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Biotic Generation of Arsenic(III) in Metal(loid)-Contaminated Freshwater Lake Sediments

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Sediments of Coeur d'Alene Lake, ID, are heavily contaminated with mine tailings that contain high levels of arsenic, iron, lead, and other trace elements. Maximal abundance of redox-active elements such as As and Fe is generally found close to the sediment−water interface, whereas peak abundance of less redox-active elements such as Pb is found at >25 cm. The suggestion that As is mobile within reduced sediments led us to characterize the sediment microbiota with regard to organisms whose activities favor As mobilization. Most probable number (MPN) estimates reveal that the densities of cultivable sulfate-, iron-, and arsenate-reducing bacteria approach 10^6, 10^5, and 10^4 cells g^{-1} wet weight sediment, respectively. Because As is considered more mobile in environments that produce As(III), we measured aqueous As(III) generation within As(V)-amended sediment microcosms. In organic acid-stimulated microcosms, >50% of a 10mM As(V) amendment is transformed to As(III), compared to 30% and 5% in unstimulated microcosms and abiotic controls, respectively. In microcosms amended with an inhibitor of SRB metabolism (molybdate), As(V) reduction was in some cases diminished, suggesting that SRB may contribute to As(V) reduction. The capacity for biotic As(V) reduction clearly exists in CDAL sediments, and the profile of As abundance may be partly attributed to metal(loid)-transforming bacteria.

Introduction

During the first two-thirds of the 20th century, mining in northern Idaho produced over 1 billion troy ounces of silver and 8 million tons of lead (*1*). Much of this activity took place along the South Fork of the Coeur d'Alene River (CDAR), a region that came to be known as the Silver Valley. As a result, mine tailings containing high concentrations of lead, zinc, arsenic, and other trace elements accumulated in stream banks and bars throughout the lower CDAR Valley. Although tailings ponds were constructed in the late 1960s, spring runoff and periodic flooding have continued to transport these materials downstream, heavily contaminating Coeur d'Alene Lake (CDAL). CDAL is a natural lake of glacial origin covering ca. 130 km^2 and having an average depth of roughly 64 m. CDAL is dimictic, regularly undergoing two periods of thermal stratification and a fall and spring overturn. The lake appears to be variable in trophic status as inferred from total nitrogen and phosphorus levels. Based on 1991−1992 data, the southern end near Lake Chatcolet is mesotrophic-eutrophic, while the rest of the lake is oligotrophic-mesotrophic (*2*).

On the basis of a surface sediment survey (*3*), 85% of CDAL sediments appears to be enriched with Ag, As, Cd, Cu, Fe, Hg, Mn, Pb, Sb, and Zn. Field measurements of sediment E_h consistently reveal a steep E_h gradient and the presence of a redox boundary within 10 cm of the sediment−water interface (*4*). Total metal analysis of deep sediment cores reveals that iron and trace elements in CDAL exhibit two distinct patterns of distribution (*4*). Maxima for total abundance of redox-active elements such as iron and manganese are observed near the sediment−water interface (<10 cm), whereas maxima for less redox-active elements such as lead occur more deeply (>25 cm). We have postulated that biotic and abiotic transformations of arsenic, iron, and sulfur account in large measure for these patterns. In this paper, we demonstrate that the capacity for microbially mediated redox transformation of arsenic exists in CDAL sediments.

Arsenic is toxic to both plants and animals, and its trivalent form is considered to be much more toxic than its pentavalent form (*5*). Both forms readily accumulate in living tissues due to their affinity for proteins, lipids, and other cellular components (*6*). In many aqueous systems, arsenite (As(III)) has been shown to predominate under reducing conditions (*7, 8*). Moreover, compared to arsenate (As(V)), arsenite demonstrates greater mobility in both sediment and groundwater systems (*6*−*10*). This difference in mobility has been attributed to the high affinity of As(V) for insoluble species such as hydrous ferric or manganese oxides (*10*). Some researchers postulate that arsenite has a lower affinity for these compounds to the extent that binding only occurs consequent to the oxidation of As(III) to As(V). This transformation is thought to be mediated either by the iron or manganese oxyhydroxides themselves (*10, 11*) or by microorganisms (*12*). Alternatively, the greater apparent mobility of As(III) under reducing conditions in neutral or slightly acidic sediments may result from the fact that these conditions favor reduction of both iron and manganese (hydr)oxide sorbents as well as reduction of As(V). In fact, some recent studies suggest that, at circumneutral pH, As(III) may actually have a higher affinity for iron (oxyhydr)oxides than As(V) (*13*).

