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Biogeochemical transformations of arsenic in circumneutral freshwater sediments

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Abstract

Arsenic is a wide-spread contaminant of soils and sediments, and many watersheds worldwide regularly experience severe arsenic loading. While the toxicity of arsenic to plants and animals is well recognized, the geochemical and biological transformations that alter its bioavailability in the environment are multifaceted and remain poorly understood. This communication provides a brief overview of our current understanding of the biogeochemistry of arsenic in circumneutral freshwater sediments, placing special emphasis on microbial transformations. Arsenic can reside in a number of oxidation states and complex ions. The common inorganic aqueous species at circumneutral pH are the negatively charged arsenates ($H_2As^VO_4^-$ and $Has^VO_4^{2-}$) and zero-charged arsenite ($H_3As^{III}O_3^0$). Arsenic undergoes diagenesis in response to both physical and biogeochemical processes. It accumulates in oxic sediments by adsorption on and/or co-precipitation with hydrous iron and manganese oxides. Burial of such sediments in anoxic/suboxic environments favors their reduction, releasing Fe(II), Mn(II) and associated adsorbed/coprecipitated As. Upward advection can translocate these cations and As into the overlying oxic zone where they may reprecipitate. Alternatively, As may be repartitioned to the sulfidic phase, forming precipitates such as arsenopyrite and orpiment. Soluble and adsorbed As species undergo biotic transformations. As(V) can serve as the terminal electron acceptor in the biological oxidation of organic matter, and the limited number of microbes capable of this transformations are diverse in their phylogeny and physiology. Fe(III)-respiring bacteria can mobilize both As(V) and As(III) bound to ferric oxides by the reductive dissolution of iron-arsenate minerals. SO_4^{2-}-reducing bacteria can promote deposition of As(III) as sulfide minerals via their production of sulfide. A limited number of As(III)-oxidizing bacteria have been identified, some of which couple this reaction to growth. Lastly, prokaryotic and eukaryotic microbes can alter arsenic toxicity either by coupling cellular export to its reduction or by converting inorganic As to organo-arsenical compounds. The degree to which each of these metabolic transformations influences As mobilization or sequestration in different sedimentary matrices remains to be established.

The biological relevance of arsenic is twofold and relates primarily to its toxicity and/or its capacity to serve in oxidation-reduction reactions that conserve energy in microbial metabolism. Various studies suggest that humans are particularly susceptible to arsenic poisoning (Byron et al. 1967; Heywood & Sortwell 1979). Arsenic toxicity can manifest in every major organ system, and a growing body of evidence indicates that As can act as a potent carcinogen (Morton & Dunnette 1994). In 1996, the U.S. Congress amended

the Safe Drinking Water Act of 1974, and directed the Environmental Protection Agency to propose new standards for As in drinking water. In January, 2001, the final ruling was published, setting that standard at 0.01 mg/L. Public water systems have until 2006 to comply. These observations and developments underscore the need to better understand geochemical and biological factors that influence mobility and bioavailability of arsenic in the environment.

Arsenic is widely distributed but relatively rare, ranking 20th in elemental abundance in the continental crust (Cullen & Reimer 1989). Over 99% of arsenic occurs in rocks (Mackenzie et al. 1979). Sedimentary rocks often contain much higher concentrations than igneous or metamorphic rocks (Bhumbla & Keefer 1994), with the former varying from ∼2–400 mg As/kg, while the latter seldom surpass more than about ∼1.5–3 mg As/kg (Bhumbla & Keefer 1994; Smith et al. 1998). This arsenic is most often associated with sulfur compounds principally arsenopyrite (Boyle & Jonasson 1973). Arsenic associated with sulfide ore deposits can be highly elevated above these "background" values (1000s of mg/kg to a few wt. %). Much of the remaining arsenic reservoir resides in soils and the oceans. Arsenic concentrations in uncontaminated soils generally range from 5–10 mg/kg (Bhumbla & Keefer 1994). However, naturally elevated concentrations ranging from 250–500 mg/kg can occur in soils derived from shales (Colbourn et al. 1975; Bhumbla & Keefer 1994). In soils either adjacent to or derived from sulfide ore, concentrations can reach upwards of 8000 mg/kg (Levander 1977).

The primary source of arsenic in soils and sediments is the parent material from which they are derived (Yan-Chu 1994). Anthropogenic inputs to the environment also contribute to the redistribution of the global inventory. Chilvers & Peterson (1987) estimated that the ratio of natural-to-anthropogenic emissions of arsenic to the atmosphere is ca. 60 : 40, while Nriagu & Pacyna (1988) estimated a 30 : 70 ratio. Anthropogenic contributions include the industrial use of arsenic trioxide; the milling, smelting and/or weathering of Pb, Zn, Cu and Au ores; the production of fly and bottom ash from coal combustion; and the historical applications of inorganic arsenical compounds as pesticides and herbicides (for brief reviews, see Smith et al. 1998; Chilvers & Peterson 1987; Bhumbla & Keefer 1994).

Speciation critically determines arsenic toxicity and bioavailability (see Figure 1). For example, of the inorganic arsenic species, As(III) is considered more toxic than As(V), owing to its disruptive effect on protein disulfide bonds (Morton & Dunnette 1994). Evidence continues to accumulate implicating biotic transformations, specifically those in which bacteria utilize arsenic and iron as either electron donors or acceptors, as key factors that influence As distribution and speciation. Given public health concern over arsenic, its transformations in freshwater systems are of particular interest. In this communication, we provide a brief review of arsenic biogeochemistry as it pertains to circumneutral freshwater sediments. Special emphasis is placed on the influence of microbial metal(loid) dissimilation on arsenic speciation in iron-rich soils and sediments.

Arsenic transformations

Arsenic is a group 5A element that is isoelectric with nitrogen and phosphorus. It exhibits stability while in oxidation states +5, +3, 0 and −3. This variable charge allows the formation of a number of complex arsenic species (see Figure 1). The common inorganic aqueous species at circumneutral pH are the negatively charged arsenates ($H_2As^VO_4^-$ and $HAs^VO_4^{2-}$) and zero-charged arsenite ($H_3As^{III}O_3^0$). Inorganic and/or microbially-mediated redox reactions can transform inorganic arsenic species to methylated forms. Mono- and di-methyl As(V) species are the most common organic aqueous forms of arsenic. With continued reduction these can be reduced to the volatile and very toxic methyl-arsines. Arsenic metal (As^0) rarely occurs in nature, and arsine (AsH_3) does so only at very low pE values (Ferguson & Gavis 1972). Inorganic oxidation of arsenite by oxygen in natural waters would be slow without microbial catalysis and/or other redox agents. However, in the presence of redox pairs such as Fe^{3+}/Fe^{2+}, Mn^{4+}/Mn^{2+}, SO_4^{2-}/HS^-, or HCO_3/CH_4, significant oxidation of As(III) or reduction of As(V) can occur at rates that are environmentally relevant (Cherry et al. 1979).

