



Photochemical oxidation of arsenic(III) in ferrioxalate solutions and elk exposure to arsenic in Yellowstone's geothermal environments
by Benjamin David Kocar

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Resources and Environmental Sciences '

Montana State University

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Abstract:

Photochemical reactions generate products, such as $\text{OH}^\bullet$, that are responsible for the chemical transformation of many natural water constituents. However, little work has been performed studying the mechanisms of arsenite (As(III)) photochemical oxidation, and no work has been performed which directly links the oxidation of As(III) to $\text{OH}^\bullet$. Ferrioxalate, a compound found in atmospheric and surface waters, produces $\text{OH}^\bullet$ upon irradiation and has been used to study the mechanisms of photochemically-induced oxidation for a variety of environmentally relevant compounds. Consequently, ferrioxalate solutions with varying concentrations of As(III) , Fe(III) and 2-propanol were irradiated with a quartz tungsten halogen lamp to determine rates and mechanisms of As(III) oxidation. Results indicate that rates of oxidation in the ferrioxalate system are rapid ($0.5\text{--}254.0\ \mu\text{M hr}^{-1}$). Furthermore, experiments using 2-propanol to scavenge $\text{OH}^\bullet$ demonstrate that As(III) is directly oxidized by $\text{OH}^\bullet$. Finally, significant rates of As(III) oxidation (3.7 and $5.6\ \mu\text{M hr}^{-1}$) were observed in a solution containing natural DOC, indicating that photochemical oxidation of As(H) may significantly influence arsenic (As) cycling in natural waters.

Elk (*Cervus elaphus*) residing in the MF watershed, YNP are exposed to elevated levels of As, primarily through ingestion of high-As aquatic and terrestrial plants, sediments, and algae. Plants and soils collected from dry, terrestrial environments contained low As concentrations, and Madison, Firehole, and Gibbon River water contained low As relative to aquatic and terrestrial plants and sediment samples. Consequences of exposure via these routes include elevated levels of As in elk tissues, rumen content and feces compared to a control population; some of these levels approach or exceed levels found in dosing studies where ruminants exhibited signs of chronic arsenic toxicosis. Analysis of As species in selected plant and elk samples indicate that the ingested forms of As are predominately inorganic, and that M-F elk may be detoxifying As via methylation. Increasing As:creatinine in elk urine over the course of high total snow water equivalence (SWE) winters indicate seasonally driven As exposure, with As:creatinine reaching peak levels between the months of February and March. Finally, average As:creatinine values are positively correlated with total winter SWE for several years, implying that snow depth is an important variable governing overall elk exposure to As.

PHOTOCHEMICAL OXIDATION OF ARSENIC(III) IN FERRIOXALATE
SOLUTIONS AND ELK EXPOSURE TO ARSENIC IN YELLOWSTONE'S
GEOTHERMAL ENVIRONMENTS

by

Benjamin David Kocar

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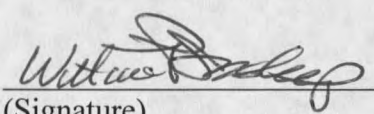
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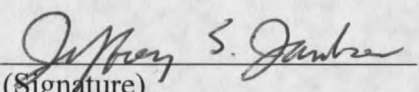
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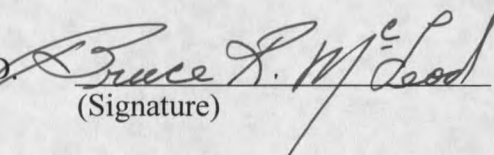
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TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	viii
ABSTRACT	ix
1. INTRODUCTION	1
REFERENCES CITED	5
2. PHOTOCHEMICAL OXIDATION OF AS(III) IN FERRIOXALATE SOLUTIONS	7
Introduction	7
Materials and Methods	10
Reacting Solutions	10
Experimental Apparatus and Irradiation Source	11
Analytical Methods	13
Effect of 2-Propanol on Initial As(III) Oxidation Rate	14
Oxidation of As(III): H ₂ O ₂ and Fe(II) Dark Controls	15
Apparent Quantum Yield	15
Photochemical Oxidation of As(III) in a Natural Water	16
Results and Discussion	17
Photochemical Oxidation of As(III)	17
As(III) Oxidation Rate Dependence on pH	21
Oxidation Rate Dependence on Initial As(III) and Fe(III)	21
Quantum Yields	25
Mechanism of Photochemical Oxidation of As(III)	26
As(III) Oxidation in the Presence of DOC	30
Environmental Implications	32
REFERENCES CITED	35
3. ELK EXPOSURE TO ARSENIC IN YELLOWSTONE'S GEOTHERMAL ENVIRONMENTS	40
Introduction	40
Methods	44
Site descriptions	44
Sample collection	46
Urine collection and creatinine analysis	47
Total As analysis	48

TABLE OF CONTENTS-CONTINUED

Soil As extraction.....	49
Extraction and analysis of As species in selected samples	49
Statistical analysis.....	51
Estimation of As accumulation and excretion.	52
Results.....	52
As concentrations in elk samples.....	52
As in forage mixtures and individual plant species	56
Soil and water As	59
As speciation in rumen content, fecal matter, plants, soil and water..	60
Seasonal and yearly fluctuations in urine As.....	62
Discussion.....	65
Elk As ingestion from plants, soil and water	65
Estimates of As Exposure via Forage.....	67
Seasonal As exposure and potential toxicological effects	69
Summary.....	71
REFERENCES CITED.....	73
4. SUMMARY.....	78

LIST OF TABLES

Table	Page
1.1 Appearance/disappearance rates and quantum yields of constituents in irradiated solutions.....	19
1.2 Compilation of reactions and corresponding rate constants concerning production and/or consumption of free radical Species in Fe-As-H ₂ O Systems.....	23
1.3 Comparison of apparent half-lives for the oxidation of As(III) via abiotic and biotic pathways.....	34
2.1 Mean concentration (mg kg ⁻¹) of total As in hair, skin, bone, feces and rumen of Madison-Firehole or Sun Ranch control elk populations.....	54
2.2 Concentrations of total As (mg kg ⁻¹ , DW) measured in elk forage mixtures sampled from the Madison-Firehole watershed and from control locations within the Northern Range and Sun Ranch.....	57
2.3 Concentrations of total As (mg kg ⁻¹ , DW) in individual plant species sampled within the Madison-Firehole watershed.....	59
2.4 Distribution of As species in selected plants, elk rumen content and elk feces determined using methanol extraction and analysis via HPLC-ICP-MS	61
2.5 Mean As:C ratios of radiocollared elk within the MF watershed by month for three winters exhibiting a wide range in total snowfall.	63

LIST OF FIGURES

Figure	Page
1.1 Calculated (GEOCHEM) distribution of aqueous Fe(III) species as a function of pH in the presence (A) and absence (B) of 1 mM oxalate	12
1.2 Disappearance of As(III) in (A) irradiated ferrioxalate solutions and in (B) dark or irradiated controls.....	18
1.3 Log initial rates of As(III) oxidation (M hr^{-1}) as a function of (A) pH (B) initial As(III) and (C) initial Fe(III)	22
1.4 Oxidation rate of As(III) and formation rate of 2-propanone ($\mu\text{M hr}^{-1}$) as a function of initial [As(III)].....	27
1.5 Percent of As(III) oxidized as a function of Fe(II) in the presence of 1.35 μM initial As(III) and 10 mM initial H_2O_2 in the dark (Fenton's Reaction).....	28
1.6 Disappearance of As(III) in the presence of 0.86 mM dissolved organic C (Hyalite natural water) upon exposure to sunlight.....	31
2.1 Map of the Madison-Firehole (MF) study area in Yellowstone National Park showing plant, soil and water sampling locations, carcass locations, and home ranges of elk from which urine samples were collected.	45
2.2 Concentrations of total As in skin, hair, bone, rumen contents, and fecal matter of Madison-Firehole (MF) and Sun Ranch Control (SRC) elk (mg kg^{-1} , dry weight).....	53
2.3 Concentrations of total As (mg kg^{-1} , dry weight) in forage mixtures collected from the MF watershed and the Northern Range control sites.....	58
2.4 Average As:C ratios in elk urine of radiocollared animals as a function of total winter snow water equivalent (SWE).....	63
2.5 Individual As:C ratios in elk urine ($n = 140$) as a function of time across three winters.....	64
2.6 Estimated concentration of As in elk skin and bone (mg kg^{-1}) as a function of forage As..	68

ABSTRACT

Photochemical reactions generate products, such as $\text{OH}^\bullet$, that are responsible for the chemical transformation of many natural water constituents. However, little work has been performed studying the mechanisms of arsenite (As(III)) photochemical oxidation, and no work has been performed which directly links the oxidation of As(III) to $\text{OH}^\bullet$. Ferrioxalate, a compound found in atmospheric and surface waters, produces $\text{OH}^\bullet$ upon irradiation and has been used to study the mechanisms of photochemically-induced oxidation for a variety of environmentally relevant compounds. Consequently, ferrioxalate solutions with varying concentrations of As(III) , Fe(III) and 2-propanol were irradiated with a quartz tungsten halogen lamp to determine rates and mechanisms of As(III) oxidation. Results indicate that rates of oxidation in the ferrioxalate system are rapid ($0.5\text{--}254.0\ \mu\text{M hr}^{-1}$). Furthermore, experiments using 2-propanol to scavenge $\text{OH}^\bullet$ demonstrate that As(III) is directly oxidized by $\text{OH}^\bullet$. Finally, significant rates of As(III) oxidation (3.7 and $5.6\ \mu\text{M hr}^{-1}$) were observed in a solution containing natural DOC, indicating that photochemical oxidation of As(III) may significantly influence arsenic (As) cycling in natural waters.

Elk (*Cervus elaphus*) residing in the MF watershed, YNP are exposed to elevated levels of As, primarily through ingestion of high-As aquatic and terrestrial plants, sediments, and algae. Plants and soils collected from dry, terrestrial environments contained low As concentrations, and Madison, Firehole, and Gibbon River water contained low As relative to aquatic and terrestrial plants and sediment samples. Consequences of exposure via these routes include elevated levels of As in elk tissues, rumen content and feces compared to a control population; some of these levels approach or exceed levels found in dosing studies where ruminants exhibited signs of chronic arsenic toxicosis. Analysis of As species in selected plant and elk samples indicate that the ingested forms of As are predominately inorganic, and that M-F elk may be detoxifying As via methylation. Increasing As:creatinine in elk urine over the course of high total snow water equivalence (SWE) winters indicate seasonally driven As exposure, with As:creatinine reaching peak levels between the months of February and March. Finally, average As:creatinine values are positively correlated with total winter SWE for several years, implying that snow depth is an important variable governing overall elk exposure to As.

CHAPTER 1

INTRODUCTION

Arsenic (As), the 20th most abundant element in the earth's crust (1), is a known toxin and human carcinogen (2). A major health crisis has arisen in a multitude of countries around the world where As in drinking water supplies exceed $10 \mu\text{g L}^{-1}$, the current minimum contaminant level suggested by the World Health Organization (3). In Bangladesh alone, the estimated potential As-exposed population is 30 million, and drinking water concentrations may surpass 2 mg L^{-1} (4). Consequently, thousands living in these regions are afflicted with a variety of As-associated maladies, including hyperpigmentation, keratosis, skin lesions, and a variety of cancers (5). Although information regarding As poisoning is circulating in stricken locations, millions of inhabitants consume tainted well-water irregardless of the associated health risks due to severe shortages of sanitary drinking water. Unfortunately, water treatment processes and facilities designed to remove high levels of As are costly, so As poisoning is especially prominent in the impoverished. Most people living in As-impacted locations struggle to simply meet daily nutritional requirements (5), let alone pay for water treatment technology; thus a dire need exists for cheap, simple and effective treatment processes that may be implemented on a large scale.

Well-waters in Bangladesh and other countries that contain high levels of As often contain high levels of ferrous iron (Fe(II)), which is rapidly oxidized (on a time scale of minutes) to ferric iron (Fe(III)) and amorphous Fe(III) oxyhydroxides upon exposure to air (6). This process is beneficial, since arsenate (As(V)) adsorbs strongly to amorphous Fe(III)-oxyhydroxide (7), resulting in a decrease of As_{ts} in well-water that is allowed to oxygenate and settle over a period of hours. Unfortunately, arsenite (As(III)) is the dominant species of As pumped from groundwater, which adsorbs only weakly to Fe(III)-oxyhydroxides (7), and may persist without being oxidized (by dissolved O₂) to As(V) for many days (6,8). Consequently, an initial As(III) oxidation step is needed to facilitate As adsorption to amorphous Fe(III)-oxyhydroxides. Photooxidation is a process which has recently been used to oxidize As(III) in simulated Bangladesh well-water and in acid mine drainage containing high concentrations of Fe(III) (milligrams L⁻¹) (9). However, few studies have been performed directly linking photochemical reaction mechanisms to As(III) oxidation (9,10), and very little data exists to date which accurately describe mechanisms of photochemical oxidation of As(III) in environmentally relevant systems (i.e. surface and atmospheric waters). Furthermore, no studies have been performed that describe the potential influence of photochemistry on biogeochemical cycling of As(III) in the environment.

Photochemical processes generate important reaction products that are responsible for the chemical transformation of elements and carbon compounds found in atmospheric and surface waters. During a cloudless, summer noon hour, surface waters receive approximately 1 kW m⁻² of sunlight; equivalent to about 2 moles of photons per square

meter within wavelengths 300-500 nm (11). A large portion of these photons are absorbed by dissolved organic carbon (DOC), such as oxalate, to form excited DOC intermediates, which may further react with other dissolved constituents such as Fe(III) and dissolved O_2 to produce oxidative products (13). Some of these products include superoxide anion ($O_2^{\cdot -}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) (11). Of these, $OH^{\cdot}$ has been found to oxidize a variety of environmentally relevant compounds, such as herbicides (14), and has also been shown to strongly influence the oxidation state and resulting biogeochemistry of several elements such as Fe (11,12). Although As(III) is oxidized by $OH^{\cdot}$ generated via pulse irradiation (15), no studies have been performed directly linking oxidation of As(III) by $OH^{\cdot}$ generated via irradiation of DOC. Hence, the objectives of the study in Chapter 2 were to (1) evaluate oxidation rates of As(III) in irradiated ferrioxalate (Fe(III) + oxalate) solutions as a function of pH, (2) identify probable mechanisms of photochemical As(III) oxidation, and (3) evaluate the oxidation of As(III) in a representative natural water containing dissolved organic carbon.

In addition to using As laden well-water for drinking and cooking, inhabitants of Southeast Asia and other As-afflicted regions often use it to water livestock and irrigate crops (16). This may result in widespread transfer of As into soils, surface waters, and food chains existing in these locations. Unfortunately, there is a paucity of data regarding the distribution of As in biota inhabiting As-impacted ecosystems, and there is no data describing the concentration of As in large mammals that frequent such areas. Since many human inhabitants of these locations depend on ruminants as a source of food (17),

it is important to determine the biological endpoints of As in ruminants which frequent As-impacted locations.

One environment that contains naturally elevated levels of As and a large population of free-ranging ruminants is the Madison-Firehole (M-F) watershed in Yellowstone National Park (YNP), Wyoming, USA. Concentrations of As in M-F surface waters may exceed 3 mg L^{-1} (18), providing a unique opportunity to elucidate the ecotoxicity of As across different trophic levels in a model system. Specifically, the biological endpoints of As may be observed in elk (*Cervus elaphus*) with permanent home-ranges (19) located in the M-F watershed, providing insight regarding ingestion routes and seasonal exposure of As. Hence, the objectives of my study in Chapter 3 were to: (1) determine concentrations of As in the tissues, excreta, and rumen contents of elk residing in the upper Madison River basin (2) evaluate potential exposure pathways responsible for elevated levels of As in elk and (3) correlate As exposure to elk with frequency of habitation in high-As thermal locations.

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CHAPTER 2

PHOTOCHEMICAL OXIDATION OF AS(III) IN FERRIOXALATE SOLUTIONS

Introduction

The speciation of arsenic (As) in soils and natural waters is an important factor controlling the environmental fate and subsequent toxicology of this metalloid (1,2). The two common inorganic forms of As present in surface waters are arsenate (H_2AsO_4^- , HAsO_4^{2-}) and arsenite (H_3AsO_3^0), and transformation rates between these two valence states may be mediated by both chemical and microbiological processes (3). The reduction of As(V) can occur chemically by dissolved sulfide at low pH (4), and via microbial processes including dissimilatory reduction (5,6) and detoxification via As-induced *ars* genes (7). Likewise, the oxidation of As(III) can be due to microbiological (8,9) and chemical processes. Chemical species common in natural waters that may contribute to the oxidation of As(III) include $\text{Mn}^{\text{IV}}\text{O}_2(\text{s})$, Fe(III), and H_2O_2 (10-14). Of these, $\delta\text{-MnO}_2$ is capable of rapid rates of As(III) oxidation under conditions typical of natural water systems (e.g. pH values ranging from 4-9). Oxidation rates of As(III) by Fe(III) are only significant at low pH (<3) and at high concentrations of Fe(III) (13).

