.48E-02	1.22E-05	3.43E-08	0.00829
INOC_c	NA	-4.34E-03	2.18E-05	-3.49E-07	
AsL_a	0.001	4.66E-04	2.95E-04	2.28E-06	
AsL_b	0.001	4.79E-04	3.94E-04	1.12E-06	0.358
AsL_c	0.001	3.97E-04	2.74E-04	1.64E-06	
AsH_a	1	9.90E-01	9.85E-01	6.24E-05	0.00708
AsH_b	1	9.44E-01	9.39E-01	7.23E-05	
AsH_c	1	9.45E-01	9.39E-01	7.58E-05	
INOC-AsL_a	0.001	3.70E-04	4.10E-04	-5.35E-07	
INOC-AsL_b	0.001	3.81E-04	4.30E-04	-6.55E-07	
INOC-AsL_c	0.001	4.23E-04	4.65E-04	-5.66E-07	0.0265
INOC-AsH_a	1	9.09E-01	9.04E-01	6.57E-05	0.00750
INOC-AsH_b	1	8.88E-01	8.88E-01	NA	
INOC-AsH_c	1	9.86E-01	9.86E-01	NA	

[1] Sample a, b and c indicate replicates.

TABLE 12. Data from the BG-11 Modification Experiment

Sample Name[1]	pH [2] Initial Day 0	Final Day 20	OD Final	Arsenate Spike (μg/L) Initial	Final	Average Error
N-0.1BG-11_a	7.92	7.51	NA	100	100.5	
N-0.1BG-11_b	8.00	7.26	NA	100	108.6	105.3
N-0.1BG-11_c	7.91	7.17	NA	100	106.8	4.28
I-0.1BG-11_a	7.96	7.15	0.280	NA	NA	
I-0.1BG-11_b	8.04	9.27	0.483	NA	NA	NA
I-0.1BG-11_c	8.02	9.65	0.674	NA	NA	NA
N-0.01BG-11_a	7.97	7.22	NA	100	118.2	
N-0.01BG-11_b	8.07	7.29	NA	100	113.6	113.9
N-0.01BG-11_c	7.98	4.46	NA	100	109.6	4.28
I-0.01BG-11_a	7.96	7.37	0.200	NA	NA	
I-0.01BG-11_b	7.99	9.68	0.592	NA	NA	NA
I-0.01BG-11_c	7.95	9.91	0.677	NA	NA	NA
N-0.1-TMS,Fe_a	7.90	5.98	NA	100	99.7	
N-0.1-TMS,Fe_b	8.03	7.55	NA	100	99.2	98.40
N-0.1-TMS,Fe_c	8.04	7.53	NA	100	96.3	1.82
I-0.1-TMS,Fe_a	8.04	8.39	0.230	NA	NA	
I-0.1-TMS,Fe_b	7.98	9.76	0.543	NA	NA	NA
I-0.1-TMS,Fe_c	7.97	9.76	0.677	NA	NA	NA
N-0.01Fe_a	7.98	7.39	NA	100	95.5	
N-0.01Fe_b	7.90	7.68	NA	100	94.0	95.17
N-0.01Fe_c	7.96	7.45	NA	100	96.0	1.07
I-0.01Fe_a	8.01	9.21	0.518	NA	NA	
I-0.01Fe_b	8.02	9.75	0.685	NA	NA	NA
I-0.01Fe_c	8.05	9.91	0.762	NA	NA	NA

[1] Sample a, b and c indicate replicates.
[2] pH adjusted to 8±0.1 on days 6, 13 using 0.25 M NaOH and 0.1 M HNO_3

Arsenate Uptake and Sequestration

Cyanobacteria in arsenate-spiked solutions grew at a rate consistent with the arsenate-free cyanobacteria control (Fig. 15). The consistent growth allows reliable comparisons between the 3 arsenate spike conditions. Oxygen depletion caused the cell count to decline after time step 3. The vent caps were replaced with foam plugs to allow better transmission of oxygen and growth resumed.

Growth in the preliminary and uptake and sequestration experiments indicate arsenate toxicity does not affect the cyanobacterium as it does more complex photoautotrophs (Paivoke and Simola, 2001). Plant growth was stunted when subjected to 73.3 mg of sodium arsenate/kg dry weight while *Anabaena* sp. Strain PCC 7120 showed increased growth when cultured in the phosphate-deficient 74.9 mg arsenate/L BG-11 growth media in the preliminary uptake experiment. The increased growth is similar to a green alga isolated from an arsenic-contaminated lake (Knauer and Hemond, 2000). Knowing *Anabaena* sp. Strain PCC 7120 has no prior arsenate exposure, it is possible that other cyanobacteria have a developed a resistance to arsenate toxicity.

Arsenate uptake and sequestration is evident in the 1.0 μM arsenate experiment (Fig. 16). Approximately 10% of the total arsenate is sequestered in the cyanobacterial cells at the final time step (day 43). The percentage of arsenic varies in solution over the final 3 time steps (days 32, 37, 43). The mass of arsenic sequestered appears to reach an equilibrium after day 13 of the

experiment. Paerl (1988) notes persistent cyanobacteria bloom lasting up to 4 months which is enough time for arsenic sequestration to reach equilibrium.

Arsenate uptake and release by the cyanobacteria can explain the variation in arsenic concentration over the last time steps. In this scenario, arsenate enters the cell where several things may occur. Arsenate may be excreted back into solution. Cyanobacteria may biotransform the arsenate by reducing it to arsenite, similar to the green alga, *Chlorella* sp. (Knauer and Hemond, 2000). They may also methylate the arsenate, similar to another green alga, *Chlorella vulgaris* (Kuroiwa et al., 1994). At this point, the arsenic may be sequestered in the cell or released back into solution.

Arsenate uptake and sequestration is not evident in the 10 *μ*M arsenate experiment (Fig. 17). Uptake may be occurring similar to the rate in the 1.0 *μ*M arsenate experiment. If this is true, error in measurement would mask any possible uptake by cyanobacteria.

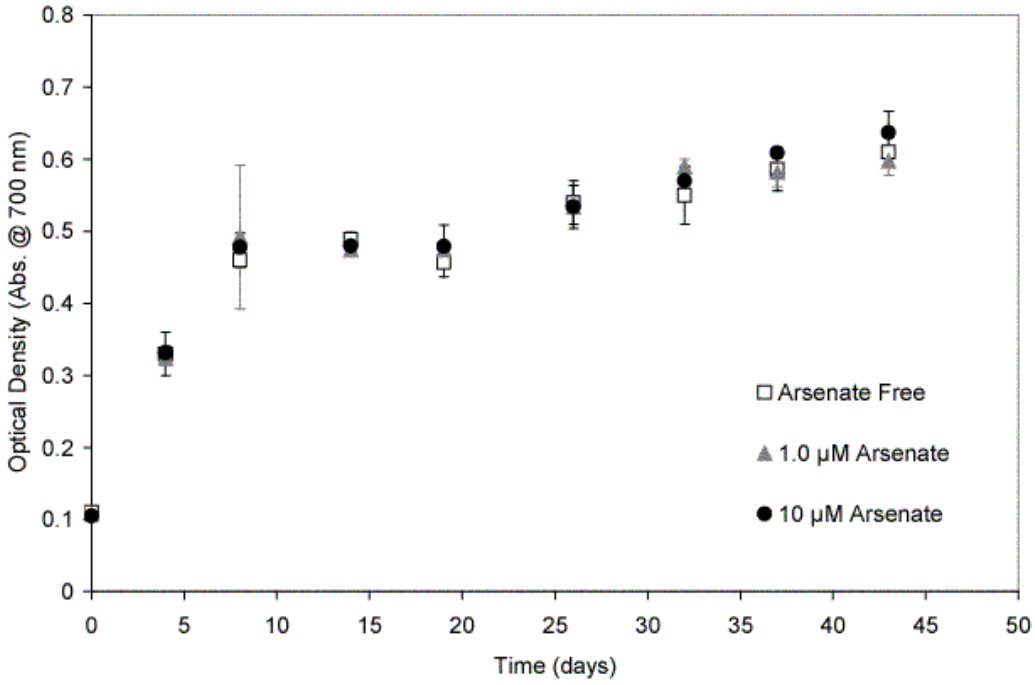


FIGURE 15. Cyanobacteria growth in the arsenate uptake experiment. Error is the standard deviation of triplicate samples. Error on Day 8 (1.0 *µ*M As) can be attributed to one mis-spiked sample.

