

Processes controlling arsenic solubility and mobility in soils
by Clain Alan Jones

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
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Abstract:
The practice of liming to remediate contaminated soils and mine tailings has the potential to mobilize arsenic (As), due to the pH dependence of As sorption reactions on oxide minerals and layer silicate minerals. Two low pH samples (pond tailings and reprocessed tailings) collected near Anaconda, Montana were subjected to unsaturated column transport experiments before and after liming. Soluble As concentrations measured in column effluent increased by factors of 10 (reprocessed tailings) to 400 (pond tailings) following liming. Based on the experimental data collected, increased mobility of As with liming appears to be consistent with the pH dependence of sorption reactions of As on Fe oxide minerals rather than dissolution-precipitation reactions involving As.

Groundwater As concentrations in the northern portion of the Lower Madison River Valley, Montana, are above the state human health standard (0.24 μM) and correlate with high soluble As concentrations in overlying soils. Sixteen paired (irrigated and nonirrigated) sites were sampled in four major regions adjacent to the Madison and Upper Missouri Rivers to determine if the contamination was caused by irrigation. There were no significant differences (a=0.05) in total or soluble As concentrations between irrigated and nonirrigated soils within any of the four regions. In addition, sorption coefficients (Kd values) in six paired soils selected for additional chemical characterization were not significantly different (a=0.05) between irrigated and nonirrigated sites. Based on the suite of data, it was determined that irrigation history has not significantly affected As solubility or attenuation capacities in soils of the study area.

Reduction of arsenate [As(V)] to arsenite [As(III)] influences the mobility and toxicity of As, yet the mechanisms controlling the rate of reduction in soils and natural waters are poorly understood. Reduction experiments were conducted anaerobically in serum bottles with a range of glucose and As(V) concentrations. At As(V) concentrations ranging from 6 to 600 μM, the rate of As(V) reduction was first-order with respect to initial As(V) and highly dependent on the number of As(V) reducers. In a parallel study, reduction rates of As(V) with the unenriched population were evaluated in the presence of goethite or ferrihydrite. The results indicate that rates of As mobilization during reduction are strongly controlled by oxide surface area and As surface coverage.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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TABLE OF CONTENTS

TABLE OF CONTENTS vi

LIST OF TABLES viii

LIST OF FIGURES x

ABSTRACT xiii

1. INTRODUCTION 1

2. ARSENIC TRANSPORT IN CONTAMINATED MINE TAILINGS FOLLOWING LIMING 5

Introduction 5
Materials and Methods 7
Sample Collection and Characterization 7
Column Experiments 9
Results and Discussion 11
Sample Characterization 11
Column Experiments 17
Conclusions Experiments 17
Conclusions 22

3. EFFECTS OF LONG-TERM IRRIGATION ON SOIL ARSENIC MOBILITY OF THE MADISON AND UPPER MISSOURI RIVER VALLEYS, MONTANA 24

Introduction 24
Materials and Methods 28
Sample Collection 28
Soil Characterization 28
Batch Sorption Isotherms 29

Sequential Extractions 31
Analysis for Solid Phases 32
Saturated Column Experiments 33
Results and Discussion 33
Sample Characterization 33
Sorption of As in Irrigated vs. Nonirrigated Soils 36
Partitioning of As in Selected Soils 39
Saturated Column Experiments 41
Conclusions 43

4. MICROBIAL REDUCTION RATES OF SOLUBLE AND SORBED PHASE ARSENATE 45

Introduction 45
Materials and Methods 48
Reduction Rates of Soluble As(V) 48
Reduction Rates of As(V) in Presence of Fe Oxides 50
Analytical Techniques 52
Results and Discussion 54
Effect of Carbon on As(V) Reduction Rates 54
Effects of As Enrichment and Microbial Growth on As(V) Reduction 55
Effect of As(V) Concentration on As(V) Reduction Rate 58
Proposed Metabolic Pathways 59
Reduction of As(V) in the Presence of Goethite 62
Reduction of As(V) in the Presence of Ferrihydrite 66
Conclusions 68

5. SUMMARY 70

REFERENCES CITED 73

APPENDICES 81

Appendix A - Results from Batch Hydride Generation Speciation Technique ... 82
Appendix B - Results from Extraction Procedures Designed to Quantify Sorbed Arsenate and Arsenite 89
Appendix C - Supplemental Data to Chapter 3 94
Appendix D - Arsenate Oxidation-Reduction Experiments with Soils and Solid Phases 99
Appendix E - Biotic Arsenate Reduction Rate Experiments in Solution, Supplement to Chapter 4 104

LIST OF TABLES

Table

1. Total arsenic and metal concentrations of six mine samples determined by X-ray fluorescence (XRF) 11

2. Soil water chemistry of saturated paste extracts for six mine soils and saturation indices with respect to selected solid phases 13

3. Molar ratios of S/Fe and As/Fe in particles of pond tailings and reprocessed tailings as determined by energy dispersive analysis of X-rays (EDAX) 16

4. Soil sorption coefficients (K_d) of six paired Madison River Valley soils 36

5. Average chemical and physical characteristics from the top 20 cm of six paired Madison River Valley soils 38

A1. Compilation of As speciation data from 1/96 – 2/97 83

A2. Results from As speciation tests using batch hydride generation in SSE nutrient solution 84

A3. Efficiency of arsine generation from As(III) standards (148 μM) made in SSE-R2 85

A4. As(V) recoveries using batch hydride speciation (see Chap. 4, Materials and Methods) for a range of reagent volumes and length of speciation 86

A5. As(III) recoveries using batch hydride speciation (see Chap. 4, Materials and Methods) for a range of reagent volumes and length of speciation 87

A6. Recoveries and efficiencies of batch hydride speciation method using 20% 2M TRIS, 20% $NaBH_4$ reagent 88

B1. Recovery of As(V) and As(III) sorbed to 2-line ferrihydrite (50 m*M* as Fe) with 20 mL of 1 *M* K_2HPO_4 extractant (pH ≅ 9.0) 90

B2. Recoveries of As(V) from a 1 m*M* As(V) treatment (20 mL) on 2-line ferrihydrite (25 m*M*) following 1 hr extractions with P buffers (20 mL)..... 90

B3. Solution As, extracted As, and As recoveries from 2-line ferrihydrite (25 m*M*) using 0.01, 0.1, and 1 *M* P extractants on As(V), As(III), and mixed 1 m*M* standards 91

B4. Levels of As extracted from a 2 g L^{-1} suspension of Al-oxide C (Degussa Corp., Ridgefield Park, NJ) with 1 *M* NaOH for 2hr @ 70C..... 92

C1. Total and soluble arsenic concentrations for surficial (top 20 cm) paired Madison River Valley soils..... 95

C2. Soil sorption coefficients and selected characteristics of six paired Madison River Valley soils 96

C3. Arsenic recovered from each sequential extract in selected surficial (top 20 cm) Madison River Valley soils..... 97

C4. Solution levels of As(V) and As(III) following a 96 hr batch sorption isotherm for six selected Madison and Missouri River sites..... 98

D1. Solution iron (Fe) concentrations in goethite suspensions at both a high and a low As:Fe ratio 103

E1. Effect of enrichment, NO_3^-, and N_2-purging on the extent of As(V) reduction in serum bottles 105

LIST OF FIGURES

Figure

1. Percentage of total As recovered from each sequential extractant for both the reprocessed tailings and pond tailings 15

2. Arsenic concentrations and pH levels in column effluent for limed and unlimed treatments of pond tailings (Fig. 2A) and reprocessed tailings (Fig. 2B)..... 18

3. Solubility diagrams for scorodite ($FeAsO_4 \cdot 2H_2O$) and $Ca_3(AsO_4)_2$ compared to activities of AsO_4^{3-}, Fe^{3+}, and Ca^{2+} in column effluent. 21

4. Median surface water arsenic (As) concentrations (Nimick et al., 1998) and sampling locations in the Upper Madison (UMad; Sites A1-A4), Lower Madison South (LMadS; Sites B1-B4), Lower Madison North (LMadN; Sites C1-C4), and Upper Missouri (UMis; Sites D1-D4). 25

5. Total and soluble As concentrations in the top 20 cm of soil for the Upper Madison (UMad), Lower Madison South (LMadS), Lower Madison North (LMadN), and Upper Missouri (UMis)..... 35

6. Predicted versus measured K_d for twelve Madison River soils..... 37

7. Concentrations of As recovered from each sequential extractant for three irrigated (I) and nonirrigated (NI) soils. 40

8. Increase in soil arsenic as a function of water loading in saturated soil columns (5 cm long, 3 cm diameter) 42

9. Changes in As(V) concentration and optical density (A_{500}) in serum bottles as a function of initial glucose concentration (10, 30, and 100 m*M* as C). 54

10. Extent of As(V) reduced (A, B) and growth curves (C, D) versus time for a range of initial As(V) concentrations in serum bottles inoculated with either CN-0 or CN-8. 55

11. Extent of As(V) reduction as a function of optical density in serum bottles inoculated with either CN-0 (A) or CN-8 (B). 57

12. Effect of initial As(V) concentration on As(V) reduction rate in serum bottles inoculated with CN-0 or CN-8. 58

13. Electrons transferred per serum bottle in a solution containing 5 m*M* As(V) inoculated with CN-8 61

14. Concentrations of soluble As(T), As(V), and As(III) versus time as Pt electrode E_H decreased in a stirred goethite suspension with a high As:Fe molar ratio (0.04). 63

15. Concentrations of soluble As(T), As(V), and As(III) versus time as Pt E_H decreased in a stirred goethite suspension with a low As:Fe molar ratio (5.3×10^{-4}). 65

16. Effect of decreased Pt electrode E_H (A) on As and Fe concentrations (B) in a stirred ferrihydrite suspension at an As:Fe molar ratio of 0.012. 66

B1. Sorbed vs. extracted As from Al-oxide C (2 g L^{-1}) using either A) 1m*M* PO_4/1m*M* PO_3 (pH=5.5) or B) 100 m*M* PO_4 (pH 7.0). 93

D1. Measured redox potential (E_H) and As concentrations versus time in a slurry (9:1 solution:solid ratio) made from soil C3-I at pH 9.0 and pH 7.5 100

D2. Concentrations of As in 9:1 SSE-R2:solid slurries of A) Soil C3-I and B) Soil C3-NI during reduction. 101

D3. Arsenic levels in a filtered (0.2-μ) C3-NI extract (3:1 D.I. H_2O:soil ratio) before and after spiking solutions with As(III) 102

E1. Effect of initial glucose levels on As(V) reduction with the CN-5 population in anaerobic serum bottles at pH 6.3. 106

E2. Evidence that the CN-5 population was fermenting glucose and reducing H^+. . 107

E3. Effect of low C levels (as glucose) on As(V) reduction with CN-7 in serum bottles. 108

E4. Effect of CN-8 growth (assumed to correlate with optical density) on the extent of As(V) reduction. 109

E5. Concentrations of dissolved organic carbon (DOC) over time in serum bottles inoculated with CN-8 at three As(V) concentrations. 110

E6. Fate of glucose in serum bottles inoculated with CN-8..... 111

E7. Microbial growth (based on optical density changes) over time in anaerobic serum bottles. 112

E8. The effect of initial As(V) concentration on As(V) reduction rate with organisms extracted from soil C3-NI. 113

ABSTRACT

The practice of liming to remediate contaminated soils and mine tailings has the potential to mobilize arsenic (As), due to the pH dependence of As sorption reactions on oxide minerals and layer silicate minerals. Two low pH samples (pond tailings and reprocessed tailings) collected near Anaconda, Montana were subjected to unsaturated column transport experiments before and after liming. Soluble As concentrations measured in column effluent increased by factors of 10 (reprocessed tailings) to 400 (pond tailings) following liming. Based on the experimental data collected, increased mobility of As with liming appears to be consistent with the pH dependence of sorption reactions of As on Fe oxide minerals rather than dissolution-precipitation reactions involving As.

Groundwater As concentrations in the northern portion of the Lower Madison River Valley, Montana, are above the state human health standard (0.24 μM) and correlate with high soluble As concentrations in overlying soils. Sixteen paired (irrigated and nonirrigated) sites were sampled in four major regions adjacent to the Madison and Upper Missouri Rivers to determine if the contamination was caused by irrigation. There were no significant differences ($\alpha = 0.05$) in total or soluble As concentrations between irrigated and nonirrigated soils within any of the four regions. In addition, sorption coefficients (K_d values) in six paired soils selected for additional chemical characterization were not significantly different ($\alpha = 0.05$) between irrigated and nonirrigated sites. Based on the suite of data, it was determined that irrigation history has not significantly affected As solubility or attenuation capacities in soils of the study area.

Reduction of arsenate [As(V)] to arsenite [As(III)] influences the mobility and toxicity of As, yet the mechanisms controlling the rate of reduction in soils and natural waters are poorly understood. Reduction experiments were conducted anaerobically in serum bottles with a range of glucose and As(V) concentrations. At As(V) concentrations ranging from 6 to 600 μM, the rate of As(V) reduction was first-order with respect to initial As(V) and highly dependent on the number of As(V) reducers. In a parallel study, reduction rates of As(V) with the unenriched population were evaluated in the presence of goethite or ferrihydrite. The results indicate that rates of As mobilization during reduction are strongly controlled by oxide surface area and As surface coverage.

CHAPTER 1

INTRODUCTION

Arsenic (As) is the twentieth most common element found in the earth's crust (Cullen and Reimer, 1989). Concentrations of As in many soils and waters are elevated due to geothermal activity, pesticide applications, and mining operations (Stauffer and Thompson, 1984; Peryea, 1991; Neuman et al., 1993a). High concentrations of As have been found to cause toxicity in plants, animals, and microorganisms (Tamaki and Frankenberger, 1992; Pontius et al., 1994; Jiang and Singh, 1994). Therefore, understanding the processes controlling the toxicity, solubility, and mobility of As should prove useful in developing management strategies designed to minimize impacts of As on living organisms.

The oxidation state, or valence, of As affects both its toxicity and mobility. The primary oxidation states (and chemical forms) of As are: +5 (arsenate), +3 (arsenite), 0 (elemental arsenic), -3 (arsine). Arsenate, As(V), is thermodynamically stable in oxidized environments and arsenite, As(III), is thermodynamically stable in reduced environments. At neutral pH, As(V) species are negatively charged (e.g. $H_2AsO_4^-$, $HAsO_4^{2-}$), whereas As(III) is uncharged ($H_3AsO_3^o$). Methylation of As(III) leads to the formation of organic As acids (e.g. methylarsonic acid, dimethylarsinic acid). In highly

reduced environments, As solid phases (As^{o}, As_2S_3, AsS) and arsine gases (AsH_3, $(CH_3)_3As$) are thermodynamically stable.

Crop yields have been found to be significantly decreased ($\alpha=0.05$) by As application rates of 25 mg kg^{-1} for rice (Onken and Hossner, 1995) and 50 mg kg^{-1} for ryegrass and barley (Jiang and Singh, 1994). As(III) had a significantly greater effect than As(V) on yield of ryegrass ($\alpha=0.001$) and barley ($\alpha=0.01$), yet no significant effect on rice yield ($\alpha=0.05$). Growth of microorganisms has been found to be inhibited at 13 μM As(III), whereas the same population tolerated 1,000 fold more As(V) (Tamaki and Frankenberger, 1994). Mammalian toxicity is also dependent on As species based on the LD_{50} (dose that is lethal for 50% of test animals). For example, the LD_{50} in rats for As(V) and As(III) are 20 and 14 mg kg^{-1}, respectively, whereas the LD_{50} for dimethylarsinic acid ranges from 700 to 2,600 mg kg^{-1} (Tamaki and Frankenberger, 1992). The observed differences in toxicity between As species for plants, microorganisms, and animals are likely due to differences in mechanisms causing toxicity. Synthesis of adenosine triphosphate (ATP) is inhibited by As(V) due to its similarity to phosphate, whereas As(III) inactivates enzymes by reacting with protein sulfhydryl groups (Tamaki and Frankenberger, 1992).

Chronic toxic effects of As exposure in humans include cancers of the skin, lung, bladder, and liver (Pontius et al., 1994). Much of the existing epidemiological data regarding chronic toxicity in humans was gathered from 60 villages in Taiwan, where As concentrations in drinking water range up to 13 μM (Brown and Chen, 1995).

A risk assessment conducted with these data found that the current EPA drinking water standard of 0.67 μM (50 $\mu g\ L^{-1}$) results in a 0.9 – 1.7% risk of death from cancer assuming an intake of 1 $L\ d^{-1}$ (Pontius et al., 1994). However, a second evaluation of the data found no increased risk of cancer from drinking water containing less than 1.34 μM As (Brown and Chen, 1995). Although uncertainties exist regarding relationships between As concentration in drinking water and cancer risks, the compiled toxicological data demonstrate that As may be harmful to living organisms at concentrations commonly observed at contaminated sites (Korte and Fernando, 1991; Moore, 1994; Nimick et al., 1998).

Equilibrium solution concentrations of As(V) in soils are generally controlled by sorption processes, rather than precipitation/dissolution reactions, due to the high solubility of metal arsenates (Robins, 1981; Sadiq et al., 1983; Dove and Rimstidt, 1985; Nishimura et al., 1987). Iron, manganese, and aluminum oxides have been found to sorb 10 to 1,000 fold more As than layer silicates (montmorillonite, kaolinite) or calcite (Hingston et al., 1971; Oscarsson et al., 1981; Goldberg and Glaubig, 1988). Sorption of As(V) to iron and aluminum oxides has been found to decrease at high pH (Goldberg, 1986; Darland and Inskeep, 1997a; Raven et al., 1998); thus, it was hypothesized that liming As-contaminated soils may increase As mobility. Liming is a common practice used to raise pH and promote plant reestablishment in acidic, contaminated mine tailings, yet the effects of liming on As transport are not well established. Therefore, a study was undertaken to assess the effects of liming on As

mobility in mine tailings (Chap. 2). Results from this study should prove useful to mine remediation specialists in designing mitigation strategies for contaminated sites.

Geothermal hot springs in Yellowstone National Park have resulted in elevated concentrations of As in the Madison and Missouri Rivers (Nimick, 1998). A previous study suggested that high concentrations of As in soil porewaters of the Lower Madison River Valley, Montana were caused by evapoconcentration of As-rich irrigation water withdrawn from the Madison River (Sonderegger and Ohguchi, 1988). Therefore, a study (Chapter 3) was conducted to i) determine the effects of long-term irrigation on As solubility and ii) evaluate processes affecting As sorption and solubility in soils of the Madison and Upper Missouri Rivers.

Although As(III) does not sorb as strongly as As(V) to Fe oxides at low As:Fe ratios (Pierce and Moore, 1982), recent work shows that As(III) sorbs more strongly than As(V) at high As:Fe ratios (Manning et al., 1998; Raven et al., 1998; Sun and Doner, 1998). In sulfidic sediments, As(III)-sulfide solid phases (e.g. As_2S_3, AsS) may control the solubility of As (Moore et al., 1988). Thus, the oxidation-reduction (redox) status of soils and waters may strongly control the mobility of As. Although the effects of redox potential on the thermodynamic stability of As(V) and As(III) species have been well established (Ferguson and Gavis, 1972), less is known about the rates of As(V) reduction to As(III) in either soils or waters. Therefore, a third study (Chapter 4) was undertaken to i) evaluate processes controlling microbial reduction rates of soluble As(V) and ii) determine the effects of reduction on As solubility in the presence of Fe oxides.

CHAPTER 2

ARSENIC TRANSPORT IN CONTAMINATED MINE TAILINGS FOLLOWING LIMING

Introduction

Liming is an established, effective method for immobilizing trace metals, elevating pH, and promoting plant reestablishment in low pH, contaminated mine tailings (Neuman et al., 1993b); however, increases in soil pH may result in arsenic (As) mobilization due to the pH dependence of sorption reactions. This raises the concern of contaminating drinking water supplies following liming in soils or tailings containing elevated levels of As that are hydraulically connected to groundwater or surface water. The U.S. EPA may reduce the maximum contaminant level for As in drinking water from 0.67 μM to between 0.0067 and 0.134 μM, due to recent evidence that the current criteria may result in human cancer risks as high as 13 in 1000 (Pontius et al., 1994). Consequently, there is significant interest in processes controlling the transport of As into surface or ground waters.

Previous studies have investigated the pH effects of As sorption on soils or well-characterized solid phases (Hingston et al., 1971; Goldberg and Glaubig, 1988).

Goldberg and Glaubig (1988) found that the sorption of arsenate by montmorillonite and kaolinite was highest near pH 5 and dropped significantly between pH 7 and 9. Hingston et al. (1971) determined that arsenate sorption on both goethite and gibbsite decreased with increasing pH. Goldberg (1986) reported good correlations between experimental and modeled arsenate sorption on Fe and Al oxides using a constant capacitance model, which assumed a ligand exchange mechanism. Jacobs et al. (1970) showed that the elimination of amorphous Fe and Al by treatment with oxalate substantially reduced or eliminated arsenate sorption capacity in 24 Wisconsin soils, implying soil arsenate was primarily sorbed to oxides of Fe and Al. In contrast, arsenate solid phases are generally believed to be nonexistent in surface soils, due to their relatively high solubility, except at high arsenate levels (Robins, 1981; Sadiq et al., 1983; Dove and Rimstidt, 1985; Nishimura et al., 1987). For example, Dove and Rimstidt (1985) found that at an Fe activity of 1 m*M*, arsenate activities needed to be at least 0.01 *M* for scorodite ($FeAsO_4 \cdot 2H_2O$) to be stable, which is much higher than typically found in soil porewater. Thus, the effects of pH on arsenate solubility in soils are due primarily to surface complexation reactions rather than precipitation-dissolution reactions.

One of the recommended liming strategies for maximum metal immobilization in mine tailings of the Clark Fork River Basin of Montana consists of raising the soil pH to near 10, using a mixture of $Ca(OH)_2$ and $CaCO_3$ and allowing the soil to equilibrate for approximately one year prior to plant reestablishment. During

equilibration, the pH generally declines to values near 8 (Schafer et al., 1989). Field research at contaminated mining sites in the Clark Fork Basin has shown that this liming strategy results in metal immobilization, yet may lead to As mobilization (Neuman et al., 1993a).

The objectives of the present study were to (i) determine the extent of As mobilization in low pH mine tailings after liming and (ii) evaluate probable mechanisms for increased As transport after liming. To accomplish these objectives, several representative mine tailings and metal-contaminated soils were characterized and two of the tailings were subjected to transport experiments with and without liming.

Material and Methods

Sample Collection and Characterization

Six samples (0 to 0.30-m depth) were collected within two km of an abandoned copper smelter located in Anaconda, Montana, and included pond tailings (PT), smelter stack deposits (SSD), and reprocessed tailings (RT). All sampling locations were within a site that was placed on the National Priority List in 1983 due to metal contamination following 96 years of copper smelting (Neuman et al., 1993b). Samples were collected in triplicate at two locations. Concentrations of total As and total cations (Ca, Cu, Fe, Mn, Pb, and Zn) were determined using X-ray fluorescence (Jones, 1982) and particle size analysis was performed using the hydrometer method

(Gee and Bauder, 1986). Saturated pastes (Rhoades, 1982) were centrifuged at 10,000 *g* and filtered through 0.20-μm nylon filters to characterize soil solution chemical content. The resulting filtrates were analyzed for dissolved organic carbon (DOC) using a Dohrmann DC 190; cations, B, and S by inductively coupled plasma spectrometry (ICP); and Cl^-, NO_3^-, SO_4^{2-}, and PO_4^{3-} by ion chromatography (IC). Total alkalinity was measured by HCl titration to an inflection point near pH 4.5. These titrated samples were then sparged with N_2 (g) for more than 15 min, titrated to the original pH with NaOH, and retitrated to the inflection pH with HCl in order to calculate carbonate and noncarbonate alkalinity. Continuous-flow hydride generation atomic absorption spectrophotometry (HGAAS) was utilized to analyze As. Samples were acidified to 3*M* HCl, prereduced with 1% KI, and treated with both 5*M* HCl and 0.6% sodium borohydride ($NaBH_4$) in 0.5% NaOH. Flow rates were approximately 7 and 1 mL min^{-1} for samples and reagents, respectively. Generated arsine was quantified at 193.7 nm in an air-acetylene flame (Perkin Elmer 3100). The As detection limit, estimated as three standard deviations of 50 absorbance blank readings, was 3.4 n*M*. Free ion activities were estimated using the aqueous chemical equilibrium model, GEOCHEM (Sposito and Mattigod, 1979) as modified by Parker et al. (1987). Stability constants for pertinent aqueous complexes were obtained from compiled thermodynamic equilibrium data (Sadiq and Lindsay,1979; Sposito and Mattigod, 1979; Nordstrom and May, 1989).

Each sample was subjected to a sequential extraction for As (Johnston and Barnard, 1979; Ganje and Rains, 1982) based on developed procedures for extraction of phosphorus (Olsen and Sommers, 1982) and organically bound metals (Tessler et al., 1979). Although sequential extractions yield only qualitative information on As fractionation, this procedure was useful for assessing the relative lability of As among the different samples. The method consisted of sequential extractions in the following order: 1 *M* NH_4Cl, 0.1 *M* NaOH, 0.3 *M* citrate bicarbonate (CB), 0.3 *M* citrate dithionate bicarbonate (CDB), 1 *M* HCl and 30% H_2O_2/0.8 *M* NH_4OAc. All samples were analyzed for As with HGAAS; the CDB and H_2O_2 extracts were also analyzed with ICP. The residual solids were digested using a four acid heat-treatment (Welsch et al., 1990) and As levels in the resulting digests were determined using HGAAS.

Selected grains of pond tailings (PT) and reprocessed tailings (RT) were affixed to Al stubs, coated with C and viewed with a scanning electron microscope (SEM). The grains were mapped for 15 elements using energy dispersive analysis of x-rays (EDAX) to identify particles enriched in As, Fe, or S. Particles that contained high levels of these elements were analyzed in further detail to determine semiquantitative atomic ratios using standardless analysis with PROZA correction (Goldstein et al., 1992).

Column Experiments

Unsaturated column experiments were conducted on limed and unlimed PT and RT. Liming treatments consisted of adding a mix of 60% $CaCO_3$/40% $Ca(OH)_2$ at

concentrations of 3.0 and 25.6 g kg^{-1} of PT and RT, respectively. The $CaCO_3/Ca(OH)_2$ ratio and liming concentrations were determined from acid-base accounting as part of a remedial investigation study (Schafer et al., 1989). The PT (25% sand, 56% silt, 19% clay) was diluted 3:1 with 40-200 mesh SiO_2 (Fluka) to assure adequate flow, whereas the RT (95% sand, 3% silt, 2% clay) was left undiluted. Columns (5 cm diameter, 20 cm long PVC pipe) were sealed with rubber O-rings in polycarbonate end caps. A plastic porous plate (Soil Measurement Systems, Las Cruces, New Mexico) was inserted within each bottom end cap to control soil matric potential. Soil columns were packed by tapping lightly between successive soil additions and then were saturated from the bottom with 5 m*M* K_2SO_4. Upon complete wetting, a 20 kPa vacuum was applied to the bottom of the column, and 5 m*M* K_2SO_4 was delivered to the top of the column at 10 mL hr^{-1} with a syringe pump. This flow rate equates to a pore water velocity of approximately 1.7 cm hr^{-1}. The vacuum was adjusted over the course of the experiments to maintain volumetric water contents (θ_V) between 30-36% and 23-28% for the PT and RT, respectively. These water contents were well below the saturated water contents in each respective column. Under these conditions the columns remained aerated and it was assumed that soluble As was present as arsenate (As(V)) rather than arsenite (As(III)). Unlimed control columns were sampled for approximately 23 and 100 pore volumes for the PT and RT, respectively, then treated with a high pH influent (0.1 *M* $KHCO_3$, adjusted to pH 10.1 with KOH, and bubbled with air to maintain the pH level). All effluent samples were analyzed for pH, EC, As,

and alkalinity. Selected samples were also analyzed for Fe, Ca, S, Cu, and Zn, and a smaller subset analyzed for K, Mn, Al, Na, and Mg. Analysis methods were identical to those outlined previously. Free ion activities were calculated using GEOCHEM on those samples analyzed for the complete parameter set, as well as selected samples that produced reasonable cation/anion balances without data on K, Mn, Al, Na, and Mg.

Results and Discussion

Sample Characterization

Total arsenic (As) contents of the six mine samples (Table 1) ranged from 48 to 3421 mg kg^{-1} (10-700 fold higher than typical background levels of 5 mg kg^{-1}; Nriagu, 1994). Iron represented 15-21% of the reprocessed tailings (RT), indicative of

Table 1. Total arsenic and metal concentrations of six mine samples determinedby X-ray fluorescence (XRF).