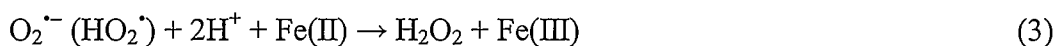
Although the production of H_2O_2 in natural water has been well documented (15-20), significant rates of As(III) oxidation via H_2O_2 require pH values greater than the pKa

for H_3AsO_3^0 (e.g. $\text{pH} > 9.3$), and high concentrations of H_2O_2 relative to As(III) (13,14). However, H_2O_2 is an important reactant involved in the production of free radical species (e.g. $\text{OH}^\bullet$, $\text{HO}_2^\bullet$), which have been reported to oxidize As(III) (21,22). Specifically, the oxidation of As(III) has been reported in low pH (<2.5) irradiated ferric perchlorate solutions (21), and attributed to the production of free radical species, $\text{OH}^\bullet$ and $\text{Cl}_2^{\bullet-}$. In Fe(III) -citrate solutions, $\text{O}_2^{\bullet-}$ was hypothesized to be the important free radical species responsible for the photochemical oxidation of As(III) (22). Although different mechanisms of As(III) oxidation were suggested in these studies, it is clear that free radical species such as $\text{O}_2^{\bullet-}$ and $\text{OH}^\bullet$ generated from photochemical reaction products, such as H_2O_2 , are responsible for As(III) oxidation in these systems.

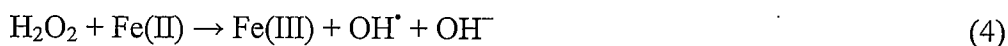
The photochemical formation of H_2O_2 in natural waters is thought to occur as a result of disproportionation of hydroperoxyl ($\text{HO}_2^\bullet$; $\text{pK}_a = 4.8$) and superoxide ($\text{O}_2^{\bullet-}$) radicals, formed from the capture of light energy by dissolved organic carbon (DOC) and subsequent reduction of $\text{O}_{2(\text{g})}$ (17,26,27):



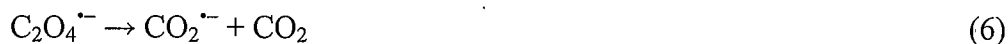
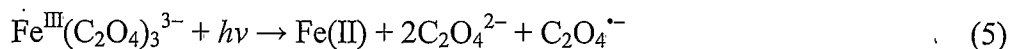
The absorption of light by appropriate chromophore(s) of DOC results in an excited state intermediate DOC^* , which transfers electrons to O_2 to form the superoxide radical $\text{O}_2^{\bullet-}$ followed by disproportionation of $\text{HO}_2^\bullet$ to form H_2O_2 (28). Alternatively, H_2O_2 may be produced via the reaction of superoxide with reduced metals such as Fe(II) :



Once H_2O_2 is formed, it can react further with Fe(II) (Fenton's Reaction) in the dark to yield the hydroxyl radical ($\text{OH}^\bullet$), a strong oxidant capable of oxidizing many organic compounds and other environmentally relevant species (23,29,30):



The formation of H_2O_2 and $\text{OH}^\bullet$ has also been studied extensively in irradiated ferrioxalate solutions (18,31-33). Not only is the ferrioxalate system a classic model for the study of photochemical formation of H_2O_2 and $\text{OH}^\bullet$ (33,34), oxalate is also a common natural and anthropogenic compound found in nearly all natural waters including soil pore waters, surface waters, and atmospheric water (18,35). Oxalate has a high affinity for ferric iron, and even at molar oxalate:Fe ratios as low as 1:1, the Fe(III) -oxalato complexes are the dominant solution species of Fe(III) at pH values < 7 . It is thought that photolysis of the tri-oxalato ferrioxalate species yields the free radical $\text{CO}_2^{\bullet-}$ via the spontaneous decarboxylation of the oxalyl radical anion, $\text{C}_2\text{O}_4^{\bullet-}$ (36):



The oxalyl radical has a short lifetime before decarboxylation to form $\text{CO}_2^{\bullet-}$, thus preventing its participation in other reactions (36). Under aerobic conditions, $\text{CO}_2^{\bullet-}$ reacts quickly with O_2 (24):



which, depending on pH, results in the formation of either the superoxide radical, $\text{O}_2^{\bullet-}$ or the hydroperoxyl radical, $\text{HO}_2^\bullet$ (Table 1.2). Once the superoxide radical is formed, the

formation of H_2O_2 and $\text{OH}^\bullet$ in the ferrioxalate system proceeds as described in eqs 2-4. Importantly, the exclusion of O_2 via bubbling with N_2 (g) has been shown to effectively curb the production of $\text{O}_2^{\bullet-}$ and the subsequent formation of H_2O_2 (18).

The photochemical production of $\text{OH}^\bullet$ free radicals in natural waters either via DOC or oxalate pathways may contribute to As(III) oxidation occurring in surface waters of lakes, oceans and rivers. Consequently, my objectives were to (1) evaluate oxidation rates of As(III) in irradiated ferrioxalate solutions as a function of pH, (2) identify probable mechanisms of photochemical As(III) oxidation, and (3) evaluate the oxidation of As(III) in a representative natural water containing dissolved organic C (DOC). Our results indicate that the photochemical oxidation of As(III) in ferrioxalate solutions can be rapid (half-lives ranging from 0.01 to 1 h), and that photochemical oxidation of As(III) may also be important in natural waters containing Fe(III) and DOC.

Materials and Methods

Reacting Solutions

Reaction mixtures (total volume = 0.2 or 0.4 L) were prepared under a red safelight in a 1.0 L glass vessel via the sequential addition of analytical grade KCl, FeCl_3 , $\text{K}_2\text{C}_2\text{O}_4$, and NaH_2AsO_3 stock solutions (fresh NaH_2AsO_3 stock solutions prepared every 3 days). All solutions were prepared in a background of 0.01 M KCl or KClO_4 and the concentration of initial oxalate in the reaction vessels was kept constant at 1 mM. With

the exception of experiments designed to determine the influence of Fe(III) concentration, experiments were conducted at 18.0 μM Fe(III). At these ratios of oxalate:Fe(III), over 99% of the total soluble Fe(III) existed as oxalate complexes, of which the tri-oxalato species was dominant (Figure 1.1; See [18] for log K values). The distribution of aqueous species was estimated with GEOCHEM (48), using equilibrium constants for soluble Fe(III)-complexes as presented by Zuo and Hoigne (18). In one set of experiments at pH 5.0, initial As(III) concentrations were varied from 1.3 μM to 13.5 mM As(III) to determine the rate dependence of As(III) photooxidation on initial As(III) concentration. The influence of pH on As(III) photooxidation was evaluated at pH values ranging from 3 to 7 at initial As(III) concentrations of 17.4 μM . In addition, one set of experiments was conducted at 133 μM As, but at variable Fe(III) concentrations ranging from 0.02 to 18 μM .

Experimental Apparatus and Irradiation Source

Ferrioxalate reaction mixtures were exposed to light emitted from a 250 W Quartz Tungsten Halogen lamp (QTH, Oriel Instruments). The effective photon flux of the lamp between wavelengths 300-500 nm was determined to be approximately 97 $\mu\text{E cm}^{-2} \text{ h}^{-1}$ using ferrioxalate actinometry (37). The temperature of all reaction mixtures was held constant at $25 \pm 2^\circ\text{C}$ with a circulating water bath connected to a jacketed reaction vessel. The pH of the reacting solutions was held constant during irradiation

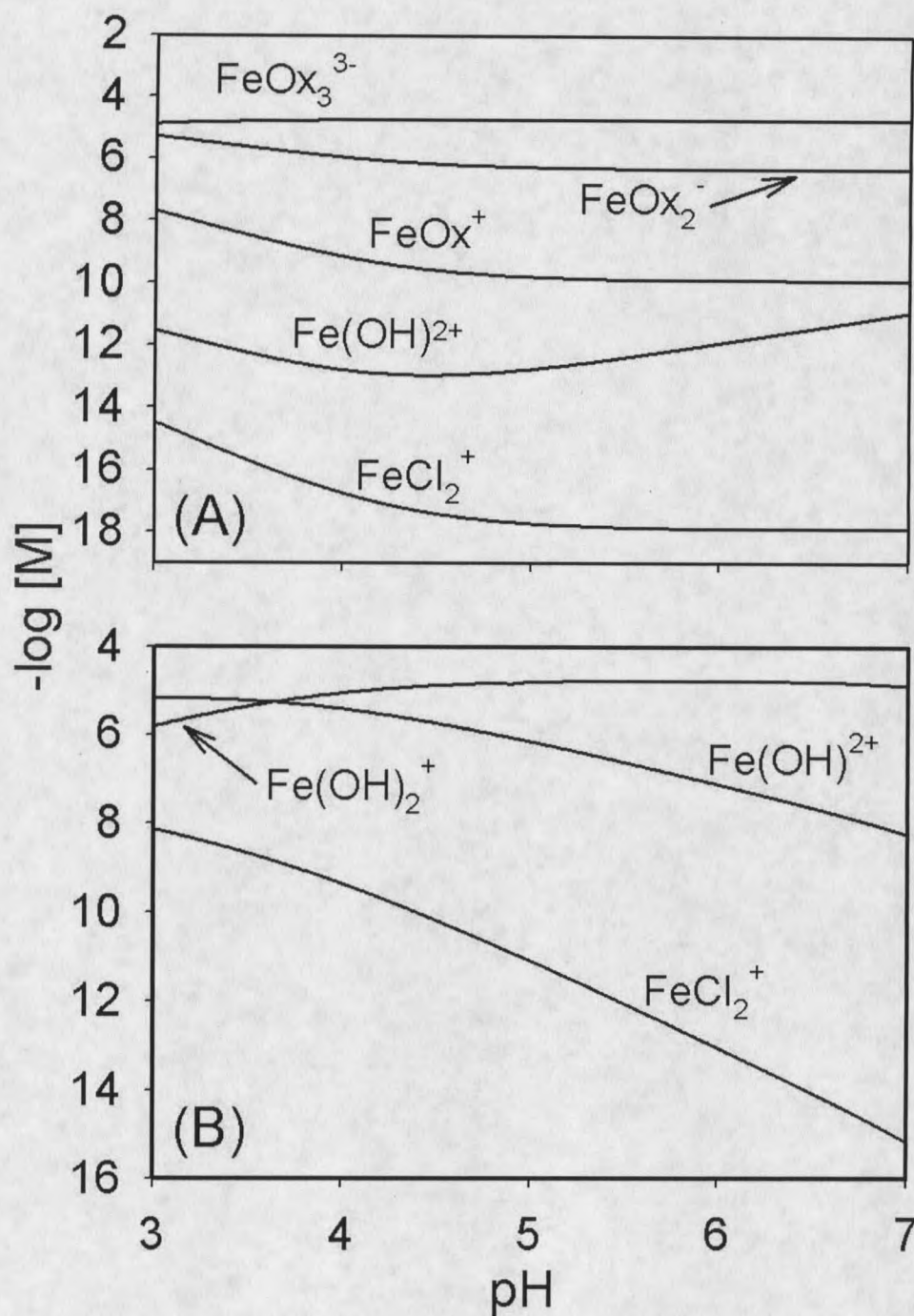


FIGURE 1.1. Calculated (GEOCHEM) distribution of aqueous Fe(III) species as a function of pH in the presence (A) and absence (B) of 1 mM oxalate (0.01 M KCl, 18 μM Fe(III), 18 μM As(III) and 10 mM KCl).

using an autotitrator (Radiometer, Copenhagen) operating in pH-stat mode while being constantly stirred with a Teflon bar and vigorously bubbled with compressed air.

Reaction mixtures were sampled as a function of time (generally for periods up to 30 min) and analyzed for total soluble As(III), As(V), Fe(II), Fe(III), and H_2O_2 (methods discussed below). The majority of irradiation experiments discussed in the current study were performed in triplicate.

Analytical Methods

Determination of As(V) was performed by adding 5 mL of reaction mixture to a 15 mL HDPE bottle containing 1 mL of 2.0 M TRIS buffer ((hydroxymethyl) aminomethane). While sparging the mixture with N_2 , 1 mL of 0.025 M NaOH and 1.59 M NaBH_4 was added in 0.5 mL increments over 7 min to reduce As(III) to arsine gas. The sample was then sparged for an additional 7 min to purge arsine. Concentrations of As(III) were determined by difference between As(ts) and As(V) (5,38) measured using continuous flow hydride generation atomic adsorption spectrometry (HG-AAS)(5). Samples were acidified with 3M HCl, pre-reduced with 1% potassium iodide (KI), and mixed with 0.6% NaBH_4 in 0.5% NaOH. Subsequent emission of arsine gas was quantified at 193.4 nm in a quartz cuvet immersed in an air-acetylene flame (Perkin Elmer model 3100 atomic adsorption spectrophotometer). The detection limit for As using this method was 3.4 nM. Concentrations of H_2O_2 were determined using the N,N-

diethyl-1,4-phenylenediamine method developed by Bader et al (39), and concentrations of Fe(II) and Fe(III) were determined using the o-phenanthroline method (40).

Effect of 2-Propanol on Initial As(III) Oxidation Rate

Reaction mixtures of 0.01 M KCl, 18 μM Fe(III), 1 mM oxalate, 13.5 mM 2-propanol, and varying amounts of As(III) (0-13.5 mM) were irradiated to determine the initial rate of $\text{OH}^\bullet$ formation (when $[\text{As(III)}] = 0$) and to determine the effect of an $\text{OH}^\bullet$ scavenger on initial As(III) oxidation rates. These reaction mixtures were sampled as a function of time for a total of 30 min, and analyzed for 2-propanone using gas chromatography (Varian Gas Chromatograph, Model 3400, Walnut Creek CA, operating under flame ionization mode). In the presence of excess 2-propanol relative to other potential $\text{OH}^\bullet$ scavengers, the production of 2-propanone can be used to estimate the formation rate of $\text{OH}^\bullet$ (33):

$$R_o (\text{OH}^\bullet \text{ formation}) = R_o (\text{2-propanone formation})/0.87 \quad (8)$$

where R_o is initial rate ($\mu\text{M h}^{-1}$) and 0.87 represents the fraction of 2-propanol molecules attacked by $\text{OH}^\bullet$ that ultimately result in the formation of 2-propanone (33). In experiments designed to determine the affect of an $\text{OH}^\bullet$ scavenger on initial As(III) oxidation, ferrioxalate solutions containing three concentrations of As(III) (0.135, 1.35, and 13.5 mM) were irradiated in the presence of 13.5 mM 2-propanol. Reaction mixtures were sampled as a function of time for a total of 30 min, after which samples were analyzed for 2-propanone and As(III)/As(V) as described above.

Oxidation of As(III): H₂O₂ and Fe(II) Dark Controls

Solutions of 2.66 μM As(III) and 20 mM H₂O₂ were prepared under a red safelight and sampled as a function of time for 10 min. An equal volume solution containing either 3.6, 7.2, 17.8 or 35.8 μM Fe(II) was then added, and the reaction mixture sampled as a function of time for an additional 20 min for a total of 30 min per experiment. Samples were analyzed for Fe(II)/Fe(III), As(III)/As(V), and H₂O₂ as described above. Both solutions were prepared at pH 2.7, had a background ionic strength of 0.01 mM KCl and were constantly bubbled with air. Care was taken not to exceed the amount of Fe(II) in solution which upon oxidation to Fe(III), would result in the precipitation of amorphous Fe(III) hydroxide. The solubility limit of Fe(III) was estimated using a $\log K = 3.54$ for the reaction $\text{Fe}(\text{OH})_3 (\text{s}) + \text{H}^+ = \text{Fe}^{3+} + 3\text{H}_2\text{O}$ (41). The maximum estimated solubility of Fe(III) at pH 2.7 was 70.5 μM , significantly greater than the concentration of Fe(II) used in our solutions. In one experiment, As(III) oxidation was measured in the presence of 13.5 mM 2-propanol to confirm the affect of an OH $\cdot$ scavenger on the oxidation of As(III).

Apparent Quantum Yield

Apparent quantum yields of As(V) and H₂O₂ were determined from the rates of formation of As(V) and H₂O₂. The apparent quantum yield (Φ_a) was defined as the number of moles of As(V) or H₂O₂ formed per mole of photon absorbed by the solution:

$$\Phi_a = \frac{d[X]/dt}{I_0' (1-10^{-Abs})/l} \quad (9)$$

where $d[X]/dt$ represents the rate of formation of As(V) or H_2O_2 ($\mu M h^{-1}$) at the wavelengths of irradiation, I_0' is the photon flux through the irradiated cell ($97 \mu E cm^{-2} h^{-1}$), $1-10^{-Abs}$ is the average fraction of light absorbed over wavelengths 300-500 nm, and l is 3.46 cm, the average path length of the jacketed reaction vessel.

Photochemical Oxidation of As(III) in a Natural Water

To determine whether photochemical oxidation of As(III) may occur under sunlight conditions in natural waters containing dissolved organic C (DOC), a water sample was collected from a pristine wetland (pH = 6.5) at an elevation of 2000 m in Hyalite Canyon located 25 km south of Bozeman MT. The natural water sample was filter-sterilized ($0.22 \mu M$) following collection, then refrigerated in an autoclaved vessel until use. The amount of DOC was determined using a DC-80 carbon analyzer (Tekmar-Dohrmann, Cincinnati, OH) and found to equal 0.86 mM C. Total and non-carbonate alkalinity were determined by titration with standardized 0.025 M HCl, using unpurged and purged ($N_{2(g)}$) samples. Since the Hyalite water sample was found to contain below detectable Fe(III) or Fe(II) ($<0.45 \mu M$), it was spiked to a concentration of $18 \mu M$ Fe(III) one hour prior to irradiation under natural sunlight at 12 p.m., August 22, 2000. Natural sunlight intensity was measured in the photosynthetically relevant wavelengths using a

quantum sensor (LiCOR, Model 190, Lincoln, NE) and total solar irradiance was measured with a solarimeter (Kipp and Zonen Model CM5, Delft, Netherlands). Experiments involving the Hyalite natural water sample were performed in duplicate, and concentrations of As(III), As(V) and H_2O_2 were determined as described previously.

