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Biogeochemical Processes Affecting Arsenic (As) Release and Bioavailability Near Abandoned Mine Wastes

Cherie L. DeVore
University of New Mexico - Main Campus

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Cherie L. DeVore

Candidate

Civil, Construction & Environmental Engineering

Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Jose M. Cerrato , Chairperson

Lucia Rodriguez-Freire

Abdul-Mehdi S. Ali

Bruce Thomson

Kerry J. Howe

BIOGEOCHEMICAL PROCESSES AFFECTING ARSENIC (AS) RELEASE AND BIOAVAILABILITY NEAR ABANDONED MINE WASTES

by

CHERIE LYNN DEVORE

B.S., Environmental Science, University of New Mexico, 2011
M.W.R., Hydroscience, University of New Mexico, 2015

DISSERTATION

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Cherie L. DeVore

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Master of Water Resources in Hydroscience 2015

Ph.D. in Civil Engineering, 2020

ABSTRACT

The aim of this dissertation was to investigate the biogeochemical processes affecting the mobilization and bioavailability of arsenic (As) from mining-impacted sediments to surface water sources and plants. The Cheyenne River Watershed has been affected by mining legacy for several decades. The biogeochemical cycling of As on tribal land is not well understood, which justified the following research objectives: 1) Investigate the effect of competing anions on the release of As from sediments exposed to oxidizing conditions; 2) Evaluate chemical and microbiological processes affecting the release of soluble As, Fe, and Mn from contaminated sediments under laboratory controlled aerobic and anaerobic conditions; 3) Evaluate As uptake in *Schizachyrium scoparium* inoculated with endophytic fungi using hydroponic experiments. Field and laboratory approaches used in this work contribute novel information about As speciation and reactivity. Results will be useful to identify potential exposure pathways and enhance risk reduction strategies for nearby communities.

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Chapter 1

1.1 Introduction

The western part of the United States has a legacy of over 160,000 abandoned mines and represents a particular area of exposure to mine wastes[1]. For instance, uranium, arsenic and vanadium in drinking water sources exceeding United States Environmental Protection Agency (EPA) and World Health Organization (WHO) standards have been identified in many Navajo Nation and Pueblo communities in the western U.S[2-4]. Metal combinations that may impact human health for Native American people were also found to be correlated to mine source distance[5]. The Cheyenne River watershed is a location that has experienced As release to surface water due to gold mining legacy during the Black Hills gold rush in the 1870s[6, 7]. Physical and chemical processes have resulted in transport and deposition of As-enriched materials within Whitewood Creek, the Belle Fourche, and Cheyenne River. A map of the study area can be found in Chapter 3, **Figure 1**. Deposits from the mine wastes, which include sulfide and iron minerals, associated with gold ore and 9.5 metric tons of As in the form of arsenopyrite (FeAsS), were found along Whitewood Creek in the 1970s when production ceased at the gold mine[8]. The Cheyenne River flows to the Missouri River, through the lands of the Cheyenne River Sioux Tribe (CRST), located 241 km downstream of the Homestake Mine[9]. There are concerns about potential metal exposures in this community[10]. The ability to adequately assess potential metal exposures among Native American communities living near abandoned mines is limited by the knowledge of mechanisms driving release at these sites.

The goal of this research was to investigate the biogeochemical processes affecting the mobilization and bioavailability of arsenic (As) from mining-impacted sediments to surface water sources and vegetation on Cheyenne River Sioux (CRST) tribal land in North-central South Dakota. Results from this initiative will be useful to limit potential exposure pathways and enhance risk reduction strategies for communities living near abandoned mines.

More than half of the abandoned hard rock mines in the United States are located near federal and tribal lands, which presents a risk to Native Americans living near these sites. The environmental fate of As related to its speciation and biogeochemical cycling on tribal lands is not well understood. Thus, the current state of knowledge can be improved by integrating controlled laboratory experiments with solid and aqueous speciation measurements to evaluate arsenic retention and release from mine wastes. Previous field and laboratory investigations point to the importance of As speciation and its reactivity under environmentally relevant pH and redox conditions. The specific objectives for this project were: *i)* Investigate the effect of competing anions on the release of As from sediments exposed to surface oxidizing conditions; *ii)* Investigate the effect of microbial redox processes on the release of arsenic from mine wastes; *iii)* Evaluate As uptake in *Schizachyrium scoparium* inoculated with endophytic fungi using controlled laboratory hydroponic experiments. The overall objective was to integrate multidisciplinary research and outreach to investigate the chemical and biological processes that affect arsenic speciation and mobility from mine waste samples located near Native American communities using aqueous speciation chemistry, spectroscopy, microscopy, microbiology and diffraction analyses.

The research performed in this dissertation was used to differentiate between the chemical, microbiological and physical-chemical processes affecting the release and bioavailability of As from a mining impacted watershed that has historically been overlooked. This dissertation is decided into five chapters and two appendices. Chapter 2 is a literature review on the topics covered by this dissertation, including the occurrence of As in the environment, a background on legacy mining activities, biogeochemical processes influencing As mobility and plant tolerance to As. The dissertation ends with a review of fundamental research gaps in the proposed field of study. Chapters 3, 4 and 5 are the main body of work of the dissertation and each is formatted as a research paper.

Chapter 3 has been published in *Environmental Science: Processes and Impacts* (https://doi.org/10.1039/C8EM00461G) and relates to the investigation of environmentally relevant anions on the release of As from solids collected from the Cheyenne River watershed exposed to surface oxidizing conditions. Chapter 3 includes a discussion of the aqueous chemistry and spectroscopic results before and after reaction with phosphate and bicarbonate, which are identified as environmentally relevant to the watershed.

Chapter 4 builds on our understanding of the chemical processes responsible for As mobilization. The manuscript resulting from this chapter will be submitted to the journal *Environmental Science & Technology*. Chapter 5 focuses on understanding the effects of root endophytes on As uptake by relevant agricultural plants in Native American communities using laboratory hydroponic experiments. DNA sequencing and microscopy analyses were conducted to obtain information about localization of endophytes in roots in the presence and absence of As. The role of endophytes on As tolerance and the decoupling of chemical and biological influences on As release from mine waste in the study area are currently not well understood. Thus, the fundamental knowledge gained from this research will lay the foundations for future studies and bioremediation scenarios by integrating molecular biology and environmental chemistry. Appendices A, B and C contain supplementary information for the chapters 3,4 and 5 respectively.

Chapter 2: Literature Review

2. Background

2.1 Arsenic chemistry in environmental systems. There are than 300 As minerals that include approximately 60% arsenates, 20% As-sulfur minerals, and 10% As-oxides with the rest composed of arsenites, arsenides, and metal alloys.[1] Inorganic rsenic can occur as As(III) and As(V) under oxidation-reduction potential (E_h) and pH conditions that are relevant to most surface and groundwaters[2]. As in these two oxidation states are referred to as arsenite (As(III)) and arsenate (As(V)), and the As speciation is important due to the higher toxicity and increased mobility of As(III).[3] Several organic As species exist (DMA, MMA, TMAO, TMA) and account for less than 5% of the total As concentrations[4]. Complex geochemical processes affect the mobilization of As in the environment. Arsenic release from solids can result from oxidation of sulfide minerals and from the dissolution of iron oxyhydroxides and other metal oxides[5, 6]. Under reducing conditions, soluble As concentrations can be affected by desorption of As following dissolution of iron (Fe) and manganese (Mn) oxides and the sorption or co-precipitation of As with carbonates and sulfide minerals.[7] These geochemical factors differ from those influencing soluble As concentrations under oxidizing conditions. For example, arsenopyrite is unstable under surface oxidizing conditions. The most frequently observed product of arsenopyrite weathering is scorodite ($FeAsO_4 \cdot 2H_2O$). Other products of arsenopyrite oxidation are arseniosiderite [$Ca_2Fe_3(AsO_4)_3O_2 \cdot 3H_2O$] and jarosite ($KFe_3(SO_4)_2(OH)_6$).[8] Amorphous hydrous ferric arsenate-sulfate, pharmacosiderite [$KFe_4(AsO_4)_3(OH)_4 \cdot 6–7H_2O$], and arsenolite ($As_2O_3$) are less common products.[9] Arsenic can be associated with jarosite, which is thermodynamically unstable at circumneutral pH. Jarosite dissolution can cause the release of metals to water at specific pH and redox conditions.[10, 11]

Interactions at the mineral-water interface play a fundamental role in controlling As solubility and transport. Arsenate can form inner-sphere surface complexes on both amorphous aluminum (Al) and Fe oxide, while arsenite forms both inner- and outer-sphere surface complexes on amorphous Fe-oxides.[12] In circumneutral reducing conditions, the reduction of arsenate to arsenite can be important for As release, especially since arsenite adsorbs less strongly to Fe and Mn oxides.[13, 14] Additionally, the presence of similar high-affinity anions for surface sites on Fe-oxides, such as phosphate, silicic acid, and bicarbonate can contribute to the mobility of As.[15-17] Surface complexation models and adsorption studies have been used to predict As mobilization in groundwater environments.[18-20] Some have confirmed these models with experimental data,[21] but there is a lack of field studies supported by experimental data.

2.2. Significance: Mining Legacy near Native American Communities. The western part of the United States has a legacy of over 160,000 abandoned mines and represents a particular area of exposure to mine wastes.[22] For instance, uranium, arsenic and vanadium in drinking water sources exceeding United States Environmental Protection

Agency (EPA) and World Health Organization (WHO) standards has been identified in many Navajo Nation and Pueblo communities in the western U.S.[23-25] Metal combinations that may affect human health are found to be correlated to mine source distance.[26]

The Cheyenne River watershed is a site that has experienced As release to surface water due to gold mining legacy during the Black Hills gold rush in the 1870s.[8, 27] Physical and chemical processes have resulted in substantial transport and deposition of As-enriched materials within Whitewood Creek, the Belle Fourche, and Cheyenne River. Mine wastes, which include sulfide and iron minerals, associated with gold ore and 9.5 million metric tons of As in the form of arsenopyrite (FeAsS), occur along Whitewood Creek in the 1970s when production ceased at the gold mine.[28] The Cheyenne River flows to the Missouri River, through the lands of the Cheyenne River Sioux Tribe (CRST), located 241 km downstream of the Homestake Mine. There are existing concerns about potential metal exposures in this community. The ability to adequately assess potential metal exposures among Native American communities living near abandoned mines is limited by the knowledge of mechanisms driving release at these sites.

2.3. Background on Study Area. The Cheyenne and Moreau River are the largest rivers crossing the Cheyenne River Sioux Tribe (CRST) Reservation and their watersheds lie within the Great Plains physiographic province (**Figure 1**, Chapter 3). The Cheyenne River Indian Reservation lies on a gently northwest dipping flank of the Williston watershed in north-central South Dakota. The area of the Reservation is approximately 10,956 km^2 and encompasses Dewey and Ziebach Counties. The topography of the

Reservation is largely rolling upland that has been eroded by the Missouri River and its tributaries.[29] About 88 percent of the land area is covered by native grass and is used primarily for grazing livestock.

The Cheyenne River forms the southern boundary of the Reservation and is the largest tributary to the Missouri River within South Dakota (drainage area of about 66,045 km^2). The Cheyenne River originates in eastern Wyoming, flows southeast around the southern Black Hills, and then flows northeast across South Dakota to its mouth at Lake Oahe. The Cheyenne River is joined by the Belle Fourche River approximately 24 km southwest of the Reservation boundary. The Belle Fourche River flows northeast in Wyoming and then flows southeast around the northern Black Hills to its confluence with the Cheyenne River in South Dakota.[29] The Belle Fourche River is the largest tributary to the Cheyenne River and drains about one-third of the entire Cheyenne River Watershed.[30] Whitewood Creek is a major tributary of the Belle Fourche River.

2.4. Biogeochemical processes influencing As mobility and bioavailability. Transport of arsenic and other constituents in the environment can be the result of physical, chemical and/or biological processes. As(V) is the predominant species in oxidizing environments and As(III) is predominant in reducing conditions.[3] Understanding chemical and redox transformations of As are important for predicting its fate in the environment, as well as to achieve remediation of As-contaminated water and soils. Arsenic dissolution into the aqueous phase may be caused by four main processes in the soil environment: i) competitive ion displacement[15], ii) limited adsorption onto media or desorption[31], iii) arsenate reduction to arsenite[32] and iv) reductive mineral dissolution from many forms of oxyhydroxides.[33] Investigations of mining impacts have suggested that chemical leaching and physical processes can also be release mechanisms of mine waste material associated with Mn- and Fe- hydroxide material.[34]

Chemical stabilization and adsorption of arsenic compounds is often discussed as one of the mechanisms for removal to reduce As mobility and bioavailability.[35] In addition, numerous groundwater investigations provide useful insights on the biological mechanisms contributing to arsenic release and transport from anoxic sediments.[36-38] However, these conditions may not explain As mobility from solids that experience transport and resuspension in a riverbank environment. Though As has been recognized as a toxic element, some microorganisms can use it as an electron donor for autotrophic growth or as an electron acceptor for anaerobic respiration.[39] In addition, As detoxification systems in which As is oxidized to the less toxic form or reduced for subsequent excretion are distributed widely in microorganisms.[4, 40, 41] Microorganisms, fungi and plants can also methylate arsenic to less toxic forms under strongly reducing conditions, which affects As bioavailability. Arsenic transformation by methylation-demethylation convert inorganic arsenic into organic forms contributing to its bioavailability in soil and plants, particularly in the rhizosphere.[42] To our knowledge, limited studies exist to evaluate the role of the rhizosphere on As bioavailability from local plants grown in the Cheyenne River Watershed.

2.5. As tolerance in Plants. There are various mechanisms of As tolerance/detoxification in plants, including low rate of As(V) uptake, efflux of accumulated As, transformation of inorganic As to less toxic organic forms and complexation and sequestration to non-reactive locations.[43] The mechanisms for tolerance in plants vary in different plants. For example, a hyper-tolerant plant utilizes various strategies to reduce total As load in shoots, while a hyperaccumulator has efficient As uptake and translocation and little efflux in order to increase As accumulation in shoots.[44] Further, plants may utilize one or multiple strategies for tolerance. Plants growing in heavily As-contaminated soils (such as *Holcus lanatus* and *Cytisus striatus*) achieve As hyper-tolerance by decreasing the rate of As infiltration. Hyper-tolerant plants can suppress As(V) uptake by the high affinity phosphate As(V) transport system.[45, 46] Arsenate is an analogue of phosphate and thus enters the plant system through phosphate transporters.[47] Arsenite exists as a neutral molecule ($As(OH)_3$) at neutral pH and under reducing conditions, and uptake can occur via aquaglyceroporin channels, which can also transport other neutral molecules like silicic acid, and boric acid.[48]

Additionally, volatilization or efflux of the accumulated As can reduce total As present in a plant.[49] These mechanisms occur in bacteria and fungi and are proposed to exist in plants as well. In bacteria and fungi, enzymes are known to convert As(III) to the gaseous trimethylarsine (TMA).[50] Arsenic methylation has been experimentally demonstrated and a variety of organic methylated As compounds have been detected in rice and other submerged plants.[51, 52] The efflux of As in the form of As(III) has also been demonstrated via rice silicon transporters.[53] In contrast, in hyperaccumulators, As uptake and transport are highly efficient and As efflux is not a major detoxification mechanism.[54] In addition, complexation can affect the translocation of As either from root to shoot or from root to the growing medium.[55]

2.6. Effects of fungi and endophytes on plant uptake. Microbial colonization of the mycorrhizosphere – the zone influenced by the plant roots and the mycorrhizal fungus– represents a natural process commonly found in various type of soils including those affected by mining and agricultural activities. Symbiotic interactions between bacteria and fungi can affect As speciation in the rhizosphere and consequently As uptake by the plant. For example, colonization of rice roots with arbuscular mycorrhizal fungi increased the ratio As(III)/As(V) in the roots.[56] Mycorrhizal symbiosis can change the affinity of phosphate transporters in the roots potentially affecting the affinity of the roots for As.[57]

The methylation and associated detoxification of As due to Arbascular mycorrhizal symbiosis has been reported in literature.[58] For instance, arsenic resistant plant growth promoting rhizobacteria have been identified in the contaminated rhizosphere which reduced phytotoxicity in rice seedlings grown in arsenic impacted soil.[59] Many arsenic resistance studies highlighting volatilization and accumulation mechanisms have focused on rice grains.[59, 60] Arbascular mycorrhizal (AM) increased arsenic translocation into the Chinse brake fern in hydroponic experiments.[61] Other studies with AM colonized on plant roots illustrate the same effect.[62, 63]

However, As translocation into plants inoculated with a mycorrhizal symbiont depends both on the specific plant species and choice of fungal symbiont. Some fungal isolates found in gold mining impacted sediment have been found to volatilize or accumulate arsenic.[64] In another gold mining site, ascomycota was the most abundant fungal phylum in the study areas.[65] Ascomycota isolates are known to have mycorrhizal associations with over 90% of the terrestrial plant community.[66] Fungal colonization of 46 grass species in the Rocky Mountain area of the United States was investigated and Ascomycota were found to associate with some of these plants.[67] Though fungi can associate with many plants, the mechanisms of arsenic uptake in native grasses from prairielands located in central United States are understudied. This information is relevant to understand mechanisms that affect accumulation of As in plants and potential exposure routes of As to community members that use these plants either for consumption or cultural purposes.

The colonization of the rhizosphere represents a natural process commonly found in various type of soils including those affected by agricultural and mining activities.[64, 68] For example, mycorrhizal symbiosis can catalyze the oxidation and reduction of As, transform inorganic As into an organic form, and enhance the growth of plant biomass to dilute the concentration of accumulated As in plant tissues.[40, 62] Fungi can affect the bioavailability and toxicity of As by modifying soil chemistry of the rhizosphere.[69] Symbiotic interactions between bacteria and fungi can affect As speciation in the rhizosphere and As uptake by the plant. Thus, these relationships should be explored further to better understand As translocation to plant parts used by local communities.

2.7. Gaps in the Literature and Research Objectives.

2.7.1. Limitations Identified in Previous Studies. This research addressed a current gap in the literature related to understanding the complex biogeochemical cycling of arsenic from mine wastes to sediment and native plants in the study area. The distinction between chemical and biological processes on the cycling of arsenic from abandoned mine wastes remains a challenge. More focus on transformations of arsenic from microbially mediated processes using speciation and microbiological techniques is needed. Microbial methylation has been highlighted in laboratory experiments as a key process in this discussion and associated toxicity characteristics are important in speciation studies. However, differentiating between chemical and microbial mechanisms is often challenging due to the complex nature of environmental samples, but the need to characterize their roles in mine waste transport still exists. The speciation and bioavailability of arsenic from contaminated soils focuses on reducing, groundwater environments in the current literature. However, more work needs to be done under oxidizing conditions and under redox conditions that better reflect transport and resuspension in the environment. Additionally, understanding how arsenic speciation affects bioavailability is also poorly understood. Pure culture studies of As methylation have shown that the extent of methylation can vary widely between microorganisms and plants. However, plant species native to Asian countries have been the predominant focus of these investigations. It is necessary to examine the rhizosphere of native plants and effects of fungi on As uptake since the role of fungi-plant symbiosis is not well understood.

To our knowledge, limited studies have evaluated the role of the rhizosphere on As bioavailability from native plants grown in the Cheyenne River Watershed. The colonization of plant roots with endophytic fungus represents a natural process commonly found in various type of soils including those affected by agricultural and mining activities[64, 68]. The predominant focus has been on mycorrhizal symbiosis, which can catalyze the oxidation and reduction of As, transform inorganic As into organic forms, and enhance the growth of plant biomass to dilute the concentration of As accumulated in plant tissues[70]. Mycorrhizal fungi can affect the bioavailability and toxicity of As by modifying soil chemistry of the rhizosphere[69]. Symbiotic interactions between bacteria and fungi can affect As speciation in the rhizosphere and As uptake by the plant. Thus, these relationships should be explored further to better understand As translocation to plant parts used by local communities.

2.7.2. Research Objectives

This research provides new mechanistic insights on the bioavailability of As from mining-impacted sediment governed by biogeochemical redox cycling. We investigated the role of biologically mediated release of As and other metals from As-enriched material. Laboratory experiments target the differentiation between chemical and microbiological processes affecting arsenic mobilization from mining impacted sediments, which remains as an unresolved challenge. Figure 1 in Chapter two summarizes selected mechanisms for As release that are addressed by this dissertation

The overall goal of this. research was to understand the processes that enhance the mobility of arsenic (As) from riverbank solids to surface water and plants impacted by legacy gold mining. This research seeks to identify the biogeochemical mechanisms that drive mobilization of As under relevant pH and redox conditions.

The specific **objectives and hypotheses** are:

Objective 1. Investigate the effect of competing environmentally relevant anions on the release of As from sediments collected in a mining impacted watershed exposed to surface oxidizing experimental conditions.

Hypothesis 1. Arsenic associated with outer-sphere surface complexes is displaced under oxidizing conditions by high concentrations of HCO_3^- (20 mM) in the Cheyenne River.

Objective 2. Differentiate between chemical and microbiological processes affecting the release of soluble As, Fe, and Mn from contaminated sediments under laboratory controlled aerobic and anaerobic conditions.

Hypothesis 2. Anaerobic conditions enhance the methylation and microbial reduction of As(V) to As(III), mobilizing As from mine waste sediments with urea as an electron donor.

Objective 3: Evaluate As uptake in *Schizachyrium scoparium* inoculated with endophytic fungi using hydroponic experiments.

Hypothesis 3. Fungal isolates associated with little bluestem grass decrease arsenic translocation into grass shoots.

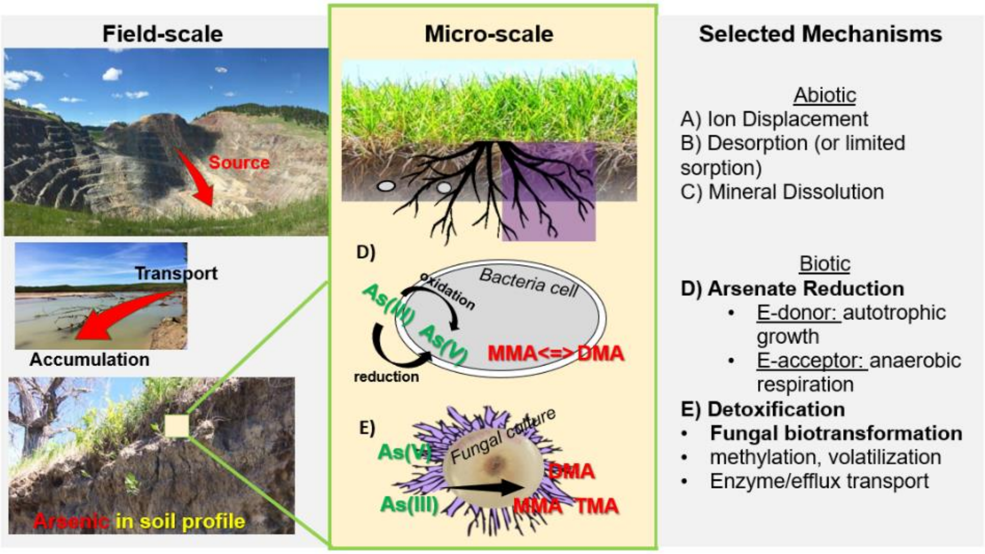

Figure 1. Schematic of selected processes governing arsenic release.