	Pond Tailings (PT)	Smelter stack deposits (SSD)			Reprocessed Tailings (RT)	
Element	A	B	C	D	E	F
	mg kg^{-1}					
As	248	2358	48	1506	3421	2338
Ca	ND †	27900	21100	20700	14800	5300
Cu	274	1331	126	616	2386	2382
Fe	13100	46800	42800	35400	215800	152300
Mn	626	1283	1203	885	914	ND
Pb	704	301	ND	147	881	813
Zn	103	816	205	466	341	671

† ND=none detected

high initial pyrite (FeS_2) content. Total metal concentrations of Cu, Pb, and Zn were also generally elevated, consistent with the composition of mine tailings in this region. Stabilization of these barren tailings through revegetation has been a primary goal of mine reclamation efforts in order to reduce erosion and prevent migration of these contaminants to receiving waters.

The chemical content of saturated paste extracts for the six mine tailing samples is shown in Table 2. The smelter stack deposits (SSD) had saturated paste pH values ranging from 7.8 to 8.0, whereas the pond tailings (PT), and RT had pH levels of 3.5-4.1. As expected, soluble concentrations of nearly all metal ions were highest in those samples with low pH, with SO_4^{2-} being the principal anion. High soluble concentrations of Zn, Cu, Ni, Cr, and Cd in the low pH samples document the need for liming to reduce potential soluble metal migration and phytotoxicity. Conversely, the SSD had relatively low soluble metal concentrations, balanced primarily with the anions HCO_3^-, SO_4^{2-}, and $HAsO_4^{2-}$. Soluble As concentrations for the six soils spanned more than four orders of magnitude, and were positively correlated with pH. The relationship between soluble As and pH among all samples is consistent with the pH dependence of As sorption reactions on oxide minerals and layer silicates (Goldberg, 1986; Goldberg and Glaubig, 1988). Total As did not correlate well with soluble As; for example, RT samples had the highest total As but the lowest soluble As.

Charge balance calculations performed after GEOCHEM speciation yielded errors ranging in magnitude from 1.1 to 35.9% (Table 2). A comparison of ion activity

Table 2. Soil water chemistry of saturated paste extracts for six mine soils and saturation indices† with respect to selected solid phases.

Constituent	Pond Tailings (PT)	Smelter Stack Deposits (SSD)			Reprocessed Tailings (RT)	
	A	B	C	D	E	F
% Saturation (mass basis)	37	29	27	25	24	24
pH	4.1	7.8	8.0	7.8	3.5	3.9
E.C. (dS m^{-1})	1.52	0.62	0.35	0.36	2.79	2.56
DOC (m*M* as C)	3.8	2.5	1.7	0.8	0.3	0.4
Non-carb. alk. (m*M* charge)	0	2.0	0.3	0.9	0	0
Cations						
K (m*M*)	0.31	0.17	0.11	0.04	0.08	0.19
Ca (m*M*)	5.19	2.08	0.85	1.14	13.68	13.97
Mg (m*M*)	1.28	1.08	0.47	0.43	0.41	0.40
Na (m*M*)	0.48	0.80	0.71	0.66	0.10	0.12
Al (µ*M*)	149	<7	<7	<7	1067	360
Fe (µ*M*)	26	0.4	<0.30	<0.30	375	899
Mn (µ*M*)	269	1.8	<0.05	<0.05	73	39
Zn (µ*M*)	304	0.9	0.5	0.2	746	767
Cu (µ*M*)	510	2.8	1.0	1.8	528	502
Pb (µ*M*)	<0.41	<0.41	<0.41	<0.41	0.62	1.20
Ni (µ*M*)	0.97	<0.37	<0.37	<0.37	6.00	3.53
Cr (µ*M*)	0.35	<0.27	<0.27	<0.27	0.56	0.65
Cd (µ*M*)	1.09	<0.05	<0.05	<0.05	0.96	1.61
Anions (m*M*)						
As	0.0007	2.32	0.02	0.64	0.0003	0.0001
B	0.007	0.005	0.003	0.007	0.018	0.019
Cl	0.27	0.10	0.14	0.05	0.06	0.05
HCO_3	0.00	1.33	2.11	0.74	0.00	0.00
NO_3	0.33	0.40	0.46	0.19	0.07	0.05
PO_4	ND ‡	0.03	ND	0.01	ND	ND
SO_4	8.26	1.51	0.26	0.67	18.22	13.30
Charge balance §						
Σcationic charge (m*M*(+))	12.36	5.07	3.81	3.40	20.85	21.80
Σanionic charge (m*M*(-))	12.06	5.13	2.44	2.41	27.19	21.28
Net charge (m*M*)	0.30	-0.06	1.37	0.98	-6.34	0.52
% error (as cationic chg.)	2.4	-1.1	35.9	29.0	-30.4	2.4
Saturation indices						
$Al(AsO_4)$	-5	-8	-10	-8	-7	-7
$Ca_3(AsO_4)_2$	-23	-5	-9	-6	-26	-25
$Cu_3(AsO_4)_2$	-9	-1	-7	-2	-13	-12
$Fe(AsO_4)$	-4	-3	-8	-5	-4	-3
$Mg_3(AsO_4)_2$	-23	-4	-8	-6	-28	-27
$Mn_3(AsO_4)_2$	-16	-4	-13	-11	-22	-22
$Zn_3(AsO_4)_2$	-17	-7	-11	-9	-20	-19

† saturation index = log [IAP(ion activity product)/K_{sp}]: K_{sp} values from Wagemann (1977).

‡ ND = none detected

§ charge balance determined from distribution of ion species (GEOCHEM)

products (IAPs) for seven possible metal arsenates with their respective literature K_{sp} values (Wagemann, 1977), indicated that all six saturated pastes were undersaturated with respect to metal arsenates. Given discrepancies in literature K_{sp} values (Robins, 1981; Sadiq and Lindsay, 1981; Robins, 1987), and uncertainty regarding the extent that true equilibrium was reached during the 24 hr saturated paste extraction, it cannot be definitively concluded from IAP calculations that metal arsenates did not exist in these samples. Dissolution studies with metal arsenates (Ca, Fe, Mg) show that As and metal activities can vary up to approximately two-fold after 24 hours from calculated equilibrium activities (Robins, 1981). At most, this could account for errors in log (IAP/K_{sp}) of 0.6 to 1.5 for Ca, Fe, Mg arsenates, thus still suggesting the saturated pastes were undersaturated with respect to these metal arsenates (Table 2). The saturation indices coupled with direct SEM/EDAX observations (discussed later) suggest that discrete metal arsenate phases were not controlling aqueous As activities.

The PT sample and the lower pH RT sample (Soils A and E, respectively, Table 2) were chosen for further sample characterization and column experiments because their low pH has apparently prevented natural revegetation in the field, and liming is therefore the likely remediation strategy (Neuman et al., 1993b). Sixty percent of As in the RT was found in the residual fraction (Fig. 1), with 32% of the remainder in the NaOH fraction (nonoccluded Fe/Al oxides; Olsen and Sommers, 1982). Conversely, only 9% of the As in the PT existed in the residual fraction, while 66% was found in the NaOH fraction. Although 0.1 *M* NaOH could also extract As bound as Fe or Al

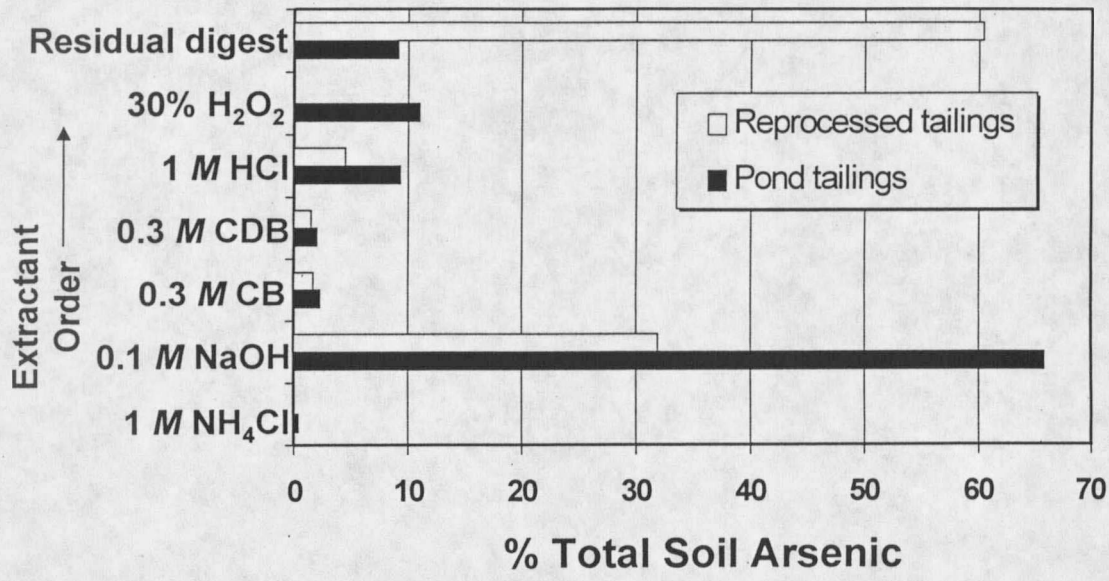


Figure 1. Percentage of total As recovered from each sequential extractant for both the reprocessed tailings and pond tailings.

arsenates, the saturated paste data suggested that these materials were undersaturated with respect to these metal arsenate phases. The large difference in readily extractable As between these two samples suggests a significant difference in As lability. Arsenic in the PT sample may be considered more susceptible to mobilization compared to the RT, as will be discussed further.

Analysis of PT and RT using SEM-EDAX revealed no solid phases concentrated in As. Most particles contained As near or below the detection limit of the instrument (approximately 0.2% by atom), although some particles contained as much as 6% As. Particles containing high levels of Fe, S, or As were further analyzed for possible identification of primary or secondary minerals. The S/Fe ratios in both samples were

generally well below elemental ratios for pyrite, and fell closer to expected ratios for jarosite (Table 3). Although some of the PT particles had S/Fe ratios near arsenopyrite, As/Fe ratios were all well below the 1:1 ratio found in either arsenopyrite (FeAsS) or scorodite ($FeAsO_4 \circ 2H_2O$). It is difficult to invoke from EDAX analyses alone the presence of specific primary or secondary minerals of Fe, S, or As. The low S/Fe ratios in most of these samples (as compared to pyrite) suggest conservation of Fe (likely as Fe oxyhydroxides) relative to S. The formation of jarosite would not be unexpected in these samples, given that it is a common secondary mineral formed from the oxidation of pyrite (Dinauer, 1977); however, we found no evidence for the presence of residual arsenopyrite or secondary formation of scorodite.

Table 3. Molar ratios of S/Fe and As/Fe in particles of pond tailings and reprocessed tailings as determined by energy dispersive analysis of X-rays (EDAX). For comparison, ratios of S/Fe and As/Fe are shown for several possible mineral phases.

	Pond Tailings		Reprocessed Tailings	
Particle	S/Fe	As/Fe	S/Fe	As/Fe
1	0.05	0.11	0.52	0.01
2	0.89	0.13	0.25	0.02
3	0.67	0.04	0.18	0.02
4	1.80	0.36	0.53	0.02
5	0.35	0.11	0.16	0.03
6	1.15	0.08	0.12	0.03
7	1.04	0.07	0.40	0.04
8	0.93	0.48	0.31	0.05
Mean (SD)	0.86 (.53)	0.17 (.16)	0.31 (.16)	0.03 (.01)
Mineral				
Arsenopyrite	1	1	1	1
Pyrite	2	---	2	---
Jarosite	0.67	---	0.67	---
Scorodite	---	1	---	1

It is likely, based on the strong affinity of As for Fe oxyhydroxides (Hingston et al., 1971; Goldberg, 1986; Fuller et al., 1993), that the majority of As was sorbed to these minerals.

Column Experiments

Effluent As concentrations of the unlimed PT column reached steady-state levels near $10^{-6.5}$ *M* within several pore volumes (Fig. 2A). Liming the PT resulted in an effluent As concentration that was approximately two orders of magnitude higher than the unlimed column. After 13 pore volumes, effluent from the limed PT column peaked at approximately 10^{-4} *M*; four hundred fold higher than in the unlimed column. The amount of As mobilized from the limed column during the experiment (26 pore volumes) represented 28% of the initial column As. Effluent pH values of the unlimed PT column were relatively stable at approximately 4.5 to 5 after several pore volumes. Liming resulted in an initial effluent pH near 8, followed by a gradual climb to pH values near 10.0. Increases in effluent pH of the limed column corresponded to increases in effluent As (Fig. 2A), consistent with the pH dependence of As sorption reactions on oxide minerals (Goldberg, 1986). Addition of 0.1 *M* $KHCO_3$ (pH 10.1) to the unlimed PT column at 23 pore volumes resulted in an increase in effluent pH and As; however, after three additional pore volumes, the hydraulic conductivity decreased to near 0, likely due to dispersion of colloidal material, and no further samples could be obtained.

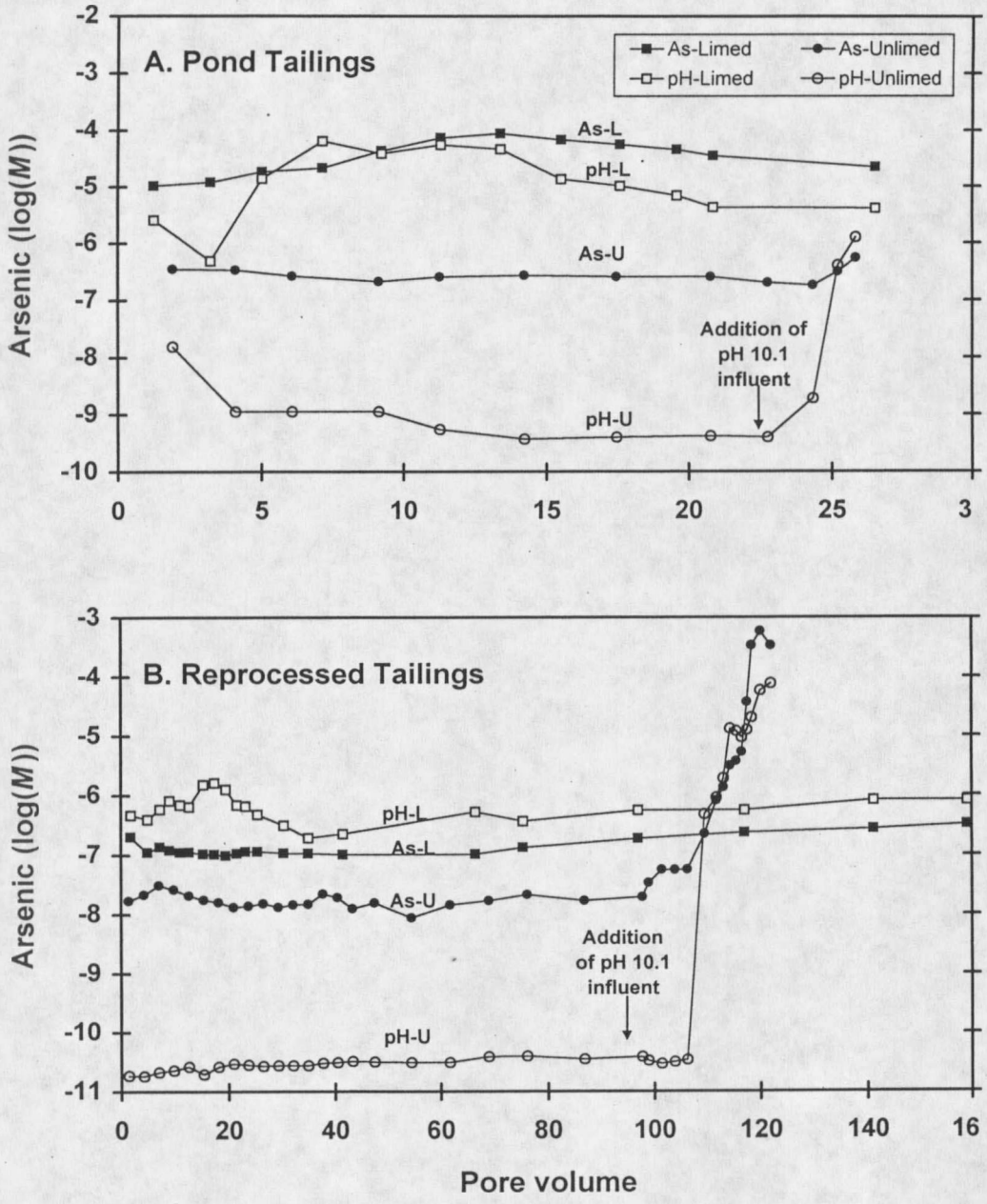


Figure 2. Arsenic concentrations and pH levels in column effluent for limed and unlimed treatments of pond tailings (Fig. 2A) and reprocessed tailings (Fig. 2B). The arrow indicates the point of addition of 0.1 *M* $KHCO_3$ (pH 10.1) influent to the unlimed column.

Effluent As concentrations remained near $10^{-7.8}$ *M* at pH levels near 3.5 in the unlimed RT column for 100 pore volumes (Fig. 2B). The liming treatment resulted in an increase in effluent pH to values near 8 and effluent As to values near 10^{-7} *M*. Although the liming amendment increased effluent As concentrations by almost one order of magnitude in the RT sample, As concentrations ranged from two to three orders of magnitude lower than those found in the PT after liming. The total As content of the PT column was only 62 mg kg^{-1} (following dilution with SiO_2), compared with approximately 3400 mg kg^{-1} for the RT column. Therefore, the soluble/total As ratio was approximately 5000-fold higher in the limed PT column than in the RT column.

Differences in effluent As concentrations between the two limed samples were consistent with differences in the "lability" of bound As (Fig. 1). The NaOH-extractable As fraction is thought to represent As associated with Al or Fe and would likely be more susceptible to desorption with increases in pH following liming. Presumably, the large residual fraction in the RT (60%) would not be as "labile" after liming. Differences in As mobilization between the two samples may have also been due to differences in pH values after liming. Effluent pH values in the PT column reached 9.8 within 7 pore volumes and remained near 9 for almost 20 pore volumes. Conversely, pH values in the RT column remained near 8 for the majority of the column experiment. Based on the pH dependence of As sorption reactions on oxide minerals (Goldberg, 1986), higher pH values in the PT column likely contributed to

more As desorption. In fact, when the pH of the unlimed RT column was raised to near 10, effluent As increased from approximately $10^{-7.8}$ *M* to $10^{-3.2}$ *M* within 20 pore volumes. The fact that a high ionic strength/high pH influent solution (0.1 *M* $KHCO_3$) was necessary to mobilize substantial amounts of As from the RT column is consistent with the higher residual As fraction in this sample. Results from the column experiments presented here are consistent with the pH dependence of surface complexation reactions of As on solid phase minerals such as goethite, ferrihydrite, gibbsite, Al hydroxides, and layer silicates.

Prior to liming, it was demonstrated that IAPs of PT and RT saturated paste extracts were undersaturated with respect to several metal arsenate solid phases (Table 2); however, after liming, changes in pore water chemistry necessitated a reanalysis of IAPs, especially considering the potential for Ca-arsenate to form in the presence of calcite (Sadiq et al., 1983). Solubility curves for both scorodite and Ca-arsenate were compared to selected effluent data for each of the three treatments (Fig. 3). All effluent solutions were found to be undersaturated with respect to these two solid phases. Although the possibility of an Fe-arsenate phase with lower solubility than that assumed for scorodite cannot be ruled out (Nordstrom and Parks, 1987), such phases have not been commonly observed in other studies. In addition, other possible solid phase Ca-arsenates such as $Ca(H_2AsO_4)_2$, $CaHAsO_4$, and $Ca_5H_2(AsO_4)_4$ can exist in a Ca-As-H_2O system. Each of these solid phases is more soluble than $Ca_3(AsO_4)_2$ (Nishimura et al.,

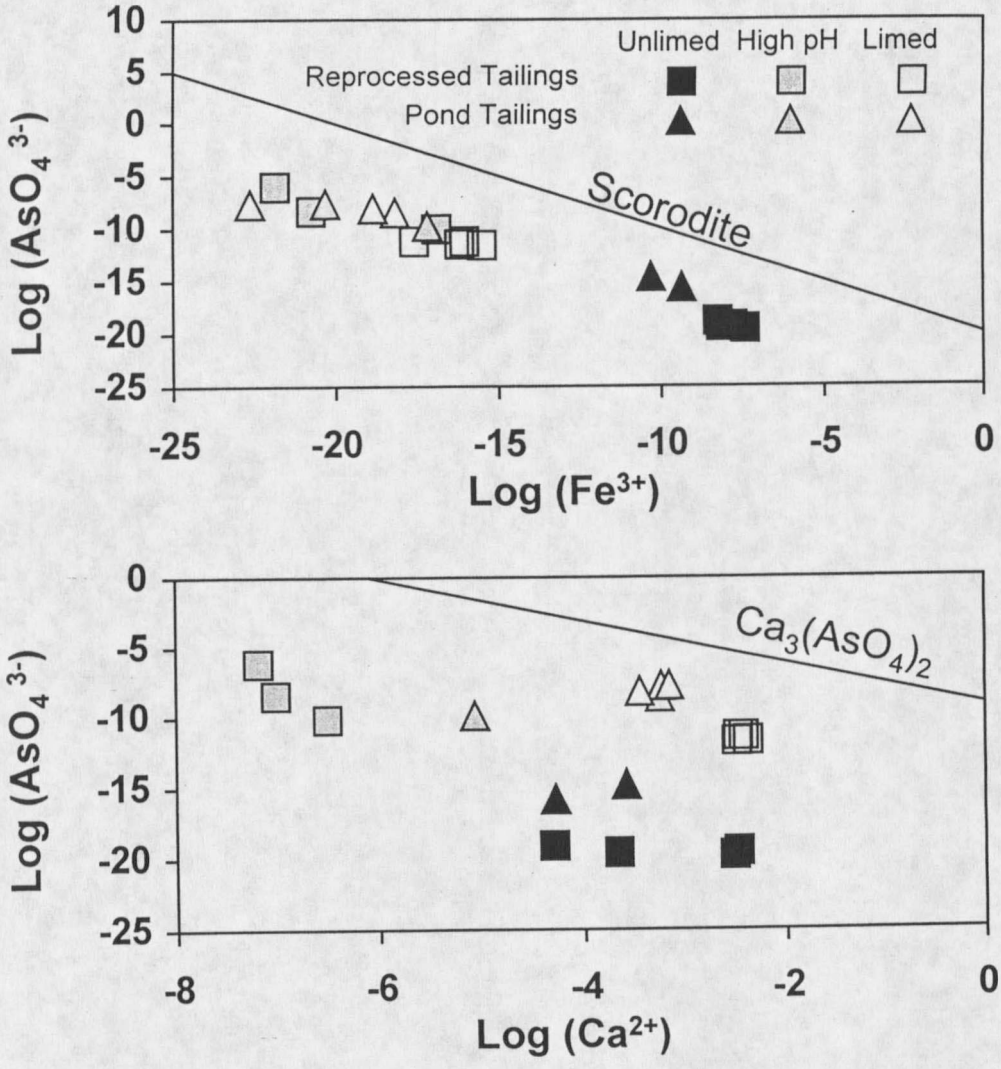


Figure 3. Solubility diagrams for scorodite ($FeAsO_4 \bullet 2H_2O$) and $Ca_3(AsO_4)_2$ compared to activities of AsO_4^{3-}, Fe^{3+}, and Ca^{2+} in column effluent. Data points below the solubility lines indicate these solutions were undersaturated with respect to the given solid phase.

1987), hence the effluent solutions were undersaturated with respect to these solid phases as well. These data, combined with the EDAX results, support the contention that the behavior of As after liming is not controlled by equilibrium with respect to

solid phase arsenates, but is related to the pH dependence of As sorption reactions typically observed on oxide minerals.

Conclusions

Characterization of several representative mine tailing samples showed that soluble As levels were not correlated with total As, but were more strongly correlated with soil solution pH. Furthermore, saturated pastes of all mine tailing samples were undersaturated with respect to known metal arsenate solid phases. The mobility of As in two selected mine tailing samples was shown to increase substantially in unsaturated laboratory columns following a recommended liming treatment ($CaCO_3/Ca(OH)_2$). Adsorption-desorption reactions on oxide minerals are believed to be responsible for the pH dependence of soluble As concentrations, rather than precipitation-dissolution reactions of solid metal arsenates. The concentration of As in column effluent after liming was not correlated with total As in the two samples chosen for detailed column experiments. Sequential extractions showed substantial differences in "labile" As between the two samples, consistent with results from column experiments after liming. Consequently, the distribution of soil-bound As is important for determining the susceptibility of mine tailings to As mobilization following liming. In addition, the pH of the post-limed tailings is important for determining the amount of As mobilized after liming. Remediation of acidic mine tailings or other As-contaminated soils using lime

amendments should be evaluated with respect to the potential effects on As mobilization, especially at contaminated sites hydraulically connected to surface or ground waters.

CHAPTER 3

EFFECTS OF LONG-TERM IRRIGATION ON SOIL ARSENIC MOBILITY OF THE MADISON AND UPPER MISSOURI RIVER VALLEYS, MONTANA

Introduction

Geothermal discharge in Yellowstone National Park provides a continual source of As to the Madison and Missouri River system (Stauffer and Thompson, 1984; Nimick et al., 1998). Concentrations of As in 850 km of the Madison-Missouri mainstem (Fig. 4) exceed the State of Montana human health standard of 0.24 μM, with concentrations approaching 5 μM in the Madison River headwaters (Nimick et al., 1998). In addition, As levels exceed the EPA drinking water standard of 0.67 μM in approximately 70 km^2 of groundwater in the Lower Madison River Valley (Nimick, 1998) and As in saturated paste extracts from soils overlying this area range up to approximately 130 μM (Keith, 1995). A previous study suggested that evapoconcentration of applied irrigation water was increasing As levels in soils, groundwater, and irrigation return flows of the Lower Madison River Valley (Sonderegger and Ohguchi, 1988). Based on this theory, the State of Montana implemented controls in 1992 to prevent new irrigation projects that would raise As

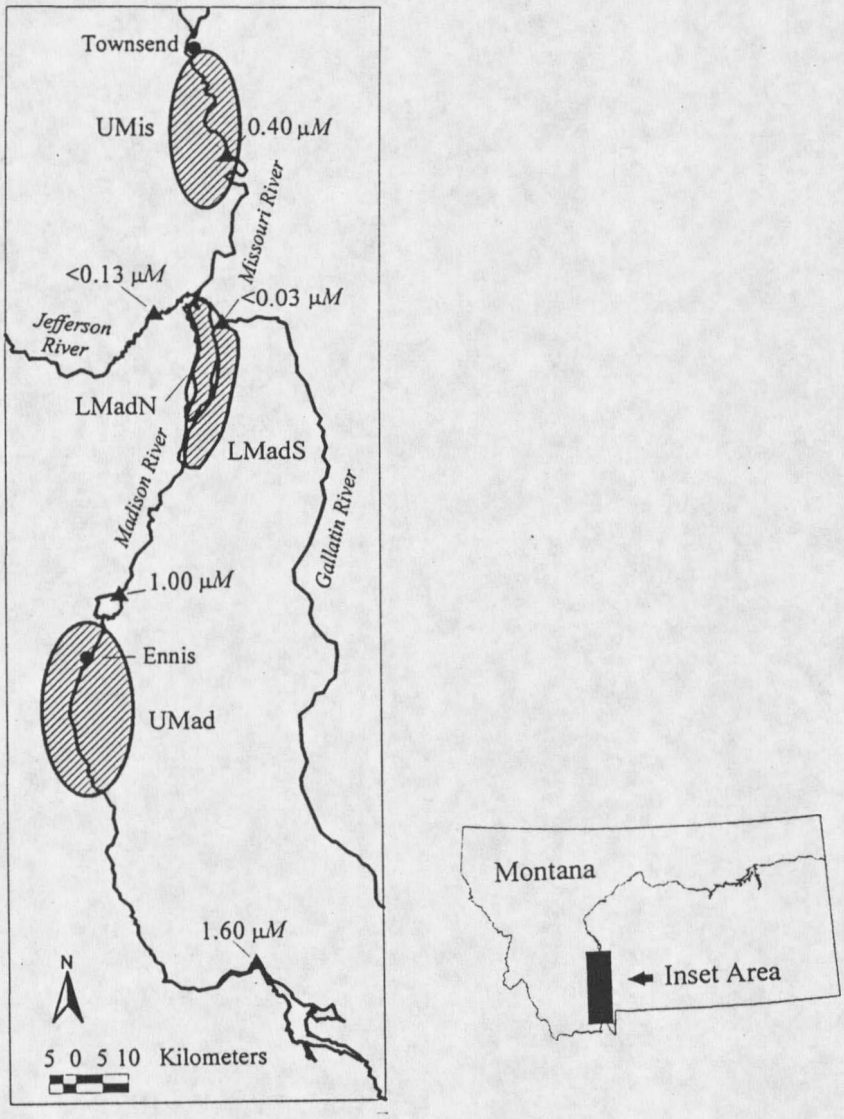


Figure 4. Median surface water arsenic (As) concentrations (Nimick et al., 1998) and sampling locations in the Upper Madison (UMad; Sites A1-A4), Lower Madison South (LMadS; Sites B1-B4), Lower Madison North (LMadN; Sites C1-C4), and Upper Missouri (UMis; Sites D1-D4). Levels of As in the alluvial aquifer underlying the LMadN exceeded the federal drinking water standard of 0.67 μ*M* As, yet were below this level in all other sampled groundwater (Tuck et al., 1997).

levels in downstream waters. To predict the effects of current and future irrigation projects in the Madison-Missouri River Basin, it is necessary to understand processes controlling As transport in both irrigated and nonirrigated soils.