Results and Discussion

Photochemical Oxidation of As(III)

At pH 3.0, the oxidation of $17.4 \mu\text{M}$ As(III) in irradiated ferrioxalate solutions was complete within 10 min (Figure 1.2A), corresponding to an initial oxidation rate of $255 \pm 16 \mu\text{M h}^{-1}$ (Table 1.1). The rate of As(III) oxidation in irradiated solutions decreased with increasing pH, falling to $14 \mu\text{M h}^{-1}$ at pH 7.0 (Figure 1.2A, Table 1.1). To verify that the measured oxidation of As(III) occurred as a direct result of photochemical processes resulting from the irradiation of Fe(III)-oxalate solutions, irradiated experiments were compared to appropriate dark controls (Figure 1.2B). The dark control containing identical concentrations of Fe(III), oxalate and As(III) showed no oxidation of As(III) (Figure 1.2B) during a 30 min incubation. Further insights regarding the mechanism of As(III) oxidation were obtained from controls in the presence of H_2O_2 or Fe(III) at pH 3.0 (Figure 1.2B). Insignificant oxidation of As(III) was observed in dark or irradiated controls containing $300 \mu\text{M}$ H_2O_2 . An additional control was performed at a

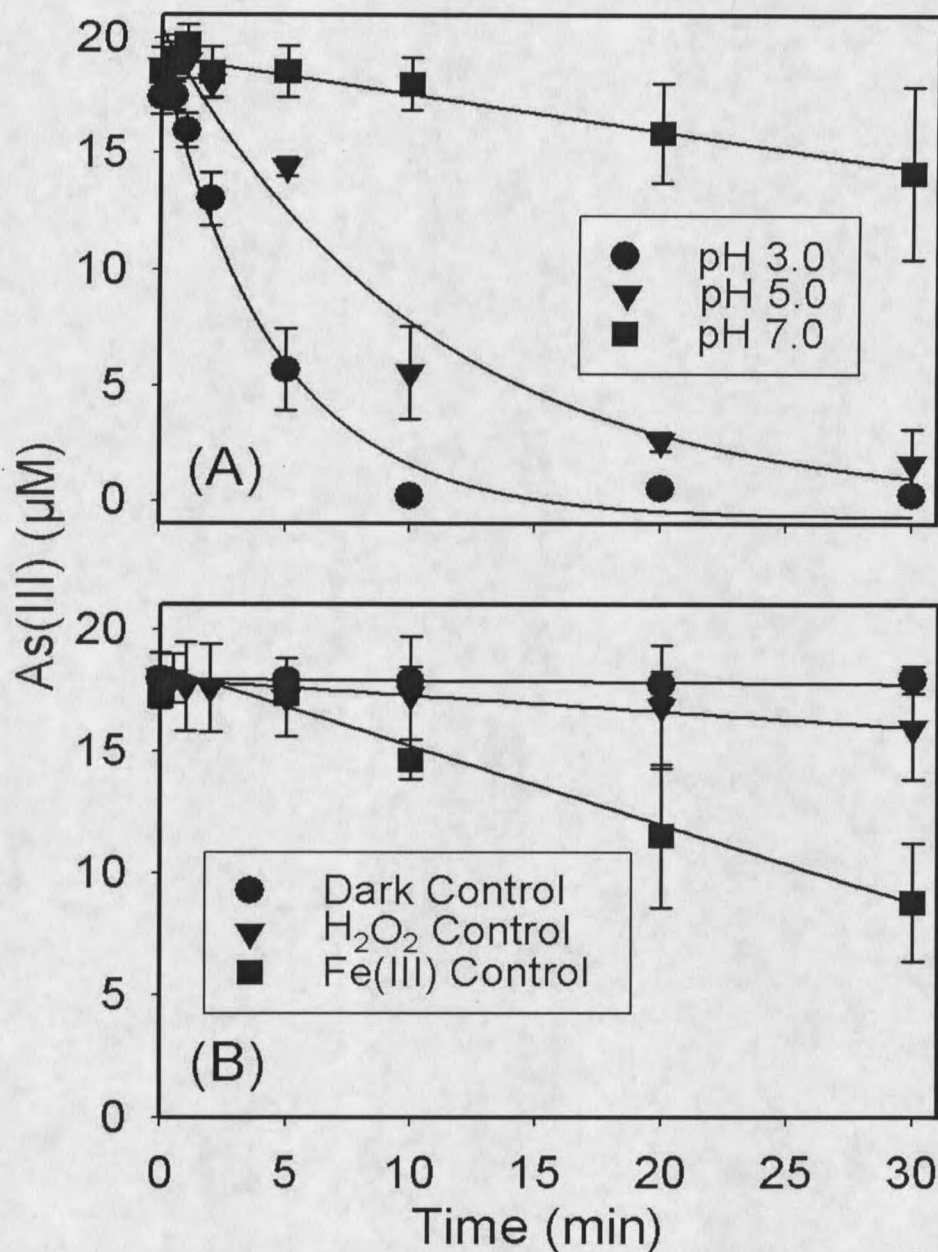


FIGURE 1.2. Disappearance of As(III) in (A) irradiated ferrioxalate solutions and in (B) dark or irradiated controls. Irradiated ferrioxalate solutions and the dark control contained $18\ \mu\text{M}$ As(III), $18\ \mu\text{M}$ Fe(III), and $1\ \text{mM}$ oxalate. The H_2O_2 control contained $18\ \mu\text{M}$ As(III) and $300\ \mu\text{M}$ H_2O_2 , while the Fe(III) control contained $18\ \mu\text{M}$ As(III) and $18\ \mu\text{M}$ Fe(III). Background ionic strength of all solutions = $0.01\ \text{M}$ KCl.

TABLE 1.1. Appearance/Disappearance Rates and Quantum Yields of Constituents in Irradiated Solutions

Varying Condition	Constant Condition ^a	-d[As(III)]/ dt	d[C ₃ H ₆ O]/ dt ^b	d[H ₂ O ₂]/ dt	-d[H ⁺]/ dt	Φ As(V) ^d	Φ H ₂ O ₂	Φ C ₃ H ₆ O (b)
μM hr ⁻¹								
pH								
7	17.4 μM	14 (4) ^c		15 (10)	0 (0)	0.009	0.01	
6	As(III)	91		193	902	0.06	0.12	
5		118 (21)		259 (13)	630 (261)	0.07	0.16	
4	18 μM	157		794	1279	0.1	0.49	
3	Fe(III)	255 (16)		861 (37)	1236 (60)	0.16	0.53	
[As(III)]								
1.3	pH 5.0	12 (2)		229 (17)	542 (59)	0.007	0.14	
9.3	18 μM	66 (4)				0.04		
17.4	Fe(III)	118 (21)		259 (15)	630 (261)	0.07	0.16	
135		174 (2)		261 (16)	369 (42)	0.11	0.16	
1350		157 (6)		^e	892 (30)	0.10		
13500		218 (22)		^e	833 (63)	0.13		
KClO ₄								
	pH 5.0							
	18 μM	127 (12)		237 (17)	761 (62)	0.08	0.15	
	Fe(III)							
	17.4 μM As(III)							
[Fe(III)]								
0.02	pH 5.0	0.5 (0.2)		2.6 (0.5)	0 (0)	0.0003	0.002	
0.1	135 μM	3.3 (0.2)		3.3 (0.2)	0 (0)	0.002	0.002	
0.2	As(III)	4.9 (1.2)		2.8 (0.5)	0 (0)	0.003	0.002	
1.8		156 (11)		48 (16)	102 (37)	0.1	0.03	
[As(III)]								
0	pH 5.0		147 (10)	170 (6)	1123 (44)		0.11	0.09
135	18 μM	16 (5)	74 (5)	260 (20)	1052 (19)	0.01	0.16	0.05
1350	Fe(III)	61 (10)	63 (9)	^e	948 (35)	0.04		0.04
13500	13.5 mM 2-propanol	196 (15)	47 (8)	^e	953 (65)	0.12		0.03
Hyalite								
Sunlight	pH 5.0	5.6 (0.3)		bd ^f	24 (7)	2e ⁻⁶		
QTH lamp	18 μM	3.7 (0.2)		bd ^f	0 (0)	3e ⁻⁶		
	Fe(III)							

^a All solutions were prepared in 0.01 M KCl with the exception of experiments where 0.01 M KClO₄ was used. All solutions contained 1 mM oxalate, with the exception of the Hyalite natural water samples. ^b C₃H₆O = 2-propanone. ^c Values in parentheses are standard errors of triplicate analysis. ^d Φ = apparent quantum yield. ^e concentrations > 1350 μM As(III) interfere with H₂O₂ measurement. ^f bd = below detection.

higher $\text{H}_2\text{O}_2\text{:As(III)}$ ratio of 10 mM H_2O_2 and 1.3 μM As(III) and no oxidation was observed within 10 min. The H_2O_2 controls indicated that direct oxidation of As(III) via H_2O_2 was insignificant under the current solution conditions. This is important because oxidation rates of As(III) via H_2O_2 can be significant at pH values greater than 8.0, and at high ratios of $\text{H}_2\text{O}_2\text{:As(III)}$ (13).

The irradiated control experiment conducted at pH 3.0 containing only Fe(III) and As(III) yielded an As(III) oxidation rate of approximately 13 $\mu\text{M h}^{-1}$ (Figure 1.2B), roughly 5% of the As(III) oxidation rate observed in the presence of Fe(III) and oxalate (Table 1.1). The oxidation of As(III) in the absence of oxalate may be due to the production of $\text{OH}^\bullet$ from photoreduction of Fe(III):



where $\text{Fe}(\text{OH})^{2+}$ is an important photo-reactive species in acidic solutions (Figure 1.1) (42). Although photochemical reactions with $\text{Fe}(\text{OH})^{2+}$ may represent a significant contribution to As(III) oxidation at low pH, the rate of As(III) oxidation was considerably faster (20 times) in the presence of ferrioxalate complexes. Emmett and Khoe (20) suggested that $\text{Cl}_2^{\bullet-}$ may be a potential oxidant of As(III) in Fe(III) and Cl^- containing solutions. However, the concentration of FeCl_2^+ was 2-3 orders of magnitude lower than $\text{Fe}(\text{OH})_2^+$ under our conditions (Figure 1.1). Furthermore, the rate of As(III) oxidation was identical in experiments where KClO_4 was substituted as a background electrolyte (Table 1.1), ruling out $\text{Cl}_2^{\bullet-}$ as an important oxidant of As(III).

As(III) Oxidation Rate Dependence on pH

The rate of photochemical As(III) oxidation in the ferrioxalate system was highly pH dependent (Figure 1.3A) with initial oxidation rates ranging from $255 \pm 16 \mu\text{M h}^{-1}$ at pH 3.0, to $14 \mu\text{M h}^{-1}$ at pH 7.0. The measured production rate of H_2O_2 also declined with increasing pH (Table 1.1), which is consistent with results from previous work in ferrioxalate systems (18). This pH dependence reflects the mechanism of H_2O_2 formation via $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$, where at pH values below 4.8 (pK_a for $\text{HO}_2^\bullet$), $\text{HO}_2^\bullet$ is the dominant species favoring the formation of H_2O_2 via both eqs 2 and 3. At higher pH, $\text{O}_2^{\bullet-}$ becomes the dominant species, which is thought to react more quickly with Fe(III) to form O_2 as opposed to reacting with Fe(II) to form H_2O_2 (Table 1.2). Although H_2O_2 is not the species directly responsible for the oxidation of As(III), the formation rate of free radical $\text{OH}^\bullet$ is proportional to the formation rate of H_2O_2 (eq 4).

Oxidation Rate Dependence on Initial As(III) and Fe(III)

The rate of As(III) oxidation was evaluated as a function of initial As(III) concentration under constant solution conditions [$\text{Fe(III)} = 18 \mu\text{M}$, $\text{pH} = 5.0$, oxalate = 1 mM], and found to be approximately first-order with respect to As(III) from 1.3 to 17.4 μM initial As(III) (Figure 1.3B). Under the reaction conditions employed in this study, the oxidation rate plateaued at concentrations of $\text{As(III)} \geq 135 \mu\text{M}$. Under constant solution conditions resulting in constant H_2O_2 production rates (Table 1.1), the psuedo

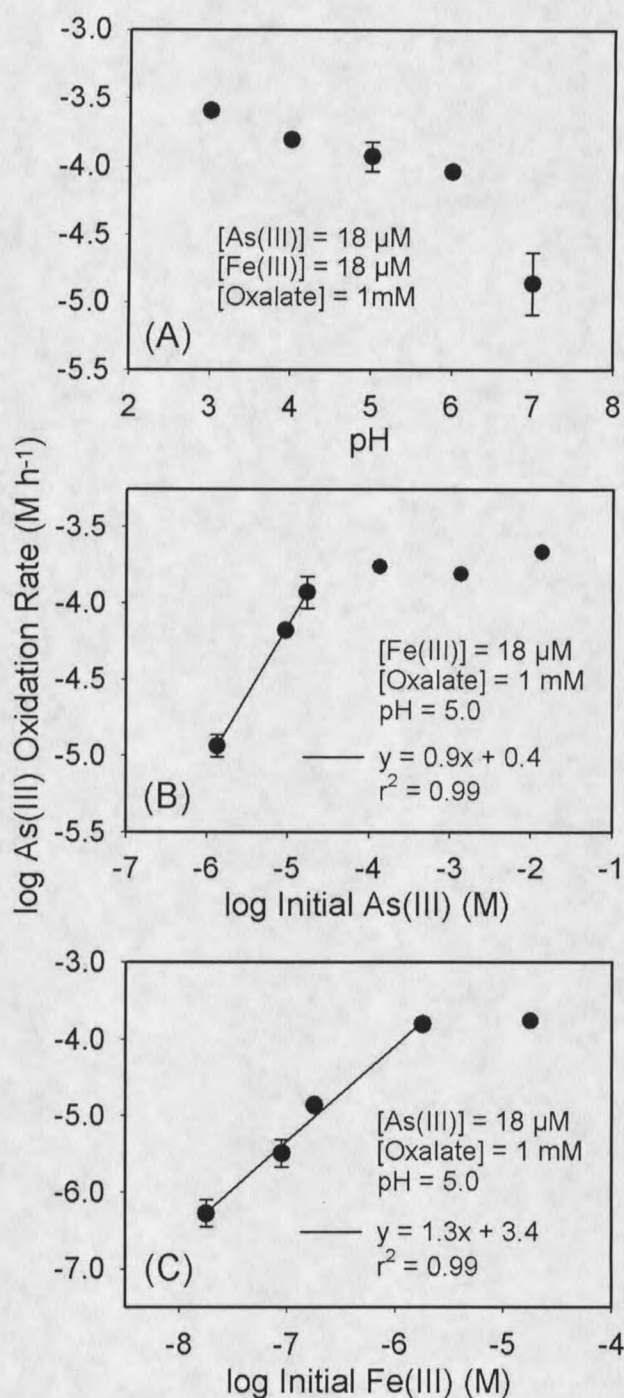


FIGURE 1.3. Log initial rates of As(III) oxidation (M hr^{-1}) as a function of (A) pH (B) initial As(III) and (C) initial Fe(III). Background ionic strength of all solutions = 0.01 M KCl.