Some redox controls involve multiple transformations. For example, nitrate can control aqueous arsenic concentrations by oxidizing ferrous iron to ferric oxides. Ferric oxides can then co-precipitate with arsenic, removing it from the water column (Senn & Hemond 2002). Soluble arsenic commonly partitions into the solid phase of soils and sediments. Numerous studies attribute this to binding with hydrous oxides of iron, manganese and aluminum (Aggett & O'Brien 1985; Oscarson et al. 1983; Mok & Wai 1994), clays

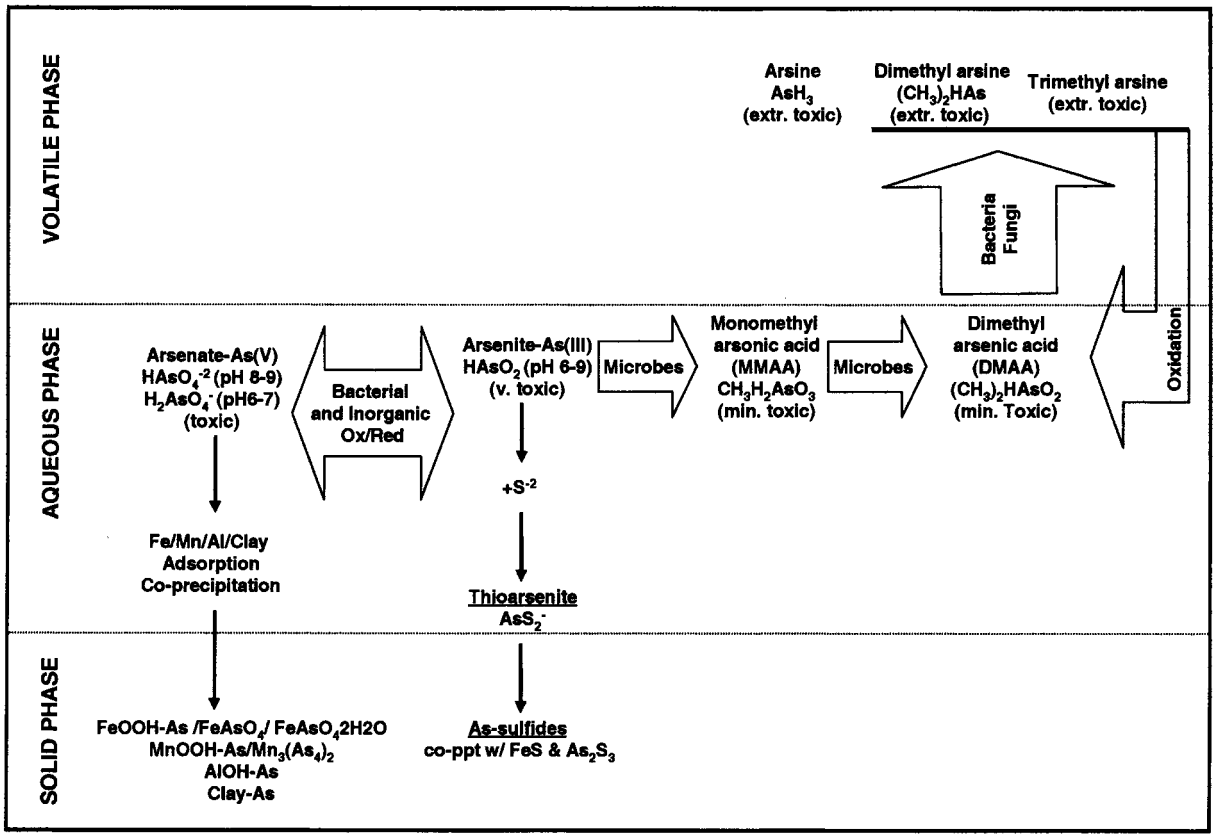

Figure 1. Hypothetical cycling of arsenic in a freshwater system. (Modified from Bhumbla & Keefer 1994.)

(Frost & Griffin 1977), and sulfides (Moore et al. 1988). The dominant mechanism by which arsenic accumulates in oxic sediments is believed to be adsorption on and/or co-precipitation with hydrous iron oxides (Aggett & O'Brien 1985; Mok & Wai 1994). Some authors have reported that arsenate adsorbs onto iron hydroxides more strongly than does arsenite (Pierce & Moore 1982; DeVitre et al. 1991). However, recent laboratory studies suggest that, at circumneutral pH, arsenite and arsenate can be retained equally well by iron oxide surfaces (Sun & Doner 1996; Manning et al. 1998; Jain et al. 1999).

Hydrous manganese oxides can also adsorb arsenic and catalyse arsenic redox reactions. Certain manganese oxide minerals readily oxidize arsenite to arsenate (Oscarson et al. 1980, 1981, 1983). This capability is dependent on mineral structure. Birnessite, a manganese oxide with a clay-mineral layered structure, sorbs arsenite into the interlayer where it autocatalytically reacts with Mn^{4+} to form arsenate (Moore et al. 1990). Arsenate is partially incorporated into the mineral structure and some released to the aqueous phase along with interlayer cations. These combination redox-adsorption reactions can occur quite rapidly (minutes to a few hours).

When buried in anoxic/suboxic environments, iron and manganese oxyhydroxides can be reduced and dissolve, releasing Fe^{2+} and Mn^{2} and associated adsorbed/coprecipitated arsenic. The upward advection of pore water carries these cations and arsenite into the overlying oxic zone where arsenic co-precipitates with manganese and iron oxides (Masscheleyn et al. 1991; Aggett & O'Brien 1985; Ferguson & Gavis 1972). Because Mn^{2+} oxidizes much more slowly than Fe^{2+}, it can also migrate into higher layers than iron (Balzer 1982). This "chromatagraphic" separation likely explains the different associations of arsenic with Mn and Fe observed in soils and sediments (Murray & Brewer 1977). A repartitioning of bound arsenic from iron and manganese (oxy)hydroxides to the sulfidic phase very often arises as sediments shift from oxic to anoxic, sulfidic environments (Moore 1988). Diagenetic sulfides scavenge desorbed As(III), forming precipitates such as FeAsS and As_2S_3 (Rittle et al.

1995; Newman et al. 1997) or adsorb onto iron-sulfide surfaces (Davis 1984). Anoxic sediment can therefore become an arsenic sink. However, upon re-establishment of oxic conditions, either by a shift in the redox boundary or a physical disturbance, sulfides can be oxidized and the complexed arsenic released (Moore et al. 1988).

The speciation and mobility of arsenic can exhibit seasonal and annual variability, much of which may be owed to biotic transformations (Kuhn & Sigg 1993; Spliethoff et al. 1995). Unfortunately, the relative contributions of microbial processes to arsenic cycling have only recently begun to be elucidated. This is especially true for the methylated species of arsenic. Additionally, there is an incomplete understanding of the biogeochemical controls on arsenic speciation and transformation among reservoirs (sediment/soil $\leftrightarrow$ aqueous $\leftrightarrow$ atmosphere). However, it appears clear that many of these transformations are driven and accelerated by microbial metabolism.