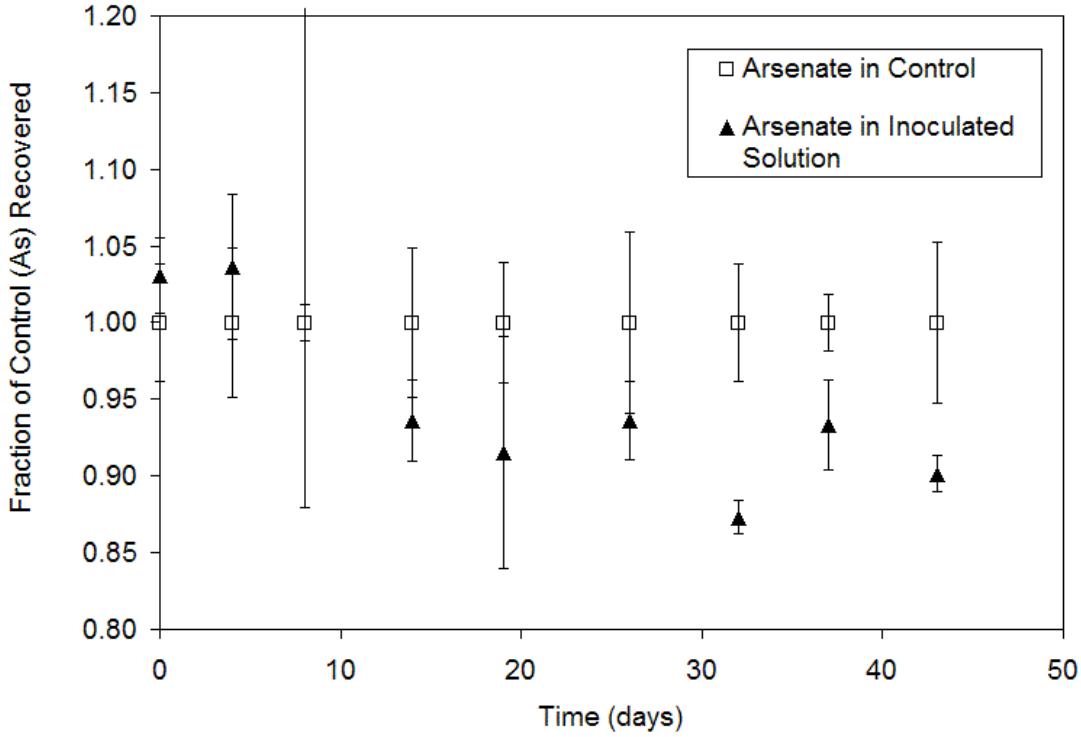


FIGURE 16. Arsenate uptake in the 1.0 *μ*M As solution. Error is the standard deviation of triplicate samples. Error on Day 8 (1.0 *μ*M As) can be attributed to one mis-spiked sample.

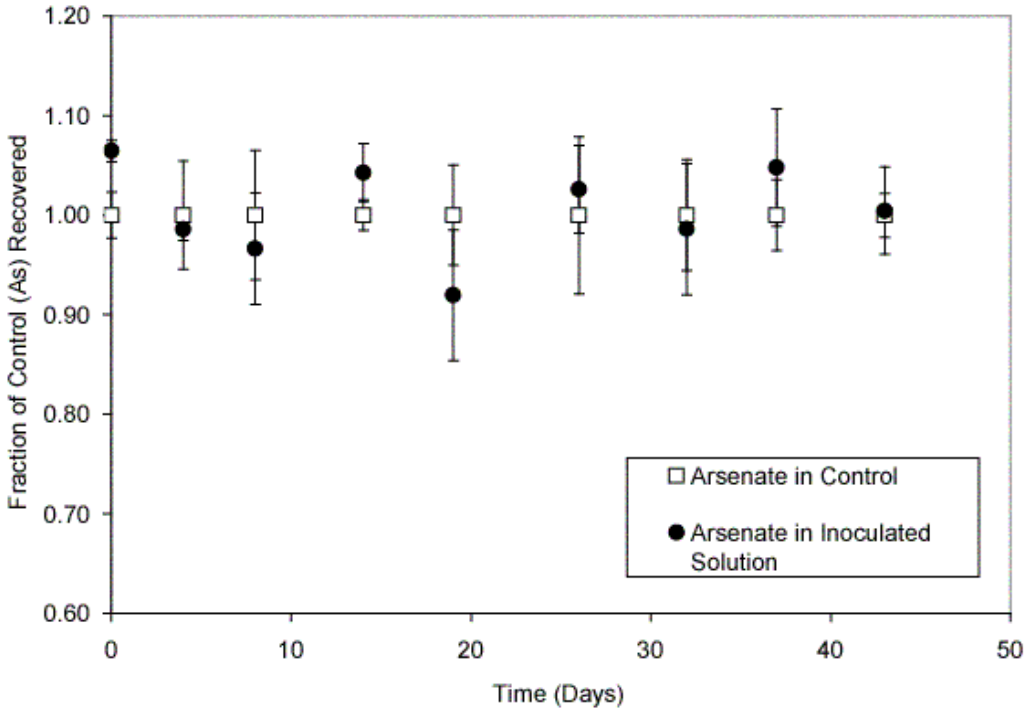


FIGURE 17. Arsenate uptake in the 10 *μ*M As solution. Error is the standard deviation of triplicate samples.

The experimental pH was 7.2 ±0.27. Under oxic conditions, $HAsO_4^{2-}$ is the dominant arsenic species. HPLC was used to determine arsenic speciation. The difference between the chromatograms theoretically illustrates the difference in arsenic speciation between the initial arsenate-spiked BG-11 with final arsenate spiked BG-11 (Fig. 18). Curve BG11AsH shows the initial arsenate peaks. Curve BG11cbacAsH shows the final, altered arsenic peaks. There is an obvious difference between the 2 chromatograms indicating the initial arsenate was altered by the cyanobacteria. The shifts of intensity and time of the peaks in the chromatogram may indicate the presence multiple arsenic species. The sporadic nature of the peaks makes it difficult to identify specific arsenic species (arsenate, arsenite, organoarsenicals).

Cellular Arsenic Partitioning

Data showing arsenic partitioning can be seen in Table 13. Most of the arsenic remained in solution. No arsenic was found in the EDTA rinse for both

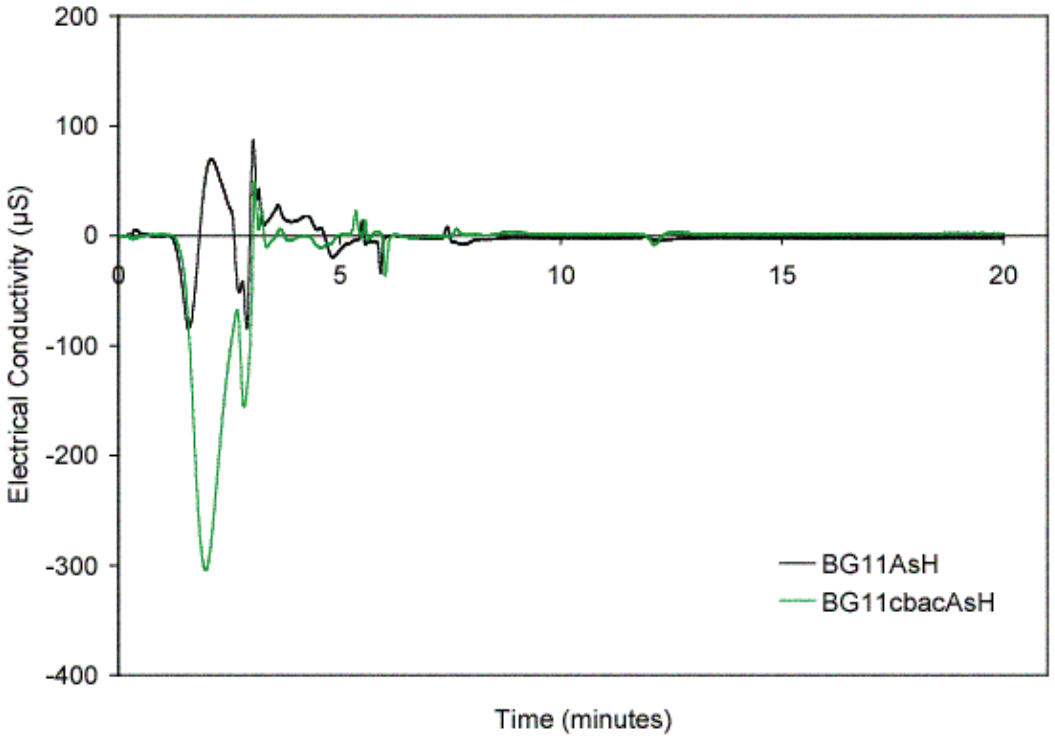


FIGURE 18. Arsenic speciation using HPLC. Each condition is the average of triplicates. Background subtractions theoretically illustrate differences in arsenic speciation as affected by cyanobacteria. The sporadic chromatogram makes species identification impractical.

the inoculated samples and the control solution. One sample (As-INOC_c) in the nitric acid solution showed significant arsenic in the cells. Samples As-INOC_a and As-INOC_b show low cellular arsenic due to incomplete transfer of the cells from the filter to the nitric acid. Residual EDTA solution on the filters of these samples enabled some sample leakage. Looking at As-INOC_c, approximately 5.2 % (21 ppb) of the 400 ppb arsenic solution is present in the cells.

The lack of arsenic in the EDTA rinse indicates no arsenic sorbed to the cell walls. Therefore, arsenate sorption to cells is not a viable arsenate sink as is arsenate sorption to iron oxyhydroxide minerals (Raven et al., 1998). The intracellular arsenic concentration is similar to that of a freshwater green alga, isolated from an arsenic-contaminated system and measured for arsenic content (Kuroiwa et al., 1994). The uptake and sequestration experiment shows equilibrium in 13 days. The green alga had a much greater arsenate exposure time, yet had intracellular arsenic the same order of magnitude as the cyanobacterium.

TABLE 13. Cellular Arsenic Partitioning Data

Sample[1]	Arsenic (μg)					Recovery
	Calculated	Measured				
		Solution	Sorbed	Intracellular	Total	
As_a	8	9.3	0.12	0	9.4	1.17
As_b	8	8.9	0	0	8.9	1.11
As_c	8	9.4	0	0	9.4	1.18
INOC-As_a	8	9.0	0	0.028	9.1	1.13
INOC-As_b	8	9.2	0	0	9.2	1.15
INOC-As_c	8	8.9	0	0.49	9.4	1.18

[1] Sample a, b and c indicate replicates.