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Chapter 3

3. Effect of bicarbonate and phosphate on arsenic release from mining-impacted sediments in the Cheyenne River watershed, South Dakota, USA

Cherie L. DeVore,[a] Lucia Rodriguez-Freire,[b,] Abdul Mehdi-Ali,[c] Carlyle Ducheneaux,[d] Kateryna Artyushkova[e], Zhe Zhou,[f] Drew E. Latta,[f] Virgil W. Lueth[g,] Melissa Gonzales[h], Johnnye Lewis,[i] and José M. Cerrato[a*]

[a]Department of Civil Engineering, MSC01 1070, University of New Mexico, Albuquerque, New Mexico 87131, USA. E-mail: jcerrato@unm.edu; Fax: (001) (505) 277-1918; Telephone: (001) (505) 277-0870

[b]Department of Civil and Environmental Engineering, 266 Colton Hall, University Heights, New Jersey Institute of Technology, Newark, New Jersey 07102

[c]Department of Earth and Planetary Sciences, MSC03 2040, University of New Mexico, Albuquerque, New Mexico 87131, USA

[d]Department of Environmental and Natural Resources, Cheyenne River Sioux Tribe, Eagle Butte, South Dakota 57625

[e]Department of Chemical and Biological Engineering and Center for Microengineered Materials, University of New Mexico, Albuquerque, New Mexico 87131, USA

[f]Department of Civil and Environmental Engineering/IIHR, The University of Iowa, 4105 Seamans Center, Iowa City, Iowa 52242, USA

[g]New Mexico Bureau of Geology & Mineral Resources, New Mexico Tech, 801 Leroy Place, Socorro, New Mexico 87801

[h]School of Medicine, Department of Internal Medicine, University of New Mexico Health Sciences Center, MSC10 5550, Albuquerque NM 87131
[i]Community Environmental Health Program, College of Pharmacy, MSC09 5360, 1 University of New Mexico, Albuquerque, USA

Abstract. The mobilization of arsenic (As) from riverbank sediments affected by gold mining legacy in north-central South Dakota was examined using aqueous speciation chemistry, spectroscopy, and diffraction analyses. Gold mining resulted in the discharge of approximately 109 metric tons of mine waste into Whitewood Creek (WW) near the Homestake Mine and Cheyenne River at Deal Ranch (DR), 241 km downstream. The highest concentrations of acid-extractable As measured from solid samples was 2,020 mg kg-1 at WW and 385 mg kg-1 at DR. Similar sediment mineralogy between WW and DR was identified using XRD, with the predominance of alumino-silicate and iron-bearing minerals. Alkalinity measured in surface water at both sites ranged from 1,000 to 2,450 mg L-1 as CaCO3 (10-20 mM HCO3- at pH 7). Batch laboratory experiments were conducted under oxidizing conditions to evaluate the effects of NaHCO3 (0.2 mM and 20 mM) and NaH2PO3 (0.1 and 10 mM) on the mobilization of As. These ions are relevant for the site due to the alkaline nature of the river and nutrient mobilization from the ranch. The range of As(V) release with the $NaHCO_3$ treatment was 17-240 µg L^{-1}. However, the highest release (6,234 µg L^{-1}) occurred with 10 mM NaH2PO3, suggesting that As release is favored by competitive ion displacement with PO_4^{-3} compared to HCO3. Although higher total As was detected in WW solids, the As(V) present in DR solids was labile when reacted with $NaHCO_3$ and NaH_2PO_3, which is a relevant finding

for communities living close to the river bank. The results from this study aid in a better understanding of As mobility in surface water sites affected by mining legacy.

3.1 Introduction

Arsenic (As) can naturally occur at a wide range of concentrations in waters world-wide.[1] Additionally, anthropogenic activities such as mining can impact surface waters and associated sediments, causing the occurrence of elevated concentrations of As and other co-occurring metals.[2] The western part of the United States has a legacy of over 160,000 abandoned mines and represents a particular area of exposure to mine wastes[3]. A variety of adverse health effects ranging from skin and bladder cancers to cardiovascular and neurological diseases, have been associated with As exposure, primarily from ingestion of As-contaminated drinking water [4-6]. The World Health Organization and the United States Environmental Protection Agency have set a standard of 10 µg As L^{-1} for drinking water [7, 8].

The Cheyenne River watershed is a specific site that has experienced As release to surface water due to gold mining legacy [4]. Mineral extraction and waste disposal near Lead, South Dakota directly discharged spent mine tailings into Whitewood Creek from 1876 until 1977. Approximately 109 million metric tons of tailings containing As and other heavy metals have been deposited downstream of Whitewood Creek along the Belle Fourche and Cheyenne Rivers[9]. Whitewood Creek drains the area of Homestake Mine and is a tributary of the Belle Fourche, which flows into the Cheyenne River[10]. Physical and chemical processes have resulted in substantial transport and deposition of As-enriched materials within Whitewood Creek, the Belle Fourche, and Cheyenne River[11, 12]. Deposits from the mine wastes, which include sulfide and iron minerals, associated with gold ore and 9.5 metric tons of As in the form of arsenopyrite (FeAsS), were found along Whitewood Creek in the 1970s when production ceased at the gold mine[13]. The Cheyenne River flows to the Missouri River, through the lands of the Cheyenne River Sioux Tribe (CRST), located 241 km downstream of the Homestake Mine. There are existing concerns about potential metal exposures in this community[3].

Complex geochemical processes affect the mobilization of As in the environment. Arsenic release in source waters can result from the dissolution of iron oxyhydroxides and other metal oxides [14]. Arsenic can occur as As(III) and As(V) under oxidation-reduction potential (E_h) and pH conditions that are relevant to most surface and groundwaters[1, 15]. Usually, these oxidation states of As are arsenite (As(III)) and arsenate (As(V)), and the As speciation is important due to the higher toxicity and enhanced the mobilization of As(III)[1, 16]. Under reducing conditions, soluble As concentrations can be affected by desorption of As from dissolution of iron (Fe) and manganese (Mn) oxides and the sorption or co-precipitation of As with carbonates and sulfide minerals. These geochemical factors differ from those influencing soluble As concentrations under oxidizing conditions. For example, arsenopyrite is unstable under surface oxidizing conditions[17]. The most frequently observed product of arsenopyrite weathering is scorodite ($FeAsO_4{\cdot}2H_2O$). Other products of arsenopyrite oxidation are arseniosiderite [$Ca_2Fe_3(AsO_4)_3O_2{\cdot}3H_2O$] and jarosite ($KFe_3(SO_4)_2(OH)_6$).[12] Amorphous hydrous ferric arsenate-sulfate, pharmacosiderite [$KFe_4(AsO_4)_3(OH)_4{\cdot}6\text{–}7H_2O$], and arsenolite ($As_2O_3$) are less common products[11]. Arsenic can be associated with jarosite, which is thermodynamically unstable at circumneutral pH. Jarosite dissolution can cause the release of metals in water at specific pH and redox conditions.[18, 19]

Interactions at the mineral-water interface play a fundamental role in controlling As transport. Arsenate can form inner-sphere surface complexes on both amorphous aluminum (Al) and Fe oxide, while arsenite forms both inner- and outer-sphere surface complexes on amorphous Fe-oxides[20]. In circumneutral environmental conditions, the reduction from arsenate to arsenite can be important for As release, especially since arsenite adsorbs much less strongly to Fe and Mn oxides[21, 22]. Additionally, the presence of similar high-affinity anions for surface sites on Fe-oxides, such as phosphate, silicic acid, and bicarbonate can contribute to the mobility of As[16, 23-25]. Phosphate is particularly effective at competing with arsenate for sorption sites on Fe oxide minerals[26]. Previous adsorption studies with carbonate and phosphate have focused on groundwater environments with the assumption that native phosphate in the subsurface is very low, so in these cases, equimolar phosphate with As(V) will not interfere with the adsorption of As(V) in groundwater systems[27]. However, phosphate concentrations suppressed As(V) adsorption onto alluvial sediments when it was present at concentrations that were 10 times higher than that of As (V).[27] Thus, competitive ion displacement and mobilization of As in the presence of phosphate represents a mechanism of release and transport into the aqueous phase[28]. Areas with nutrient mobilization via agricultural runoff and phosphate-based fertilizers can be at risk for As mobilization[29]. In addition, carbonate ions can play a role in contaminant retention and release via competitive adsorption. An investigation of bicarbonate(HCO_3^-) in groundwater found that concentrations of released As from subsurface core sediment strongly depended on concentrations of HCO_3^-, with enhanced mobility at higher concentrations[30]. In addition, carbonate concentrations modeled after typical values found in Bangladesh (0.01M) after receiving considerable attention in the past two decades[24], is only half the concentration of carbonate observed in South Dakota due to the alkaline nature of regional soils.[11]

Surface complexation models and adsorption studies have been used to predict As mobilization in groundwater environments[28, 30, 31]. Some have combined these models with experimental data[32], but there is still a lack of field studies confirmed by experimental data. While there is extensive literature on As mobilization, many of the experimental and environmental conditions do not apply to the Cheyenne River. First, many studies have focused on the mobilization of As from enriched sediment and pore waters at the E_h range found in groundwaters[22, 33-37]. Arsenic release into groundwater can be due to anoxic conditions that may interfere with the stability of Fe-oxides[15, 38-40]. However, fewer studies have emphasized better understanding As mobilization in surface oxidizing environments. Second, leaching of As into groundwater by carbonation of As sulfide minerals was reported, but this process may not apply to both the surface water conditions and specific As minerals present in mining ore deposits along the Cheyenne River[25]. This river is a relevant surface water source for the Cheyenne River Sioux Tribe and has high alkalinity values ranging from 1000 mg L^{-1} (10 mM) to 2,400 mg L^{-1} (24 mM) as $CaCO_3$ near the mine waste source. There are not many field or laboratory studies in the literature that have been conducted to evaluate the effect of well-buffered surface waters at such high alkalinity levels which justifies the need for the present study.

The objective of this study is to investigate the effect of competing environmentally relevant anions on the release of As from solids collected from the Cheyenne River watershed exposed to surface oxidizing conditions. We integrated laboratory experiments with aqueous chemistry and spectroscopy measurements to investigate As speciation and mobilization in sediment collected from Whitewood Creek (near the mine source) and Cheyenne River (241 km downstream on tribal land). A novel aspect of this study consists on the investigation of chemical mechanisms that affect As release under surface oxidizing conditions with high concentrations of HCO_3^- (20 mM), attempting to represent the alkaline nature (10-20 mM) of the Cheyenne River and Whitewood Creek. We also investigated the competing effect of phosphate (PO_4^{-3}) on As mobilization in As-bearing soils collected from land that is used for agriculture and livestock. The results of this study have relevant implications for informing tribal and regulatory decision makers, as well as environmental risk assessments and mitigation efforts for communities located near abandoned mine wastes with similar characteristics to those observed in the Cheyenne River.

Study Area

The Cheyenne and Moreau River watersheds lie within the Great Plains physiographic province. The Cheyenne River Indian Reservation lies on a gently northwest dipping flank of the Williston watershed in north-central South Dakota. The area of the Reservation is approximately 10,956 km^2 and encompasses Dewey and Ziebach Counties. The topography of the Reservation is largely rolling upland that has been eroded by the Missouri River and its tributaries.[41] About 88 percent of the land area is native grass and is used primarily for grazing livestock. The Cheyenne and Moreau Rivers are the largest rivers traversing the Cheyenne River Sioux Tribe (CRST) Reservation.

The Cheyenne River forms the southern boundary of the Reservation and is the largest tributary to the Missouri River within South Dakota (drainage area of about

66,045 km^2). The Cheyenne River originates in eastern Wyoming, flows southeast around the southern Black Hills, and then flows northeast across South Dakota to its mouth at Lake Oahe. The Cheyenne River is joined by the Belle Fourche River approximately 24 km southwest of the Reservation boundary. The Belle Fourche River flows northeast in Wyoming and then flows southeast around the northern Black Hills to its confluence with the Cheyenne River in South Dakota. The Belle Fourche River is the largest tributary to the Cheyenne River and drains about one-third of the entire Cheyenne River Watershed.[42] The Whitewood Creek is a major tributary of the Belle Fourche River. Throughout the study, we refer to the upstream source as WW (Whitewood Creek) and downstream sample on tribal land as DR (Deal Ranch) (**Figure 1**).

3.2 Materials and Methods

3.2.1 Materials

During 2016, we collected water and sediment samples from the study area in the locations indicated in the map in **Figure 1**. In June 2016, two water samples from the Cheyenne River (impacted by the Homestake Mine), and two samples from the Moreau River (not impacted by the Homestake Mine) were analyzed for pH, alkalinity, anions, and total and dissolved elements (see section 3.2 for details on methods for anions and total and dissolved elements analyses). A second sampling trip was conducted in September 2016 to collect water samples upstream of the Cheyenne River, Belle Fourche River and Whitewood Creek close to the Homestake Mine. For each sample, one liter of surface water was collected using a clean polypropylene Nalgene bottle and preserved with 2% trace metal grade nitric acid (HNO_3^-). Water samples were filtered using a 0.45 µm Basix filter®, and a portion of the volume was filtered using a 0.22 µm Basix filter®

in the laboratory. Bottles were rinsed three times with sample water in the field before filling and sealing with no headspace. Samples were cooled to 4°C and shipped for analyses to the University of New Mexico (UNM). Field parameters were measured using a YSI 650 MDS pH/temp/conductivity probe and calibrated on the day of sample collection. The global positioning system (GPS) coordinates were collected at each site location (see **Table S1** in Appendix A).

Sixteen sediment samples were collected from the riverbank profile at co-located sites in June 2016 from **Figure 1**. Ten additional sediment samples were collected upstream of the Cheyenne River, Belle Fourche River and Whitewood Creek in close proximity to the Homestake Mine in September 2016. A hand trowel was rinsed with 10% nitric acid solution and used to collect half a gallon of sediment from the riverbank and bed. Samples were placed in one-gallon plastic bags and cooled to 4°C for shipment with water samples to UNM. Throughout the study, we refer to the upstream source as WW (Whitewood Creek) and downstream sample on tribal land as DR (Deal Ranch). The authors were escorted by the CRST Department of Environment and Natural Resources staff while conducting sampling. Travel and time constraints to South Dakota by UNM collaborators reduced the number of sampling events and possible sampling locations.

3.2.2 Solution Chemistry Analyses

Aqueous elemental analyses for this study were performed using inductively coupled optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 5300DV) and inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin Elmer NexION 300D-Dynamic Reaction Cell) in aqueous solutions for trace element content (As, Cr, Fe, Mn, and U). Samples for ICP-MS were filtered using a 0.45 µm Basix filter and preserved with 2% HNO_3^-. A ThermoFisher Scientific Ion Chromatogram (ICS-1100) was used to analyze 0.45 µm non-acidified water samples for major anion concentrations. Arsenic speciation was characterized using a Flexar 400 High-Performance Liquid Chromatography (HPLC) coupled with ICP-MS for As(III) and As(V) species. Samples were stabilized with a mobile phase solution (0.5 mM EDTA, 1 mM TBAoH and 1% MeOH) adjusted to pH 7 (±0.01). A CAPCELL PAK C18 column (250 mm × 4.6 mm, 5 µm particle size) was used for the separation of target As species. All reagents used in the experiments and analyses were analytical grade unless otherwise mentioned. Additional information about the HPLC-ICP-MS analyses can be found in Supplementary Information (**Table S2**).

3.2.3 Solid Characterization Analyses

Sediment samples were dried for 12 h at 60 ^oC in a controlled–temperature oven. Dried sediment samples were crushed and homogenized using a shatterbox. One gram of the dried sediment was weighted and added to a 50 mL digestion tube. All samples were acid digested (2.0 mL HNO_3 (UHP) + 1 mL HCl (UHP)) in triplicate to determine extractable elemental concentrations. The mixture was digested for 60 min at 65° C and then for an additional 60 min at 80° C. Digested samples were diluted with deionized water to 25 mL. The digested and diluted samples were filtered through a 0.45 µm filter to remove any particulate matter for aqueous elemental analyses. Loss on Ignition (LOI) was used to estimate the organic matter content in sediment samples. Five grams of sample were weighed and dried at 104°C for 12 hours. A calculation was made for mass loss difference after drying temperature increase from 104°C to 550°C for 5 hours (h).

Dried solid samples from field and laboratory analyses were analyzed using X-ray photoelectron spectroscopy (XPS), X-ray powder diffraction (XRD), X-ray fluorescence (XRF) and Mössbauer spectroscopy. Measurements of X-ray fluorescence (XRF) were conducted to determine bulk elemental composition using a Rigaku ZSX with a rhodium X-ray rube that can be operated from 200 to 4000 watts (End window, Rh-anode, 4 kW, 60 kV). A Kratos AXIS-Ultra DLD X-ray Photoelectron Spectrometer was used to acquire elemental composition and oxidation states at the near surface (5-10 nm) of the solids. Pure (99%) Au powder was put onto samples for charge correction. Three areas per sample were analyzed using monochromatic Al K α source and charge neutralization. CASAxps was used to process the spectra. The spectra were calibrated by adjusting the position of Au 4f spectra to 84 eV. Shirley background was used for Fe 3p and As 3d spectra processing. Fe 3p spectra were fitted with two symmetrical peaks due to Fe(II) and Fe(III). As 3d spectra were fitted with two symmetrical peaks due to As(III) and As(V) with each of them having a doublet separated by 0.77 eV. The positions of the peaks was determined previously from references materials analyzed and reported[43].

A PanAnalytical XPert Pro Diffractometer was used for X-ray diffraction (XRD) analyses using CuK radiation for mineral identification of sediment samples. Samples were mounted in a rotating stage and were scanned from 5 to 70 degrees using 0.008 (°2θ) step size and 40.0 s scan step time. The XRD patterns were interpreted using PANALYTICAL X’PERT HIGHSCORE PLUS software. Mössbauer spectra were collected in transmission geometry with a constant acceleration drive system (SEE Co., Inc.) and a ^{57}Co(Rh) source. The temperature was controlled in a Janis gas-exchange closed cycle cryostat down to a sample temperature of 15K. Data were calibrated with an α-Fe foil at room temperature and fit with the Recoil software package using Voigt lineshapes. Quadrupole shift parameters are reported as 2ε, which is twice the parameter epsilon_0 in Recoil.

3.3 Laboratory experiments

Laboratory batch experiments were performed to evaluate the reaction of As in sediments from WW and DR with bicarbonate and phosphate concentrations relevant to the environment of the Cheyenne River Watershed. Batch reactors were operated in triplicate using deionized water, sodium bicarbonate ($NaHCO_3$) stock solutions (0.2 mM and 20 mM) and sodium phosphate (NaH_2PO_3) stock solutions (0.1 mM and 10 mM), which were prepared based on aqueous chemistry results and literature review of the Cheyenne River.[11] Five 100 mL centrifuge tubes were labeled for 20 mM $NaHCO_3$; fifteen 100 mL for 0.2 mM $NaHCO_3$ and 5 for DI water control. Five grams of dried WW and DR sediment was added per 100 mL centrifuge tube. A total of fifteen 100 mL centrifuge tubes was placed on a bench rotator for 96 h at 30 rpm. A volume of 3 mL of sample was collected at t = 0.5, 1, 2, 4, 6, 12, 24 and 96 h. Volume and pH were recorded at each time step. Samples were filtered using a 0.22 µm filter and cooled to 4 °C. 0.5 mL of sample was immediately placed in a 1.5 mL plastic HPLC vial and preserved in mobile phase solution to keep stable until aqueous elemental analyses were conducted.

3.4. Results and Discussion

Elemental composition of solid and water quality data from the site

Elevated As concentrations were measured in sediments in Whitewood Creek (WW) near the Homestake Mine, but also 241 km downstream in the Cheyenne River Sioux Tribe

Reservation. Arsenic concentrations as high as 2,020 mg kg^{-1} were measured in the sediments at WW. The WW flows into the Belle Fourche River and eventually into the Cheyenne River where high As concentrations were measured in the riverbank sediments. Arsenic concentrations in riverbank sediments more than 161 km downstream of the mine on tribal land at Deal Ranch (DR) and Cherry Creek were in the range of 90 – 394 mg kg^{-1} (**Table 1**). The As concentrations in these sediments exceed the regionally established background concentration of 10 mg kg^{-1}, and it is in agreement with a 1989 USGS study which reported similar values in the Belle Fourche (>3,500 mg kg^{-1}) and the Cheyenne River (>530 mg kg^{-1}) solids[4]. However, low As concentrations were measured in the Moreau River, ranging from 7.4-14 mg kg^{-1}, which was chosen as a reference location because it is not hydrologically connected to the Homestake Mine. Elemental bulk composition measured in sediments by XRF detected higher Fe and As content than acid extractable concentrations. Silicates are not readily dissolved by Aqua regia,[44] which might explain the differences between XRF and acid digestion analyses for Fe in the samples (**Table 2**). The organic matter content in our samples was estimated by LOI and the mass loss observed in our sample was 2.23-2.99 (±0.005-0.006)%. The mass loss is likely attributed to the volatilization of organic matter within the sample. A previous study measured LOI at three sites along the Cheyenne River and were in the range of 0.5-1.0%[11], similar to the measurements in this study. The organic matter content found near the Cheyenne River is lower than what has been observed in other environmental systems. For instance, the LOI in agricultural soils is reported to be 65% and it ranges from 7% to 9% in soils that have been exposed to wildfires.[45, 46]. These results suggest that, although mining operations have ceased for many decades, accumulation of As-impacted sediments has occurred on tribal land.

While the sediment concentration of As in the sediments was elevated, relatively low As concentrations were detected in surface waters at different locations along the Whitewood Creek, Belle Fourche River, Cheyenne River and Moreau River (**Table 2**). For instance, As measurements in surface water ranged from 2.25-62.2 µg L^{-1} in Whitewood Creek (WW) near the mine waste, and ranged from 3.74-5.84 µg L^{-1} (DR, CC) downstream along the Cheyenne River. The As concentrations in waters from the Moreau River ranged from 1.38-2.06 µg L^{-1}. Additional samples were collected between WW and DR along the Belle Fourche River and were comparable to values measured in the Cheyenne River[11, 47]. The results from this study at Whitewood Creek are consistent with As measurements in a previous study, where concentrations vary between about 20 and 80 µg L^{-1} during the year in response to variations in groundwater inflow and dilution[4]. As a reference for freshwater sources, aquatic life chronic level criterion continuous concentration is 150 µg As L^{-1}.[48]

Additional water quality data were collected in these surface waters that are relevant to describing conditions that may mobilize metals in the environment. The pH in Whitewood and Belle Fourche Rivers closer to the mine waste ranged from 8.21-8.90 and alkalinity values ranged from 1,342-2,650 mg L^{-1} as $CaCO_3$. The pH in the Cheyenne River 241 km downstream of Homestake Mine ranged from 7.69-8.32, with alkalinity 580-1,000 mg L^{-1} as $CaCO_3$ (**Table 3**). These alkalinity values are relatively high considering that this is a surface water source; the range of alkalinity values measured in this study are characteristic of waters associated with alkaline soils from this region of South Dakota[11]. The major-ion composition of the water results from the cumulative effects of the interaction with alluvial aquifers, underlying shale units and transported mine waste. Sodium and calcium are the predominant cations, and sulfate is the predominant anion. Sulfate concentrations exceed the secondary drinking-water standard of 250 mg L^{-1} at all sampling sites[42], and range from about 1,100 to more than 2,500 mg L^{-1}. The sources of sulfate in the alluvium can be attributed to both the shale and mine tailings[49].