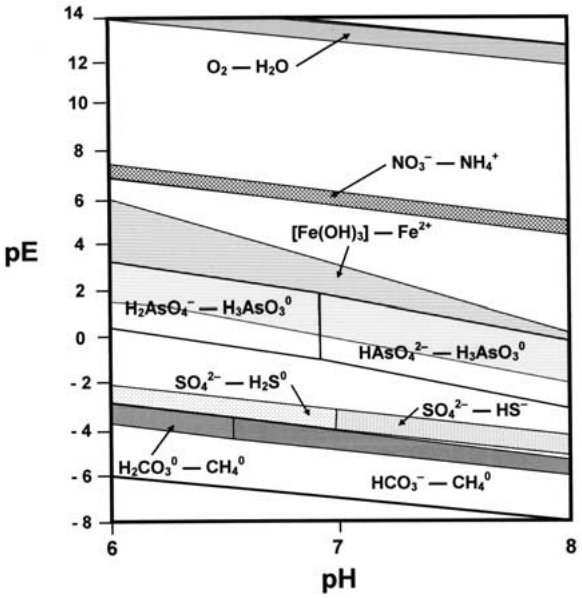

Figure 2. Redox windows for important redox species compared to arsenic species used by microbes in typical circumneutral aquatic environments. Modified from Cherry et al. (1979). Diagram assumes concentrations of the species from the analytical detection limit to common values found in fresh water. pE represents the negative logarithm of electron activity, and is related to the thermodynamic redox potential by the expression pE = (F/2.3RT)E_h.

Microbial arsenate reduction

Viewed from a thermodynamic perspective, heterotrophs help resolve chemical disequilibria that arise primarily from oxygenic photosynthesis. These disequilibria persist because of the non-uniform distribution of redox-active materials in the environment and the slow kinetics of many energetically favorable redox reactions (Fenchel & Finley 1995). Heterotrophic metabolism results in oxidation of biologically-reduced carbon, and is constrained by the relative abundance of suitable electron acceptors. Based upon the relative $-\Delta G$ of their redox couples, microbial respiratory preferences should sequentially deplete $O_2 \leftrightarrow H_2O$, $NO_3^- \leftrightarrow N_2$, $Mn^{4+} \leftrightarrow Mn^{2+}$, $NO_3^- \leftrightarrow NH_4^+$, $Fe^{3+} \leftrightarrow Fe^{2+}$, $SO_4^{2-} \leftrightarrow HS^-$, and $CO_2 \leftrightarrow CH_4$ (Scott & Morgan 1990). Biotic arsenate reduction is also feasible, with the free energy of $As^{5+} \leftrightarrow As^{3+}$ falling between the oxidation of ferric iron and sulfate (Sadiq 1990). However, in natural systems the distribution of any terminal electron accepting process is strongly dependent on local pH and redox status (see Figure 2), as well as the speciation and the relative abundance of all potential electron acceptors.

The first description of microbial arsenic metabolism was made by Green (1917), who sought to determine why arsenical cattle dips lost their efficacy over time. Green isolated an apparent arsenate-reducer, *Bacterium arsenreducens*, and an arsenite-oxidizer, *B. arsenoxydans* (Green 1917). The former was a facultative anaerobe. In mixed cultures grown in minimal media arsenite oxidation prevailed, whereas arsenate reduction prevailed in media amended with glycerine, glucose or fresh livery manure. These observations suggest that *B. arsenreducens* reduced As(V) via a detoxification mechanism, not respiration. Likewise, Woolfolk & Whiteley (1962) demonstrated the coupling of arsenate reduction to the oxidation of molecular hydrogen by cell extracts of *Micrococcus lactilyticus* (*Veillonella alcalescens*). These authors concluded that the activity was neither assimilatory nor energy-conserving.

Arsenate-reducing bacteria have been isolated from arsenic-rich soils and sediments using anaerobic minimal media where As(V) serves as the sole terminal electron acceptor. Both primary enrichments from contaminated sediments and pure culture isolates have been shown capable of reducing up to several millimoles per liter of arsenate per day (Ahmann et al. 1994; Dowdle et al. 1996; Newman et al. 1998). Jones et al. (2000) reported that in an un-enriched soil community arsenate reduction commenced largely after microbial growth had ceased. From this community, an arsenic-reducing bacterium, strain CN8, was isol-

ated using glucose as electron donor. Because growth rate and yield were independent of initial As(V) concentration and mass of As(V) reduced, and because the stoichiometry of H_2 and butyric acid production indicated their use as terminal electron acceptors, Jones et al. (2000) concluded that strain CN8 reduces As(V) *via* a detoxification mechanism wherein As(V) is reduced to As(III) prior to export. It is noteworthy that the rates of As(V) reduction by CN8 were 5- to 10-fold lower than reported rates of dissimilatory As(V) reduction (e.g., Laverman et al. 1995).

Under anoxic conditions, arsenate has can serve as a terminal electron acceptor in the biological oxidation of organic matter. Accordingly, a number of microbes have been recently isolated on the basis of their ability to respire arsenate. The first to be discovered, *Sulfurospirillum arsenophilum* strain MIT-13, was isolated by Ahmann et al. (1994) from arsenic-contaminated sediments of the Aberjona watershed in eastern Massachusetts. Strain MIT13 grows in anaerobic medium with lactate as the substrate and As(V) as the electron acceptor. For example, provided 2 mM lactate and 10 mM arsenate, populations double about every 14 h. Nitrate and fumarate can also serve as terminal electron acceptors (Stolz et al. 1999).

The microaerophile *Sulfurospirillum barnesii* strain SES-3 can also respire arsenate, in addition to selenate, Fe(III), thiosulfate, elemental sulfur, Mn(IV), nitrate and nitrite, trimethylamine oxide and fumarate (Laverman et al. 1995; Stolz et al. 1999). Interestingly, while both selenate-grown and nitrate-grown SES-3 demonstrate the constitutive ability to reduce arsenate, selenate- and nitrate-reduction cannot occur simultaneously. This observation suggests competitive inhibition and implies that nitrate and selenate reductases may also catalyze As(V) reduction. Experiments by Zobrist et al. (2000) confirm these observations, as well as the simultaneous reduction of ferric iron and arsenate. This last activity of SES-3 makes possible dissimilatory Fe(III)- and As(V)-reduction of ferrihydrite to which arsenate is adsorbed. Respiration of arsenate adsorbed onto aluminum hydroxide demonstrates that SES-3 can reduce As(V) prior to its dissolution (Zobrist et al. 2000).