Accumulation of arsenic at the redox boundary has been repeatedly observed in both stratified water columns and sediments (*9, 14, 15*). Under the highly reduced, neutral pH conditions characteristic of deeper strata, arsenic can be precipitated by sulfide (*14, 16*). Under the oxidizing conditions that prevail nearer the surface, scavenging and precipitation of arsenic by iron and manganese oxyhydroxides limits upward diffusion (*10, 11, 17*). Near the redox boundary, however, arsenic is more likely to occur as As(III) and appears to be more mobile (*7*−*9, 17*). Over time, diffusion of soluble arsenic released by chemical or bacterial activity leads to its accumulation within this layer of the sediment.

Detailed examination of the vertical profile of As(V) vs As(III) in stratified sediments or water columns, however, has sometimes revealed unexpected speciation profiles. Where strongly reducing conditions exist, As(III) should be favored over As(V) from thermodynamic considerations alone. However, in several systems, such as the meromictic Lake Pavin in France as well as lakes in the Aberjona Watershed, As(V) is found at unexpectedly high levels in

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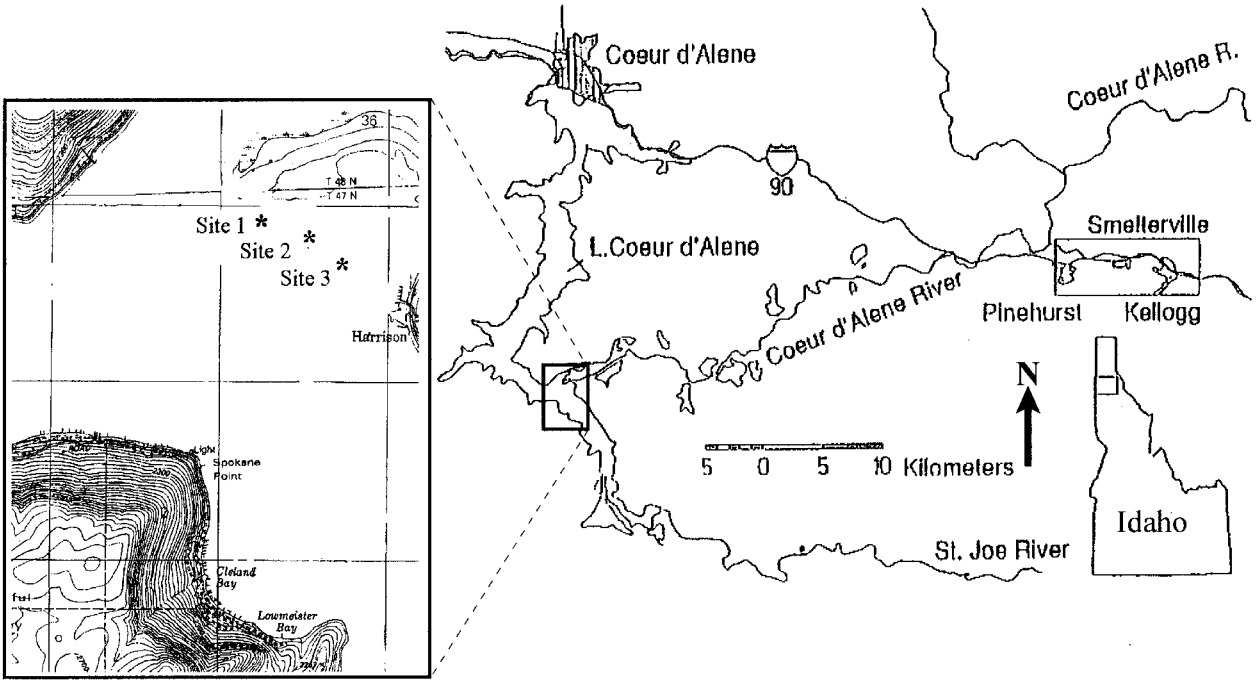

FIGURE 1. Coeur d'Alene Lake located in northern Idaho. Study sites in the box are located in the contaminated Coeur d'Alene River delta. South of this delta, heavy metal contamination decreases along a gradient toward the unimpacted St. Joe River.

permanently anaerobic regions of the lake (*14*, *18*). In other systems, As(III) is found to predominate in aerobic regions of lakes (*11*, *15*) even though the thermodynamically favored As(V) species should be prevalent. These anomalies have been variously interpreted as being caused by the presence or absence of diagenetic forms of iron or manganese, sulfide, or key microbial transformants.