TABLE 1.2. Compilation of Reactions and Corresponding Rate Constants Concerning Production and/or Consumption of Free Radical Species in Fe-As-H₂O Systems

	eq #	k (L mol ⁻¹ sec ⁻¹)	Ref.
As(III) Oxidations			
$\text{H}_3\text{As}^{\text{III}}\text{O}_3 + \text{OH}^\bullet \rightarrow \text{As}^{\text{IV}}(\text{OH})_4$	(13)	8.5×10^9	(43)
$\text{H}_3\text{As}^{\text{III}}\text{O}_3 + \text{O}_2^{\bullet-} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{As}^{\text{IV}}(\text{OH})_4 + \text{H}_2\text{O}_2$		3×10^6	(22)
$\text{H}_3\text{As}^{\text{III}}\text{O}_3 + \text{Cl}_2^{\bullet-} + \text{H}_2\text{O} \rightarrow \text{As}^{\text{IV}}(\text{OH})_4 + 2\text{Cl}^- + \text{H}^+$		Unknown	(21)
$\text{H}_3\text{As}^{\text{III}}\text{O}_3 + \text{Fe species} \rightarrow ? \rightarrow \text{H}_2\text{As}^{\text{V}}\text{O}_4^-$		Unknown	(44,45)
As(IV) Oxidations			
$\text{As}^{\text{IV}}(\text{OH})_4 (+\text{H}^+) + \text{O}_2 \rightarrow \text{H}_2\text{As}^{\text{V}}\text{O}_4^- + (\text{HO}_2^\bullet)/\text{O}_2^{\bullet-} + 2\text{H}^+$		1.1×10^9	(43)
$\text{As}^{\text{IV}}(\text{OH})_4 + \text{O}_2 \rightarrow \text{As}^{\text{IV}}(\text{OH})_4\text{-O}_2$		$\sim 1 \times 10^9$	(43)
$\text{As}^{\text{IV}}(\text{OH})_4\text{-O}_2 \rightarrow \text{H}_2\text{As}^{\text{V}}\text{O}_4^- + (\text{HO}_2^\bullet)/\text{O}_2^{\bullet-} + 2\text{H}^+$		$\sim 1 \times 10^{10}$	(43)
2-Propanol Oxidation and Follow-up			
$\text{OH}^\bullet + \text{HROH} \rightarrow \text{ROH}^\bullet + \text{H}_2\text{O}$		1.9×10^9	(23)
$\text{ROH}^\bullet + \text{O}_2 \rightarrow \text{RO} + \text{HO}_2^\bullet$		4.1×10^9 ^a	(24)
Ferrioxalate Photolysis and Follow-up Reactions			
$\text{Fe}^{\text{III}}(\text{C}_2\text{O}_4)_3^{3-} + h\nu \rightarrow \text{Fe(II)} + 2\text{C}_2\text{O}_4^{2-} + \text{C}_2\text{O}_4^{\bullet-}$	(5)	$\Phi_{\text{Fe(III)}}N_a/V$ (M s ⁻¹) ^b	(33)
$\text{C}_2\text{O}_4^{\bullet-} \rightarrow \text{CO}_2^{\bullet-} + \text{CO}_2$	(6)	2×10^6	(36)
$\text{CO}_2^{\bullet-} + \text{O}_2 \rightarrow \text{O}_2^{\bullet-} + \text{CO}_2$	(7)	4.2×10^9	(24)
$\text{O}_2^{\bullet-}/\text{HO}_2^\bullet$ Reactions			
$\text{HO}_2^\bullet \leftrightarrow \text{O}_2^{\bullet-} + \text{H}^+$		$\text{pK}_a = 4.8$	(28)
$2\text{HO}_2^\bullet \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	(2)	8.3×10^5	(28)
$\text{HO}_2^\bullet + \text{H}^+ + \text{Fe(II)} \rightarrow \text{H}_2\text{O}_2 + \text{Fe(III)}$	(3)	1.2×10^6	(25)
$\text{HO}_2^\bullet + \text{Fe(III)} \rightarrow \text{O}_2 + \text{Fe(II)} + \text{H}^+$		3.6×10^5	(28)
$\text{HO}_2^\bullet + \text{O}_2^{\bullet-} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 + \text{OH}^-$	(16)	9.7×10^7	(28)
$\text{O}_2^{\bullet-} + 2\text{H}^+ + \text{Fe(II)} \rightarrow \text{Fe(III)} + \text{H}_2\text{O}_2$	(3)	1×10^7	(25)
$\text{O}_2^{\bullet-} + \text{Fe(III)} \rightarrow \text{Fe(II)} + \text{O}_2$		1.5×10^8	(25)
$\text{H}_2\text{O}_2 + \text{Fe(III)} \rightarrow \text{O}_2^{\bullet-} + \text{Fe(II)} + 2\text{H}^+$		2.6×10^{-3} pH 5	(46)
•OH Reactions			
$\text{OH}^\bullet + \text{OH}^\bullet \rightarrow \text{H}_2\text{O}_2$		5.2×10^9	(23)
$\text{OH}^\bullet + \text{O}_2^{\bullet-}/\text{HO}_2^\bullet \rightarrow \text{H}_2\text{O} + \text{O}_2$		6.6×10^9 pH 0.5-6.75	(23)
$\text{OH}^\bullet + \text{H}_2\text{O}_2 \rightarrow \text{O}_2^{\bullet-}/\text{HO}_2^\bullet + \text{H}_2\text{O}$		3.3×10^7 pH 3-5	(46)
$\text{OH}^\bullet + \text{Fe(II)} \rightarrow \text{Fe(III)} + \text{OH}^-$		3.2×10^8 pH 3-5	(46)
Fenton Reaction and Fe(OH)^{2+} Photolysis			
$\text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^\bullet + \text{OH}^-$	(4)	63, 5.7×10^2 pH 3,5	(47,46)
$\text{Fe}^{\text{III}}(\text{OH})^{2+} + h\nu \rightarrow \text{Fe(II)} + \text{OH}^\bullet$	(10)	6.3×10^{-4}	(42)

^a Average of three values given in ref 24 ^b value based on photochemical transformation of ferrioxalate. $\Phi_{\text{Fe(III)}}$ = quantum yield of Fe(III) generation (typical value ~ 1.2), N_a = absorbed photon flux (einstein sec⁻¹) and V = volume irradiated (L)

first-order rate expression describing the oxidation rate dependence on initial As(III) can be written:

$$-d[\text{As(III)}]/dt = k_{\text{As-obs}}[\text{As(III)}]^x \quad (11)$$

where in our studies the fitted values of x and $k_{\text{As-obs}}$ were 0.90 and 2.31 h^{-1} , respectively. The apparent rate constant ($k_{\text{As-obs}}$) is a lumped parameter containing information related to the initial reaction conditions, such as light intensity and initial concentrations of Fe(III), oxalate, and $\text{O}_2(\text{g})$.

The dependence of As(III) oxidation rate on the initial concentration of Fe(III) was tested under constant initial solution conditions of $\text{pH} = 5.0$, $\text{As(III)} = 134 \text{ }\mu\text{M}$, and 1 mM oxalate (Figure 1.3C). The rate of As(III) oxidation increased with increasing concentrations of initial Fe(III) from 0.02 to 18 μM , however, the initial rate plot ($\log R_o$ vs. $\log [\text{Fe(III)}]_o$) was linear only over the range from 0.02 to 1.8 μM Fe(III) ($\log [\text{Fe(III)}]_o = -7.75$ to -5.75). Under constant solution and irradiation conditions, this relationship can also be described using a pseudo-first order rate expression:

$$-d[\text{As(III)}]/dt = k_{\text{Fe-obs}}[\text{Fe(III)}]^z \quad (12)$$

where $k_{\text{Fe-obs}} = 1475 \text{ h}^{-1}$ and $z = 1.24$ over the range of initial Fe(III) concentrations from 0.02 to 1.8 μM . Again, the apparent rate constant, $k_{\text{Fe-obs}}$ is a lumped rate parameter dependent on the initial reaction conditions of the irradiated ferrioxalate solutions. The influence of initial Fe(III) on the oxidation rate of As(III) during irradiation of ferrioxalate solutions is consistent with previous work showing that higher concentrations of Fe(III) result in increasing photolysis rates of oxalate with corresponding increases in H_2O_2 production (18).

Quantum Yields

Photochemical oxidation of As(III) in ferrioxalate solutions was more efficient compared to photochemical oxidation in the natural DOC sample, reflecting the higher rate of H_2O_2 production and subsequent higher generation rate of oxidative free radical species in irradiated ferrioxalate solutions and (Table 1.2). At pH 5.0, the maximum values of $\Phi_{\text{As(V)}}$ achieved in ferrioxalate solutions at high As(III) concentrations ($> 135 \mu\text{M}$) were 0.11-0.13, corresponding to an approximate ratio of 1 mole As(V) formed per 8 moles of photons absorbed. Although the $\Phi_{\text{As(V)}}$ in the irradiated natural water sample was considerably lower (2×10^{-6}), the rate of As(III) oxidation (Table 1.1, $5.6 \mu\text{M h}^{-1}$) in this sample suggests that As(III) photochemical oxidation may be significant in natural water samples.

Apparent quantum yields for 2-propanone were less than those found by Hislop and Bolton (12), who reported $\Phi_{2\text{-propanone}}$ values greater than unity in the ferrioxalate system. These differences are likely due to the fact that Hislop and Bolton (12) used over two orders of magnitude greater Fe(III), four times more oxalate, and 10 mM initial H_2O_2 in their system. However, $\Phi_{\text{H}_2\text{O}_2}$ was higher in the ferrioxalate system than in natural waters. Values of $\Phi_{\text{H}_2\text{O}_2}$ ranging from 0.09×10^{-4} to 17×10^{-4} have been reported (24) in irradiated natural water samples, compared to values observed in this study ranging from 1.6×10^{-3} at $0.02 \mu\text{M}$ Fe(III) and pH 5.0, to 0.53 at $18 \mu\text{M}$ Fe(III) and pH 3.0 (Table 1.1).

Mechanism of Photochemical Oxidation of As(III)

Although H_2O_2 is not the important species responsible for the rapid oxidation of As(III) observed in the current study, H_2O_2 is a necessary reactant for the formation of the strong oxidant, $\text{OH}^\bullet$. Hislop and Bolton (33) used 2-propanol as a $\text{OH}^\bullet$ scavenger and measured the subsequent formation of 2-propanone to study the reactions responsible for the formation of $\text{OH}^\bullet$ in irradiated ferrioxalate solutions. Likewise, we conducted a series of experiments in the absence and presence of excess 2-propanol (13.5 mM) where As(III) ranged from 0-13.5 mM. In irradiated ferrioxalate solutions containing no As(III) and 13.5 mM 2-propanol (pH 5.0), the production rate of 2-propanone was found to be $147 \pm 10 \mu\text{M h}^{-1}$ (Table 1.1), corresponding to an $\text{OH}^\bullet$ production rate of $169 \mu\text{M h}^{-1}$ (Eq. 9). Under identical solution conditions containing 13.5 mM As(III) and 0 mM 2-propanol, the maximum rate of As(III) oxidation was $218 \pm 22 \mu\text{M h}^{-1}$ (Table 1.1). These results suggest an approximate 1:1 mole ratio of $\text{OH}^\bullet$ produced to As(III) oxidized. To further examine the role of $\text{OH}^\bullet$, rates of 2-propanone formation and As(III) oxidation were quantified in competition experiments containing both As(III) and 2-propanol as $\text{OH}^\bullet$ scavengers. At ratios of 2-propanol:As(III) > 10 , the rate of As(III) oxidation decreased compared to the maximum rate observed in the absence of 2-propanol ($218 \mu\text{M h}^{-1}$, Figure 1.4). However, at equimolar $[\text{As(III)}]$ and $[\text{2-propanol}] = 13.5 \text{ mM}$, the rates of As(III) and 2-propanol oxidation were $196 \mu\text{M h}^{-1}$ and $47 \mu\text{M h}^{-1}$, respectively, yielding a rate ratio of 4.2 (Table 1.2). The rate constants for the oxidation of As(III) and 2-propanol by $\text{OH}^\bullet$ have been estimated to be 8.5×10^9 and $1.9 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ (Table

1.2), respectively, yielding a ratio of rate constants of 4.5. These data show clearly that As(III) and 2-propanol compete for $\text{OH}^\bullet$ and that the relative rates of As(III) versus 2-propanol oxidation are consistent with the values of reported rate constants for these two species. Hug et al (22) recently suggested that $\text{OH}^\bullet$ was not the dominant oxidant of As(III) in Fe(III)-citrate solutions at pH 7; however, our results suggest that $\text{OH}^\bullet$ is the dominant oxidant of As(III) in the ferrioxalate system at pH 5.0.

To further verify the importance of $\text{OH}^\bullet$ in the oxidation of As(III), experiments were conducted in the dark containing only Fe(II) and H_2O_2 (Fenton's Reaction) as an

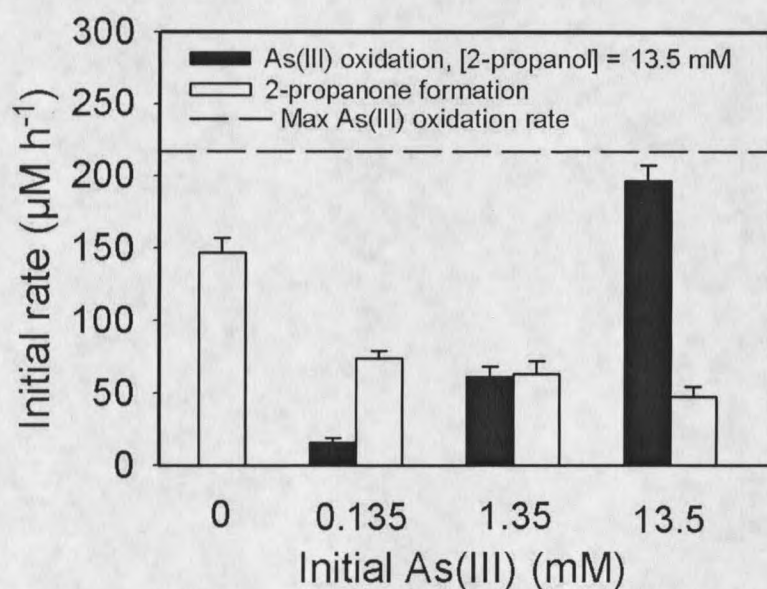


FIGURE 1.4. Oxidation rate of As(III) and formation rate of 2-propanone ($\mu\text{M hr}^{-1}$) as a function of initial [As(III)] (initial conditions: 1 mM oxalate, 18 μM Fe(III), 0.01 M KCl) in the presence of 13.5 mM 2-propanol. Dashed line represents the maximum As(III) oxidation rate observed in the absence of 2-propanol.

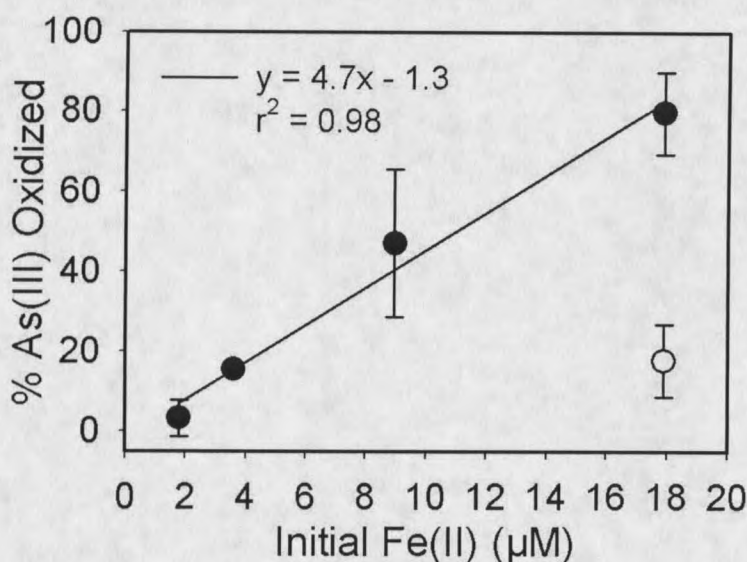


FIGURE 1.5. Percent of As(III) oxidized (●) as a function of Fe(II) in the presence of 1.35 μM initial As(III) and 10 mM initial H_2O_2 in the dark (Fenton's Reaction). Oxidation of As(III) in the presence of 13.5 mM 2-propanol is shown (○) at $\text{Fe(II)} = 18 \mu\text{M}$.

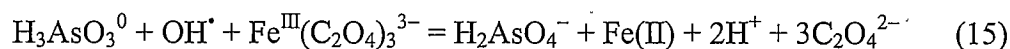
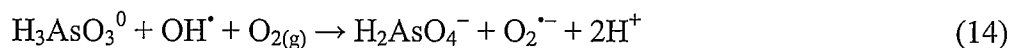
alternative method of generating reactive $\text{OH}^\bullet$ species. When solutions containing variable concentrations of Fe(II) (2-18 μM) were added to an equal volume of solution containing 2.7 μM As(III) and 20 mM H_2O_2 , maximum As(III) oxidation rates were achieved within 2 min (Figure 1.5) (no oxidation of As(III) by H_2O_2 occurred in the 10 min prior to mixing). However, the oxidation of As(III) was inhibited in the presence of 13.5 mM 2-propanol, yielding approximately 10% oxidation of As(III) compared to 80% in the absence of 2-propanol (Figure 1.4). These data suggest that although $\text{OH}^\bullet$ is responsible for the majority of As(III) oxidation during the Fenton Reaction, other

oxidants hypothesized to form via both the thermal and photo-Fenton reaction (44,45) may oxidize As(III) as well.

As mentioned above, oxidation of As(III) in irradiated ferrioxalate solutions and in dark reactions containing Fe(II)/H₂O₂ likely occurs as a result of attack by the OH[•] free radical, which is generated by the reduction of H₂O₂ with Fe(II) (Eq. 4). Klaning (43) proposed the following elementary reaction mechanism describing the oxidation of As(III) by OH[•]:

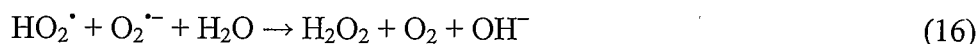


where the product, As(IV), is rapidly oxidized by a secondary oxidant such as O₂ to yield As(V) (Table 1.2). The summary reaction describing the oxidation of one mole of As(III) to As(V) (2 electron transfer) at pH 5.0 by one mole of OH[•] and an additional oxidant, such as O₂ or Fe(III) can be described as:



It is important to note that the As(III) oxidation steps proposed in reactions 14 and 15 generate H⁺. This is in apparent conflict with the pH dependence discussed in Figure 1.1, where the oxidation of As(III) clearly increases with increasing concentration of H⁺. Furthermore, the observed H⁺ consumption rates necessary to maintain constant pH during irradiation experiments show that the overall irradiation process consumes H⁺ (Table 1.1). The primary proton consuming reactions in the ferrioxalate system relate directly to the formation of H₂O₂ via reactions 2 and 3, and to the formation of OH[•] via reaction 4.

The empirical data on proton consumption at pH 3.0 in irradiated experiments suggest that 1.4 moles of H^+ were consumed per mole of H_2O_2 produced (see experiment at $[\text{As(III)}] = 17.4 \text{ uM}$, Table 1.1). This ratio increases to 2.4 moles of H^+ consumed per mole of H_2O_2 produced when the pH increases to 5.0 (near the pK_a of $\text{HO}_2^\cdot$) as a result of the following reaction:



where the production of OH^- increases the moles of H^+ consumed per mole H_2O_2 generated. However, at a constant pH = 5.0, the ratio of H^+ consumed per mole of H_2O_2 produced drops from 2.4 to approximately 1.4 at higher concentrations of initial As(III), consistent with the additional H^+ generated via reactions 14 and 15. In summary, the net effect of irradiating ferrioxalate solutions results in H^+ consumption; clearly the production of H_2O_2 and subsequent formation of free radical $\text{OH}^\cdot$ increases with decreasing pH. Under the solution conditions employed here, this pH dependence is responsible for higher As(III) oxidation rates at lower pH despite the fact that the suggested oxidation steps of As(III) via reactions 14 and 15 are proton generating.