Laboratory Batch Experiment: reactivity of As with bicarbonate

The release of As was investigated in batch experiments reacting sediments (WW, DR) using HCO_3^- (0.2 mM and 20 mM) and PO_4^{-3} (0.1 mM and 10 mM) under surface oxidizing conditions. Note that concentrations used in previous studies for bicarbonate found in typical groundwaters are too low to be representative of Cheyenne River's surface water environment.[24, 27] Thus, the bicarbonate concentrations and pH selected for this study are characteristic of WW and DR surface waters. Also, phosphate was added to the experiment as a proxy to evaluate the effect of nutrient inputs into the Cheyenne River watershed resulting from livestock and agricultural activities in the area. The goal of these experiments is to use controlled laboratory settings to better understand processes that could be relevant to the mobilization of As in the study site.

Increasing bicarbonate concentrations promote As mobilization from the sediments (**Figure 2**). Low bicarbonate concentration (0.2 mM) representing typical values found in drinking waters (0.2-2.0 mM as $CaCO_3$) released a maximum of 2.4 µg L^{-1} As(V) in WW near the mine (**Figure 2A**) and a maximum of 48 µg L^{-1} as As(V) downstream of the mine at DR (**Figure 2B**) over a period of 96 h. No As(III) was released with either treatment and fluctuations within 1 pH unit (7.34-8.23) were observed (**Figure S1**). A high bicarbonate concentration (20 mM) emulating the high alkalinity measured in the Cheyenne River (1,000 to 2,100 mg L^{-1} as $CaCO_3$) mobilized a maximum of 14 µg L^{-1} As(V) from WW sediments (**Figure 2A**) and a maximum 204 µg L^{-1} As(V) was released from DR sediments (**Figure 2B**). Additionally, a control without bicarbonate (only DI water) was conducted in parallel and As(V) concentrations were below 100 µg L^{-1}. Hence, the results from this experiment suggest that the presence of bicarbonate in surface water could cause As mobilization from the sediments.

The presence of carbonates in groundwater systems with adsorbed As has been examined in previous studies that integrated controlled laboratory experiments with surface complexation modeling[21, 26, 50, 51]. A slight decrease in arsenate adsorption was observed at pH 7.75-8.00 in early reaction times in one study. This decrease in As adsorption could be due to the increased negative charge at the surface of ferric oxides such as hematite caused by adsorbed carbonate[52]. In addition, the importance of carbonate-complexes on up to 70% of ferrihydrite surface sites in the subsurface was illustrated in surface chemistry models[30]. There are relevant implications for As mobility in groundwaters where these complexes can either enhance or suppress adsorption[31, 53]. The competitive effect of carbonates has been mostly studied in batch experiments reacting synthetic solid samples with solutions having chemical characteristics similar to those in groundwater[25, 30, 54]. These observations are important to consider, given the abundant carbonate minerals interacting with the alluvium and overlying surface water[49]. Future studies are necessary to investigate surface complexation reactions in natural surface water systems. Additional experiments were conducted to evaluate the release of As after reaction with phosphate, a known chemical analog of arsenate (note the similarities in pK_a values indicated in **Table S3**) and a proxy for nutrient inputs into the surface waters evaluated in this study.

Batch Experiment: reactivity of As with phosphate

The release of As(V) was at least 10-times higher after reacting mine waste solids with phosphate, compared to the experiments with bicarbonate. Our experiments were conducted with a low (0.1 mM) and high (10 mM) phosphate concentration. The low concentration was selected because phosphate measured in the surface water was not detectable during our field sampling at sites shown in **Figure 1**. However, previous monitoring activities conducted by the CRST Environment Department have reported total phosphorus concentrations in the range of 0.17-3.4 mg L^{-1} (0.01-0.24 mM)[41]. Most of the land base in the Cheyenne River watershed is dedicated to livestock purposes and several irrigation areas exist within the basin. Previous studies have used a range of 0.57-16.67 mM PO_4^{-3} in microcosm studies with P amended contaminated soil to understand the influence of phosphate on As leaching.[29, 55] The 0.1 and 10 mM concentrations selected for our study are similar to the treatments in the batch and column experiments with P and As-amended soils from these previous studies[29, 55] A maximum release of 1.6 µg L^{-1} As was observed in experiments reacting 0.1 mM phosphate with WW solids (**Figure 3B**) and the range of pH changes during this experiment were 6.90-8.25 (±0.01-0.16) (**Figure S2**). The release of As in this experiment is a relatively low concentration as it only represents 0.03% of the total As in the solids. However, a maximum concentration of 244 µg L^{-1} As was observed in experiments reacting 0.1 mM phosphate with DR solids (**Figure 3D**), which is more than two orders of magnitude higher than the As released from WW solids.

Experiments reacting sediments with a higher phosphate concentration (10 mM) released a maximum of 6,056 µg L^{-1} from WW (**Figure 3A**) and 3,939 µg L^{-1} from DR (**Figure 3C**). More than 95% of the total As was in the form As(V). A minor fraction was released as As(III) with the phosphate treatment (10 mM) and the range of pH during this experiment was 5.58-6.59 (**Figure S1**). Compared to the bicarbonate treatment where no As(III) was detected, phosphate released As(III) in the range of 1-5 µg L^{-1}. A maximum of 4.89% As(V) of the solid concentration was released with the PO_4^{-3} treatment from WW solids compared to 0.025% with bicarbonate. A maximum of 33% As (V) was released with the PO_4^{-3} treatment from DR solids, compared to 1.33% with the bicarbonate treatment **(Table 4**). Although more total As concentration (2,040 mg kg^{-1}) was measured in WW sediments, solids from DR (285 mg kg^{-1}) mobilized more As(V) on a mass basis. Our results using field solids suggest that As(V) is more easily released from DR solids compared to WW solids after reaction with DI water, bicarbonate and phosphate solutions. Additional analyses of the solids are discussed in later sections to help us understand As release under these experimental conditions.

It is known that the adsorption of As(V) and As(III) onto Fe-oxides can be influenced by the presence of competing anions in the system and their relative binding affinities. Competition for surface sites between similar anions influence adsorption processes by changing electrostatic charges at the solid surface[28]. Our results indicate that As release is favored by competitive ion displacement with 10 mM PO_4^{-3} compared to 20 mM HCO_3^-. This is likely due to the displacement of $HAsO_4^{-2}$ related to the higher affinity of the solid surface for the dominant species HPO_4^{-2}, which has a more negative charge than HCO_3^-**.** Since less than 5% As (III) was released into solution, the As likely occurs as weakly bound As(V) at the surface of the sediments. It is possible that inorganic phosphorus associated with livestock runoff could be an important factor for mobilizing surface-bound As in Cheyenne River sediments, mainly after storm events. The potential for nutrient runoff to affect As release is an important factor to consider based on the results from these experiments with phosphate.

Previous studies have shown the effects of phosphate on the decrease of arsenate adsorption by reacting synthesized iron oxide solids with As solution concentrations synthesized in the laboratory.[28, 53] The dominance of As(III) released using similar concentrations of competitive anions used in this study (22.7 mM CO_3 and 0.25 mM PO_4^{-3}) was measured. Solids from one study were synthetic iron coated sands operated in controlled column experiments. The distribution of As species released from this study can be used to show the effects of different surface sites from field derived solids compared to synthesized materials. Experimental data using a synthesized iron oxide-based sorbent showed the individual and competitive adsorption of As(V) and HPO_4^{-226}. The addition of 129 μM phosphate to a suspension with 6.67 μM As(V) (926 μg L^{-1}) induced desorption of As(V). In addition, a 10-fold molar mass of phosphate to arsenate suppressed arsenate adsorption in experiments conducted with unsaturated alluvial sediments from a recharge pond in Antelope Valley, CA.[27]

Chemical equilibrium modeling (MINEQL) used to simulate aqueous species (solids not considered) in this system under relevant laboratory and field conditions indicated that $HPO_4^{2-,}$ $HAsO_4^{2-}$, $H_2AsO_4^-$, and HCO_3^- were the predominant species. Considering the complex speciation of As(V) in sediments and its reactivity with naturally occurring anions, modeling results suggest that HCO_3^- and HPO_4^{2-} should be considered to understand As mobilization in the Cheyenne River and other environments with similar characteristics at pH 7. Additional analyses were conducted to further investigate chemical characteristics of unreacted and reacted solids from these experiments.

Solid analyses

X-ray diffraction (XRD). Patterns from XRD analyses showed that the predominant mineralogy of the sediments are very similar between the two sample areas (WW and DR) and contain predominately alumino-silicate and iron-bearing minerals including, 75-77% quartz (SiO_2), 6% chlorite ($(Mg,Fe^{2+},Fe^{3+},Mn,Al)_{12}[Si,Al)_8O_{20}(OH)_{16}$), 7% biotite ($(K_2(Mg,Fe)_{6-4}(Fe,Al,Ti)_{0-2}O_{2-3}O_{20}](OH,F)_4$), 4-8% feldspar ($((Na, K, Ca)Al_{1-2}Si_{2-3}O_8])$, and 2% grunerite ($((Mg,Fe,Mn)_7[Si_8O_{22}](OH)_2$) (**Figure 4**). These minerals are important for understanding anion retention on solid surfaces since As has an affinity to react with alumino-silicate and iron oxide minerals as reported in other studies.[56] Although the mineralogy from these sites is similar, some differences were observed as seen in the small amounts of gypsum (~4%) at WW which is lacking at DR and the presence of calcite (~2%) at DR not seen at WW. In addition, the DR site contains about twice as much feldspar in the sediment (~8%) compared to WW. Additional analyses were conducted to better understand As and Fe solid speciation in the solids.

X-ray photoelectron spectroscopy. Solids were analyzed using XPS for detecting changes in oxidation state in the top ~5 nm of the surface before and after reaction with the higher PO_4^{-3} concentration (10 mM) in batch experiments over 96 h. The presence of PO_4^{-3} influenced change in As speciation in the surface of the sediments (**Figure 5**). Before the reaction with PO_4^{-3}, a similar distribution of oxidation state was observed for both sediments with 70 % As(V) and 30% As(III). These analyses indicate that As(V) is the predominant oxidation state in the near surface region of these sediments. Arsenate and PO_4^{-3} can form similar bonds at the solid surface because of their similar chemistry[28]. These XPS results coupled with results obtained from the batch experiments suggest that As(V) is more weakly bound to DR solids and can be more easily mobilized than WW with PO_4^{-3}, even though WW has a higher solid concentration of As. Although the relative affinity of As(V) and As(III) can depend on solution composition, the characteristics of iron oxides must also be taken into consideration.[16] The content of Fe(II) ranged from 64-72% in sediments from WW, indicating the predominance of pyrite and other solids containing reduced Fe close of the mine as shown in another study.[11] However, the Fe(III) observed in DR was two-fold higher compared to WW sediments, indicating that the oxidizing conditions in the Cheyenne River could cause the prevalence of Fe(III) downstream of the mine. An increase in Fe(III) was detected by XPS after reaction with phosphate . These XPS data indicate that Fe(III) is more prevalent in the surface of these solids due to competitive displacement between As(V) and PO_4^{-3}. Additional analyses were pursued to better understand Fe speciation in sediments.

Mössbauer Spectroscopy. To characterize the Fe-bearing minerals in the Whitewood Creek and Deal Ranch sediments, we collected Mössbauer spectra on the bulk sediments (**Figure 6**). Fitting the Mössbauer spectra (**Table 5**) of the WW and DR samples revealed that the Fe mineralogy was similar in both sediment samples, with each sample having a Fe(II) doublet (center shift (CS) = 1.26 mm/s, quadrupole splitting (QS) = 2.8 mm/s), a broad Fe(III) doublet (CS = 0.45 mm/s, QS = 1.6 and 0.88 mm/s, respectively), and a magnetically ordered Fe(III) sextet (CS = 0.5 mm/s, $2\varepsilon = -0.22$ to -0.26, and the hyperfine field (H) ~ 42-43 T). The Fe(II) and Fe(III) doublets have Mössbauer spectra consistent with a number of minerals, including Fe in clay minerals and primary silicates, as well as magnetically unordered amorphous or nanocrystalline Fe(III) phases[57, 58]. This is consistent with the identification of aluminosilicate and Fe-bearing minerals with XRD. The major differences between the two spectra are that the WW sediment contains more of a Fe(III) sextet (63%) with Mössbauer parameters closely matching goethite than the Deal Ranch sediment (56% goethite).[58] A larger relative area of Fe(II) doublet (27% for WW, and 18% for DR) concomitant with a decreased area of Fe(III) doublet (10% in WW and 27% in DR) in the Whitewood Creek sediment was also measured. In contrast with surface specific Fe speciation provided from the top 2-3 nm by XPS, the WW sample has much lower Fe(II) in the bulk as determined by Mössbauer (27%) in comparison to 73% of Fe(II) detected at the surface. The DR sample has more homogeneous Fe chemistry with a similar amount of Fe(II) detected at the surface by XPS (26%) and in the bulk by Mössbauer (18%).

The presence of goethite identified by Mössbauer spectroscopy in sediments from WW and DR is relevant as this could explain the association of As in these sediments. For example, the association of As and goethite has been widely reported in the literature.[16, 59-62] Given the similar bulk mineralogy of the two sediments, as observed by Mössbauer spectroscopy, the following explanations for As release are plausible and include: (*i*) different As-binding minerals present in the two sediments (e.g. a different surface or Fe-As mineral), (*ii*) different surface Fe-to-PO_4/CO_3 ratios in the two sediments during extraction, and (*iii*) greater potential for As(V) occlusion within Fe minerals or Fe mineral aggregates in the WW sample making As poorly extractable[63]. Additionally, the potential for Fe and As influenced by interactions with natural organic matter (NOM) is possible.

However, detailed mechanistic studies of NOM are challenging in environmental systems due to the heterogenous nature of NOM and the estimated organic content in our samples is only 2-3%.[64]. Based on our present data, we cannot definitely conclude which of these or other potential mechanisms affect anion-induced As release in the WW and DR sediments.

3.5 Summary and conclusions

This study evaluated the concentration and reactivity of As in sediments impacted by an abandoned gold mine (Homestake Mine), in the Whitewood Creek (near the mine) and in the Cheyenne River (241 km downstream and inside the Cheyenne River Sioux Tribe reservation). This study suggests that the presence of environmentally relevant ions, bicarbonate and phosphate, can favor the release of As, through competitive ion displacement. More specifically, the highest As release was observed in batch experiments after reaction of mine waste solids with 10 mM sodium phosphate, which released 30 times as much as 20 mM sodium bicarbonate. However, a considerable release of As was observed after reaction with 20 mM bicarbonate, which has important implications for water quality conditions of the Cheyenne River.

These results are relevant given that As release to water sources continues to be a concern in CRST and in many other communities located near mine waste sites. For instance, the high alkalinity (ranging from 1,000 to 2,100 mg L^{-1} as $CaCO_3$) in the Cheyenne River and Whitewood Creek could affect As reactivity. In the current study, the reaction of mine waste sediments with 20 mM bicarbonate in batch experiments resulted in a maximum release of 240 μg L^{-1} of As(V). In addition, the role of inorganic and organic phosphorus on As release into surface water sources is an important consideration. For example, in the current study, the reaction of 10 mM phosphate with mine waste solids resulted in the release of up to 6,900 μg L^{-1}As(V). Land use within CRST boundaries includes 85% livestock use and agricultural activities, so the potential for the effect of organic and inorganic phosphorus on the release of As from sediments after runoff events should be considered. More research is necessary to better understand the effect of agricultural inputs on As reactivity in sediments from CRST.

Though As can be released by changes in associated Fe mineralogy and reductive dissolution, this study also suggests that the mobilization of As can be affected by phosphate and to some extent bicarbonate by competitive ion displacement of weakly bound As(V) at the surface of the solids. This is an interesting finding given that, although the DR solids have lower total As, the concentrations of As(V) mobilized after reaction with bicarbonate and phosphate are considerable. The presence of labile As(V) from sediments is relevant to consider in the assessment of As exposure to the local community. Existing literature has indicated that As has the affinity to associate with goethite in environmental systems. Given that Mössbauer analyses indicate that goethite is present in samples from WW and DR, it is likely that goethite plays an important role in As binding in these sediments. However, additional research is necessary to better understand the specific binding mechanisms for As in sediments from our system.

The Cheyenne River watershed continues to receive sediment loads from upstream, and the alterations of As as a function of sediment burial and redox transformations can be an important component of As release. Therefore, future investigations of redox processes can aid in our understanding of As mobility in the riverbank profile along the Cheyenne River, where the community of CRST harvests fruiting trees and medicinal plants.

Additional studies are necessary to better understand the effect of microbial and plant processes on As mobility. The results of this investigation on As reactivity have important implications for environmental exposure and risk assessments.

Site ID	Site	River	Distance from Mine (km)	As (mg kg^{-1})
WW	Whitewood Creek	Whitewood Creek	0.49	2,040 (±118)
BF/WW	Belle/Whitewood	Belle Fourche	5.70	18.2 (±1.52)
DR	Deal Ranch	Cheyenne River	230	235 (±119)
CC	Cherry Creek	Cheyenne River	237	394 (±54.7)
RL	Ross Lawrence	Moreau River-Reference	*NC	14.1(±1.85)
TB	Thunder Butte	Moreau River-Reference	*NC	7.40 (±0.79)

***NC=** Not hydrologically connected to Cheyenne River

Table 1. Summary of As concentrations in sediments (mg kg^{-1}) downstream of the Homestake Mine.

WW XRF (mg kg-^{1})	WW acid-extractable (mg kg-^{1})	DR XRF (mg kg-^{1})	DR acid-extractable (mg kg-^{1})

Fe	199,143 (±2,578)	34,541 (±12186)	95156 (±256)	244 (± 9.10)
As	4,584 (±20)	2,040 (±118)	1,008 (±8.5)	235 (±119)

Table 2. Summary of Fe and As concentrations in sediments measured by XRF and acid digestions.

Site ID	**Site Name**	**River**	**Total As (μg L^{-1})**	**Alkalinity mg L^{-1} (mM) as $CaCO_3$**	**pH**

WW	Whitewood Creek	Whitewood Creek	62.16	2,650 (2.7)	8.50
BF	Belle Fourche River	Belle Fourche	19.0	2867 (28.7)	8.21
BF/WW	Belle Fourche/Whitewood	Belle Fourche	2.25	1342 (13.4)	8.90
WA	Wasta	Cheyenne River	6.68	1647 (16.5)	7.39
BR	Bridger	Cheyenne River	6.10	1464 (14.6)	8.30
DR	Deal Ranch	Cheyenne River	5.14	720(7.2)	7.82
CC	Cherry Creek	Cheyenne River	5.84	1,000(10)	7.69
RL	Ross Lawrence	Moreau River	1.47	580 (5.8)	8.32
TB	Thunder Butte	Moreau River	2.05	164 (1.6)	8.23

Table 3. Summary of As and alkalinity concentrations, and pH in surface water from Whitewood Creek to Cherry Creek

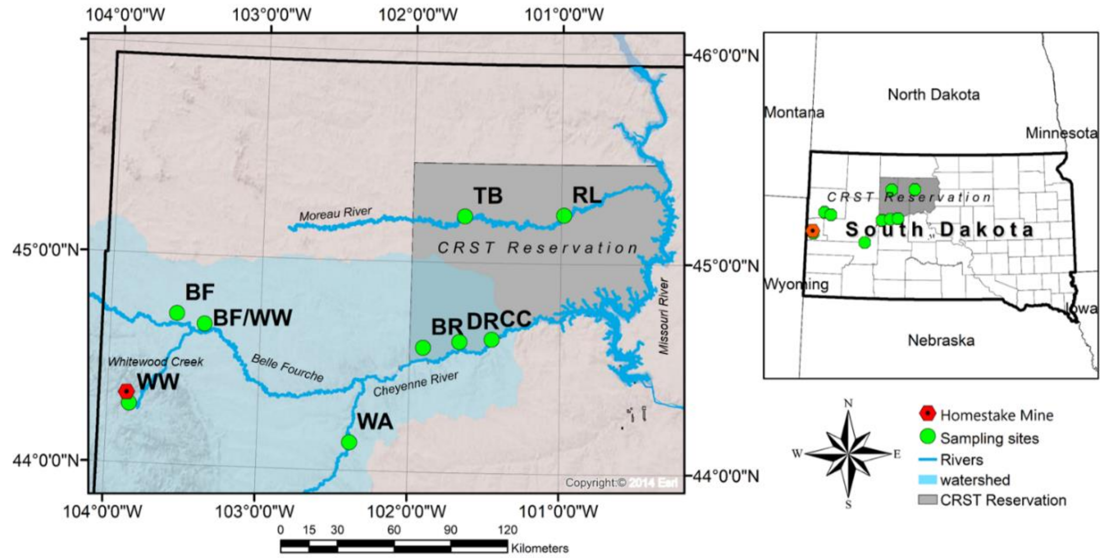


Figure 1. Map of study area in North Central South Dakota.

	Total As ($mg\ kg^{-1}$)	**% mobilized with HCO_3**	**% mobilized with PO_4^{-3}**	**% mobilized DI Control**
WW	2,040	0%	4.9%	0%
DR	235	1.3%	33%	0.7%

Table 4. Percentage of As mobilized from Whitewood Creek (WW) and Deal Ranch (DR) sediments after reaction with bicarbonate, phosphate and de-ionized water.