Arsenate Reduction

Arsenate uptake and reduction was conducted using increasing N:P ratios. Fig. 19 shows the predicted speciation of arsenic in solution using the Eh and pH values measured from solution. For the Eh measurements, the Zobell reference solution was reading 27 mV low which is within the range of the symbol. The diagram predicts the arsenate species ($HAsO_4^{2-}$) in all solutions. Figure 20 shows uptake and speciation data. There is minimal net uptake throughout the experiment. This can be interpreted by applying the luxury uptake concept to the increasing N:P ratios. The uptake and sequestration experiment was conducted in a fixed-nitrogen deficient media. Though nitrogen limited cell growth, luxury uptake of phosphate continued. Arsenate uptake also continued,

possibly due to the physicochemical similarity between arsenate and phosphate (Campos, 2002).

Cyanobacteria growth is consistent within each treatment, though growth was directly related to the N:P ratios. Increased growth is attributed to increased N:P ratios rather than arsenate toxicity at low N:P ratios. *Anabaena variabilis* showed effects of arsenate toxicity (25 mM As, 50 mM As, 75 mM As) in a phosphate-deficient media (Theil, 1988). *Anabaena* sp. Strain PCC 7120 showed increased growth when exposed to 1.0 mM arsenate in phosphate-deficient media, though this concentration is much lower than the arsenate concentrations for *Anabaena variabilis*. However, both concentrations are high (3 orders of magnitude) when compared to surface water arsenic concentrations in South Texas making arsenate toxicity unlikely to affect inhabitant cyanobacteria.

The control solutions show minimal arsenite while the inoculated solutions showed complete arsenite, with error in the MidN:P-INOC solution attributed to a low sample (MidN:P-INOCa) reading. This indicates faster arsenate gross uptake rates than the uptake and sequestration experiment suggest. In these samples, arsenate reduction occurred faster than oxidation in solution, which allowed arsenite accumulation. Turpeinen et al. (2002) report arsenate biotransformation in acid soils with little accumulation of these products. This suggests the makeup of microbial community plays a major role in arsenic speciation. During phytoplankton blooms, the nuisance species can account for

up to 99% of the bloom's composition (Paerl, 1988). Therefore, arsenate biotransformation should still be a major arsenic control under *Anabaena* sp. bloom conditions.

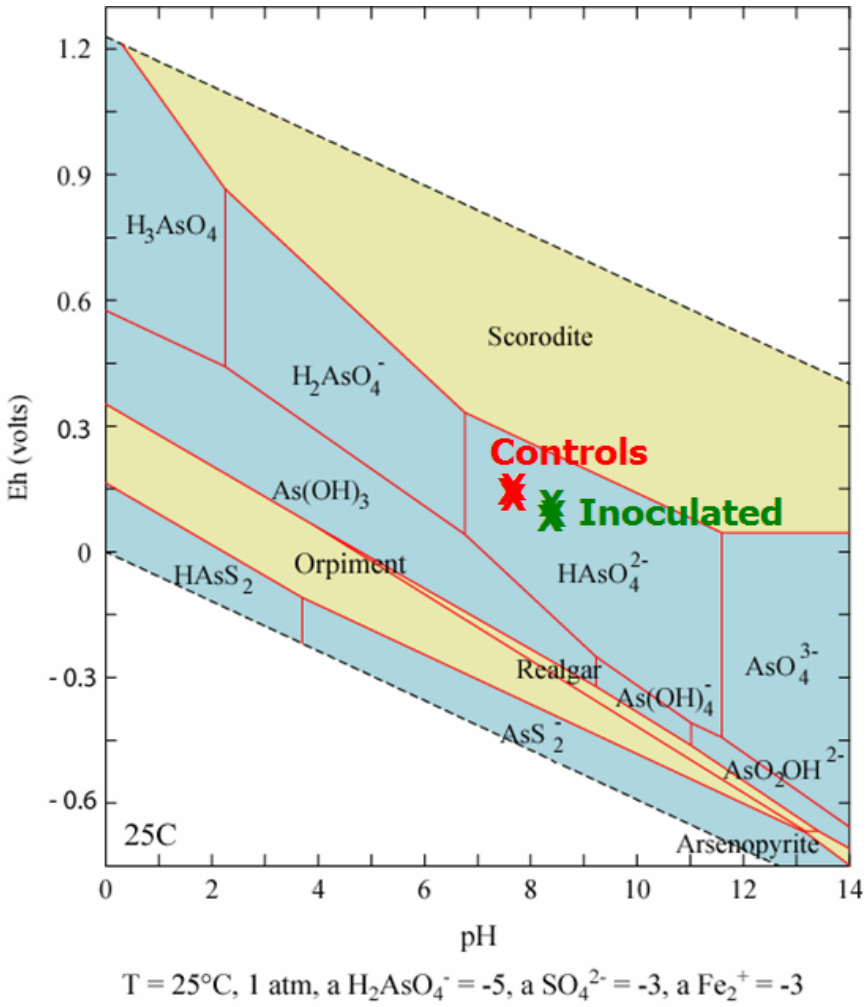


FIGURE 19. Predicted arsenic speciation. Added points show Eh and pH data collected in the N:P Ratio experiments. This system includes the influence of iron and sulfur. (Figure by Misun Kang).

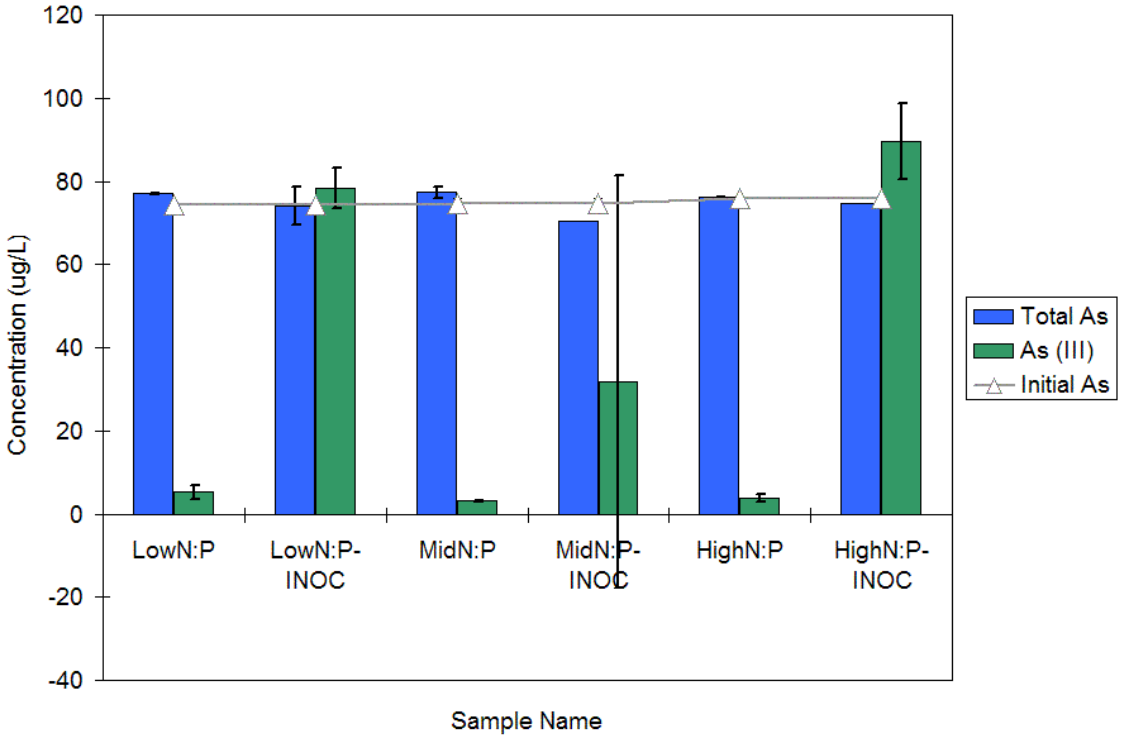


FIGURE 20. Arsenate sequestration and reduction. Initial As was measured in triplicate, Total As and As(III) were measured in duplicate. Error is the standard deviation of the replicats. Error in initial As is smaller than the symbols. Error in MidN:P-INOC is due to differing As(III) measurements (A= 89 ppb, B= 3.1 ppb).

Cyanobacteria Effect on Arsenate Bioavailability

Elevated pH and low N:P ratios favor development of *Anabaena* sp. blooms (Paerl, 1988). An N:P ratio of 85 is considered high, while a ratio of 5 is considered low (Sterner and Elser, 2002). Field data indicates elevated pH while

the USGS data shows average N:P ratios of 11.4 ±15 suggesting conditions favoring blooms.

Results from the arsenate experiments and the favorable conditions for *Anabaena* sp. bloom development highlight the potential increased impact cyanobacteria have on the fate and transport of arsenic in the environment. At high environmental arsenic concentrations (As ≥ 10 *μ*M), the role of cyanobacteria arsenate reduction as only a small percentage of arsenate would be sequestered. However, cyanobacterium reduction rates of arsenate at these higher concentrations is still undefined. The importance of cyanobacteria as an agent of sequestration increases as arsenic concentrations decrease. This is evident when comparing 10 *μ*M As uptake experiment with the 1.0 *μ*M As uptake experiment. A higher percentage of arsenate was sequestered in the 1.0 *μ*M As uptake experiment, a concentration which relates better to surface water concentrations in South Texas.

Sohrin et al. (1997) report an increase in dimethylarsonic acid in summer in a eutrophic lake, and arsenite increases in spring and fall. Iron and manganese concentrations were elevated in summer via anoxia caused reductive-dissolution. Evapoconcentration and reductive-dissolution of arsenic-enriched sediments increase the total arsenic concentration in the summer months. Cyanobacteria would have a greater influence in South Texas surface waters, given the low potential for sequestration by reactive iron (Raven et al., 1998). *Anabaena* sp. Strain PCC 7120 reaches an intracellular arsenic

equilibrium after approximately 13 days at which point accumulation ceases to be the main control on arsenate and reduction becomes increasingly important.