As(III) Oxidation in the Presence of DOC

The rate of photo-induced oxidation of As(III) was significant in the Hyalite natural water sample containing DOC (Figure 1.6). At pH 5.0 and 18 uM Fe(III), rates of As(III) oxidation were 3.7 uM h^{-1} under the QTH light source and 5.6 uM h^{-1} under natural sunlight.

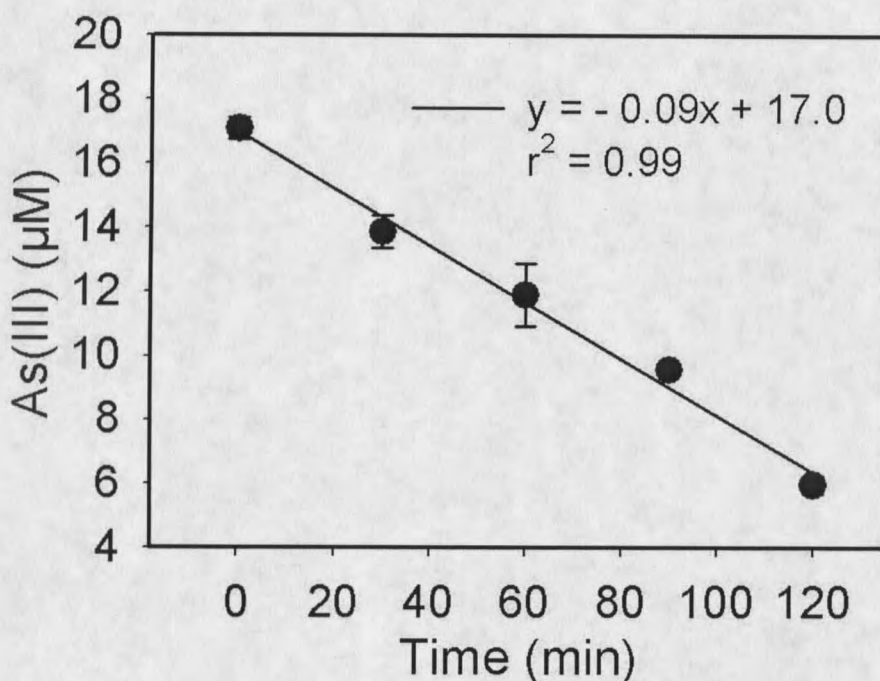


FIGURE 1.6. Disappearance of As(III) in the presence of 0.86 mM dissolved organic C (Hyalite natural water) upon exposure to sunlight (photosynthetically active radiation = $1538 \pm 22.6 \mu\text{E m}^{-2} \text{sec}^{-1}$ and total solar irradiance = $787.4 \pm 11.0 \text{ W m}^{-2}$). Initial conditions: 18 μM As(III), 18 μM Fe(III), pH 5.0

The amount of photosynthetically relevant and total solar radiation measured at the surface of the reaction solution under sunlight was $1538 \pm 22.6 \mu\text{E m}^{-2} \text{s}^{-1}$ and $787.4 \pm 11.0 \text{ W m}^{-2}$, respectively.

Although it has been hypothesized that DOC acts as a sink for $\text{OH}^\bullet$ (50), it has also been suggested that $\text{O}_2^{\bullet-}$ is produced directly from DOC upon irradiation (eq 1). This reaction then leads to the production of H_2O_2 and $\text{OH}^\bullet$ from $\text{O}_2^{\bullet-}$ through aforementioned mechanisms. Characterization of the Hyalite water sample revealed a noncarbonate

alkalinity of 0.09 mM. This translates to 1 mole charge:10 moles C, and is consistent with the range expected for aquatic DOC (51). The ratio of moles charge to moles Fe was 5:1 in our experiment and would have resulted in significant complexation of Fe(III) with organic functional groups (phenolic and carboxylic).

Environmental Implications

The photochemical oxidation of As(III) to As(V) has been shown to occur at significant rates in both irradiated ferrioxalate solutions and in a natural water sample containing low levels of DOC. The mechanism of As(III) oxidation in irradiated ferrioxalate solutions ranging from pH 3 to 7 appears to be due primarily to the generation of $\text{OH}^\bullet$ radicals from the reaction of H_2O_2 and Fe(II). In ferrioxalate solutions, Fe(II) and H_2O_2 are initial reaction products formed from the photochemical decomposition of ferrioxalate, which react further via the Fenton reaction to yield $\text{OH}^\bullet$. Oxidation of As(III) and inhibition by excess 2-propanol ($\text{OH}^\bullet$ scavenger) were observed in the dark Fenton reaction where $\text{OH}^\bullet$ is generated upon addition of Fe(II) and H_2O_2 without irradiation. In the natural water sample, addition of Fe(III) to native DOC resulted in lower, yet significant rates of As(III) oxidation upon irradiation. In addition to clarifying the role of $\text{OH}^\bullet$ in the oxidation of As(III), the results presented here suggest that the photochemical oxidation of As(III) may be extremely important in surface waters containing dissolved Fe(III) and DOC.

The rates of As(III) oxidation observed in the presence of irradiated ferrioxalate solutions or natural DOC are comparable to rapid rates of As(III) oxidation measured for

other oxidative pathways, including inorganic electron transfer reactions and microbially mediated processes (Table 1.3). For example, apparent half-lives describing photochemical oxidation of As(III) in irradiated ferrioxalate solutions and in natural water containing DOC fall within the same range observed for oxidation of As(III) via microorganisms using pure cultures under log growth conditions and in suspensions containing $\text{MnO}_2(\text{s})$. Further, the photochemical oxidation rates observed in the current study are roughly three to four orders of magnitude faster than oxidation rates attainable in the presence of O_2 or H_2O_2 . Although the oxidation of As(III) by H_2O_2 has been shown to be significant at pH values $> 8-9$ (13), rates at $\text{pH} < 7$ are likely too slow to be of significance in natural water systems. Given the ubiquity of Fe(III) and DOC in surface waters, we expect that the photochemical oxidation of As(III) represents an additional pathway responsible for As(III) oxidation in natural water systems, following mechanisms similar to those described here for irradiated ferrioxalate solutions. Future work should emphasize the potential role of photochemical processes on As(III) oxidation-reduction reactions important to As cycling in natural waters.

TABLE 1.3. Comparison of Apparent Half-Lives for the Oxidation of As(III) via Abiotic and Biotic Pathways

Oxidant/Process	Half-life (h)	Ref.
Irradiated ferrioxalate solution (pH 5.0, 18 uM Fe(III), 17.4 uMAs(III))	0.15	(this study)
Irradiated Hyalite sample	1.6	(this study)
O ₂	8640	(50)
Fe(III) (pH 5.0)	227	(12)
H ₂ O ₂ (pH 7.5)	38.5	(13)
Synthetic δ -MnO ₂	0.15-0.35	(11)
O ₃	0.07	(52)
TiO ₂ (Suspended) + $h\nu$	0.04	(53)
Microbial oxidation	0.01, 0.3	(8,9)

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CHAPTER 3

ELK EXPOSURE TO ARSENIC IN YELLOWSTONE'S GEOTHERMAL ENVIRONMENTS

Introduction

Arsenic (As) is a toxic and ubiquitous metalloid found in soils, waters, and biota throughout the world. Anthropogenic activity, including smelting operations, and manufacturing represent sources of As contamination in the environment (1), but natural hydrologic processes, such as percolation of water through As-rich geologic materials also contribute a significant flux of As into the surface environment. For example, in the Madison-Firehole (MF) watershed of Yellowstone National Park (WY, USA), groundwater mixes with hydrothermal fluids containing high concentrations of As. Hence, many geothermal springs in YNP contain As concentrations which far exceed $10 \mu\text{g L}^{-1}$, the current drinking water standard suggested by the World Health Organization, and in some cases, approach or exceed 3.4 mg L^{-1} (2,3). Consequently, the Firehole, Gibbon, and Madison Rivers within YNP all contain As concentrations exceeding $100 \mu\text{g L}^{-1}$ (4), and As concentrations in the Madison River exceed $10 \mu\text{g L}^{-1}$ farther than 150 km downstream from West Yellowstone, MT (5,6)

Despite elevated levels of As in the MF drainage of YNP, no work has been performed to date describing As exposure to native ungulate species, including populations of elk (*Cervus elaphus*) and bison (*Bison bison*) which frequent geothermally-influenced locations. Moreover, there is a scarcity of data linking As accumulation in ruminants with exposure to natural sources of As. Two cases of As poisoning in cattle were attributed to geothermal sources in New Zealand (7), and low concentrations of As were observed in livers and kidneys of free-ranging deer from several locations in Oklahoma (8). One case of acute As poisoning in deer has been reported, but this was presumably caused by the ingestion of an arsenical herbicide (9). Numerous studies have reported acute As poisoning in domestic ruminants, usually from point sources such as arsenical herbicides (10,11), wood preservatives (i.e. copper-chromium-arsenate) (12), and As dips used for controlling insects, such as lice (13). Unfortunately, these studies are of little use for understanding the ecotoxicology of As in natural systems where long term chronic exposure of As is occurring.

Acute symptoms of inorganic As exposure in ruminants include intense abdominal pain, salivation, bloody diarrhea and urine, staggering during ambulation, extreme weakness and death (14). In contrast, chronic symptoms of prolonged As exposure include depression, weight loss, and gastrointestinal distress (15). Adverse biochemical effects are generally caused by the interaction of As(III) with sulfhydryl (-SH) groups, which are essential to enzyme function and cellular metabolism (16). As(V) does not react with -SH, although it is an analog of phosphate, and decouples biochemical reactions such as substrate level phosphorylation (16). Tissues rich in -SH,

such as skin, accumulate As at a high rate and thus may serve as excellent indicators of exposure to As. Organo-arsenicals may also be toxic, but large quantities must be ingested. For example, the LD₅₀ of dimethylarsonate (DMA) in mice is 1,200 mg kg⁻¹ (body weight), compared to a LD₅₀ of 34.5 mg kg⁻¹ for As₂O₃ (17).

Arsenite and arsenate are the two primary inorganic forms of As present in soils and waters. Arsenate is less acutely toxic than arsenite, a relationship which is most pronounced in lower organisms, such as Cladoceran (*Daphnia pulex*). Dosing studies involving Cladoceran have demonstrated 50% immobilization in 48 hours with 49.6 mg L⁻¹ arsenate, whereas arsenite induced the same effects at 3 mg L⁻¹ (18). However, little difference was observed in mice dosed with arsenite (LD₅₀ = 35 mg kg⁻¹, [17]) or arsenate (LD₅₀ = 41 mg kg⁻¹). Furthermore, in ruminants, low concentrations of ingested arsenate are likely reduced to arsenite by the rumen microbial community (19) resulting in similar toxicity for arsenate and arsenite. It has been hypothesized that members of the rumen microbial community detoxify As via methylation (20), ultimately resulting in the production of DMA, which is almost two orders of magnitude less toxic than arsenite. This detoxification mechanism is also known to occur in the tissues of mammals, primarily in the liver.

In addition to DMA, other naturally occurring forms of As are far less toxic than inorganic species, including monomethylarsonate (MMA), trimethylarsine oxide (TMAO), arsenocholine (AC), arsenobetaine (AB), and arsenosugars (all LD₅₀'s > 1,000 mg kg⁻¹ in mice [17]). Some aquatic and terrestrial plants have been found to methylate inorganic As to MMA and DMA, and aquatic plants in particular have been found to

incorporate inorganic As into carbon skeletons to produce innocuous arsenosugars.

Arsenocholine (AC) and arsenobetaine (AB) are generally found in marine organisms, although trace amounts of AB have also been found in ant-hills and earthworms located in sites contaminated with As (21,22).

The elevated levels of naturally occurring As in the MF watershed provide a unique opportunity for understanding the ecotoxicology of As across different trophic levels. Specifically, the biological endpoints of As may be observed in elk with permanent home-ranges located in the MF watershed, providing insight regarding ingestion routes and seasonal exposure of As. Hence, the objectives of my study were to (i) determine concentrations of As in the tissues, feces, and rumen contents of elk residing in the upper Madison River basin, (ii) evaluate potential exposure pathways responsible for elevated levels of As in elk, and (iii) correlate As exposure to elk with frequency of habitation in thermal areas. Results show that As concentrations in tissues, feces and rumen contents of elk in the MF watershed are elevated with respect to a control population, which is attributable to the ingestion of forage containing high As. Analysis of selected soil, water, plant, rumen content, and fecal samples suggest that the major ingested forms of As contributed by several plant species, soil and water are inorganic, and detection of DMA in rumen contents and feces suggest that elk are detoxifying As. Concentrations of As in urine of radiocollared elk increased throughout the winter months and were higher during a winter with greater snow water equivalence (SWE). These observations are consistent with documented behavioral patterns of MF elk

where many individuals concentrate winter feeding within or near the MF watersheds containing high As-forage.

Methods

Site descriptions

The MF study area is located on the western edge of YNP, WY, in the upper Madison River drainage (Figure 2.1). Approximately 26,800 ha of the drainage serve as winter range for 500-800 elk (54), which are nonmigratory and remain within the boundaries of YNP year-round (23). Numerous geyser basins and associated geothermally heated soils in the MF watershed result in significant land area with reduced snow cover and unique terrestrial and aquatic plant communities that provide forage throughout the winter (24). These geyser basins also host a multitude of springs that vary widely in chemical composition, but generally contain high concentrations of As, ranging from approximately 1-3 mg L⁻¹ (3).

Control samples of elk, plants, soil and water were collected from the Northern Range, YNP (NR) and the Sun Ranch, MT (SR). The NR is located in the northeast corner of YNP and encompasses the watersheds of the Lamar and Yellowstone Rivers. The SR is located approximately 50 km NW of West Yellowstone, MT, and represents winter range for approximately 2500-3000 elk (25). The different geology of the NR, composed mainly of andesitic rock with some metamorphic and sedimentary rocks,

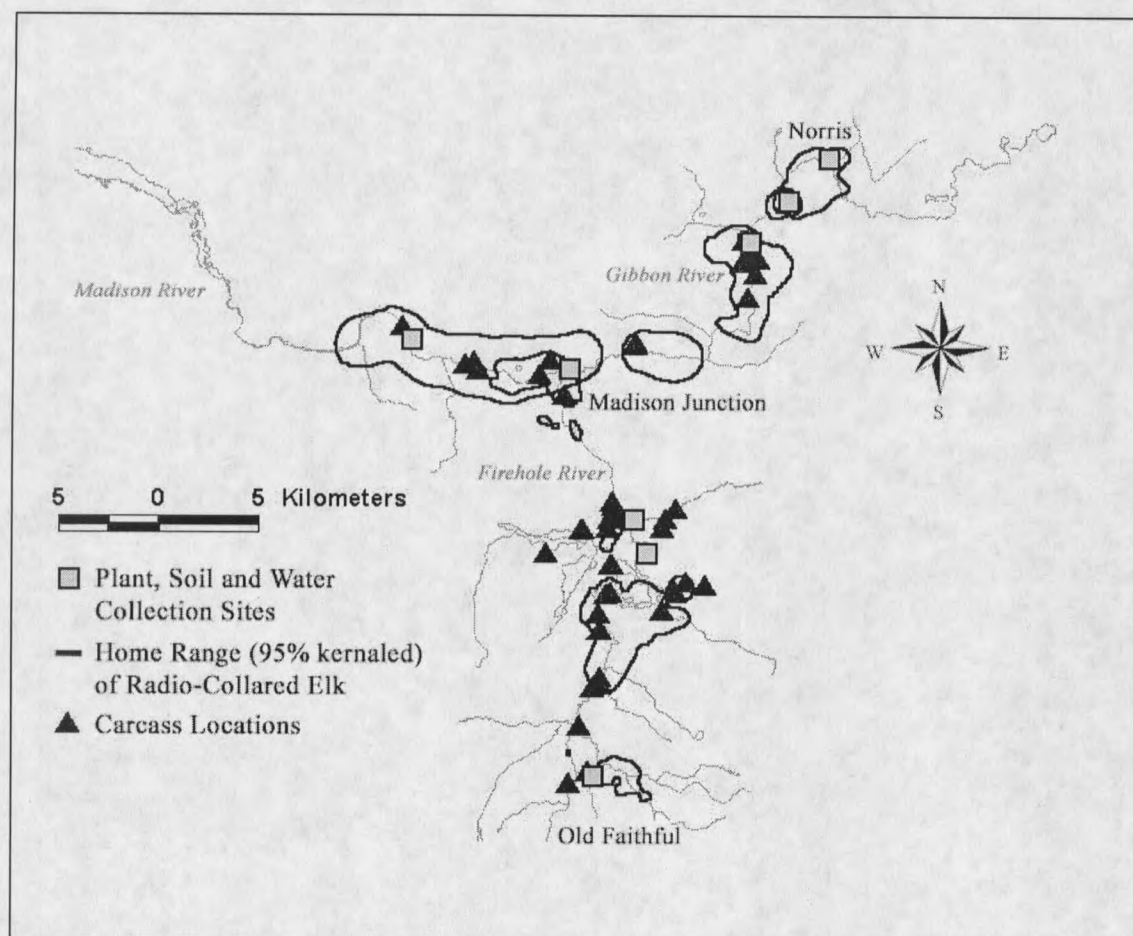


FIGURE 2.1. Map of the Madison-Firehole (MF) study area in Yellowstone National Park showing plant, soil and water sampling locations, carcass locations, and home ranges of elk from which urine samples were collected. Telemetry data was gathered year-round over a period of 11 years and kernalled (95%) to produce elk home ranges (53).

results in low surface water As concentrations compared to the MF study area, which exists in the Yellowstone Caldera and consist of Quaternary rhyolitic lavas and ash flow tuffs (26) containing high As concentrations. The geology of the SR is Tertiary valley fill composed of sandstone, siltstone, claystone, volcanic ash and coarse-grained

conglomerate (5). Surface water As concentrations on the SR were less than $2 \mu\text{g L}^{-1}$ (this study). Hence, both the NR and the SR represent control locations that are not significantly impacted by As.

