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The methylation of arsenic in marine sediments

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Laboratory studies have shown that microorganisms present in both natural marine sediments and sediments contaminated with mine-tailings are capable of methylating arsenic under aerobic and anaerobic conditions.

Incubation of sediments with culture media produced volatile arsines [including AsH_3, $(CH_3)AsH_2$, and $(CH_3)_3As$] as well as the methylarsenic(V) compounds $(CH_3)_nAs(O)(OH)_{3-n}$ (n = 1, 2, 3). The concentration of the arsines increased and then decreased in a growth and decay pattern reminiscent of the methylation and demethylation of mercury. Thus, arsenic speciation varied with time, being controlled by the biochemical activity of the dominant microbe(s) at the time of sampling, and changing in response to the ecological succession within the microbial community.

The analysis of the interstitial waters of sediments collected from several British Columbia (Canada) coastal sites gave results that were consistent with the culture experiments, in that the methylarsenicals were ubiquitous, but present only in small amounts. It is estimated that methylarsenic(V) species account for less than 1% of the arsenic present in porewaters. The actual proportion was dependent on a number of factors but, contrary to prevailing viewpoints, there was no relationship to the organic content of the sediments, nor did methylation occur only in the presence of high arsenic concentrations. Instead, all of the evidence was consistent with *in situ* microbial methylation and demethylation processes that are similar to the arsenic transformations that occur in soil ecosystems.

The results are discussed in terms of the cycling of arsenic in the marine environment and within the marine food web.

Keywords: Arsenic methylation, arsenic speciation, methylarsenicals, sediment, mine-tailings, biomethylation, demethylation

INTRODUCTION

Organoarsenic compounds are present in the environment due to their natural occurrence, their direct input, and as a consequence of their *in situ* production.[1–3] Anthropogenic sources of organoarsenicals are limited, consisting mainly of herbicides and pesticides used in agriculture. However, the conversion of inorganic arsenic compounds to organic derivatives is carried out by a variety of organisms.[1,4] The known,[1–4] exocellular, products of such transformations are restricted to the compounds displayed in boldface in Fig. 1. These include volatile arsenic(III) species (upper line, Fig. 1), which are commonly referred to as arsines, as well as the methylarsenic(V) compounds MMAA, DMAA and TMAO (see Fig. 1 for abbreviations).

The first report[5] of the biological production of an arsine appeared in 1893 and involved the formation of 'Gosio gas'. In this case molds growing on household wallpaper transformed inorganic arsenic compounds present as pigments to trimethylarsine (TMA).[4] Subsequent studies[1,4,6–8] have demonstrated that the ability to produce trimethylarsine is widespread amongst yeasts and fungi. However, bacterial transformations of arsenic have been identified only relatively recently. It was not until 1971 that McBride and Wolfe[9] demonstrated that a volatile compound with a garlic odor [probably $(CH_3)_2AsH$][1] was produced under anaerobic conditions by the methanobacterium strain MoH. It is now known that a wide range of anaerobic and aerobic bacteria are capable of methylating arsenic.[1,10–12] The major difference between fungi and bacteria appears to be the greater variety of arsines that are produced by bacteria.[1] Even in culture experiments involving a single bacterial strain that has been treated with arsenate, several products, $(CH_3)_nAsH_{3-n}$ ($n = 1-3$), are found.[1, 10–12] In a limited number of examples arsine (AsH_3) itself is produced.[1,13]

Monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO) are likely intermediates in these biomethylation reactions, a view that is substantiated by the formation of similar methylarsines when cultures are inoculated with MMAA, DMAA or TMAO.[1,4] However, the presence of these species as exocellular metabolites in the biomethylation of inorganic arsenic has rarely been reported. This is partly an anlytical problem, as the procedures would usually have been incapable of their detection.[1] Under appropriate circumstances it is possible that these compounds will be found. For example, a recent report[14] has described the production of DMAA, together with MMA and TMA, when *C. humicola* and *S. brevicaulis* were treated with $[(CH_3)AsO]_x$.

Monomethylarsonic and dimethylarsinic acids (or their appropriate salts) are the predominant organoarsenicals in other systems.[1-3] These compounds have been found in the urine[1,15] of humans, rats and mice, in association with algae growing *in culture*[16] and in the photic zone of natural waters,[17-20] and in soil porewaters.[21] TMAO was reported to be present in one study[22] of human urine but has otherwise been more elusive. This is probably due to its rapid microbiological conversion to trimethylarsine,[23] and to the special care that must be taken for its detection by conventional analytical procedures.[24]

The stability of methylarsenicals towards common environmental reagents suggests that the products of arsenic biomethylation should exist for some time in the environment.[1] Even the air-sensitive methylarsines are converted by O_2 to stable *methyl*arsenic(V) compounds (Fig. 1). However, soil[11,12] and aquatic bacteria[25] as well as yeasts[26] are known to *de*methylate methylarsenicals. Concurrent biomethylation and bio-demethylation have been observed even when only one organism is present.[1] Demethylation may provide some control over the concentrations of organoarsenicals in the environment. Nevertheless, methylarsenicals have been found[1-3] in the atmosphere, in soils and in natural waters, and are believed[27] to play a role in the global cycling of arsenic. However, relatively little is known about the occurrence of such species in sediments.

Wood first proposed[28] an arsenic biological cycle that was based on the original observation[9] of arsenic methylation by methanobacteria under anaerobic conditions. Consequently, he suggested that

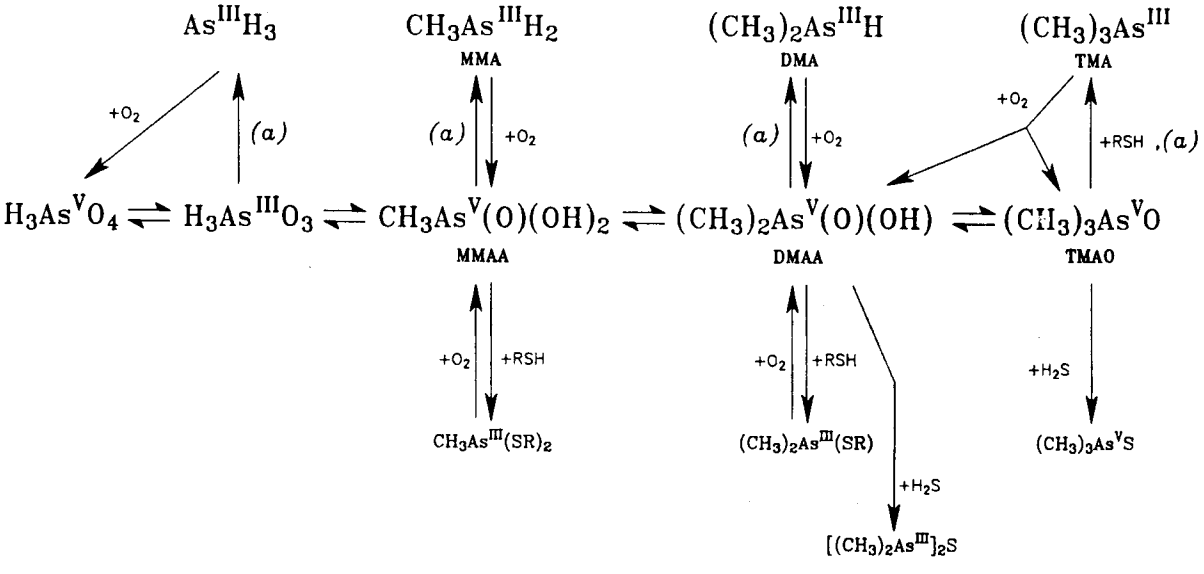

Figure 1 Interrelationships amongst some environmentally important arsenic compounds (modified from Ref. 1). Known exocellular arsenicals are depicted in boldface. No mechanism is implied and, for clarity, only fully protonated species are shown. (a) indicates biological or chemical (e.g. reaction with borohydride) conversions to more volatile arsines. Abbreviations are those used in the text.