Microorganisms capable of direct As(III) oxidation and direct As(V) reduction have been described (*12*, *19*−*22*). In particular, two physiologically defined taxa, the dissimilatory iron-reducing bacteria (DIRB) and the sulfate-reducing bacteria (SRB), contain representatives that can either directly transform arsenic or act upon inorganic compounds that transform arsenic. In an iron-rich environment, iron oxyhydroxides bind arsenic (*10*). However, DIRB can utilize iron oxyhydroxides as terminal electron acceptors, releasing bound trace elements. In addition, certain DIRB have also been shown to reduce As(V) (*20*). Production of aqueous sulfide by SRB can directly reduce both arsenate and iron hydroxides (*17*, *22*). When aqueous sulfide is not in excess, these reactions can increase arsenic solubility; where sufficient levels of soluble sulfide exist, arsenic sulfides can form (*17*). It is therefore reasonable to suppose that in freshwater sediments DIRB and SRB play essential roles in the cycling of arsenic under low redox conditions.

We have hypothesized (*4*), based on sequential extraction data, redox profiles, and the strength of correlation between arsenic and other contaminant metals in CDAL, that As is being mobilized toward the sediment−water interface while, in contrast, lead and zinc remain buried in deeper strata. In this paper, we report that the microbial elements capable of performing the necessary transformations of As, Fe, and S to account for this phenomenon are present in CDAL sediments. We also demonstrate the occurrence of significant biotic As(V) reduction in replicate sediment microcosms.

Materials and Methods

Sediment Core Removal and Sampling. Cores were retrieved from sediments along a transect extending from one edge of the Coeur d'Alene River Delta toward the town of Harrison, ID (Figure 1). At the times of sampling, the overlying water column in this region ranged between 1 and 2 m. Capped 3-m sections of 1-in. (i.d.) PVC pipe were inserted into the water column until the end of the pipe touched the sediment, at which point the cap was removed, and the PVC was pressed into the sediment ~60 cm. The cap was then replaced, the core removed, and the pipe cut in a manner that left 10 cm of lake water overlying sediments. Pipe ends were sealed, and the cores were transported to the laboratory and stored upright in a plastic container. The container was kept in a 4 °C cooler and flushed continuously with N_2 gas. Sediment cores were extruded from the bottom up onto a waxed paper-coated processing board and immediately transferred into an anaerobic chamber containing premixed N_2:CO_2:H_2 gas in a ratio of 80:15:5. Pore water was expelled from subsamples of 8-cm core sections in an acid-washed, plastic-lined compression chamber at 0.5 P N_2 (g), and passed through 0.2-μm nylon filters.

Analysis of Iron and Trace Elements. Trace element abundance was determined using a Thermo Jerell Ash (Franklin, MA) IRIS ICP. Analyses were performed on aqua regia-digested sediments and pore water extracted as described above. Every 20th sample was duplicated, and less than 10% error was detected. As(III) was analyzed by hydride gas generation followed by ICP spectrometry. The hydride gas generation method of Glaubid and Goldberg (*23*) was modified by an addition of oxalic acid/sodium oxalate buffer (to obtain a pH of 4.5) and 0.35% sodium borohydride/0.30% sodium hydroxide solution (*24*) and utilized a gas−liquid separator (Bausch and Lomb ARL341) hydride gas generation unit.