CHAPTER IV

IMPLICATIONS AND SUMMARY

The focus of this experiment analyzes the effect cyanobacteria have on arsenic fate and transport when geochemistry (Eh, pH, oxy-hydroxides) is not the main control. To achieve this, iron was limited in the BG-11 growth media without any adverse effect on cyanobacteria growth.

Cyanobacteria uptake, sequester and reduce arsenate in solution. The relative importance of arsenate uptake and sequestration increases as the arsenic concentration decreases. This realization is applicable when relating this experiment to the Nueces River and San Antonio River watersheds. This is a region deficient in non-silicate bound iron with elevated freshwater arsenic concentrations in the lower range (As < 1.0 μM). It is important to note that this cyanobacterium was not been previously exposed to arsenic yet arsenate uptake still occurred. This suggests uptake may be a process common to all cyanobacteria.

The cyanobacteria were shown to reduce arsenate. This is important for 2 reasons. Reducing arsenate increases mobility and potential toxicity (Jain and Ali, 2000). Also, the presence of arsenite in an oxic environment may be a biomarker of an organism's exposure to arsenic. Areas where cyanobacteria

blooms are present will likely have moderately reduced bioavailable arsenic due to sequestration, though arsenite concentrations may be elevated. *Anabaena* sp. Strain PCC 7120 reaches an intracellular arsenic equilibrium after approximately 13 days at which point accumulation ceases to be the main control on arsenate and reduction becomes increasingly important.

The peaks in the HPLC chromatogram indicate many arsenic species.The cyanobacteria may biotransform inorganic arsenic to organoarsenicals similar to other photosynthetic organisms (Koch et al., 1999). This is a much different scenario than simply reducing arsenate as organoarsenicals are much less toxic and less mobile than arsenite. Organoarsenicals are a more reliable biomarker of arsenic toxicity than arsenite in oxic waters.

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APPENDIX A

DITHIONITE-CITRATE-BICARBONATE IRON EXTRACTION DATA

A.1. Recorded Iron Data (GFAAS)

Sample ID	Conc (*μ*g/L)	RSD%	Absorbance		Readings		
			Mean	Background	One	Two	Three
FeCAL ZERO	0	18.5	0.0889	0.0081	0.1071	0.0843	0.0752
FeSTANDARD 1	20	7	0.154	0.0082	0.1587	0.1617	0.1417
FeSTANDARD 2	40	2.9	0.2949	0.0092	0.2983	0.2852	0.3012
FeSTANDARD 3	60	2.4	0.392	0.0095	0.4028	0.3889	0.3845
FeSTANDARD 4	80	1.5	0.491	0.0086	0.4977	0.483	0.4923
FeSTANDARD 5	100	1.1	0.5387	0.0087	0.5456	0.5341	0.5363
Feblank DDW	6.947	1.4	0.0535	-0.0022	0.0541	0.0526	0.0538
FeRC-281	15.159	4.5	0.1167	-0.0018	0.1206	0.1108	0.1189
FeLgC-534	15.328	3.5	0.118	-0.0036	0.1228	0.1163	0.115
FeNR-59	25.496	2.3	0.194	-0.0026	0.1953	0.1977	0.189
FeSpC-281	28.915	1.7	0.2183	-0.0029	0.2182	0.2222	0.2146
FeNR-281	7.369	6.7	0.0567	-0.0037	0.0611	0.0553	0.0538
FeSC-1358	11.569	6.1	0.0891	-0.0044	0.0954	0.0858	0.0861
Feblank DDW	-5.16	4.6	-0.0397	-0.0041	-0.0386	-0.0419	-0.0387
FeSC-2049ext	-0.451	>100	-0.0035	-0.0036	0.0008	-0.0073	-0.0039
FeAR-99	4.759	27	0.0367	-0.0039	0.0471	0.0274	0.0354
FeLCC-534	1.871	21.1	0.0144	-0.0042	0.0126	0.0179	0.0127
FeNR-151	7.563	11.6	0.0582	-0.004	0.0648	0.0514	0.0585
FeWC-233a	-0.346	78.9	-0.0027	-0.0041	-0.0011	-0.0018	-0.0051
FeLP-791	13.212	4.5	0.1017	-0.0032	0.1059	0.0968	0.1025
Feblank DDW	-5.636	6.5	-0.0434	-0.0045	-0.0404	-0.0437	-0.0461
Fe60 ppb	63.28	0.5	0.4076	0.0003	0.4098	0.4058	0.4072
FeEcC-627	15.293	4.2	0.1178	-0.0035	0.1216	0.1194	0.1123
FeSA-80	13.577	6.5	0.1046	-0.0043	0.1123	0.1014	0.1
FeSA-72	19.064	6.7	0.1468	-0.0032	0.1579	0.1389	0.1437
FeEC-181	3.205	9	0.0247	-0.0045	0.0265	0.0253	0.0222
FeSA-123	14.474	5.2	0.1115	-0.0029	0.1178	0.1103	0.1063
FeCBC-887	30.942	2.1	0.2326	-0.003	0.2379	0.232	0.228
Feblank DDW	-2.218	>100	-0.0171	-0.0034	-0.0417	-0.0362	0.0266
FeWC-233b	-2.235	6.6	-0.0172	-0.004	-0.0185	-0.0168	-0.0163
FeWater blank	-4.848	4.7	-0.0373	-0.0038	-0.0359	-0.0393	-0.0368

A.2. Calculation for determining iron in South Texas sediment

Sample ID	Conc. (μg/L)	Extract Dilution	Extract (μg Fe/L)	Iron (μg Fe/27.5 mL)	Sediment (g)	Iron (mg Fe/ kg sed)
Feblank DDW	6.947	na				
FeRC-281	15.159	0.00005	303180	8337.5	2.504	3333.2
FeLgC-534	15.328	0.00005	306560	8430.4	2.5035	3369.2
FeNR-59	25.496	0.00005	509920	14023.	2.5037	5608.20000
FeSpC-281	28.915	0.00005	578300	15903	2.5037	6363.1
FeNR-281	7.369	0.00005	147400	4053	2.5043	1622
FeSC-1358	11.569	0.00005	231380	6362.9	2.5037	2545.9
Feblank DDW	-5.16	na				
FeSC-2049ext	-0.451	0.00005	-9020	-248	2.5046	-99.2
FeAR-99	4.759	0.00005	95180	2617	2.5043	1047
FeLCC-534	1.871	0.00005	37420	1029	2.5042	411.7
FeNR-151	7.563	0.00005	151260	4160	2.504	1664
FeWC-233a	-0.346	0.00005	-6920	-190	2.5032	-76.2
FeLP-791	13.212	0.00005	264240	7266.6	2.5039	2906.2
Feblank DDW	-5.636	na				
Fe60 ppb	63.28	na				
FeEcC-627	15.293	0.00005	305860	8411.2	2.5033	3366.2
FeSA-80	13.577	0.00005	271540	7467.4	2.5039	2987.9
FeSA-72	19.064	0.00005	381280	10485	2.5036	4192.1
FeEC-181	3.205	0.00005	64100	1763	2.5041	704.9
FeSA-123	14.474	0.00005	289480	7960.7	2.504	3183.6
FeCBC-887	30.942	0.00005	618840	17018	2.5041	6807.8
Feblank DDW	-2.218	na				
FeWC-233b	-2.235	0.00005	-44700	-1229	2.5032	-491.9
FeWater blank	-4.848	na				

APPENDIX B

ARSENATE UPTAKE EXPERIMENT: SUMMARY DATA

B.1. Data for BG-11

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0	0	7.04	0.06	0.05	0
4	0	0	7.01	0.03	0.05	0
8	0	0	7.04	0.02	0.05	0
14	0	0	7.55	0.34	0.052	0
19	0	0	7.14	0.04	0.041	0
26	0	0	7.11	0.04	0.04	0
32	0	0	7.26	0.05	0.04	0
37	0	0	7.00	0.30	0.04	0
43	0	0	7.18	0.10	0.04	0

B.2. Data for INOC

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0	0	7.16	0.04	0.11	0
4	0	0	8.09	0.02	0.33	0.03
8	0	0	7.37	0.06	0.46	0.01
14	0	0	7.34	0.1	0.489	0.01
19	0	0	7.45	0.07	0.457	0.02
26	0	0	7.36	0.16	0.54	0.03
32	0	0	7.36	0.09	0.55	0.04
37	0	0	6.52	0.02	0.586	0.03
43	0	0	7.36	0.11	0.61	0

B.3. Data for AsL

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0.781	0.03	7.09	0.04	0.051	0
4	0.82	0.04	7.02	0.05	0.051	0
8	0.817	0.01	7.07	0.01	0.051	0
14	0.611	0.03	7.1	0.05	0.051	0
19	1.014	0.04	7.05	0.03	0.041	0
26	0.847	0.05	7.03	0.01	0.041	0
32	1.054	0.04	7.28	0.07	0.041	0
37	1.093	0.02	6.58	0.02	0.04	0
43	0.954	0.05	7.12	0.03	0.039	0

B.4. Data for INOC AsL

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0.805	0.02	7.18	0.04	0.107	0
4	0.85	0.04	8.12	0.02	0.324	0.01
8	1.01	0.36	7.29	0.04	0.492	0.1
14	0.572	0.015	7.14	0.15	0.475	0
19	0.928	0.07	7.57	0.08	0.475	0
26	0.793	0.02	7.46	0.04	0.534	0.01
32	0.92	0.01	7.37	0	0.59	0.01
37	1.02	0.03	6.53	0.05	0.582	0.02
43	0.86	0.01	7.37	0.04	0.598	0.02