methylarsines would be generated in sediment porewaters and in the anoxic zones of lakes and oceans. However, Andreae found no evidence of arsines in porewaters of coastal and open-ocean sediments, nor in oxic and anoxic seawater samples.[2,17] In a study of the anaerobic biomethylation of arsenate by methanobacteria found in sewage sludge, compost, rumen fluid and marine mud, McBride *et al.*[29] found that only the marine mud failed to produce methylarsines. As a result of these observations, there has been a general acceptance that micro-organisms in sediments do not normally produce volatile arsines.[2] In fact, methylarsenicals of any kind are presumed to be absent.[2] A report by Wong *et al.*[30] that methylarsenic(V) compounds, and in some cases DMA and TMA, were produced during the aerobic incubation of freshwater sediments with nutrient broth, has been unconfirmed. The sediments were contaminated with arsenic ($200-500\,mg\,kg^{-1}$) and the results have been interpreted[2] as a bacterial response to extreme arsenic stress. More recently, mono- and di-methyl arsenic(V) compounds have been detected in estuarine porewaters[31] and in the extracts of estuarine sediments.[32] However, in these cases the authors could not conclusively discriminate between the *in situ* production of these arsenicals or their direct input from anthropogenic sources via adjoining rivers. In one case *in situ* production was favored, but the methylarsenicals were thought to be formed by the degradation of organic detritus,[31] rather than by the biomethylation of inorganic arsenic precursors.

It is necessary to resolve this issue. Sediments act as reservoirs for arsenic,[1,3] and biomethylation may provide a mechanism by which arsenic is remobilized, or assimilated by benthic organisms, or both.[1] The lack of evidence for *in situ* methylation has also been noted as an important gap in our understanding of the transformation of endocellular arsenic compounds found in the marine food chain.[33] Furthermore, the presumed absence of methylarsenicals prompted at least one[34] of the few groups studying sedimentary arsenic speciation[31–35] to analyze only for inorganic species.

We have recently reported[24] the occurrence of organoarsenicals in marine interstitial water collected from the surface sediments of several British Columbia coastal sites. A strong positive correlation between the sum of the methylarsenic compounds and the total dissolved arsenic concentrations was interpreted in terms of *in situ* microbial methylation. Here the results of a multi-year investigation of deeper sediment cores and of sediment culture experiments are described. Collectively, these data support the concept that methylarsenicals are produced *in situ* and that their distributions are determined by a balance between microbial methylation and demethylation.

EXPERIMENTAL

1 Sediment samples

Sediments were collected from a variety of British Columbia (Canada) coastal sites (Fig. 2). A short core was obtained by divers at a water depth of 20 m in Sooke Basin. A ship-deployed corer was used to sample the sediments in Saanich Inlet (215 m water depth) and Satellite Channel (80 m). The same coring device was used in a multi-year investigation of four stations located in Alice Arm: A3 (September 1984; 80 m), D3 (September 1984; 356 m), A12 and A5 (September 1984; October 1985, 1986, and 1987; 81 and 220 m, respectively). For brevity, only the A12 and A5 results are discussed in detail; A3 and D3 were similar to A5.

2 Sampling

Sediment cores were obtained using a modified benthos corer fitted with a polyacrylic tube (122 cm long × 9.0 cm diameter) to which a stainless-steel cutter/catcher assembly was mounted. The corer was gravity-deployed and then recovered by means of a hydraulically powered winch located on the research vessel. The water was removed from the top of the sediment, the corer disassembled, and the sediment secured in the tube by means of a fitted cap and plunger. These precautions permitted the tube to be laid horizontally with a minimum of disturbance of the sediment profile. For cruises conducted in 1985 or later, the tube was wrapped in an insulating blanket and a cooling jacket, which maintained the sediment at or below the bottom water temperature – usually about 5°C (max. deviation ±5°C).

For porewater studies, the sediment was extruded in 5 cm sections into squeezers (modified after Reeburgh and described elsewhere)[24] located in a nitrogen-filled glove bag. Filled squeezers were

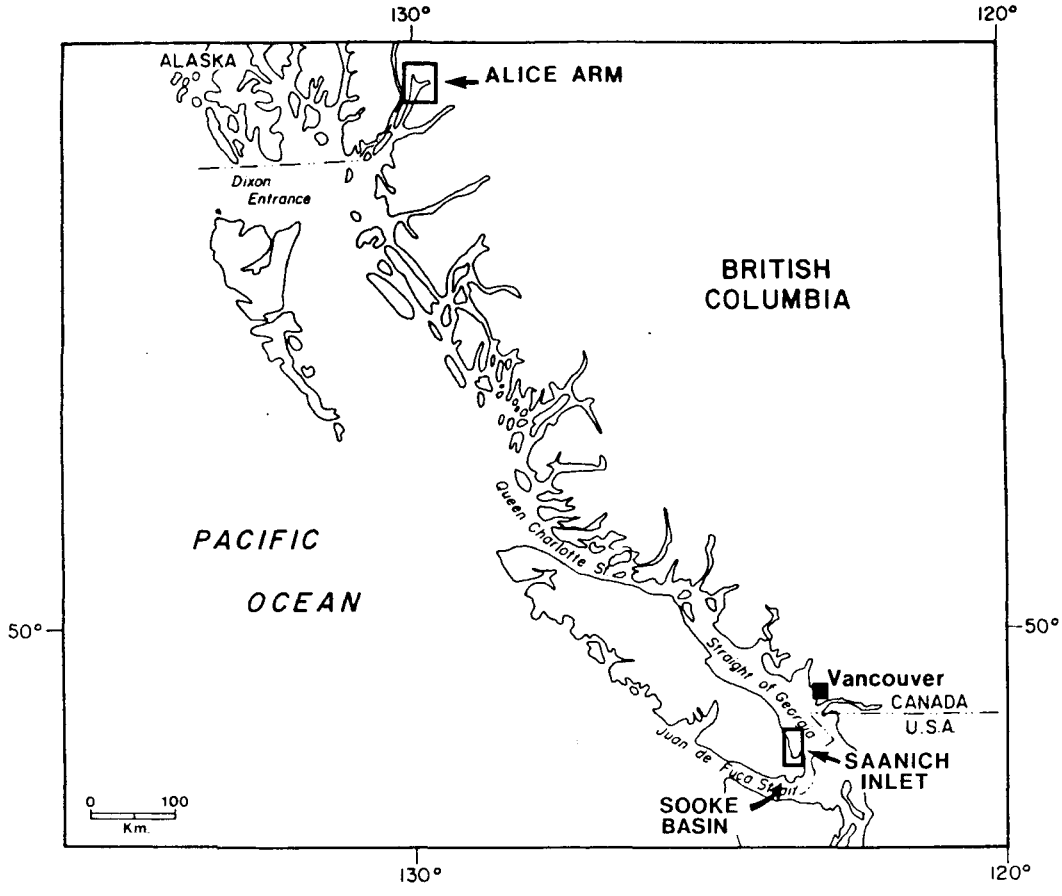

Figure 2 Map of British Columbia, Canada, coastline showing major study areas.

removed from the glove bag and mounted in a 'C' clamp on a squeezing rack. Porewater was expressed through a 0.45 or 0.22 μm filter upon application of nitrogen pressure (138–207 kPa) to the squeezers and collected in sterile cooled (0°C) polyethylene containers (Evergreen) under a continuous flow of nitrogen. The porewater was subsampled and sealed under nitrogen, preserved by rapid freezing on dry ice, and stored at −20°C prior to analysis. The squeezed sediments were sealed in plastic bags and stored at −20°C.