Most Probable Number (MPN) Analysis. Abundance of cultivable microbes belonging to one or more physiologically defined taxa was estimated by a set of replicate terminal dilution enrichments. Sample processing for microbiological analyses was conducted entirely within an anaerobic chamber containing a N_2:CO_2:H_2 atmosphere in a ratio of 80:15:5. One gram of sediment was removed from the center of the extruded core at depth intervals of 2, 18, and 35 cm. Sediments were placed into 10-mL serum vials containing

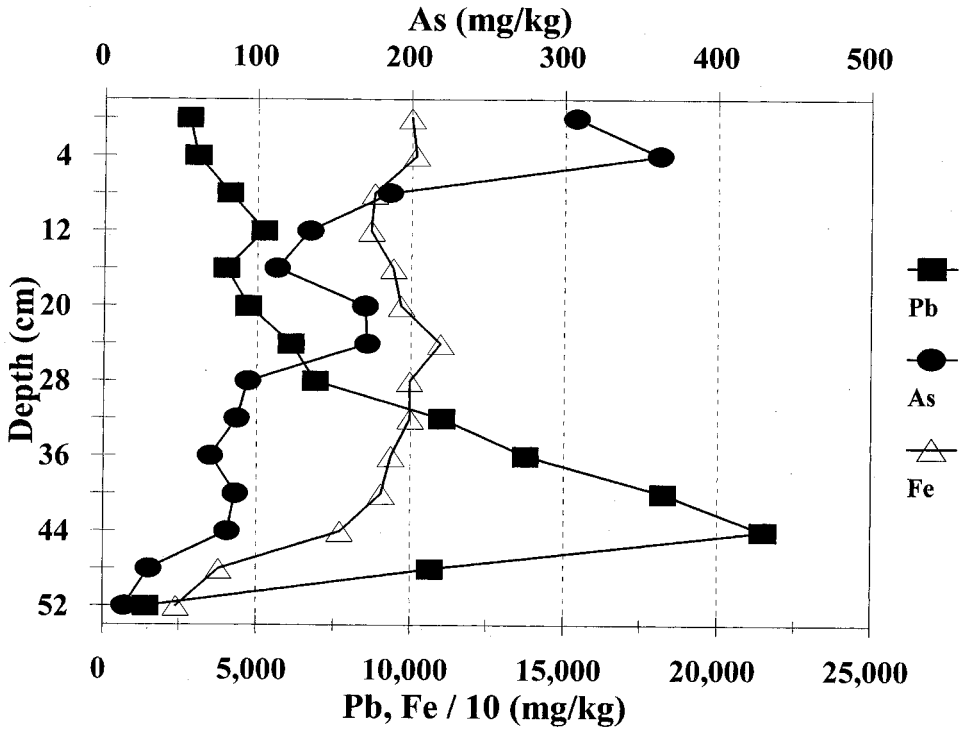

FIGURE 2. Vertical profile of total metal abundance at site 3. Note the surficial arsenic peak, the intermediate iron peak, and the deep lead peak. These patterns are consitstent across sites and consistent with results reported previously (*4*).

9 mL of 1/2X Ringers solution (per liter: 4.0 g of NaCl, 0.5 g of $NaHCO_3$, 0.125 g of KCl, and 0.13 g of $CaCl_2$) (*25*), then sealed in the chamber by crimping. A 0.01% cetyl-trimethylammonium bromide (CTAB) was added to promote formation of uniform sediment suspensions (*26*). Vials were shaken at 200 rpm on a gyrorotary platform maintained at room temperature for 1−2 h. Sterile, 96-well BioBlock containers (Rainin, Woburn, MA) were used for evaluating MPN of cultivable bacteria. Each of the 96 2-mL wells was filled with 0.9 mL of media described below.

Abundance of SRB was estimated using Pfennig medium modified by the addition of 0.93 g/L lactate and 7.33 mg/L $FeSO_4$; the media contained all recommended electron donors except palmitic acid (*26*). Abundance of cultivable arsenate-tolerant SRB was estimated using modified Pfennig media amended with 1 mM sodium arsenate (0.312 g/L final concentrated). Abundance of arsenic reducers was estimated using Pfennig media further modified by replacing sulfate with 10 mM sodium arsenate. The addition of dithionite and sodium sulfide to Pfennig media was eliminated in this last determination in order to avoid abiotic reduction of arsenate. One milliliter of sediment suspension was removed in the anaerobic chamber using a 1-mL syringe fitted with a 19-gauge needle; 0.1 mL was added to each of the first four wells of the first row of the appropriately marked BioBlock. These suspensions were taken through six successive 10-fold serial dilutions using a multichannel pipettor; the final well thus represented a 10^{-7} dilution. Sediment dilutions were incubated in the dark at room temperature for 30 d before they were scored for blackening, indicative of sulfidogenesis. All MPNs were calculated using techniques and tables of the American Public Health Association (*27*). MPN for As reducers was calculated after cultures were assayed for As(III) formation using hydride-generation ICP (measured sensitivity <100 ppb). Wells were scored positive for As(V) reduction if As(III) levels exceeded the 95% confidence interval around the mean of the abiotic controls.