Sample collection

Terrestrial and aquatic plant mixtures containing the dominant elk forage species were collected from 8 sites across the MF study area where elk were known to graze (Figure 2.1), and from 8 NR and 3 SR sites containing similar plant species. Plant material was cropped to the ground to simulate elk feeding behavior and not washed to determine total ingested As from both plants and soil contamination. In addition, individual plant species were gathered from the same locations and gently washed with doubly de-ionized water (DDW) prior to processing. Forage mixtures and individual plant species were then freeze-dried, ground in a steel mill, and passed through a $200 \mu\text{m}$ sieve.

Soil samples were collected from the MF drainage and SR across several plant habitats (riverbank, wet meadow, transition meadow, dry meadow), and placed into 50 mL Falcon tubes (Fisher Scientific). Within 24 hours they were freeze dried and passed through a 2 mm sieve. Water samples were collected from the Gibbon, Firehole, and Madison rivers, as well as two small streams on the SR, and passed through a $0.22 \mu\text{m}$ nylon filter (MSI, inc.) into a sterile 50 mL Falcon tube with minimal headspace. All soil and water samples were gathered in duplicate at the same locations where terrestrial and aquatic elk forage mixtures were collected.

Samples of skin, hair, bone, rumen content, and fecal matter were collected from recently deceased elk (cows and calves) over the winters of 1999-00 and 2000-01. These samples were gathered as part of an elk mortality study (R. Garrott, Dept of Ecology, MSU) within YNP, focused on predator/prey population dynamics. Control samples of skin, hair, bone, rumen contents and fecal matter were gathered at the SR during a winter elk hunt in January, 2001 (J. Gude, Dept. of Ecology, MSU). Care was taken to avoid contamination by using As-free latex gloves, instruments, and storage bags. Samples were dried for 2 days at 80 °C, ground in a steel mill, and passed through a 2 mm sieve. Prior to processing, bone samples were washed with H₂O₂ (30%) for several hours to dissolve soft tissue and marrow, which were otherwise difficult to remove.

Urine collection and creatinine analysis

Snow-urine samples were collected from 10 radiocollared MF elk from the winters of 1991-92, 1993-94, and 1996-97 (n = 140, all years) whose permanent home-ranges were known to overlap As-impacted locations (Figure 2.1). All urine samples were collected and handled using protocols described by DelGiudice et al. (27). The absolute concentration of As in elk urine is influenced by hydration state (which varies over the course of a winter); consequently, As concentrations were normalized by dividing by the concentration of creatinine, a metabolic by-product excreted at a relatively constant rate (28). Creatinine concentrations were determined by using a modified version of the colorimetric Jaffe kinetic method (29). Briefly, 50 µL aliquots of

urine sample were added to 50 μL of DDW water in a 96-well plate, followed by the addition of 50 μL of 0.3 M NaOH and saturated picric acid solution. After 15 minutes of color development, absorbance of the stable creatinine-picric acid complex was read at 500 nm with a 96-well plate reader (Fisher Scientific, Labsystems Multiskan Plus).

Total As analysis

Acid digestion was used to determine total As in animal tissue, urine and plant material by aggressive tissue dissolution and oxidation of organo-arsenical species to detectable inorganic form. Briefly, a modified three-acid tissue digestion procedure was used (30), where 0.25-0.50 grams of plant or animal tissue were weighed into 75 mL Pyrex digestion tubes. Samples were then pre-digested in 5.0 mL of concentrated HNO_3 for 12 hr at room temperature. Concentrated H_2SO_4 and 70% HClO_4 were added to each tube in 1 mL aliquots, and the samples digested on an aluminum block (Tecator, model 1015), using the following heating protocol: 115 $^\circ\text{C}$ for 1 hour, 170 $^\circ\text{C}$ for 1 hour, and 215 $^\circ\text{C}$ for 2.5 hours. A ramp time of 45 min between the last two temperature steps (170 and 215 $^\circ\text{C}$) was used to avoid rapid heating of HClO_4 , which can cause an explosion. A glass boiling chip was added to each tube, and a glass funnel was used to facilitate acid-reflux and prevent splattering of sample upon heating. After cooling, samples were brought to 30 mL with 3N HCl, mixed well and refrigerated until analysis (see below). A mussel tissue control sample obtained from the National Institute of Standards and Technology (NIST, Standard Reference Material 2976) was analyzed for As between

October, 2000 and April, 2002 and found to contain $13.2 \pm 0.2 \text{ mg kg}^{-1}$ ($n=20$), in close agreement with the published value of $13.3 \pm 1.8 \text{ mg kg}^{-1}$.

Continuous flow hydride generation atomic adsorption spectrophotometry (HG-AAS) was used to analyze As in aqueous samples, extracted solutions, and acid digests (31). Previously acidified samples (3N HCl) were pre-reduced with 1% KI, and mixed with 0.6% NaBH_4 in 0.5% NaOH. Subsequent emission of arsine gas was quantified at 193.4 nm in a quartz cuvet immersed in an air-acetylene flame (Perkin Elmer model 3100 atomic adsorption spectrophotometer). The detection limit for As using this method was 3.4 nM ($0.25 \mu\text{g L}^{-1}$).

Soil As extraction

Soil extractable As was determined using the ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) as outlined in soil and plant analytical methods of the Western States Proficiency Program (32). Briefly, 10 g of soil and 20 mL of extracting solution containing 1.0 M NH_4HCO_3 and 0.005 M DTPA (pH 7.6) were measured into 40 mL HDPE centrifuge tubes, shaken at ≈ 240 oscillations/min for 15 min, filtered (Whatman #1 ashless) and analyzed for As using HG-AAS.

Extraction and analysis of As species in selected samples

A small subset of plant tissue, rumen content, and fecal matter samples were analyzed for As(III), As(V), MMA, DMA, TMAO, AB, AC, and several arsenosugars

using a methanol water extraction procedure (21.) Freeze dried samples were weighed (0.2 g) into 50 mL HDPE tubes and extracted with 30 mLs of a 9:1 methanol:water solution, shaken at ≈ 120 oscillations/min for 14 hours. After shaking, sample slurries were centrifuged for 10 min at 2000 rpm and the supernatant decanted into a 250 mL round-bottom flask. The residue present in the HDPE tube was washed with 30 mL of the 9:1 methanol water solution, centrifuged at 2000 rpm, and the supernatant was once again decanted into the same round bottom flask; this washing procedure was repeated 3 times. The combined supernatants were evaporated with a Rota-Vap (Buchi, model R110) to near dryness, then resuspended to a final volume of 10 mL with DDW H₂O. Aliquots of each extract were used for analysis of total As by HG-AAS (discussed above), and for various As species using high pressure liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) at the National Water Quality Laboratory, USGS (Boulder CO). Briefly, As species were separated via liquid chromatography (Waters 600-MS system controller) on an AS-7 (Dionex, Sunnyvale, CA) column using a 2.5 to 50 mM HNO₃ (in 0.5% methanol) step gradient over 525 sec, in series with an ICP-MS (PE Sciex Elan 6100) using a cross-flow nebulizer (PE Sciex). The ICP-MS system was operated at 1100 W, data was acquired at a dwell time of 500 ms, and ion intensity was measured at m/z 75. Standards of AB, AC, TMAO, and arsenosugars I-IV were obtained from W.R. Cullen, University of British Columbia, Canada. Arsenite, arsenate, MMA and DMA were purchased from J.T. Baker (Philipsburg, NJ) and EM Science (Gibbstown, NJ) chemical companies.

Statistical analysis

Bootstrapping and a simple test statistic, hereafter referred to as Bootstrapping, were used to compare As concentrations in elk tissue, rumen content, feces, soil and plant samples between the control and study sites. Briefly, a number of random samples equal to the population size were taken separately from each population (MF and control) with replacement. The means of the random samples from each population were compared, and a value of 1 was added to a counter if the first population mean was greater than the second. After this process was iterated 1000 times, a two tailed p -value was generated by dividing the number stored in the counter by the number of iterations and multiplying the result by 2. In this study, a two-tailed p -value less than 0.05 represented a significant difference between the two populations. This method of statistical analysis is less dependent on the need for normality in data distributions and homogeneity of variation among treatments than other statistical methods, such as analysis of variance (ANOVA), and is commonly applied in ecology (33).

Differences in mean urine arsenic:creatinine (As:C) ratios among winter months of a given year and across three years of varying snowfall accumulation were evaluated using ANOVA and Fisher's pairwise comparison. All As:C data were transformed with a square root function to meet ANOVA assumptions.

Estimation of As accumulation and excretion.

Concentrations of As in elk hair and bone were estimated as a function of As dose using absorption rate coefficients determined in domestic blackface sheep. A very thorough ^{73}As dosing experiment was performed previously (34) to accurately determine rate coefficients describing As transfer among body tissue and excreta. Kinetic modeling software (CKS, IBM Almaden Laboratories) was used to predict dose response using a zero order rate constant (mg As day^{-1}) to simulate a constant dose of As into the rumen. Linear regression was then used to express skin and bone As concentration as a function of dosing rate. Observed concentrations of As in skin and bone of elk were then used to estimate potential As dosing rates. Dosing rates were converted to As concentration (mg kg^{-1}) in elk forage by assuming a daily dry matter intake of 27.5 g kg^{-1} (body weight) day^{-1} (35).

Results

As concentrations in elk samples

Concentrations of As in elk samples from the MF watershed were highly variable (Figure 2.2), but were significantly elevated with respect to the SR population ($p < 0.01$, Table 2.1). Mean As values in MF elk were generally 1 to 2 orders of magnitude higher than SR elk, and numerous individuals exhibited As levels $> 10 \text{ mg kg}^{-1}$ in skin, hair, rumen, and feces. Within the MF group, As levels in hair and skin were significantly ($p < 0.01$) higher than bone samples in agreement with the fact that As accumulates more

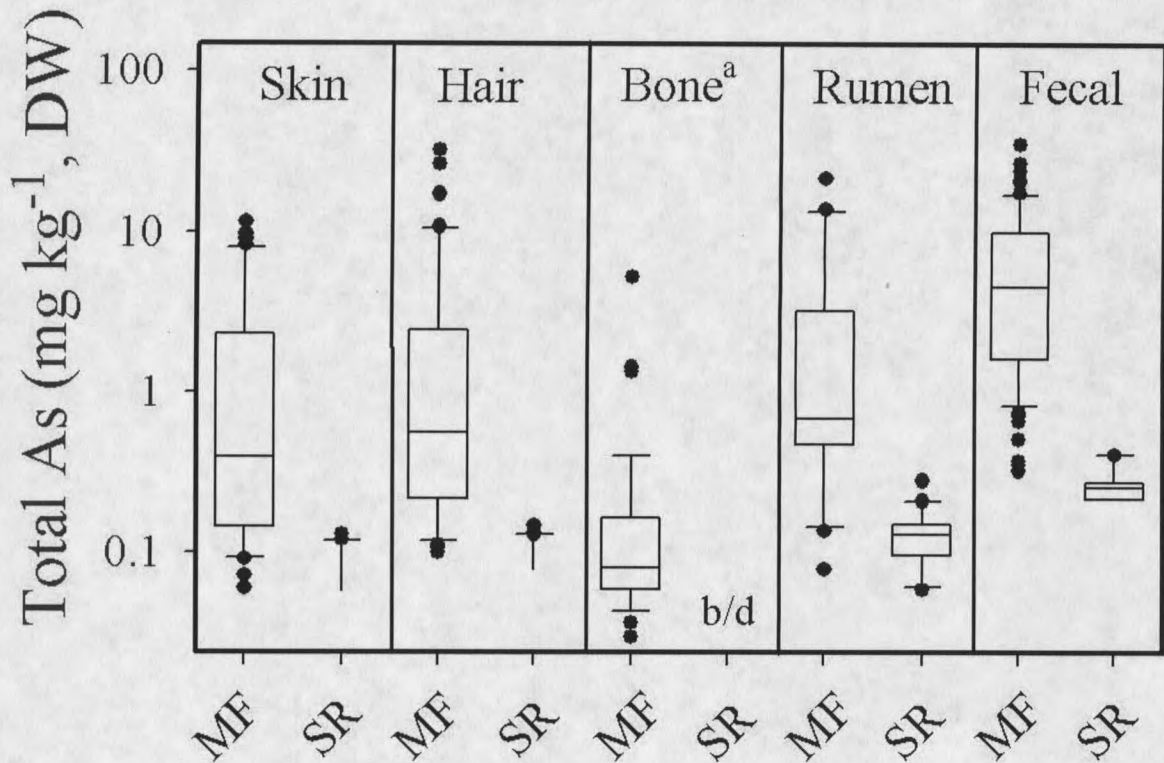


FIGURE 2.2. Concentrations of total As in skin, hair, bone, rumen contents, and fecal matter of Madison-Firehole (MF) and Sun Ranch Control (SRC) elk (mg kg^{-1} , dry weight). Boundary of the box closest to zero represents the 25th percentile, the line within the box represents the median, and boundary of the box farthest from zero represents the 75th percentile. Whisker caps represent the 10th and 90th percentile, and dots represent outliers below and above whisker caps. ^a All bone samples collected from Sun Ranch elk were below detection (b/d).

rapidly in -SH rich tissues than in bone (16). Arsenic concentrations in all tissues, rumen contents, and fecal matter were not significantly different between adult cows and calves (calve gender was typically unknown). In addition, there was no correlation ($r^2 < 0.001$, linear regression) between the age of an individual and As concentrations for the tissues analyzed in this study. Concentrations of As in MF elk samples may be compared to

TABLE 2.1. Mean Concentration (mg kg^{-1}) of Total As in Hair, Skin, Bone, Feces and Rumen of Madison-Firehole or Sun Ranch Control Elk Populations

Population	Sample Type				
	Hair	Skin	Bone	Feces	Rumen
Madison-Firehole	3.4 ± 1.1^a (42) ^b	2.1 ± 0.5 (38)	0.41 ± 0.2 (26)	7.1 ± 0.9 (61)	3.5 ± 1.6 (16)
Sun Ranch	0.05 ± 0.01 (16)	0.03 ± 0.01 (17)	< 0.015 (17)	0.23 ± 0.03 (15)	0.13 ± 0.02 (16)
Bootstrap <i>p</i> -value ^c	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

^a $\pm$ values are standard errors. ^b values in parenthesis = sample number, ^c *p*-value obtained when two populations from different sites are compared via Bootstrapping

concentrations found in cases of chronic and acute As poisoning in other ruminants. For example, hair samples of several elk in the MF watershed contained approximately 18 mg kg^{-1} As (Figure 2.2). This exceeds the As concentration found in the hair of goats that were dosed with 20% of the As(III) ALD₅₀ per day for 84 days, a treatment which eventually resulted in 100% mortality of the animals due to chronic As toxicosis (36). In addition, concentrations of As in cattle hair ranging from $0.8\text{--}3.4 \text{ mg kg}^{-1}$ have been observed 17 days post-exposure to As-contaminated feed (37). All individuals developed acute hemorrhagic diarrhea, and 2 of 11 head ultimately died as a result of acute As toxicosis. The highest concentrations of As was 3.4 mg kg^{-1} , equivalent to the mean value of hair As concentrations observed in this study. Although As concentration in hair is one indicator of overall environmental exposure to As, hair is particularly sensitive to external contamination from As-laden soil and water (38), a possible contributor within

the geothermally impacted watersheds of YNP. Consequently, high As levels in hair may not necessarily reflect As exposure exclusively through ingestion of As-containing feed.

Since skin and bone are less prone to environmental contamination than hair, As concentrations in these samples are likely more reflective of As exposure through ingestion. For comparative purposes, skin dry weight (DW) As concentrations were converted to fresh weight (FW) concentrations using tissue-specific percent water content (39), and found to range from 0-3.5 mg kg⁻¹. This range is below the average skin As concentration in As-exposed goats of 23.2 mg kg⁻¹ (FW) (36). However, many skin As concentrations observed in this study far exceeded the FW value of 0.4 mg kg⁻¹ measured in cattle dosed with 1.25 mg kg⁻¹ day⁻¹ As(V) (41), a treatment that produced no gross or microscopic signs of As toxicity. Bone As concentrations of 0.2 mg kg⁻¹ were also reported for cattle (40), and these values are slightly less than the mean bone As concentration (converted to FW) observed in this study of 0.28 mg kg⁻¹, again suggesting that elk are ingesting greater amounts of As than administered in chronic toxicity studies.

A wide range of rumen As concentrations have been reported in ruminant animals following As exposure. For example, As concentrations ranging from 4-176 mg kg⁻¹ were observed in the rumen contents of cattle receiving a lethal dose of As₂O₃ (As(III)) (7). In contrast, As concentrations ranging from 2-4 mg kg⁻¹ were observed in cattle receiving a chronic dose of As (37), and rumen contents of As-dosed goats contained 23.4 mg kg⁻¹ As (36). Consequently, it is not clear whether As concentrations ranging from 0.08-21.8 mg kg⁻¹ found in the rumen contents of elk are indicative of As

poisoning. The only value found in the literature for As concentrations in ruminant feces was 4.4 mg kg^{-1} reported by Peoples (40), in cattle dosed with low levels of As. This value is approximately half the mean As concentration observed in elk feces of 7.1 mg kg^{-1} , suggesting that the mean dose of As to MF elk may be higher than the $1.25 \text{ mg kg}^{-1} \text{ day}^{-1}$ used in the cattle study (40). However, the amount of fecal matter produced by a ruminant per day varies based on the intake and digestibility of feed (41), thus As concentrations in feces also vary based on diet.