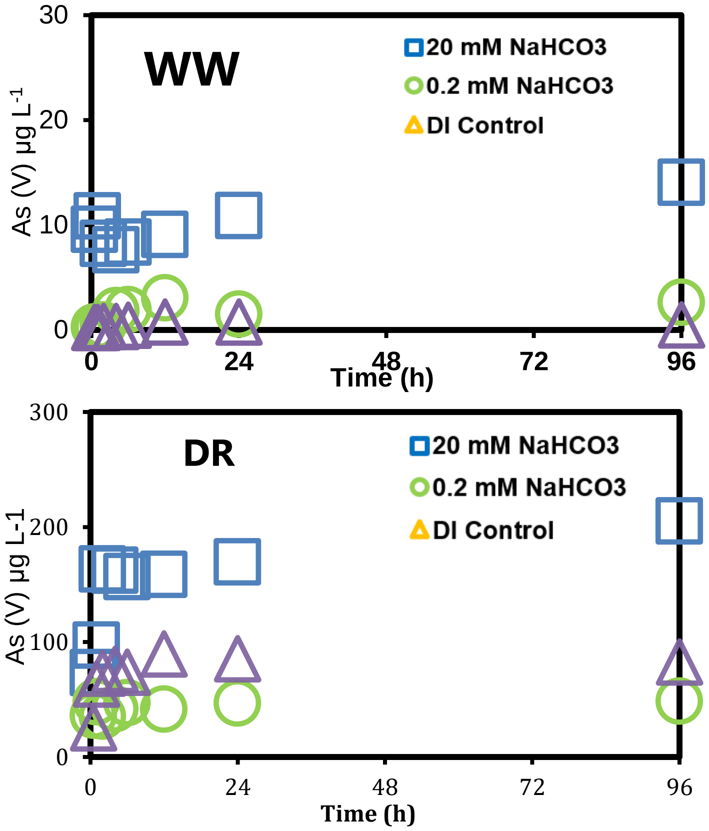


Figure 2. The release of As(V) after reaction of solids reacted with de-ionized water (DI Control), 0.2 mM, and 20 mM sodium bicarbonate solutions for: **A)** Whitewood Creek (WW) and **B)** Deal Ranch (DR).

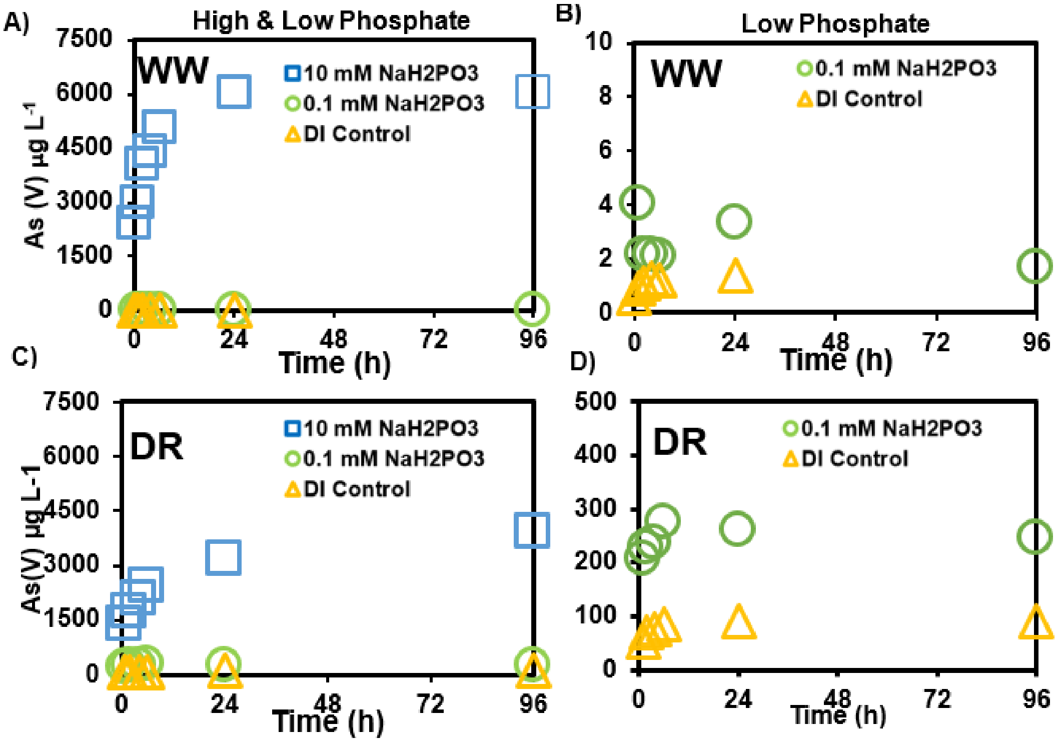
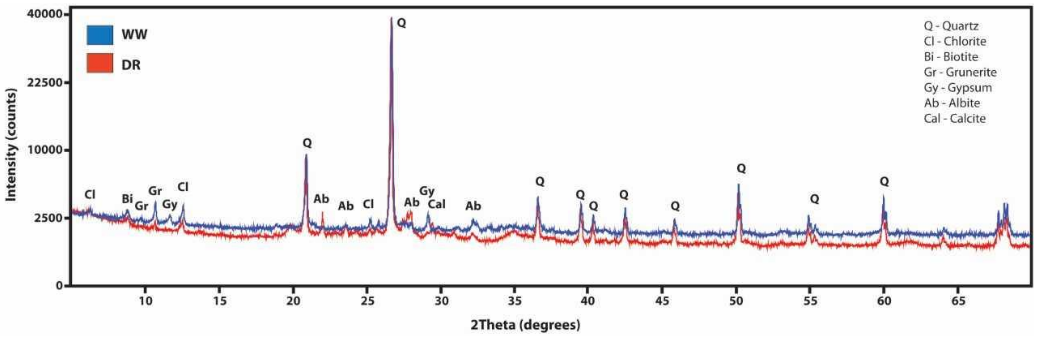
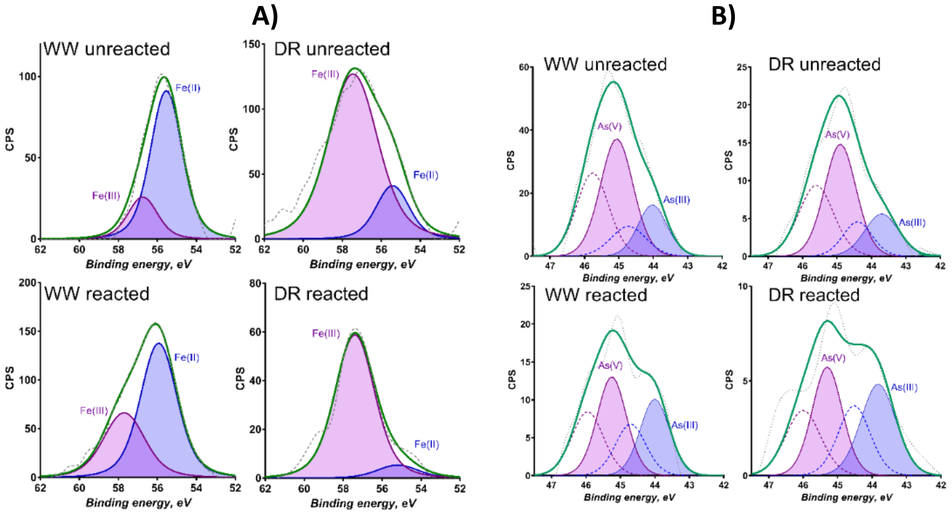


Figure 3. Release of As(V) after reaction of solids with de-ionized water (DI Control), 0.1 mM, and 10 mM sodium phosphate solutions for: **A)** Whitewood Creek (WW) high & low phosphate **B)** Whitewood Creek (WW) zoomed into low phosphate **C)** Deal Ranch (DR) high & low phosphate and **D)** Deal Ranch (DR) zoomed into low phosphate



Compound name	WW (Atomic Wt %)	DR (Atomic Wt %)
Quartz	77%	75%
Gypsum	4%	-
Biotite	7%	7%
Chlorite	6%	6%
Calcite	-	2%
Feldspar	4%	8%
Grunerite	2%	2%

Figure 4. XRD Patterns and Atomic Weight % of Whitewood Creek (WW) and Deal Ranch (DR) sediments.



Sample		% Fe(II)	% Fe(III)
WW	unreacted	72.7	27.3
	reacted	64.8	35.2
DR	unreacted	26	74
	reacted	8.7	91.3

Sample		% As(III)	% As(V)
WW	unreacted	28.1	71.9
	reacted	43.6	56.4
DR	unreacted	36.2	63.8
	reacted	29	71

Figure 5. High-resolution XPS spectra for oxidation state percent content in solid samples from Whitewood Creek (WW) and Deal Ranch (DR) before and after reaction with phosphate for: **A)** Fe (based on fitting of Fe 3p high-resolution spectra); and **B**) As (based on fitting of As 3d high-resolution spectra).

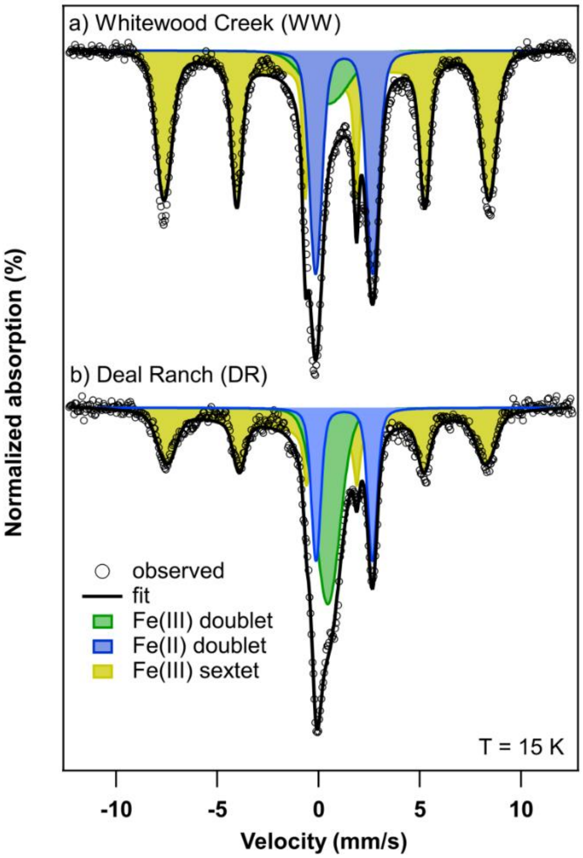


Figure 6. Mössbauer spectra of **a)** Whitewood Creek (WW) and **b)** Deal Ranch (DR) sediments collected at a temperature of 15 K.

Sample	Component	Relative area (%)	Center shift, CS (mm/s)	Quadrupole splitting, QS or 2ε [b] (mm/s)	σ(Δ)[c] (mm/s)	Hyperfine field, H (Tesla)	σ(H)[d] (Tesla)	χ_v^2
Whitewood Creek (WW)	Fe(II) doublet	27 (0.2)	1.26	2.82	0.51	N/A	N/A	3.72
	Fe(III) doublet	10 (0.4)	0.45	1.63	1.23	N/A	N/A	
	Fe(III) sextet	63 (0.4)	0.50	-0.22		43.4	13.66	
Deal Ranch (DR)	Fe(II) doublet	18 (0.1)	1.27	2.78	0.35	N/A	N/A	1.22
	Fe(III) doublet	27 (0.3)	0.45	0.88	0.67	N/A	N/A	
	Fe(III) sextet	56 (0.4)	0.51	-0.26		42.3	20.91	

a value in parenthesis reflects the error (1σ) in determination of the relative area for each component

b 2ε = quadrupole shift parameter in sextet

c σ(Δ) = standard deviation of quadrupole splitting component

[d] σ(H) = standard deviation of hyperfine field component

* = parameter held constant during fitting

Table 5. Summary of Mössbauer spectral parameters derived from fitting the 15 K sediment spectra.

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Chapter 4

4. Release of As, Fe and Mn from contaminated sediment in aerobic and anaerobic conditions: chemical or microbiological?

Cherie L. DeVore[1], Lucia Rodriguez-Freire[2], Noelani Villa[1], Maedeh Soleimanifar,[2] Jorge Gonzalez-Estrella[1], Abdul Mehdi Ali[3],, Juan Lezama-Pacheco[4], Carlyle Ducheneaux[5], José M. Cerrato[1]

[1] Department of Civil, Construction & Environmental Engineering, MSC01 1070, University of New Mexico, Albuquerque, New Mexico 87131, USA

[2] Department of Civil & Environmental Engineering, New Jersey Institute of Technology, Newark, New Jersey

[3] Department of Earth and Planetary Sciences, MSC03 2040, University of New Mexico, Albuquerque, New Mexico 87131, USA

[4] Department of Earth Systems Science, Stanford University

[5] Department of Environment and Natural Resources, Cheyenne River Sioux Tribe, Eagle Butte, South Dakota

ABSTRACT: We integrated aqueous chemistry, spectroscopy, and microbiology to identify chemical and microbial processes affecting the release of arsenic (As), iron (Fe), and manganese (Mn) from sediments contaminated exposed to aerobic and anaerobic conditions. The Cheyenne River Sioux Tribe in South Dakota has dealt with mining legacy for decades. Arsenic concentrations in contaminated sediments ranged from 96 to 259 mg kg^{-1}, and were found to co-occur with Fe and Mn. The highest release of As, Fe, and Mn was obtained when transitioning from aerobic to anaerobic conditions in batch

experiments reacted with glucose, compared to those reacted with phosphate. Pseudo-first-order leaching rates of As, Fe and Mn released were in the range of (0.522 to 0.859 days^{-1}) after cycling to anaerobic conditions in glucose amendments. Reduction of Fe and As was confirmed by aqueous speciation and by XPS and XANES analyses. Glucose acts an electron donor to support microbial reductive dissolution caused by anaerobic respiration and should be considered an important process for metal mobilization in systems transitioning from aerobic to anaerobic conditions. These results are relevant for natural systems, for surface and groundwater exchange or other systems in which metal cycling is influenced by chemical and biological processes.

4.1 Introduction

Arsenic (As) is a toxic and redox active metalloid that can affect humans by ingestion and inhalation pathways.[1, 2] A previous investigation of the Cheyenne River Watershed in South Dakota reported arsenic As concentrations in mining-impacted sediments ranging from 96-285 mg kg^{-1}, which are above the regional crustal average of 9-10 mg kg^{-1} As.[3-5] Although chemical mechanisms for arsenic release in the Cheyenne River Watershed were previously investigated,[3, 5, 6] the contribution of microbiological processes on arsenic release from the solids remains unknown. Differentiating between chemical and microbial mechanisms responsible for the release of As and other co-occurring metals, such as Fe and Mn, is difficult due to the complex nature of the environment. Thus, investigating the biogeochemical factors impacting the mobilization of As and other co-occurring metals from contaminated sediment is necessary for informing risk assessments.

Biogeochemical processes can affect the transport of As in the environment, influencing speciation in a range of oxidizing and reducing conditions. For example, As(V) ($H_2AsO_4^-$ and $HAsO_4^{2-}$) is the predominant species in solution in oxidizing environments, while As(III) (H_3AsO_3 and $H_2AsO_3^-$) is predominant in reducing environments. The redox transformations of As are important due to the higher toxicity and increased mobility of As(III). Arsenic solubilization from contaminated solids can result from the following main processes: i) competitive ion displacement with ions such as phosphate and silicate[3, 7], ii) limited adsorption onto media or desorption[6, 8, 9], iii) arsenate reduction to arsenite[10-12], and iv) reductive mineral dissolution from many forms of oxyhydroxides[13, 14] v) oxidation of As containing minerals. Arsenic co-occurs with other metals such as Fe and

Mn oxides.[10, 15] Microbiological mechanisms for As, Fe, and Mn reduction can occur by respiration or detoxification[16, 17].[18] Numerous microorganisms can withstand a wide range of oxidation-reduction potentials and use As, Fe, Mn and other metals as electron acceptors[14, 19, 20] Additionally, the transition to anaerobic conditions is a key driver in promoting As desorption through the reduction of As (V) or reductive dissolution of Fe-oxides[21]. Mn and Fe oxides have been reported to affect As mobilization in numerous studies by adsorption or oxidation of arsenite[22]. For instance, the coupled influences of As(V) reduction and reductive dissolution of iron (hydr)oxides is believed to be a principal control in porewater As concentrations[10]. The organic carbon source used by microbes as an electron donor for anaerobic respiration is an important factor influencing As(V), Fe(III), and Mn(VI) reduction[23].

Arsenic mobilization and exposure has gained attention from groundwater investigations related to naturally occurring As under anaerobic conditions in Vietnam, China and Bangladesh[24-27]. Completely aerobic or anaerobic conditions are described in experimental and field conditions. However, research on As release due to the transition between aerobic to anaerobic redox conditions with contaminated soil is limited. Environments that have surface and groundwater exchange, waterlogging, burial and resuspension provide an important context for these redox conditions. Labile organic matter in these zones can enhance the reduction and subsequent dissolution of Fe-oxides, which can release adsorbed As[28]. While the reduction of As, Fe, Mn have been investigated separately,[29-33] their simultaneous reduction in contaminated soils by native microorganisms can be further explored. Understanding these mechanisms will improve our knowledge of contaminant transport in contaminated environments.

The objective of this study was to evaluate chemical and microbiological processes affecting the release of soluble As, Fe, and Mn from contaminated sediments under laboratory controlled aerobic and anaerobic conditions. We used microbiological, chemical and spectroscopic techniques to understand microbially mediated As release from reacted sediments incubated under aerobic to anaerobic conditions in the presence of an electron donor. A novel aspect of this study consists of identifying the simultaneous As-Fe-Mn release under reducing conditions in contaminated sediments with the *in-situ* microbial community. While many redox investigations employ single microbial strains, the use of a mixed culture of native organisms from contaminated sediment provides environmental relevance.

4.2 Materials and Methods

4.2.1 Sample Collection. Contaminated sediment samples were collected from Cherry Creek riverbank coinciding with sample collection from a previous investigation in the Cheyenne River Watershed[3]. A hand trowel was rinsed with 10% nitric acid solution and used to collect half a gallon of sediment from the riverbank and bed. Samples were placed in one-gallon plastic bags and cooled to 4 C for shipment with water samples to UNM.

4.2.2 Solid chemistry. Sediment samples were dried for 12 h at 60°C in a controlled temperature oven. Dried sediment samples were crushed and homogenized using a shatter box. One gram of the dried sediment was weighted and added to a 50 mL digestion tube. All samples were acid digested (2.0 mL HNO_3 and 3 mL HCl) in triplicate to determine extractable elemental concentrations. The mixture was digested for 60 min at 65°C and then for an additional 60 min at 90°C. Digested samples were diluted with deionized water to 25 mL. The digested and diluted samples were filtered through a 0.45 µm filter to remove any particulate matter for aqueous elemental analyses. Dried solid samples from field and laboratory analyses were analyzed using X-ray photoelectron spectroscopy (XPS), X-ray fluorescence (XRF) and X-ray Adsorption Spectroscopy (XAS).

<u>X-Ray adsorption spectroscopy.</u> Oxidation and structural states of solid-phase arsenic were determined using XANES. The As K-edge XANES spectra were analyzed by linear combination fitting by using As(III), and As(V) standards. X-ray measurements for As were performed at Beamline 7-3 at the Stanford Synchrotron Radiation Laboratory. Samples were measured at the As K Edge in fluorescence mode using a 32 element Ge detector and a double crystal Si(220) Monochromator, calibrated at the first inflection point of, and Au metal foil absorption at, 11919.0 eV. Measurements were performed at room temperature. Powdered samples were placed on Al holders between two layers of Kapton tape. No beam damage was observed between measurements as spectra was consistent throughout different scans.

4.2.3 Aqueous chemistry. Aqueous chemical analyses were performed using inductively coupled optical emission spectrometry (ICPOES) (Perkin Elmer Optima 5300DV) and inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin Elmer NexION 300D-Dynamic Reaction Cell) in aqueous solutions for trace element content (As, Fe, and Mn). Samples for ICP-MS were filtered using a 0.45 µm Basix filter and preserved with 2% HNO_3. A Thermo Fisher Scientific Ion Chromatogram (ICS- 1100) was used to analyze 0.45 µm non-acidified water samples for major anion concentrations. Arsenic speciation was characterized using a Flexar 400 High-Performance Liquid Chromatography (HPLC) coupled with ICP-MS for As(III) and As(V)species. Samples were stabilized with a mobile phase solution (0.5 mM EDTA, 1 mM TBAoH and 1% MeOH) adjusted to pH 7(+0.01).

A CAPCELL PAK C18 column (250 mm 4.6 mm, 5 mm particle size) was used for the separation of target As species. Aqueous Fe(II) was measured by the ferrozine assay, and Fe(II) standards were prepared from ferrous ammonium sulfate (GFS Chemicals). All reagents used in the experiments and analyses were analytical grade.

4.2.4 Redox cycling experiments. To better understand the chemical and microbial driven release of As, Fe and Mn in the sediments, a series of batch experiments were performed. Batch reactors were conducted in 160 mL serum bottles sealed with butyl rubber stoppers and aluminum crimps. The bottles were inoculated with 135 mL basal medium and 10 g of riverbank sediment under aerobic and anaerobic conditions. Bottles for aerobic conditions were covered with a sterile cotton ball and gauze. Bottles for anaerobic conditions were flushed with a gas mix of N_2/CO_2 (80:20). Anaerobic conditions were confirmed by measuring Fe(II) and reduced H_2S using spectroscopic methods. Bottles were supplied with an exogenous electron donor at a concentration of 0.125 g COD/L as glucose. Control bottles (or reactors) were filled only with de-ionized water and basal medium. Additional assays were supplied with 10 mM PO_4 to evaluate the chemically mediated release as described in a previous study[3]. The fraction of As, Fe and Mn released was calculated by using the mass of the unreacted sediment, volume of solution in serum bottles and concentration of metals in sediment. All treatments were conducted in triplicate. All bottles were incubated for 336 h at 25°C with mechanical shaking at 130 rpm. Sampling was conducted using sterile needles and syringes.

4.2.5 DNA extractions. Sediment samples from bottles supplied with glucose were taken at 168 h and at the end of the experiment. One gram was also taken from the original soil sample. Details of DNA extraction have been previously described[34]. The analyses were performed by MR DNA Laboratory.

4.2.6 Data Analysis. Pseudo-first-order kinetics were used to represent microbial As, Fe and Mn release during batch experiments that after transitioning from aerobic to anaerobic conditions.

$$\frac{d[Metal\,released]}{dt} = k_1[Metal\,released] \qquad \text{(Eqn. 1)}$$

After separation of variables and integration of Equation (1):

$$ln[Metal\ Released] = k_1 * t \qquad \text{(Eqn. 2)}$$

concentrations of As, Mn and Fe with time were used to determine the pseudo-first order rate constant; points in the lag phase were excluded. Pseudo-first-order rate analysis was performed for measurements where three or more points were obtained. Previous studies have also used pseudo-first-order kinetics to represent rates of microbial transformation and release.

4.3 Results and Discussion

4.3.1 Initial Analyses of Contaminated Solids. Arsenic concentrations measured in contaminated sediment using acid digestions ranged from 96-259 mg kg^{-1}. These values exceed the regional background concentration of 10 mg kg^{-1} and are consistent with concentrations measured in a previous study near the site[3]. Acid-extractable Fe concentrations ranged from 21,000-22,005 mg kg^{-1} and Mn 682-703 mg kg^{-1}. XRF measurements show an abundance of Si, Ca, Al and Fe elements. The mineralogy of the solids for this study have similar characteristics from a study done by our group,[3] in which, the primary XRD patterns (**Figure S1**) were consistent with Quartz (SiO_2), Albite ($AlSi_3NaO_8$) and Calcite ($CaCO_3$). \As(V) was detected in these solids using XANES.