Arsenic Reduction Assays. Three sets of microcosm experiments were initiated on the basis of their position relative to the sediment−water interface. Sediments taken from the 2 cm depth were representative of the region containing the surficial As peak, whereas sediments from 18 and 35 cm were representative of those regions containing the principal Fe peak and the maxima for Pb and Zn, respectively (see Figure 2).

A three-tenths milliliter aliquot of the same sediment suspension used to initiate the MPN was added to 9 mL of 10 mM (sodium) arsenate-containing buffer in 10-mL serum vials. This phosphate-based buffer (*20*) consisted (per liter) of 0.34 g of K_2HPO_4, 0.34 g of KH_2PO_4, 0.46 g of NaCl, 0.12 g of $MgCl_2 \cdot 6H_2O$, and 0.06 g of $CaCl_2 \cdot 2H_2O$. Sediments were subjected to six treatments run in triplicate for each depth assayed: Pfennig organic acids (final concentrated per liter, 2 g of sodium acetate, 0.7 g of propionic acid, 0.8 g of butyric acid, 0.5 g of benzoic acid, 0.93 g of lactic acid), Pfennig organic acids + Na-molybdate (10 mM final concentrated), organic acids + formaldehyde (1% final concentrated), molybdate alone, formaldehyde alone, and no amendment. The total volume of all vials was adjusted to 10 mL with additional anaerobic phosphate buffer (pH 7.2), whereupon vials were sealed and crimped. All manipulations were carried out anaerobically. Serum vials were incubated at room temperature in the dark for 30 d, after which time reactions were terminated by the addition of 0.1 mL of 10% w/v ascorbic acid (*28*). Serum vials were stored at 4 °C until ICP analysis.

Results and Discussion

Coeur d'Alene Lake sediments in areas that experience relatively low flow from the CDAR show maximum arsenic abundance within 10 cm of the sediment−water interface. Iron is extremely abundant throughout CDAL sediments; its maximum abundance generally occurs at depths ≤25 cm. By contrast, the maxima for lead and zinc occur at depths >25 cm (Figure 2). These patterns hold across all three sites and are consistent with previously reported results (*4*).

Using sequential extraction methods, we have established that regardless of depth ~70% of total arsenic in CDAL

TABLE 1. Iron and Trace Element Abundance in CDAL Waters

	element (μg/L)			
	As	Fe	Pb	Zn
lake waters				
concn range[a]	<1−1	NA[d]	<1−41	<10−390
median	<1	NA	3	99
no. of samples (near surface and near bottom waters)	145	NA	145	146
sediment pore waters				
concn range in CDAL pore water[b]	40−350	1800−40 330	40−1540	300−1550
median	180	4270	250	490
mean ± SD (n = 12)	160 ± 98	7687 ± 10 755	185 ± 104	560 ± 346
federal water quality standards				
drinking water standards[c]	50	300	50	5000
concn considered chronically toxic to aquatic FW life	342	NA	2000	25 000

[a] Ref *38*. [b] This study. [c] Ref *4*. [d] NA, not applicable.

sediments is associated with sulfides (*4*). Approximately 15−20% of solid-phase As is bound to amorphous or crystalline iron/manganese (hydr)oxides. Arsenic in the $MgCl_2$-exchangeable fraction ranges from 0 to 6 mg/L (*29*), while As concentrations in sediment pore waters range from 0.35 mg/L to below detection limit (Table 1; Table 2 in Supporting Information). Since sequential extraction information is based on operational definitions, these values should be interpreted with caution. Nevertheless, metal(loid)s found in $MgCl_2$-exchangeable and pore water fractions are considered to be most bioavailable as well as most easily transported from sediments into overlying waters (*7*).