B.5. Data for AsH

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0.868	0.02	7.02	0.02	0.052	0
4	0.917	0.05	7.02	0.07	0.05	0
8	0.922	0.06	7.14	0.03	0.051	0
14	0.656	0.01	7.06	0.05	0.052	0
19	0.995	0.05	7.09	0.02	0.04	0
26	0.887	0.07	7.03	0.04	0.04	0
32	1.078	0.06	7.1	0.08	0.04	0
37	1.128	0.04	6.69	0.11	0.039	0
43	0.905	0.02	7.16	0.04	0.04	0

B.6. Data for INOCAsH

Time (Day)	Arsenic (μM)	Error	pH	Error	OD (Abs. @ 700 nm)	Error
0	0.924	0.01	7.08	0.05	0.105	0
4	0.904	0.01	8.12	0.05	0.331	0.01
8	0.891	0.05	7.42	0.07	0.478	0.02
14	0.684	0.02	7.21	0.06	0.48	0.01
19	0.915	0.06	7.61	0.03	0.479	0.03
26	0.91	0.04	7.45	0.1	0.534	0.03
32	1.063	0.07	7.32	0.08	0.57	0.02
37	1.182	0.07	6.65	0.05	0.609	0
43	0.909	0.04	7.44	0.06	0.637	0.03

APPENDIX C

ARSENATE UPTAKE EXPERIMENT: AAS DATA

Arsenate Uptake and Sequestration

C.1. Time 0

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	32.3	0.0027	0.0867	0.0035	0.0028	0.0018
AsSTANDARD 1	25	4.3	0.1482	0.0876	0.1535	0.15	0.1412
AsSTANDARD 2	50	1.9	0.2834	0.0889	0.2783	0.2829	0.289
AsSTANDARD 3	75	2	0.4424	0.0898	0.4441	0.4502	0.4329
AsSTANDARD 4	100	3.1	0.5497	0.0917	0.5298	0.5612	0.558
Asblank-BG11	-0.482	93.5	-0.0029	0.0917	-0.001	-0.0017	-0.0059
As1a0	1.32	21.3	0.0078	0.0789	0.0089	0.0087	0.0059
As1b0	-1.078	17.7	-0.0064	0.0774	-0.0059	-0.0077	-0.0056
As1c0	-1.121	24.3	-0.0066	0.0796	-0.0049	-0.0081	-0.0069
As2a0	-1.254	10.3	-0.0074	0.0811	-0.0073	-0.0068	-0.0083
As2b0	-1.509	11.1	-0.0089	0.0786	-0.0099	-0.0091	-0.0079
As2c0	-1.344	5.2	-0.008	0.0804	-0.008	-0.0084	-0.0075
As3a0	56.167	2.7	0.3215	0.0864	0.3117	0.3271	0.3256
As3b0	60.777	2.2	0.3504	0.0857	0.344	0.348	0.3591
As3c0	58.604	1.7	0.3367	0.0894	0.3311	0.3367	0.3424
As100 ppb	96.018	1.5	0.5354	0.0873	0.5331	0.5443	0.5289
Asblank-BG11	-0.629	44.3	-0.0037	0.084	-0.004	-0.0053	-0.002
As4a0	61.16	2.1	0.3528	0.0864	0.3445	0.3552	0.3586
As4b0	61.119	5.1	0.3525	0.0811	0.3326	0.3576	0.3675
As4c0	58.676	1.2	0.3371	0.0921	0.3394	0.3323	0.3397
As5a0	67.008	5	0.3902	0.0998	0.3798	0.4126	0.378
As5b0	64.607	4.8	0.3747	0.0903	0.3705	0.3593	0.3944
As5c0	63.389	5	0.367	0.0909	0.3535	0.3594	0.388
As6a0	68.144	0.9	0.3975	0.0955	0.3987	0.3935	0.4003
As6b0	70.303	1.9	0.4115	0.0954	0.4138	0.4029	0.418
As6c0	69.114	1.5	0.4038	0.0907	0.3967	0.4067	0.408
As100 ppb	102.67	1.2	0.5603	0.0893	0.5669	0.5604	0.5536
Asblank-BG-11	-0.327	55.5	-0.0019	0.0917	-0.0019	-0.003	-0.0009
As3a0-UF	35.077	1.6	0.2042	0.0904	0.2058	0.2005	0.2063

C.2. Time 1

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	7.3	0.008	0.1017	0.0074	0.008	0.0085
AsSTANDARD 1	25	2.9	0.1177	0.0904	0.1159	0.1155	0.1216
AsSTANDARD 2	50	1.1	0.2556	0.0896	0.257	0.2524	0.2572
AsSTANDARD 3	75	4.6	0.3844	0.0947	0.4043	0.3784	0.3704
AsSTANDARD 4	100	1.5	0.4843	0.091	0.4783	0.482	0.4926
Asblank-BG11	0.458	38.9	0.0022	0.0935	0.0024	0.0028	0.0012
As1a1	0.118	>100	0.0006	0.0854	0.0027	-0.0009	-0.0002
As1b1	0.242	55.5	0.0011	0.085	0.0016	0.0004	0.0014
As1c1	0.049	>100	0.0002	0.0845	0.001	0.0001	-0.0004
As2a1	0.056	>100	0.0003	0.0822	0.0016	0.0006	-0.0015
As2b1	-0.343	19.5	-0.0016	0.0859	-0.0014	-0.0015	-0.002
As2c1	-0.588	7.7	-0.0028	0.082	-0.0026	-0.003	-0.0027
As3a1	61.864	2	0.3166	0.0879	0.3092	0.3209	0.3196
As3b1	64.022	0.9	0.3277	0.0863	0.3248	0.3277	0.3306
As3c1	58.474	1.2	0.2991	0.0853	0.2951	0.3001	0.3022
As100 ppb	93.587	2.5	0.4614	0.0982	0.4603	0.4735	0.4503
Asblank-BG11	0.738	58.7	0.0035	0.0896	0.0012	0.004	0.0052
As4a1	59.873	0.1	0.3063	0.0877	0.306	0.3067	0.3064
As4b1	64.644	0.7	0.3309	0.0894	0.3322	0.3283	0.3322
As4c1	65.793	0.6	0.3368	0.0891	0.3356	0.3391	0.3358
As5a1	65.628	1.1	0.336	0.1084	0.3365	0.3394	0.3321
As5b1	67.88	1.5	0.3476	0.098	0.3535	0.3442	0.345
As5c1	72.441	0.5	0.3711	0.0996	0.3714	0.3728	0.3692
As6a1	68.4	3.6	0.3503	0.0947	0.3361	0.3549	0.3598
As6b1	67.215	3	0.3442	0.0948	0.3343	0.3434	0.3548
As6c1	67.45	4.1	0.3454	0.0978	0.3292	0.3507	0.3563
As100 ppb	94.265	1.3	0.4639	0.092	0.4574	0.4694	0.4649
Asblank-BG-11	1.35	37	0.0064	0.0925	0.0073	0.0081	0.0037

C.3. Time 2

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	13.1	0.0096	0.0844	0.0088	0.011	0.0089
AsSTANDARD 1	25	4	0.1247	0.0874	0.1275	0.1189	0.1277
AsSTANDARD 2	50	4.9	0.2689	0.0869	0.2558	0.2686	0.2824
AsSTANDARD 3	75	2.7	0.3793	0.0864	0.3706	0.3904	0.3767
AsSTANDARD 4	100	2.2	0.5143	0.0864	0.5025	0.5249	0.5154
Asblank-BG11	0.422	38.7	0.0021	0.086	0.002	0.003	0.0014
As1a2	0.121	>100	0.0006	0.0889	0.0016	-0.0018	0.002
As1b2	-0.232	>100	-0.0012	0.0868	0	-0.0027	-0.0008
As1c2	-0.674	47	-0.0034	0.0891	-0.0035	-0.0049	-0.0017
As2a2	-0.162	92.9	-0.0008	0.086	-0.0015	0	-0.001
As2b2	-0.571	67.9	-0.0028	0.0869	-0.0035	-0.0043	-0.0007
As2c2	-0.447	>100	-0.0022	0.0871	-0.0035	-0.0049	0.0016
As3a2	61.496	1.5	0.3249	0.0904	0.3203	0.3244	0.3299
As3b2	60.637	4	0.321	0.092	0.3161	0.3115	0.3354
As3c2	61.358	2.2	0.3242	0.0927	0.3171	0.3313	0.3243
As100 ppb	96.765	2.9	0.4966	0.0841	0.506	0.4798	0.5039
Asblank-BG11	0.325	51.1	0.0016	0.0819	0.0021	0.0007	0.0021
As4b2	59.277	3.5	0.3148	0.0892	0.3023	0.3189	0.3232
As4c2	60.515	4.8	0.3204	0.0946	0.3059	0.3365	0.3189
As5a2	64.592	3	0.3384	0.0858	0.3358	0.3296	0.3497
As5b2	74.06	5.2	0.3758	0.0862	0.3565	0.3756	0.3954
As5c2	68.451	2.7	0.3543	0.0862	0.3509	0.3468	0.3653
As6a2	63.519	4.7	0.3338	0.0852	0.3158	0.3431	0.3423
As6b2	65.612	3.4	0.3427	0.0853	0.3411	0.332	0.3549
As6c2	71.071	4.5	0.3646	0.0864	0.3472	0.3669	0.3797
Asblank-BG-11	-0.033	>100	-0.0002	0.0816	0.0028	0.001	-0.0043
As4a2	106.57	4	0.5505	0.1098	0.5285	0.551	0.5721
As100 ppb	94.484	1.7	0.4841	0.0986	0.476	0.4839	0.4924