Although the number of validated species capable of dissimilatory arsenic reduction is limited, these organisms, considered as a group, show considerable phylogenetic and metabolic diversity (see Figure 3 and Table 1, respectively). *S. arsenophilum* and *S. barnesii* belong to the ε-subdivision of the Proteobacteria. Arsenate-reducing members of the δ-subdivision have also been identified. For example, Macy et al. (2000) isolated *Desulfomicrobium* strain Ben-RB and *Desulfovibrio* strain Ben-RA from an arsenic-contaminated reed bed in Bendigo, Australia. Both strains ally with sulfate-reducing lineages. Each can reduce sulfate and arsenate concomitantly, but only *Desulfomicrobium* strain Ben-RB appears capable of employing arsenate as sole electron acceptor. This was attributed to *Desulfomicrobium* strain Ben-RB maintaining a membrane-bound cytochrome (or an enzyme associated with one), and *Desulfovibrio* strain Ben-RA a chromosomal homologue of *arsC*. Although *Desulfomicrobium* strain Ben-RA has yet to be adequately characterized, Leu et al. (1999) reported that *Desulfomicrobium* isolates collected from two different marine oil fields are also able to reduce sulfite and thiosulfate, but not nitrate.

Several low G+C, gram-positive arsenate reducers have been collected. Newman et al. (1997) isolated the spore-forming, sulfate-reducer *Desulfotomaculum auripigmentum* strain OREX-4 from Upper Mystic Lake, Woburn, Massachusetts. OREX-4 preferentially respires arsenate over sulfate. Although expected thermodynamically, this property may result from sulfate reduction being inhibited by the presence of arsenate. Consistent with this idea, molybdate was shown to diminish the reduction of both arsenate and sulfate, although evidence directly linking these pathways was not provided. Newport & Nedwell (1988) suggested that the inhibitory effect of molybdate on SRB requires an active sulfate reduction pathway. Their addition of thiosulfate to cultures of *Desulfotomaculum ruminis*, *Desulfovibrio vulgaris* and *D. desulfuricans* grown in lactate/sulfate medium resulted in two- to four-fold increases in the minimum MbO_4 concentration required for inhibition (Newport & Nedwell 1988). Moreover, in media lacking sulfate, *D. ruminis* and *D. vulgaris* grew in the presence of 10 mM MbO_4.

Niggemyer et al. (2001) isolated spore-forming *Desulfitobacterium* strain GBFH from Lake Coeur d'Alene, Idaho. This bacterium affiliates closely with *Desulfitobacterium frappieri* strain PCP-1 and *Desulfitobacterium hafniense* strain DCB-2, and it has been suggested that the three may comprise a single species. They exhibit similar carbon source utilization profiles and nearly identical terminal electron acceptor preferences, the notable difference being the inability of GBFH to reduce nitrate. All three can respire As(V), along with Fe(III), Se(VI), Mn(IV), sulfite,

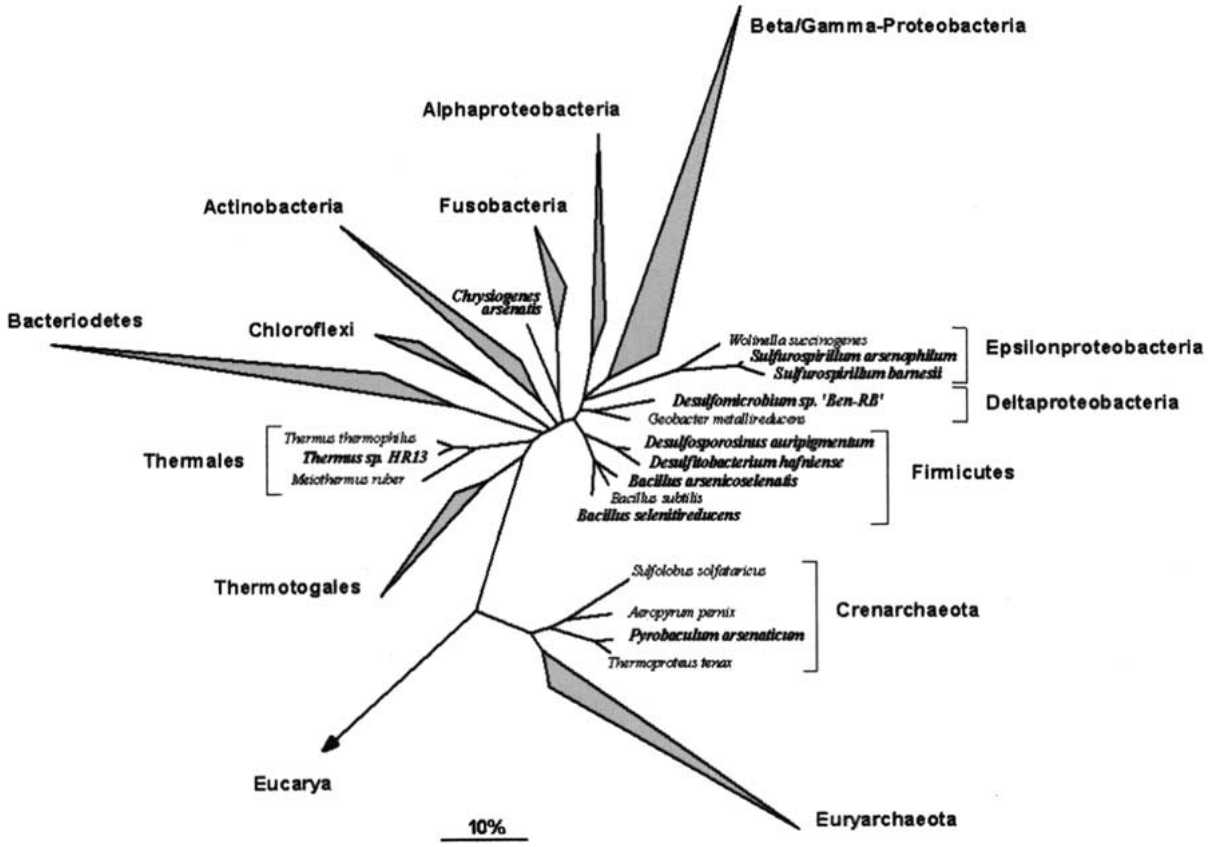

Figure 3. Unrooted phylogenetic tree showing the positions of currently recognized As(V)-reducing microorganisms (in bold) among the major lineages of prokaryotes. The tree is based upon an optimized global tree reconstructed from almost full-length SSU rRNA gene sequences belonging to all three domains using various treeing programs including in the ARB package (http://www.arb-home.de/). The bar indicates 10% estimated sequence divergence.

thiosulfite, elemental sulfur and fumarate. Blum et al. (1998) isolated *Bacillus arsenicoselenatis* strain E1H and *Bacillus selenitireducens* strain MLS10 from Mono Lake, California. Both couple the oxidation of lactate to the reduction of arsenate. The strict anaerobe *B. arsenicoselenatis* also reduces Se(VI) to Se(IV), and the microaerophile *B. selenitireducens* Se(IV) to Se(0). These activities enable the complete reduction of Se(VI) to Se(0) when the strains are grown in co-culture. These bacteria showed limited ability to grow on alternative electron donors and accepters.