In most cases, culture experiments were initiated on the ship at the time of sampling. These sediments were extruded directly into the appropriate containers and amended as described in the following section. Exceptions to this procedure were the chemofocusing experiments (see Section 3, Culture work, Method 2) with Alice Arm sediments – these sediments had been previously frozen. Sooke Basin sediments were stored at 5°C prior to being used in culture work.

3 Culture work

The methylation of arsenic by the mixed microbial communities present in sediments was examined by a series of experiments. Typically, sediment (50–100 g) was introduced into a 1 dm^3 Teflon flask (Cole–Parmer, USA) fitted with an air-tight machined Teflon cap equipped with two valve-controlled ports. Filtered and autoclaved seawater [containing $\sim 1.2\ ng\ g^{-1}$ arsenate] and an equal volume (max. 100 cm^3 each) of culture media (Difco marine culture broth) were then added. Where appropriate, the flasks were spiked with a solution of known arsenic concentration. For aerobic sediments all of the above operations were carried out in air; a nitrogen or argon atmosphere was used for

anoxic sediments. The resulting mixtures were incubated in the dark at 5–10°C, and analyzed for the presence of volatile arsines by one of the methods described below. No arsines were found, by any of the techniques, in the following control and reference experiments: media and seawater, no sediment; autoclaved sediment + media + seawater; sediment + seawater, no media.

Method 1

The intense, garlic-like odor of the arsines is a good indicator of their presence. The odor threshold of TMA is reported to be 0.002 $\mu g\,kg^{-1}$ in aqueous solution.[36] Therefore, a qualitative evaluation of arsine production was carried out by cautious sniffing of a sample of the head-space of the culture flasks. This procedure has been successfully used in several studies.[1,23]

Method 2

Mass spectroscopy can be used to identify TMA that has been chemofocused onto a mercuric-chloride-impregnated filter.[37] The experiments were conducted as described in the literature,[37] but the glass fiber filters were washed with 30% hydrochloric acid (HCl), rinsed several times with distilled water and dried overnight prior to being soaked in 5% aqueous mercuric chloride ($HgCl_2$). The resulting mercuric chloride traps were suspended above the sediment mixture with Teflon thread. Mass spectroscopic analysis (AEI-MS50 Varian–Atlas CH4 spectrometers) of pieces of the filters was performed at the University of British Columbia. In the case of positive results, TMA was identified by a peak at $m/z = 120$ and by a fragmentation pattern identical to an authentic sample. Other peaks could be attributed to Hg^+, $HgCl^+$ and $HgCl_2{}^+$.

Method 3

In some experiments the volatile arsenicals produced in the culture flasks were identified using the gas chromatographic/atomic absorption (GC AA) system that is described in detail under Analyses (Section 4) below. In these cases, the arsines were first concentrated cryogenically (cryofocused). To accomplish this the head-space was swept from the culture flask using a flow of helium. The effluent gases were passed through two U-tube traps connected in series and immersed in liquid nitrogen. Each U-tube was equipped with two Teflon valves and a side-port fitted with a Teflon-lined septum. A 15 min purge was sufficient to transfer the arsines quantitatively to the traps where they were frozen. The valves were closed and the traps removed from the gas stream. The culture flasks were purged with nitrogen, sealed and the incubation continued as appropriate. The traps were warmed to room temperature and a sample of their contents removed, via the septum, using a gas-tight syringe. This sample was then injected into the reaction vessel of the GC AA system.

4 Analyses

Interstitial water metabolites

The interstitial water from sediment cores was analyzed for ammonium ($NH_4{}^+$), phosphate (HPO_4^{2-}) and sulfate (SO_4^{2-}) ions, either by ion chromatography (Analytical Services Unit, Queen's University, Canada), or by standard literature methods.[38] Care was taken not to expose the samples to air. Detection limits were: phosphate, 2 $\mu mol\,dm^{-3}$; sulfate, 0.1 $mmol\,dm^{-3}$; ammonia, 0.01 $mmol\,dm^{-3}$. Triplicate runs on the same samples were reproducible to ±2%.

Arsenic

Analytical standards were prepared fresh each day in Teflon containers from stock solutions containing 1000 $\mu g\,g^{-1}$ (ppm) arsenic of the appropriately purified arsenical obtained as follows: arsenite from arsenic oxide (As_2O_3), Alfa, USA; arsenate ($Na_2HAsO_4 \cdot 7H_2O$), Sigma, USA; MMAA ($Na_2(CH_3)AsO_3 \cdot 6H_2O$), Pfaltz and Bauer, USA; DMAA [$Na(CH_3)_2AsO_2 \cdot xH_2O$], Sigma, USA. TMA [$(CH_3)_3As$] (Strem, USA) standards were prepared using standard vacuum-line techniques. These same arsenicals were used in the sediment culture experiments.

Arsenic analyses employed the gas chromatographic (GC) separation and atomic absorption (AA) detection of arsine (AsH_3) and the volatile methylarsenic(III) compounds MMA, DMA, and TMA introduced either directly or produced by means of pH-selective hydride generation (HG) from arsenate or arsenite and the appropriate methylarsenic(V) species. For interstitial water collected in 1984, and the analysis of samples processed by the Method 3 procedure described above, the apparatus was the same as that described previously.[24] Subsequent samples were analyzed by

means of a modified system, which is now described.

Aliquots of interstitial water were introduced into a Teflon reaction vessel and buffered to pH 6 with Tris–HCl, prepared from tris(hydroxymethyl)aminomethane (Ultrapure, Aldrich, USA) and hydrochloric acid (Analar, BDH, USA). Prolonged flushing with helium released any volatile arsines present in the sample. Slow addition of 4% potassium borohydride (KBH_4) (Sigma, USA) converted arsenite only to arsine. After the analyses of the liberated arsine by the system described below, the sample was acidified to pH 1 by the addition of 4 mol dm^{-3} HCl. At this pH, *further addition* of KBH_4 reduced the arsenate to AsH_3, MMAA to MMA, DMAA to DMA, and TMAO to TMA.

The arsines so produced were swept from the reaction vessel by a helium flow through a lead acetate trap [to remove hydrogen sulfide (H_2S)], and a −78°C, dry-ice/alcohol Teflon water trap, and collected in a Teflon coil immersed in liquid nitrogen. After trapping the sample, the Teflon coil was removed from the liquid nitrogen and immersed in a 50°C water bath. A gas-sampling valve was switched, and the arsines were separated on a rigorously silanized (Silyl-8, Chromatogrpahic Specialties, Canada) Porapak P-S (Chromatographic Specialties, Canada) packed Teflon column by means of careful temperature programming (Varian 3700 GC). The effluent from the GC was swept to an Instrumentation Laboratories 351 atomic absorption spectrophotometer, where it was combusted in a hydrogen–air flame in a quartz cuvette. The signal was monitored at 193.7 nm and processed using a Hewlett–Packard 3390 A integrator. For head-space analysis, gaseous samples were introduced via a septum into an empty reaction vessel.