Solubility of As in soils can be controlled by sorption/desorption, precipitation/dissolution, and oxidation/reduction reactions. Sorption of arsenate (As(V)), the thermodynamically favored species in oxidized environments, has been found to decrease with increased pH (Goldberg and Glaubig, 1988; Darland and Inskeep, 1997a; Jones et al., 1997; Raven et al., 1998). Sorption of As(V) to oxide materials has been shown to occur via a ligand exchange mechanism based on good correlations between experimental and modeled As(V) sorption on Fe and Al oxides using a constant capacitance model (Goldberg, 1986). Surface complexation of As(V) at Fe oxide surfaces occurs primarily as a bidentate innersphere surface complex, although monodentate surface complexes are also possible based on x-ray absorption fine structure spectroscopy (Waychunas et al., 1995). Phosphate additions have been shown to substantially mobilize As(V) (Davenport and Peryea, 1991; Peryea, 1991; Darland and Inskeep, 1997a) due to competition for binding sites at the oxide surface. At low redox potentials ($<$20 mV at pH 7), arsenite (As(III)) is the thermodynamically stable species. Many studies have demonstrated that As(III) does not sorb as strongly as As(V) (Frost and Griffin, 1977; Pierce and Moore, 1982; Xu et al., 1991), and total soluble As concentrations often increase with decreased redox potential (Masscheleyn et al., 1991a; McGeehan and Naylor, 1994). Precipitation/dissolution reactions involving As

are often less important than sorption/desorption reactions in surface soils because As(V) solid phases are generally too soluble to control equilibrium As(V) concentrations (Robins, 1981; Sadiq et al., 1983; Dove and Rimstidt, 1985; Nishimura et al., 1987). Conversely, As(III)-sulfide solid phases, such as amorphous As_2S_3 and orpiment, are stable in highly reduced systems (Webster, 1990; Eary, 1992) and may control As solubility in saturated soils and sediments.

Irrigation with Madison River water, containing approximately 0.6 to 1.3 μM of As (Nimick, 1998) has the potential to alter soil solution As levels through a variety of mechanisms. For example, sorbed As levels may increase due to irrigation, thereby supporting higher equilibrium solution As levels. Increased pore water velocities from irrigation may also affect solution As concentrations due to kinetic limitations of As sorption. Darland and Inskeep (1997b) found that nonequilibrium conditions existed at pore water velocities as low as 0.2 cm h^{-1} in As transport studies; therefore, sorption equilibrium may not be achieved during irrigation events. Finally, flood irrigation may decrease redox potentials, favoring the formation of As(III), and possibly decreasing As sorption. In summary, irrigation could have a variety of effects on As solubility depending on specific site characteristics. Consequently, the objectives of the present study were to: i) determine effects of long-term irrigation on accumulation of soil profile As, ii) identify soil characteristics that control As sorption, iii) compare As lability between irrigated and nonirrigated sites, and iv) determine the potential for continued As attenuation in soils of the Madison and Upper Missouri River Basins.

Materials and Methods

Sample Collection

Soil samples were collected during 1993 and 1994 at sixteen paired (irrigated and nonirrigated) sites adjacent to the Madison and Missouri Rivers, Montana (Keith, 1995). Sampling sites were grouped into four major regions (Fig. 4): Upper Madison (Sites A1-A4), Lower Madison South (Sites B1-B4), Lower Madison North (Sites C1-C4), and Upper Missouri (Sites D1-D4). Sampling was concentrated in the Lower Madison because groundwater in this vicinity has elevated As levels (0.25 – 2.3 μM; Nimick, 1998). Irrigated (I) sites had been irrigated with river water for more than 20 yr, and at least two sites (B1 and C2) had been irrigated for approximately 110 yr. Nonirrigated (NI) sites had never been irrigated and were selected based on textural and topographical similarities with the paired irrigated sites. The paired sites were less than 60 m apart at each sampling location. Soils were collected at discrete depth increments (0-5, 5-10, 10-20, 20-30, 30-60, 60-90, 90-150, 150-250, 250-350 cm), and samples were stored on ice during transport to the laboratory.

Soil Characterization

Soil samples were passed through a 2-mm sieve, and a subsample of the fine earth fraction was dried at 70°C for one week. Particle size analysis was performed

using the hydrometer method (Gee and Bauder, 1986) and organic matter (O.M.) was analyzed using a modified Walkley-Black procedure (Nelson and Sommers, 1982). Saturated pastes of air-dried soils (Rhoades, 1982) were centrifuged at 6,000 *g* for 30 minutes and filtered (0.2-μm) prior to analysis for soluble constituents. Soluble Ca was analyzed using atomic absorption spectrophotometry (AAS), and soluble As was analyzed with continuous flow hydride generation atomic absorption spectrophotometry (HGAAS). Samples for As analysis were acidified to 3 *M* HCl, pre-reduced in 1% KI, and mixed in a reaction coil with 0.6% $NaBH_4$/0.5% NaOH. Flow rates were 7 and 1 mL min^{-1} for sample and reagent, respectively. Generated arsine was quantified in an air-acetylene flame (Perkin-Elmer 3100) at 193.7 nm. Total soil As was analyzed using a four acid heat digestion (Welsch et al., 1990), and As levels in the resulting digests were quantified using HGAAS. Ammonium oxalate extractable (AOE) Fe, Mn, and Al were analyzed to estimate "free" oxide fractions (Loeppert and Inskeep, 1996), and Olsen P was extracted using the $NaHCO_3$ method of Olsen and Sommers (1982).

Batch Sorption Isotherms

Soils from six paired sites (A1,B1,B4,C2,C3,D1) were selected for further analysis based on soluble and total As levels. Depth-weighted composites were formed by mixing the 0-5, 5-10, and 10-20 cm soil samples in a 1:1:2 mass ratio. Batch sorption isotherm experiments for As(V) were performed in duplicate, using a 2:1 solution:solid ratio (i.e. 20 mL 0.01 *M* KCl:10 g soil). Initial As concentrations were 0,

1.3, 4.0, 13, 40, 133, and 1330 μM, and each soil slurry was shaken on a horizontal shaker at approximately 120 cycles min^{-1} for 96 hr. Equilibration time was determined by conducting a kinetic sorption study at 13 μM As using soils from sites B1-NI and C2-NI. Samples were centrifuged at 10,000 *g* for 10 min, the supernatant filtered (0.2-μm), and the filtrate analyzed for As using HGAAS (described previously). Isotherm results were fit to Langmuir and Freundlich models, and r^2 values were found to be greater than 0.999 for all 24 samples. Linear regressions were also performed, and the resulting distribution coefficients (K_d values), describing the linear portion of As sorption, were regressed against pH, O.M., clay content, AOE metals (Fe, Mn, Al), Olsen P, and total As.

A subsequent batch sorption experiment was conducted to determine the extent of As(V) reduction during the 96 hr batch isotherm experiments. Initial As(V) concentrations of 0 and 13 μM were used for each of the 12 soils. As(V) and As(III) were determined using the following method modified from Masscheleyn et al. (1991b). Five mL of filtered sample were added to 0.5 mL of 2 *M* TRIS buffer (pH = 6.5). While bubbling this buffered solution with N_2 (g), a solution of 3% $NaBH_4$/0.1% NaOH was added incrementally (0.1 mL over 30 s, 3 min wait, 0.4 mL over 30 s, 3 min wait) to liberate arsine from any As(III) in the sample. The original sample (As(T)) and the speciated sample (As(V)) were both analyzed for As, and As(III) concentration was calculated by difference. Analysis of duplicate standards made from $NaAsO_2$ and

$Na_2HAsO_4 \circ 7H_2O$ salts yielded As(III) and As(V) recoveries of 97 ± 2% and 103 ± 1%, respectively.

Sequential Extractions

Paired surface soils (sites A1, C2, and C3), representing the full range of experimentally determined K_d values, were subjected to sequential extractions to estimate the distribution of soil-bound As based on a compilation of procedures (Johnston and Barnard, 1979; Tessier et al., 1979; Ganje and Rains, 1982; Olsen and Sommers, 1982). The method consisted of sequential extractions in the following order: 1 *M* NH_4Cl (exchangeable), 0.1 *M* NaOH (non-occluded in Al and Fe oxides), 1 *M* HCl (Ca-bound and occluded in Al and Fe oxides), and 30% H_2O_2/0.8 *M* NH_4OAc (organically-bound). Although sequential extractions yield only qualitative information on As fractionation, this procedure helped assess relative As lability among irrigated and nonirrigated soils. Extracted samples were centrifuged at 10,000 *g* for 10 min and filtered through 0.2-μm nylon filters. The H_2O_2 extracts were analyzed for As with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and As in the remaining extracts was quantified with HGAAS. Total residual As was determined using heat digestion as described previously. Quality assurance/quality control consisted of the following: all samples were extracted in duplicate; percent recoveries were calculated by comparing summed extract concentrations with total As; interferences were tested by adding a known quantity of As to each C3-I extract (matrix spikes) and

to each extraction blank (extraction spikes); HGAAS accuracy was assessed with EPA certified standards obtained from Spex Certiprep, Inc. (Metuchen, NJ). The mean relative standard deviation (RSD) of duplicate analyses that had As values above the detection limit was 11.4%. Total sequential extraction recoveries for the six samples averaged 95%, matrix spike recoveries ranged from 98 to 125%, extractant spike recoveries ranged from 96 to 109%, and the average recovery on duplicate EPA standards was 99.5%.

Analysis for Solid Phases

Ion activity products (IAPs) for scorodite ($FeAsO_4 \cdot 2H_2O$) and $Ca_3(AsO_4)_2$ were calculated by assuming the activity of Fe^{3+} was controlled by an amorphous $Fe(OH)_3(s)$ ($\log K_{sp} = -39.3$) and that $I=0.013EC$, where I = ionic strength in mol L^{-1} and EC=electrical conductivity in dS m^{-1} (Lindsay, 1979). Ion activities were calculated using the aqueous chemical equilibrium model, GEOCHEM (Sposito and Mattigod, 1979) as modified by Parker et al. (1987). Stability constants for relevant aqueous complexes were compiled from thermodynamic equilibrium data (Sadiq and Lindsay, 1979; Sposito and Mattigod, 1979; Nordstrom and May, 1990). Grains of sample C3-NI, the soil with the highest soluble and total As concentrations of the twelve chosen for further analyses, were affixed to Al stubs, coated with C, and viewed with a JEOL-6100 scanning electron microscope (SEM). The grains were mapped for As, Fe, Al, S, and Ca using energy dispersive analysis of x-rays (EDAX) and those grains containing

elevated levels of As were more closely investigated to determine presence of discrete As solid phases.

Saturated Column Experiments

Column transport experiments were conducted on composited surface samples from soils B1-I, B1-NI, C2-I, and C2-NI to determine additional As sorption capacities. Both B1-I and C2-I had been flood-irrigated from 1882 to 1976 at approximately 60 cm yr^{-1}, and are currently sprinkler-irrigated at approximately 30 cm yr^{-1}. Soils were packed into acrylic columns (3 cm diam., 5 cm length) with bulk densities of 1.15-1.25 g cm^{-3}. The soils were saturated from the bottom with 0.01 *M* KCl, then treated with influent (0.01 *M* KCl/1.3 μ*M* As(V)) at a pore water velocity (saturated conditions) of 1.7 cm hr^{-1} until the cumulative loading approached 900 cm (approximately 30 yr of simulated irrigation). Column effluent was passed through 0.45-μm in-line filters prior to sample collection and analysis. Effluent solution As concentrations were measured with HGAAS and the increase in soil As calculated by difference.

Results and Discussion

Sample Characterization

Total As levels in Madison River irrigated (I) soils were calculated to be 1.5 - 7.5 mg kg^{-1} higher than in nonirrigated (NI) soils assuming an irrigation history of 100

yr at 60 cm yr^{-1}, an As concentration of 1 μM in irrigation water, and 50% As retention in the upper 20 cm. Average measured differences in total As levels between irrigated and nonirrigated soils were within the calculated range at sites in both the Upper Madison (UMad; Sites A1-A4) and Lower Madison South (LMadS; Sites B1-B4); although neither difference was significant at $\alpha=0.05$ (Fig. 5). A reversed trend was observed in the Lower Madison North (LMadN; Sites C1-C4) where average total As levels were approximately 12 mg kg^{-1} lower in irrigated than in nonirrigated soils. Although the measured difference in As between irrigated and nonirrigated soils in the LMadN was not significant ($\alpha=0.05$), the lower As levels in irrigated soils suggest irrigation may be leaching As out of the soil profile. Total As levels in soils within LMadN were two to three fold higher than in the other three sampling areas, again demonstrating the uniqueness of LMadN soils. Total As levels in the Upper Missouri (UMis; Sites D1-D4) were similar between irrigated and nonirrigated soils, as expected due to relatively low As levels (~0.4 μM) in irrigation water. Soluble As concentrations followed the same trends as total As levels among regions and between irrigated and nonirrigated soils (Fig. 5). Most notably, average soluble As levels in nonirrigated soils of the LMadN were five fold higher than in irrigated soils, again suggesting that irrigation with lower Madison River water ([As] ~ 0.9 μM) is decreasing naturally occurring high levels of As by dilution and flushing. Average soluble As concentrations in the nonirrigated LMadN soils were approximately 30 μM, more than 50 fold higher

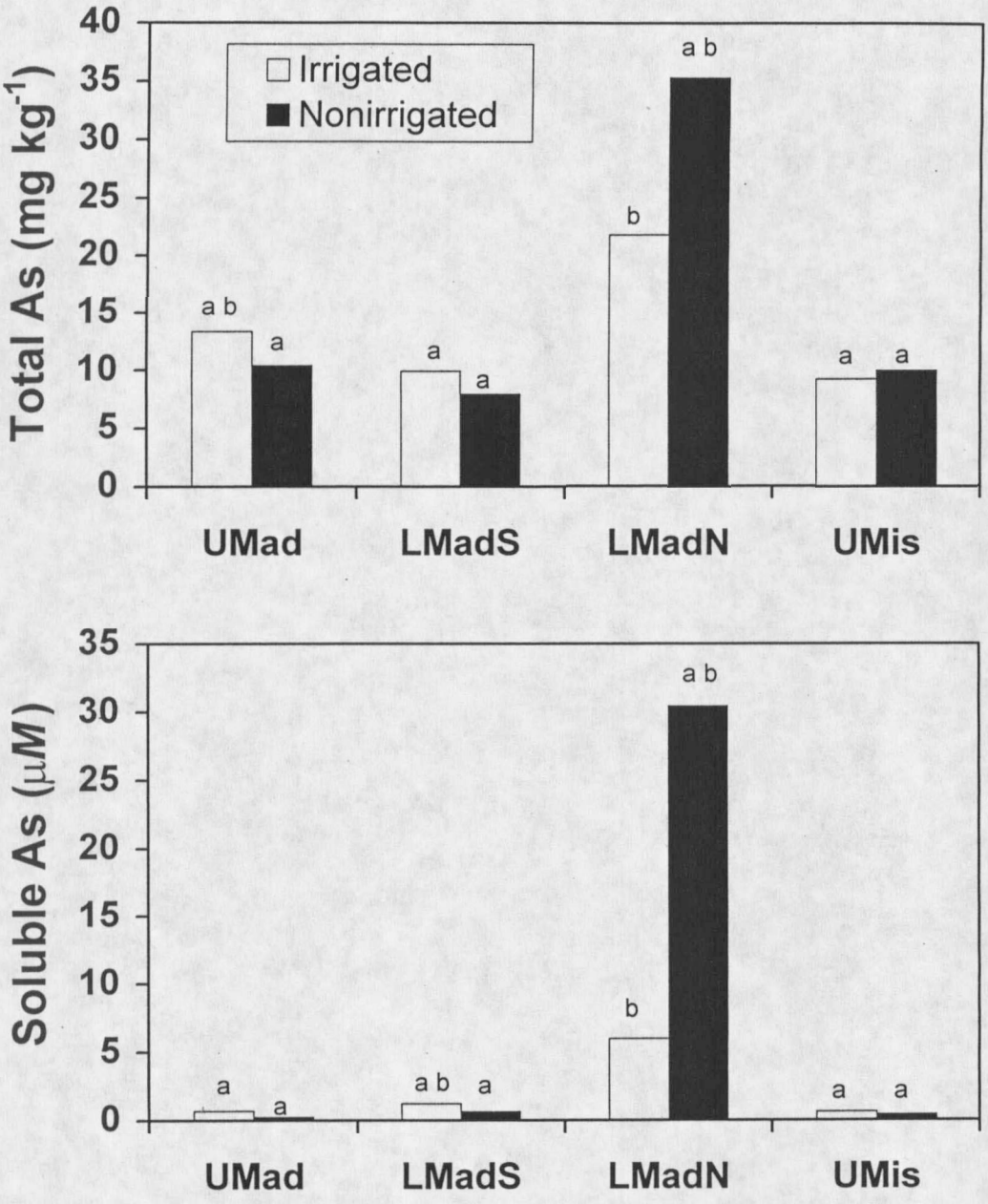


Figure 5. Total and soluble As concentrations in the top 20 cm of soil for the Upper Madison (UMad), Lower Madison South (LMadS), Lower Madison North (LMadN), and Upper Missouri (UMis). Each value is an average of depth-weighted As levels from four sampling sites. Bars containing the same letter are not significantly different (α=0.05). The lack of significance between irrigated and nonirrigated As levels in the LMadN region is due to high variability among nonirrigated sites.

than concentrations in the other three nonirrigated areas. High soluble and total As in soils of the LMadN are possibly caused by upward transport of As from shallow, more As-rich, groundwater in this vicinity (Nimick, 1998). In summary, irrigation has not significantly increased soil profile As levels in the four sampling regions (α=0.05), and soils in the LMadN region have uniquely high levels of both total and soluble As.

Sorption of As in Irrigated vs. Nonirrigated Soils

Distribution coefficients (K_d), describing the linear portion of As sorption isotherms, ranged from 3.4 L kg^{-1} in C2-I to 33.2 L kg^{-1} in A1-NI (Table 4), indicating substantial differences in As sorption characteristics among soils. Although the mean K_d for irrigated soils was somewhat lower than for nonirrigated soils (14.7 $\pm$ 12.0 L kg^{-1} vs. 17.4 $\pm$ 8.8 L kg^{-1}), the difference was not significant (α=0.05). Therefore,

Table 4. Soil sorption coefficients (K_d) of six paired Madison River Valley soils.

	Linear K_d †	
Site	Irrigated	Nonirrigated
	----------L kg^{-1}----------	
A1	30.2 ± 1.0	33.2 ± 2.8
B1	7.9 ± 0.3	12.5 ± 1.7
B4	12.2 ± 0.02	8.2 ± 1.0
C2	3.4 ± 0.1	19.9 ± 0.1
C3	5.2 ± 0.7	17.7 ± 1.6
D1	29.3 ± 2.3	13.0 ± 0.8
Mean	14.7	17.4

† K_d values are from the linear portions of duplicate isotherms.

irrigation has not significantly lowered the capacity for additional As sorption in the tested soils. A regression analysis between K_d and each of eight variables [pH, O.M., clay content, AOE metals (Fe, Al, Mn), Olsen P, and total As] was performed to determine which of these soil characteristics were correlated to As sorption coefficients. A three variable multiple linear regression described the majority of variation in K_d values among samples (Fig. 6). Colinearity was assessed between variables, and none of the three variables were significantly related ($\alpha = 0.05$). The equation,

$$K_d = 0.015(\text{AOE Fe}) + 0.038(\text{AOE Mn}) - 0.62(\text{Olsen P}) + 3.3 \quad (r^2 = 0.92),$$

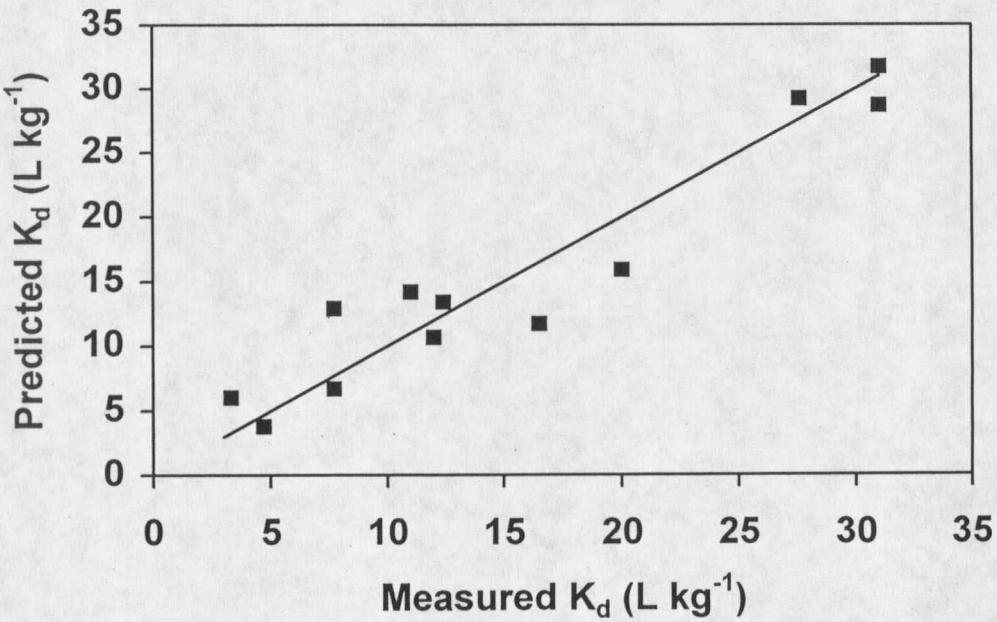


Figure 6. Predicted versus measured K_d for twelve Madison River soils. Measured K_d values were calculated from the linear portion of each sorption isotherm. The predicted K_d is based on a multiple linear regression with AOE (ammonium oxalate extractable) Fe, AOE Mn, and Olsen P as independent variables.

suggests that levels of oxide materials and exchangeable P, rather than irrigation history, control As sorption in these soils. It has been well documented that As sorbs strongly to oxides of Fe (Hingston et al., 1971; Raven et al., 1998) and Mn (Oscarson et al., 1981), and that P competes for As sorption sites (Roy et al., 1986; Peryea, 1991; Darland and Inskeep, 1997a). Although irrigation could decrease As sorption by decreasing levels of Fe and Mn oxides, or increasing P levels, there were no significant differences ($\alpha=0.05$) between irrigated and nonirrigated means for any of these three variables (Table 5).

Table 5. Average chemical and physical characteristics† from the top 20 cm of six paired Madison River Valley soils.

Parameter	Irrigated	Nonirrigated	P-value	Significance
K_d (L kg^{-1}) ‡	14.7 ± 12.0	17.4 ± 8.8	0.66	NS §
pH	8.0 ± 0.1	8.0 ± 0.2	0.51	NS
O.M.(%)	2.8 ± 1.9	3.1 ± 2.7	0.79	NS
clay(%)	20.7 ± 9.1	17.3 ± 8.5	0.56	NS
AOE ¶ Fe (mg kg^{-1})	656 ± 479	455 ± 162	0.35	NS
AOE Mn (mg kg^{-1})	292 ± 163	322 ± 179	0.77	NS
AOE Al (mg kg^{-1})	828 ± 246	789 ± 277	0.80	NS
Olsen P (mg kg^{-1})	15.3 ± 9.2	8.3 ± 3.0	0.11	NS
Total As (mg kg^{-1})	14.9 ± 7.9	20.0 ± 21.1	0.59	NS

† The pH, O.M., clay, and total As values are depth-weighted averages (Keith, 1995), and the remaining results are for depth-weighted composites.
‡ K_d values were calculated from the linear portion of duplicate isotherms.
§ NS - Not significant ($\alpha=0.05$)
¶ AOE - Ammonium oxalate extractable (Loeppert and Inskeep, 1996)

Determination of As(V) and As(III) species following the batch isotherm experiment revealed that over 50% of the As(V) was reduced to As(III) in only C2-I and C3-I, the soils with the lowest K_d values. This suggests that K_d may also be a function of As valence state due to generally accepted differences in sorption between As(V) and As(III) (Pierce and Moore, 1982; Xu et al., 1991); although it has recently been demonstrated that ferrihydrite can sorb similar amounts of As(III) and As(V) at high As surface coverages (Raven et al., 1998). When data from the two reduced samples were excluded from the regression analysis, the coefficients remained virtually the same, suggesting the production of As(III) in the two samples did not grossly affect the regression model. In summary, levels of oxide materials and $NaHCO_3$-exchangeable P apparently account for the majority of the variability in K_d values among sites.

Partitioning of As in Selected Soils

Irrigation has not consistently altered the partitioning of As in three soils from the Upper and Lower Madison regions based on a comparison of sequential extraction data between irrigated and nonirrigated soils (Fig. 7). More notable differences in As partitioning and lability exist between sites. For example, the 1 *M* NH_4Cl and 0.1 *M* NaOH fractions account for approximately 10 mg kg^{-1} in both C3-I and C3-NI, yet less than 2.5 mg kg^{-1} in the other four soils. These differences in the two most labile fractions are consistent with the high soluble As levels found in C3-I (12 μM) and C3-NI (20 μM) compared to soluble As levels of less than 3 μM found in the other four

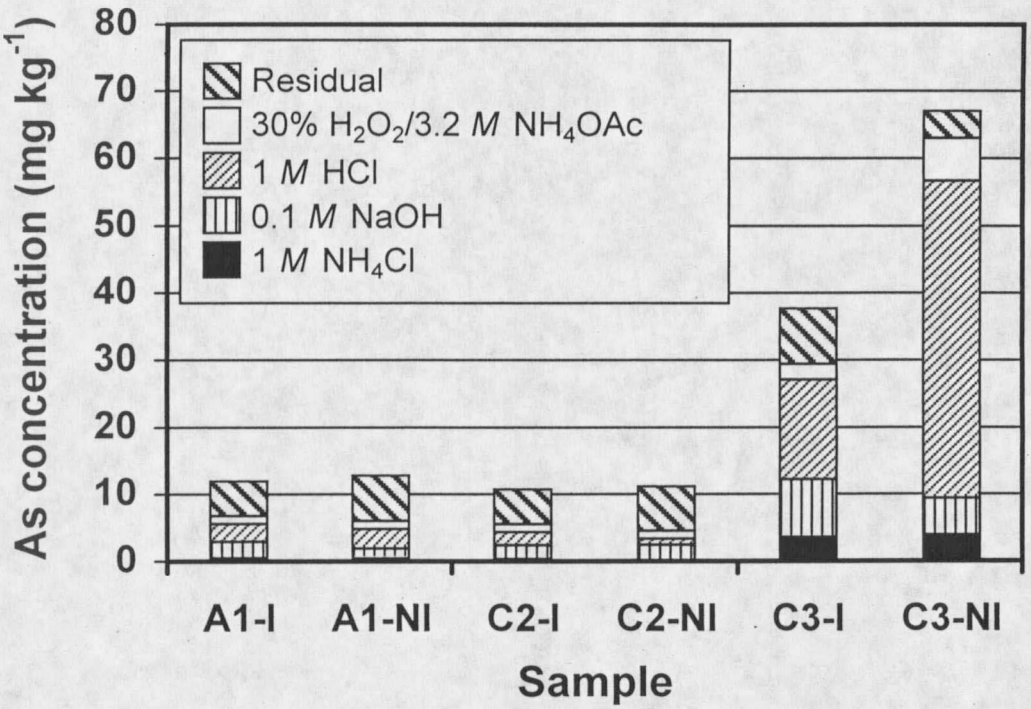


Figure 7. Concentrations of As recovered from each sequential extractant for three irrigated (I) and nonirrigated (NI) soils. Values are averages of results from extractions performed on duplicate samples.

soils. In addition, residual fractions account for less than 20% of the total As in C3, yet approximately 50% of total As in A1 and C2, also indicating large differences in As lability between sites. Soils from C2 and C3 are both from the LMadN region, again demonstrating the high degree of heterogenity in As distribution within this region. In summary, irrigation history has not substantially altered As distribution at three selected sites.