As in forage mixtures and individual plant species

Arsenic concentrations in each MF forage mixture were highly variable, but the majority were significantly greater than levels observed in control sites (NR or SR) (Table 2.2, Figure 2.3). Dry meadow MF forage mixtures were not significantly different compared to either the NR or SR samples ($p = 0.41, 0.51$ respectively). Within the MF watershed, concentrations of As were highest in aquatic plants and decreased as a function of distance from the primary watershed; aquatic, riverbank, sedge meadow, and transition meadow forage mixtures all contained elevated As levels compared to the dry meadow forage mixtures ($p < 0.01$). Individual plant species representing the dominant members of forage mixtures were analyzed for total As (Table 2.3). High As concentrations were found in aquatic plants compared to terrestrial plants, which generally contained 1-2 orders of magnitude less total As ($p < 0.01$).

TABLE 2.2. Concentrations of Total As (mg kg^{-1} , DW) Measured in Elk Forage Mixtures Sampled from the Madison-Firehole Watershed and from Control Locations within the Northern Range and Sun Ranch.

Site	Forage Regime				
	Aquatic	Riverbank	Sedge Meadow	Transition Meadow	Dry Meadow
Madison-Firehole	224.5 ± 110.0^a (4) ^b	22.6 ± 13.5 (5)	7.7 ± 0.8 (7)	3.9 ± 2.0 (4)	0.4 ± 0.3 (3)
Northern Range	1.1 ± 1.0 (2)	0.17 ± 0.02 (4)	0.22 ± 0.11 (7)	0.06 ± 0.02 (4)	0.05 ± 0.02 (4)
Sun Ranch	—	—	0.19 ± 0.06 (3)	—	0.07 ± 0.01 (3)
Bootstrap p -value ^c	^d ^e < 0.01	< 0.01	< 0.01 < 0.01	< 0.01	0.41 0.50

^a $\pm$ values are standard errors, ^b values in parentheses = sample number, ^c p -value obtained when two populations from different sites are compared via Bootstrapping, ^d comparison between Madison-Firehole population and Northern Range control population, ^e comparison between Madison-Firehole and Sun Ranch control population

Arsenic concentrations found in MF aquatic plants were similar to values of 316 mg kg^{-1} dry weight (DW) observed in *Potamogeton* growing in water containing approximately $100 \mu\text{g L}^{-1}$ As (42), and were considerably higher than total As concentrations of $2.5\text{--}78 \text{ mg kg}^{-1}$ observed in aquatic plants growing in an As-impacted ecosystem (43). Concentrations of As were highly variable within individual plant species sampled from different habitats (riverbank, wet meadow, etc.) (Table 2.3), with

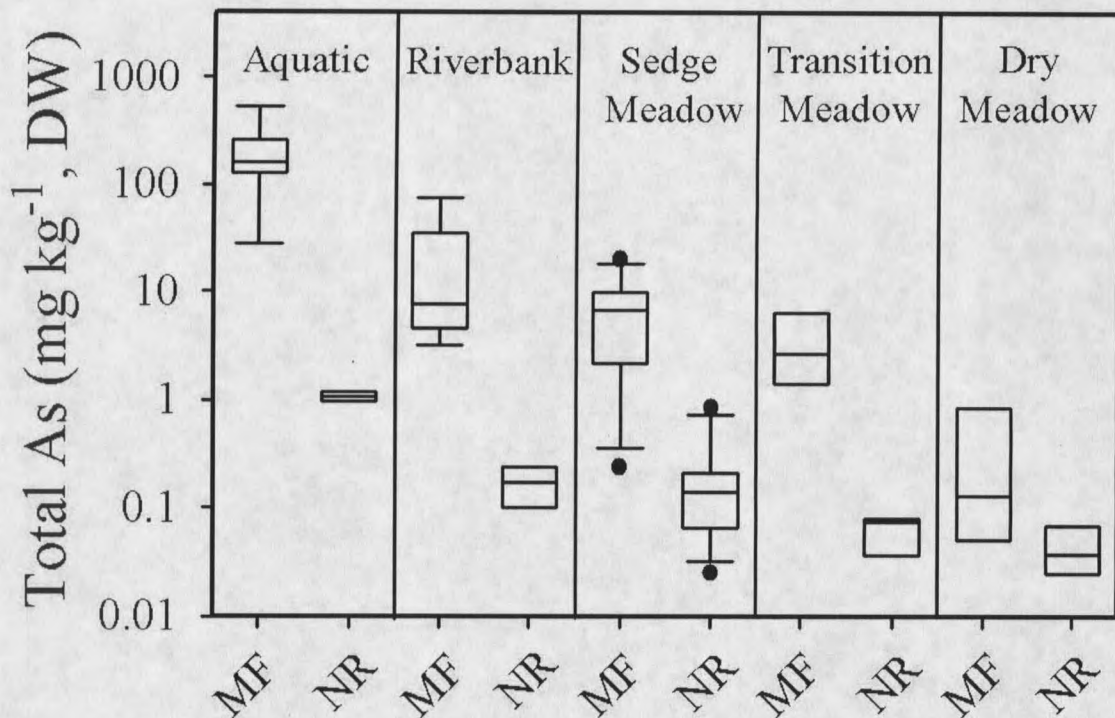


FIGURE 2.3. Concentrations of total As (mg kg^{-1} , dry weight) in forage mixtures collected from the MF watershed and the Northern Range control sites. Boundary of the box closest to zero represents the 25th percentile, the line within the box represents the median, and boundary of the box farthest from zero represents the 75th percentile. Whisker caps represent the 10th and 90th percentile, and dots are outliers below and above whisker caps.

higher values generally observed in plants sampled from the wetter environments.

Consequently, elk that consume aquatic and terrestrial forage (with the exception of dry meadow vegetation) in the MF watershed are likely exposed to significant levels of As.

TABLE 2.3. Concentrations of Total As (mg kg^{-1} , DW) in Individual Plant Species Sampled Within the Madison-Firehole Watershed

Latin name	Common name	n	mean (S.E.) ^a
<u>Terrestrial</u>			
<i>Agropyron caninum</i>	Slender wheatgrass	5	2.1 (1.3)
<i>Aster sp.</i>	Aster	1	22.2
<i>Calamagrostis canadensis</i>	Marsh reedgrass	1	0.4
<i>Calamagrostis inexpansa</i>	Northern reedgrass	1	5.9
<i>Carex aquatilis</i>	Water sedge	5	2.5 (1.0)
<i>Carex utriculata</i>	Beaked sedge	5	1.3 (0.3)
<i>Deschampsia cespitosa</i>	Tufted hairgrass	10	3.8 (1.4)
<i>Eleocharis palustris</i>	Creeping spikerush	2	5.0 (4.0)
<i>Eleocharis rostellata</i>	Beaked spikerush	9	16.2 (11.2)
<i>Hordeum sp.</i>	Barley	3	1.3
<i>Juncus balticus</i>	Wire rush	3	1.5 (0.6)
<i>Juncus tweedyi</i>	Tweedy's rush	1	4.9
<i>Muhlenbergia filiformis</i>	Pull-up muhly	2	0.9 (0.1)
<i>Phleum pretense</i>	Field timothy	4	2.4 (1.4)
<i>Pinus contorta</i>	Lodgepole pine	3	0.8 (0.02)
<i>Poa palustris</i>	Fowl bluegrass	6	1.4 (0.3)
<i>Senecio hydrophilus</i>	Water groundsel	1	2.8
<i>Triglochin sp.</i>	Slender arrow-grass	5	28.5 (11.5)
<u>Aquatic</u>			
<i>Berula erecta</i>	Water Parsnip	1	126.0
<i>Chara/Nitella sp.</i>	Stonewort	4	162.1 (61.8)
<i>Hippuris vulgaris</i>	Common mare's tail	3	122.8 (31.1)
<i>Myriophyllum sp.</i>	Water-Milfoil	3	118.9 (38.4)
<i>Potamogeton sp.</i>	Pondweed	5	619.9 (291.0)
<i>Sparganium sp.</i>	Bur reed	1	287.5
<i>Ranunculus aquatilis</i>	Water buttercup	1	258.5

^a S.E. = standard error where appropriate ($n > 1$)

Soil and water As

The mean concentration of AB-DTPA extractable As in soils sampled across the MF drainage was $170 \pm 43 \mu\text{g kg}^{-1}$ ($n = 13$, range = 31.2 - $504.5 \mu\text{g kg}^{-1}$), compared to only $14.5 \pm 2.8 \mu\text{g kg}^{-1}$ As in soils from the SR ($n = 9$, $p < 0.01$). In addition, sediment

samples collected directly from the riverbeds of the Gibbon and Firehole Rivers contained 853 and 1302 $\mu\text{g kg}^{-1}$ AB-DTPA extractable As, respectively. These values are higher than AB-DTPA extractable As concentrations of 50 $\mu\text{g kg}^{-1}$ obtained from a mine-waste impacted site containing 53 mg kg^{-1} total As (44). Values of As obtained via the AB-DTPA extraction method do not represent total As concentrations, but rather the amount of As which is removed from sorption sites on mineral phases (32). Hence, the AB-DTPA extraction more closely represents the bioavailable fraction of As existing within soil and sediments. Water samples collected from the Firehole River at the Lower Geyser Basin contained 295 $\mu\text{g L}^{-1}$ As, while water samples collected from the Gibbon River at Gibbon Meadow and near the Norris campground contained 66 and 21 $\mu\text{g L}^{-1}$, respectively. In addition, a sample collected at Nez Perce Creek contained 161 $\mu\text{g L}^{-1}$ As. For comparison, water samples taken from several small streams running through the Sun Ranch contained only $1.1 \pm 0.8 \mu\text{g L}^{-1}$ As ($n=5$), and indicate the substantial difference in potential As exposure between the two locations.

As speciation in rumen content, fecal matter, plants, soil and water

A subset of rumen content, fecal matter, and plant species samples were analyzed for various As species using HPLC-ICP-MS. As(III) was the dominant species found in all samples, followed by As(V) and DMA (Table 2.4). Rumen contents contained a small amount of DMA (3% of extractable), suggesting that methylation is occurring in the rumen and is consistent with previous hypotheses that microbial rumen communities are

TABLE 2.4. Distribution of As Species ^a in Selected Plants, Elk Rumen Content and Elk Feces Determined Using Methanol Extraction and Analysis via HPLC-ICP-MS ^b.

Sample ^c	% EE ^d	DW total [As], mg kg ⁻¹	Arsenic Species		
			% As(III)	% As(V)	% DMA
Rumen Contents	44.6 (13.6)	3.1 (3.2)	88.3 (3.4)	8.8 (5.8)	2.9 (2.63)
Feces	33.2 (17.1)	17.2 (11.3)	70.8 (11.8)	9.5 (1.6)	19.7 (10.2)
<i>Deschampsia cespitosa</i>	71.9 (48.6)	7.2 (4.6)	86.0 (6.9)	14.0 (6.9)	
<i>Carex aquatilis</i>	75.8 (22.9)	4.4 (1.9)	88.2 (11.8)	11.8 (11.6)	
<i>Potamogeton sp.</i>	1.75 (1.2)	640.9 (353.7)	64.2 (14.5)	34.5 (13.4)	1.2 (1.1)
Algal slime	0.44	2156.6	50.4	49.6	

^a As species analyzed included arsenite (As(III)), arsenate (As(V)), monomethylarsonate, dimethylarsonate (DMA), trimethylarsine oxide, arsenobetaine, arsenocholine, and several arseno-sugars, ^b Values in parentheses are standard errors, ^c all samples: n = 2. ^d Percent extraction efficiency = percent of total As extracted using methanol:water.

capable of detoxifying inorganic As via methylation (20). Fecal matter contained higher quantities of DMA (20% of extractable), again suggesting methylation during digestion prior to excretion. The inorganic forms of As (arsenate and arsenite) were the dominant forms found in surface waters and soil samples. Although small amounts of methylated As species have been found in a eutrophied lake (45) and in soils (46), the predominant forms of As in soil and surface waters are generally inorganic. The terrestrial plants *Carex aquatilis* and *Deschampsia cespitosa* contained predominantly inorganic As (Table 2.4), consistent with previous reports of only small amounts of methylated species in *Carex* (43), and only trace amounts of organo-arsenicals in *Deschampsia* (52). Although our sample of *Potamogeton sp.* contained small amounts of DMA, the dominant

extractable As species were inorganic (Table 2.4). However, the low methanol:water extraction efficiency (< 2%) for *Potamogeton* sp. and the algal slime precludes definitive conclusions about the actual distribution of As species. Low methanol:water extraction efficiencies have also been observed in earthworms (21).

Seasonal and yearly fluctuations in urine As

Mean urine As:C ratios were significantly correlated with SWE across three disparate winters (Figure 2.4). Values of SWE were obtained from a SNOTEL site at Madison Plateau (operated by the Natural Resources Conservation Service, Portland, Oregon) and represent the cumulative daily water content (cm) in the snow column over the entire winter. Thus, these values serve as an excellent index of winter harshness (55). In addition, significant differences in mean As:C ratios were observed across months for the more severe winter of 1996-97 (Table 2.5). These data support the hypothesis that elk exposure to As increases as deeper snowpack encourages feeding in aquatic and riparian habitats (47). To further examine this relationship, all individual urine As:C ratios (n= 140) were plotted and fit to quadratic equations describing increases in As:C during winter progression (Figure 2.5). The most pronounced increases in As:C over the winter months occurred during the winter with the highest SWE (1996-97, Table 2.5). Essentially no increases in As:C were observed over the winter months during the winter with the lowest SWE (1993-94, Table 2.5).

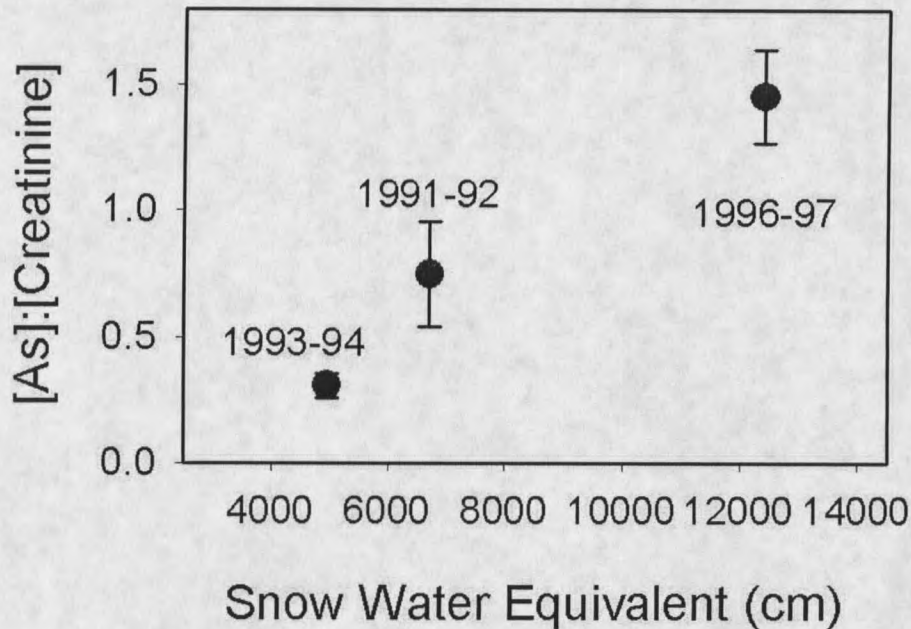


FIGURE 2.4. Average As:C ratios (x1000) in elk urine of radiocollared animals as a function of total winter snow water equivalent (SWE). Values of SWE were compiled from SNOTEL (NRCS) data taken from the west edge of YNP (Madison Plateau).

TABLE 2.5. Mean As:C Ratios of Radiocollared Elk within the MF Watershed by Month for Three Winters Exhibiting Increasing Snow Water Equivalent.

Months	Winter (snowfall)					
	1993-94 (4956 cm)		1991-92 (6715 cm)		1996-97 (12404 cm)	
Dec	0.005	a ^a (12) ^b	—		0.015	a (10)
Jan	0.009	ab (5)	0.019	ab (11)	0.032	b (14)
Feb	0.015	b (15)	0.016	a (11)	0.036	b (15)
Mar	0.018	b (10)	0.030	ab (9)	0.051	c (9)
Apr	0.018	b (11)	0.039	b (4)	0.037	cb (4)

^a means followed by different letters within a given year were significantly different using Fisher's pairwise comparisons ($\alpha = 0.05$). ^b Values in parentheses = n.