Absolute detection limits were 0.12 ng (of arsenic) for arsenite and 0.25 ng for arsenate and the methyl arsenicals. Aqueous sample volumes for inorganic arsenic determinations were typically 2–5 cm^3, giving concentration limits of 0.3 and 0.7 nmol dm^{-3} for arsenite and arsenate respectively. The organo-arsenicals were usually present in much lower concentrations and were therefore determined from a separate, larger (30–40 cm^3) aliquot. Concentration limits in porewaters were 0.1 nmol dm^{-3} for MMAA, DMAA and TMAO. The reproducibility of all standards and duplicate samples was ±5%. For experiments employing the Method 3 sampling procedure, the volume of head-space gas was identical (~50 cm^3) in each of the incubation flasks. The concentrations of volatile arsines are therefore reported in arbitrary units that are self-consistent with respect to each other and to the controls/standards.

Caveats regarding HG GC AA analyses were noted previously,[24] but several points need to be emphasized. It has been suggested[39] that any mono-, di-, or tri-methylated arsenical capable of reaction with borohydride at pH 1 could be responsible for the methylarsines detected by this procedure. However, as no such compounds have been identified to date,[1] we have reported our results in terms of MMAA, DMAA, and TMAO. There is evidence of other methylarsenicals, possibly methylarsenic(III) species, that produce volatile products at neutral pH. This is discussed in a later section. Finally, we have followed the common convention[1–3] of referring to the methylarsenic(V) acids, $(CH_3)As(O)(OH)_2$ and $(CH_3)_2As(O)(OH)$, as fully protonated. It should be noted that the pK_a values[1] (MMAA: 3.6, 8.2; DMAA: 6.2) clearly indicate that the anions, or their salts, will dominate at the pH values (7.5–8.2) typical of interstitial waters. Similarly, arsenate will be present as $As(O)(OH)(O^-)_2$ and arsenite as $As(OH)_3$.

Arsenite compounds bound to the sediment and not dissolved in the pore water are collectively defined here as solid-phase arsenic. Total solid-phase arsenic concentrations were determined as follows. Dried sediments were digested in a mixture of deionized water (10 cm^3) and aqua regia (12 cm^3 of 3:1, v v, hydrochloric–nitric acids, $HCl:HNO_3$) for 3 h in a 80°C water bath with occasional agitation. The samples were then centrifuged and the supernatant liquid was analyzed for arsenic [as arsenate] by the hydride generation method described above. Calibration curves were established by treating National Research Council of Canada sediment standard (BCSS-1) with an identical procedure and checks were run by including standards and blanks with each batch of samples. The range of values obtained for each batch of samples was typically ±5% for triplicate runs of the same samples.

RESULTS AND DISCUSSION

1 Study areas and sediment characterization

In order to identify the factors influencing the origin and distribution of methylarsenic compounds, experiments were conducted with sediments collected

from a number of locations and encompassing a range of oceanographic conditions. Thus, the sites varied in the input of lithogenic and organic matter and in the properties (oxic versus anoxic) of the water column overlying the sediments. As a result, the sedimentary environment differed greatly, both with geographical origin and with depth in the same sediment core. Experiments with sediments consisting in whole or part of mine-tailings (the finely ground waste material from ore processing) provided insight into the influence of anthropogenic activity.

The sediments were characterized by determination of the arsenic and organic carbon content, and by identification of the redox zones (Table 1). This last parameter can be determined from the profile of the interstitial water metabolites (HPO_4^{2-}, NH_4^+, H_2S, and SO_4^{2-}) and is a consequence of the microbial degradation of organic matter.[40,41] In this process, oxidants are utilized in order of decreasing free energy availability. In ideal conditions oxygen (O_2) is consumed first (oxic zone) releasing NO_3^-, HPO_4^{2-}, and HCO_3^- to the porewater. When oxygen consumption exceeds the rate at which it can be resupplied by diffusion from the overlying water, the next available oxidants are employed. This creates a suboxic zone characterized by the appearance in pore waters of NH_4^+, Mn^{2+}, Fe^{2+} (as well as HPO_4^{2-} and HCO_3^-) due to the successive reduction of NO_3^-, manganese(IV) and iron(III) oxides. Sulfate depletion and H_2S production can be used to identify the next, anoxic, layer. Below this, methane formation occurs. The thickness of these zones depends on the availability of each of the oxidants, the sedimentation rate, and the organic content of the sedimenting material. The zones can be disturbed by burrowing organisms (bioturbation) and it is common for microenvironments to exist within a larger redox zone. Nevertheless, these zones differ in the prevailing redox potential, and in the general nature of the microbial community. A full range of these environments was sampled in this work. The complete characterization of the porewater metabolites will be reported separately,[42] and only the general features are discussed here.

Saanich Inlet provided samples of completely anoxic sediments. The physical oceanography of this fjord, which is located at the southern end of Vancouver Island (Fig. 2), has been well documented.[43] It is relevant to note here that water circulation to the Inlet is restricted by a 70 m sill at its mouth (Fig. 3), and by limited freshwater input at its head. These factors, together with a large input of organic matter from the biologically productive surface waters, result in waters below 135 m being anoxic, with only partial, periodic, intrusions of oxygenated water. Thus, the sediments sampled from a depth of 215 m were homogeneous, black, rich in H_2S and organic carbon (Table 1), and had no evidence of bioturbation. No oxygen could be detected in the water overlying the sediment and porewater sulfate concentrations ranged from 6 to 0.1 mmol dm^{-3} from the sediment–water interface to a depth of 50 cm. Sooke Basin sediments were also anoxic with a similar organic carbon and arsenic composition.

Satellite Channel, consisting of shallower waters (80 m) located adjacent to Saanich Inlet, is characterized by strong tidal currents. These result in fully

Table 1 Sediment characteristics[a]

Origin	Depth (cm)[b]	C_{org} (%)[d]	As (ppm)[e]	Redox zone[f]
Saanich Inlet	0–50	4	5	Anoxic
Satellite Channel	0–35	1	9	Oxic/suboxic
	35–80	1	6	Anoxic
Alice Arm				
A5	0–15[c]	1	30	Oxic/suboxic
	15–75[c]	<0.4	35	Anoxic
A12	0–15	1	12	Oxic/suboxic
	15–80	1	8	Suboxic

[a]Average values for indicated depth zone. [b]Depths are those most relevant to the sediment sections used for culture experiments and are measured from the sediment–water interface. [c]Surface sediments consisted of a mixture of natural sediments and mine tailings. Below 15 cm, there were exclusively finely ground mine-tailings. [d]Organic carbon content of solid sediments. [e]Solid-phase arsenic determined by acid digestion (HCl/HNO_3) and HG AA. [f]According to classification scheme described in Refs 40 and 41. Also see text. Oxic zones generally restricted to $\ll$5 cm.