Ion activity products (IAPs) calculated for the upper three depth increments (0-5 cm, 5-10 cm, and 10-20 cm) at each site were compared to K_{sp} values for $Ca_3(AsO_4)_2$ and scorodite (Wagemann, 1977). IAPs were lower than K_{sp} values by at least 6 and 9 orders of magnitude for $Ca_3(AsO_4)_2$ and scorodite, respectively, indicating saturated pastes from these soils were undersaturated with respect to both solid phases. Given inconsistencies in literature K_{sp} values (Sadiq and Lindsay, 1981), and uncertainty that true equilibrium was reached during the 24 hr saturated paste extraction, it cannot be definitively concluded from IAP calculations that metal arsenates did not exist in these samples. Although insufficient data were available to test for presence of other metal arsenates, analysis of C3-NI using SEM-EDAX revealed no solid phases concentrated in As. The saturation indices coupled with the SEM-EDAX observations suggest that discrete metal arsenate phases were not controlling aqueous As activities. Therefore, sorption/desorption reactions rather than precipitation/dissolution reactions likely control As levels in soils of the study area.

Saturated Column Experiments

The two soils (B1 and C3) chosen for saturated column experiments had been irrigated for approximately 110 yr with As-rich Madison River water; yet both soils had additional capacities to retain As. Soil As concentrations increased between 3 mg kg^{-1} (C2-I) and 7.5 mg kg^{-1} (C2-NI) following approximately 900 cm of cumulative loading with a 1.33 μM As influent (Fig. 8). Soil C2-I had the lowest K_d (3.4 L kg^{-1}) of

the twelve soils selected for further analysis (Table 4); therefore, 3 mg kg^{-1} likely represents the lower boundary of additional As retention capacities in these soils. Increases in soil As in the saturated column experiments appeared to be positively related to 96 hr batch K_d values, suggesting that As retention observed in the column experiments was also controlled by levels of oxide materials and Olsen P. Removal efficiencies of As during the first five pore volumes ranged from 48% (C2-I) to 85% (B1-I); however, by the end of this experiment, the soils were removing less than 22% of the influent As. The total mass of As attenuated under field conditions will be higher because the column experiments were only performed with 5 cm columns. Differences

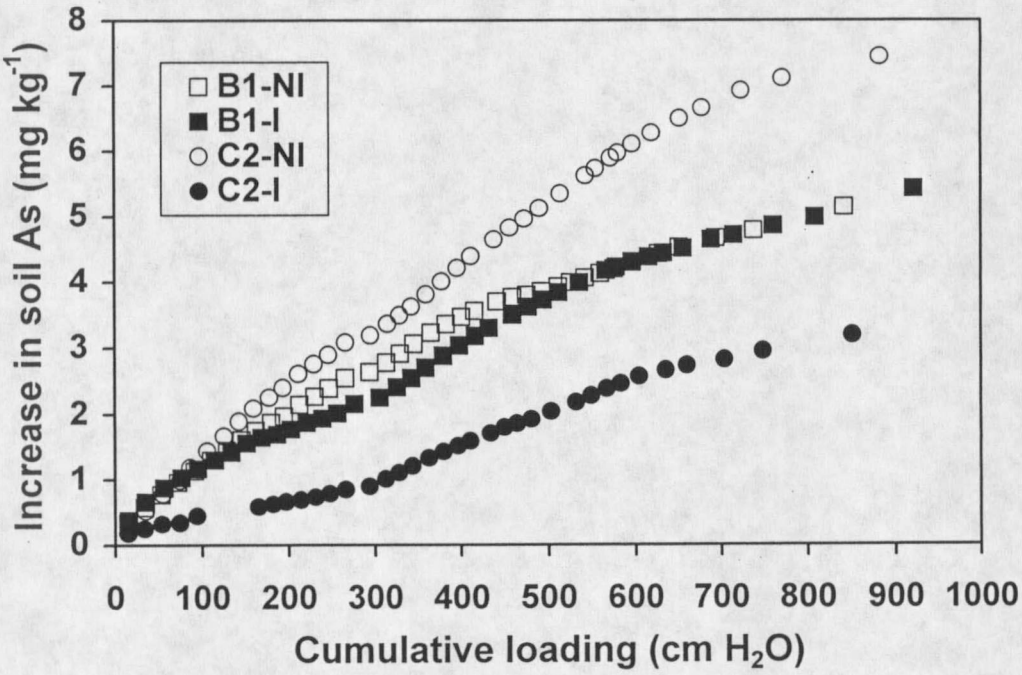


Figure 8. Increase in soil As as a function of water loading in saturated soil columns (5 cm long, 3 cm diameter). Influent (1.33 μM As in a 0.01 M KCl background solution) was pumped at a pore water velocity of 1.7 cm hr^{-1} in both irrigated (I) and nonirrigated (NI) soil columns.

in pore water velocity (PWV) between this experiment (PWV = 1.7 cm h^{-1}) and field conditions may also have a large effect on As transport. In a packed sand column (Darland and Inskeep, 1997b), As recovery in the effluent after 10 pore volumes was 7.2, 35.6, and 53.3% at PWVs of 0.2, 1, and 10 cm h^{-1}, consistent with sorption-related nonequilibrium. Therefore, PWVs are expected to affect As attenuation, and actual As retention is expected to be higher than measured due to anticipated slower average pore water velocities in the field. In addition, anticipated flow conditions in the field (transient, unsaturated flow) are expected to result in greater As removal, compared to laboratory column experiments (steady-state, saturated flow). Conversely, there is a lower likelihood for preferential flow in column experiments due to generally fewer macropores in repacked soil columns compared to field conditions. Despite differences between laboratory and field conditions, the column transport studies on soils from sites B1 and C2, combined with earlier results (Darland and Inskeep, 1997b), support the contention that As transport in these soils is likely controlled by As sorption to oxide materials and pore water velocities.

Conclusions

Soils adjacent to the Madison and Missouri River Valley were studied to determine if long-term irrigation was affecting soil profile As levels, sorption coefficients, lability, or mobility. Total and soluble As levels in the upper soil profile (top 20 cm) were slightly higher in irrigated sites than in nonirrigated sites of the Upper

Madison and Lower Madison South regions, suggesting some retention of applied As. This observed trend was reversed in the Lower Madison North where average soluble and total As levels were 6 and 1.5 fold higher, respectively, in nonirrigated soils than in irrigated soils. The differences imply that irrigation has lowered naturally occurring high levels of As in soils from the Lower Madison North through dilution and flushing. Average sorption coefficients were 16% lower in irrigated than in nonirrigated soils although this difference was not significant ($\alpha=0.05$). Sorption coefficients were positively correlated with measured levels of 'free' Fe and Mn oxides, and negatively correlated with $NaHCO_3$-extractable P. Therefore, dissolution of oxides or additional inputs of P would both be expected to decrease As sorption. Sequential extractions showed no major increases in "labile" As between irrigated and nonirrigated soils, consistent with solution As data. Saturated transport studies demonstrated that surface soils that have been irrigated with As-rich water for more than 100 yr have additional As sorption capacities of at least 3 mg kg^{-1}, and these capacities are positively correlated with sorption coefficients. Although As removal efficiencies fell from more than 48% to less than 22% following a simulated 30 yr of irrigation in 5 cm columns, attenuation of As is expected to be higher in the field due to deeper soil profiles and slower average pore water velocities. In summary, soil profile As levels and sorption characteristics have not been significantly affected by up to 110 yr of irrigation with As-rich Madison and Upper Missouri River water. Due to the large variability in soil profile As levels and sorption capacities between sampling sites, potential impacts from irrigation projects should be assessed on a site-specific basis.

CHAPTER 4

MICROBIAL REDUCTION RATES OF SOLUBLE AND SORBED PHASE ARSENATE

Introduction

Soils and sediments contaminated with arsenic (As) can pose significant health risks due to the toxicity of As. Redox potential (E_H) can affect As toxicity and mobilization in contaminated systems by altering As valence state. For example, reduction of arsenate (As(V)) to arsenite (As(III)) generally increases As toxicity and mobility (Cullen and Reimer, 1989; Korte and Fernando, 1991). Although As(V) and As(III) concentrations have been well studied under equilibrium conditions, less is known about the kinetics of As(V) reduction. Therefore, understanding the factors controlling the rates of As(V) reduction is necessary to predict the effects of E_H fluctuations on As mobility and toxicity at As-contaminated sites.

The effects of pH and E_H on the thermodynamic stability of As(V) and As(III) species have been well established (Ferguson and Gavis, 1972; Sadiq, 1997). At a pH of 6 and E_H values above approximately 100 mV, As(V) species ($H_2AsO_4^-$ and $HAsO_4^{2-}$) are stable, whereas As(III) ($H_3AsO_3^0$) is stable from roughly -125 to 100 mV. Below an E_H of -125 mV, As(III)-sulfide soluble species and As solid phases predominate.

However, concentrations of As(III) have been found to exceed As(V) concentrations in surface waters containing detectable concentrations of O_2 (Aurillo et al., 1994; Abdullah et al., 1995; Sohrin and Matsui, 1997), suggesting nonequilibrium conditions. The presence of O_2 indicates E_H values were well above 100 mV, yet the rate of As(V) reduction apparently exceeded the rate of As(III) oxidation in these surface waters. Prevalence of As(III) was generally correlated with high phytoplankton abundance, indicating the non-equilibrium conditions were microbially-mediated. These results emphasize the importance of processes controlling rates of As(V)/As(III) cycling in natural systems.

Sorption of As(V) to oxide materials has been shown to occur via a ligand exchange mechanism based on good correlations between experimental and modeled As(V) sorption on Fe and Al oxides using a constant capacitance model (Goldberg, 1986). Surface complexation of As(V) at Fe oxide surfaces occurs primarily as bidentate-binuclear and bidentate-mononuclear innersphere surface complexes, although monodentate complexes are possible especially at low As:Fe ratios based on x-ray absorption fine structure spectroscopy (Waychunas et al., 1995; Fendorf et al., 1997). As(III) adsorption at Fe oxide surfaces has only been found to occur as a bidentate, binuclear innersphere surface complex (Manning et al., 1998). As(III) does not sorb as strongly as As(V) at low As surface coverages (Pierce and Moore, 1982), although As(III) sorbs more strongly than As(V) at high surface coverages (Manning et al., 1998; Raven et al., 1998; Sun and Doner, 1998). Decreasing soil E_H through flooding has been shown to initially increase soluble As concentrations (Onken and Hossner, 1995), apparently due to differences between As(V) and As(III) sorption to solid phases and the reductive dissolution of Fe and Mn oxides (Masscheleyn et al., 1991a;

McGeehan et al., 1998). Extended flooding may decrease soluble As concentrations due to precipitation of As-sulfide solid phases (Rittle et al., 1995).

Microbial reduction of As(V) to As(III) may occur as either a detoxification mechanism or by dissimilatory reduction. Detoxification is facilitated by *ars* genes that encode for As(V) reduction via an As(V) reductase, followed by As(III) removal from the cell with an efflux pump (Tamaki and Frankenberger, 1992; Cervantes et al., 1994). As(V) has also been demonstrated to serve as the sole electron acceptor coupled with lactate oxidation in two environmental isolates (Ahmann et al., 1994; Laverman et al., 1995). Dissimilatory As(V) reduction rates with pure cultures of these isolates have been found to be as high as 10 m*M* d^{-1} at an initial As(V) concentration of 5 m*M* (Laverman et al., 1995); however, little is known about rates of soluble As(V) reduction at lower As concentrations. In addition, rates of soluble As(V) reduction with unenriched microbial populations may be substantially different than with enriched pure cultures.

Reduction rates of sorbed phase As(V) may be much lower than reduction rates of soluble As(V), due to lower bioavailability of sorbed phase As. Bioavailability of substrates may often be limited by sorption because rates of desorption-diffusion reactions are generally lower than rates of substrate utilization. Reduction of sorbed phase As(V) is expected to affect As solubility due to observed differences in sorption characteristics between As(V) and As(III).

In summary, little is known about the mechanisms controlling rates of As(V) reduction. Consequently, the objectives of this study were to i) assess the effects of carbon, As enrichment, microbial growth, and initial As(V) concentrations on microbial reduction rates of soluble As(V), (ii) determine the influence of Fe oxides on reduction rates of soluble and sorbed phase As(V), and (iii) evaluate As solubility in the presence

of Fe oxides under reducing conditions. Results from the current studies show that microbially mediated As(V) reduction rates were first-order with respect to As(V) concentrations, and were correlated with microbial growth occurring via glucose fermentation. The solubilization of sorbed phase As(V) via microbial reduction appears to be controlled by desorption of As(V) or reductive dissolution of Fe oxide rather than by reduction rates of soluble As(V).

Materials and Methods

Reduction Rates of Soluble As(V)

All microorganisms used in this study were obtained from the upper 20 cm of a fine loamy, frigid Typic Calciaquoll, an agricultural soil (designated C3-NI) that contained naturally elevated concentrations of both soluble (20 μM) and total As (840 μmol kg^{-1}; Keith, 1995; Jones et al., in review). Organisms from the soil were collected using the methods described by Kingsley and Bohlool (1981). Briefly, soil samples were extracted with a buffer composed of 0.1 M $(NH_4)_2HPO_4$/0.1% (w/v) hydrolyzed gelatin (9.5:1 buffer:soil ratio) by shaking (120 cycles min^{-1}) for 30 min. The slurry was clarified by flocculation with $CaCl_2$, and the supernatant centrifuged at 5,000 g for 10 min. The microorganisms obtained by this procedure were washed twice in nutrient solution (described below) to yield the unenriched population (hereafter referred to as CN-0). The CN-0 population was enriched sequentially with up to 300 μM As(V) in serum bottles containing N_2(g)–purged nutrient solutions. Transfers were performed aseptically with sterile syringe needles. The seventh transfer (CN-7) was diluted to extinction with 1:10 dilutions, and the enriched population (CN-8) was obtained from the most dilute suspension verified to reduce As(V). The nutrient

solution used for enrichments and As(V) reduction experiments was modified from that described by Angle (1991) and consisted of the following: NH_4Cl (6.0 m*M*), $MgCl_2$ (2 m*M*), $CaSO_4$ (1 m*M*), KH_2PO_4 (5 μ*M*), NaOH (2.5 m*M*), H_2SO_4 (1 m*M*), $FeCl_2$ (5 μ*M*), Sigma Select yeast extract (0.2 g L^{-1}), $KHCO_3$ (20 m*M*), and glucose (30 m*M*, unless specified otherwise). The medium was supplemented with 100 μL L^{-1} of micronutrient solution (Skerman, 1967).

The effects of C concentration on reduction rate were evaluated by varying glucose concentrations from 10 to 100 m*M* (as C) at 300 μ*M* As(V). C concentrations were maintained at 30 m*M* in experiments evaluating the effects of initial As(V) concentrations (6 μ*M* to 5 m*M*). As(V) reduction experiments were conducted in serum bottles (70 mL) containing 50 mL of nutrient solution. The bottles were capped with butyl rubber septa, crimp-sealed, and purged with N_2(g) (10 min at approximately 60 mL min^{-1}). The bottles were subsequently acidified with 0.15 mL of 6 *M* HCl to attain a pH of 6.5, autoclaved at 121°C for 20 min, and inoculated using a sterile syringe and needle. Bottles were inoculated to attain an initial cell density of 10^6 cells mL^{-1}, as determined using an empirically-developed relationship between cell enumeration on YEPG (yeast extract, peptone, glucose) agar media and optical density at 500 nm (A_{500}) of cell suspensions. Bottles were agitated on a horizontal shaker (120 cycles min^{-1}) at 25°C, and aseptically sampled with sterile syringes and needles. Microbial growth was estimated by measuring optical density (A_{500}), and a duplicate sample was filtered (0.22-μm) for analysis of As and dissolved organic carbon (described below). Reduction rates of soluble As(V) were determined by performing linear regressions between As(V) concentration and time for the linear portion of As(V) reduction curves.

The fate of glucose-C in the 0.6, 2, and 5 m*M* As treatments with CN-8 was evaluated by spiking each serum bottle with ^{14}C–labeled glucose, to attain an initial concentration of 1.7×10^2 Bq mL^{-1}. Both filtered (0.2-μm) and unfiltered samples were acidified with concentrated HCl (1% v/v) and purged of CO_2 prior to analysis with a Packard liquid scintillation counter (Meriden, NJ). Oxidized C was assumed to equal the difference in C between an initial sample and each unfiltered, purged sample.

Reduction Rates of As(V) in Presence of Fe Oxides

Microbial reduction rates of As(V) in the presence of Fe oxides were evaluated and compared to reduction rates of soluble As(V). An unenriched CN-0 population, described above, was used as an inoculum source in each experiment. Goethite and 2-line ferrihydrite were prepared using the methods of Schwertmann and Cornell (1991). The ferrihydrite suspension was washed with deionized H_2O until NO_3^- was not detectable (with ion chromatography) in the suspension and the goethite was dialyzed until the electrical conductivity (E.C.) in the goethite suspension equaled the E.C. of deionized H_2O. Both suspensions were lyophilized and ground with mortar and pestle, and the ferrihydrite was passed through a 125-μm sieve. The identities of the solid phases were confirmed with x-ray diffraction, and surface areas determined with three-point BET analysis using $N_2(g)$ as the adsorbate. Measured surface areas were 25 $m^2\ g^{-1}$ and 363 $m^2\ g^{-1}$ for goethite and ferrihydrite, respectively. The percentage of Fe in the ferrihydrite was determined by 6 *M* HCl digestion (24 hr) and in the goethite by assuming a molecular formula of FeOOH. A batch sorption isotherm experiment with As(V) and As(III) was conducted with 2-line ferrihydrite to select an As(V) loading level for the reduction experiments. In addition, equilibrium solution As concentrations

were used to estimate the predominant valence of sorbed As species in the microbial reduction experiment with ferrihydrite. Suspensions of ferrihydrite (25 m*M* as Fe) were treated with a range of initial As(V) and As(III) concentrations (0, 0.03, 0.1, 0.3, 1, 3, 10, and 30 m*M*) in the following pH 6 nutrient solution: NH_4NO_3 (2.5 m*M*), $MgCl_2$ (2 m*M*), $CaSO_4$ (4 m*M*), KH_2PO_4 (5 μ*M*), KOH (0.5 m*M*), NaOH (2.5 m*M*), HNO_3 (3.5 m*M*), H_2SO_4 (1 m*M*), and micronutrients (as above). Suspensions were purged with N_2(g), shaken for 96 hr on a horizontal shaker (72 cycles min^{-1}), and filtrates (0.2-μm) analyzed for As (described below). Equilibration time was determined by conducting a kinetic sorption study at 1 m*M* As(V).

As(V) reduction experiments were conducted for both goethite (at 2.5 m*M* Fe/100 μ*M* As and 25 m*M* Fe/13.4 μ*M* As) and ferrihydrite (25 m*M* Fe/300 μ*M* As) in 2 L of the same nutrient solution described above. Experiments were conducted in a controlled E_H/pH chamber (6 in diam., 2.4 L capacity) that was fitted with air-tight ports for combination pH and platinum (Pt) redox electrodes, a fritted glass nebulizer, and a gas outlet (modified from Patrick et al., 1973). During periods of active microbial growth, the controller (Model 05656-05, Cole Parmer, Vernon Hills, IL) could maintain the solution E_H to within approximately ± 25 mV by sparging the solution with 99% O_2(g)/1% CO_2(g) and/or 99% N_2(g)/1% CO_2(g) (certified by Air Liquide, La Porte, TX). Both gases were mixed with 1% CO_2 to imitate a typical soil environment. The redox electrodes were initially tested in ferrous-ferric and quinhydrone reference solutions (ASTM, 1993), and recalibrated periodically during the experiments. The difference between the measured potential and the hydrogen electrode potential in quinhydrone reference solution (pH 6.86) was added to each measured potential to obtain E_H values. Initially, the Fe oxide, As(V), and nutrient

solution mixture was continually stirred and sparged with 99% O_2(g)/1% CO_2(g) for two to four d to obtain equilibrium solution concentrations of As. Each mixture was subsequently inoculated with a CN-0 cell suspension and sparged for an additional 24 hr with the O_2(g)/CO_2(g) mixture prior to sparging with 99% N_2 (g)/1% CO_2 (g). To decrease redox potential via microbial reduction, glucose was periodically added at average rates ranging from 2.3 – 5.2 mmol C d^{-1}. The solution pH was held constant by delivering small aliquots of HCl (0.1 - 3 *M*) or KOH (0.1 - 1 *M*) either manually or with a Dosimat 655 pH controller (Brinkmann Instruments, Inc., Westbury, NY). Samples were filtered (0.2-μm) for determination of As(T), As(V), As(III), S(-II), Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}, alkalinity, EC, Fe, and cations (described below).

Analytical Techniques

Soluble As was analyzed with continuous flow hydride generation atomic absorption spectrophotometry (HGAAS) by acidifying samples to 3 *M* HCl, pre-reducing in 1% KI, and mixing with 0.6% $NaBH_4$/0.5% NaOH in a reaction coil. Flow rates were 7 and 1 mL min^{-1} for sample and $NaBH_4$ reagent, respectively, and generated arsine was quantified in an air-acetylene flame (Perkin-Elmer 3100) at 193.7 nm. The average measured concentration of a 0.267 μ*M* EPA certified standard (Spex Certiprep, Inc., Metuchen, NJ) was 0.268 ± 0.025 μ*M* (n=69). As(V) and As(III) were determined for the solution studies using the following method modified from Masscheleyn et al. (1991b). Five mL of filtered sample were added to 1 mL of 2 *M* TRIS buffer (pH 6.0). While sparging the buffered solution with N_2(g), a solution of 3% $NaBH_4$/0.1% NaOH was added incrementally (0.2 mL over 15 s, 3 min wait, 0.8 mL over 45 s, 3 min wait) to liberate arsine from any As(III) in the sample. Samples

for As determination were preserved in 1% concentrated HCl until analyzed. The original sample, As(T), and the speciated sample, As(V), were both analyzed for As with HGAAS, and the As(III) concentration was calculated by difference. Analysis of standards made from $NaAsO_2$ and $Na_2HAsO_4 \cdot 7H_2O$ salts yielded recoveries of 93 ± 10% (n=7) and 110 ± 5% (n=8) for As(III) and As(V), respectively. Quantification of As(III) and As(V) in the solid phase experiments was similar to the above procedure except 0.5 mL of both TRIS and $NaBH_4$ were used, yielding As(III) and As(V) recoveries of 94 ± 14% (n=17) and 110 ± 16% (n=18), respectively.

Dissolved organic carbon (DOC) was analyzed with a DC-80 carbon analyzer (Tekmar-Dohrmann, Cincinnati, OH); cations and S by inductively coupled plasma spectrometry (ICP); and Cl^-, NO_3^-, SO_4^{2-}, and PO_4^{3-} by ion chromatography (IC). Total alkalinity was measured by HCl titration to an inflection point near pH 4.5. Titrated samples were then sparged with $N_2(g)$ for more than 15 min, titrated to the original pH with NaOH, and retitrated to the inflection pH with HCl in order to calculate carbonate activity. Fe was analyzed with the phenanthroline method and S(-II) was determined by either iodometric titration (only for the high As:Fe goethite experiment) or the methylene blue method (APHA, 1989). Fe, S(-II) and A_{500} were quantified with a Hitachi U-2000 spectrophotometer. Ion activities were calculated using the aqueous chemical equilibrium model, MINTEQ (Alison et al., 1991). Gas partial pressures in serum bottle headspaces were determined using a Carle (Tulsa, OK) Series 100 gas chromatograph and total headspace pressures were measured with a pressure transducer (Tensimeter, Soil Measurement Systems, Las Cruces, NM). Total headspace gas volumes were calculated by multiplying the gas partial pressure by the total headspace pressure, and moles of headspace gas were calculated with the ideal gas law.

Results and Discussion

Effect of Carbon on As(V) Reduction Rates

The effects of initial glucose concentration on microbial reduction rates of soluble As(V) were determined in serum bottle experiments using an As enriched culture (CN-7). Reduction of As(V) occurred simultaneously with growth at all glucose concentrations, indicating that reduction was microbially-mediated (Fig. 9). Maximum microbial growth rates and rates of As(V) reduction were similar across all glucose concentrations. However, total biomass accumulation and extents of As(V) reduction were C-limited at glucose concentrations of 10 m*M* C. Therefore, all subsequent serum bottle experiments were conducted with 30 m*M* C (as glucose).

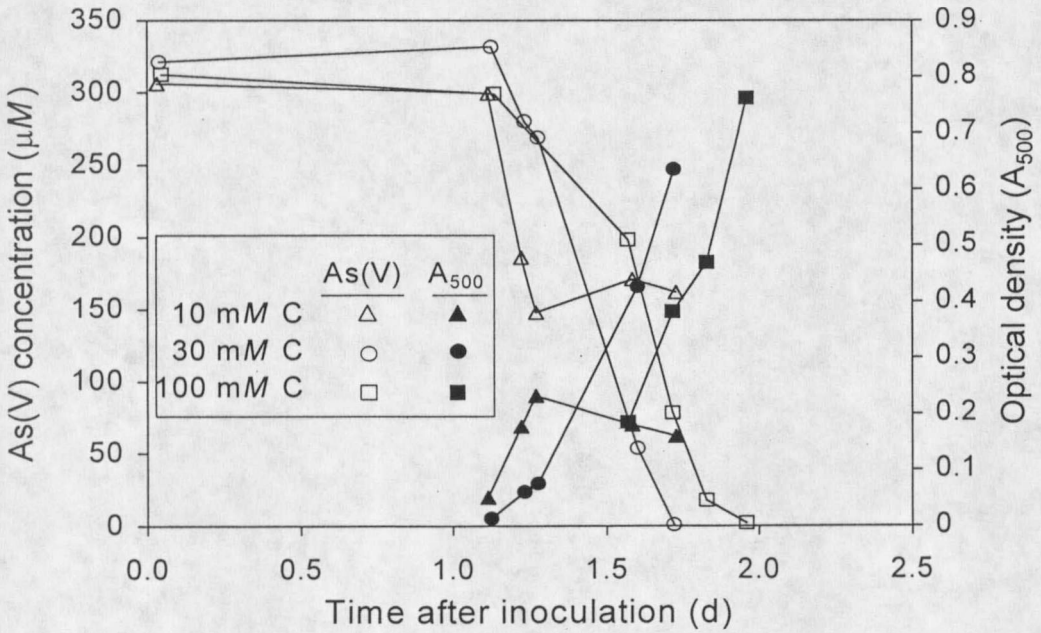


Figure 9. Changes in As(V) concentration and optical density (A_{500}) in serum bottles as a function of initial glucose concentration (10, 30, and 100 m*M* as C). Each bottle was inoculated with CN-7, targeting an initial cell density of 10^6 cells mL^{-1}. Total soluble As concentrations in each treatment remained at approximately 300 μ*M* throughout experiment (data not shown).