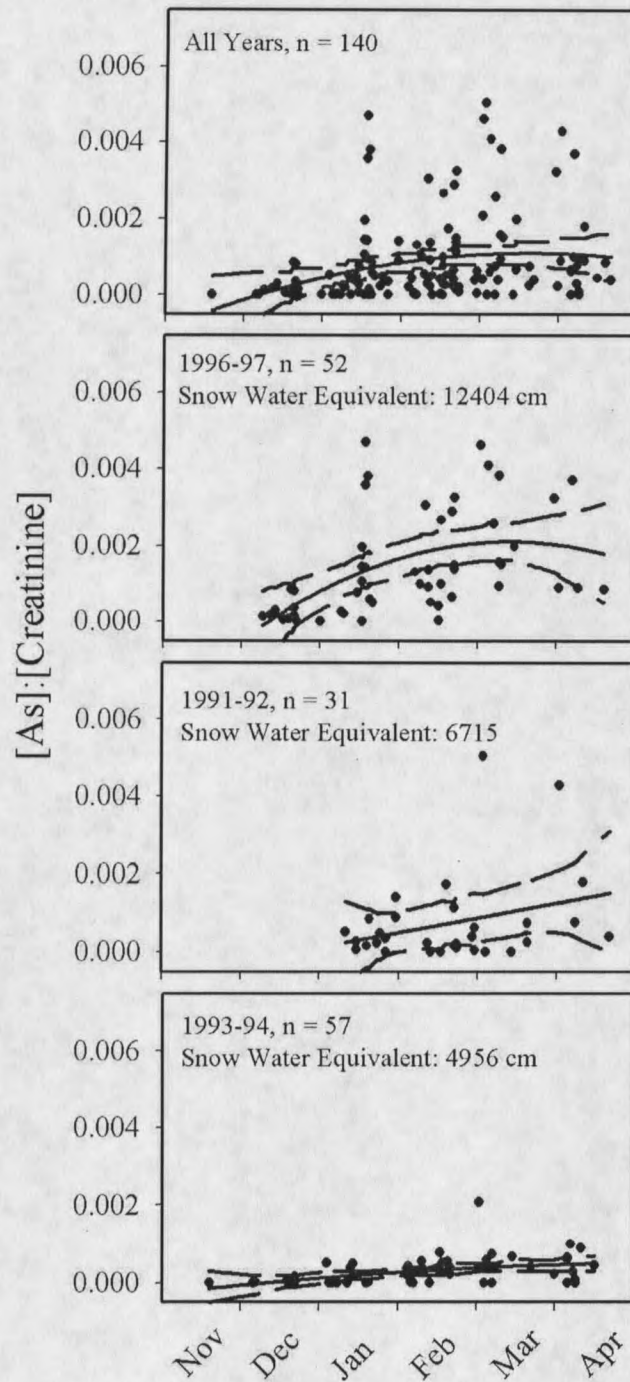


FIGURE 2.5. Individual As:C ratios in elk urine ($n = 140$) as a function of time across three winters. Urine samples came from 10 radiocollared, nonmigratory cow elk from the MF watershed (see Figure 2.1 for home range data). Solid lines are fitted quadratic equations, and dashed lines represent 95% confidence intervals.

Discussion

Elk As ingestion from plants, soil and water

Elevated levels of As in tissues, excreta, and rumen contents of elk residing in the MF drainage (YNP) are associated with ingestion of high As forage in geothermal and aquatic habitats. The wide variation in As concentrations of MF elk is likely a result of variation in As forage concentrations within the geothermal and aquatic habitats (Figure 2.3), and the variability among individual feeding patterns (48). The average As concentration in elk forage mixture ranged from 0.4 – 224.4 mg kg⁻¹. Assuming an elk consumption rate of approximately 27.5 mg As kg⁻¹ (body weight) day⁻¹ dry forage material (35), and an average adult cow elk weight of approximately 250 kg (49), it was estimated that elk may ingest between 0.1-6.2 mg kg⁻¹ (body weight) day⁻¹. A dose rate of 4.5 mg As(III) kg⁻¹ day⁻¹ in lambs resulted in weight loss, refusal of feed, and gross signs of hepatic damage associated with As toxicosis (50). However, it has been reported that dosing cows with 1.25 mg kg⁻¹ day⁻¹ As(V) resulted in no outward signs of As toxicosis after 56 days (40). Consequently, the impact of low-level As consumption based on past studies in domestic ruminants ranges from no effect to chronic toxicity.

Elk likely consume significant quantities of soil and water with vegetation while feeding. Consequently, soil and water samples were analyzed to address the possibility that co-ingestion with plants might increase As exposure. Soil samples taken from riverbank, wet meadow, and dry meadow environments and extracted with AB-DTPA did not contain high levels of extractable As (range = 31-505 µg kg⁻¹ dry weight) in

comparison to plant total As concentrations, suggesting that soil ingestion does not represent a significant As exposure pathway. However, total As concentrations in two MF soil samples were 12 and 60 mg kg⁻¹, indicating a potentially larger amount of As present in these soils that is not AB-DTPA extractable. Sediment samples collected from the Gibbon and Firehole rivers contained elevated levels of extractable As compared to soil samples (853 and 1302 µg kg⁻¹, respectively), but were still relatively low compared to total As concentrations in aquatic plant species. A large number of sediment samples have been analyzed by Chaffee (USGS, unpublished data), who observed total As values ranging from 2.5-530 mg kg⁻¹ (mean = 40.4 mg kg⁻¹, n=45) in a variety of springs, streams, and rivers in the MF watershed. In comparison, sediments analyzed from the Northern Range (in non-geothermally influenced areas) contained 0-6.5 mg kg⁻¹ total As (mean = 2.5 mg kg⁻¹, n=58, Chaffee, unpublished data). Consequently, significant quantities of As may be ingested when MF elk feed on aquatic plants associated with sediment containing high As.

The Firehole, Gibbon, and Madison Rivers all contained As concentrations less than 300 µg L⁻¹. Although As concentrations found in these rivers are responsible for the accumulation of high As found in aquatic forage, they are not the likely cause of high As concentrations found in elk tissues. For example, elk would have to consume over 3 L of Firehole River water to receive a 1 mg dose of As. This value is quite low when compared to ingestion of 1 kg of high As-containing aquatic forage containing over 200 mg kg⁻¹ As, and similar to the As dose obtained when ingesting only 0.4 kg of

transitional meadow forage. However, direct ingestion of As from drinking water may contribute to exposure over extended periods of time.

Although soil, sediment and water samples analyzed in the current study contained lower levels of As compared to plants, a variety of springs and minerals exist in the MF drainage which may represent significant sources of As to elk. For example, As concentrations significantly higher than 3 mg kg^{-1} have been observed (3) in selected MF hot springs, and Schaufelberger (51) describes the formation of As-rich minerals near hydrothermal features consisting of orpiment (As_2S_3) and realgar (As_4S_4). Although elk have not been observed ingesting waters or minerals directly associated with thermal springs, they have been observed consuming plants in areas where these springs and minerals abound, making it possible that these high As minerals contribute to As exposure in MF elk.

Estimates of As Exposure via Forage

There is a paucity of information regarding As accumulation and excretion rates in ruminant tissues. However, rate coefficients describing As tissue accumulation and excretion were obtained in an ^{73}As labeling experiment in blackface sheep (34). Using kinetic parameters from this study, As concentrations in skin and bone were predicted as a function of As dose (Figure 2.6). Actual elk skin As concentrations ranged from 0-3.5 mg kg^{-1} , suggesting forage As levels ranging from 0-23 mg kg^{-1} . Actual bone As concentrations ranged from 0-1.0 mg kg^{-1} , corresponding to As levels in forage of 0-20

mg kg^{-1} . Interestingly, these ranges of forage As concentrations fall within observed As values in terrestrial forage mixtures, but were considerably less than aquatic mixtures. Although many elk are seen to directly forage on aquatic vegetation containing high As, concentrations of As in tissue samples analyzed in this study suggest these MF elk fed primarily on vegetation containing less As (i.e. terrestrial forage mixtures).

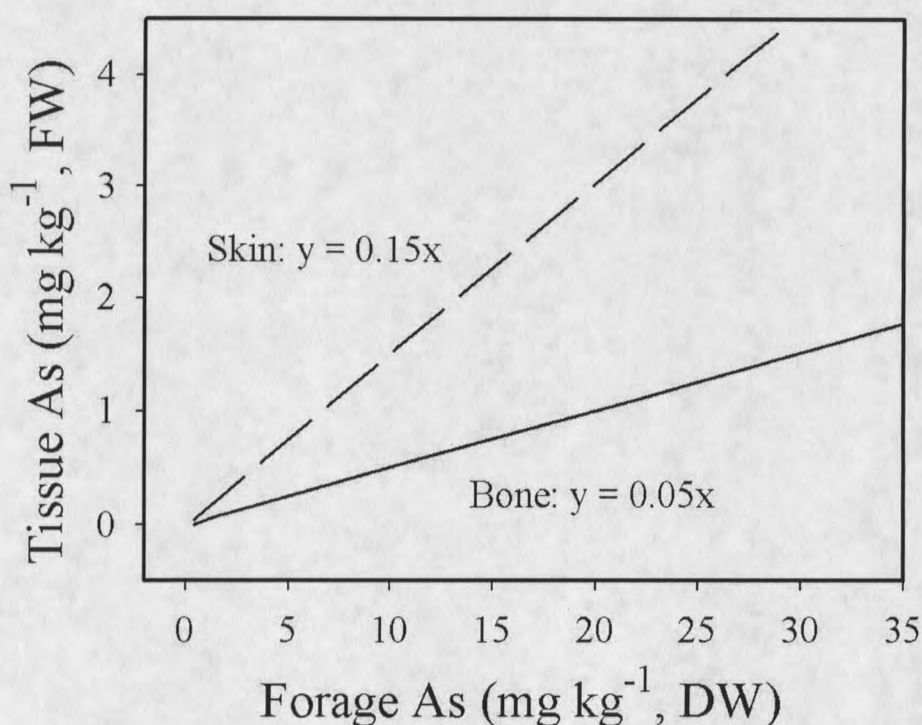


FIGURE 2.6. Estimated concentration of As in elk skin and bone (mg kg^{-1}) as a function of forage As. The dose response relationships were derived using rate coefficient data for As accumulation/excretion (34). Forage concentrations were calculated by converting the daily As dose ($\text{mg kg}^{-1} \text{ day}^{-1}$) used in the accumulation/excretion calculations to mg kg^{-1} in forage by assuming a dry forage intake of $27.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ for an average cow elk (250 kg).

Seasonal As exposure and potential toxicological effects

As winter snow depths increase, some elk have a greater propensity to forage in geothermal and aquatic environments. It is not uncommon to observe elk in mid-winter feeding directly in the primary rivers and in geothermal locations where some plant species are photosynthesizing year-round. These foraging patterns minimize energy expended in deep snow, and make it easier to maintain normal body temperature in warmer thermal and river environments. Consequently, elk that migrate into thermal locations over the course of a winter show increasing concentrations of As in urine samples (Figure 2.5). These data suggest that there is a very strong seasonal influence with respect to As exposure in MF elk; exposure is likely minimal in late spring through mid-fall, when non-geothermally influenced forage is succulent and plentiful, but increases mid-fall through late spring when this forage senesces and becomes snow covered. This is consistent with the positive correlation between As concentration in elk urine and total winter SWE (Figure 2.4), demonstrating that As exposure increases in years with deeper average snowpack.

In mammals, As is generally detected in urine within 48 hr after ingestion of high As feed (16). Given that elk urine As concentrations were highest in late winter, especially in high total SWE years, it is logical to assume that clinical signs of chronic As toxicosis would be most prevalent at this time. Unfortunately, signs of chronic As toxicosis, including depression, refusal of feed, and staggering during ambulation (14) are easily masked by the clinical signs of starvation, which occurs often in late winter in MF elk, regardless of the desirable forage and beneficial climatic conditions of aquatic and

geothermal habitats. Consequently, it is not yet possible to conclude that MF elk are affected by chronic As toxicosis in the MF drainage. However, the elevated level of As present in some tissue, rumen and fecal samples suggests that chronic As toxicosis may be occurring in some animals, and could be a significant contributor to environmental stresses that culminate in reduced animal life span (47).

The average life expectancy of elk residing in the MF watershed (15 years) is significantly lower than elk residing in the Northern Range (25 years) (47). Interestingly, geothermal features represent the only major habitat difference between the Northern Range and MF watersheds; consequently, the unique geochemistry of MF thermal features likely plays a pivotal role in decreased life expectancy. Strong evidence has been provided that lower life expectancy in the MF elk population is associated in part to increased tooth wear caused by chronic ingestion of fluoride, another trace element found in elevated levels within geothermal watersheds of YNP (47). It is therefore unclear whether As poisoning may contribute to the lower life expectancy of MF elk. However, this study has shown that the elevated levels of As found in various exposure pathways (plants, sediments, etc.) may provide potentially toxic doses of As to ruminants.

Since ancestors of nonmigratory elk currently residing in the MF watershed have likely inhabited this region for thousands of years, it is possible that some individuals have evolved enhanced As resistance or detoxification mechanisms that allow them to forage on aquatic and terrestrial plants containing elevated levels of As. These mechanisms may include a digestive tract (i.e. rumen) microbial community which is capable of methylating significant quantities of inorganic As, or adaptations in elk

physiology providing greater capability to detoxify As. Future work is needed to test these hypotheses, including a thorough examination of freshly killed MF elk for signs of As toxicosis in diagnostic tissues, such as the liver and kidney, collection of fresh rumen content for use in microbially-driven As transformation studies, and perhaps a comparison of As dosing effects on farm-raised elk with and without rumen microbial inoculations derived from MF elk.

Summary

Elk residing in the MF watershed, YNP are exposed to elevated levels of As through several major pathways, including aquatic and terrestrial plant material, algae and sediments. Minor pathways representing lower As exposure include MF waters, terrestrial soils, and dry meadow vegetation. As a consequence of exposure via these major and minor pathways, MF elk contain elevated levels of As in their tissues, rumen content and feces compared to a control population; some As levels approach or exceed levels found in dosing studies where ruminants exhibited signs of chronic As toxicosis.

The analysis of various As species in selected plant, rumen and fecal samples indicate that the ingested forms of As represented by *Deschampsia cespitosa*, *Carex sp.*, *Potamogeton sp.* and algal slime are predominately inorganic, and that As is methylated during digestion, perhaps via microbial transformation or other physiological detoxification pathways. However, extraction efficiencies for algal slime and *Potamogeton sp.* samples were too low to definitively comment on the distribution of As

species in these samples. Further analyses of plant, rumen content, and fecal matter samples for various arsenic species is needed to better understand As exposure pathways to elk and subsequent rumen and/or physiological mechanisms of potential As detoxification.

Elk urine concentrations of As:creatinine were correlated with total winter SWE and increased over the course of a high total SWE winter, peaking between the months of February and March. The toxicological impacts of seasonal As exposure are confounded with effects of potential starvation and chronic fluoride exposure. However, As exposure yields elevated As levels in elk tissues and may contribute to the low life expectancy of MF populations, although evolutionary mechanisms may allow MF elk to resist or detoxify inorganic As present in MF forage. Finally, even though several studies exist describing the affect of high As levels on microbial life in YNP, additional studies are needed to describe the exposure and possible affect of As on other macrobiota closely associated with the geothermally impacted MF watershed.

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CHAPTER 4

SUMMARY

In Chapter 2, the rates of photochemical As(III) oxidation in the ferrioxalate system and in a natural water sample were investigated. An approximate 1:1 molar ratio of $\text{OH}^\bullet$ produced vs. As(III) oxidized indicated that $\text{OH}^\bullet$ and a secondary electron acceptor, such as O_2 , were the reaction constituents responsible for oxidation.

Competition experiments where solutions containing high concentrations of As(III) and 2-propanol confirmed this observation; the rate ratios of As(III) and 2-propanol oxidation by $\text{OH}^\bullet$ were consistent with rate constant data found in the literature.

Oxidation rates of As(III) in the ferrioxalate system vary from $0.5\text{--}254.0 \mu\text{M hr}^{-1}$ depending on a variety of factors, including pH, initial As(III) concentration, and initial Fe(III) concentration. Rates of As(III) oxidation increase with decreasing pH, due to an increase in the rate of H_2O_2 formation. The rate of As(III) oxidation at low pH may also increase due to the presence of photo-labile Fe(III)-hydrolysis species, increasing the rate of $\text{OH}^\bullet$ formation. A near psuedo first-order relationship exists between initial As(III) concentration and initial rate of As(III) oxidation, and initial Fe(III) concentration and initial rate of As(III) oxidation under some of the solution conditions employed. The rate

of As(III) oxidation in a natural water sample was 5.6 $\mu\text{M}/\text{hour}$ in sunlight, suggesting that photo-chemical processes may indeed play a significant role in the oxidation of As(III) in surface waters.

In Chapter 3, it was observed that elk residing in the MF watershed, YNP are exposed to elevated levels of As, primarily through ingestion of high-As aquatic and terrestrial plants, sediments, and algae. Plants growing in dry, terrestrial environments and soils collected from adjacent locations contained low As concentrations, and Madison, Firehole, and Gibbon River water contained low As relative to aquatic and terrestrial plants and sediment samples, indicating that the ingestion of dry-land plants, soils and water were less significant routes of As exposure to elk, although they may represent a small fraction of total ingested As. A consequence of exposure via these routes includes elevated levels of As in elk tissues, rumen content and feces compared to a control population; some of these levels approach or exceed levels found in dosing studies where ruminants exhibited signs of chronic arsenic toxicosis. Speciation of selected plant and elk samples indicate that the ingested forms of As are predominately inorganic, and that M-F elk may be detoxifying As via methylation. However, the distribution of As species in *Potamogeton sp.* and an algal slime sample is unknown due to low As extraction efficiencies. Increasing As:creatinine in elk urine over the course of high total SWE winters indicate seasonally-driven As exposure, with As:creatinine reaching peak levels between the months of February and March. Finally, average As:creatinine values are positively correlated with total winter SWE, implying that total winter snowfall is an important variable governing overall elk exposure to As.

The work presented in Chapter 2 will contribute to the field of environmental photochemistry and perhaps wastewater treatment. To date, very few data have been presented describing mechanisms of photochemical oxidation of As(III) in the presence of DOM. Furthermore, photochemistry may also play a very important role with respect to As redox cycling in surface waters; it is the author's hope that information presented herein will encourage further research in this topic. The work presented in Chapter 3 will contribute to the field of ecotoxicology, in which there is currently no data describing As accumulation in ruminants exposed to low levels of As that may induce potentially chronic effects. Thus, this work will serve to provide more data concerning As exposure routes and As tolerance in higher organisms inhabiting As-impacted watersheds.

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