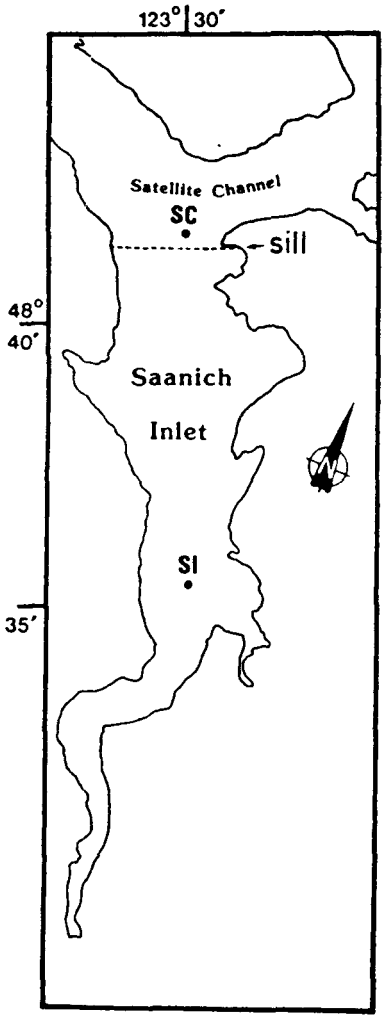

Figure 3 Map of Saanich Inlet/Satellite Channel showing positions of sediment core stations, SI and SC, respectively.

oxygenated bottom waters and a low sedimentation rate. As a result, sulfate depletion was observed at a depth of 40 cm (22 mmol dm^{-3} SO_4^{2-}) and continued to the bottom of the core (80 cm; 4.5 mmol dm^{-3}). Above 40 cm, the Mn^{2+} and Fe^{2+} concentration profiles and the greenish color of the sediments were consistent with a suboxic zone; these profiles, together with a shallow, brown, surface layer, also confirmed that oxygen penetration was restricted to 2–5 cm, as is typical for coastal systems.[44]

Alice Arm, a glacially fed fjord located about 100 km inland from the Pacific coast on the northwestern mainland of British Columbia (Fig. 2), provided sediments of both natural and anthropogenic origin, each with high total (solid-phase) arsenic content. This region (Fig. 4) has been the focus of extensive mining activities since the turn of the century, most recently with operations located at Kitsault.[24] BC Molybdenum operated at the site from 1966 to 1972, during which period tailings were discharged to Alice Arm surface waters via Lime Creek. The mine was redeveloped by Amax of Canada, which released effluent via a submerged outfall located adjacent to Roundy Creek. During the operation of the mine (April 1981–October 1982), approx. 4×10^6 tonnes of mine-tailings were discharged. Numerous investigations have demonstrated that these have been distributed throughout the region to at least as far as Hans Point. Run-off, principally via the Kitsault River, results in a natural sedimentation rate of about 1–2 cm y^{-1}.[45] The tailings material found at stations A3, D3, and A5 was low in organic carbon, having presumably only adsorbed organic matter during rapid sedimentation. However, being finely ground, the tailings were tightly packed and were characterized by high porewater concentrations of Fe^{2+} and Mn^{2+} and low SO_4^{2-}. Variable amounts of brown natural sediment, with no sulfate depletion, were found throughout the multi-year sampling period. The depths of these zones were consistent with the natural sedimentation rate, but it is likely that the predominantly natural sediments were slightly contaminated by light tailings particles that had been resuspended from the sides of the fjord. Station A12 provided mainly natural sediments, which displayed no sulfate depletion and gave Mn^{2+} and Fe^{2+} values that were consistent with suboxic redox behavior from 5 to 80 cm. In latter parts of the sampling program there was evidence of slight contamination of the surface sediments by tailings material carried over the sill from the main body of Alice Arm.[42]

2 Culture studies – volatile arsenic(III) compounds

It was of interest to determine if micro-organisms present in sediments were capable of producing volatile arsenic(III) compounds. Experiments were therefore carried out in which sediments containing a range of arsenic concentrations were incubated at typical bottom-water temperatures (5–10°C), and the production of volatile arsines monitored by one of three different techniques. The results, which are

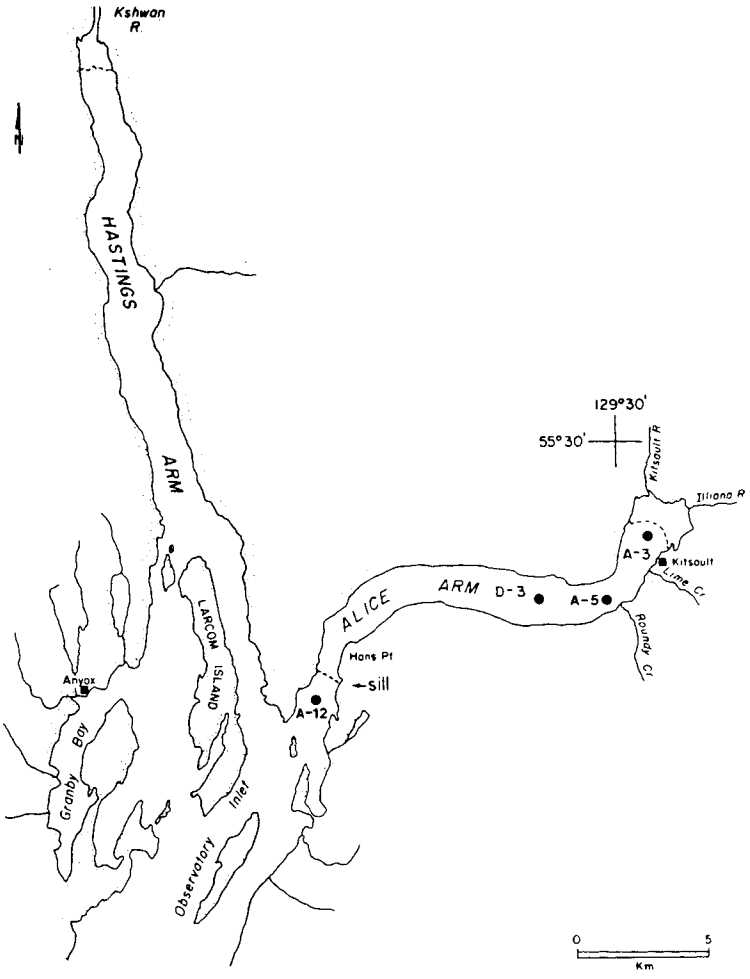

Figure 4 Map of Alice Arm showing sediment core stations.

summarized in Tables 2 and 3, and Figs 5–7, indicate that the volatilization of arsenic by sediment micro-organisms is a general phenomenon.

Several points are apparent from the results of the qualitative (Table 2) and semiquantitative (Table 3) experiments.

(1) No arsines were detected from incubations involving sediment itself, a mixture of autoclaved sediment and culture media (nutrient broth), or sterilized culture media inoculated with inorganic arsenic. Thus, arsenic volatilization was dependent on the growth of naturally occurring sediment micro-organisms.

(2) Also clear is the absence of any correlation between the organic carbon content of the sediment (Table 1) and the ability to produce volatile arsines. Positive results were obtained, for example, with both the anoxic, organic-rich, Saanich Inlet mud, as well as the organic-deficient mine-tailings material from the anoxic zone of the Alice Arm A5 core. As will be noted later, this observation also extends to the occurrence of methylarsenic(V) compounds in porewaters. A proposal that methylarsenicals will only be found in sediments as a result of the hydrolytic degradation of algal detritus is therefore not supported by the data reported here.

(3) The production of arsines was not restricted to cases of high solid phase or porewater arsenic concentrations. The arsenic content (Tables 1

and 3) of all of the samples, including the tailings, falls in a range typical of natural sediments. Thus, volatilization was not just a response to extreme arsenic stress.

(4) Arsines were found when sediment was incubated under both anaerobic (Tables 2 and 3) and aerobic (Table 3) conditions in contradiction to assumptions[2] based on Wood's cycle.[28]

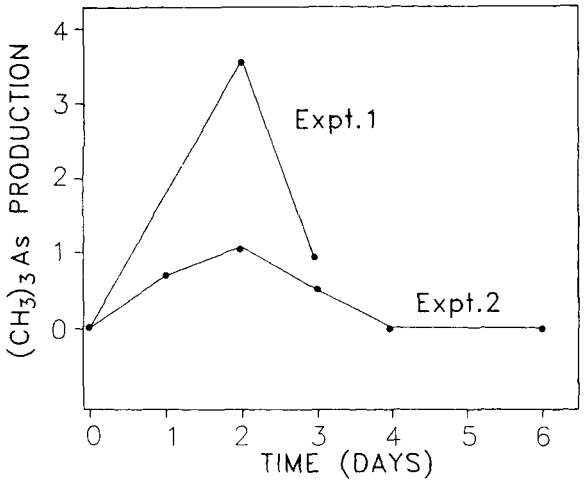

Figure 5 TMA $[(CH_3)_3As]$ production from the anaerobic incubation of Sooke Basin sediments. No TMA was found in Expt 3. Relative concentrations, where 0 represents the detection limit; points at 0 indicate no detection.