Three additional clades contribute to the currently recognized diversity of arsenate-respiring bacteria (Figure 3). Macy et al. (1996) isolated *Chrysiogenes arsenatis* strain BAL-1 from a reed bed at Ballarat Goldfields, Australia. *Chrysiogenes arsenatis* appears to represent a new and deeply branching lineage of the Bacteria (Macy et al. 1996). Using acetate as a substrate, growth occurred with arsenate and nitrate as electron acceptors, but not sulfate, thiosulfate or Fe(III). BAL-1 also reduced arsenate bound to iron oxide. Gihring and Banfield (2001) isolated *Thermus* strain HR13 from Growler Hot Spring in northern California. This microbe appears to carry out both arsenite oxidation and arsenate respiration. Experiments investigating the ability of HR13 to oxidize arsenite in unaerated cultures revealed cyclic transformations of As(III) to As(V) and back again. The authors suggested that this remarkable cycling might either have resulted from the alternation of detoxification and respiration, or from vacillating oxygen levels. Energy was not conserved by arsenite oxidation, however, As(V) respiration did accompany lactate oxidation. Although not yet thoroughly investigated in HR13, studies of *Thermus* strain SA-01 demonstrated use of nitrate, Fe(III) and elemental sulfur as electron acceptors (Kieft et al. 1999). Huber et al. (2000) isolated *Pyrobaculum arsenaticum* – a hyperthermophilic ar-

Table 1. Summary of relevant physiological characteristics of previously published DasRB[a]

Physiological parameters	Previously characterized DasRB									
	GBFH^T	MIT-13^T	SES-3^T	BAL-1^T	ORTEX-4^T	E1H^T	MLS-10^T	BEN-RB[b]	PZ6^T	HR13[b]
Electron donors										
Lactate	+	+	+	+	+	+	+	+		+
Pyruvate	+	+	+	+	+	−	+			
H_2 + acetate	−	+	+	+	+	−	−			
Fumerate	+	+	+	+	−					
Malate	−	[a]		+	+	+	−			
Succinate	−		+	+	−	−	−			
Citrate	−		+	−		+	−			
Butyrate	−	+[c]			+					
H_2	−	−	−	−	+				+	
Formate	+	+[c]	+[c]	−	−	−	−			
Acetate	−	−	−	+	−	−	−		−	
Glucose	−			−	−	−	+			
Glycerol	−				+					
Ethanol	−				+	−	−			
Galactose	−					−	+[d]			
Methanol	−			−	−	−	−			
Electron acceptors										
As(V)	+	+	+	+	+	+	+	+	+	+
Nitrate	−	+	+	+	−	+	+		−	
Nitrite	−	+	+	+		−	+			
Se(VI)	+	−	+	−		+	−		+	
Se(IV)	−	−	−		−	−	+		−	
Sulfate	−	+	+	−	+	−	−	+		
Thiosulfate	+	+	+	−	+	−	−		+	
Sulfite	+		−		+	−	−			
S_0	+	+	+						+	+
Fe(III)	+		+	−	−	+	−			+
Mn(IV)	+	−	+		−					
Fumarate	+	+	+		+	+	+			
O_2	−	+	+[c]	−	−	−	+[e]		−	
Estimated optima										
Temp. (°C)	37	20	33	27	25–30				95	75
PH	7.5	7.5	7.5		6.4–7.0	8.5	9.5			7
Doublings (h)	3.5	14	5	4				9	1.3	

[a] Blank cells indicate that no data are available with regard to the specific variable. See text for references to DasRB isolates.
[b] Physiological characterization is minimal with no formal survey of electron donors or acceptors.
[c] Growth occurs only in the presence of acetate as a carbon source.
[d] Growth is fermentative rather than respiratory.
[e] Growth is microaerophilic.

chaeon – from a hot spring at Pisciarella Solfatara, Naples, Italy. Growing chemolithoautotrophically, *P. arsenaticum* used CO_2 as a carbon source, H_2 as an electron donor, and arsenate, thiosulfate and elemental sulfate as electron acceptors. Growing organotrophically, selenate was also respired. A previously catalogued strain of *P. aerophilum* (Volkl et al. 1993) has also been shown capable of respiring arsenate and selenate, both chemolithoautotrophically and organotrophically.

The extent to which microbial respiration of arsenic actually accounts for its mobilization in contaminated sediments has yet to be resolved. Dowdle et al. (1996) observed dissimilatory reduction of ar-

senic in anoxic slurries collected from a San Francisco Bay salt marsh and from Lahontan Reservoir in Nevada. Upon amendment with 10 mM arsenate, live sediments completely removed As(V) from solution in under a week; amendment with either lactate, H_2 and glucose considerably enhanced rates of arsenate reduction. No such effect was detected in either heat-sterilized or formalin-killed cultures. Moreover, treatment with inhibitors/uncouplers of respiration such as dinitrophenol, rotenone or 2-heptyl-4-hydroxyquinoline *N*-oxide also stopped arsenate reduction. As(V) reduction was not inhibited by molybdate, suggesting that the process was independent of SRB-mediated sulfidogenesis.

Ahmann et al. (1997) observed the rapid catalysis of arsenic from iron arsenate in sediments collected from the Halls Brook Storage Area in eastern Massachusetts. Aqueous As(V) dropped from ~165 μM to 0.85 μM in the first two days' incubation of live sediments. Reduction of solid ferric arsenate continued to occur after soluble As(V) was depleted, and within two weeks concentrations of dissolved As(III) increased to ~725 μM. This was accompanied by a proportional increase in aqueous Fe(II). Negative controls consisting of heat- or formalin-killed sediments did not accumulate soluble As(III) and Fe(II). To investigate the involvement of iron-reducing bacteria, parallel cultures were incubated using a form of ferrous arsenate that does not support iron dissimilation. Consistent with the lower solubility of ferrous arsenate, aqueous As(V) remained low; but, comparable accumulations of As(III) still took place.

Similar patterns have been observed in heavy metal(loid)-contaminated sediments of Lake Coeur d'Alene, Idaho (Harrington et al. 1998). In anaerobic sediment slurries amended with 10 mM As(V) and incubated 30 days, ~30% of As(V) was transformed to As(III). Amendment with organic acids resulted in a 2–3-fold increase in As(V) reduction, while levels of As(III) and As(V) in formalin-killed cultures changed little. Most probable number (MPN) estimates of Lake Couer d'Alene sediments revealed that densities of sulfate-, iron- and arsenate-reducing bacteria were approximately 10^6, 10^5, and 10^4 cells g^{-1} wet weight sediment, respectively (Harrington et al. 1998; Cummings et al. 2000). Inhibition of the sulfate-reducing community by the addition of molybdate diminished As(V) reduction in some cultures, suggesting that at least some SRB in Lake Coeur d'Alene are also capable of reducing arsenate. Ferrous iron production occurs in this environment, and dissimilatory iron reducers are a conspicuous feature of the resident microbial community (Cummings et al. 1999, 2000). However, the relative contribution made by FeRB, SRB and AsRB to arsenic transformation and mobilization remains to be determined.