Iron is extraordinarily abundant throughout CDAL sediments (5−10% by dry weight), and more than 70% of Fe occurs as iron sulfide (*4*). However, iron also occurs in forms that are more readily bioavailable. Iron in the $MgCl_2$-exchangeable fraction ranges from approximately 800 mg/kg (dry weight) in surficial sediments to 300 mg/kg at depths below 30 cm (*29*). Iron pore water concentrations range from approximately 2 to greater than 40 mg/L (Table 1; and Table 2 in Supporting Information).

Previous work has shown that total microbial abundance in CDAL sediments ranges from 10^5 to 10^8 cells/g wet weight sediment (*4*). Our MPN estimates indicate that cultivable SRB range in abundance from 10^3 up to 10^6 cells/g wet wt sediment (Figure 3). Because we have imperfect knowledge of the substrate preferences and micronutrient requirements of all SRB, these numbers necessarily provide a minimum estimate of SRB abundance. Our estimates of 10^6 cells/g in zones of peak SRB abundance rank among the highest reported recoveries of this taxon in the literature (*30, 31*). Approximately 10% of the Pfennig media incubations that contained 1 mM sodium arsenate were sulfidogenic. Thus, a significant fraction of the CDAL sulfate reducer community may be described as arsenic-tolerant (Figure 3).

Dissimilatory arsenic-reducing organisms have been recently isolated in pure culture (*19*−*22*). The physiology and 16S-ribosomal subunit phylogeny of one such organism places it within the sulfate-reducer group (*22*). We therefore designed a medium to estimate the MPN of arsenate reducers that could also be sulfate reducers. The basal medium used in this assay supports growth by all currently recognized SRB genera (*26*) but replaces sulfate with arsenate, an alternate terminal electron acceptor whose structural similarity may enable it to behave as a metabolic analogue. Using this medium, we estimate that the abundance of cultivable As(V) reducers in CDAL sediments ranges between 10^3 and 10^5 cells/g wet weight sediment (Figure 3).

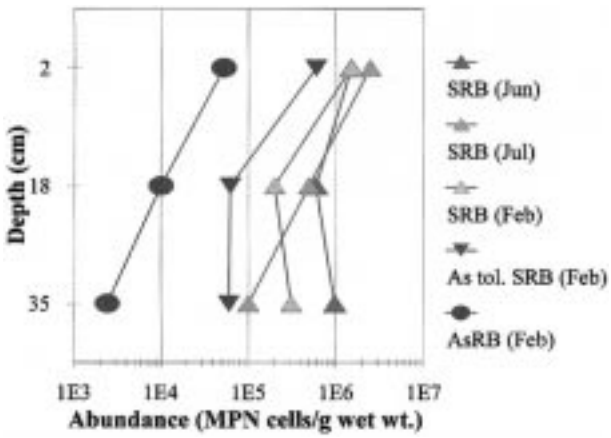

FIGURE 3. Most probable number (MPN) analysis of sulfate and arsenate reducers in CDAL sediments. SRB were assayed in three different months at three different sites, As-tolerant SRB and As(V)-reducers were assayed only at the February sampling. Reported MPN for each depth is a mean of data from the three sites depicted in Figure 1.

Work in progress on CDAL sediments provides evidence for an active dissimilatory iron-reducing bacterial (DIRB) community (*29*). These studies show that profiles of reduced iron begin at approximately 550 mg/kg at the sediment−water interface and increase with depth to levels exceeding 2800 mg/kg. Incubation of anaerobic sediment microcosms for 39 d with 2 mM sodium acetate (final concentrated 0.16 g/L) stimulates ferrous iron production, and serial dilutions incubated under iron-reducing conditions have blackened iron gel media to dilutions of 10^{-5}. Altogether, these observations indicate that certain strata of CDAL sediments support active and abundant DIRB populations.

The total arsenic load delivered to CDAL sediments over the last century has been estimated to exceed 1500 tons (*32*). Examining the potential for dissimilatory As reduction in this environment has special relevance in light of this fact; moreover, we have observed that total As concentration can exceed 500 mg/kg within 10 cm of the sediment−water interface (*4*) (Figure 2). Currently, Federal drinking water standards restrict acceptable As concentrations to 50 μg/L (*5*). Pore water As concentrations an order of magnitude greater than these limits can be found within 20 cm of the water column (Table 1; Table 2 in Supporting Information).

