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Methylated arsenic species in estuarine porewaters

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Inorganic arsenic, monomethylarsenic and dimethylarsenic species have been observed in samples of sediment porewater collected from the Tamar Estuary in South-West England. Porewater samples were collected using *in situ* dialysis. The arsenic species were separated by hydride generation and concentrated by liquid nitrogen trapping, prior to analysis by directly coupled gas chromatography–atomic absorption spectroscopy. The predominant dissolved arsenic species present was inorganic arsenic (5–62 $\mu g\,dm^{-3}$). However, this is the first time significant concentrations of methylated arsenic species have been quantified in estuarine porewaters (0.04–0.70 $\mu g\,dm^{-3}$), accounting for between 1 and 4% of the total dissolved arsenic. The presence of methylated arsenic compounds in porewaters is attributed to *in situ* environmental methylation, although the possibility of methylated arsenic species being derived from biological debris cannot be excluded.

Keywords: Inorganic arsenic, methylated arsenic, porewaters, dialysis membrane filtration, hydride generation, liquid nitrogen trapping, coupled gas chromatography–atomic absorption spectroscopy, arsenic methylation

INTRODUCTION

Biologically mediated methylation of As under environmental conditions was first reported by Challenger,[1,2] who studied the ability of the mould *Scopulariopsis brevicaulis* to methylate inorganic forms of arsenic. Subsequently, various workers have reported the ability of both aerobic micro-organisms (e.g. Wong *et al.*[3]) and anaerobic micro-organisms (e.g. McBride and Wolfe[4]) to methylate arsenic. However, the aquatic environment remains largely unexplored despite the fact that in many cases there is a huge array of micro-organisms, some of which have the potential for arsenic methylation. The lack of sensitive and selective analytical methods has been the major obstacle to such work. Thus, it is only recently that suitable methods have been developed[5–9] and reports of the existence of methylated arsenic species, particularly in the marine environment, have begun to appear in the literature.

Johnson and Braman[10] were the first to report concentrations of methylated arsenic species in various members of the pelagic sargassum community. Additional detailed studies of methylated arsenic concentrations have also been carried out on macro-algal samples from North American coastal waters[11] and UK estuaries.[12] More recently Howard *et al.*[13,14] have exploited the high productivity of the Beaulieu Estuary, UK, to study the predominance of dissolved methylated arsenic species. These authors show that arsenic methylation is both temperature- and salinity-dependent, with the highest concentrations of both monomethylarsenic (MMA) and dimethylarsenic (DMA) being produced when temperatures were greater than 12°C and salinities greater than 24‰. The source of this methylated arsenic has not been thoroughly investigated although it has been suggested that porewaters of anoxic sediments may constitute an important reservoir.[15] To date, there has been no positive identification of methylated species in marine porewaters even though attempts have been made to detect them.[16,17]

In this work we report the first observations of methylated arsenic species in estuarine porewaters. The sampling was carried out in the Tamar Estuary, South-West England (see Fig. 1), which is noted for its high arsenic concentrations

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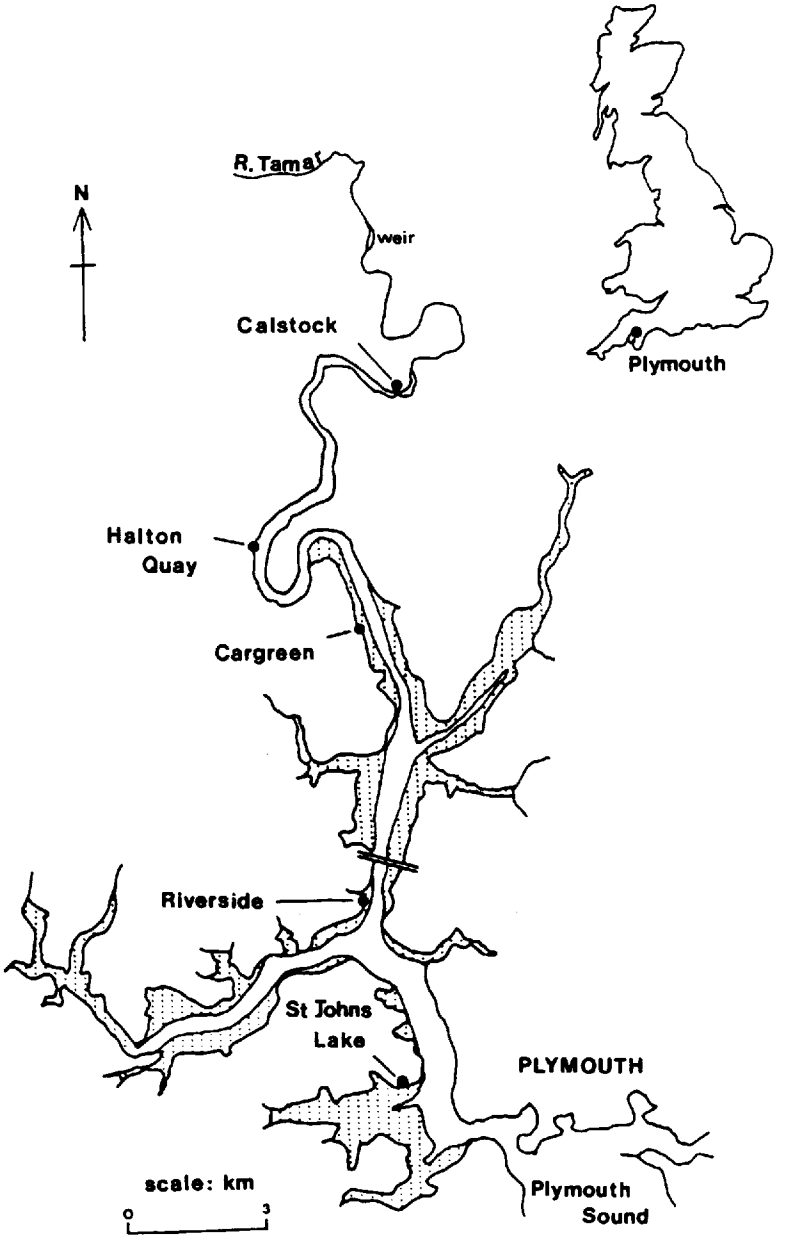