Mixtures of 52% As(V) and 48% As (III), and 80% Fe(III) and 20% Fe(II) oxidation states were detected in the near surface (5-10 nm) by XPS. The differences in percentages of As(V) versus As(III) are due to the fact that XANES detects bulk while XPS detects the near surface and these can be often different as shown in other studies.[3, 34, 35] Microbiological characterization of the sediment was conducted to better understand potential mechanisms for metal release.

4.3.2 Illumina DNA Sequencing of Microbial Community in Contaminated Solids.

The microbial community in sediments from Cherry Creek shows a high diversity of common soil microorganisms (*Clorophexi* 3%, *Actinobacteria* 3%, and *Bacillus* 1%) including nitrogen fixing (*Bradyrhizobium*, 1%) and ammonia oxidizers (*Nitrososphaera*, 3%) among the most predominant species. A number of arsenic utilizing bacteria have been also identified in mixed cultures collected from sediments from Cheyenne River Watershed, accounting for 0.3% of the total microbial communities. *Bacillis, Acidobacteria* and *Actinobacteria* are major phyla in meta-analyses of contaminated soils across the world[36, 37 38].

4.3.3 Redox Cycling Experiments.

Metal release: Reaction with De-ionized Water and Phosphate. Arsenic concentrations in solution measured from batch experiments reacting contaminated sediments with phosphate were at least five times higher than those reacted with de-ionized water under aerobic conditions. For instance, limited release of As (2.5 µM) was detected in batch experiments in which contaminated sediments were mixed with de-ionized water under aerobic conditions. However, a maximum concentration of 17 µM As was released from batch reactors supplied with 10 mM phosphate under aerobic conditions. Less than 2.7%

of the total As in the solids was released when mixed with de-ionized water, while a maximum of 43% of the total As was released after reaction with 10 mM phosphate under aerobic conditions (**Figure 3A, 3D**). Once release for As was obtained under aerobic conditions, the concentrations did not change substantially when batch reactors were transitioned to anaerobic conditions.

The maximum aqueous concentrations of Fe and Mn were comparable for batch experiments reacting contaminated sediments with de-ionized water (6.8 µM Fe and 1.8 µM Mn) and 10 mM phosphate (8.9 uM Fe and 0.8 µM Mn) under aerobic conditions. The co-occurring release of Fe and Mn increased after transitioning to anaerobic conditions, ranging from 20-61 µM (**Figure 2A, 2C**). Soluble Fe(II) measured in glucose amendments after cycling from aerobic to anaerobic conditions were in the range of 5.5-968 µM. The highest concentration coincided with high releases of total Fe from glucose amendments after cycling to anaerobic conditions. A total 0.04% Fe and 0.2% Mn was released from the sediments after reaction with de-ionized water, while a maximum release of 0.1% of the total Fe and 7.2% of the total Mn was released after reaction with 10 mM phosphate after transitioning to anaerobic conditions (**Figure 3A, 3D**).

Phosphate-induced release of As from contaminated sediments was higher than DI water and glucose solutions under aerobic conditions due to competitive ion displacement, which is a chemical mechanism studied in previous work by our group[39]. The mobilization of As can be explained by the presence of PO_4 under aerobic conditions since they are competing oxyanions for surface sites with similar pKas[3, 7, 40]. Lower magnitudes of soluble Fe and Mn release from phosphate incubations and de-ionized water confirm that ion displacement is the mechanism for As release.

Microbial metal release as a result of Glucose addition. The addition of glucose as an electron donor for metal reduction resulted in maximum concentrations of As released from contaminated sediments in experiments cycled from aerobic to anaerobic conditions. A considerable release of Fe and Mn was observed in experiments amended with glucose under anaerobic conditions only, or when transitioning from aerobic to anaerobic conditions. Metal concentrations in solution increased under reducing conditions due to microbial respiration and were at least 10 times higher than those obtained from the chemical experiments explained in the previous subsection. For example, maximum concentrations of 69 µM As, 1,559 µM Fe and 276 µM Mn were obtained in experiments amended with glucose cycled from aerobic to anaerobic conditions. In addition, the fraction of metals released were at least twice as high as those released following addition of phosphate (**Figure 3**).

Limited metal release was detected in batch experiments reacted with glucose under aerobic conditions. The metal release observed in experiments reacted with glucose was comparable to that obtained after reaction with de-ionized water under aerobic conditions. Both glucose and de-ionized water are weak chemical reactants under aerobic conditions, as suggested by the maximum percentage released in **Figure 3.** Total metal release (As, Fe, Mn) ranged from 0.46-61 µM from phosphate experiments compared to glucose experiments (4.7-1,558 µM). Overall, glucose amendments released more metals from the contaminated solid compared to released following phosphate addition.

The transition from aerobic to anaerobic conditions enabled the microbial community in the contaminated sediments to use As, Mn, and Fe as electron acceptors with glucose as an electron donor. In addition, several field experiments[41-43] and laboratory microbial batch incubations under anaerobic conditions[25, 33, 44-46] have shown that addition of biodegradable organic matter, such as glucose, to aquifer sediments leads to arsenic release to groundwater. Our results indicate that glucose, an exogenous electron donor, can support the reductive dissolution of mineralized forms of As and Fe from contaminated sediments.

Pseudo-first-order rate constants were obtained for As, Fe, and Mn released in experiments cycled from aerobic to anaerobic conditions amended with glucose. The pseudo-first-order rate constants (k_1) were only obtained for experiments cycled from aerobic to anaerobic conditions amended with glucose which yielded the highest metal release for all experiments conducted in this study. The k_1 values obtained for As, Fe, and Mn range from 0.552 to 0.859 (**Figure S2**). The R^2 values obtained for the regressions for the pseudo-first-order rate analysis of Ln[Metals] were all greater than 0.95. The k_1 values obtained for this study were comparable within the same order of magnitude to those measured in previous microbial reduction studies for Fe and Mn[47,18,20]. For instance, the kinetics of microbial Fe(III) reduction during anaerobic incubation of wetland sediments followed first order rates (0.075-0.138 d^{-1}) in the presence of organic matter[48]. Recently, similar magnitudes of As(V) microbial reduction rates were obtained using a kinetic model for systems with microorganisms and Fe-oxides[49]. Time-dependent microbial reduction rate coefficients in experiments with As, Fe oxides and microorganisms highlight the importance of differentiating between chemical, microbial and physical mechanisms for kinetic investigations[50].

The highest release of As occurred during experiments with glucose amendments and cycling conditions compared to unamended or non-cycled incubations. Iron concentrations remained stable until being cycled to anaerobic conditions, after which a maximum of 1335 μM was reached. The mobilization of As and co-occurring metals from anaerobic environments have been previously investigated using natural field samples and controlled laboratory incubations[51-53]. Additional analyses were conducted to assess the speciation of As in reacted sediments.

As speciation in reacted contaminated solids amended with glucose. The results from linear combination fitting of As K-edge XANES spectra indicate that As reduction occurred in the solids after exposure to anaerobic conditions. The unreacted sample is dominantly As(V), as suggested by linear combination fits using Scorodite (FeAsO4) and adsorbed As(V). Additionally, linear combination fits were performed to the Control, Anaerobic and Aerobic-Anaerobic samples using the unreacted sample and As(III)-sorbed as end members. to better understand the transformation from the unreacted As(V) to As(III). After exposure to anaerobic conditions, 64% As(III) was detected and was comparable to 51% in the aerobic-anaerobic scenario, especially given that the error in the linear combination fitting of XANES spectra is within 10%. The reduction of As observed in the solid analyses is consistent with the release of As and Fe observed in the aqueous chemistry analyses which supports that anaerobic respiration can cause the reductive dissolution of As and Fe minerals in contaminated sediments.

4.4 Biogeochemical Considerations.

Previous studies have reported that microbial reduction of As, Fe and Mn bearing solids can result in the release of these metals under various redox conditions. The potential processes driving metal release from the contaminated sediment are: 1) the transition from aerobic to anaerobic conditions induces microbial reduction of As, Fe and Mn from contaminated sediment in experiments amended with glucose, an electron donor; and 2) the influence of competitive ion displacement can release surface bound As under aerobic conditions. The release of As from contaminated sediments by microbial reduction, was 74%, which was higher than that observed by ion displacement as observed in experiments reacted with phosphate (43%). The release of As, Fe, and Mn reacted with water and glucose are comparable under aerobic conditions because glucose is a very soluble neutral molecule and not adsorbed to mineral surfaces.. Therefore, the concentrations released aerobically (0-48 h) after reaction with glucose and water represent a labile fraction.

The transition from aerobic to anaerobic conditions supports microbial reduction of As, Fe and Mn. For instance, the release of As vs. Fe follows a linear trend (R^2=0.967) in the anaerobic experiments cycled from aerobic to anaerobic conditions amended with glucose, suggesting that the reductive dissolution of As- and Fe-bearing minerals occurred (**Figure S3A**). Reduction from As(V) to As(III) of the contaminated sediments was confirmed in the bulk solids using XANES. Microbial reductive dissolution of As, Fe and Mn can depend on oxidation-reduction potential, electron donor source, microbial community composition and solution chemistry. A wide range of Fe, Mn and As reductive dissolution rates have been reported.[54-56] (add mn paper and 1-2 citations) The glucose incubations from our experiments released a higher proportion of Fe compared to As under completely anaerobic conditions. Similarly, another study reported a higher release of Fe compared to As concentrations released from sediments obtained from the Mekong delta in batch experiments amended with glucose due to reductive mineral dissolution.[57] The extent of As release in completely anaerobic conditions can be doubled by the addition of glucose by reduction of As(V) and Fe(III) oxides, compared to incubations without an electron donor.[58] These reductive processes are known to be key drivers of As release to solution from the solid phase[10, 59, 60].

In agreement with our findings, the reductive dissolution and increases in metal release have been reported in redox-sensitive studies amended with electron donors such as acetate, glucose and lactate.[19, 46, 49, 61] For example, As, Fe and Mn release rates obtained from a contaminated sediment under anaerobic for a system amended with lactate had a similar release.[52]. However, the order of redox cycling of anaerobic to aerobic was the opposite of that evaluated in our study (aerobic to anaerobic as shown in **Figures 1** and **2**). Similar to the microbial reduction illustrated in our study, dissimilatory As reduction mediated by microorganisms (e.g. *Shewanella, Geobacter*) can contribute to As enrichment in groundwater or other reduced zones[21, 23, 49, 61].

In contrast, the release of As and Fe did not follow a linear trend in experiments reacting contaminated sediments with 10 mM phosphate (R^2=0.357) (**Figure S3B).** In a previous study, As was preferentially released compared to Fe, indicating that secondary ion displacement was a predominant process for release under aerobic conditions.[3] The limited release of As and Fe was also not linear in experiments reacting contaminated sediments with glucose under aerobic conditions (R^2=0.1994), confirming that the release of As and Fe corresponds to a labile fraction. This result is consistent with other studies that reacted de-ionized water and weak extractants with sediments from the watershed[3, 5, 62]. Limited Fe and Mn was released in experiments reacting contaminated sediments with de-ionized water, glucose and phosphate under aerobic conditions, corresponding to a labile fraction.

Many oxyanions, like phosphate, can interact with the surface of Fe-oxides and affect adsorption of As. Secondary ion displacement of As(V) with phosphate is a proposed mechanism of As release from Fe-oxide phases under aerobic conditions.[63-65] Competitive ion displacement due to reaction with phosphate accounted for 43% As released compared to 76% released during glucose amendments. The presence of similar high-affinity anions for surface sites on Fe-oxides, such as phosphate, silicic acid, and bicarbonate can contribute to the mobility of As under oxidizing conditions.[7, 66-68] However, release of As, Fe and Mn due to reductive dissolution is observed in various studies, compared to only aerobic As release due to secondary ion displacement[42, 49, 66].

The higher release of Fe can be partially explained by the high initial concentration of Fe in the sediment compared to Mn and As (**Figure 3**). However, although the Fe concentrations were the highest in solution compared to As and Mn, only 7% of the total Fe was present as identifiable mineral phases, such as albite and grunerite, as indicated by XRD (**Figure S1**). Although the As concentrations released in solution were lower compared to Fe and Mn, 74% of the As was released after the biogeochemical conditions evaluated in this study. These results indicate that As was the most amenable to release under the chemical and microbiological conditions tested in this study. The limited Fe and Mn release from phosphate incubations in our study compared to glucose amendments confirm that different processes were affecting the releases of As, Fe and Mn.

4.5 Environmental Implications

High concentrations of As in riverbank sediment is a concern for nearby communities given that the Cheyenne River watershed receives sediment loads from the Homestake Mine upstream. This study investigates the role of microorganisms on tribal land, which, to our knowledge, has not been previously studied. The transformation of complex metal mixtures influenced by the *in-situ* microbial community can be overlooked by using synthetic solids or well-known reducing microbial isolates. The conditions investigated in this study highlight the environmental relevance of microbial redox cycling in contaminated sediments from the Cheyenne River watershed on tribal land. In addition to chemical mechanisms affecting As release as reported in previous studies,[5, 6, 62] the reductive dissolution of As, Fe, and Mn catalyzed by native microorganisms and redox cycling in contaminated sediments is also important to consider. The potential for reductive dissolution of As and other metals is also suggested in other studies as a driving mechanism from reduced water-logged soils.[52, 69] Future studies are necessary to investigate the role of plants and organic matter in soils to better understand pathways of exposure to As.

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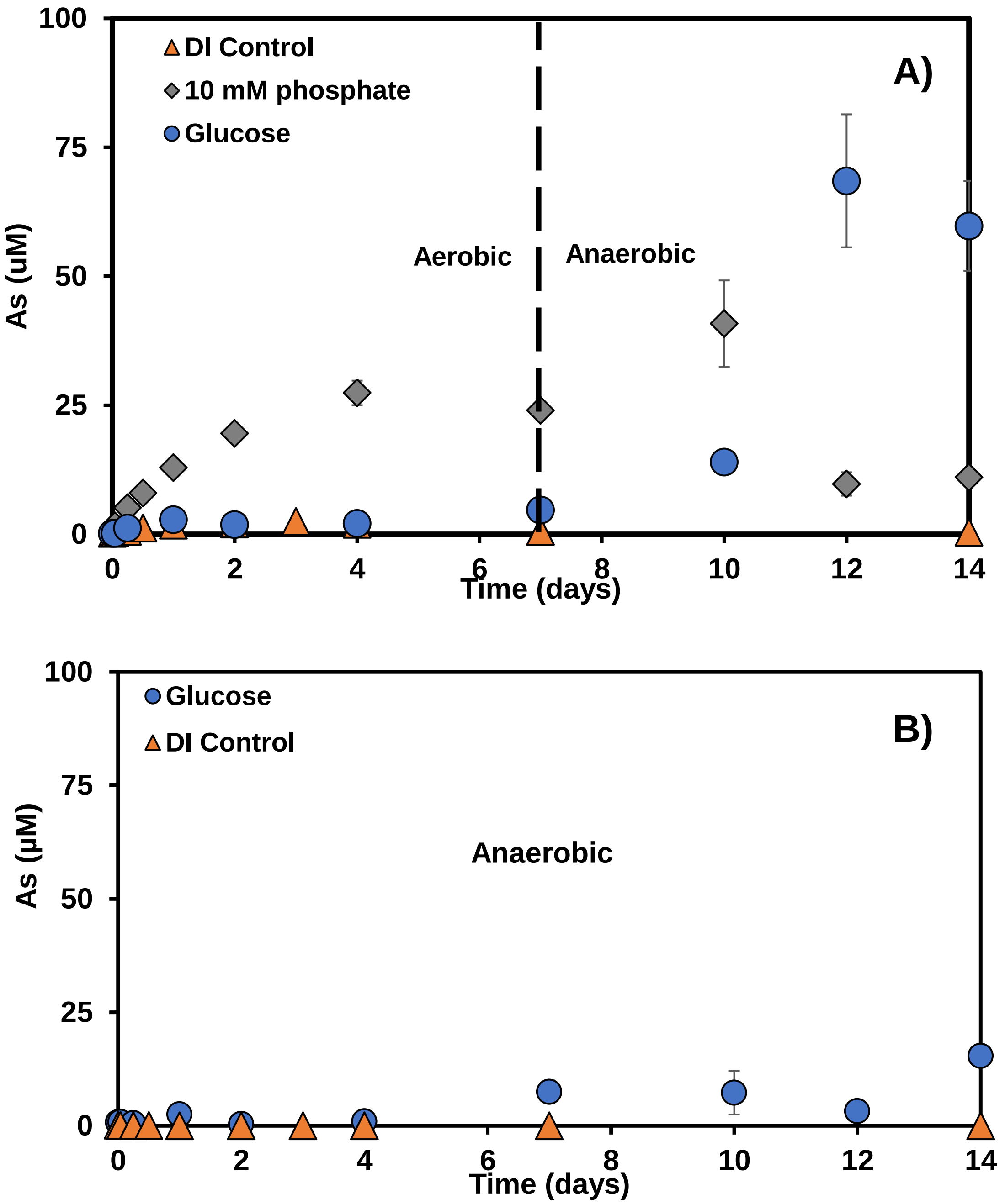


Figure 1. As release under **A)** Aerobic to Anaerobic cycling conditions and **B)** Completely Anaerobic Conditions

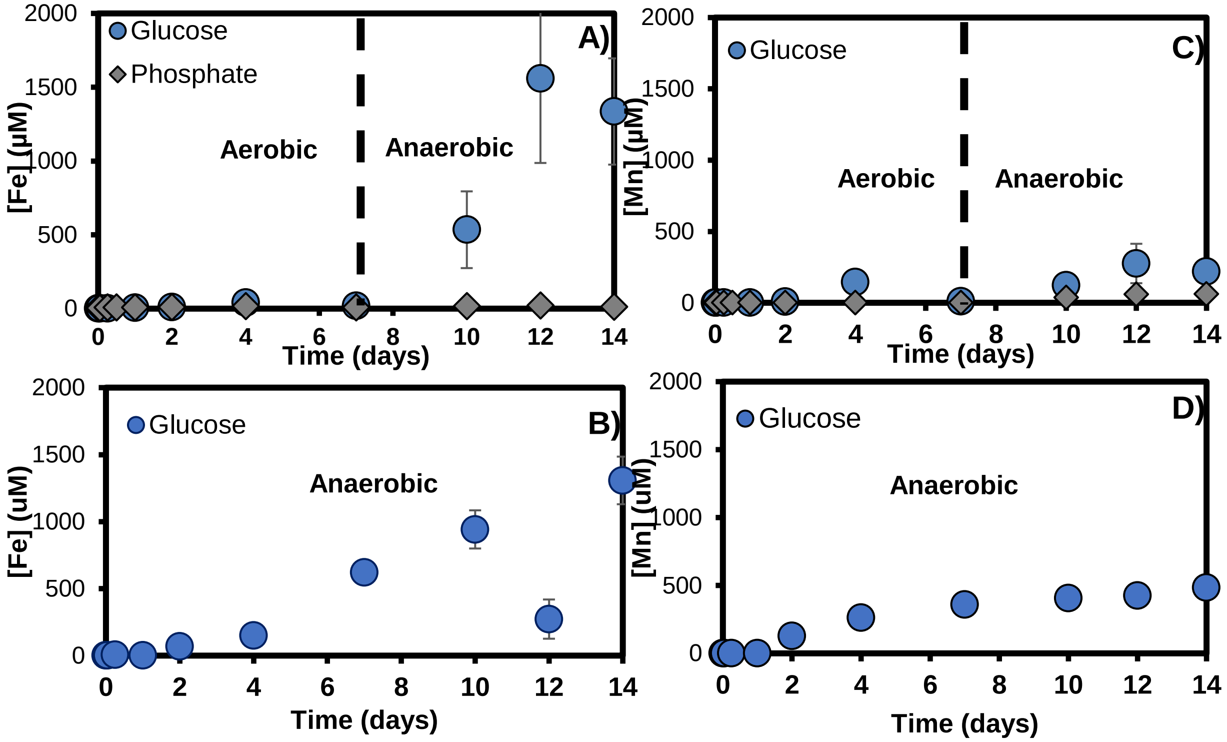
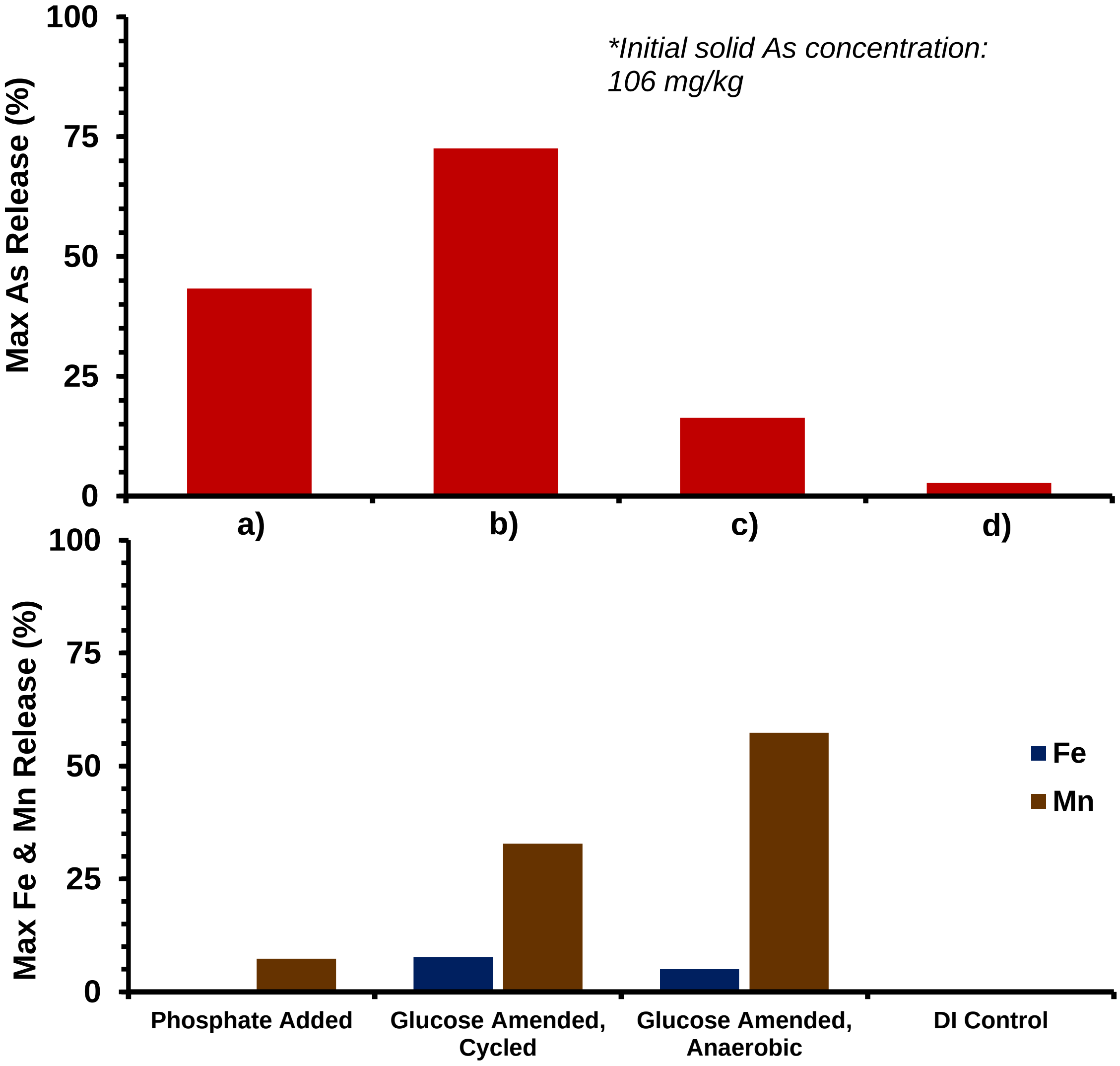