Effects of As Enrichment and Microbial Growth on As(V) Reduction

The effect of prior As enrichment on As(V) reduction was evaluated by inoculating solutions with either CN-0 (unenriched) or CN-8 (enriched) microbial populations. No reduction was observed in sterile controls, confirming the hypothesis that As(V) reduction was biologically mediated (Fig. 10). Reduction of As(V) with

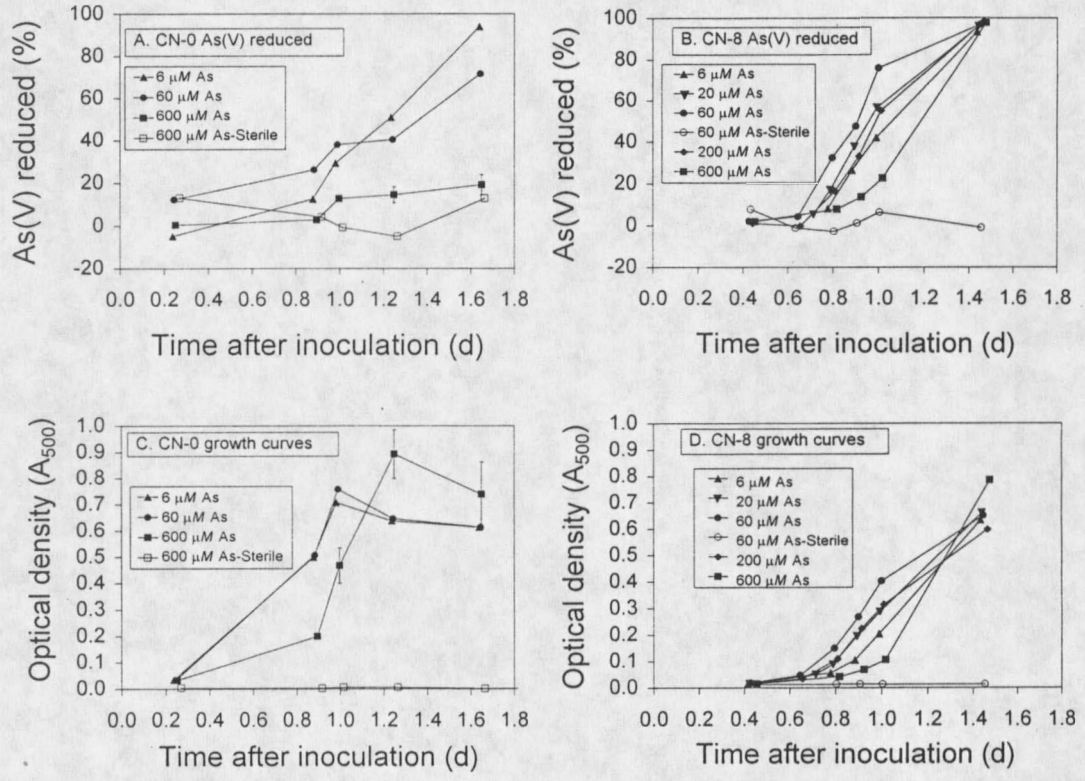


Figure 10. Extent of As(V) reduced (A, B) and growth curves (C, D) versus time for a range of initial As(V) concentrations in serum bottles inoculated with either CN-0 or CN-8. Glucose concentration was 30 m*M* (as C) for each treatment. Experiment with CN-0 and an initial As(V) concentration of 600 μ*M* was conducted in duplicate and error bars denote one standard deviation about the mean. Error bars smaller than symbols are not shown. As(V) reduced (%) = $100[1 - (As_V/As_T)]$.

CN-8 was considerably faster and more complete than with CN-0, indicating that the eight sequential enrichments selected for an As(V) reducing population (or isolate). Reduction of As(V) with CN-0 primarily occurred after microbial growth had ceased (≅1 d). This is in sharp contrast to As(V) reduction in the CN-8 incubations, where As(V) reduction occurred simultaneously with microbial growth. In both populations, initial growth was somewhat delayed at 600 μ*M* As compared to lower As concentrations, suggesting As toxicity, yet growth rates were uninhibited in the high As treatments. The CN-0 population reduced significantly lower amounts of As(V) than CN-8 during the growth phase, implying As(V) reducers represented a minor portion of the CN-0 population (Fig. 11). The extent of As(V) reduction with the CN-8 population was positively correlated ($r^2 = 0.95$) to A_{500} when all data from 6 to 600 μ*M* As were grouped (n=36), implying that As(V) reduction was dependent on growth of the CN-8 population. Although As(V) reduction correlated with microbial growth in CN-8, microbial growth rates were independent of initial As(V) from 6-600 μ*M* As. At 2-5 m*M* As, growth rates were somewhat lower than at As(V) concentrations below 2 m*M* (data not shown), yet final optical densities were comparable in all As treatments (6 μ*M* – 5 m*M*). These results show that the presence of As(V) was not required for microbial growth, and did not enhance microbial growth rates in either the enriched or unenriched populations. Although we cannot rule out dissimilatory reduction of As(V), our results suggest that As(V) reduction may have occurred via an alternate mechanism such as detoxification (Tamaki and Frankenberger, 1992; Cervantes et al., 1994).

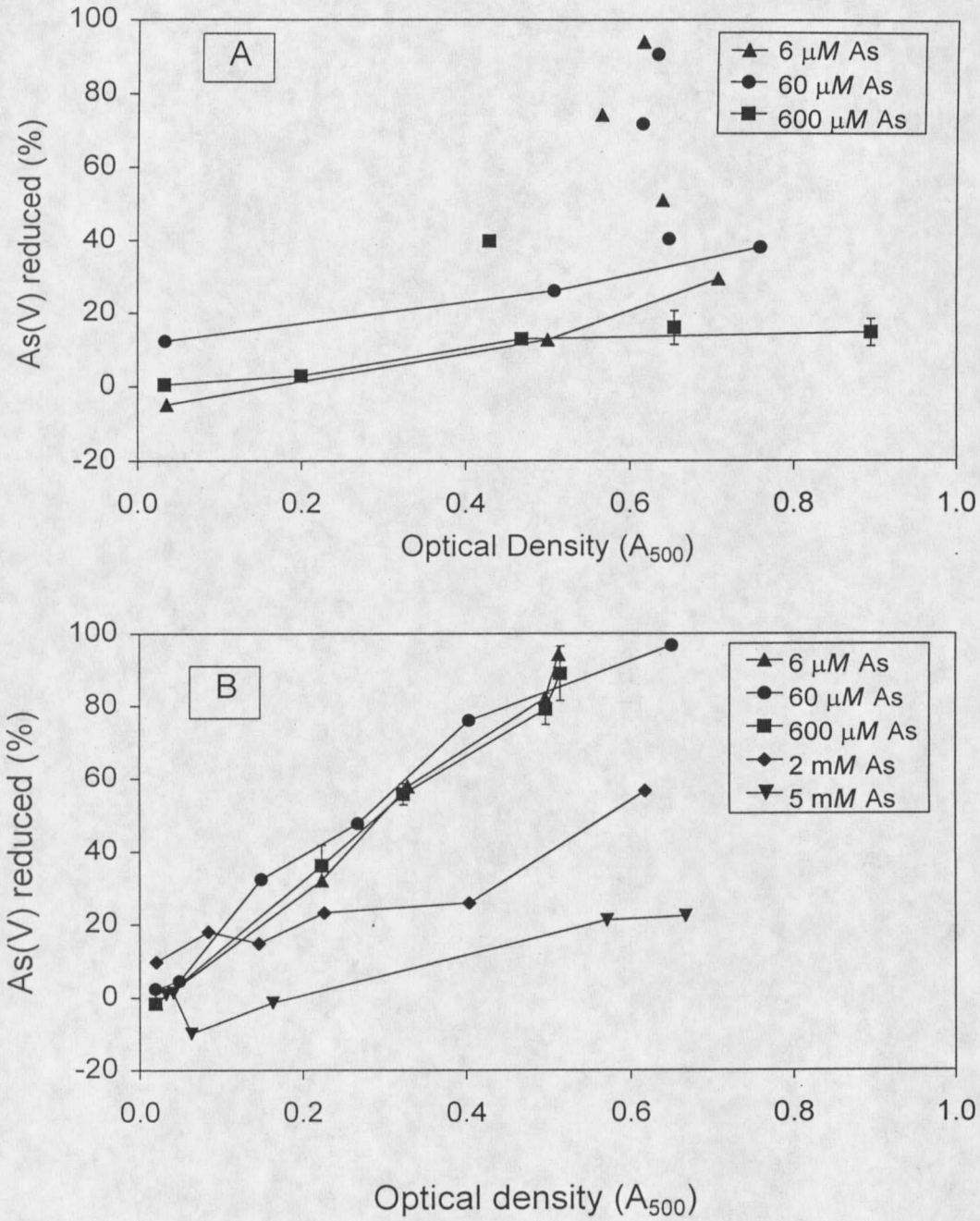


Figure 11. Extent of As(V) reduction as a function of optical density in serum bottles inoculated with either CN-0 (A) or CN-8 (B). Data for symbols that are not connected by lines were collected after growth phases (Fig.10). Experiments with initial As(V) concentrations of 600 μ*M* were conducted in duplicate and error bars denote one standard deviation about the mean. Error bars smaller than symbols are not shown. As(V) reduced (%) = $100[1 - (As_V/As_T)]$.

Effect of As(V) Concentration on As(V) Reduction Rate

The effects of initial As(V) concentration on rates of As(V) reduction ($M\ d^{-1}$) were evaluated for both the unenriched and enriched soil microbial populations (Fig. 12). Rates of As(V) reduction with the CN-8 population were positively correlated ($r^2 = 0.999$) with initial As(V) concentrations ranging from 6 to 600 μM. The relationship is nearly first-order ($n=0.91 \pm 0.01$) with respect to initial As(V) concentration,

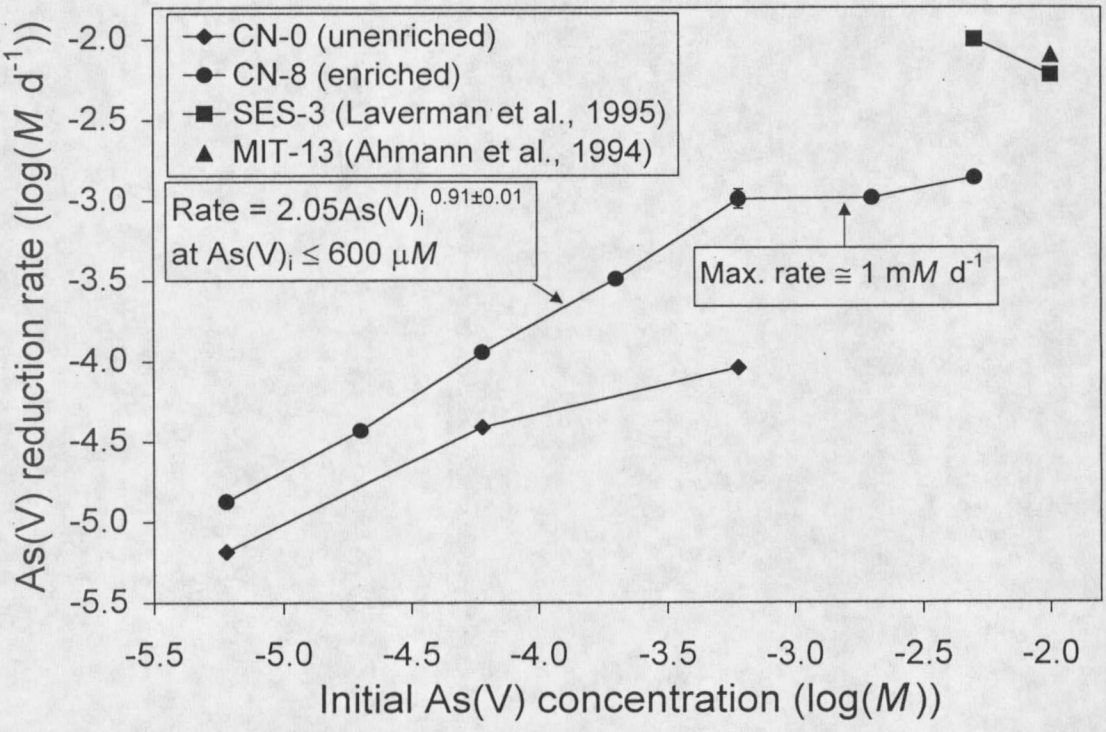


Figure 12. Effect of initial As(V) concentration on As(V) reduction rate in serum bottles inoculated with CN-0 or CN-8. Rates of As(V) reduction for two environmental isolates from other studies are shown for comparison purposes. Experiments with initial As(V) concentrations of 600 μM ($\log(M) \cong -3.2$) were replicated (triplicates on CN-8, duplicates on CN-0) and error bars denote one standard deviation about the mean. Error bar for reduction rate with CN-0 is smaller than symbol. Reduction rates with CN-8 were positively correlated ($r^2=0.999$) with initial As(V) concentrations from 6 to 600 μM As.

demonstrating that rates of As(V) reduction increased proportionally with initial As(V) concentrations. Reduction rates of As(V) reached a maximum of 1 mM d^{-1} above 600 μM As(V), suggesting As(V) reduction was enzyme-limited. By comparison, the rates of dissimilatory As(V) reduction by strains MIT-13 (Ahmann et al., 1994) and SES-3 (Laverman et al., 1995) were five to ten fold higher than the maximum rate obtained by CN-8. Both MIT-13 and SES-3 utilized As(V) as the sole electron acceptor for growth. Conversely, the CN-8 population apparently did not use As(V) as the sole electron acceptor based on similar microbial growth rates and final optical densities regardless of initial As(V) concentration (Fig. 11). Rates of As(V) reduction with CN-0 were lower than with CN-8 by approximately 2 fold at 6 μM As and 10 fold at 600 μM As. Growth rates were comparable between CN-0 and CN-8 (Fig 10); therefore, the lower As(V) reduction rates with CN-0 were likely due to a smaller population of As(V) reducers. In summary, at initial As(V) concentrations $\leq$ 600 μM, the As(V) reduction rate was first-order with respect to initial As(V) and highly dependent on the number of As(V) reducers.

Proposed Metabolic Pathways

Previous sections indicated that neither CN-0 nor CN-8 populations were coupling As(V) reduction with C oxidation based on similar total growth regardless of initial As concentrations. The detection of $H_2(g)$ in serum bottle headspaces (up to 0.11 atm with CN-8 and 0.015 atm with CN-0) indicated that both populations were fermenting glucose (Brock et al., 1994). The populations also produced no detectable $CH_4(g)$ ($< 4 \times 10^{-4}$ atm) or S(-II) (detection limit $\cong 10^{-7}$ M), indicating glucose

fermentation was not methanogenic and that SO_4^{2-} was not utilized as a major electron acceptor.

Examples of possible fermentation pathways that result in $H_2(g)$ production follow (Brock et al., 1994).

$$C_6H_{12}O_6 + 2H_2O \Rightarrow 2CH_3COOH \text{ (acetic acid)} + 2CO_2(g) + 4H_2(g) \quad [1]$$

$$C_6H_{12}O_6 \Rightarrow C_4H_7O_2H \text{ (butyric acid)} + 2CO_2(g) + 2H_2(g) \quad [2]$$

$$C_6H_{12}O_6 \Rightarrow 2CH_3COH \text{ (acetaldehyde)} + 2CO_2(g) + 2H_2(g) \quad [3]$$

A detailed C budget using ^{14}C-labeled glucose for the 5 m*M* As(V) treatment revealed that CN-8 produced similar quantities of $CO_2(g)$ (0.17 mmol) and $H_2(g)$ (0.15 mmol), in agreement with the stoichiometry of Eq. [2] and [3]. Dissolved organic C (DOC) obtained after glucose disappearance in CN-8 incubations averaged 21 m*M* C, independent of initial As concentration (0.6-5 m*M* As). Given an initial DOC concentration of 30 m*M* (as glucose), the amount of C remaining was also consistent with the stoichiometry of the fermentation reactions presented in Eq. [1] - [3]. Although the exact fermentation pathway cannot be determined without analysis of organic endproducts, the results suggest that a reduced organic compound (e.g. acetaldehyde, butyric acid) was produced during glucose fermentation. Currently, we are attempting to identify organic acids produced by CN-8 during glucose fermentation with ion chromatography and mass spectroscopy. Regardless of the organic acid(s) formed, the electron budget with CN-8 demonstrates that even at a high As concentration (5 m*M*), As(V) reduction accounted for a minor portion of the total electrons transferred (Fig. 13). Glucose fermentation apparently accounted for the remainder of transferred electrons with CN-8 based on the electron budget.

The difference in primary metabolic pathways among CN-8 (fermentation) and isolates MIT-13 and SES-3 (respiration) may be responsible for differences in observed rates of As(V) reduction (Fig. 12). Several organisms capable of fermentation (*E. coli,*

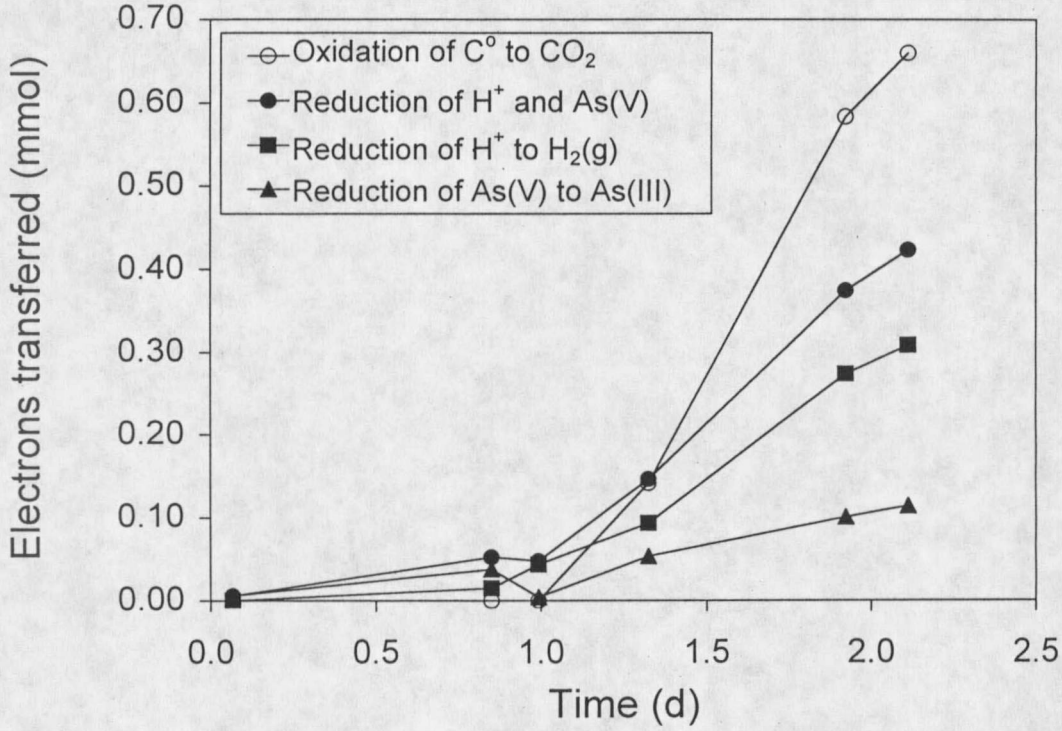


Figure 13. Electrons transferred per serum bottle in a solution containing 5 m*M* As(V) inoculated with CN-8. SO_4^{2-} did not account for significant amounts of accepted electrons based on below detectable levels of S(-II) at each sampling point (detection limit $\cong 10^{-7}$ *M*). The difference between the total electrons transferred during oxidation vs. during reduction was attributed to C reduction during glucose fermentation.

Staphylococcus aureus, Staphylococcus xylosus) have been found to invoke As(V) resistance by reducing As(V) to As(III) with an As(V) reductase, and subsequently excreting As(III) from the cell with an efflux pump (Cervantes et al., 1994; Silver et al., 1994). Although the specific mechanism for As(V) reduction with CN-0 and CN-8

cannot be definitively elucidated with the data collected, these results have important implications for understanding As(V) reduction in soils and natural water systems. The reduction of As(V) via detoxification occurs without utilization of As(V) as a terminal electron acceptor coupled to respiration. If detoxification is an important mechanism of microbially-mediated As(V) reduction in soils and natural waters, then predictions of As(V) reduction in soils based solely on redox status may be problematic.

Reduction of As(V) in the Presence of Goethite

Incubation studies were also conducted in the presence of Fe oxides in controlled E_H/pH chambers to 1) determine if a ubiquitous electron acceptor in soils (Fe(III)) inhibits reduction of soluble As(V) and 2) determine rates of As solubilization from Fe oxides as a result of microbially induced reduction. In a goethite suspension with a high As:Fe molar ratio (0.04) inoculated with a CN-0 suspension, soluble As(V) was reduced to As(III) as Pt E_H decreased from 450 to 200 mV (Fig. 14). The peak rate of reduction, 110 μM d^{-1} at an initial As(V) concentration of 60 μM, was within the range of reduction rates obtained by CN-0 and CN-8 in the absence of a solid phase (Fig. 12). Although the studies in the presence and absence of goethite were conducted under somewhat different conditions, the results imply reduction of soluble As(V) was not inhibited by the presence of goethite.

Total soluble As concentrations gradually declined as As(V) was reduced (Fig. 14), possibly due to recent observations that As(III) sorbs more strongly than As(V) to goethite at pH 7.5 and high As:Fe ratios (Sun and Doner, 1998). When the Pt electrode E_H further decreased to 0 mV, total soluble As first increased, then decreased sharply after S(-II) was first detected, implying an As(III)-sulfide solid phase may have precipitated. Ion activity products (IAPs) were lower than K_{sp} values for amorphous

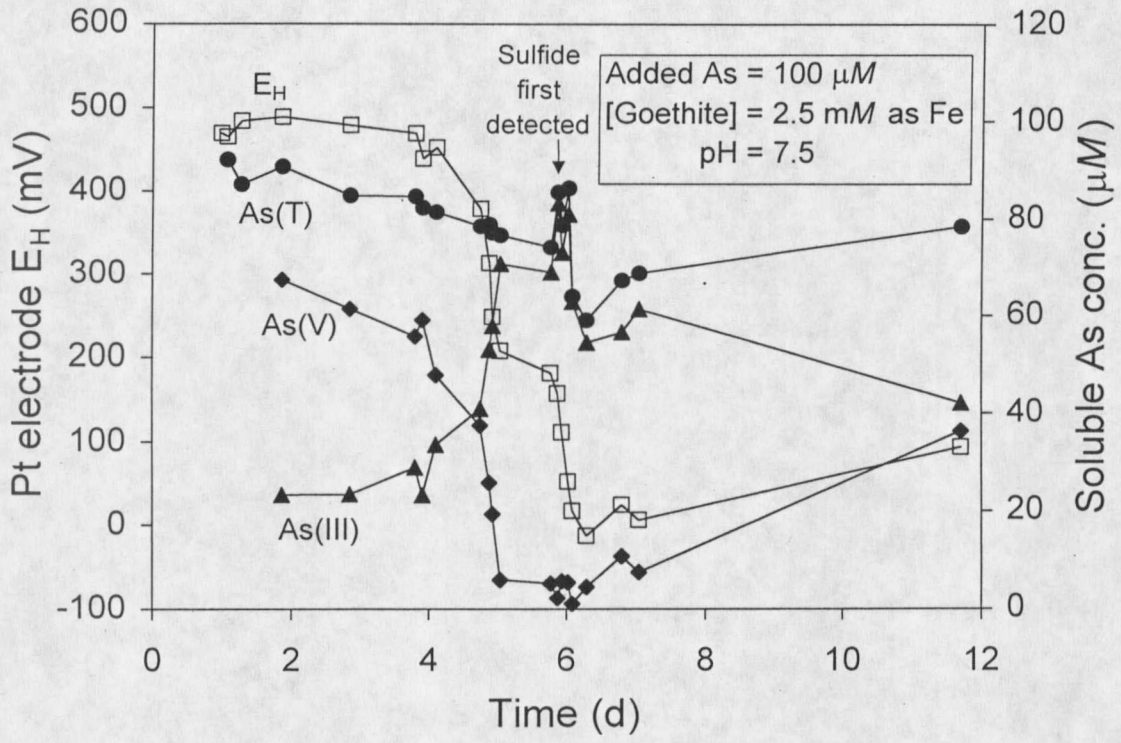


Figure 14. Concentrations of soluble As(T), As(V), and As(III) versus time as Pt electrode E_H decreased in a stirred goethite suspension with a high As:Fe molar ratio (0.04). Suspension was inoculated with a CN-0 population at 3.8 d into experiment and O_2(g) was replaced with N_2(g) on day 4 to promote reduction. Sulfide levels ranging from $5x10^{-6}$ to $8x10^{-5}$ *M* were detected at day 6.

As_2S_3 (Eary, 1992) and orpiment (Webster, 1990) by factors of at least 8.3 and 1.6, respectively. The results indicate the samples were undersaturated with respect to both solid phases; however, it can not be conclusively stated that As(III)-sulfide solid phases did not precipitate given discrepancies in literature equilibrium constants (Sadiq and Lindsay, 1979). Both As(III) and S(-II) were detected at higher Pt electrode E_H levels than expected based on equilibrium calculations. In fact, measured Pt E_H levels were higher than calculated E_H levels by 100 to 440 mV based on measured S(VI)/S(-II) and As(V)/As(III) couples and literature equilibrium constants (Lindsay, 1979; Sadiq et

al., 1983). Disparity between E_H values measured with Pt electrodes and those calculated from activities of oxidized and reduced species has been well documented (Stumm, 1984). These results demonstrate that As(V) and S(VI) reduction may occur at substantially different Pt electrode E_H levels than predicted by thermodynamic equilibrium models.

The initial soluble As concentration represented 85% of the total As in the high As:Fe treatment. To evaluate effects of reduction in a system dominated by sorbed, rather than soluble As, an experiment was conducted in a goethite suspension with a low As:Fe ratio (5.3×10^{-4} mol mol^{-1}). Initial soluble As concentrations represented only 0.5% of total As in the low As:Fe treatment, yet increased seven fold over 25 d as Pt E_H decreased from 500 to −100 mV (Fig. 15). The increase in soluble As concentrations (measured predominantly as As(III)) was in agreement with weaker sorption of As(III) versus As(V) on Fe oxides at low As:Fe molar ratios (Pierce and Moore, 1982). The microbial reduction of initial soluble As(V) to As(III) occurred within 2 d after inoculation, consistent with time scales observed in the absence of Fe oxides. Rates of As solubilization averaged 0.01 $\mu M\ d^{-1}$, considerably slower than soluble As(V) reduction rates (12 to 1000 $\mu M\ d^{-1}$). The contrast between rates of As solubilization and rates of soluble As(V) reduction suggest that sorption/desorption reactions rather than reduction rates of soluble As(V) control rates of increase in soluble As.

Reductive dissolution of goethite could have accounted for a portion of the increase in soluble As concentrations. Although the CN-0 population was found to ferment glucose, organisms within the population may also be capable of reducing Fe^{3+}. In addition, Fe^{3+} reduction has been found to couple abiotically with the oxidation of organics (Macur et al., 1991). To evaluate the significance of reductive

dissolution on As solubility, soluble Fe concentrations were measured and found to average less than 13 μM (0.05% of total Fe) during the experiment (unpublished data). Although this result suggests that reductive dissolution was not a significant cause of the 7 fold increase in soluble As concentrations, Fe(II) surface phases have been shown to represent a significant fraction of total Fe(II) in reduced goethite systems (Roden and Zachara, 1996). Therefore, it is difficult to elucidate the relative effect of reductive dissolution on changes in soluble As(V) concentrations based on our data. In summary, As solubilization rates were likely controlled either by desorption rates or reductive dissolution rates, but were apparently not controlled by soluble As(V) reduction rates.

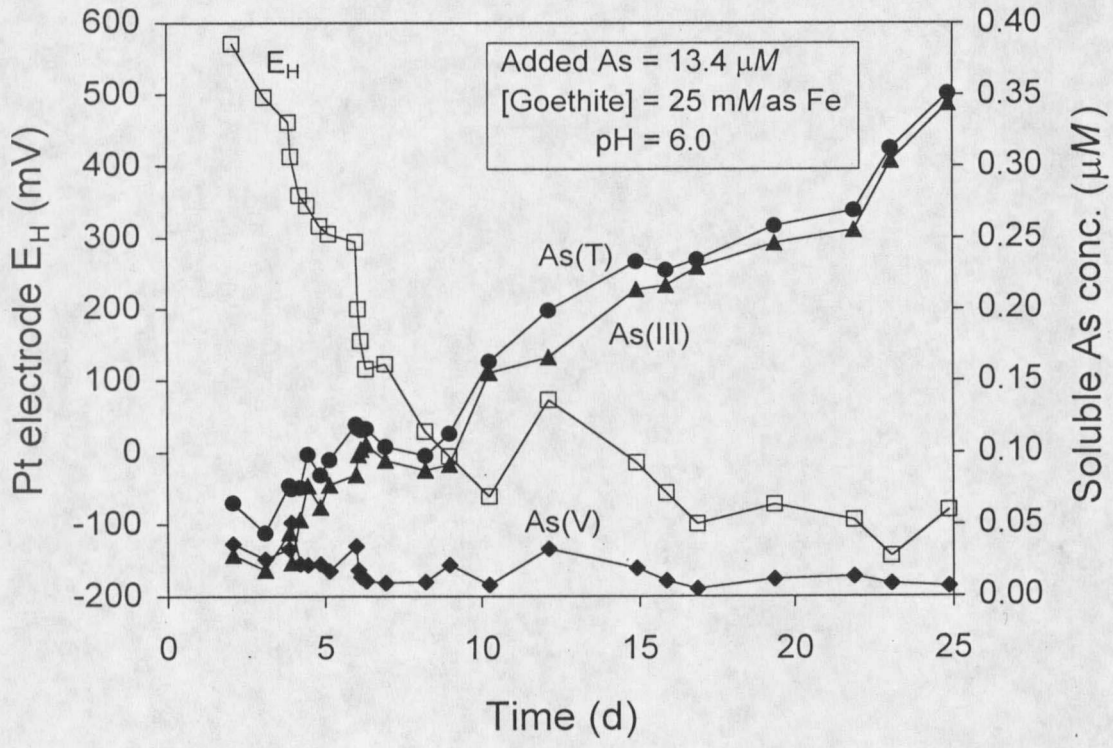


Figure 15. Concentrations of soluble As(T), As(V), and As(III) versus time as Pt E_H decreased in a stirred goethite suspension with a low As:Fe molar ratio (5.3×10^{-4}). Suspension was inoculated with a CN-0 population at day 2 and O_2(g) was replaced with N_2(g) on day 3.