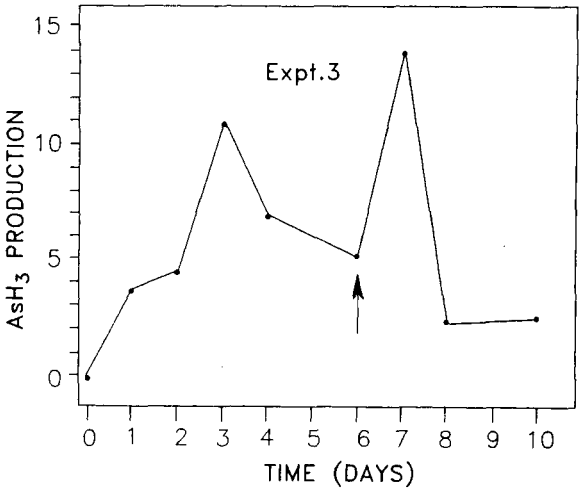

Figure 6 Arsine (AsH_3) production from the anaerobic incubation of Sooke Basin sediments. Arrow indicates the addition of MMAA. No AsH_3 was detected in either Expt 1 or 2. Relative concentration units; 0 represents the detection limit.

The intense garlic odor of the head-space of all of the culture flasks was consistent with the product of volatile arsines (Tables 2 and 3) and TMA was unequivocally identified (chemofocusing/mass spectroscopy) as a component of this phase in experiments with Alice Arm sediments (Table 3). Cryofocusing of the gases liberated by Sooke Basin sediments permitted the identification, by GC AA (Method 3), of several additional arsenicals. Demethylation was also apparent in these experiments, which are now described.

Arsine, MMA and TMA were all produced when

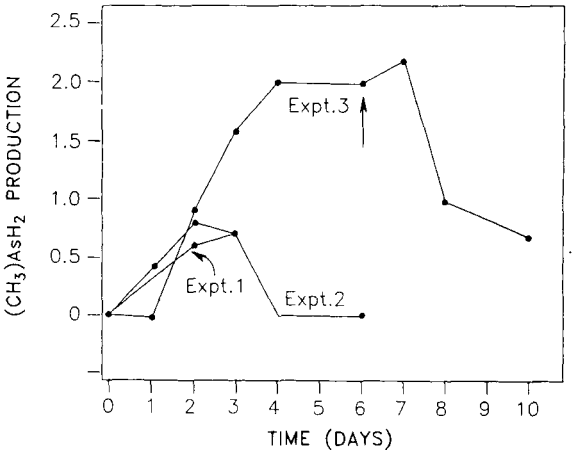

Figure 7 MMA $[(CH_3)AsH_2]$ production from the anaerobic incubation of Sooke Basin sediments. Expts 1, 2 and 3 were monitored for 3, 6 and 10 days, respectively. Vertical arrow (Expt 3) indicates the addition of MMAA. Relative concentration units; 0 represents the detection limit.

Table 2 Production of volatile arsines from sediments, Method 1: odor detection[a]

Sediment origin[b]		Result[c]	Incubation period (days)
Saanich Inlet,	15–27 cm	+	50
Satellite Channel			
Surface,	0–15 cm	+	50
Deep,	35–45 cm	–	50
Alice Arm			
A5,	25–35 cm	+	65
A12,	25–35 cm	+	65

[a]Samples handled under inert atmosphere and incubated in sealed flasks. [b]See Table 1 for sediment characteristics. Each flask contained the indicated sediment plus culture broth. [c]–, Not detected; +, detected. No arsines detected in experiments using identical sediments, but without culture media. Similarly negative results for autoclaved samples of sediment plus culture media incubated for identical times.

Table 3 Production of trimethylarsine from Alice Arm sediments, Method 2: chemofocusing/MS detection[a]

Experiment type	Sample[b]	Arsenic concentration (nmol dm^{-3})[c]		Result[d]
		Arsenite	Arsenate	
Reference	Culture media; no sediment; inoculated with arsenicals	1600	1360	–
	A12, 0–15 cm, autoclaved	7	52	–
Anaerobic	A5, 40–55 cm	1600	1360	+
	A12, 40–55 cm	27	51	+
Aerobic[e]	A5, 10–15 cm	293	867	+++
	A12, 0–15 cm	7	52	++++

[a]See Methods section for experimental details. Incubation period, 10 days. [b]Table 1 provides sediment characteristics. Culture media were added to each flask. [c]Arsenic concentrations are those of the original interstitial water. [d]–, Not detected; +, detectable with number indicating relative strength [e]Flasks were fitted with sterilized porous plugs.

samples of Sooke Basin sediment were incubated anaerobically with nutrient broth. The distribution of these arsenicals was dependent on the length of the incubation, and on the age of the sediment sample. Three experiments (indicated as Expt 1, Expt 2 and Expt 3) were carried out under identical conditions, but with different duration times (3, 6 and 10 days, respectively). The sediments used for each experiment were removed from a homogeneous sample of a Sooke Basin core stored at 5°C. Thus, Expts 1–3 employed successively 'older' sediment samples – a point that appears to be responsible for the dramatic differences in the experimental results. These differences are readily apparent from an examination of Figs 5–7, in which each data point is an average of a triplicate set. No analysis was carried out on day 1 (Expt 1 only); day 5 (Expts 2 and 3); day 9 (Expt 3). No arsenic was added to the sediments except as described below. Initial arsenic concentrations were similar to the average values observed for Saanich Inlet: solid phase arsenic, 5 ng g^{-1} (ppm); total dissolved arsenic, 436 nmol dm^{-3} made up of 305 nmol dm^{-3} arsenite and 131 nmol dm^{-3} arsenate.

TMA was produced in both Expts 1 and 2, with a maximum occurring after two days of incubation (Fig. 5). This maximum was then followed by a sharp decrease. No TMA was found during days 4–6 in Expt 2, and it was similarly undetectable in *any* of the Expt 3 samples. In contrast, arsine was not found in Expts 1 and 2, but was present throughout the duration of Expt 3. The AsH_3 profile peaked at day 3 (Fig. 6), but was restimulated by the addition of a small amount (concentration change of 17 nmol dm^{-3} in the culture flask) of methylarsonic acid on day 6 (indicated by arrow in Fig. 6). Arsenate inoculations (giving 25–65 nmol dm^{-3} changes in the culture flask) to similar Expt 3 samples on day 3, had no effect on arsine or TMA production. MMA was produced by all of the samples (Fig. 7). The largest concentrations were reached in Expt 3, but these were unaffected by spiking with arsenate. The addition of methylarsonic acid appeared to restimulate MMA production, but the change was small. DMA was absent in all of the experiments. As noted in the other culture work, active micro-organisms were responsible for the observations, as no arsines were found in autoclaved controls.