C.4. Time 3

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	8.5	0.0175	0.0896	0.0192	0.0167	0.0166
AsSTANDARD 1	25	5.2	0.1648	0.0858	0.1563	0.1644	0.1735
AsSTANDARD 2	50	6.1	0.2973	0.088	0.2827	0.2918	0.3175
AsSTANDARD 3	75	3.2	0.4841	0.0888	0.4889	0.4964	0.467
AsSTANDARD 4	100	3.1	0.6085	0.0893	0.6171	0.587	0.6214
Asblank-BG11	-0.284	70.9	-0.0019	0.0871	-0.0034	-0.0011	-0.0011
As1a3	-0.496	63.4	-0.0033	0.0941	-0.0013	-0.0031	-0.0054
As1b3	-0.897	17.5	-0.0059	0.0973	-0.0065	-0.0065	-0.0047
As1c3	-1.117	2.1	-0.0074	0.0937	-0.0074	-0.0072	-0.0075
As2a3	-1.077	7.5	-0.0071	0.0897	-0.0073	-0.0075	-0.0065
As2b3	-1.552	7.3	-0.0102	0.091	-0.0094	-0.0104	-0.0109
As2c3	-1.543	6.9	-0.0102	0.093	-0.0105	-0.0106	-0.0094
As3a3	45.415	0.7	0.275	0.0971	0.2756	0.2767	0.2727
As3b3	47.979	0.9	0.2876	0.0988	0.2906	0.2852	0.2869
As3c3	43.986	1	0.2678	0.0982	0.269	0.2696	0.2649
As100 ppb	86.708	0.3	0.5491	0.0846	0.5507	0.5489	0.5476
Asblank-BG11	-0.042	>100	-0.0003	0.0802	-0.0003	0.0009	-0.0014
As4a3	42.39	8.3	0.2598	0.0905	0.2496	0.2451	0.2847
As4b3	42.015	4.2	0.2579	0.0899	0.2453	0.2632	0.2651
As4c3	44.167	1.6	0.2688	0.0898	0.2692	0.2642	0.2728
As5a3	49.194	2.9	0.2934	0.0856	0.3031	0.2873	0.2899
As5b3	49.921	1.4	0.2969	0.0833	0.2939	0.3017	0.2952
As5c3	48.282	1.3	0.289	0.081	0.2903	0.2919	0.2849
As6a3	49.77	2.6	0.2962	0.0803	0.2898	0.294	0.3048
As6b3	52.726	1.3	0.3162	0.0863	0.3194	0.3115	0.3178
As6c3	51.107	2.6	0.305	0.0823	0.307	0.2962	0.3117
As100 ppb	88.117	1.7	0.556	0.087	0.5472	0.5552	0.5657
Asblank-BG-11	-1.023	34.9	-0.0067	0.0781	-0.0041	-0.0078	-0.0084

C.5. Time 4

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	4.7	0.0202	0.0743	0.0208	0.0191	0.0206
AsSTANDARD 1	25	7.4	0.0933	0.0774	0.1008	0.0919	0.0873
AsSTANDARD 2	50	15.5	0.2095	0.0628	0.2449	0.2025	0.181
AsSTANDARD 3	75	2.2	0.3009	0.0819	0.3058	0.2935	0.3033
AsSTANDARD 4	100	1.5	0.3921	0.0829	0.3976	0.3857	0.3932
Asblank-BG11	-0.655	58.8	-0.0024	0.0788	-0.0036	-0.0029	-0.0008
As1a4	0.423	>100	0.0016	0.0855	-0.0004	0.0085	-0.0034
As1b4	-0.135	>100	-0.0005	0.0833	-0.0063	0.0004	0.0044
As1c4	0.077	>100	0.0003	0.0816	-0.0006	-0.0026	0.0041
As2a4	-1.695	31.3	-0.0063	0.0884	-0.0067	-0.0042	-0.0081
As2b4	-1.164	20	-0.0043	0.0847	-0.0048	-0.0048	-0.0033
As2c4	-1.493	65.7	-0.0056	0.0866	-0.0014	-0.0072	-0.0081
As3a4	79.1	2.9	0.3157	0.0877	0.3259	0.3085	0.3128
As3b4	74.791	0.7	0.3001	0.0865	0.3007	0.2979	0.3018
As3c4	74.032	1.5	0.2975	0.0887	0.2953	0.2944	0.3027
As100 ppb	96.983	2.1	0.3809	0.0815	0.3725	0.3816	0.3886
Asblank-BG11	-1.387	50.6	-0.0052	0.0784	-0.0023	-0.0059	-0.0074
As4a4	63.819	5.4	0.261	0.0929	0.2479	0.2757	0.2593
As4b4	69.729	5.4	0.2822	0.0903	0.2649	0.2942	0.2877
As4c4	74.918	0.5	0.3006	0.089	0.3	0.2995	0.3023
As5a4	76.726	2	0.3071	0.079	0.3084	0.3003	0.3127
As5b4	70.617	4.8	0.2854	0.0803	0.2743	0.281	0.3009
As5c4	76.206	0.6	0.3052	0.0795	0.3061	0.3065	0.303
As6a4	63.771	3.1	0.2608	0.0786	0.2545	0.2698	0.2581
As6b4	73.495	1.6	0.2956	0.0812	0.2906	0.2999	0.2962
As6c4	68.228	2.9	0.2769	0.0797	0.2687	0.2772	0.2848
As100 ppb	90.781	2.4	0.3581	0.0826	0.368	0.3547	0.3517
Asblank-BG-11	-2.75	25	-0.0103	0.0787	-0.0129	-0.0078	-0.0102

C.6. Time 5

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	39.7	0.0126	0.0623	0.0183	0.0099	0.0095
AsSTANDARD 1	25	6.9	0.1961	0.0601	0.2114	0.1859	0.1911
AsSTANDARD 2	50	0.6	0.3882	0.0607	0.3904	0.388	0.3862
AsSTANDARD 3	75	6	0.5563	0.0612	0.5291	0.5938	0.5461
AsSTANDARD 4	100	2	0.7177	0.0606	0.7257	0.7258	0.7015
Asblank-BG11	-0.732	33.3	-0.0057	0.0588	-0.0043	-0.0079	-0.005
As1a5	-0.729	>100	-0.0057	0.0616	0.0019	-0.0073	-0.0118
As1b5	-1.294	42.9	-0.0101	0.0631	-0.0057	-0.0103	-0.0144
As1c5	-1.342	15.4	-0.0105	0.0621	-0.0119	-0.0088	-0.0109
As2a5	-1.135	52.4	-0.0089	0.0629	-0.0099	-0.013	-0.0038
As2b5	-0.85	53.2	-0.0067	0.0644	-0.0028	-0.0073	-0.0098
As2c5	-1.747	19	-0.0137	0.0648	-0.0132	-0.0114	-0.0165
As3a5	59.472	2.9	0.4554	0.0652	0.455	0.4687	0.4424
As3b5	65.496	3.3	0.4959	0.0687	0.4789	0.4972	0.5117
As3c5	65.427	0.9	0.4955	0.068	0.4989	0.497	0.4905
As100 ppb	91.768	0.9	0.6649	0.0637	0.6648	0.6708	0.6593
Asblank-BG11	-1.694	4.9	-0.0133	0.0609	-0.013	-0.0129	-0.014
As4a5	57.958	2.9	0.4449	0.0748	0.432	0.458	0.4446
As4b5	59.361	2.4	0.4546	0.0756	0.4552	0.4652	0.4434
As4c5	60.937	1.6	0.4654	0.0768	0.4576	0.4725	0.4661
As5a5	68.001	1.2	0.5123	0.0645	0.5073	0.5103	0.5193
As5b5	70.405	1.9	0.5277	0.0664	0.5301	0.5363	0.5167
As5c5	66.284	2.9	0.5011	0.0652	0.5177	0.4943	0.4914
As6a5	65.553	1.2	0.4963	0.0679	0.4892	0.4999	0.4998
As6b5	71.639	1	0.5355	0.0671	0.5378	0.5292	0.5395
As6c5	67.314	0.7	0.5078	0.0671	0.5039	0.5096	0.5099
As100 ppb	84.527	0.6	0.6183	0.0665	0.6192	0.6144	0.6213
Asblank-BG-11	-2.433	13.5	-0.0191	0.0661	-0.0167	-0.0187	-0.0218