Dissimilatory arsenic reduction appears to proceed via the activity of respiratory arsenate reductases associated with cytochromes (Stolz & Oremland 1999). To date, only the systems of *Sulfurospirillum barnesii* strain SES-3 and *Chrysiogenes arsenatis* have been appreciably described. Newman et al. (1998) suggested that the arsenate reductase of SES-3 should span the cytoplasmic membrane, with the active site facing the cytoplasm. This assertion was supported by the observation that arsenic trisulfide particles had been shown to accumulate intracellularly along the cytoplasmic membrane in respiring *Desulfotomaculum auripigmentum* (Newman et al. 1997). Oremland & Stolz (2000) subsequently reported the purification of the enzyme – a membrane bound, ~120 kDa trimeric complex. This protein has an α subunit of 65 kDa, a β subunit of 31 kDa, and a γ subunit of 22 kDa. A *b*-type cytochrome appears to complement membrane fractions. Stolz et al. (1997) demonstrated that membrane fractions from cells grown only on nitrate, fumarate, selenate or thiosulfate are capable of reducing all of these substrates; however, enzyme activity was greatest for the substrate on which the cells had been originally grown. Newman et al. (1998) reported estimates of V_{max} for this arsenate reductase that are several-fold greater than values observed for the ArsC enzymes of *Escherichia coli* and *Staphylococcus aureus*. K_m values were said to be about an order of magnitude lower, as well. Unlike SES-3, the soluble arsenate reductase of *C. arsenatis* appears to reside in the periplasm (Krafft & Macy 1998). Likely a heterodimer, this protein is composed of two subunits having masses of 87 kDa (ArrA) and 29 kDa (ArrB), and contains molybdenum, iron, acid-labile sulfur and zinc as cofactors. Interestingly, nitrate, sulfate, selenate, and fumarate cannot serve as electron acceptors for this species. And, as observed for SES-3, the estimated values for V_{max} and K_m of the *C. arsenatis* arsenate reductase were higher and lower, respectively, than those reported for ArsC.

Other microbial arsenic transformations

Dissimilatory iron reduction

Although both As(V) and As(III) can bind strongly to hydrous ferric oxides under oxic conditions (Fendorf et al. 1997; Manning et al. 1998), adsorbed arsenic can be mobilized by the activity of dissimilatory iron-reducing bacteria (FeRB) upon establishment of anoxia. Cummings et al. (1999) showed that the dissimilatory iron-reducer *Shewanella alga* strain BrY promotes arsenate mobilization from both synthetic scorodite ($FeAsO_4 \cdot 2H_2O$) and As-contaminated sediments collected from Lake Coeur d'Alene, Idaho. Respiratory reduction of Fe(III) to Fe(II) accounted for arsenic mobilization, as no reduction of As(V) to As(III) occurred. This ability to couple the reduction of Fe(III) with growth is broadly distributed. Phylogenetic analyses of small-subunit ribosomal gene sequences indicate FeRB representatives within the *Proteobacteria*; endospore-forming, low G+C, gram-positive bacteria; thermophilic *Archaea*; and several other distinct clades within the *Bacteria* (for reviews, see Lonergan et al. 1996; Lovley et al. 1997; Lovley 2000; Cummings et al. 2001). Given the abundance of iron in many soils and sediments, it is imperative that we better understand the role(s) dissimilatory iron-reducing bacteria play in solubilizing iron arsenate minerals.

Dissimilatory sulfate reduction

Arsenic often re-partitions from iron and manganese oxyhydroxides and organics in oxidized, surficial sediments to the sulfidic phase situated below the redox interface (Moore et al. 1988). However, at surface temperatures and pressures, this shift requires that the reduction of sulfate to sulfide be conducted by sulfate-reducing bacteria, or SRB (Goldhaber & Kaplan 1974). Rittle et al. (1995) and Castro et al. (1997) demonstrated that stimulation of the sulfate-reducing community facilitated deposition of arsenic as metal sulfides. In microcosms containing mining-contaminated sediments from the Milltown Reservoir in western Montana (Rittle et al. 1995) and the Summer Camp Pit in northern Nevada (Castro et al. 1999), organic carbon amendments were associated with steep declines in soluble arsenic, iron and sulfate. At least some of the arsenic apparently precipitated as arsenopyrite or an amorphous equivalent. In related work, Dvorak et al. (1992) used simple anaerobic reactors filled with mushroom compost to treat metal-contaminated waters from two Pennsylvania coal mines; iron, nickel, cadmium and zinc were efficiently removed from solution as monosulfides.

Direct biomineralization of arsenic has also been observed. Newman et al. (1997) provided evidence that *Desulfotomaculum auripigmentum* can precipitate orpiment, As_2S_3. Both As(V) and S(VI) can serve as electron acceptors, but the possibility that As_2S_3 formation also functions as a detoxification mechanism still exists. Although orpiment remains stable over a fairly limited range of pH values and S(II) concentrations (Newman et al. 1997), the formation of authigenic sulfides can apparently serve to sequester large amounts of trace elements, including arsenic (Moore et al. 1988).

Microbial arsenic oxidation

The oxidation of arsenic sulfide minerals such as orpiment, realgar and arsenopyrite releases As(III) into the aqueous phase. As the chemical oxidation of arsenite can be quite slow, the reaction proceeds most typically by means of a microbial or surface catalyst (Ferguson & Gavis 1972; Cherry et al. 1979). Two members of the Proteobacteria, *Pseudomonas arsenitoxidans* (Ilyaletdinov & Abdrashitova 1981) and *Agrobacterium/Rhizobium*-like NT-26 (Santini et al. 2000), have been shown to grow chemolithoautotrophically with arsenite, oxygen and carbon dioxide.

The remaining few arsenite-oxidizing bacteria (AsOxB) hitherto described are heterotrophs, and appear to engage this activity only as a detoxification measure. Nevertheless, field studies suggest that rates of naturally occurring arsenite oxidation can be quite rapid. In Hot Creek, a tributary of the Owens River in California, Wilkie & Hering (1998) observed the almost complete oxidation of As(III) within $\sim$1200 m of its geothermal source. Conservative transport of total dissolved arsenic prevailed over the reach, with the half-life of the in-stream oxidation calculated at $\sim$0.3 h. In batch kinetic studies, oxidation was not observed after sterile filtration of the creek water or after the application of antibiotics. In a thermal spring located in the Hundred Springs Plain of Norris Geyser Basin, Yellowstone National Park, Langner et al. (2001) also observed the rapid oxidation of As(III) immediately following its discharge into shallow surface waters. The rate greatly surpassed that measured at Hot Creek, displaying a half-life of about 0.6 min. EDS spectra of the microbial mat coinciding with the

region of As(III) oxidation revealed co-precipitation of iron(III) oxyhydroxide with As(V).