Figure 1 The Tamar Estuary, South-West England and the five porewater sampling sites.

as a result of previous mining.[18] In addition, the estuary has a high biological activity and has been well characterized chemically.[19] It is, therefore, a suitable site to investigate methylation processes.

EXPERIMENTAL

Reagents and glassware

All chemicals were of analytical reagent grade unless otherwise stated and glassware was

cleaned by soaking overnight in 10% (v/v) AnalaR nitric acid, followed by rinsing in de-ionized, doubly-distilled water and drying.

Stock solutions containing 1000 mg dm^{-3} of As were prepared from Na_2HAsO_4 (AnalaR grade, BDH Chemicals, Poole, Dorset), monomethylarsonic acid (disodium salt) and dimethylarsinic acid (sodium salt), respectively. These latter methylated arsenic standards (99% pure) were obtained from Dr K.J. Irgolic (Texas A&M University, USA). Low concentration standards (< 10 mg dm^{-3}) were prepared by the dilution of the stock solutions immediately prior to use. Sodium tetrahydroborate (99% pure) (Aldrich Chemicals, Gillingham, Dorset) was used to prepare a stabilized 4% w/v solution in 0.1 M-NaOH, which was filtered prior to use.

Apparatus

Estuarine porewater samples were analysed for both inorganic and methylated arsenic compounds. Gaseous covalent hydrides were formed from disodium hydrogen arsenate, monomethylarsonic acid and dimethylarsinic acid standards or samples, and the hydrides formed were collected in a liquid nitrogen cooled trap as described by Howard and Arbab-Zavar.[7] The trap was then connected to a gas chromatograph (Series 104, Pye Unicam, Cambridge) via a switching valve and the trap was heated with hot water. The separated hydrides were then detected by a directly coupled gas chromatography–atomic absorption spectroscopy system (GC–AAS).[8,9] Figure 2 shows schematically the two stages of this analysis step. Standards of Na_2HAsO_4, monomethylarsonic acid and dimethylarsinic acid or environmental samples were pumped by peristaltic pump at 2.5 cm^3 min^{-1}, with a flow of 1 mol HCl dm^{-3} and $NaBH_4$ (4% w/v in 0.1 mol NaOH dm^{-3}) also at 2.5 cm^3 min^{-1} via a mixing coil into a gas–liquid separator. The liberated arsines were dried with NaOH pellets and swept by nitrogen, via an ice-bath to trap moisture, to the cryogenic trap for collection on to glass beads (40-mesh) held at -196°C (i.e. a liquid nitrogen trap).

In the second stage of analysis the cryogenic trap was connected to the GC–AAS system via a four-way valve as described by Chau *et al.*[20] The Dewar flask containing the liquid nitrogen was removed and replaced by a hot water bath (80°C). The volatilized arsines were flushed into the GC by a flow of nitrogen at 30 cm^3 min^{-1} The effluent from the GC exited via a heated interface, held isothermal with the GC, and met a small flow of hydrogen at a glass-lined T piece. The resultant gas mixture was burnt and the As atoms produced were introduced into a ceramic tube of recrystallized alumina heated by an air/acetylene flame.[8] The ceramic atom cell was aligned in the light path of the spectrometer (SP9, Pye Unicam, Cambridge) and the detection of arsenic was achieved by monitoring the 193.7 nm As line and using a hollow cathode lamp (HCL) as the light source. The output from the spectrometer was processed by an electronic integrator (Model 339 0A, Hewlett Packard) which reported information on peak height and area, in addition to retention times. The optimum instrumental conditions used for the analyses of arsenic are given in Table 1.