Figure 2. Fe release under **A)** Aerobic to Anaerobic cycling conditions and **B)** Completely Anaerobic Conditions; Mn release **C)** Aerobic to Anaerobic cycling conditions and **D)** Completely Anaerobic Conditions



Initial Solid concentration		
As (*mg/kg*)	Fe (*mg/kg*)	Mn (*mg/kg*)
106	22,000	693

Figure 3. Max release (%) under a range of biogeochemical conditions: **a)** Aerobic cycled to anaerobic with 10 mM phosphate; **b)** Aerobic cycled to anaerobic amended with 0.125 g/L glucose as an electron donor; **c)** Completely Anaerobic amended with 0.125 g/L glucose; **d)** Aerobic cycled to anaeobic with de-ionized water

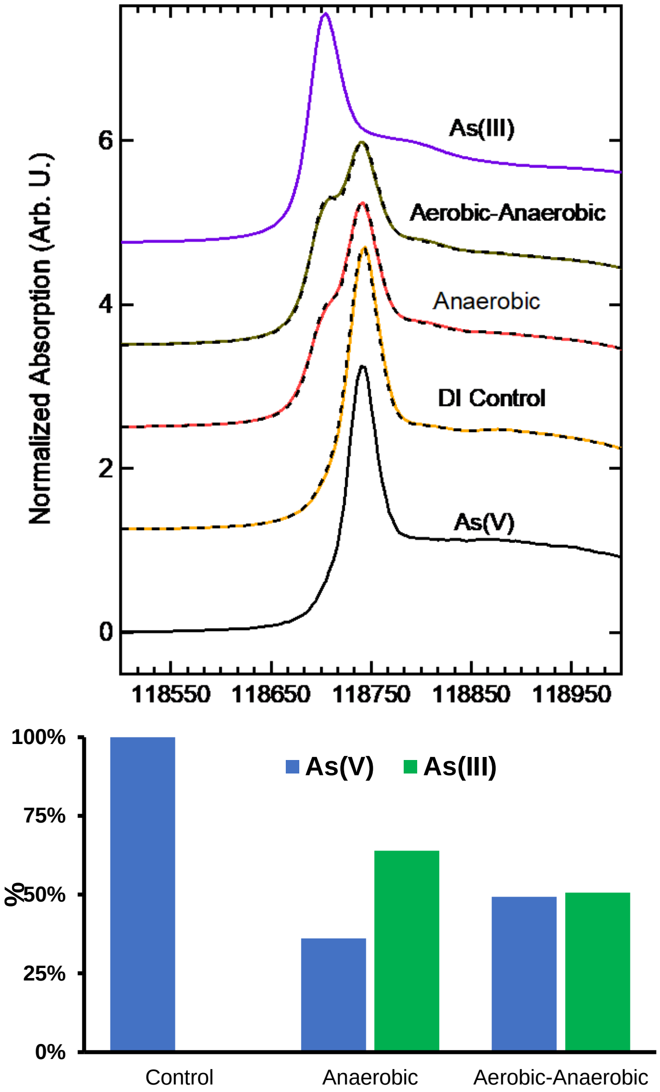


Figure 4. As-K edge XANES analysis for control, anaerobic, and aerobic-anaerobic conditions.

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Chapter 5

5. Arsenic uptake in hydroponically grown *Schizachyrium scoparium* *(*Little Bluestem) amended with root colonizing endophytes

Cherie L. DeVore[1*], *Eliane El Hayek*[1], *Taylor Busch*[1], *Benson Long*[1], *Paul Owen-Smith*[2], *Michael Mann*[2], *Jennifer Rudgers*[2], *Abdul Mehdi Ali*[3], *Tamara Howard*[4], *Michael Spilde*[3], *Adrian Brearley*[3], *Carlyle Ducheneaux*[5], *José M. Cerrato*[1]

[1] Department of Civil, Construction & Environmental Engineering, MSC01 1070, University of New Mexico, Albuquerque, New Mexico 87131, USA

[2] Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA

[3] Department of Earth and Planetary Sciences, MSC03 2040, University of New Mexico, Albuquerque, New Mexico 87131, USA

[4] Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, University of New Mexico, Albuquerque, New Mexico 87131, USA

[5] Department of Environment and Natural Resources, Cheyenne River Sioux Tribe, Eagle Butte, South Dakota

Abstract: The mechanisms affecting As uptake in these plants used by the community remain poorly understood. We used microscopic, spectroscopic, microbial culturing and molecular biology, and aqueous chemical techniques to evaluate arsenic (As) uptake in hydroponically grown *Schizachyrium scoparium* inoculated with endophytic fungi. After addition of 0, 0.5, and 2.5 mg L^{-1} As(V), an 80% decrease of As was detected in the hydroponic solution within the first 12 hours, indicating that As was removed from solution under all conditions investigated in this study. Confirmation of endophyte colonization of *Fusarium chlamydosporum species complex 4-b* and *Cadaphora species* were confirmed in rootlets within 14 days after inoculation using bright field microscopy and TEM. The presence of endophytes supported better external and vascular cellular structures compared to un-inoculated rootlets (control). TEM/EDS and X-Ray STEM mapping found low concentrations of As in the cellular structure of exposed plants, but it does not appear to be associated with particles. Chemical equilibrium modeling suggests that the presence of calcium phosphate minerals in the hydroponic system could lead to possible As adsorption through surface complexation and/or co-precipitation. Thus, the decrease of As concentrations may have occurred due to uptake, with or without fungi, or through adsorption and precipitation of As. The results of this study have implications for remediation and risk assessment applications for the biological and physical-chemical processes affecting the uptake of As in plants.

5.1 Introduction

Uptake of arsenic (As) by plants grown in sediments from the Cheyenne River has been a community concern due to elevated concentrations of As found in riverbank sediments. Fruiting trees and medicinal plants that grow from these sediments are used by the surrounding community for food and ceremonial practices, thus presenting possible As exposure pathways.[1, 2] However, the mechanisms affecting As uptake in these plants remain unclear. Understanding potential exposure pathways is necessary to enhance risk reduction strategies for communities living near these sites. The accumulation of As in plants can aid in developing strategies for limiting exposure routes to the environment and to humans.

There are several mechanisms of As tolerance, detoxification and accumulation in plants, including: low rate of As(V) uptake, efflux of accumulated As, transformation of inorganic As to less toxic organic forms, complexation and sequestration to non-reactive locations.[3, 4] The mechanisms for tolerance in plants varies in different plants. For example, a tolerant plant may utilize strategies to limi As uptake in shoots, while a hyperaccumulator has mechanisms to facilitate As uptake and translocation in shoots. Further, plants may utilize one or multiple strategies for tolerance. Plants growing in heavily As-contaminated soils (such as *Holcus lanatus* and *Cytisus striatus*) can achieve hyper-tolerance by decreasing the rate of As uptake. Additionally, volatilization or efflux of accumulated As can reduce total As present in a plant. Conversion of inorganic As species to volatile methylated As compounds occur in bacteria and fungi and are proposed to exist in plants as well.

The colonization of the rhizosphere by microorganisms represents a natural process commonly found in various types of soils, including those affected by agricultural and mining activities [5, 6]. For instance, plants which promote rhizobacteria have been identified in contaminated rhizosphere and reduced phytotoxicity in rice seedlings grown in As impacted soil.[7] Many As resistance studies highlighting volatilization and accumulation mechanisms have focused on rice plants because of their importance as a major food commodity, but also due to the fact that much rice is cultivated in regions where soils contain high natural As concentrations.[7, 8] Plants also interact with symbiotic organisms such as mycorrhizal fungi (ecto- and endomycorrhizae), rhizobial and endophytic bacteria, plant growth promoting rhizobacteria and endophytic fungi.[9, 10] Many studies focus on arbuscular mycorrhizal (AM) symbiosis, which is one mechanism can mediate the oxidation and reduction of As, transform inorganic As into a less toxic organic form, and enhance plant growth to dilute the concentration of accumulated As in plant tissues.[11] [5]

Endophytic fungi occur in almost every plant in the natural environment and contribute to plant growth and tolerance to environmental stress conditions. They are mutualistic symbionts that grow in the intercellular spaces of plant tissues and are transmitted by seeds.[12] For example, dark septate endophytes (DSE) are known to colonize the root tissues of plants. Endophytes can provide multiple benefits to tall grasses, such as tall fescue. These increased drought tolerance, pathogen resistance, nutrient acquisition such as phosphorous, and overall plant vitality.[13-19] These DSE in the seedlings of some grasses have been isolated but not under the environmental stress of metals such as As.

Fungi in the phylum Ascomycota dominate heavy metal contaminated sites worldwide. For example, ascomycota endophytic fungi was the most abundant fungal phylum in a gold mining site and ascomycetes generally have high abundances in contaminated sites relative to uncontaminated sites.[20-22] Several ascomycota have been studied with heavy metals that exhibit high tolerance and interact with metals through biosorption, bioprecipitation, or bioreduction and volatilization.[23-26] Commonly studied ascomycetes with As and other metals include Aspergillus, Fusarium, and Talaromyces.[25, 27-31] These taxa are also commonly found in symbiosis with plant roots.

Though fungi can associate with many plants, the mechanisms of As uptake in native grasses, such as *Schizachyrium scoparium* (little bluestem), from prairielands located in central United States are not understood. To our knowledge, limited studies exist to evaluate the role of endophytic fungi on As bioavailability from agriculturally relevant plants grown in the Cheyenne River Watershed, which justifies the need for this study. Most current As uptake and bioavailability literature focus on cereal crops and hyperaccumulating fern species.

In this study, As uptake in *Schizachyrium scoparium* was investigated because of its widespread distribution in the watershed. Many similar grasses are also used in sweat ceremonies and smudging by the local communities. The objective of this study was to determine the effect of dark septate endophytes (DSE) on As uptake using laboratory hydroponic experiments. However, we can also improve knowledge of interfacial processes that affect As uptake in economically and culturally relevant plants in the presence of endophytes since chemical and biological uptake mechanisms in *Schizachyrium scoparium* is not well understood.

5.2 Materials and Methods

Germination and Colonization of Endophytes. Plants were grown from seeds obtained from southwestern US regional seed stock (Curtis and Curtis, Clovis, NM; Western Native Seed, Coaldale, CO), with five replicates. The palea and lemma around the seeds of little bluestem (*Schizachyrium scoparium)* were first removed, which can house native fungal communities. The seedlings were internally sterilized using a 55°C water bath for 15 minutes. External sterilization consisted of using a 70% ethanol soak for 2 minutes followed by 2% NaHO soak for 3 minutes. Seeds were rinsed four times using autoclaved MilliQ water before inoculation with fungal isolates. The seeds were inoculated with two dark septate endophyte species, *Cadaphora and Talaromyces pinophilus*. In addition, five replicates of each seed were placed in a 5:1 soil slurry using sediment from the riverbank. Little bluestem is a bunchgrass with extensive root systems that need soil or sand substrate to support plant growth. Thus, the seedlings were placed in sterilized sand media for a germination period before moving to the hydroponic system. Little bluestem seeds were placed in autoclaved sand for five weeks at room temperature and watered with sterile MilliQ water.

Hydroponic experiments. After germination, the seedlings were moved to a sterile hydroponic system supplied with the required plant nutrients under 25°C (day) and 21 °C night temperature in 120h/6h light cycle for the sunflowers. A critical factor in growing little bluestem is length of day. One study in North Dakota required more than 18 hours of artificial light each day for growth of little bluestem plants during the winter months (USDA, 1983). The 6 month-old seedlings from the hydroponic system were then acclimatized to conditions relevant to the Cheyenne River for 5 days by placing each one of them separately in sterile Nalgene polypropylene (PP) bottles in 500 mL conditioning solution. Conditioning solutions were freshly prepared in sterile ultrapure water containing simplified Hoagland nutrients (MgSO4, 0.5 mM; NH4NO3, 2 mM; KCl, 1 mM; NaHCO3, 5 mM; KH2PO4, 0.12 mM and CaCl2.2H2O, 3 mM) at pH 7.0. After 5 d, plants were exposed to 0 ppm, 0.500 ppm, and 2.5 ppm Na_3AsO_4 for seven days before harvesting for chemical and microbial analyses. Aliquots were collected from the hydroponic solution at t=0, 12, 24, 48, 96 and 168 hours for chemical analyses.

Chemical Analyses. Aliquots collected from the hydroponic solution were acidified using 2% ultrahigh purity nitric acid (HNO3), and total As concentration was measured with a PerkinElmer NexION 300D (Dynamic Reaction Cell) Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), with a detection limit < 0.5 µg L-1. Laboratory blanks and QA/QC measures were taken and checked to ensure quality data. Plants from the hydroponic exposure were separated into roots and shoots at the end of the experiment and washed three times with Ultrapure MilliQ water. Whole, root and stem fresh weights were recorded before being placed in a temperature-controlled oven at 60 C for 12 hours before pulverizing for acid digestions. Dry weights were also recorded. Roots and shoots were acid digested in triplicates under progressive heating using 10 mL reagent-grade HNO_3. Following the acid digestion, samples were diluted and filtered using a 0.45 µm filter for As analyses using ICP-MS.

PCR and Illumina Sequencing. We utilized a two-step amplification protocol in which the DNA were first amplified with fungal-specific primers (ITS2 region) and then amplified with general primers appended with barcodes. The first PCR round used 25 µl reaction mixtures with 5 µL of template DNA (plant roots), 0.25 µL of 5.8S-FUN and

ITS4-FUN primers, 0.5 µlL of 10 mM dNTPs, 5 µL of Phusion HF buffer, 0.25 µL of Phusion High Fidelity DNA polymerase, 14 µL of ultrapure water. The thermocycling conditions consisted of initial denaturing at 98°C for 30 seconds, followed by 27 cycles of denaturation at 98°C for 10 s, 58°C for 10 s, 60°C for 4 min, and a final extension at 60°C for 20 minutes. The PCR products were treated with ExonucleaseI to remove first round primers and then amplified again using general primers with barcodes for multiplexing. The second PCR used 25 µl reaction mixtures with 5 µL of purified PCR products, 5 µL of barcoded primers, 0.5 µlL of 10 mM dNTPs, 5 µL of Phusion HF buffer, 0.25 µL of Phusion High Fidelity DNA polymerase, 9.25 µL of ultrapure water. The thermocycling conditions for the second round PCR were initial denaturing at 98°C for 30 seconds, followed by 7 cycles of denaturation at 98°C for 10 s, 58°C for 10 s, 60°C for 4 min, and a final extension at 60°C for 20 minutes. The PCR products were pooled and purified twice using a 4:5 ratio of Ampure volume to the library volume. Sequencing of these libraries was carried out using Illumina 2 X 300 bp extra-long-read kit on a full run of the MiSeq instrument.

Microscopy TEM/SEM. Cross section samples were prepared for Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) from the finer rootlets of the plant roots (**Figure S1**). Plants were fixed with osmium tetroxide to preserve membrane structure and increase contrast for imaging but there are known interferences with As and PO_4 detection. To account for the possible interference, tannic acid was also used as a stain and counterstained with uranyl acetate and lead citrate references. Microstructural and microanalytical studies of the FIB section were performed using a JEOL 2010F FASTEM field emission gun scanning TEM (FEGSTEM/TEM) instrument equipped with an Oxford AZtec EDS system using an Oxford X-Max N 80 mm2 ultrathin window SDD EDS detector. The FIB section was characterized using bright-field TEM (BF-TEM), high-angle annular dark-field (HAADF) STEM, electron diffraction, and STEM EDS X-ray mapping. Full spectral X-ray maps of the areas of interest were obtained and EDS spectra of regions were extracted from the X-ray maps using the AZtec software. Single spot EDS analyses were also performed on regions of interest after the STEM EDS X-ray maps were collected. Digital TEM and STEM images were acquired and processed using GATAN Digital Microscopy Suite© (DMS) imaging software. X-ray microanalyses from the STEM X-ray maps and point EDS analyses were quantified using the Oxford AZtec software utilizing the Cliff-Lorimer thin film approximation using theoretical k-factors.

<u>Chemical Equilibrium Modeling.</u> Visual MINTEQ was used to predict the expected aqueous As species and various complexes and precipitates that could form in solution during exposure. The aqueous As species were calculated using inputs based on the chemical composition of the conditioning and exposure solutions (**Table SI1**) with 0 mM (Control), 0.667 mM (low), 33.3 mM (high) As at pH 7.5.

5. 3 Results and Discussion

5.3.1 Inoculation of endophytes with plants. Colonization was observed at 14 days after inoculation with endophytes in plant growing chambers amended with sterile sand. Aseptate and septate hyphae, and arbuscules were observed within finer rootlets using Brightfield microscopy. Hyphae were the principal fungal structures observed under the cuticle, within the epidermal cells. Images also confirmed the localization of endophytes in vascular cells that varied in type and size. Overall, the presence of endophytes supported better external and vascular cellular structures compared to “control” rootlets not inoculated with endophytes (**Figure 2**).

Arsenic accumulation in hydroponic experiments. An 80% decrease in As concentration was detected in the hydroponic solution within the first 12 hours, indicating rapid As removal in all experimental systems (**Figure 3**). The decreased As concentrations could have occurred due to uptake in plants, with or without fungi, or due to adsorption and precipitation of As in the hydroponic system. The pH in hydroponic media ranged between 7.04 and 8.10 throughout the experiment. The biggest changes in hydroponic solution pH occurred in “Control” plants without fungi or soil inoculated over 168 hours, suggesting the influence of a chemical reaction on the decrease of As observed.

We used chemical equilibrium modeling to help understand relevant reactions affecting water chemistry in the hydroponic system. A summary of the aqueous chemical speciation and saturation indices using inputs based on the hydroponic solution used is provided in **Table S1**. The predominant As species was $HAsO4^{-2}$ and several Ca- and Mg-phosphate complexes were formed at the circumneutral pH range investigated in this study.

5.3.2. Microscopy results. Dark septate fungal communities associated with roots were detected in intracellular spaces of plants by TEM (**Figure 3A**). The largest clusters of endophytes were detected in grasses colonized with *Cadaphora species.* The TEM analyses of control plants without fungal inoculation also detected the presence of microorganisms, indicating that the application of heat, ethanol and hypochlorite in these control plants did not result in complete microbial sterilization (**Figure 3B**). TEM EDS analyses suggest that As may be present above background in cross-sectional rootlet samples of plants inoculated with endophytes (**Figure 4**). However, the As K line overlapped with the Os M line (Os was used for staining the plants), which makes it difficult to detect As. EDS and X-Ray STEM map show that there are low concentrations of As in the cellular structure, but it does not appear to be associated with particles. However, As was not detected in intracellular spaces from rootlet samples using SEM EDS (**Figure S1**). These results indicate that As may be heterogeneously distributed in the plant and that limited As made it into the intracellular spaces within the plant. In addition, the possible presence of a calcium phosphate precipitate in solution suggests that substitutions of PO_4^{3-} ions and other anions on the root surface can partly explain the limited As detected inside the plant. Analyses are currently being conducted on the plant root surface to further characterize the presence of As and of other solid phases that could have precipitated in the hydroponic experiments.

5.4 Mechanistic Insights. The results from this study indicate that biological and physical-chemical processes can influence As uptake in hydroponic systems. The results show that both endophytes and As exposure can affect root cell vitality and plant biomass. Seedlings inoculated with endophytes had longer root lengths and increased biomass. TEM also illustrates that plants with endophytes look healthier compared to control plants without endophytes. Control plants without endophytes exposed to high and low concentrations of As experienced inhibited root growth and in a few cases, resulted in death. When plants are exposed to toxic metalloids, the most common protection mechanism is limiting transport into the areal parts of the plant. The result is accumulation of metals primarily in the root system. Arsenate toxicity studies in plants have shown that non-tolerant species can undergo stress symptoms, which include inhibition of root growth, decreased biomass and death.[32-35] Further investigating the presence of endophytes with P can advance our understanding of As uptake and accumulation in plants.

The presence of phosphate in our hydroponic solution may also affect As uptake. The relative concentrations of P and As in the hydroponic solution is environmentally relevant. Arsenate has similar chemistry to phosphate and is taken up by plants via P transporter systems.[32, 36] Several studies have investigated arsenate uptake under P deficiency in barley, grasses, and ferns. [36-38] These studies suggest that limited P can enhance the uptake of As.

Chemical equilibrium modeling indicates that calcium phosphate minerals, such as hydroxyapatite, were likely to precipitate. The precipitation of calcium phosphate minerals can lead to As(V) immobilization based on the solution composition of the system. Several studies have showed that metallic contaminants can be immobilized due to (a) adsorption through surface complexation with phosphate, calcium and hydroxyl surface groups of hydroxyapatite, and (b) co-precipitation of new partially soluble phases.[39, 40] The possible adsorption through surface complexation and co-precipitation could affect the uptake of As in the hydroponic system.

Plants inoculated with fungi had healthier plant roots and increased biomass compared to control plants without fungal endophytes (**Table 1**), which can partly explain the tolerance to As in healthier plants due to: a) dilution and heterogeneous distribution of As throughout the plants with higher biomass; b) more surface area of rootlets in healthier plants to accommodate adsorption/precipitation reactions. More data are necessary to better understand the specific contribution of biological and physical-chemical processes on As uptake in the hydroponic systems studied.

5.4 Environmental Implications. The results from this study have relevant implications for mobility and transport of As in plants. The uptake of As was observed in all conditions tested in this study. For example, endophyte-colonized grasses removed As and were the healthiest at the end of the experiment. Thus, a similar degree of As removal was observed in all conditions, the presence of endophytes can provide favorable growth conditions for *Schizachyrium scoparium.* The precipitation of calcium phosphate minerals could also contribute to As removal. Future investigations are necessary to further validate the symbiosis of endophytes and plants and the integration with precipitation processes for As removal in contaminated field sites.