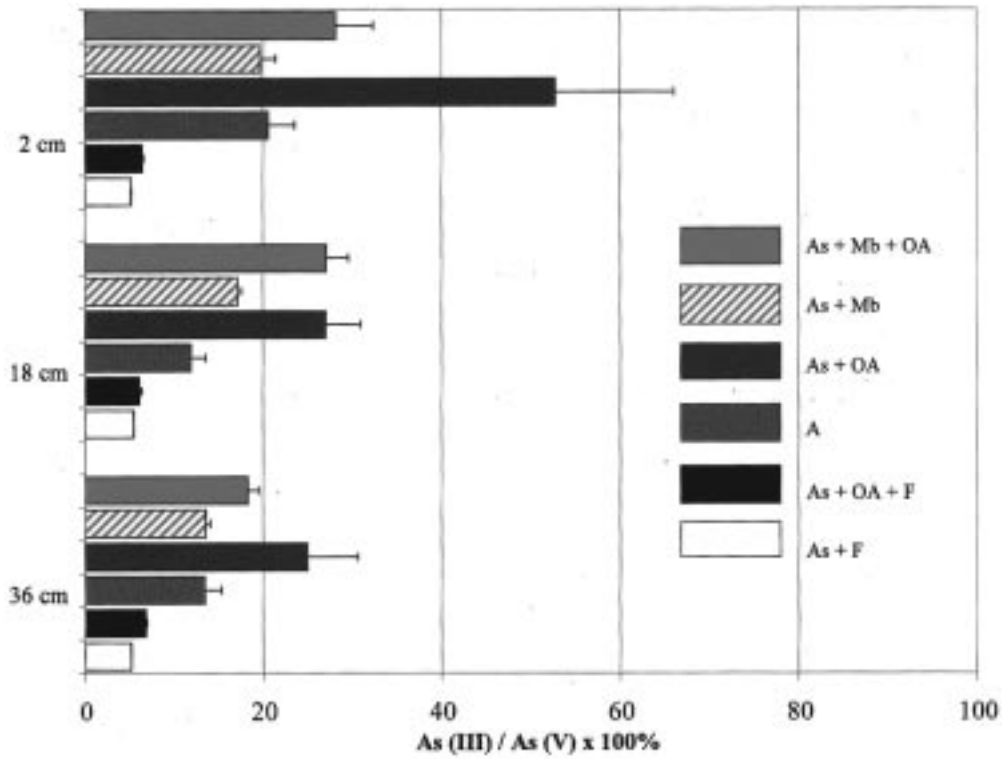

FIGURE 4. As(III) production in As(V)-amended microcosms. Error bars represent the standard error from nine replicate microcosms, three from each of three sites, at the depths represented within the core. % reduction calculated by the difference between added As(V) and recovered As(III). As = 10 mM arsenate. Mb = 10 mM molybdate. OA = modified Pfennig media organic acids. F = 1% formaldehyde. (All concentrations represent the final concentration in the formulated microcosm.)

Arsenic(V) and As(III) differ in their toxicity as well as in their respective solubilities in reduced environments such as the CDAL benthos (*7*). Our MPN estimates indicate the presence of $\sim 10^4$As(V)-reducing organisms per gram wet weight sediment. We therefore sought to establish the overall capacity of CDAL sediments for biological As(V) reduction. Our experiments were designed to investigate the fate of As(V) imported by sediment deposition or released by desorption consequent to the reduction of hydrous ferric oxides. We further sought to establish the potential for As-(III) release into overlying waters given the presence or absence of additional electron donors in the form of organic acids. Additionally, we sought to establish the contribution of specific taxa to As(V) bioreduction. Thus, 10 mM molybdate (2.06 g/L final concentrated) was added as an inhibitor of SRB metabolism (*33*). To evaluate the capacity for abiotic As(V) reduction, formaldehyde was added to sediment microcosms run with and without organic acid amendment.