Sampling procedure

During the period March to June 1986 (see Table 2 for the estuarine conditions) a series of surveys was carried out to determine the concentration of inorganic and methylated arsenic species in porewaters of the Tamar Estuary. Porewater samples were collected at strategic sites in the Tamar Estuary (see Fig. 1), using cellulose acetate dialysis bags and cylindrical perspex holders.[21] Each dialysis bag contained 20 cm^3 of deoxygenated, deionized, doubly-distilled water and the holders were buried into the surfacial sediment layer (upper 10 cm) at low tide and left to equilibrate for seven days.[22] All samples were returned to the laboratory with a small amount of sediment surrounding the holder to minimize oxidation of the sample during processing. Analysis of porewater samples was performed immediately on return to the laboratory and completed within 8 h. Chemical analyses were also carried out on the sediments retrieved from the sample sites. The procedures are detailed elsewhere.[23]

RESULTS AND DISCUSSION

The hydride derivatization technique used to determine the speciation of arsenic in this work separates species with respect to the number of methyl groups bound to As. It is, therefore, possible, if unlikely, that species other than monomethylarsonic acid and dimethylarsinic acid may also be reduced to the mono- and dimethyl-

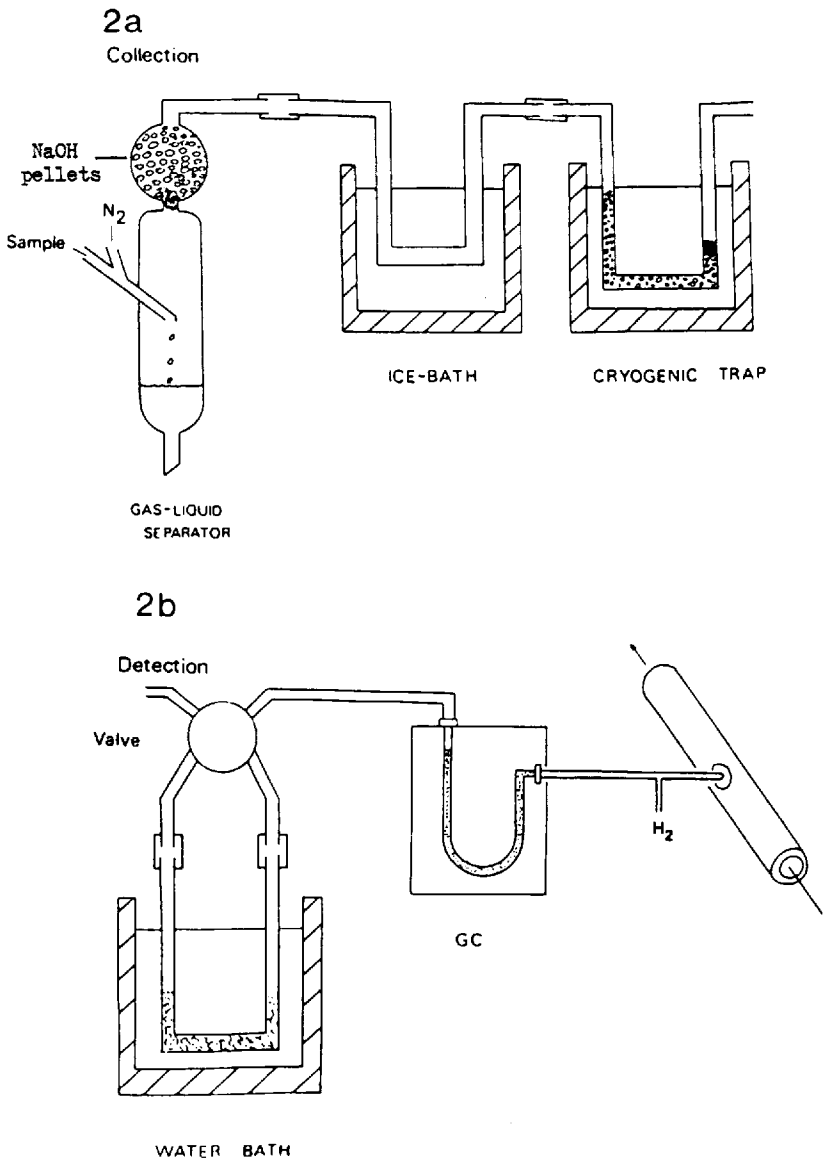

Figure 2 The collection and detection of arsenic species using the GC–AAS system.

arsine derivatives, respectively. For this reason, methylated species of arsenic reported here are classified as either MMA or DMA.