The results from this study also have important implications for risk assessments. Arsenic accumulation in grasses like *Schizachyrium scoparium* may be a concern due to its toxicity to animals and humans. The root is the first plant contact with potentially harmful elements in the rhizosphere. The simplified conditions represented in this hydroponic study represent an initial effort to understand As uptake in *Schizachyrium scoparium*. The effects of soil organic matter and redox conditions on As accumulation remain subject to future investigations. Future studies are necessary to investigate the role of plants and organic matter in soils to better understand pathways of exposure to As.

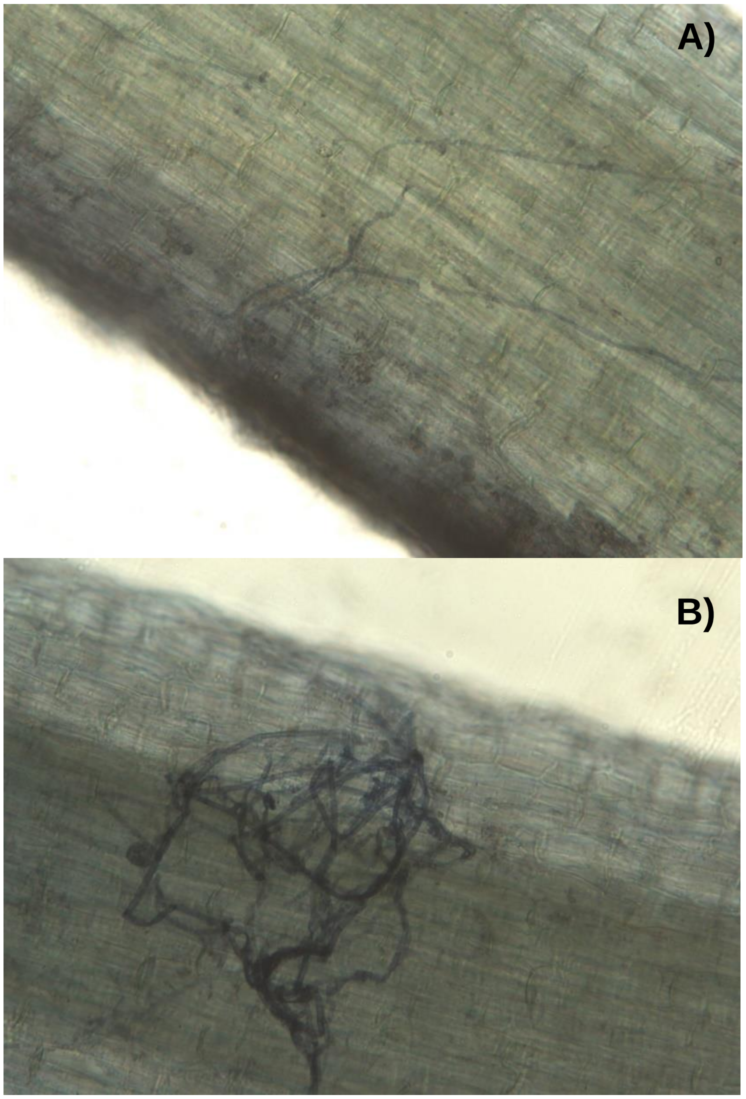

Figure 1. Light microscope image of hyphae of Dark Septate Fungi in Little Bluestem grass root (20x magnification).

Isolate	**Biomass (avg)**	**Root length (avg)**
Control, no fungi	1.60 g	22.16 cm
Fungal Isolate 1	18.15 g	60.33 cm
Fungal Isolate 2	35.33 g	53.66 cm

Table 1. Summary of biomass and root lengths of plants grown in hydroponic system.

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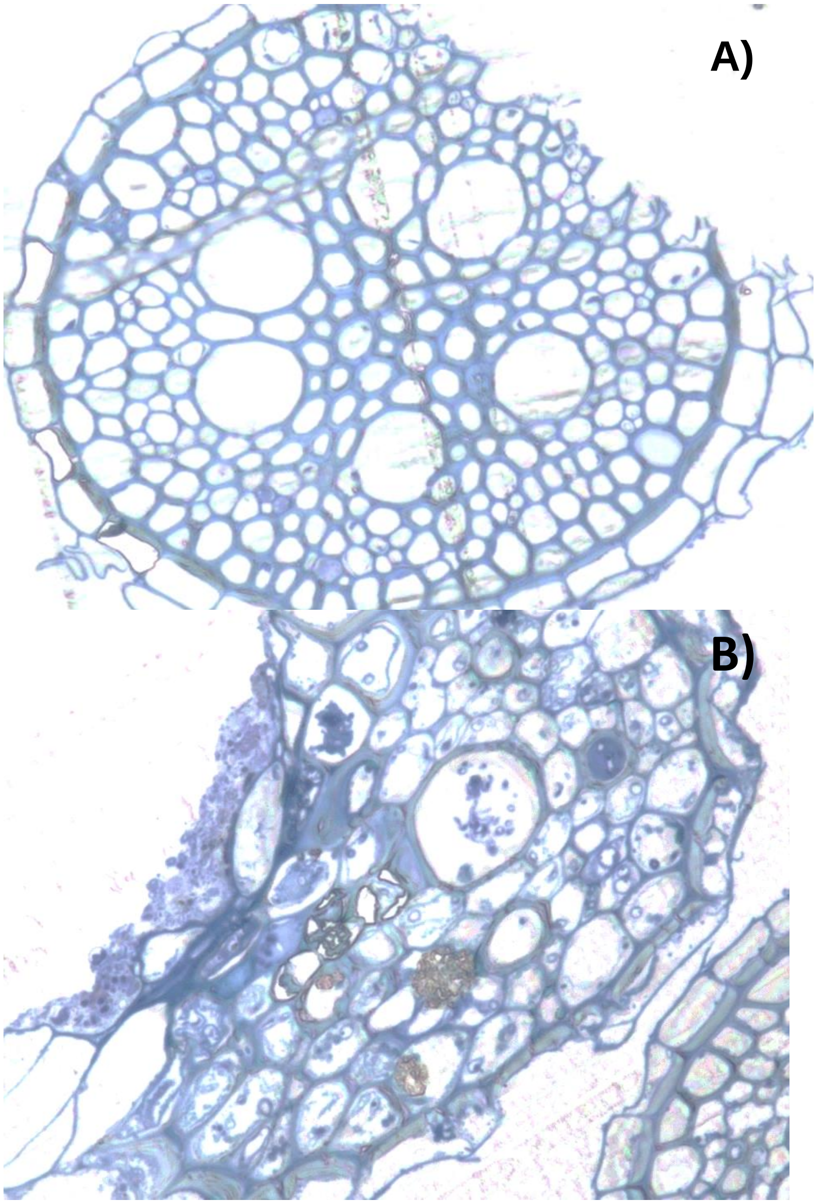

Figure 2. Brightfield microscopy images of **A)** Cross section of root inoculated with fungi; **B)** root cross section of grass without fungi

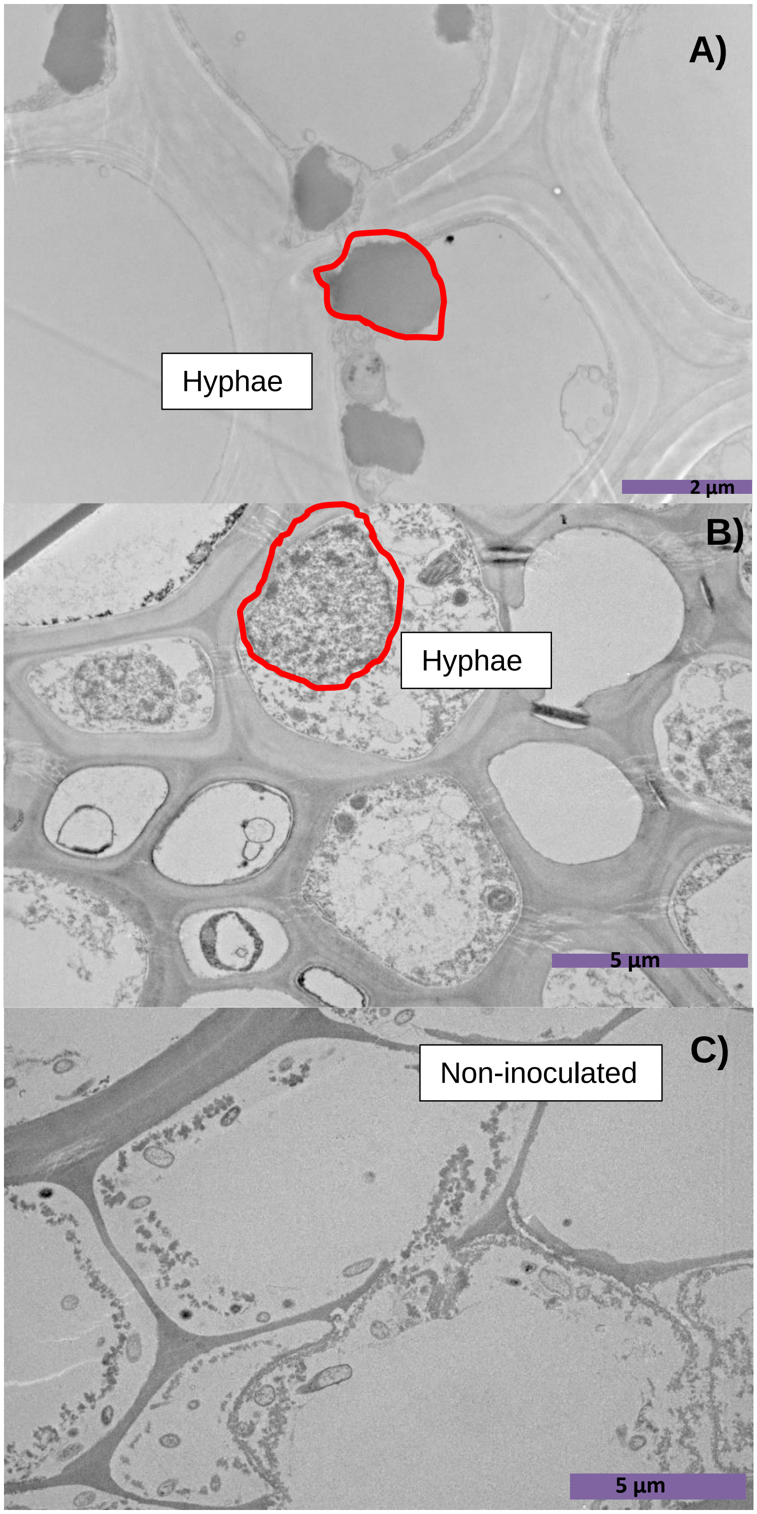

Figure 3. Transmission Electron Microscopy (TEM) image of hyphae (dark spots, red outline) in root cross sections of Little bluestem using **A)** Os stain and **B)** Tannic Acid counterstained with uranyl acetate and lead citrate **C)** Non-inoculated using Tannic Acid counterstained with uranyl acetate and lead citrate.

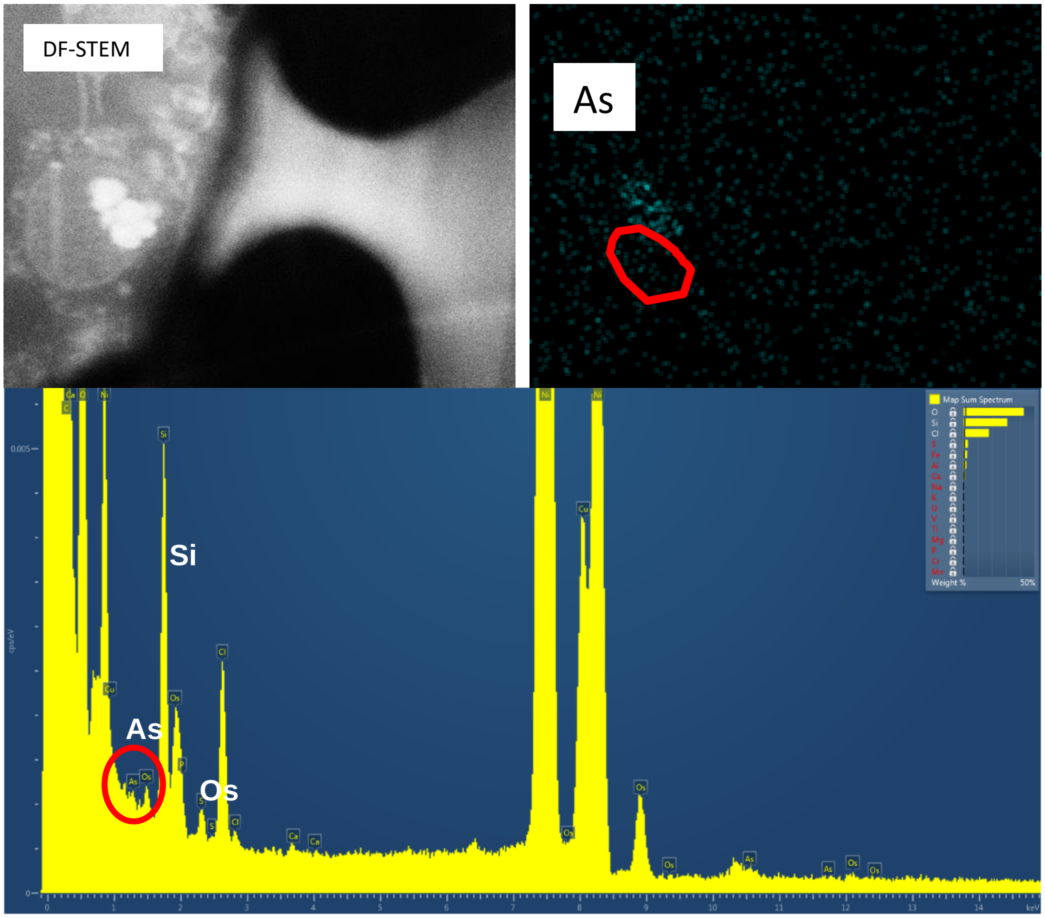

Figure 4. EDS and STEM X-ray map of grass rootlet cross-section colonized with endophyte indicating low As detected inside of root.

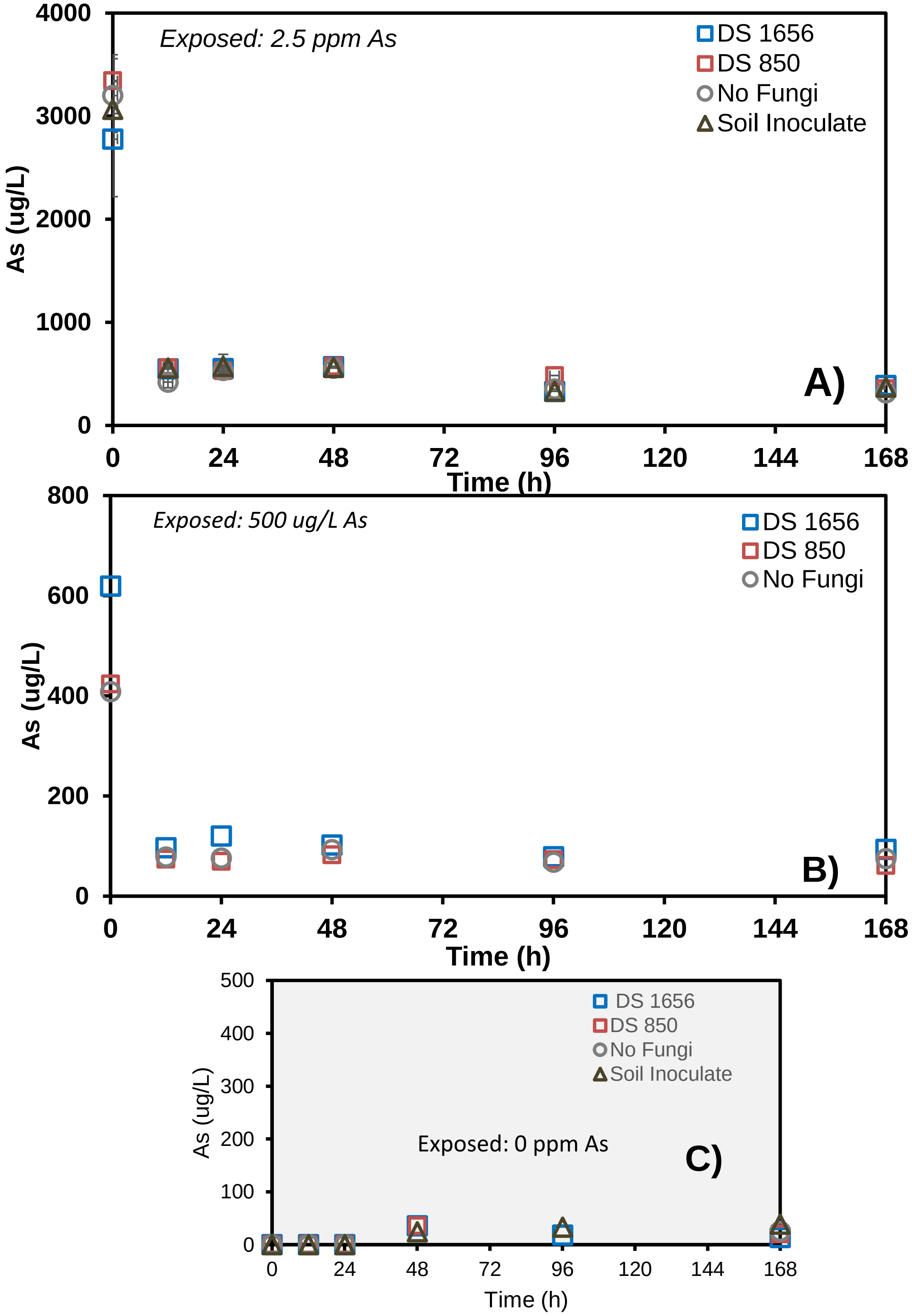

Figure 5. Arsenic concentrations measured in hydroponic solution over 168 hours from plants exposed to **A)** 2.5 mg L^{-1} **B)** 0.5 mg L^{-1} **C)** 0 mg L^{-1}.

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Chapter 6

6.1 Overall Conclusions

The results from the experiments performed in this research contribute to a better understanding of As mobility and bioavailability in water, sediment and plants. The results provide insights into the biological, chemical and physical-chemical mechanisms for mobility using advanced spectroscopy, microscopy, microbiological analytical chemistry tools. Chapter 3 of this dissertation investigated the effect of competing anions on As release under oxidizing conditions. This study evaluated the concentration and reactivity of As in sediments impacted by an abandoned gold mine (Homestake Mine), in the Whitewood Creek (near the mine) and in the Cheyenne River (241 km downstream and inside the Cheyenne River Sioux Tribe reservation). Chapter 3 results suggest that the presence of bicarbonate and phosphate, can favor the release of As, through competitive ion displacement. Though As can be released by changes in associated Fe mineralogy and reductive dissolution, this study also suggests that the mobilization of As can be affected by phosphate and to some extent bicarbonate by competitive ion displacement of weakly bound As(V) at the surface of the solids. The presence of labile As(V) from sediments may affect As exposure to local residents. Land use within CRST boundaries includes 85% livestock use and agricultural activities, so the potential for e release of As from sediments may be an exposure pathway.

Chapter 4 investigated the role of microorganisms on As, Fe and Mn stability under various redox conditions. The potential processes driving metal release from the contaminated sediment in redox cycling experiments are: 1) the transition from aerobic to anaerobic conditions induces microbial reduction of As, Fe and Mn from contaminated sediment in experiments amended with glucose, an electron donor; and 2) the influence of competitive ion displacement can cause release of surface bound As under aerobic conditions. The release of As from contaminated sediments by microbial reduction, as observed in experiments cycled from aerobic to anaerobic conditions amended with glucose was higher than that observed by ion displacement by phosphate. The limited Fe and Mn release from phosphate incubations in our study compared to glucose amendments confirm that different processes were affecting the releases of As, Fe and Mn. The transition from aerobic to anaerobic conditions triggers microorganisms to catalyze the reduction of As, Fe and Mn as electron acceptors in contaminated sediments in the presence of an electron donor (e.g. glucose). Microbial reductive dissolution of As, Fe and Mn can depend on oxidation-reduction potential, electron donor source and concentration, the composition of the microbial community and solution chemistry. The results in this study show the important role of microbial reduction in controlling metal release after transitioning from aerobic to anaerobic conditions.

In chapter 5, we investigated As uptake in a hydroponic system of plants inoculated with root colonizing endophytes. The results indicate that biological and physical-chemical processes can influence As uptake by plants grown in hydroponic systems. For plants grown in this study both endophytes and As exposure can affect root cell vitality and plant biomass. Control plants without endophytes exposed to high and low concentrations of As experienced reduced root growth and in a few cases, in death. Plants inoculated with fungi had healthier plant roots and increased biomass compared to control plants without fungal endophytes, which can partly explain the tolerance to As in healthier plants due to: a) dilution and heterogeneous distribution of As throughout the plants with higher biomass; b) more surface area of rootlets in healthier plants to accommodate adsorption/precipitation reactions. More data are necessary to better understand the specific contribution of biological and physical-chemical processes on As uptake in the hydroponic systems studied.

6.2 Environmental Implications and Future Research

The elevated concentrations of As in riverbank sediment on tribal land and in the watershed is a concern for nearby communities given that the Cheyenne River watershed receives contaminated sediment from the Homestake Mine. This study investigated the combined roles of physical-chemical and microbiological mechanisms for As release which, to our knowledge, has not been previously studied. The conditions investigated in this study highlight the environmental relevance of microbial redox cycling in contaminated sediments from the Cheyenne River watershed. In addition to chemical mechanisms affecting As release as reported in previous studies, the reductive dissolution of As, Fe, and Mn catalyzed by native microorganisms and redox cycling in contaminated sediments is also important to consider.

The results from this study have relevant implications for bioremediation applications. In our hydroponic plant uptake experiments, a similar degree of As removal was observed in all conditions and the presence of endophytes provided favorable growth conditions for *Schizachyrium scoparium.* The presence of calcium phosphate minerals and other aqueous chemistry considerations could also contribute to As removal. Future investigations are necessary to further validate the symbiosis of endophytes and plants and the integration with precipitation processes for As removal in contaminated field sites. The results from this study have relevant implications for bioremediation applications.

The insights gained from this dissertation have important implications for risk assessments. The growth of widely used grasses like *Schizachyrium scoparium* in As-contaminated regions is a concern due to its toxicity and possible bioavailability to animals and humans. The effects of soil organic matter and redox conditions on As accumulation remain subject to future investigations. The environmental samples used for this study were collected from regions of the watershed where prairie grasses cover 85% of the land base because of its economic and cultural value. Future studies are necessary to investigate the role of plants and organic matter in soils to better understand pathways of exposure to As. The effects of flow conditions and physical processes on As transport also remain subject to future investigations. The environmental samples used in batch incubations for this study were collected from regions of the watershed that house medicinal plants and food resources. Future studies are necessary to investigate the role of plants and organic matter in soils to better understand pathways of exposure to As.