The production of MMA and TMA lends further support to the work of Wong *et al.*[30] and emphasizes one of our earlier conclusions: that elevated arsenic levels are not a necessary prerequisite for arsenic volatilization. Pickett *et al.*[23] have also recently reported a noticeable odor of TMA in flasks containing natural sediments treated with TMAO and incubated 10–40 days without additional nutrients. To our knowledge, there is no precedent for arsine production by sediments. Some soils and isolated soil bacteria have been shown to produce arsine from arsenate and arsenite under anaerobic conditions.[13] Arsine can also be formed in soils by the demethylation of methylarsonate;[11,12] similar behavior is apparent in the Expt 3 samples. Further comparisons can be made with soils where it is common for mixed microbial communities and even single organisms to cause concurrent methylation, demethylation and reduction of arsenic compounds.[1,46] For example, Anderson and co-workers found that treatment of *Pseudomonas sp.* (among others) with sodium arsenate gave methylation to MMA, DMA and TMA as well as reduction to

arsenite.[10] In a related study,[11] sodium methylarsonate was reduced to MMA, methylated to DMA and demethylated to arsenate.

The micro-organisms responsible for the arsines were not identified in this study. It is known[40] that very different classes of bacteria inhabit the redox zones that are represented by the sediments used (Table 1). Even allowing for the likely possibility that the microbial communities underwent considerable compositional changes during storage and/or incubation, these results support the concept that arsenic volatilization is a phenomenon that is common to all sediment types. It is also clear that there is a greater similarity between sediment and soil ecosystems than was previously thought.

3 Culture studies – methylarsenic(V) compounds

The aqueous phase was separated (Reeburgh Squeezers) from the Alice Arm sediments used in the experiments described in Table 2, and examined for the presence of soluble methylarsenic(V) species. The results are presented in Table 4.

The porewater from the incubated, but otherwise untreated, sediments (treatment C; Table 4) contained only small amounts of methylarsenicals – principally DMAA. Incubation with culture broth (treatment A) resulted in a considerable enhancement in the concentrations of methyl species. Although DMAA was still predominant, MMAA and TMAO were also found. There were increases in the methylarsenic concentrations in some of the autoclaved controls (treatment B), but the samples were analyzed a considerable time (>65 days) after the experiment was initiated, and it is possible that microbial growth had occurred at some point. These increases were smaller than those observed in the unautoclaved samples (treatment A). We therefore conclude that sediment micro-organisms were principally responsible for the production of the methylarsenic(V) compounds found in the 'A' treatment samples.

The samples of natural sediments (A12 and A5 surface) and tailings material (deeper sections of A5) used in these experiments represented a wide range of organic carbon content as well as a variety of solid phase (Table 1) and dissolved arsenic concentrations (Table 3 lists data for similar zones). The absence of a quantitative relationship between the response of the methylarsenicals and the other sediment characteristics indicates that while the observed biomethylation was common to all the sediment types, other factors were also important. For example, physical effects, such as adsorption of the biomethylation products to the solid phase, could influence the dissolved arsenic speciation. By analogy to the volatile arsenic(III) experiments, it is also possible that both methylation and demethylation occurred during the incubation period and the observed concentrations were due to a balance between these biological processes at the time of sampling. Time-series experiments will be necessary in order to identify the exact sequence of events. However, there is evidence which suggests that net methylarsenic production could continue for some time. In similar experiments with dredged fresh- and sea-water sediments, Brannon and Patrick[47] found considerable increases in the concentrations of unidentified organoarsenic compounds in the exchangeable (water-extractable) sediment fraction. Release of these organoarsenicals persisted for several months.

The flasks were sealed during our experiments and conditions were therefore conducive to the growth of anaerobic bacteria. It is not known if similar results would have been obtained under aerobic conditions, but other evidence suggests that it is likely. For example, the methylarsenic(V) compounds are precursors to the volatile arsines, and trimethylarsine

Table 4 Methylarsenic(V) compounds in the solution phase of incubated[a] Alice Arm sediments

Station	Depth (cm)	Treatment[b]	Concentration nmol dm^{-3}		
			MMAA	DMAA	TMAO
A5	0–15	A	1.3	160	Trace[c]
		B	Trace	Trace	nd
		C	nd[d]	Trace	nd
A5	25–35	A	9.3	26.7	4.0
		B	6.8	11.6	0.9
		C	0.4	1.2	nd
A12	0–20	A	8.0	28.0	nd
		B	2.5	9.5	1.1
		C	nd	Trace	nd
A12	25–35	A	4.0	18.7	Trace
		B	nd	8.0	nd
		C	0.7	7.1	nd

[a]Same samples used in volatile detection experiments described in Table 2. Aqueous phase separated using Reeburgh squeezers as described in the Experimental section. [b]All samples incubated anaerobically at 10°C. A, sediment + culture media; B, autoclaved sediment + culture media; C, sediment only. [c]Indicates qualitative observation at detection limit. [d]Not detected.

was identified as at least one of the volatile species produced upon aerobic incubation of the same sediments (Table 3). In addition, Wong *et al.*[30] found similar distributions of MMAA, DMAA, and TMAO in their aerobic experiments with freshwater sediments. Collectively, these data strongly suggest that arsenic biomethylation to give dissolved methylarsenic(V) compounds is possible for all sediment types and environments.

The analytical results also provide evidence for the presence of other methylarsenicals. The A5 and A12 culture experiments liberated up to 3 nmol dm^{-3} of DMA and TMA in the first step (pH 7) of the hydride generation procedure. Neither methylarsine was detected if the sample was simply purged with helium and the effluent trapped and analyzed. As neither DMAA nor TMAO is reduced at neutral pH, these observations suggest the presence of other di- and trimethylarsine precursors. Possibilities include the methylarsenic(III)–sulfur species indicated in Fig. 1. These compounds are easily formed on reaction of methylarsenic(V) species with thiols;[48] alkane thiol (RSH) species are common in anoxic sediments.[49]

4 Methylarsenicals in interstitial water

The results of the culture experiments described in the previous sections clearly indicate that sediment microorganisms capable of both methylating and demethylating arsenic compounds are present in marine sediments. This is consistent with our previous study[24] in which we detected MMAA, DMAA and TMAO in the interstitial waters of marine sediments from Pacific Coastal waters. During the course of the work described above, we resampled several of our original stations. The total (solid-phase and dissolved) arsenic concentrations of the natural sediments were comparable with those obtained previously. However, there were dramatic differences in the concentrations of methylarsenicals. The individual methylated species were present, and in similar relative amounts, but the overall levels were much lower than those previously reported.[24] In many cases there were only trace amounts (i.e. just observable at the detection limit) indicating that the concentrations were about 10% of the values measured earlier. Seasonal variations, and other natural phenomena, or intra-site variability may be responsible for these differences. However, such influences had smaller effects on inorganic arsenic concentrations. Thus, the methylarsenicals were either more susceptible to temporal variations in the environment, or to post-sampling alteration, or to both. Our current (since 1985) shipboard practice is to maintain sediments rigorously at their original temperature throughout the porewater extraction process. Adsorption to the solid phase could account for these differences, but temperature variations could also result in changes in the balance of methylating/demethylating bacteria.

These results are reminiscent of the factors influencing the distribution of methylmercury compounds.[50] Disturbance of mercury-containing sediments by collection is known sometimes to result in a growth in the methylmercury concentration by biomethylation of the inorganic mercury reservoir. This is followed by demethylation and a return to normal, low, levels. In mercury-polluted sediments, methylmercury compounds are present at 0.1–1.5% of the total mercury content.[50] It appears that methylarsenic concentrations are similar, but are not dependent on the presence of arsenic contamination. Anthropogenically influenced sediments do, however, contain consistently higher levels of methylarsenicals but, as will be described below, this is not necessarily due to arsenic pollution.