Membrane transport of arsenic

Arsenate has been shown to compete with phosphate for uptake by microbial inorganic phosphate transport systems (Willsky & Malamy 1980). However, several mechanisms hinder its accumulation or attenuate assimilated reserves. For example, bacterial arsenic resistance (*ars*) operons encode specific efflux pumps capable of exporting intracellular arsenic. Plasmids conferring arsenic tolerance encode both an arsenate reductase (ArsC) and one of two arsenite-specific efflux pumps (ArsB or ArsAB). ArsAB functions as an anion-translocating ATPase, and ArsB a secondary carrier protein; both are capable of exporting arsenite (Rosen 1999). ArsC functions to reduce arsenate to arsenite (Martin et al. 2001), facilitating the activity of ArsAB or ArsB.

Arsenic methylation

Microbes can reduce inorganic As toxicity by converting these species to organo-arsenical compounds such as monomethylarsonic acid (MMAA) and dimethylarsinic (DMAA) acid. Phytoplankton appear to produce much of MMAA and DMAA in aquatic systems (Cullen & Reimer 1989), although bacteria are also capable of such transformations (Spliethoff 1995). Numerous authors have reported that distribution of reduced and methylated arsenic species varies seasonally in the photic zone of lakes. In samples obtained from a variety of localities throughout the United States, Andreae (1978) noted that the concentrations of arsenite and methylated arsenicals positively correlated with indicators of primary productivity such as chlorophyll levels and ^{14}C-uptake. Anderson & Bruland (1991) also observed elevated DMAA during summer and fall in Davis Creek Reservoir in northern California. Additionally, water samples from a number of lakes, mostly in California, showed measurable concentrations of methylated arsenic, ranging from 1–59% of total arsenic. In Lake Biwa, Japan, Hasegawa (1997) related an increase in DMAA to the yearly maximum of water temperature. Sohrin et al. (1997) confirmed this observation, while also noting a rise of As(III) in summer and fall, prior to and following the summer peak of methylated arsenic. In both natural and experimental systems, DMAA(V) comprises most of the methylarsenicals produced by phytoplankton (Cullen & Reimer 1989). Methylarsenic(III) species can be produced, but are typically oxidized following their excretion (Hasegawa et al. 2001).

The relative contributions made by algae, plants, fungi, and perhaps even some animals to arsenic flux in the environment have yet to be evaluated. Pawlik-Skowronska (2001) showed that filamentous green algae of the genus *Stigeoclonium* isolated from both mining-contaminated and unpolluted waters were capable of producing comparable amounts of phytochelatins upon exposure to heavy metal(loid)s. However, the magnitude of algal contribution to overall arsenic sequestration was not evaluated. Likewise, the biosynthesis and release of arseno-sugars has been recognized as a pathway by which aqueous As could be redistributed. To date, investigations of this phenomenon have been largely focused on marine systems (e.g., Geizinger et al. 2001). Regardless of uncertainty regarding organoarsenical compounds in freshwater systems, it is noteworthy that much, if not most, of the organic reservoir resides in the photic zone. Moreover, any incorporation of arsenic by freshwater biota requires its prior mobilization from sediment and/or input from the terrestrial environment.

Concluding remarks

Many watersheds worldwide regularly experience severe arsenic loading. This can occur as a result of weathering and secondary transport of soils naturally enriched with arsenic (Bhumbla & Keefer 1994). Arsenic contamination of groundwater in Bangladesh provides an unfortunate reminder of the scale this problem can attain. As reported by the British Geological Survey (BGS Technical Report WC/00/19, Vol. 1 (2001); [http://www.bgs.ac.uk/arsenic/Bangladesh]), water from 27% of shallow tube-wells exceed the Bangladesh standard for arsenic in drinking water of 50 μg/L; 46% exceed the World Health Organization standard of 10 μg/L. Altogether, 1.5–2.5 million wells are estimated to be contaminated with arsenic at levels that surpass the Bangladeshi standard, exposing approximately 35–57 million people to tainted drinking water. This arsenic is of natural origin, and is thought to be released from iron oxides which had earlier scavenged As from river water during its transport in the normal river load (Nickson et al. 1998).

Arsenic loading can also occur as a result of the influx of soils and sediments contaminated by the mining of As-rich ores, agricultural use of arsenical pesticides, and industrial practices that

promote As release (for brief reviews, see Smith et al. 1998; Chilvers & Peterson 1987). Indeed, estimates suggest that most arsenic encountered in the environment is of anthropogenic origin (Nriagu & Pacyna 1988). An exhaustive study (National Research Council 1999) of health risks associated with low-level As recently prompted the U.S. EPA to revise Federal standards for As in drinking water from 50 to 10 μg/L (40 CFR Parts 9, 141 and 142; [http://www.epa.gov/safewater/ars/arsenic_finalrule.pdf]). Public water systems have until 2006 to comply. Current EPA estimates suggest that roughly 5–6% of the 54,000 community and 20,000 non-transient, non-community water systems will need to take measures to meet this new standard (see [http://www.epa.gov/safewater/ars/ars_rule_techfactsheet.html]). The EPA estimates that total annual cost for implementation of the rule will be about $181 million; however, the American Water Works Association (AWWA) puts the cost at $600 million annually with $5 billion in initial capital outlays ([http://www.awwa.org/pressroom/pr/010111.htm]). These developments underscore the need to better understand the factors that influence the fate and stability of arsenic in freshwater systems.

Various observations suggest that in permanently submerged sediments, arsenic predictably undergoes diagenesis (Aggett & O'Brien 1985; Fergusen & Gavis 1972; Oscarson et al. 1983; Mok & Wai 1994; Frost & Griffin 1977; Moore et al. 1988). In aerobic waters and surficial sediments, dissolved arsenic adsorbs onto and precipitates with hydrous iron and manganese oxides. Subsequently, microbial degradation of organic matter and burial by sedimentation combine to deplete oxygen and establish reducing conditions that favor use of oxidized iron, manganese, and arsenic as terminal electron acceptors. Under conditions where pore water undergoes advection, soluble products of these transformations may be translocated towards the sediment-water interface. Under oxic conditions these will reprecipitate as oxyhydroxides. Alternatively, under reducing conditions soluble As species can become immobilized as sulfidic precipitates. The former process likely explains the patterns of As distribution observed in Figure 4 (also see Harrington et al. 1998; Cummings et al. 2000). In separate mining-impacted freshwater watersheds we have determined that total arsenic is concentrated near the sediment-water interface. For both systems the observed pattern is inconsistent with the history of As deposition. Both field E_h measurements and soluble Fe(II) concentrations indicate that As is maximally abundant near the redox boundary (data not presented).