Arsenic(V) reduction was most pronounced in uninhibited microcosms amended with organic acids (Figure 4). The capacity to reduce As(V) also decreased with increasing depth. This observation can be partly attributed to the presence of fewer As(V)-reducing bacteria at lower depths (see Figure 3). Considering that As accumulates in uppermost sediments, it is possible that the microbial community at 2 cm was simply more tolerant of the 10 mM arsenate amendment. Arsenic-(III) has been shown to be toxic to many microbes at concentrations as low as 100 μM (*34*). It is noteworthy that each microcosm not treated with formaldehyde produced As(III) well in excess of 100 μM (Figure 4).

At every depth arsenic reduction in electron donor-unamended microcosms was 2−4-fold greater than either abiotic control ($p \ll 0.001$). These results demonstrate that sufficient nutrients are present in CDAL sediments to support biological reduction of As(V). The amount of abiotic As(III) production observed (up to 10 mg/L in 30 d) likely results from reactions with reduced inorganic components (e.g., sulfides) of the sediment that we have reported previously (*4*). On the basis of published rate data for arsenate reduction by aqueous sulfide (*22*), dissolved sulfide concentrations of greater than 100 μM would be required to reduce even 10% of the added arsenate over a 30-d period, which is clearly unrealistic. Thus, biological rather than chemical pathways are required to account for the extent of arsenate reduction, a point supported by the formaldehyde-treated micocosms.

Amendment of sediment microcosms with organic acids resulted in a 2−3-fold increase in As(V) reduction as compared to unamended microcosms ($p \ll 0.001$). This observation indicates that additional carbon input to CDAL sediments would likely favor increased biological As(V) reduction. Depending on the speciation of iron in surficial sediments, the fate of the As(III) produced could include release into overlying waters, as has occurred in the Lake Ohakuri and Aberjona Watersheds (*18, 19, 35, 36*).

At some strata within the CDAL sediments, organic acid-amended microcosms showed diminished capacity to reduce As(V) when treated with molybdate (Figure 4). Because molybdate inhibits SRB metabolism (*34*), these results indicate that SRB populations may be partly responsible for biological As(V) reduction in CDAL sediments. It is also possible that some nonsulfate-reducing arsenic reducers might be molybdate sensitive; however, in other anoxic sediments molybdate has not been found to inhibit the rate or extent of As(V) reduction (*37*). We found no significant difference in As(III) production between molybdate-treated microcosms and those to which no organic acids were added ($p = 0.056$). However, when organic acids were added to molybdate-treated microcosms, significantly more reduction occurred than in microcosms amended with arsenic and molybdate only ($p < 0.02$). We therefore conclude that the

capacity to reduce As(V) is neither limited to nor exclusive to the SRB.

Metal-contaminated sediments in CDAL support an abundant and diverse microbial community. Elements of this community have the capacity to reduce arsenic. Previous studies suggest that bacterially mediated As(V) reduction contributes to As accumulation near redox boundaries in sediments and water columns (*35*, *36*). We should also bear in mind that CDAL sediments are highly enriched with iron, thus transformations of this element are likely to significantly influence sediment biogeochemistry. We hypothesize that in CDAL sediments As is initially mobilized by direct As(V) reduction and/or the release of sorbed As consequent to Fe(III) reduction. Arsenic then diffuses until it reaches the redox boundary, whereupon it is resorbed by hydrous ferric oxides in oxidized surficial sediments.

The capacity for arsenic reduction and mobilization in Coeur d'Alene Lake sediments must be understood in order to predict the conditions under which As levels could increase in surface waters. Coeur d'Alene Lake serves six community water supply systems and provides a place for outdoor recreation to thousands of people annually (*2*). Presently, these waters comply with Federal drinking water standards (Table 1). However, given that bacterial As reduction can be demonstrated in CDAL sediments, it is reasonable to suppose that certain conditions might favor an increase in surficial As concentration. SRB and DIRB are both present and active in these environments. Furthermore, such organisms can be expected to engage in reactions that directly reduce arsenate and favor the release of bound arsenicals consequent to the reduction of ferric and manganese (hydr)oxides. Because these reactions are favored under anaerobic conditions, and the reverse reactions favored under aerobic conditions, we recommend management practices for CDAL that will maintain the lake hypolimnion in an aerobic state.

Acknowledgments

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Supporting Information Available

Iron and trace element abundance in CDAL pore waters (1 page). Ordering information is given on any current masthead page.

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