Reduction of As(V) in the Presence of Ferrihydrite

Initial rates of As solubilization during reduction were much greater in a 2-line ferrihydrite suspension than in a goethite suspension, likely due to differences in reductive dissolution rates. Total soluble As concentrations increased by a factor of 20 in less than 2 d as Pt E_H decreased from 475 mV to 300 mV (Fig. 16). Further decreasing Pt E_H to 200 mV resulted in a subsequent, though smaller, increase in As

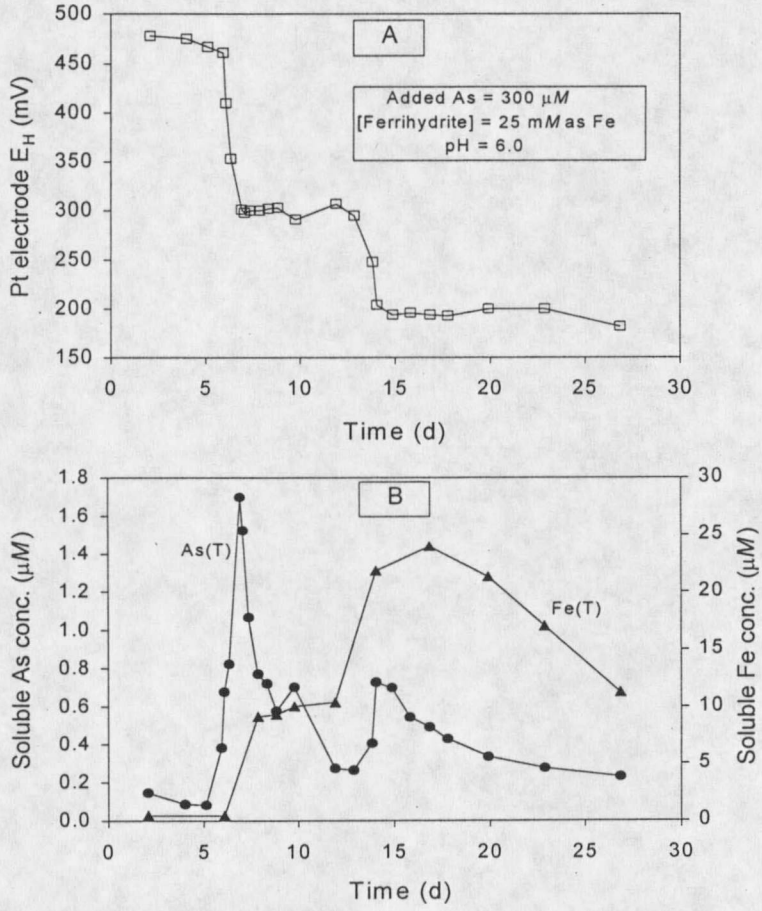


Figure 16. Effect of decreased Pt electrode E_H (A) on As and Fe concentrations (B) in a stirred ferrihydrite suspension at an As:Fe molar ratio of 0.012. Suspension was inoculated with a CN-0 population on day 4 and O_2(g) was replaced with N_2(g) on day 5 to promote reduction.

concentration. Both large spikes in As solubility were transient, however, with final soluble As concentrations representing a 2 fold increase over initial concentrations. Reductive dissolution of ferrihydrite apparently caused soluble As concentrations to increase based on concurrent increases in Fe concentrations (Fig. 16B). The rate of As solubilization (0.9 μM d^{-1}) during reduction of the ferrihydrite suspension was approximately 100 fold higher than the rate in a goethite suspension (0.01 μM d^{-1}) at similar soluble As concentrations (Fig. 15). The difference in rates was apparently due to differences in reductive dissolution rates between solid phases and a higher As loading density on ferrihydrite. When the Pt electrode E_H was stabilized at both 300 and 200 mV, soluble As concentrations declined, apparently due to As resorption. Unfortunately, As(V) and As(III) were not accurately quantified in the ferrihydrite experiment due to reagent contamination, although As(III) likely represented the majority of the soluble As based on data from the two goethite experiments (Fig. 14 and 15). Equilibrium solution concentrations of As(V) and As(III) were determined in separate batch sorption isotherm experiments with 2-line ferrihydrite suspensions at initial As concentrations of 300 μM (identical to the ferrihydrite experiment). Equilibrium solution concentrations were 0.09 and 1.9 μM for As(V) and As(III), respectively, compared to an As concentration of 0.25 μM at day 27 of the controlled E_H/pH study (Fig. 16). The equilibrium As value of 0.25 μM At day 27 was considerably lower than expected for an As(III)-ferrihydrite system suggesting that only a minority of sorbed phase As existed as As(III). Therefore, reduction rates of sorbed phase As(V) in a ferrihydrite suspension were apparently much slower than reduction rates of soluble As(V).

An attempt to quantify sorbed As(V) and As(III) by extracting ferrihydrite in 10 m*M* to 3 *M* phosphate buffers was unsuccessful due to inadequate As recoveries (< 32 %). Currently, more exhaustive extraction techniques are being developed for the quantification of sorbed phase As(V) and As(III), in hopes of more accurately quantifying reductions rates of sorbed phase As(V).

Conclusions

The effects of enrichment, microbial number, initial As(V) concentration, and presence of Fe solid phases on As(V) reduction rates were evaluated with organisms obtained from a naturally As-contaminated agricultural soil. Reduction rates with an As(V)-enriched microbial population (CN-8) were up to ten fold higher than with an unenriched population (CN-0), suggesting As(V) reducers comprised a small subset of the microbial population in the soil. At As(V) concentrations ≤ 600 μ*M* with the CN-8 population, microbial numbers and initial As(V) concentrations controlled reduction rates of soluble As(V). At initial As(V) concentrations greater than 600 μ*M*, reduction rates of soluble As(V) reached a maximum of approximately 1 m*M* d^{-1}. In summary, reduction rates of soluble As(V) are likely controlled by the growth rates of As(V) reducers and initial concentrations of soluble As(V).

Soluble As concentrations increased as E_H decreased in Fe oxide suspensions with As:Fe ratios well below sorption capacities, whereas soluble As concentrations decreased at an As:Fe ratio near the sorption capacity. The difference was attributed to the relative dependence of As(V) and As(III) sorption strengths on As surface coverage (Pierce and Moore, 1982; Manning et al., 1998; Raven et al., 1998). The net rate of As desorption from ferrihydrite during reduction was approximately 100-fold higher than from goethite at similar soluble As concentrations, due partially to differences in

reductive dissolution rates between solid phases (Roden and Zachara, 1996). Reduction rates of sorbed phase As(V) are likely much slower than reduction rates of soluble As(V) based on results from reduction experiments in low As:Fe suspensions of goethite and 2-line ferrihydrite. Therefore, rates of As sorption/desorption reactions and rates of oxide dissolution apparently control rates of As mobilization in reducing environments.

CHAPTER 5

SUMMARY

Three studies were conducted to develop a better understanding of As behavior in soils and natural waters, with special emphasis on topics that had not been adequately addressed in the scientific literature. The first study compared and contrasted the effects of liming on As transport in two unsaturated mine tailings. This study found that liming mobilized As, yet the degree of mobilization was dependent on final pH and As lability in the tailings. Based on these results, I would recommend that remediation specialists evaluate the effects of $Ca(OH)_2/CaCO_3$ ratios and loading rates on As solubility and mobility in lime-amended tailings.

The second experiment assessed the effects of irrigation on As solubility and mobility in 12 soils containing naturally-elevated levels of As. The study determined that there were no significant differences in average values of soluble As, total As, and As sorption coefficients between irrigated and nonirrigated soils. The study also determined that As sorption coefficients were positively correlated to concentrations of Fe and Mn oxides and inversely correlated with $NaHCO_3$-extractable P ("plant-available P"). Although soils of the study area will likely not be amended with Fe or Mn oxides, managers should be aware that the addition of P amendments to fields with high P and As concentrations may result in As desorption. In addition, effects of

irrigation on downstream water quality should be assessed on a site-specific basis due to the high degree of heterogenity in As concentrations found among sites. Although long-term irrigation was not found to significantly affect equilibrium concentrations of soluble and total As, irrigation events may cause transient reducing conditions, potentially increasing As mobility by reducing As(V) to As(III). This possibility led me to focus on microbial reduction rates of soluble and sorbed phase As(V) in the third study.

The results of the third study suggested that equilibrium conditions in soil systems may not be attained during a typical irrigation period (1-4 d) and that As solubility may not increase substantially during irrigation events. Additional studies with a range of soil microbial communities and naturally occurring carbon sources are necessary to determine if these conclusions are applicable to other sites. I would also recommend that reduction experiments be conducted with a range of pure isolates to minimize variable results, especially when attempting to determine mechanisms controlling As(V) reduction rates.

Another significant finding of the third study was that As(V) reduction may occur at much higher redox potentials than predicted by thermodynamic models, possibly due to detoxification reactions. If detoxification is an important pathway in unsaturated soils, the generalized assumption that As(V) predominates in surface soils and natural waters may be incorrect. To determine if As(V) reduction is a common mechanism occurring in unsaturated soil environments, As(V) and As(III) species could be quantified in soil porewaters collected from lysimeters at a range of contaminated

sites. Maintaining the sterility of lysimeters would be important due to the potential for microbially-mediated As(III) oxidation (See Appendix D). Findings from this proposed study should prove useful in demonstrating the relative importance of As(V) reduction under field conditions, and validating laboratory findings.

In conclusion, three studies were conducted to answer specific questions that had not been adequately addressed in the scientific literature. Results obtained in the current work should assist environmental managers in making sound decisions at As-contaminated sites, and promote additional research focused on understanding the processes affecting As solubility and mobility in soils and natural waters.

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APPENDICES

APPENDIX A

RESULTS FROM BATCH HYDRIDE GENERATION SPECIATION TECHNIQUE

Table A1. Compilation of As speciation data from 1/96 – 2/97 using 0.5 mL 2 *M* TRIS (pH 6.5) and 0.5 mL of 3% $NaBH_4$/0.1% NaOH per 5 mL sample (i.e. 10% of each). Goethite and 2-line ferrihydrite incubation studies (Chap. 4) were conducted from 2/96 – 10/96. See Chap. 4, Materials and Methods, for additional methodology on the speciation method. Standards were made from Na salts. All samples analyzed w/ hydride generation atomic absorption spectroscopy (HGAAS).

	As(III)		As(V)		As(T)		As(III)	Recoveries	
Date	Added	Calculated	Added	Measured	Added	Measured	Efficiency	As(III)	As(V)
	<----------μ*M*---------->								
1/9/96	1.3	1.2	0.0	0.1	1.3	1.3	92%	90%	
1/9/96	1.3	0.9	0.0	0.4	1.3		73%	71%	
1/9/96	1.3	1.2	0.0	0.1	1.3		94%	91%	
1/10/96	1.3	1.3	0.0	0.1	1.3	1.3	96%	95%	
1/10/96	1.3	1.3	0.0	0.0	1.3		97%	96%	
1/10/96	1.3	1.3	0.0	0.1	1.3		95%	94%	
1/10/96	0.0	0.1	1.3	1.4	1.3	1.5			104%
1/10/96	0.0	0.1	1.3	1.4	1.3				104%
1/10/96	0.0	0.1	1.3	1.4	1.3				104%
1/10/96	0.0	0.1	1.3	1.4	1.3				107%
1/10/96	0.0	0.1	1.3	1.4	1.3				107%
1/10/96	0.0	0.1	1.3	1.4	1.3				105%
5/29/96			0.03	0.03					115%
5/29/96			0.13	0.15					115%
10/21/96	0.0	0.0	1.3	1.3	1.3	1.3			99%
10/21/96	0.1	0.2	1.2	1.2	1.3	1.4		140%	100%
10/21/96	0.7	0.7	0.7	0.7	1.3	1.4		102%	105%
10/21/96	1.2	1.1	0.1	0.2	1.3	1.3		90%	138%
10/21/96	1.3	1.1	0.0	0.1	1.3	1.2	94%	85%	
10/21/96	0.0	-0.1	1.3	1.3	1.3	1.3			99%
10/21/96	0.1	0.1	1.2	1.2	1.3	1.4		96%	102%
10/21/96	0.7	0.7	0.7	0.7	1.3	1.4		101%	105%
10/21/96	1.2	1.0	0.1	0.2	1.3	1.2		85%	162%
10/21/96	1.3	1.2	0.0	0.1	1.3	1.2	96%	87%	
2/21/97	0.0	0.1	1.0	1.0	1.0	1.1			101%
2/21/97	0.0	0.1	1.0	1.0	1.0	1.0			99%
2/21/97	1.0	0.9	0.0	0.2	1.0	1.2	80%	93%	
2/21/97	1.0	0.9	0.0	0.2	1.0	1.1	81%	94%	
2/21/97	1.0	0.9	0.0	0.2	1.0	1.2	80%	95%	
All samples									
mean								94%	109%
std deviation								14%	16%
n								17	18
Only unmixed samples									
mean							89%	90%	105%
std deviation							9%	7%	6%
n							11	9	11

As(III) efficiency (%) = 100*(1-As(V) measured/As(T) measured), and measures the completeness of As(III) conversion to arsine gas. As recovery (%) = 100*(As measured/As added).

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Table A2. Results from As speciation tests using batch hydride generation in SSE nutrient solution (see Chapter 4, Materials and Methods, "Controlled Eh/pH experiments with Fe oxides") using 10% 2*M* TRIS, 10% $NaBH_4$. Efficiency of As(III) conversion to arsine was approximately 90%. Standards were speciated on 1/28/98 and HGAAS was used to analyze As.

	As T		As V		As III				
Standard	Added	Meas.	Added	Meas.	Added	Calc.	As III Recovery	As III Efficiency	As V Recovery
	(μ*M*)	(μ*M*)	(μ*M*)	(μ*M*)	(μ*M*)	(μ*M*)			
100:0 AsV:AsIII									
1	1.3	1.4	1.3	1.5	0.0	0			112%
2	1.3	1.3	1.3	1.4	0.0	0			106%
3	1.3	1.6	1.3	1.6	0.0	0.04			120%
4	1.3	1.5	1.3	1.3	0.0	0.22			98%
Mean	1.3	1.5	1.3	1.5	0	0.1			109%
Standard Deviation		0.1		0.1					9%
90:10 AsV:AsIII									
1	1.3	1.5	1.2	1.3	0.1	0.2	125%		110%
2	1.3	1.4	1.2	1.3	0.1	0.1	84%		110%
Mean	1.3	1.5	1.2	1.3	0.1	0.1	105%		110%
Standard Deviation		0.04		0.002					
10:90 As V:AsIII									
1	1.3	1.3	0.1	0.2	1.2	1.1	90%		178%
2	1.3		0.1	0.3	1.2	1.0	87%		205%
Mean	1.3	1.3	0.1	0.3	1.2	1.1	89%		192%
Standard Deviation				0.03					
0:100 As V:AsIII									
1	100	95.7	0	8.8	100	86.9	87%	91%	
2	100	91.2	0	8.0	100	83.2	83%	92%	
3	100	94.2	0	11.8	100	82.4	82%	88%	
4	100	91.5	0	10.5	100	81.0	81%	90%	
5	100	93.6	0	8.9	100	84.7	85%	91%	
Mean	100	92.6	0	9.8	100	82.8	83%	90%	
Standard Deviation		1.5		1.7					

Table A3. Efficiency of arsine generation from As(III) standards (148 μM) made in SSE-R2 (nutrient solution described in Chap. 4, Materials and Methods, "Reduction Rates of Soluble As(V)", without $KHCO_3$) using batch hydride speciation for a range of reagent volumes and speciation times. Speciated on 2/12/98. Measured As(V) should be 0 μM, assuming 100% efficiency of arsine generation. The results demonstrate that 20% TRIS buffer produced substantially higher efficiencies, likely due to tighter pH control. The alkaline $NaBH_4$ solution can raise the pH to a level where As(III) is not converted to arsine gas. The results also show that using 0.25 mL of $NaBH_4$ solution for 14 min, or 1 mL of the $NaBH_4$ solution for 7 min produced the highest As(III) conversion efficiencies.

Treatment (5 mL sample)	As V(μM)	efficiency (%)
1-10% TRIS, .5 mL 3% $NaBH_4$/0.1%NaOH	24	84%
2-10% TRIS, .25 mL, 14 min.	26	82%
3-20% TRIS, .25 mL, 14 min.	6	96%
4-20% TRIS, .1 mL, 7 min, .4 mL 3 min.	10	93%
5-20% TRIS, .5 mL, 1 min.	15	90%
6-20% TRIS, .5 mL, 4 min.	15	90%
7-20% TRIS, .5 mL, 7 min.	10	93%
8-20% TRIS, .5 mL, 14 min.	10	93%
9-20% TRIS, 1 mL, 7 min.	7	95%

Table A4. As(V) recoveries using batch hydride speciation (see Chap. 4, Materials and Methods) for a range of reagent volumes and length of speciation. Analysis of As was performed with ICP and all standards (0.2 m*M*) were made in SSE-R2 (Chap. 4). Recoveries are generally independent of both reagent and speciation time. Higher As(V) than As(T) measurements imply at least one of the speciation reagents enhanced the As signal. Recoveries of As(V) appear to be relatively unaffected by quantities of reagents added. Sulfide (S-(II)) did not interfere with the As analysis.

Treatment (in 5 mL sample)	Sample	As(T) measured	As(V) measured	As(V) recovery
TRIS; $NaBH_4$; min.	#	(m*M*)	(m*M*)	
40%; 0.1; 1	1A	0.20	0.22	110%
40%; 0.1; 1	1B	0.20	0.23	115%
40%; 0.1; 7	2A	0.20	0.23	116%
40%; 0.1; 7	2B	0.20	0.23	115%
40%; 0.25; 1	3A	0.20	0.23	115%
40%; 0.25; 1	3B	0.20	0.23	116%
40%; 0.25; 7	4A	0.20	0.23	115%
40%; 0.25; 7	4B	0.20	0.23	115%
20%; 0.1; 1	5A	0.21	0.24	120%
20%; 0.1; 1	5B	0.21	0.24	119%
20%; 0.1; 7	6A	0.20	0.23	115%
20%; 0.1; 7	6B	0.20	0.23	115%
20%; 0.25; 1	7A	0.21	0.23	115%
20%; 0.25; 1	7B	0.20	0.24	122%
20%; 0.25; 7	8A	0.21	0.23	115%
20%; 0.25; 7	8B	0.20	0.23	116%
40%; 0.25; 7; 1 m*M* S(-II)	9A	0.22	0.22	111%
40%; 0.25; 7; 1m*M* S(-II)	9B	0.22	0.22	111%
10%; 0.5; 7; DDIH_2O	Blank 1	0.00	0.00	
10%; 0.5; 7; DDIH_2O	Blank 2	0.00	0.00	

1. Recovery = As(V) measured/added
2. Speciation analysis performed on 2/20/98

Table A5. As(III) recoveries using batch hydride speciation (see Chap. 4, Materials and Methods) for a range of reagent volumes and length of speciation. Analysis of As was performed with ICP and standards (0.2 m*M*) were made in SSE-R2. The treatment producing the highest recoveries and efficiencies was: 20% 2 *M* TRIS, 0.25 mL 3% $NaBH_4$/0.1% NaOH, and 7 min of speciation (8A and 8B). Sulfide (1 m*M*) substantially interfered with recovery (samples 9A and 9B), possibly due to As(III)-sulfide precipitation.

Treatment (in 5 mL sample)	Sample	As(T) measured	As(V) measured	As(III) Calc	As(III) Recovery	As(III) Efficiency
Tris; $NaBH_4$; min	#	(m*M*)	(m*M*)	(m*M*)	(%)	(%)
30%; 0.1; 1	1A	0.19	0.16	0.03	15%	16%
30%; 0.1; 1	1B	0.19	0.16	0.03	17%	18%
30%; 0.1; 7	2A	0.19	0.12	0.07	34%	36%
30%; 0.1; 7	2B	0.19	0.19	0.00	-2%	-2%
30%; 0.25; 1	3A	0.19	0.09	0.10	52%	55%
30%; 0.25; 1	3B	0.19	0.11	0.09	43%	45%
30%; 0.25; 7	4A	0.19	0.05	0.14	69%	73%
30%; 0.25; 7	4B	0.20	0.05	0.14	71%	72%
20%; 0.1; 1	5A	0.19	0.13	0.06	29%	31%
20%; 0.1; 1	5B	0.19	0.12	0.07	36%	38%
20%; 0.1; 7	6A	0.19	0.11	0.08	41%	43%
20%; 0.1; 7	6B	0.19	0.07	0.12	62%	65%
20%; 0.25; 1	7A	0.19	0.07	0.12	60%	62%
20%; 0.25; 1	7B	0.19	0.07	0.13	63%	64%
20%; 0.25; 7	8A	0.20	0.04	0.15	77%	79%
20%; 0.25; 7	8B	0.20	0.04	0.16	78%	79%
40%; 0.25; 7 1 m*M* S(-II)	9A	0.04	0.01	0.02	12%	65%
40%; 0.25; 7 1 m*M* S(-II)	9B	0.02	0.03	0.00	0%	-2%

1. Total As solution for 9A was bubbled with N_2 (g) for 7 min. (sulfide had previously been found to interfere with hydride generation, and bubbling negated interference).
2. Total As solution for 9B was not bubbled.
3. As(III) efficiency (%) = 100*(1-As(V) measured/As(T) measured), and measures the completeness of As(III) conversion to arsine gas. As recovery (%) = 100*(As measured/As added).
4. Speciation performed on 2/24/98.

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Table A6. Recoveries and efficiencies of batch hydride speciation method using 20% 2*M* TRIS, 20% $NaBH_4$ reagent. All experiments studying rates of soluble As(V) reduction (Chap. 4) utilized this method. Standards were made in nutrient solution (SSE-C) described in Chap. 4. Average efficiency of As(III) speciation was close to 99%, compared to approximately 90% using 10% of each reagent (Table A1 and A2). Analyses were performed with HGAAS.

Sample	Added As T	Measured As T	Added As V	Measured As V	Added As III	Calc. As III	As III recovery	As III speciation efficiency	As V recovery
	<----------μ*M*---------->								
4/7/98									
1	294	293	294	330	0	-37			112.3%
2	294		294	337	0	-44			114.7%
3	567	574	294	309	267	265	99.1%		105.2%
4	567		294	310	267	264	98.9%		105.4%
5	267	272	0	6	267	266	99.8%	98.0%	
6/24/98									
1	300	321	300	322	0	-1			107.3%
2	300	298	300	307	0	-9			102.3%
3	300	303	150	169	150	134	89.2%		112.9%
4	300	283	150	176	150	107	71.5%		117.1%
5	300	286	0	5	300	281	93.8%	98.3%	
6	300	301	0	2	300	299	99.6%	99.2%	
mean							93.1%	98.5%	109.6%
standard deviation							10.3%	0.6%	5.3%

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APPENDIX B

RESULTS FROM EXTRACTION PROCEDURES DESIGNED TO QUANTIFY SORBED ARSENATE AND ARSENITE

Table B1. Recovery of As(V) and As(III) sorbed to 2-line ferrihydrite (50 m*M* as Fe) with 20 mL of 1 *M* K_2HPO_4 extractant (pH ≅ 9.0). Arsenic was sorbed by shaking 20 mL of As standard in ferrihydrite suspensions for 24 hr in 42 mL centrifuge tubes. Batch hydride speciation procedure used 0.5 mL 2 *M* TRIS, 0.5 mL 3% $NaBH_4$/0.1% NaOH in 5 mL sample (as outlined in Chap. 4). Sorbed concentrations were calculated by subtracting solution concentrations (prior to extraction) from total As added. Recoveries of As(III) with 0.1 m*M* total As were substantially higher than for As(V), yet As(III) and As(V) recoveries were similar at 1.0 m*M* total As, apparently due to the dependence of As surface coverage on sorption strength. A 5 min extraction provided higher As(III) recoveries than a 1 hr extraction, perhaps due to As(III) oxidation/resorption during the longer extraction. Recoveries of As in solution are provided for reference and demonstrate adequate recoveries of both species.

Sorbed standards (added to $Fe(OH)_3$)	As T measured	As T sorbed	As V measured	As V sorbed	As III calc.	As III added	Sorbed recoveries As V	Sorbed recoveries As III
	<----------mM---------->							
0.1 m*M* As III-1 hr	0.04	0.2	0.002	0	0.03	0.2		17%
0.1 m*M* As III- 5 min	0.10	0.2	0.002	0	0.09	0.2		47%
1.0 m*M* As III-5 min	0.71	2.58	0.06	0	0.66	2.58		25%
0.1 m*M* As V-5 min	0.09	0.2	0.01	0.2	0.08	0	4%	
1.0 m*M* As V-5 min	0.95	3.34	0.98	3.34	-0.03	0	29%	

Solution standards	As T measured	As T added	As V measured	As V added	As III calc.	As III added	Solution recoveries As V	Solution recoveries As III
	<----------μM---------->							
0.2 μ*M* As V	0.20	0.2	0.202	0.2	-0.002	0.0	101%	
0.2 μ*M* As V-dup		0.2	0.205	0.2	-0.005	0.0	102%	
0.2 μ*M* As III	0.18	0.2	0.030	0.0	0.154	0.2		77%
0.2 μ*M* As III-dup		0.2	0.022	0.0	0.162	0.2		81%

Table B2. Recoveries of As(V) from a 1 m*M* As(V) treatment (20 mL) on 2-line ferrihydrite (25 m*M*) following 1 hr extractions with P buffers (20 mL). There was no improvement in recovery with the stronger (3 *M*) extract, although the recovery did improve following a second (i.e. sequential) extraction.

Treatment	Sorbed μmol	Measured μmol	Duplicate μmol	Recovery
1 *M* H_2PO4 (pH = 6)	18.17	5.86		32%
3 *M* H_2PO4 (pH = 6)	18.14	5.44	5.54	30%
1st extraction (3 *M*)		5.09		
2nd extraction (3 *M*)		2.92		
Total seq. ext. (3 *M*)	18.15	8.01		44%

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Table B3. Solution As (A), extracted As (B), and As recoveries (C) from 2-line ferrihydrite (25 m*M*) using 0.01, 0.1, and 1 *M* P extractants on As(V), As(III), and mixed 1 m*M* standards. Standards were made in SSE nutrient solution (see Chapter 4, Materials and Methods, "Controlled Eh/pH experiments with Fe oxides") and As(V) and As(III) were quantified with batch hydride generation using 10% 2*M* TRIS, 10% $NaBH_4$. Extracted As(V) recoveries were approximately twice as high as As(III) recoveries and all recoveries were less than 35%.