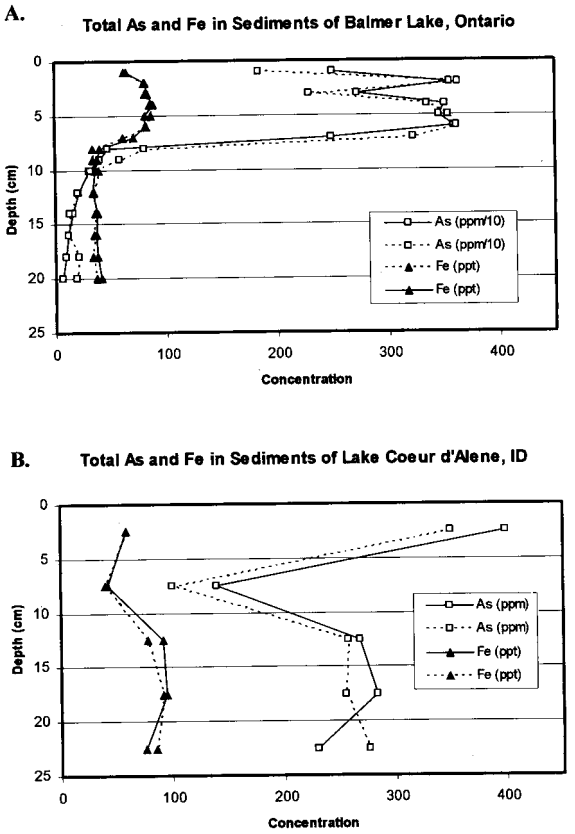

Figure 4. Vertical distribution of total arsenic and iron in the mining-contaminated sediments of (A) Balmer Lake, Ontario, and (B) Lake Coeur d'Alene, ID.

The geochemistry of arsenic is complex and lends itself to energy transformations useful for microbial metabolism. Arsenic can reside in a number of oxidation states and complex ions with negative to neutral charges. This allows a wide array of species that can be utilized during redox and complexation reactions so that arsenic undergoes a plethora of microbially-mediated transformations. Both soluble and adsorbed As(V) can serve as an electron acceptor in the bacterial oxidation of organic matter (Newman et al. 1998; Ahmann et al. 1994). Also, As(V) adsorbed onto hydrous ferric oxides can be released by the respiratory reduction of Fe(III) by dissimilatory iron-reducing bacteria (Cummings et al. 1999). The activity of sulfate-reducers favors the immobilization of arsenic as metal sulfides (Castro et al. 1997). In contrast, oxidation of arsenic sulfide minerals such as arsenopyrite and orpiment will favor arsenic mobilization (Moore et al.

1988). Finally, detoxification of inorganic arsenic can occur via its reduction and/or methylation (Rosen et al. 1999; Martin et al. 2001; Cullen & Reimer 1989; Hasegawa et al. 2001). Inasmuch as methylation reactions produce volatile arsenicals, these processes offer potential mechanisms by which sediments could be biologically depleted of contaminating arsenic.

Recent studies have firmly established that many, if not most, transformations which control arsenic flux between various compartments (sediment/soil ↔ aqueous ↔ atmosphere) are driven by and/or accelerated by microbial metabolism. However, our understanding is presently limited to the *potential* influence that microbes may exert on these fluxes. For example, we know that arsenate reduction can be mediated by fermentative bacteria that export As(III) after internalising As(V) *via* phosphate transporters. And we now appreciate that As(V) can serve as a terminal electron acceptor in energy-yielding processes mediated by dissimilatory As(V) reducers. However, it is presently unknown which process predominates in different natural settings. Our laboratory has established that abundant populations of organoheterotrophs (Wielenga et al., data not published), SRB (Harrington et al. 1998), FeRB (Cummings et al. 1999, 2000) and AsRB (Harrington et al. 1998; Niggemyer et al. 2001) actively co-exist in heavy metal(loid)-contaminated sediments of Lake Coeur d'Alene, ID. These sediments are abundant in microbially-reducible Fe(III), SO_4^{2-} and As(V), but the degree to which these microbial guilds interact to produce the observed patterns of arsenic distribution and speciation is presently unknown. Combined field and lab studies will be required to elucidate how these interactions control arsenic mobility. Such detailed understanding is essential to generating predictive models of how this community will respond to perturbations such as physical disturbance or eutrophication.

Also urgently needed is improved understanding of how key bacterial respiratory processes such as Fe(III)-, SO_4^{2-}- and As(V)-reduction interact within different matrices. Solely on the basis of their geochemical properties, sediments dominated by either iron or aluminum (Manning & Goldberg 1997; Raven et al. 1998), carbonate (Smedley et al. 2002) or silica (Davis et al. 2001) can be expected to differ markedly in their capacity to retain the various arsenic species. For example, adsorption of As(V) by both Al hydroxides and Fe hydroxides can be nearly the same below pH 7.5 (Edwards 1994). And recent evidence (Manning et al. 1998; Raven et al. 1998) suggests that at circumneutral pH As(V) and As(III) are nearly equal in their affinity for iron (oxy)hydroxides. However, As(III) does not sorb appreciably on other hydrous oxides such as those of aluminum (Manning & Goldberg 1997). Further, some aluminosilicate minerals have been shown to enhance the oxidation of dissolved arsenite – leading to the subsequent adsorption of As(V) (Manning & Goldberg 1997). Zobrist et al. (2000) approached this issue experimentally using single species of microbes that differed in their capacity to reduce As(V) and Fe(III). They demonstrated that microbially-induced reduction and dissolution of As(V) adsorbed by $Al(OH)_3$ necessarily occurred without the dissolution of the mineral phase, whereas microbial reduction of coprecipitated Fe(III) and As(V) could destabilize iron oxide aggregates, solubilizing As(V), As(III) and Fe(II). This simple, reductionist approach is the most rational way to begin testing hypotheses concerning how different respiratory processes influence As mobility. However, such experiments tell us little about how As mobility is affected by competition among species that differ in their terminal electron accepting preferences. We propose that a fertile area for future research lies in well-controlled, lab-scale studies of population-level interactions within different physical matrices. Inferences drawn from such investigations would greatly facilitate interpretation of field-scale studies aimed at understanding the dynamics of arsenic biogeochemistry in the environment.

Lastly, most research to date concerned with the biogeochemical cycling of metal(loid)s has been conducted with a view of these processes occurring on very limited spatial and temporal scales. The influence of these elements on both community succession and on the evolution of microbial species within those communities has generally been ignored. Conversely, we are only beginning to appreciate the roles that microbial succession and microbial evolution play in transforming the geochemistry and mineralogy of their physical environment (but see Newman & Banfield 2002; Reysenbach & Shock 2002). Full understanding of how the myriad metabolic and geochemical processes that potentially influence arsenic mobility actually generate specific patterns of distribution and speciation awaits investigations that integrate across broad spatial and temporal scales.

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