Appendix A

Supplementary Information from Chapter 3: Effect of bicarbonate and phosphate on arsenic release from mining-impacted sediments in the Cheyenne River watershed, South Dakota, USA

Site ID	Site name	River	As (ug L^{-1})	Pb (ug L^{-1})	U (ug L^{-1})	Fe (ug L^{-1})	Mn (ug L^{-1})
CC-CR	Cherry Creek	Cheyenne River	5.84	17.56	15.84	-	-
DR-CR	Deal Ranch	Cheyenne River	5.14	13.11	13.56	-	-
RL-MR	Ross Lawrence	Moreau River	1.47	13.40	14.42	-	-
TB-MR	Thunder Butte	Moreau River	2.05	15.67	18.43	-	-
WW	Whitewood Creek	Whitewood Creek	62.16	12.82	5.14	2010	81.3
BF	Belle Fourche	Belle Fourche	18.98	9.94	17.63	3046	93.8
BF/WW	Belle Fourche/Whitewood	Belle Fourche	2.25	7.46	15.76	3081	82.8
WA	Wasta	Cheyenne River	6.68	17.76	15.15	10861	220
BR	Bridger	Cheyenne River	6.10	6.37	6.25	29.0	6.18

Table S1. Additional Water quality data from Cheyenne River Watershed

HPLC Conditions	
As Species	As(V), As(III)
Mobile Phase	146 mg/L Ethylenediaminetetraacetic acid (EDTA) + 0.650 mL Tetrabutylammonium hydroxide (TBAoH) + 5% HPLC grade methanol (MeOH) in 1L MilliQ
Flow Rate	1 mL/min
pH	7 (±0.01)
Pressure	1000-1600 psi
Column	A CAPCELL PAK C18 column (250 mm × 4.6 mm, 5 μm particle size)
Column Temperature	50 degrees C
Injection volume	10 μL

Table S2. HPLC/ICP-MS Conditions

	Pk$_1$	**Pk$_2$**	**Pk$_3$**
Arsenate (H_3AsO_4)	2.19	6.94	11.5
Arsenite (H_3AsO_3)	9.2	14.22	19.22

Table S3. pKa values of arsenate, arsenite and phosphoric acid.

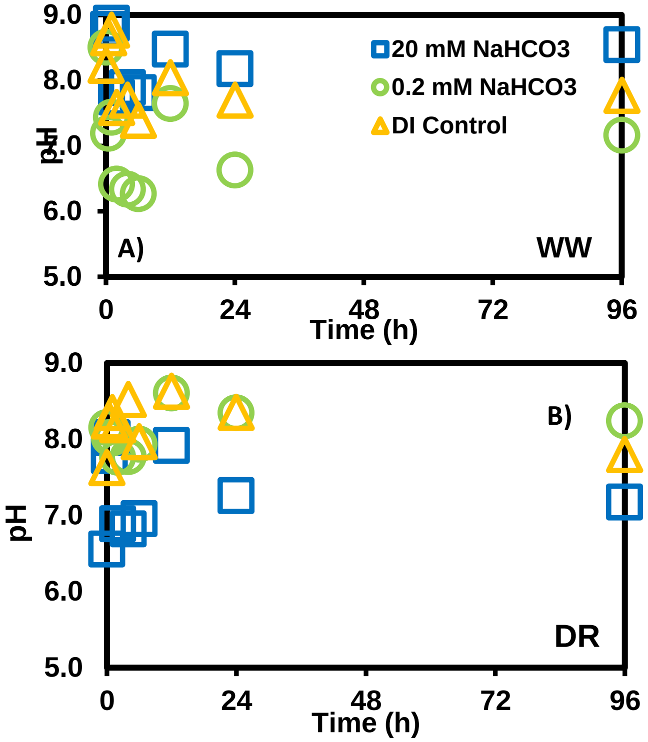


Figure S1. pH measurements during experiments with mine waste solids **A)** Whitewood Creek (WW) and **B)** Deal Ranch (DR) reacted with 0.2 mM sodium bicarbonate, 20 mM sodium bicarbonate and deionized water (DI control)

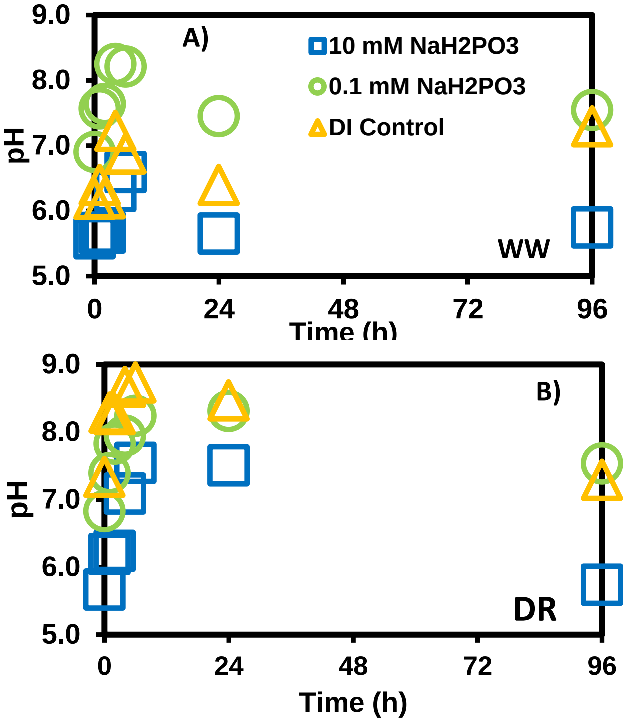
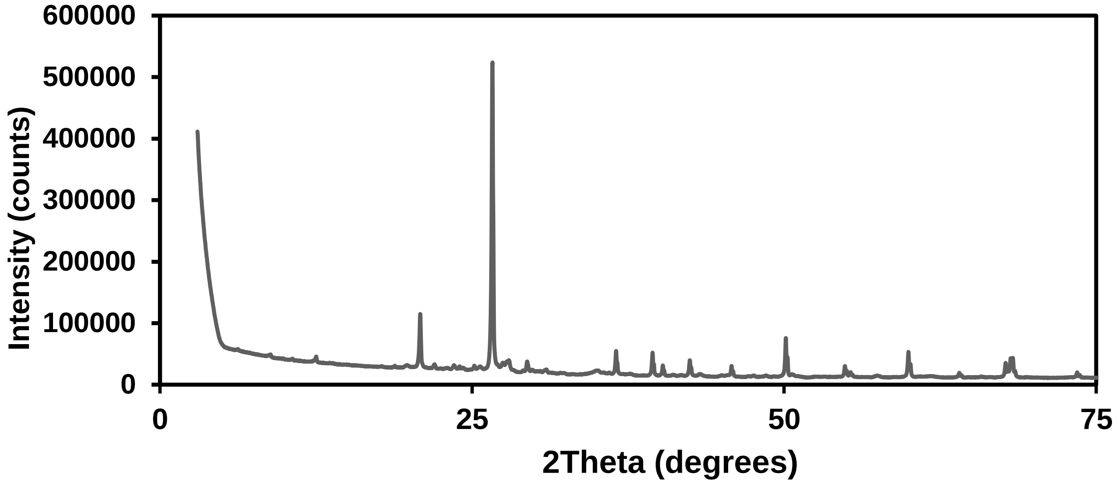


Figure S2. pH measurements during experiments with mine waste solids **A)** Whitewood Creek (WW) and **B)** Deal Ranch (DR) reacted with 0.1 mM sodium phosphate, 10 mM sodium phosphate and deionized water (DI control)

Appendix B

Supplementary Information from Chapter 4: Release of As, Fe and Mn from contaminated sediment in aerobic and anaerobic conditions: chemical or microbiological?



Compound name	CC (Atomic Wt %)
Quartz	64%
Albite	11%
Microcline	8%
Chlorite	4%
Calcite	4%
Biotite	4%
Kaolinite	2%

Figure S1. XRD Patterns and Atomic Weight % of unreacted Cherry Creek sediment.

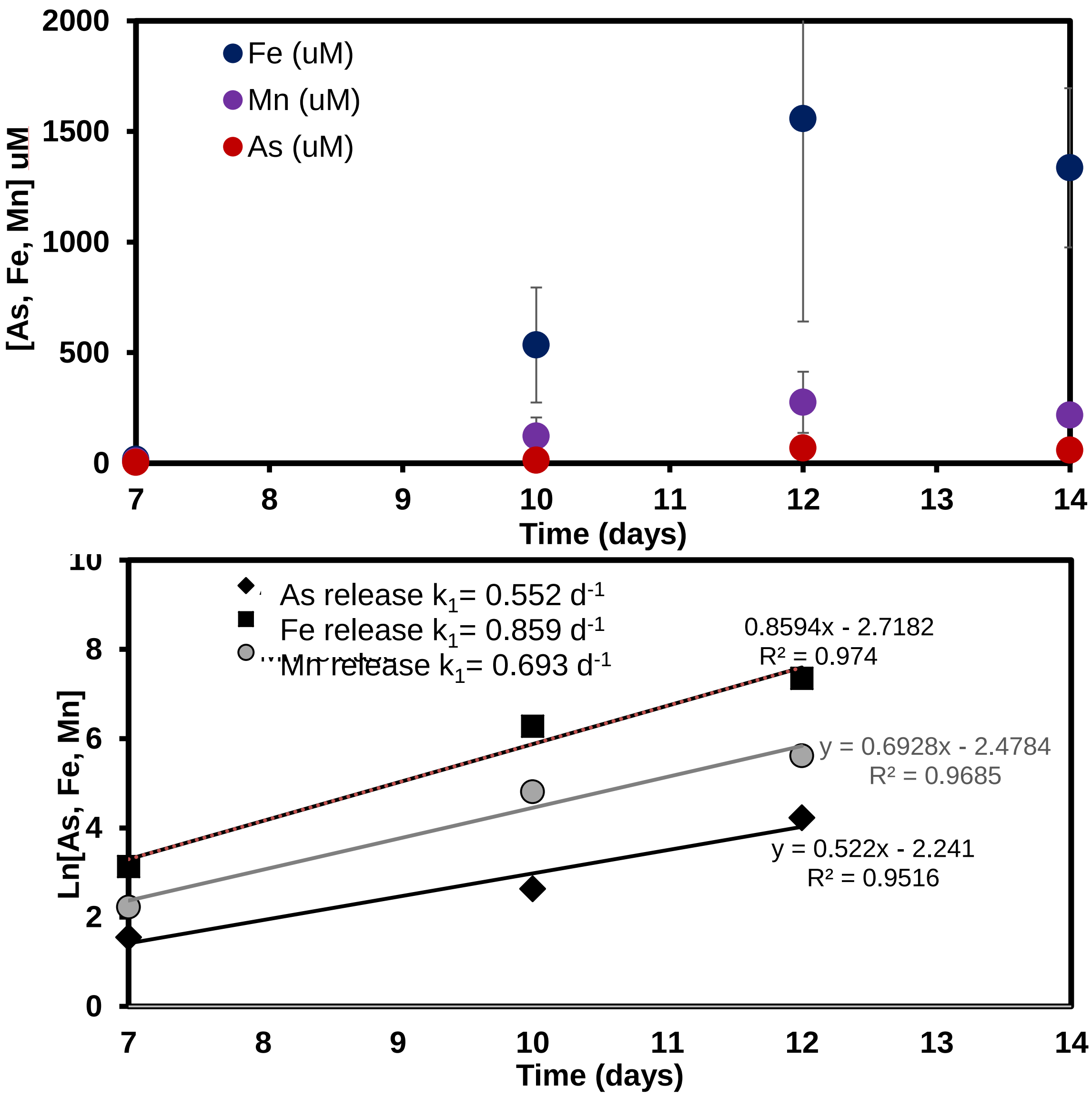


Figure S2. A) As, Fe, and Mn release (uM) in batch reactors under aerobic to anaerobic cycling conditions. B) Pseudo-first-order rate constants (k_1) for As, Fe and Mn transformed under aerobic to anaerobic cycling conditions.

Initial Solid concentration		
As (*mg/kg*)	Fe (*mg/kg*)	Mn (*mg/kg*)
106	22,000	693

Table S1. Summary of metal concentrations in unreacted sediment

			As (%)	**Fe (%)**	**Mn (%)**
a)	*Aerobic Cycle*	Glucose Amended	16.3	5.0	57.4
		10 mM Phosphate	3.0	0.2	17.2
		DI Control	2.1	0.0	0.1
b)	*Anaerobic Cycle*	Glucose Amended	72.6	7.7	32.8
		10 mM Phosphate	43.3	0.1	7.3
		DI Control	2.7	0.0	0.2
c)	*Completely Anaerobic*	Glucose Amended	16.3	5.0	57.4

Table S2. Maximum release (As, Fe, Mn) % under a range of biogeochemical conditions: **a)** Aerobically, amended with glucose, phosphate or DI **b)** Aerobic Cycled to Anaerobic, amended with glucose, phospahte or DI; **c)** Anaerobically, not cycled, amended with glucose

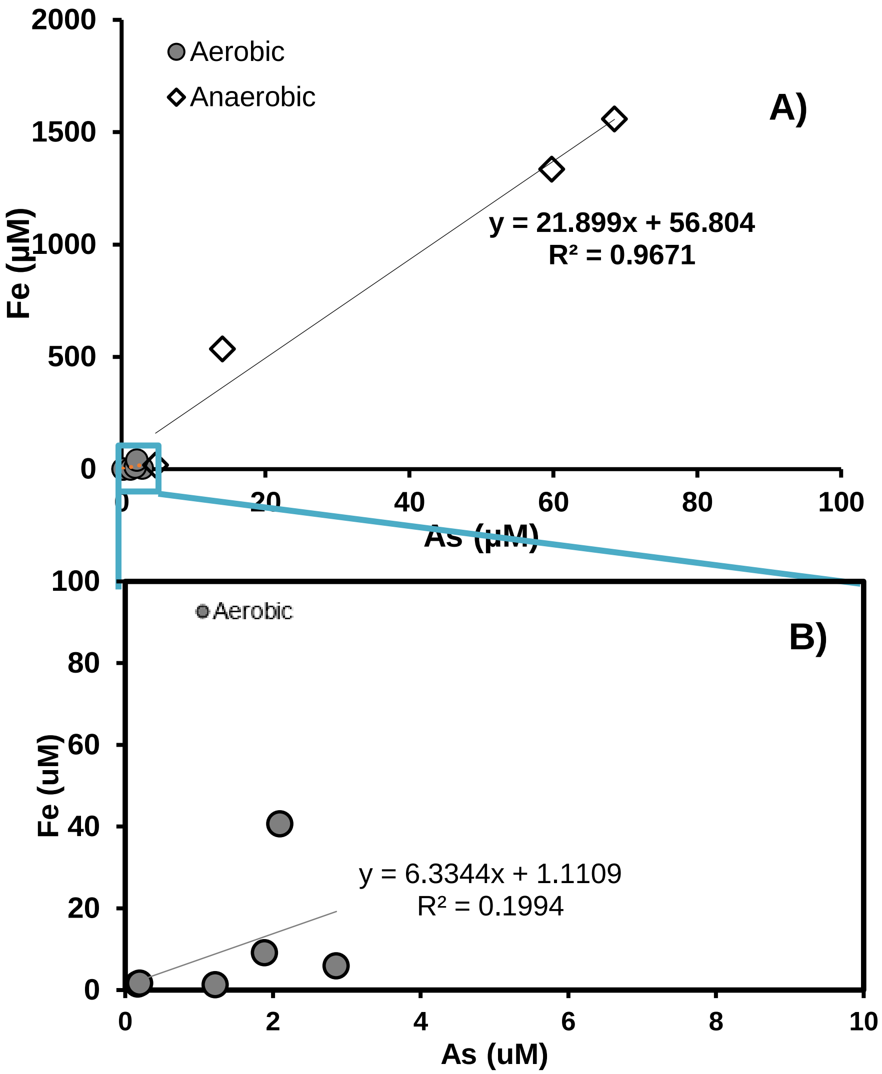


Figure S2. A) As vs. Fe release (uM) in batch reactors under aerobic to anaerobic cycling conditions amended with glucose; B) As vs. Fe release (uM) in batch reactors under aerobic to anaerobic cycling conditions amended with glucose.

<u>Appendix C</u>

Supplementary Information from Chapter 5: Arsenic uptake in hydroponically grown *Schizachyrium scoparium (*Little Bluestem) amended with root colonizing endophytes

$MgSO_4$	0.5 mM
NH_4NO_3,	2 mM
KCl	1 mM
$NaHCO_3$	5 mM
KH_2PO_4	0.12 mM
$CaCl_2.2H_2O$	3 mM

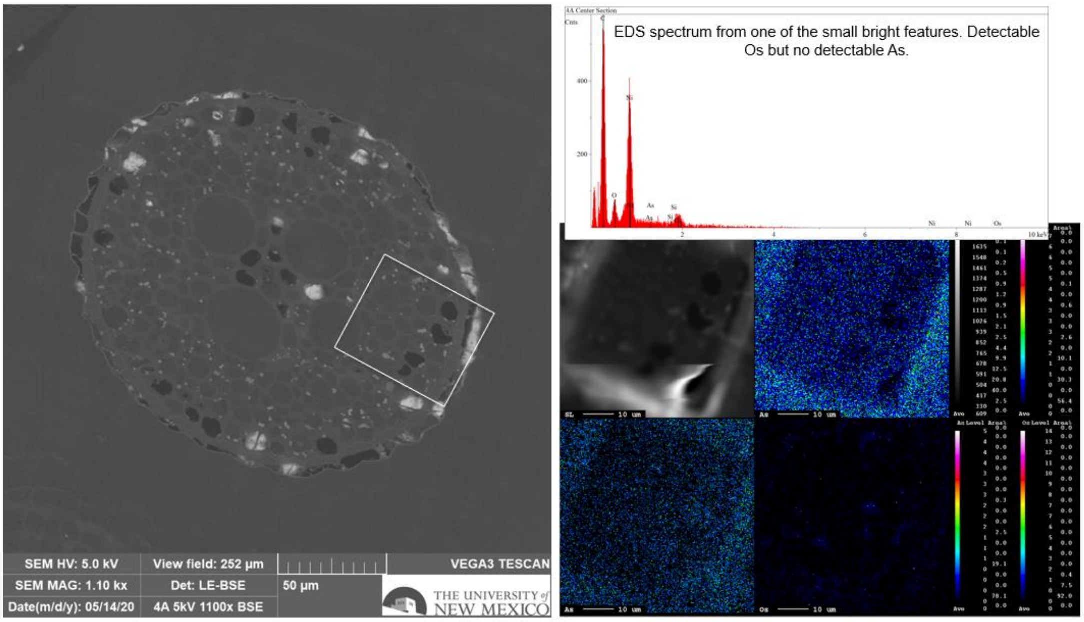
Table S1. Conditioning solution composition based on modified Hoagland Solution and Cheyenne River aqueous chemistry.

Component	% of total concentration	Species name
PO4-3	49.52	HPO4-2
	17.402	H2PO4-
	5.639	MgHPO4 (aq)
	24.179	CaHPO4 (aq)
	2.293	CaPO4-
	0.666	CaH2PO4+
	0.262	KHPO4-
	0.031	KH2PO4 (aq)
AsO4-3	82.117	HAsO4-2
	17.876	H2AsO4-
Mg+2	92.164	Mg+2
	0.914	MgCl+
	2.561	MgSO4 (aq)
	1.353	MgHPO4 (aq)
	0.289	MgCO3 (aq)
	2.708	MgHCO3+
Ca+2	90.921	Ca+2
	0.568	CaCO3 (aq)
	0.569	CaCl+
	3.18	CaSO4 (aq)
	0.358	CaNO3+
	0.967	CaHPO4 (aq)
	0.092	CaPO4-
	0.027	CaH2PO4+
	3.317	CaHCO3+

Table S2. Distribution of Aqueous species at pH 7.5.

Mineral	log IAP	Sat. index
Hydroxyapatite	-34.248	10.085
Ca3(PO4)2 (beta)	-26.909	2.011
Ca4H(PO4)3:3H2O(s)	-46.481	1.469
Ca3(PO4)2 (am2)	-26.909	1.341
Calcite	-7.987	0.493
Aragonite	-7.987	0.349
Dolomite (ordered)	-16.746	0.344
Vaterite	-7.987	-0.073
Dolomite (disordered)	-16.746	-0.206
CaHPO4(s)	-19.571	-0.296
CaHPO4:2H2O(s)	-19.571	-0.576
CaCO3xH2O(s)	-7.987	-0.843
Magnesite	-8.759	-1.299
Ca3(PO4)2 (am1)	-26.909	-1.409
Gypsum	-6.38	-1.77
Anhydrite	-6.379	-2.019
MgHPO4:3H2O(s)	-20.344	-2.169

Table S3. Saturation indices of minerals at 7.5 pH.



EDS spectrum from one of the small bright features. Detectable Os but no detectable As.

BSE close-up image of center section. Large bright features are the Ni-grid, but note there are many small, moderately bright features, about 1 um in diameter, scattered throughout the section.

WDS map of small features at right side of root in slide 10. Mapped at 5 kV & 30nA with 30 msec dwell per pixel. No apparent As although Os seems to correlate with the small bright features.

Figure S1. SEM Image of Root Cross section and BSE close up image, confirming the presence of Os correlated to carbon rich material. No As detected in cross section.

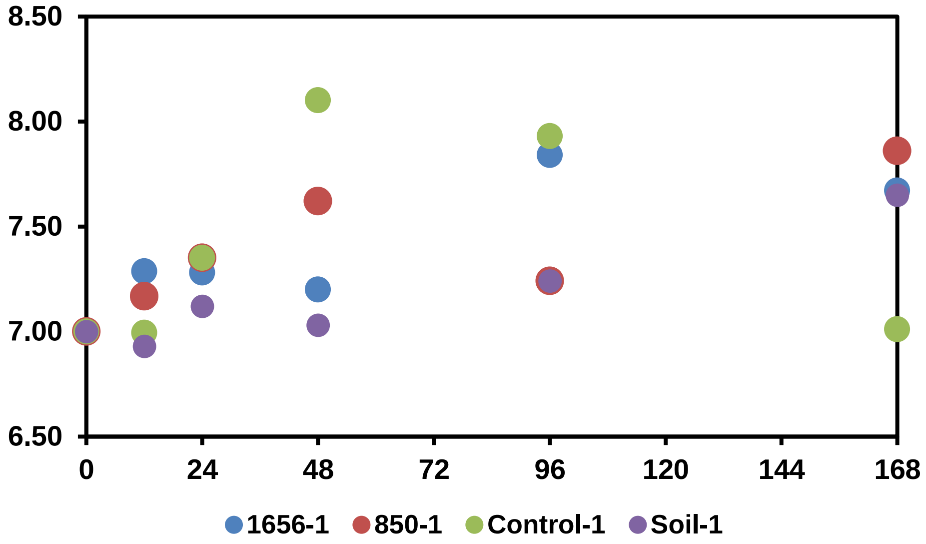


Figure S2. Changes in hydroponic pH over time