C.7. Time 6

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	11.5	0.0072	0.0781	0.0066	0.0069	0.0082
AsSTANDARD 1	25	11.9	0.1428	0.0728	0.1236	0.1487	0.156
AsSTANDARD 2	50	2.3	0.3216	0.0714	0.3259	0.3132	0.3257
AsSTANDARD 3	75	2.2	0.4698	0.0682	0.4648	0.4627	0.4819
AsSTANDARD 4	100	2.6	0.6122	0.0667	0.5999	0.6062	0.6304
Asblank-BG11	0.584	39.7	0.0033	0.0645	0.0045	0.0035	0.0019
As1a6	0.21	75.8	0.0012	0.0768	0.0005	0.0009	0.0022
As1b6	0.603	34.8	0.0034	0.0762	0.0036	0.0022	0.0046
As1c6	0.355	58.6	0.002	0.0796	0.0031	0.0007	0.0023
As2a6	0.554	10.7	0.0032	0.0797	0.0029	0.0031	0.0035
As2b6	0.305	48.3	0.0017	0.0783	0.002	0.0008	0.0024
As2c6	0.241	44.8	0.0014	0.0829	0.0007	0.0018	0.0017
As3a6	76.015	2.8	0.4757	0.0877	0.4655	0.491	0.4705
As3b6	81.325	3.4	0.5063	0.08	0.5258	0.499	0.494
As3c6	79.479	1.2	0.4957	0.0783	0.5024	0.4901	0.4945
As100 ppb	95.466	2.8	0.5867	0.0652	0.5734	0.6054	0.5812
Asblank-BG11	0.846	20	0.0048	0.0646	0.0059	0.0044	0.0042
As4a6	68.475	1.5	0.4319	0.0842	0.4292	0.4272	0.4392
As4b6	68.689	1.4	0.4331	0.0899	0.431	0.4282	0.4401
As4c6	69.377	0.5	0.4371	0.0872	0.4347	0.4389	0.4378
As5a6	80.534	1.2	0.5017	0.0656	0.4952	0.5065	0.5035
As5b6	76.146	1.1	0.4764	0.0675	0.4721	0.4823	0.4749
As5c6	85.467	0.8	0.53	0.0681	0.5267	0.5283	0.535
As6c6	73.392	3.9	0.4605	0.0681	0.446	0.4548	0.4806
Asblank-BG-11	0.751	33.9	0.0043	0.065	0.0047	0.0055	0.0027
As100 ppb	88.919	2.1	0.5497	0.0771	0.5486	0.5388	0.5616
As6a6	82.546	3	0.5133	0.08	0.4953	0.5209	0.5236
As6b6	83.027	0.1	0.516	0.0788	0.5155	0.5157	0.517

C.8. Time 7

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	15.4	0.0119	0.0755	0.0139	0.0103	0.0114
AsSTANDARD 1	25	6.5	0.1251	0.0754	0.1335	0.1246	0.1172
AsSTANDARD 2	50	1	0.2606	0.0741	0.2581	0.2604	0.2633
AsSTANDARD 3	75	1.4	0.3976	0.0735	0.3952	0.4041	0.3937
AsSTANDARD 4	100	5.8	0.5151	0.0728	0.5383	0.4813	0.5259
Asblank-BG11	0.557	21.7	0.0028	0.0708	0.0034	0.0028	0.0022
As1a7	-0.088	>100	-0.0004	0.0825	-0.005	-0.0005	0.0042
As1b7	-0.289	91.2	-0.0014	0.0846	-0.0027	-0.0001	-0.0016
As1c7	0.193	>100	0.001	0.0786	-0.001	0.0062	-0.0023
As2a7	-0.322	>100	-0.0016	0.089	-0.0055	-0.0055	0.0061
As2b7	-0.058	>100	-0.0003	0.0778	-0.0039	0.0019	0.0012
As2c7	0.255	>100	0.0013	0.0782	0.001	0.0053	-0.0025
As3a7	83.916	3.5	0.4422	0.0822	0.4351	0.4314	0.4601
As3b7	80.448	0.4	0.4252	0.0849	0.4253	0.4233	0.4269
As3c7	81.327	0.9	0.4296	0.0878	0.4279	0.434	0.4268
As100 ppb	90.282	1.2	0.4722	0.0733	0.4687	0.4694	0.4786
Asblank-BG11	-0.062	>100	-0.0003	0.0689	-0.0005	-0.0027	0.0023
As4a7	74.674	4.6	0.3959	0.0821	0.3747	0.4081	0.4048
As4b7	75.24	1	0.3989	0.0818	0.3944	0.4012	0.4011
As4c7	78.73	1.7	0.4166	0.083	0.4202	0.4211	0.4086
As5a7	81.473	1.9	0.4303	0.0757	0.4312	0.4217	0.438
As5b7	84.245	3.3	0.4438	0.0724	0.4345	0.436	0.4609
As5c7	87.791	1.5	0.4607	0.0744	0.4538	0.4604	0.4679
As6a7	87.432	3.4	0.459	0.0733	0.4544	0.4764	0.4462
As6b7	83.854	2.4	0.4419	0.0752	0.4428	0.4518	0.4311
As6c7	94.522	0.9	0.4914	0.076	0.4865	0.4922	0.4954
As100 ppb	86.615	2.7	0.4551	0.0734	0.4409	0.4604	0.4641
Asblank-BG-11	0.359	78.8	0.0018	0.0726	0.0024	0.0002	0.0028

C.9. Time 8

Sample ID	Conc (μg/L)	% RSD	Mean Abs	BG Abs	Readings 1	2	3
AsCAL ZERO	0	15.2	0.0239	0.0783	0.0245	0.0272	0.0201
AsSTANDARD 1	25	1.7	0.1525	0.0785	0.1554	0.1502	0.1519
AsSTANDARD 2	50	4.5	0.2825	0.0766	0.2772	0.2733	0.2971
AsSTANDARD 3	75	1	0.411	0.0748	0.4078	0.4098	0.4154
AsSTANDARD 4	100	2.3	0.5136	0.0743	0.5187	0.5217	0.5002
Asblank-BG11	1.521	>100	0.0093	0.0736	0.0227	0.0059	-0.0007
As1a8	0.769	>100	0.0047	0.0894	0.0044	-0.0011	0.0108
As1b8	1.256	68.5	0.0077	0.0899	0.0053	0.0137	0.004
As1c8	0.638	>100	0.0039	0.0899	0.0094	0.0031	-0.0008
As2a8	0.39	41.1	0.0024	0.0931	0.0016	0.0035	0.0021
As2b8	0.334	>100	0.002	0.0904	0.0029	-0.0092	0.0124
As2c8	-0.13	>100	-0.0008	0.0912	-0.0013	0.0043	-0.0053
As3a8	75.896	3.5	0.4151	0.0919	0.4021	0.4126	0.4307
As3b8	68.558	1	0.377	0.0906	0.3792	0.3726	0.3792
As3c8	70.01	4	0.3846	0.0972	0.3677	0.388	0.3979
As100 ppb	82.531	2	0.4446	0.0693	0.4434	0.4541	0.4365
Asblank-BG11	-0.927	79	-0.0057	0.0686	-0.0032	-0.003	-0.0108
As4a8	63.795	3.7	0.3524	0.1016	0.3391	0.3654	0.3527
As4b8	64.95	3.3	0.3583	0.0986	0.3715	0.3552	0.3483
As4c8	64.497	2	0.356	0.1052	0.3635	0.355	0.3495
As5a8	67.783	3.4	0.373	0.0716	0.3586	0.3784	0.3819
As5b8	69.296	2.2	0.3808	0.0696	0.3789	0.3899	0.3736
As5c8	66.355	2.5	0.3656	0.0689	0.3586	0.3758	0.3623
As6a8	65.286	4	0.3601	0.0706	0.3762	0.3559	0.3481
As6b8	71.209	5.1	0.3909	0.0764	0.3682	0.404	0.4004
As6c8	67.829	4.3	0.3732	0.0751	0.3602	0.3909	0.3684
Asblank-BG-11	-0.911	42.4	-0.0056	0.0732	-0.0083	-0.0042	-0.0041

Arsenic Partitioning

C.10. GFAAS Data

Sample ID	Conc µg/L	%RSD	Mean	Abs	BG	Abs	Readings
AsCALZERO	0	14.2	0.0131	0.0317	0.0116	0.0125	0.0152
AsSTANDARD1	12.5	6.7	0.0622	0.0309	0.067	0.0594	0.0603
AsSTANDARD2	37.5	10.8	0.2095	0.0278	0.2281	0.1844	0.2159
AsSTANDARD3	68.75	5.1	0.4055	0.0284	0.4237	0.3828	0.41
AsSTANDARD4	100	2.7	0.587	0.0298	0.5854	0.572	0.6035
AsBlank1	-1.507	13	-0.0075	0.0266	-0.0065	-0.0075	-0.0085
AsAsaEDTA	1.214	81.3	0.006	0.0741	0.0117	0.0039	0.0026
AsAsbEDTA	-0.256	>100	-0.0013	0.0753	0.0024	-0.0034	-0.0028
AsAscEDTA	-0.793	23	-0.0039	0.0736	-0.0035	-0.005	-0.0034
Asblank2	-0.894	23.4	-0.0044	0.0825	-0.0044	-0.0035	-0.0055
AsINOCAsaEDTA	-0.931	4.4	-0.0046	0.0743	-0.0044	-0.0047	-0.0048
AsINOCAsbEDTA	-1.125	25.5	-0.0056	0.0771	-0.0059	-0.0068	-0.004
AsINOCAscEDTA	-1.078	1.1	-0.0054	0.0747	-0.0053	-0.0053	-0.0054
AsAsaNitric	-1.334	21.6	-0.0066	0.0787	-0.006	-0.0083	-0.0056
AsAsbNitric	-1.445	2.5	-0.0072	0.0801	-0.0071	-0.0071	-0.0074

AsAscNitric	-1.226	29.1	-0.0061	0.0776	-0.007	-0.004 1	-0.0072
AsINOCAsaNitric	0.272	>100	0.0014	0.0758	-0.0019	0.004 5	0.0015
AsINOCAsbNitric	-0.833	37	-0.0041	0.0732	-0.0027	-0.004	-0.0057
AsINOCAscNitric	4.747	6.3	0.0236	0.0729	0.0242	0.022	0.0248
As100ppb	108.255	3.2	0.6346	0.0827	0.6113	0.644 4	0.6481
AsINOCAsaSoln	75.181	4.2	0.443	0.0923	0.4362	0.429	0.4639
AsINOCAsbSoln	76.625	2.2	0.4514	0.0997	0.44	0.458 4	0.4559
AsINOCAscSoln	74.386	2.3	0.4384	0.1	0.4345	0.449 8	0.4309
AsAscSoln	77.319	0.7	0.4555	0.105	0.4521	0.457 8	0.4564
AsAsbSoln	74.143	1.2	0.437	0.1084	0.4382	0.431 4	0.4413
AsAsaSoln	78.658	1.7	0.4632	0.1065	0.4549	0.464 7	0.4701