A.				Average
Treatment (20 mL)	Added As	Soln As-1	Soln As-2	Sorbed As
	<----------µmol---------->			
0.5 m*M* As III/As V	20	0.11	0.11	19.89
1 m*M* As V	20	0.17	0.10	19.87
1 m*M* As III	20	0.19	0.17	19.82

B.	As T		As V		As III calculated	
Extractant/As form	Dup1	Dup2	Dup1	Dup2	Dup1	Dup2
	<----------µmol---------->					
10 m*M* P/mixed std.	2.45	2.80	1.80	2.01	0.65	0.79
100 m*M* P/mixed std.	4.66	3.96	3.25	2.82	1.41	1.14
1*M* P/mixed std.	4.50	4.39	3.21	3.09	1.29	1.30
1*M* P/As V	5.98	6.01	5.41	4.95	0.58	1.06
1*M* P/As III	3.22	3.05	0.26	0.25	2.96	2.80

C.			
Extractant/As form	As T	As V	As III
	Average Recoveries (%)		
10 m*M* P/mixed std.	13	19	7
100 m*M* P/mixed std.	22	31	13
1*M* P/mixed std.	22	32	13
1*M* P/As V	30	26	---
1*M* P/As III	16	---	15

Table B4. Levels of As extracted from a 2 g L^{-1} suspension of Al-oxide C (Degussa Corp., Ridgefield Park, NJ) with 1 *M* NaOH for 2hr @ 70C (Sun and Doner, 1996). Samples 1-4 were from a biotic experiment (with CN-8). All As solutions were made in SSE-C (see Chap. 4). Analysis of As species was performed using batch hydride generation (20% of both reagents-see Table A6). Although total recoveries were adequate (>80%), the extraction produced large false positives for both As(V) and As(III). The large false positives for As(V) are possibly due to surface-catalyzed As(III) oxidation during the extraction (the samples were not purged of air).

Sample	<----------solution------->			<--solid phase + solution->			<---solid phase--->		
	As T	As V	As III	As T	As V	As III	As T	As V	As III
	<----------μ*M*--------->								
300 μ*M* As V-sterile	139	144	0	269	206	63	130	61	68
300 μ*M* As III-sterile	205	5	200	241	189	52	35	184	-148
300 μ*M* As V 1	200	8	192	298	228	70	98	220	-122
300 μ*M* As V 2	177	4	173	264	142	122	87	138	-51
300 μ*M* As V 3	188	9	179	217	257	-40	29	248	-219
300 μ*M* As V 4	167	5	-162	256	200	56	89	195	-106

	<-------Total recoveries------>			<--solid phase recoveries-->		
	As T	As V	As III	As T	As V	As III
	<----------%---------->					
300 μ*M* As V-sterile	90	69 false pos.		81	39 false pos.	
300 μ*M* As III-sterile	80 false pos.		17	37 false pos.		-149
300 μ*M* As V 1	99			98		
300 μ*M* As V 2	88			71		
300 μ*M* As V 3	72			26		
300 μ*M* As V 4	85			67		

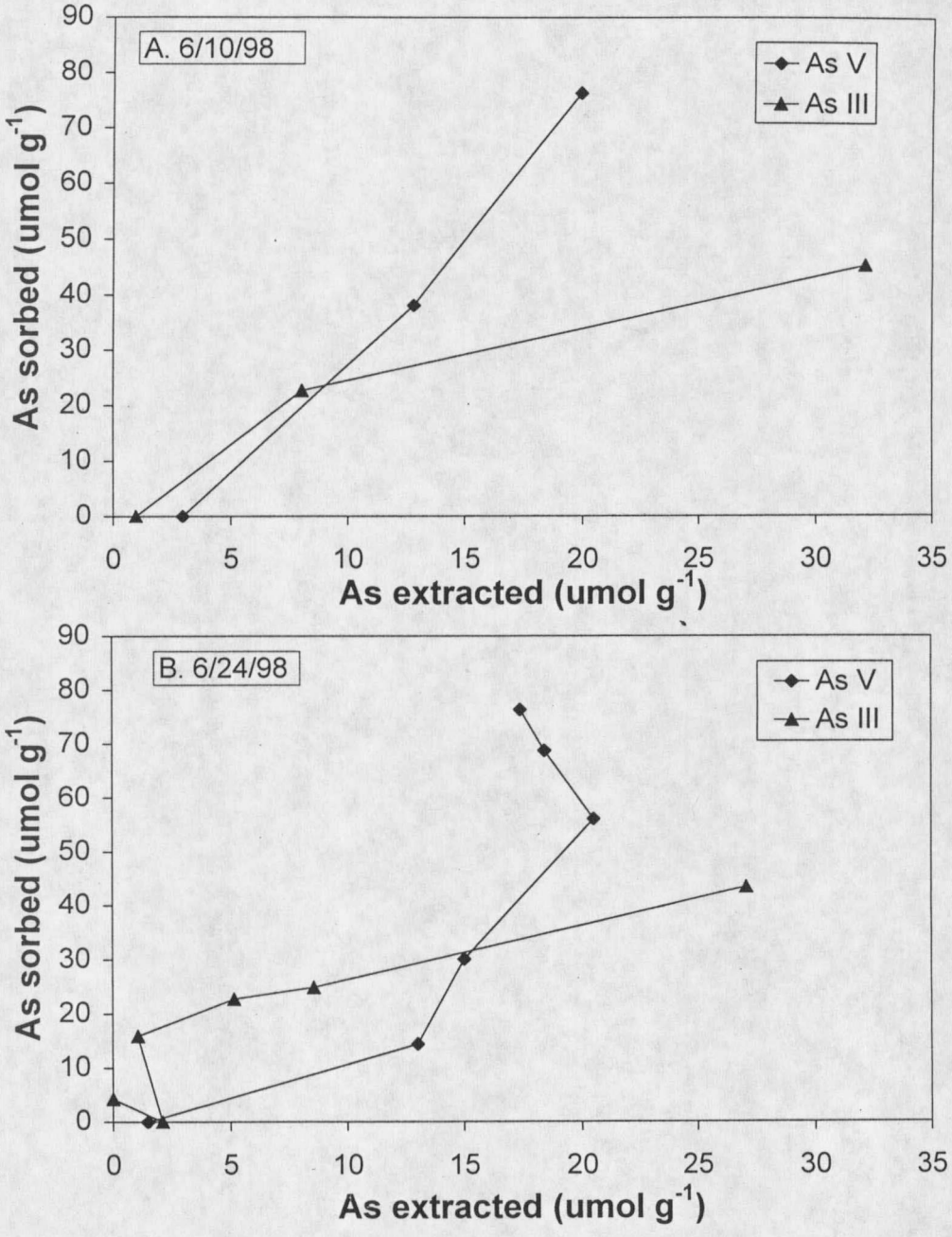


Figure B1. Sorbed vs. extracted As from Al-oxide C (2 g L^{-1}) using either A) 1m*M* PO_4/1m*M* PO_3 (pH=5.5) or B) 100 m*M* PO_4 (pH 7.0). Four mL of Al-oxide suspension (made in SSE-C) were centrifuged, decanted, vortexed for 5 min with 4 mL of extractant, filtered (0.2-μ), and analyzed for As species with batch hydride generation. Correlations between actual and measured As were relatively poor for both extractants, indicating the procedures should only be used for qualitative purposes.

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APPENDIX C

SUPPLEMENTAL DATA TO CHAPTER 3

Table C1. Total and soluble arsenic concentrations for surficial (top 20 cm) † paired Madison River Valley soils.

River Region and Site	Total Arsenic		Soluble Arsenic	
	Irrigated	Nonirrigated	Irrigated	Nonirrigated
	--------mg kg^{-1}--------		----------μM----------	
Upper Madison				
A1. Wigwam	16.5	13.6	1.0	0.4
A2. Granger W.	9.8	6.5	0.4	0.3
A3. Granger E.	19.5	13.2	0.5	0.1
A4. Valley Garden	7.7	8.3	0.9	0.2
Average	13.4	10.4	0.7	0.2
Lower Madison-South				
B1. Kilgore	7.0	8.4	0.7	0.5
B2. Anderson S.	12.4	8.0	2.4	0.5
B3. McDonnell S.	7.9	6.9	1.3	1.0
B4. McDonnell N.	12.4	8.4	0.5	0.6
Average	9.9	7.9	1.2	0.7
Lower Madison-North				
C1. Anderson N.	19.0	15.6	4.2	1.8
C2. Kammerman	11.8	14.9	2.8	0.6
C3. Van Dyk	29.8	62.8	12.3	19.8
C4. Lane	26.5	47.9	4.7	99.7
Average	21.8	35.3	6.0	30.5
Upper Missouri				
D1. Hunsaker	11.7	12.0	0.5	0.7
D2. Koehnke	8.9	8.8	0.5	0.4
D3. Hansen	9.9	10.2	0.5	0.5
D4. Bruce Seed	6.3	8.6	0.9	0.1
Average	9.2	9.9	0.6	0.4
Overall average	13.6	15.9	2.1	7.9

† Values shown are depth weighted averages of 0-5 cm, 5-10 cm, and 10-20 cm.

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Table C2. Soil sorption coefficients and selected characteristics † of six paired Madison River Valley soils. For site designations, see Table C1, and for Materials and Methods, see Chap. 3. The P-values indicate that only Olsen P was significantly correlated with K_d ($\alpha = 0.05$), yet AOE Fe, AOE Mn, and Olsen P all significantly affected K_d in a multiple linear regression (Chap. 3).

Site Designation	linear K_d ‡	pH	O.M.	clay	Ammonium oxalate extractable (AOE) Fe	Mn	Al	Olsen P	Total As	Sat. paste DOC
	L kg^{-1}		---------%---------		---------- mg kg^{-1} ----------					(mg L^{-1})
A1-I	30.2	8.0	2.31	19.5	531	596	1209	7.2	16.5	1547
A1-NI	33.2	8.0	3.61	§	526	671	1204	6.7	13.6	129
B1-I	7.9	8.1	1.93	10.5	353	336	711	23.1	7.0	262
B1-NI	12.5	8.2	1.50	7.0	314	312	546	8.4	8.4	511
B4-I	12.2	8.2	1.44	19.5	445	178	850	9.0	12.4	44
B4-NI	8.2	8.0	1.49	14.5	279	256	761	6.2	8.4	66
C2-I	3.4	7.9	3.23	16.0	503	273	1004	23.9	11.8	1047
C2-NI	19.9	7.8	1.96	16.5	360	282	1051	4.8	14.9	161
C3-I	5.2	8.0	6.33	37.5	479	220	658	23.7	29.8	324
C3-NI	17.7	7.8	8.38	30.5	690	154	581	11.6	62.8	195
D1-I	29.3	8.1	1.27	21.0	1627	149	538	4.6	11.7	25
D1-NI	13.0	8.0	1.78	18.0	560	257	593	12.1	12.0	114
Irrigated mean	14.7	8.0	2.8	20.7	656	292	828	15.3	14.9	541
Nonirrigated mean	17.4	8.0	3.1	17.3	455	322	789	8.3	20.4	196
K_d regression P-value		0.70	0.76	0.91	0.13	0.06	0.15	0.01	0.87	0.71

† The pH, O.M., clay, and total arsenic values are depth-weighted averages of measurements from Keith (1995).

‡ K_d values were calculated from the linear portion of each isotherm.

§ There was insufficient soil to analyze this sample for clay.

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Table C3. Arsenic recovered from each sequential extract in selected surficial (top 20 cm) Madison River Valley soils. All means and standard deviations were calculated from duplicates. See Chapter 3 for methods.

Soil	1 *M* NH_4Cl	0.1 *M* NaOH	1 *M* HCl	30% H_2O_2/ 3.2 *M* NH_4OAc	Residual	Sum †	Total ‡	Recovery §
	mg kg^{-1}							%
A1-I	0.472 ± 0.023	2.4 ± 0.1	2.66 ± 0.05	<1.2 ----	5.2 ± 0.5	11.4 ± 0.4	12.9	88 ± 3
A1-NI	0.414 ± 0.004	1.5 ± 0.7	2.92 ± 0.43	<1.2 ----	6.8 ± 0.1	12.2 ± 0.3	21.0	58 ± 2
C2-I	0.341 ± 0.009	2.0 ± 0.1	1.94 ± 0.13	<1.2 ----	5.2 ± 0.4	10.1 ± 0.1	9.6	106 ± 1
C2-NI	0.078 ± 0.003	2.3 ± 0.1	0.91 ± 0.03	<1.2 ----	6.6 ± 0.4	10.6 ± 0.5	10.3	102 ± 4
C3-I	3.563 ± 0.777	8.6 ± 0.3	14.87 ± 0.35	2.3 ± 0.4	8.3 ± 4.0	37.6 ± 5.0	37.2	101 ± 13
C3-NI	3.892 ± 0.147	5.5 ± 0.5	47.35 ± 10.14	6.3 ± 2.4	4.1 ± 0.2	67.0 ± 11.2	57.8	116 ± 19
Average	1.460	3.7	11.8	2.2	6.0	24.8	24.8	95

† Levels of As in samples below detection limits were set equal to 1/2 the detection limit in calculating the sum.
‡ Total digests were performed on a subsample of each soil prior to the sequential extraction.
§ Recovery (%) = 100 * (Sum/Total).

Table C4. Solution levels of As(V) and As(III) following a 96 hr batch sorption isotherm for six selected Madison and Missouri River sites (irrigated and nonirrigated). Slurries were made in 0.01 *M* KCl solutions containing either 0 or 13.4 μ*M* As(V). See Chap. 3 for additional methods. More than 50% of the As(V) was reduced only in soils C2-I and C3-I, likely due to high levels of readily available DOC (Table 2) or prevalence of As(V)-reducing populations, based on findings in Chap. 4.

	Measured As(T)	Measured As(V)	Calculated As(III)	Reported As(V)	Reported As(III)	Extent of reduction
NI-0			μ*M*			(%)
A1	0.3	0.2	0.04	0.2	0.04	16
B1	0.3	0.3	0.01	0.3	<0.02	<8
B4	0.3	0.3	0.01	0.3	<0.03	<8
C2	0.4	0.3	0.03	0.3	<0.03	<7
C3	5.6	5.5	0.11	5.5	<0.44	<8
D1	0.3	0.3	-0.00	0.3	<0.03	<8
NI-13.4						
A1	0.8	0.8	0.07	0.8	<0.06	<7
B1	1.5	1.6	-0.05	1.6	<0.13	<8
B4	2.8	2.6	0.21	2.6	<0.21	<7
C2	1.0	1.1	-0.06	1.1	<0.09	<8
C3	6.8	6.3	0.5	6.3	0.5	8
D1	2.0	2.0	0.01	2.0	<0.16	<8
I-0						
A1	0.5	0.5	-0.02	0.5	<0.04	<8
B1	0.3	0.3	0.02	0.3	<0.02	<7
B4	0.5	0.5	0.01	0.5	<0.04	<8
C2	2.4	0.1	2.3	<0.1	2.3	96
C3	11.4	0.4	11.1	0.4	11.1	97
D1	0.2	0.2	-0.01	0.2	<0.02	<8
I-13.4						
A1	1.2	1.1	0.07	1.1	<0.09	<8
B1	1.5	1.5	0.01	1.5	<0.12	<8
B4	2.4	2.2	0.14	2.2	<0.18	<8
C2	7.0	3.1	3.8	3.1	3.8	55
C3	17.4	0.4	17.1	<0.4	17.1	98
D1	1.2	1.2	0.01	1.2	<0.10	<8
As(III) std.	12.8	0.1	12.7	<0.3	12.7	
As(III) std.	13.4	0.2	13.2	<0.3	13.2	
As(V) std.	14.2	13.8	0.3	13.8	<1.1	
As(V) std.	13.9	13.6	0.3	13.6	<1.1	

As(V) detections = 2.5% of total above 6.7 μ*M*; 6 % of total below 6.7 μ*M*
As(III) detections = 2*S.D. based on summation of errors

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APPENDIX D

ARSENATE OXIDATION-REDUCTION EXPERIMENTS WITH SOILS AND SOLID PHASES

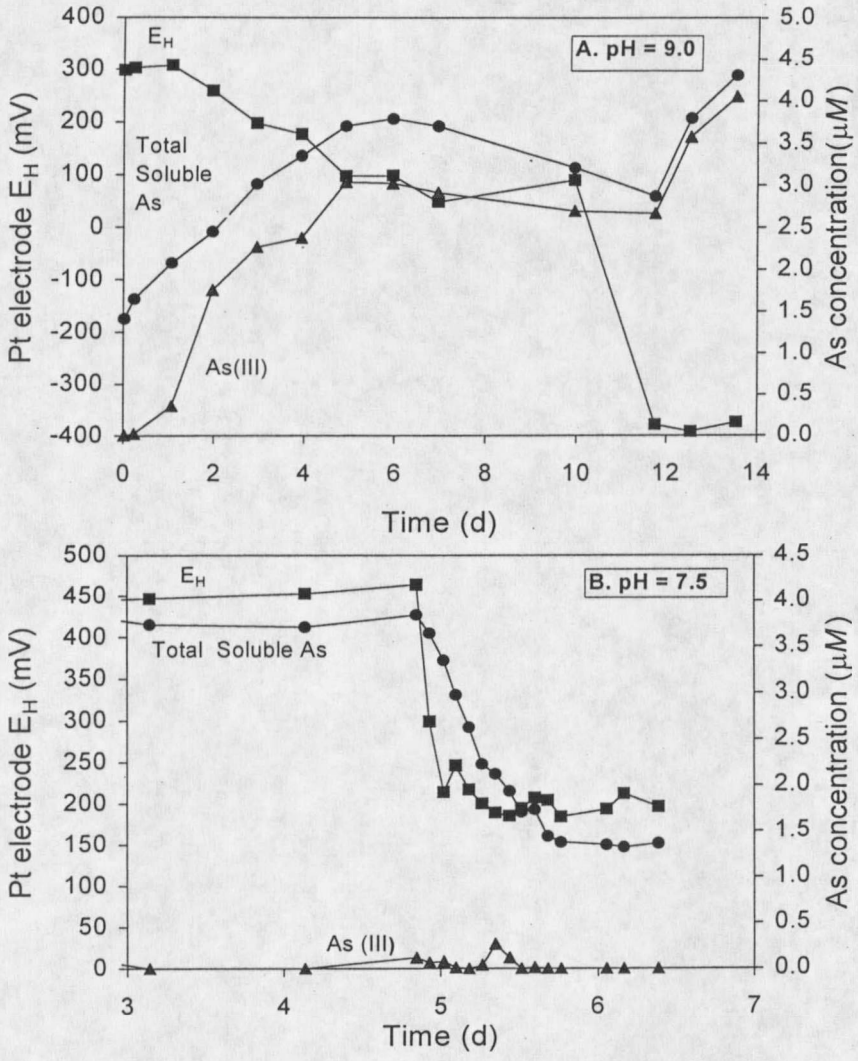


Figure D1. Measured redox potential (E_H) and As concentrations versus time in a slurry (9:1 solution:solid ratio) made from soil C3-I at pH 9.0 (A) and pH 7.5 (B). Experiments were conducted in controlled E_H/pH chambers (see Chap. 4 for details). Anaerobic conditions were attained by bubbling the pH 9.0 treatment with N_2(g) and the pH 7.5 treatment with 99% N_2/1% CO_2. The systems were stirred with a magnetic stir bar, although the stir bar became immobilized in the pH 9.0 treatment during the first d. Soluble As levels increased at pH 9.0, likely due to differences in sorption strengths between As(V) and As(III) and reductive dissolution of Fe/Mn oxides. The E_H was lowered on day 10 of the pH 9.0 experiment by adding glucose (final concentration-28 m*M* as C). As(III)-sulfide precipitation was hypothesized to have caused the decrease in soluble As in the pH 7.5 treatment. The As(III)-sulfide precipitation rate was apparently higher than the As(V) reduction rate based on lack of soluble As(III).

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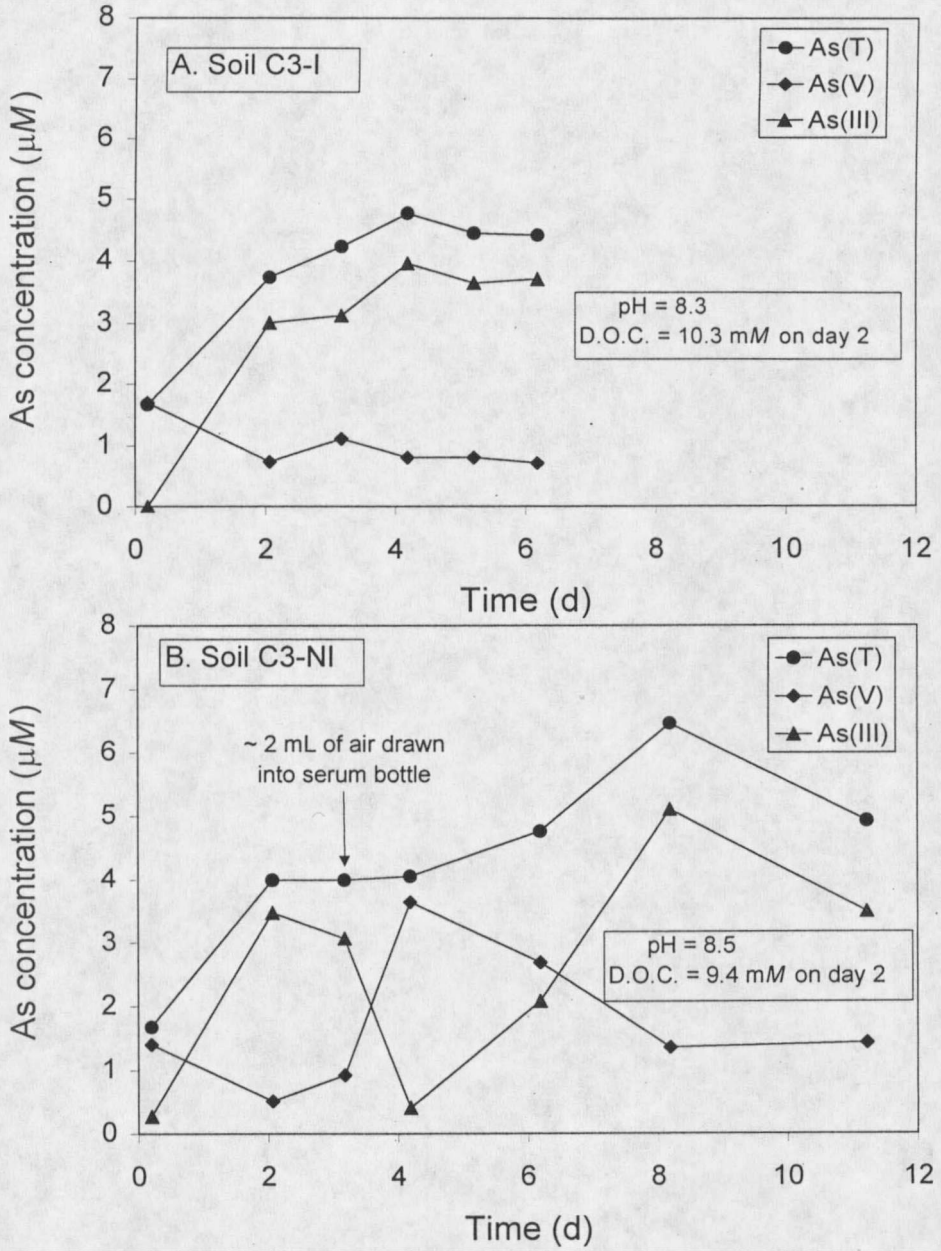


Figure D2. Concentrations of As in 9:1 SSE-R2:solid slurries of A) Soil C3-I and B) Soil C3-NI during reduction (see Appendix C and Chap. 3 for site designations and soil characterization). Initial As(V) reduction rates were not substantially lower than rates with CN-0 in the absence of soil (Chap. 4), suggesting our systems (with glucose as electron donor) did not greatly overestimate natural As(V) reduction rates with native DOC. The addition of air into the C3-NI slurry resulted in oxidation of solution As(III). Within one d after oxidation of As(III), reduction rates of As(V) resumed at rates similar to initial rates.

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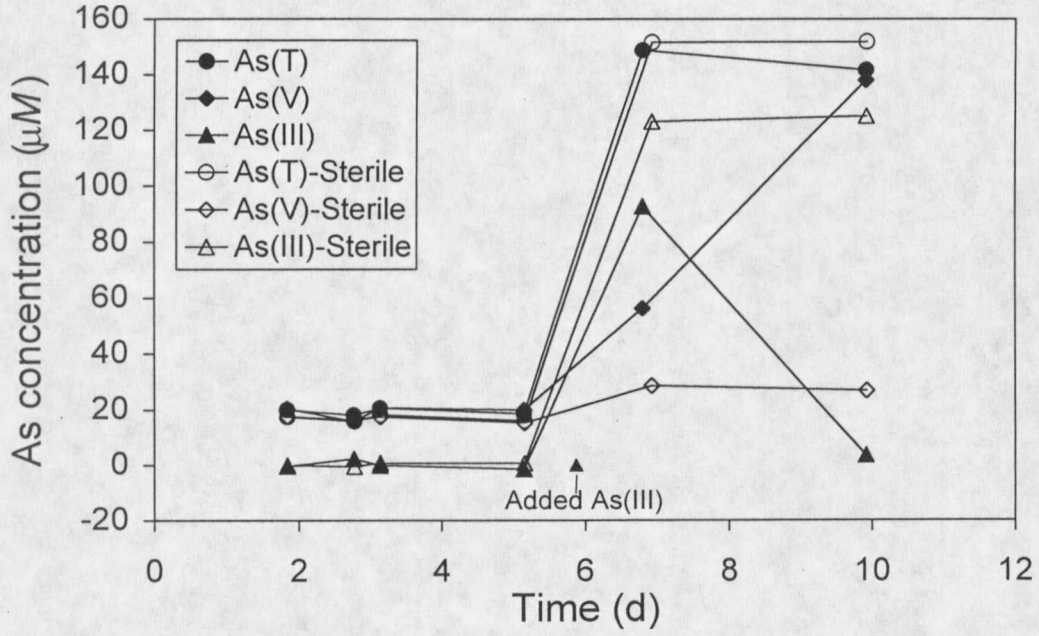


Fig. D3. Arsenic levels in a filtered (0.2-μ) C3-NI extract (3:1 D.I. H_2O:soil ratio) before and after spiking solutions with As(III). Incubations were purged with N_2(g) at approximately 60 mL min^{-1} for 10 min, inoculated with 40 g L^{-1} C3-NI soil, and then either autoclaved (open symbols) or not autoclaved (filled symbols). After verifying that As speciation was stable, As(III) was added to a concentration of 133 μ*M*. As(III) was converted to As(V) in the nonsterile treatment but remained as As(III) in the sterile treatment. Results demonstrate that As(III) oxidation is biologically mediated in C3-NI, potentially decreasing net As(V) reduction rates under field conditions. Electron acceptors that may have coupled with As(III) oxidation include NO_3^-, Mn^{+4}, or Fe^{+3}, yet because these electron acceptors were excluded in the soluble As(V) reduction studies (Chap. 4), As(III) oxidation should not have affected measured As(V) reduction rates.

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Table D1. Solution iron (Fe) concentrations in goethite suspensions at both a high and a low As:Fe ratio (Chap. 4). Total Fe concentrations were 2.5 and 25 m*M* for RC2 and RC3, respectively, indicating relatively little reductive dissolution (<0.4%) occurred in either system. Roden and Zachara (1996) found that an Fe(III)-reducing bacteria reduced 1.4% of supplied goethite over a 20 d period, and attributed the limited extent of reduction on low surface area and Fe(II) surface coatings.

RC2 (Fe(T) = 2.5 m*M*)

Time (d)	pH	Eh (mV)	Soluble FeT (μ*M*)
1.0	7.43	469	<0.54
1.9	7.47	489	<0.54
2.9	7.44	479	<0.54
3.8	7.42	469	<0.54
3.9	7.56	439	<0.54
4.8	7.57	379	<0.54
4.9	7.52	313	<0.54
4.9	7.51	248	<0.54
5.0	7.46	207	4.95
5.8	7.55	181	<0.54
5.9	7.55	111	<0.54
6.1	7.56	17	1.34
6.8	7.51	24	<0.54
11.7	7.55	93	<0.54

RC3 (Fe(T) = 25 m*M*)

Time (d)	pH	Eh (mV)	Soluble FeT (μ*M*)
0.0	6.42	609	9.90
2.1	6.02	571	99.02
3.1	6.00	496	38.87
3.9	6.05	413	<0.54
4.5	5.80	345	1.05
5.1	6.03	305	10.41
6.0	5.99	294	0.66
6.1	5.94	200	<0.54
6.1	5.93	155	3.39
6.3	5.99	116	0.54
6.9	5.97	123	1.10
8.2	6.02	29	<0.54
9.0	6.02	-5	<0.54
14.9	5.97	-14	<0.54
15.9	5.94	-56	22.16

The erratic Fe data may have been due to incomplete rinsing of acid-washed filter holders, and subsequent pH-dependent dissolution of goethite during filtration.