During both our original[24] and more recent work, we obtained reproducible results from sediments sampled at the same site and handled identically. Thus, comparisons of sediments from different sites should be possible, provided that identical sampling and work-up procedures are employed. It is therefore significant that sediments altered by mine-tailings input (e.g. A5, D3 and A3) contained consistently higher concentrations of methylarsenicals than natural sediments (e.g. A12) from the same geographical area. These anthropogenically modified sediments also displayed the greatest changes over a multi-year sampling program. These points are apparent from the results obtained from samples collected at station A5 and described below. Identical phenomena were observed for D3 and A3 samples.[42]

Figure 8(A) indicates the distribution of the total methylarsenic(V) concentration (ΣMeAs) with depth in the porewater of A5 cores collected in 1985 and 1987. Data from 1983 were reported previously,[24] but are included for comparison; 1986 results were intermediate between 1985 and 1987, but only a few depths were sampled. It is obvious that the methylarsenicals were not evenly distributed within the cores, and their concentrations varied over the

sampling period. Most apparent is the complete absence of any methylated species in the surface sediments (0–15 cm) in 1987 (and 1986 not shown). This is in direct contrast to the 1985 results, and for those obtained from 1983 samples (but these were not handled by the same procedure as the others). These variations underline the profound differences in the extent of methylation in natural sediments and tailings, as can be seen from a consideration of the changing sedimentary environment at station A5.

In 1983 visual inspection of the core indicated that the sample consisted of a thin layer (1–2 cm) of natural sediment overlying tailings material that had been deposited prior to the mine's closure the previous year. This layer of brown, loosely packed detritus extended to depths of about 6 and 9 cm in 1985 and 1987, respectively. Below those layers, the cores were uniformly grey/black in color and consisted of drier, more tightly packed, tailings to the bottom of the core.

The influence of the tailings on porewater chemistry is evident from the metabolite concentrations. Despite a low organic carbon content, bacteria were still active in the tailings, as indicated[42] by the release of HPO_4^{2-} [Fig. 8(B)] and NH_4^+ to the porewaters. Since the diffusion of oxygen from overlying waters was restricted by the low porosity of the accumulated tailings, oxic respiration was restricted to the surface natural sediments on each of the sampling dates. The suboxic zone only partially penetrated the tailings.[42] As a result, sulfate depletion was found at successively greater depths during the study: 1983, 2–5 cm; 1985, 5–10 cm; 1987, 10–15 cm [1987 results are provided in Fig. 8(B)]. Tailings are known to scavenge phosphate;[41] this is supported by the changing porewater HPO_4^{2-} profiles [Fig. 8(B)]. Significant phosphate concentrations were only found in the more recent (1987) natural, surface, sediments. The underlying tailings material displayed small, but significant, increases in HPO_4^{2-} levels over the sampling period. It is this parameter that relates most closely to the varations in ΣMeAs. Increasing $[HPO_4^{2-}]$ values were accompanied by decreasing methylarsenic concentrations.

Competitive uptake of arsenate and phosphate has been found for a number of micro-organisms.[51–53] The inverse relationship between the concentrations of exocellular methylarsenicals (which are products of arsenate transformations) and of phosphate is consistent with this competition. It is tempting to suggest that whenever phosphate concentrations fall below a certain

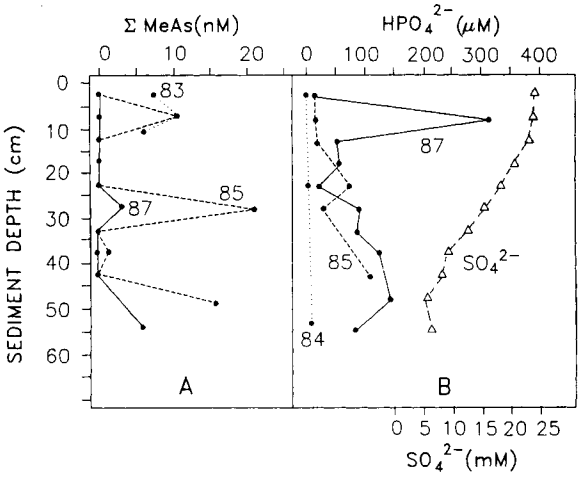

Figure 8 Profiles of interstitial water constituent concentrations with depth in sediment collected at station A5, Alice Arm, over a multi-year sampling period. Points at 0 indicate no significant signal at the detection limit of the analytical technique. A, Total methylarsenic concentrations (ΣMeAs) in 1983, 1985 and 1987. The 1985 values were always greater than, or equal to, those for 1987. B, Sulfate concentrations in 1987 and phosphate concentrations in 1984, 1985 and 1987. The 1984 $[HPO_4^{2-}]$ values were all above the detection limit (3, 9 and 16 μmol dm^{-3}).

level, methylarsenic concentrations will rise. We are currently investigating this possibility. However, other factors are also important. The differences between the natural sediments and the tailings may also be due to differences in the types of microbial communities that dominate each. By further analogy to mercury, changing physicochemical conditions can be expected to alter the ecological succession of organisms as well as their individual activities and biochemical response (methylation/demethylation).[54] We have already demonstrated that biomethylation is influenced by the total dissolved arsenic concentrations.[24] Further work is required in order to assess the relative importance of each of these factors.

CONCLUSIONS

It has been generally accepted that methylarsenicals are not produced in marine sediments, and if present at all, are introduced via the degradation of deposited organic matter or, perhaps, as a bacterial response to arsenic pollution.[2] This view is not supported by the

results presented here. Instead, methylarsenic compounds have been found to be ubiquitous in sediment porewaters, albeit in small amounts. Their concentration appears to reflect the balance between microbial methylation/demethylation at the time of sampling, and is probably subject to the complex influence of temperature, phosphate and dissolved arsenic concentrations, and other physicochemical factors.

The transformation of arsenic by micro-organisms from a variety of sedimentary environments is similar to that observed in experiments with soils[1,46] and with isolated soil bacteria.[1,10–12] This suggests a closer similarity between arsenic speciation in sediments and soil ecosystems than was previously thought. Consistent with this, nearly the full range of exocellular arsenicals (Fig. 1) found in soil systems were produced in our culture studies, the actual methylarsenic species being dependent on the microbial community dominant at the time of sampling. The release of volatile arsines from soils is believed to form an important part of the arsenic global budget even though the rates of production are small.[27] These compounds were not detected in the porewater samples, but this is not unexpected as they would be easily lost during the porewater extraction process. Arsines are also reactive to oxygen, and thus would not be expected to survive transport upwards through deep waters. However, arsenic volatilization may be important in the transport of arsenic from sediments to the water column, and may make important contributions to the arsenic budget above shallow and/or anoxic bodies of water. In this regard arsine (AsH_3) now joins stannane (SnH_4) as an example of a simple hydride with important cycling implications.[55,56]

The porewater concentrations of methylarsenic(V) compounds are estimated to be 0.1–1% of the total dissolved arsenic content of the sediments investigated over the multi-year study. This is similar to the proportions of methylmercury to inorganic mercury in polluted sediments. However, unlike methylmercurials, the organoarsenic(V) compounds are less toxic than their inorganic precursors.[1] In fact, sedimentary arsenic biomethylation may be involved in transformations of arsenic in the marine food chain.[33] It is therefore particularly exciting that there were indications that other methylarsenicals may be present in porewaters. It also serves as a reminder that hydride generation techniques, like all analytical methods, are limited to the types of compounds for which they are calibrated. Current work is focusing on the identification of additional porewater methylarsenic species that may so far have eluded detection.

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