Arsenic Reduction Data

C.11. GFAAS Data

Sample ID	Conc (μg/L)	%RSD	Mean	BG	Readings		
AsCALZERO	0	22.6	0.01	0.0936	0.0076	0.0102	0.0122
AsSTANDARD1	25	1.6	0.1331	0.091	0.1347	0.1307	0.1338
AsSTANDARD2	50	2.8	0.2886	0.0911	0.2795	0.2942	0.2922
AsSTANDARD3	75	2	0.4164	0.0893	0.4141	0.4257	0.4095
AsSTANDARD4	100	1.7	0.5323	0.0904	0.5274	0.5426	0.5268
AsblankNPW	-0.556	40.1	-0.003	0.0891	-0.0029	-0.0018	-0.0042
As5Asa	77.114	2	0.4267	0.0857	0.4344	0.4278	0.4178
As5Asb	76.892	1.8	0.4256	0.0817	0.4177	0.433	0.4261
As5AsINOCa	70.964	3.1	0.3964	0.0785	0.3827	0.4001	0.4064
As5AsINOCb	77.316	1.8	0.4276	0.0905	0.4361	0.4261	0.4206
As20Asa	78.328	1.1	0.4325	0.093	0.4345	0.4271	0.436
As20Asb	76.327	2.4	0.4228	0.0932	0.4225	0.4128	0.4333
AsblankNPW	-1.151	32.8	-0.0061	0.0921	-0.007	-0.0038	-0.0076
As85Asa	76.179	1	0.4221	0.1031	0.4262	0.4178	0.4224
As85Asb	76.023	4.8	0.4214	0.0936	0.4428	0.4031	0.4182
As85AsINOCa	55.191	85.8	0.316	0.0507	0.0129	0.5349	0.4
As85AsINOCb	47.847	83.9	0.2742	0.0661	0.0089	0.3984	0.4153
As100ppb	98.934	6.9	0.5276	0.0733	0.569	0.5004	0.5133
As20AsINOCb	70.415	1.2	0.3937	0.0907	0.3884	0.3978	0.3948
As20AsINOCa	46.708	81.4	0.2666	0.0742	0.016	0.3964	0.3875

Note: As85AsINOCa, As85AsINOCb and As20AsINOCa are not useable. Instrument error recorded little arsenic in the first of each triplicate measurement. Values used in thesis are recorded from the repeatable second and third measurements.

C.12. HGAAS Data

SAMPLE ID	RESULT TYPE	SIGNAL Abs	Rsd %	FLAGS	CONC. µg/L	TIME	DATE
Blank	Mean	-0.022	12.3		0		
Blank	Resample 1 of 3	-0.019				3:24:23	2/25/2004
Blank	Resample 2 of 3	-0.022				3:24:28	2/25/2004
Blank	Resample 3 of 3	-0.024				3:24:33	2/25/2004
Standard 1	Mean	0.023	6.9		10		
Standard 1	Resample 1 of 3	0.024				3:26:56	2/25/2004
Standard 1	Resample 2 of 3	0.025				3:27:01	2/25/2004
Standard 1	Resample 3 of 3	0.022				3:27:05	2/25/2004
Standard 2	Mean	0.085	3.8		25		
Standard 2	Resample 1 of 3	0.084				3:29:31	2/25/2004
Standard 2	Resample 2 of 3	0.088				3:29:36	2/25/2004
Standard 2	Resample 3 of 3	0.082				3:29:41	2/25/2004
Standard 3	Mean	0.195	1.1		50		
Standard 3	Resample 1 of 3	0.194				3:32:00	2/25/2004
Standard 3	Resample 2 of 3	0.197				3:32:05	2/25/2004
Standard 3	Resample 3 of 3	0.193				3:32:09	2/25/2004
Standard 4	Mean	0.33	1.9		100		
Standard 4	Resample 1 of 3	0.337				3:34:37	2/25/2004
Standard 4	Resample 2 of 3	0.327				3:34:42	2/25/2004

Standard 4	Resample 3 of 3	0.326				3:34:46	2/25/2004
5Asa	Mean	0.007	101.8		6.3279		
5Asa	Resample 1 of 3	0.014				3:37:19	2/25/2004
5Asa	Resample 2 of 3	0.005				3:37:24	2/25/2004
5Asa	Resample 3 of 3	0.001				3:37:28	2/25/2004
5Asb	Mean	-0.004	53.1		4.0317		
5Asb	Resample 1 of 3	-0.001				3:40:00	2/25/2004
5Asb	Resample 2 of 3	-0.004				3:40:04	2/25/2004
5Asb	Resample 3 of 3	-0.005				3:40:09	2/25/2004
5AsINOCa	Mean	0.273	1		74.8903		
5AsINOCa	Resample 1 of 3	0.274				3:42:44	2/25/2004
5AsINOCa	Resample 2 of 3	0.276				3:42:49	2/25/2004
5AsINOCa	Resample 3 of 3	0.27				3:42:54	2/25/2004
5AsINOCb	Mean	0.29	0.9		81.8214		
5AsINOCb	Resample 1 of 3	0.287				3:46:13	2/25/2004
5AsINOCb	Resample 2 of 3	0.291				3:46:17	2/25/2004
5AsINOCb	Resample 3 of 3	0.291				3:46:22	2/25/2004
20Asa	Mean	0.422	14.5	C	134.0193		
20Asa	Resample 1 of 3	0.485				3:48:49	2/25/2004
20Asa	Resample 2 of 3	0.42				3:48:53	2/25/2004
20Asa	Resample 3 of 3	0.363				3:48:58	2/25/2004
20AsINOCa	Mean	0.306	3.4		88.967		
20AsINOCa	Resample 1 of 3	0.307				3:52:12	2/25/2004
20AsINOCa	Resample 2 of 3	0.295				3:52:16	2/25/2004
20AsINOCa	Resample 3 of 3	0.316				3:52:21	2/25/2004
85Asa	Mean	-0.001	397.9		4.5255		
85Asa	Resample 1 of 3	0.004				3:54:49	2/25/2004
85Asa	Resample 2	-0.002				3:54:5	2/25/2004

	of 3				4	
	Resample 3				3:54:5	
85Asa	of 3	-0.006			9	2/25/2004
85Asb	Mean	-0.007	18.1	3.1761		
	Resample 1				3:57:2	
85Asb	of 3	-0.006			2	2/25/2004
	Resample 2				3:57:2	
85Asb	of 3	-0.008			6	2/25/2004
	Resample 3				3:57:3	
85Asb	of 3	-0.009			1	2/25/2004
85AsINOC						
a	Mean	0.293	5.1	83.0207		
85AsINOC	Resample 1				4:00:0	
a	of 3	0.275			3	2/25/2004
85AsINOC	Resample 2				4:00:0	
a	of 3	0.301			8	2/25/2004
85AsINOC	Resample 3				4:00:1	
a	of 3	0.302			2	2/25/2004
85AsINOC						
b	Mean	0.322	3.8	95.9718		
85AsINOC	Resample 1				4:02:4	
b	of 3	0.334			0	2/25/2004
85AsINOC	Resample 2				4:02:4	
b	of 3	0.309			5	2/25/2004
85AsINOC	Resample 3				4:02:4	
b	of 3	0.323			9	2/25/2004
BG11blank	Mean	-0.022	2.8	0.049		
	Resample 1				4:05:2	
BG11blank	of 3	-0.021			7	2/25/2004
	Resample 2				4:05:3	
BG11blank	of 3	-0.022			2	2/25/2004
	Resample 3				4:05:3	
BG11blank	of 3	-0.022			6	2/25/2004
20Asb	Mean	-0.008	2.3	3.0449		
	Resample 1				4:07:5	
20Asb	of 3	-0.008			9	2/25/2004
	Resample 2				4:08:0	
20Asb	of 3	-0.008			3	2/25/2004
	Resample 3				4:08:0	
20Asb	of 3	-0.008			8	2/25/2004
20AsINOC						
b	Mean	-0.008	1.8	3.1179		
20AsINOC	Resample 1				4:10:3	
b	of 3	-0.008			5	2/25/2004
20AsINOC	Resample 2				4:10:3	
b	of 3	-0.007			9	2/25/2004
20AsINOC	Resample 3				4:10:4	
b	of 3	-0.008			4	2/25/2004
AsV50ppb	Mean	-0.006	2.8	3.393		
	Resample 1				4:13:1	
AsV50ppb	of 3	-0.007			6	2/25/2004
AsV50ppb	Resample 2	-0.006			4:13:2	2/25/2004

	of 3				0	
	Resample 3				4:13:2	
AsV50ppb	of 3	-0.006			5	2/25/2004
20Asa-2	Mean	-0.007	2.4	3.1953		
	Resample 1				4:16:0	
20Asa-2	of 3	-0.007			0	2/25/2004
	Resample 2				4:16:0	
20Asa-2	of 3	-0.007			5	2/25/2004
	Resample 3				4:16:0	
20Asa-2	of 3	-0.007			9	2/25/2004
20AsINOC						
a	Mean	-0.007	2.9	3.1534		
20AsINOC	Resample 1				4:21:2	
a	of 3	-0.007			2	2/25/2004
20AsINOC	Resample 2				4:21:2	
a	of 3	-0.008			6	2/25/2004
20AsINOC	Resample 3				4:21:3	
a	of 3	-0.008			1	2/25/2004

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ABSTRACTS

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