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APPENDIX E

BIOTIC ARSENATE REDUCTION RATE EXPERIMENTS IN SOLUTION

SUPPLEMENT TO CHAPTER 4

Table E1. Effect of enrichment, NO_3^-, and N_2-purging on the extent of As(V) reduction in serum bottles. CN-1 reduced more As(V) than a population enriched with H_2(g), regardless of supplied nutrients or purging. Populations grown in SSE consistently reduced less As(V) than populations grown in SSER2, suggesting that NO_3^- inhibited As(V) reduction in both populations. It was unknown whether lack of NO_3^- consistently inhibited total growth because optical densities were not measured. Purging decreased As(V) reduction with the H_2(g)-enriched population, yet increased reduction with CN-1, suggesting that i) O_2(g) was necessary for As(V) reduction in the H_2-enriched population and ii) O_2(g) inhibited As(V) reduction in the CN-1 population.

Enrichment	Nut. Sol'n	N_2 Purged (P) or Non-purged (NP)	Visible Turbidity	As (V) reduction after 8 d
				(%)
H_2 bubbled, SSER2	SSE	P	+	0
H_2 bubbled, SSER2	SSE	NP	+	39
H_2 bubbled, SSER2	SSER2	P	-	2
H_2 bubbled, SSER2	SSER2	NP	+	65
N_2 purged, SSER1 (CN-1)	SSE	P	+	67
N_2 purged, SSER1 (CN-1)	SSE	NP	+	44
N_2 purged, SSER1 (CN-1)	SSER2	P	+	75
N_2 purged, SSER1 (CN-1)	SSER2	NP	+	72

1. Initial As(V) = 13.4 μM
2. H_2 bubbled systems used 1% H_2(g)/98% N_2(g)/1% CO_2(g)
3. Nutrient solutions: Supplied electron acceptors
   - SSE: SO_4^{2-} and NO_3^-
   - SSER2: SO_4^{2-}
   - SSER1: none (i.e. As(V) was only supplied electron acceptor)
4. As(V) and As(III) were quantified with 1 mL 2*M* TRIS, 0.25 mL 3% $NaBH_4$/0.1% NaOH in a 5 mL sample over 7 min. Average recoveries on prepared As(III) standards were 79% using this methodology (Table A5)

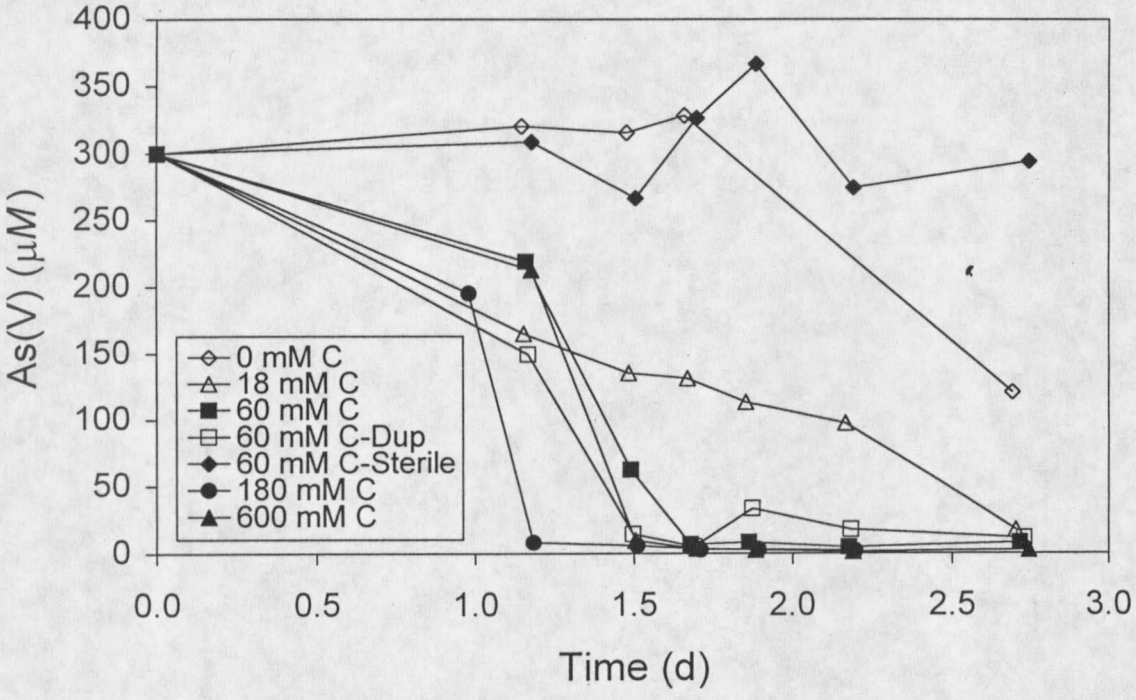


Fig. E1. Effect of initial glucose levels on As(V) reduction with the CN-5 population in anaerobic serum bottles at pH 6.3 (20 m*M* $KHCO_3$ buffer maintained the pH-see Chap. 4 for contents of nutrient solution). All treatments contained 0.2 g L^{-1} yeast extract (6.5 m*M* as C). As(V) and As(III) were quantified with batch hydride generation using 1 mL of both 2M TRIS and 1 mL of 3% $NaBH_4$/0.1% NaOH in 5 mL sample for 7 min (see Chap. 4 for details). This technique was used in all subsequent experiments . Reduction rates of As(V) were lower at C levels of 0 and 18 m*M* than at C levels above 18 m*M*, apparently due to C limitation (see Fig. E2). Although some As(V) reduction was observed in the treatment containing only yeast extract, the lag time was much greater than for treatments containing glucose.

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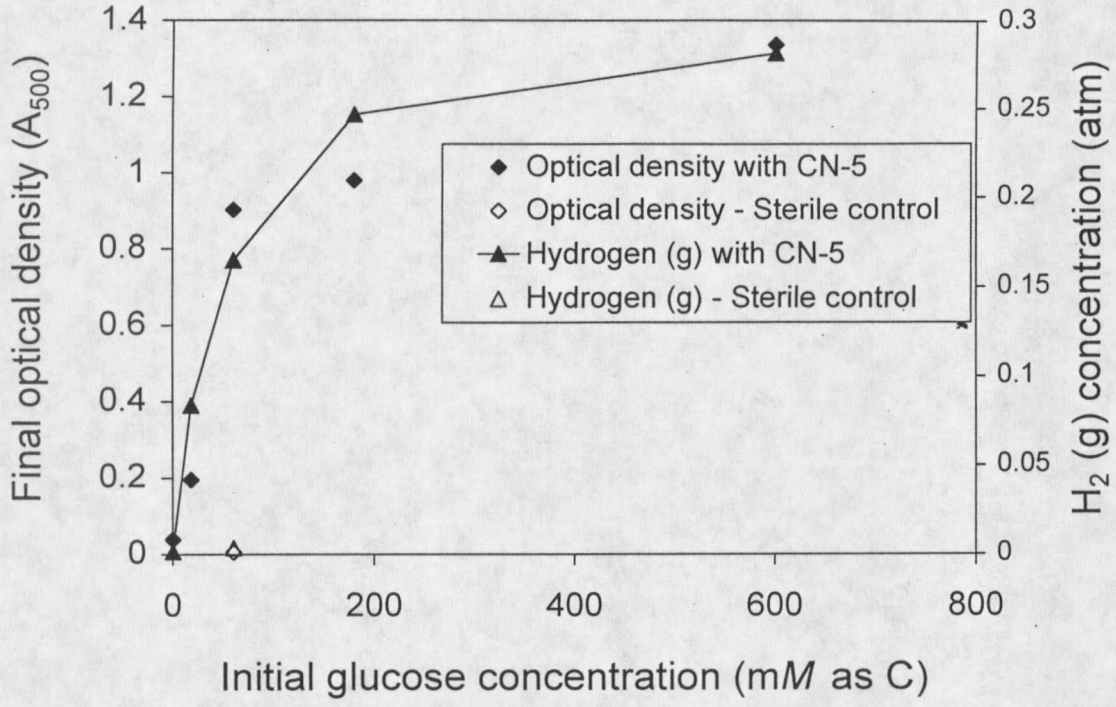


Fig. E2. Evidence that the CN-5 population was fermenting glucose and reducing H^+. The extent of growth, based on A_{500}, and H_2(g) produced were controlled by initial glucose concentrations, although the degree of control decreased above 60 m*M* C. See Fig. E1 for additional methodology.

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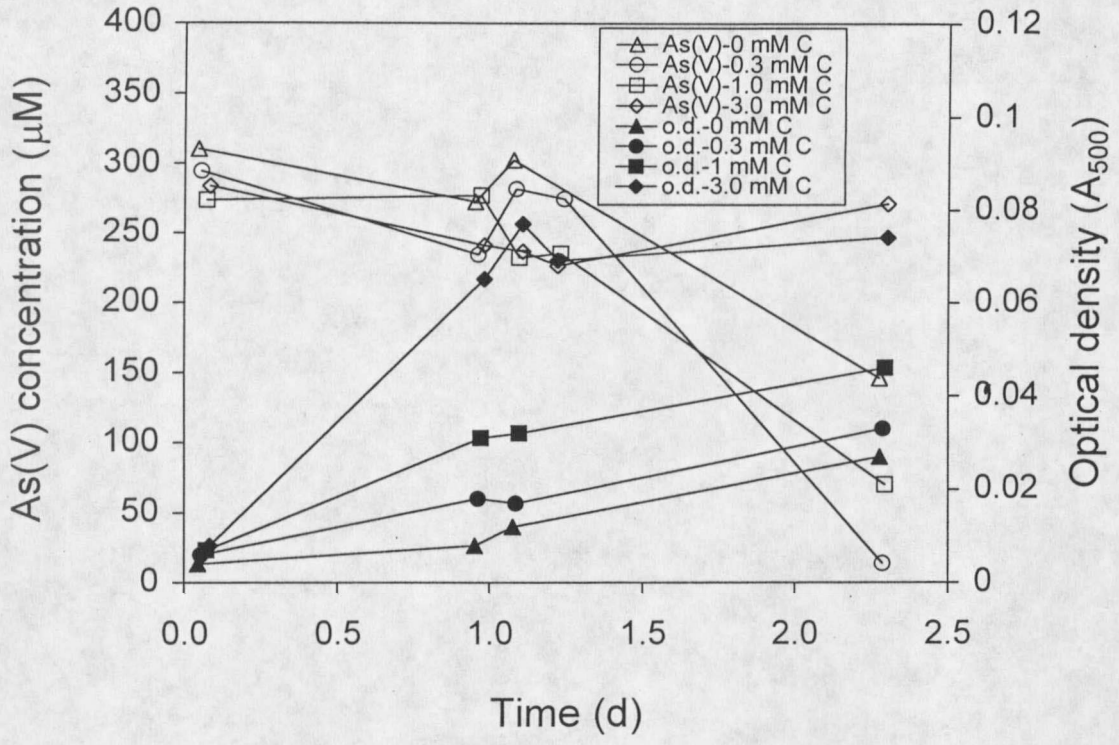


Fig. E3. Effect of low C levels (as glucose) on As(V) reduction with CN-7 in serum bottles. All treatments contained yeast extract (6.5 mM C). Growth was better correlated to initial glucose levels than to total C levels (i.e. glucose-C + 6.5 mM C), suggesting the CN-7 population could readily use glucose, but could only utilize a small fraction of the C in the yeast extract. Reduction was less than 20% complete during the initial growth phase (until 1.2 d), yet approached 95% reduction in the 0.3 mM C treatment 2.3 d into the experiment (additional growth possibly occurred on yeast extract based on similarity of growth increases). Extent of As(V) reduction was inconsistent and not well correlated with initial glucose level, although the glucose supplied in all treatments should have been sufficient to reduce all of the As(V) to As(III) assuming complete oxidation of C to $CO_2(g)$;. however, glucose fermentation was apparently the primary metabolic pathway based on $H_2(g)$ production (Fig. E2).

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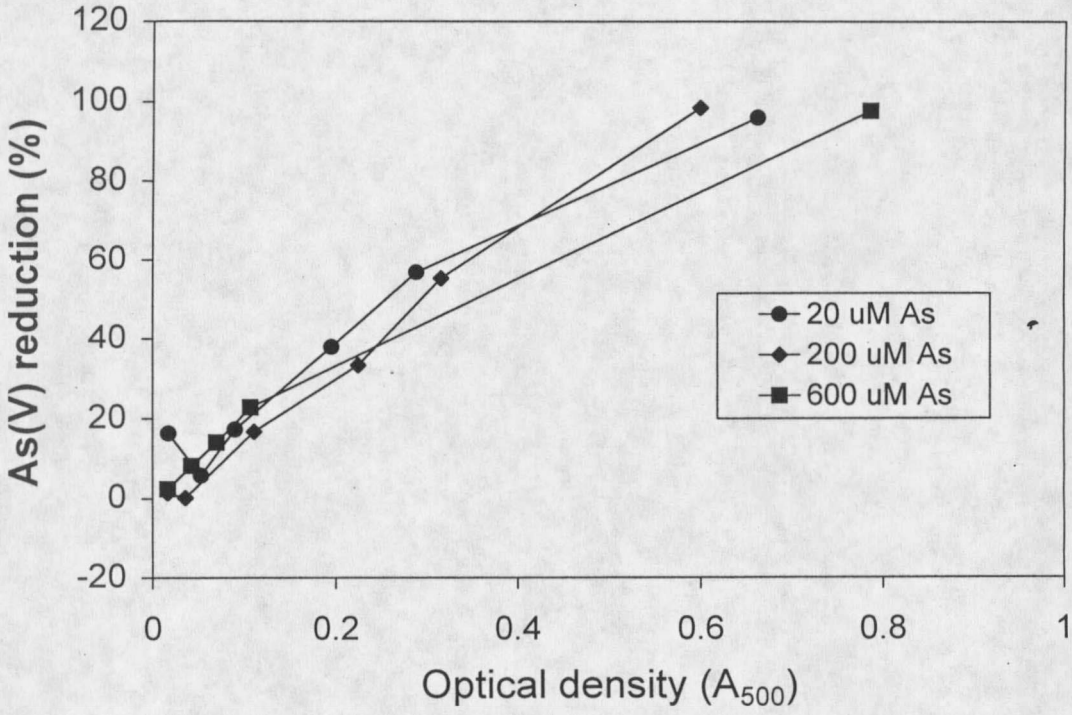


Fig. E4. Effect of CN-8 growth (assumed to correlate with optical density) on the extent of As(V) reduction. These data supplement Fig. 11 (Chap. 4) and were included in the regression between As(V) reduction and A_{500}. The three treatments shown above were inoculated together, whereas the duplicate 600 uM As treatments shown in Fig.10 were inoculated at the same time as 2 and 5 mM As treatments 12 days later. Despite different inoculations and lag times (Fig. E7), reduction rates of As(V) among the three 600 uM treatments were comparable, as shown by the small error bar in Fig. 12 (Chap. 4).

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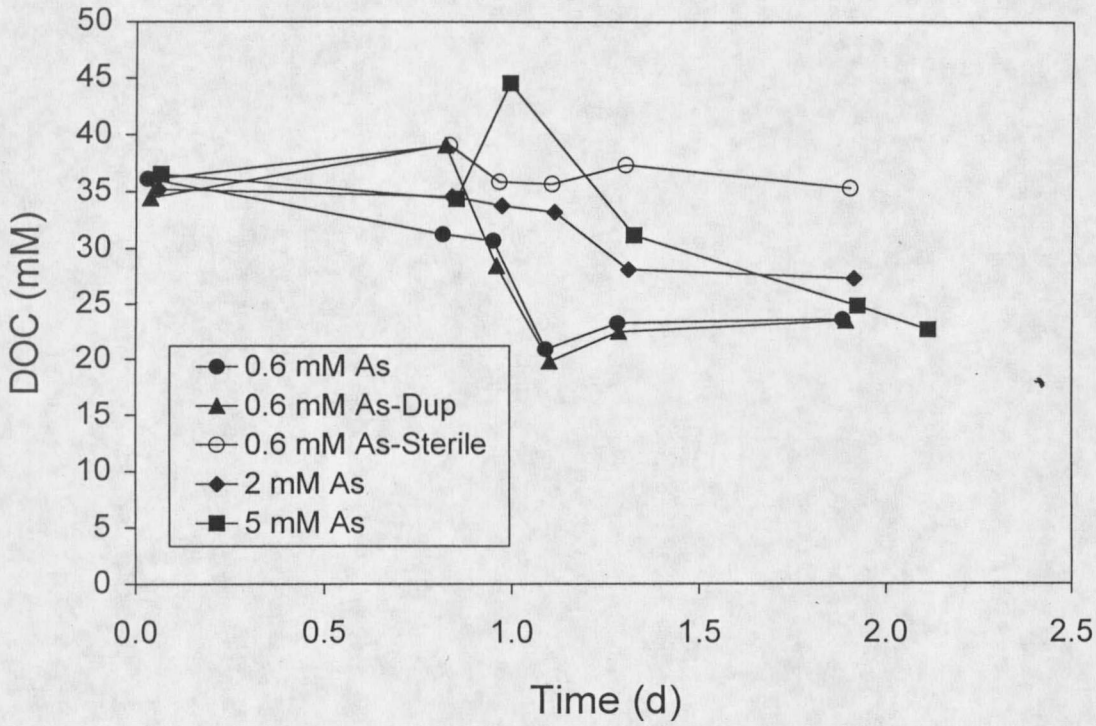


Fig. E5. Concentrations of dissolved organic carbon (DOC) over time in serum bottles inoculated with CN-8 at three As(V) concentrations. All nutrient solutions (SSE-C) initially contained glucose (30 mM C) and yeast extract (6.5 mM C). Additional methods are included in Chap. 4. Decreases in DOC coincided with both growth (Fig. E7) and As(V) reduction (Fig. 10 , Chap. 4). The lag in growth and DOC mineralization observed in the 2 and 5 mM treatments was apparently due to toxicity (As(V) prevents ATP production). The DOC levels remaining at the end of the experiment were consistent with stoichiometry of hypothesized glucose fermentation reactions (Chap. 4), and likely consisted of organic acids (e.g. acetate, butyrate).

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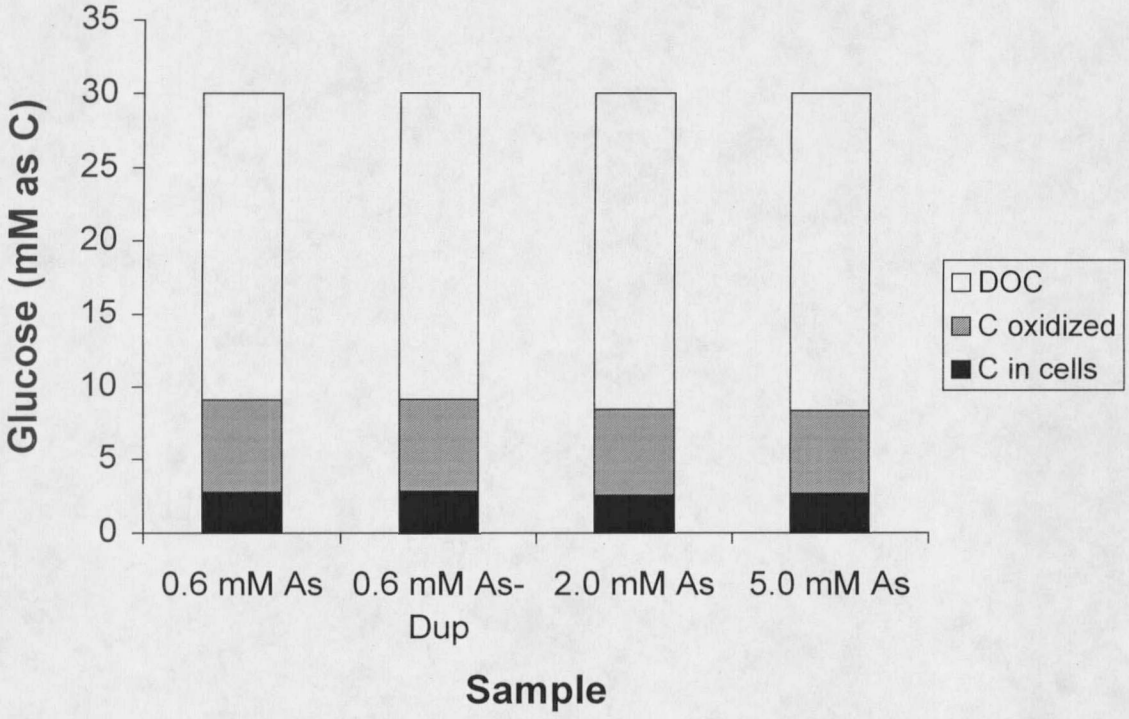


Fig. E6. Fate of glucose in serum bottles inoculated with CN-8. Each treatment was spiked with 17 uL of a ^{14}C–labeled glucose stock solution, targeting an initial concentration of 1.7×10^2 Bq mL^{-1}. Both filtered (0.2 μm) and unfiltered samples were treated with HCl and purged of CO_2 prior to analysis with a Packard liquid scintillation counter (Meriden, NJ). Cell C was assumed to equal the difference in C level between filtered and unfiltered samples; C oxidation was assumed to equal the difference between initial C and the final unfiltered sample; and the DOC level was assumed to equal C in the final filtered sample. A comparison of Fig. E5 and E6 indicates that approximately 2-5 mM C from the yeast extract (initial C = 6.5 mM) remained in each treatment. The compilation of results demonstrate that the initial As(V) level did not alter the amount of glucose mineralization or C uptake into cells, indicating that glucose fermentation rather than respiration (with As(V) as electron acceptor) was the primary metabolic pathway in this system.

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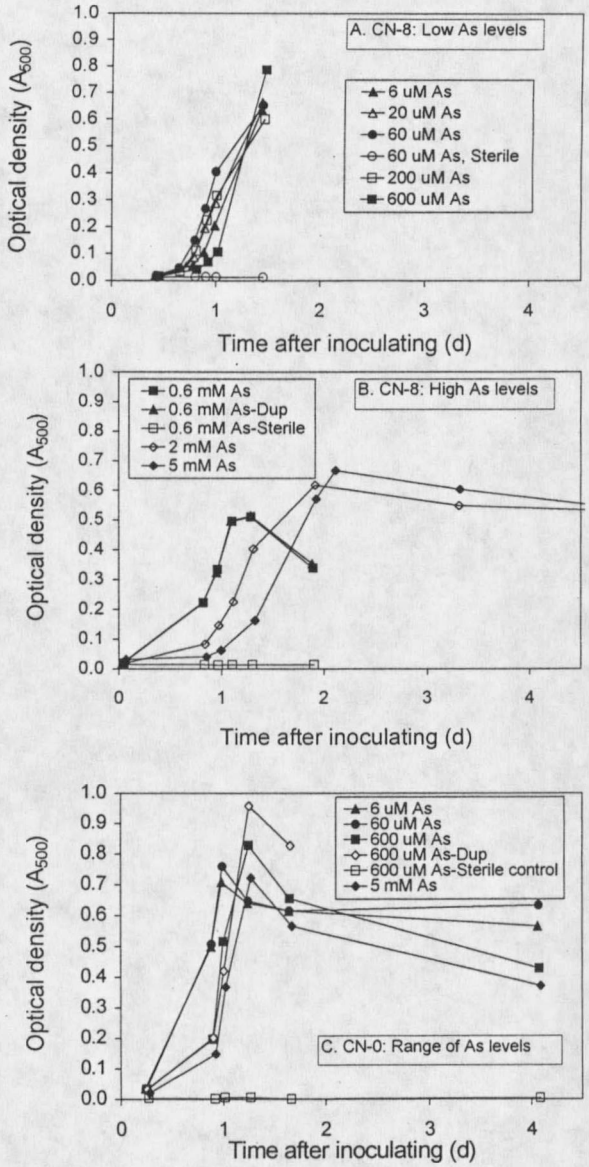


Fig. E7. Microbial growth (based on optical density changes) over time in anaerobic serum bottles for A. CN-8 at initial As(V) levels ≤ 0.6 mM, B. CN-8 at As(V) levels ≥ 0.6 mM, and C. CN-0 for the full range of As(V) levels. See Chap. 4 for detailed methodology. At As(V) levels > 0.6 mM, CN-8 lag times were longer and growth rates were lower than at lower As levels. In serum bottles inoculated with CN-0, As(V) levels > 0.06 mM apparently lengthened lag times yet did not substantially affect growth rates. The CN-0 population grew faster than the CN-8 population, especially in the 5 mM As(V) treatment, suggesting at least one organism within CN-0 was more As-tolerant than the enriched population. Total growth was relatively similar with both populations in all treatments, likely because growth was coupled with fermentation of glucose (30 mM in all treatments).

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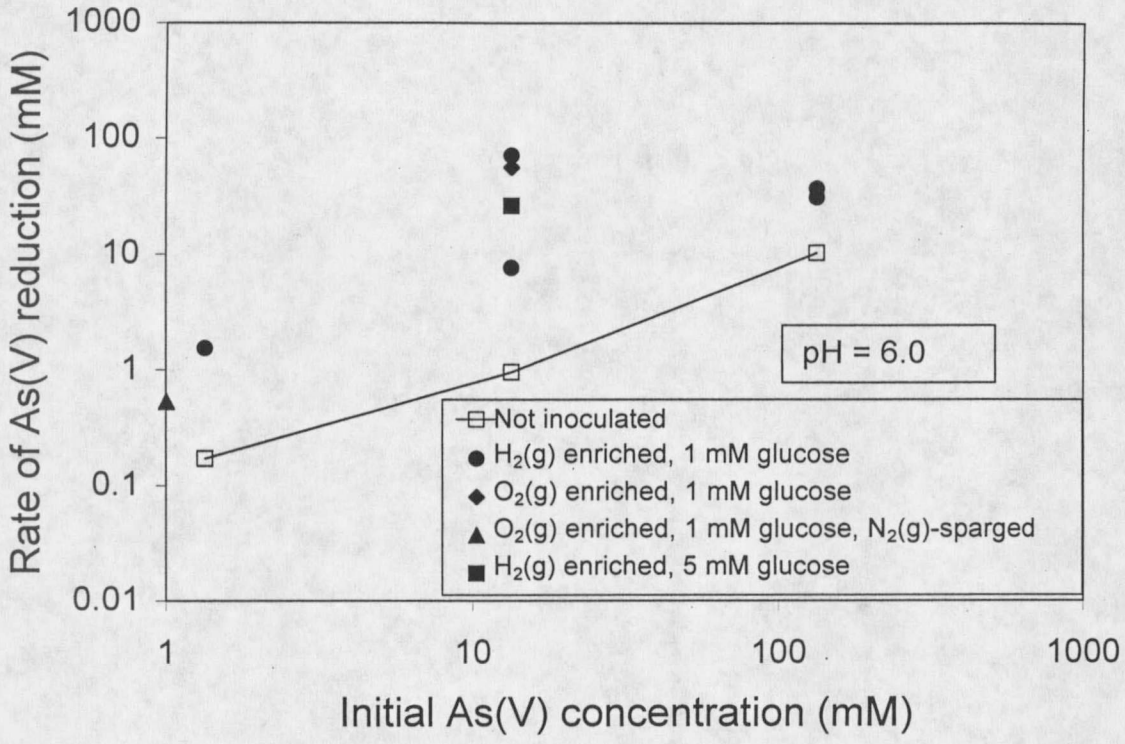


Fig. E8. The effect of initial As(V) concentration on As(V) reduction rate with organisms extracted from soil C3-NI (see Chap. 3 and 4 for more details) and enriched once as denoted above. Nutrient solutions (SSE) were added to glass jars fitted with pH and E_H electrodes. Solutions were continuously shaken (120 rpm) and sparged ($\cong$10 mL min^{-1}) with either a gas comprised of 98% Ar/1% H_2/1% CO_2, or 99% N_2/1% CO_2 (triangle symbol above). Results from eight other experiments (four with lactate, four with glucose) are not shown due to lack of As(V) reduction. The absence of As(V) reduction implies that either an alternate electron acceptor inhibited reduction, As(V) reducers were selected against in some of the enrichments, or the As(III) oxidation rate exceeded the As(V) reduction rate (see Fig. D3). Approximately 30 mM of electrons could be accepted by the NO_3^- (3.5 mM) if reduced to NH_4^+, compared to only 24 mM of electrons donated by 1 mM glucose assuming complete oxidation. Thus, NO_3^- may have inhibited As(V) reduction in some of the enrichments, although it did not consistently prevent reduction. Some contamination (10^3 – 10^5 cells mL^{-1}) was found in each treatment that was not inoculated, though the data imply As(V) reduction rates with enriched C3-NI populations using C as electron donor were generally at least 10 fold higher than rates with 1% H_2(g) as electron donor.

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