The concentrations and forms of dissolved arsenic species observed at the five sampling sites are given in Table 3. The key feature of these data is that for each of the four surveys between March and June 1986 detectable concentrations of both dissolved inorganic arsenic *and* methylated arsenic species were always present in the porewaters of the Tamar Estuary. The total dissolved inorganic arsenic concentrations ranged from 5 to 60 $\mu g\,dm^{-3}$ which compare favourably with 3–50 μg As(V) dm^{-3} and 0.3–25 μg As(III) dm^{-3} reported for Tamar Estuary porewaters by Knox *et al.*[24] These values are in contrast to soil porewater analyses carried out by Haswell *et al.*,[25] who found concentrations of As(V) between 310 and 210 $\mu g\,dm^{-3}$, for samples collected in the vicinity of Calstock. The concentrations of methylated arsenic species detected at the sampling sites were significantly lower than

Table 1 Optimal analytical conditions

GC–AAS		Hydride generation	
Column packing:	10% OV101 on Chromosorb W(80-100)	Reagents (flow rate 2.5 $cm^3 min^{-1}$):	HCl (1.0 mol dm^{-3})
Temperatures:			$NaBH_4$ (4.0% w/v)
Column	30°C isothermal		Buffer, pH 5.0 (sodium acetate/acetic acid)
Injector	50°C		
Interface	60°C	Drying agent:	NaOH pellets/ice-bath
Gas flow rates:			
N_2	30 $cm^3 min^{-1}$	Trapping system packing:	Liquid nitrogen cooled glass beads (40-mesh)
H_2	100 $cm^3 min^{-1}$	Trapping period:	5 min
Air	4.0 $dm^3 min^{-1}$		
C_2H_2	0.6 $dm^3 min^{-1}$	Purge flow (N_2):	300 $cm^3 min^{-1}$
Light source:	As (HCL), 8 mA		
Wavelength:	193.7 nm		
Bandpass:	0.5 nm		
Deuterium hollow cathode lamp Background correction applied			

Table 2 Estuarine conditions during porewater sampling

Survey date	Mean monthly river flow ($m^3 s^{-1}$)	Mean estuarine temperature (°C)	Estuarine pH range
March, 1986	13.8	8	7.0–8.0
April, 1986	24.9	10	7.0–8.1
May, 1986	20.1	14	6.0–7.2
June, 1986	9.5	15	7.0–8.2

those for total inorganic arsenic. Methylated arsenic compounds accounted for between 1.5 and 4% of the total dissolved inorganic As (see Table 3), with concentrations in the range 0.04–0.70 $\mu g\ dm^{-3}$. The methylation of arsenic is considered to be a requirement for the overall biological detoxification of the element in the environment. Micro-organisms present in sediment porewaters are able to biomethylate arsenic[3] and thus detoxify their environment of arsenic. It could be argued that the production of methylated arsenic species would be highest at sites where sediment arsenic pollution and microbial activity are maximized. Indeed the study carried out by Haswell *et al.*[25] on soil porewaters in the Tamar Valley seem to support this, since the concentrations of the methylated species (1–22 μg MMA dm^{-3}) are relatively more significant, presumably as a consequence of arsenic contamination of the soil. However, in the case of estuarine porewaters there appears to be no strong relationship between the arsenic content of the sediments (see Table 4) and the porewater concentrations of inorganic or methylated arsenic. This suggests the possibility of an additional source of porewater MMA and DMA.

The data in Table 3 reveal no consistent evidence of a time-dependent change in concentrations associated with the significant seasonal changes in river-flow, water temperature and pH (see Table 1). However, consideration of the mean values shows that the relative proportion of methylated arsenic (compared with total As) increases from about 2% in the upper estuary (Calstock; Halton Quay) to about 4% at the seaward end (St John's Lake). Within this trend the DMA porewater concentrations remain roughly constant throughout the estuary and, although the MMA concentrations are not dissimilar, they do increase by a factor of two on going down-estuary. A proportion of the additional methylated arsenic at the seaward end could arise from the influx of decaying biological material in the form of marine phytoplanktonic and macro-algal debris. Both phytoplankton (*Skeletonema costatum*) and macro-algae (*Ascophyllum nodosum*) are known to contain and release almost exclusively DMA.[12,23,26] Thus, if plant tissue is incorporated into estuarine sediments, degradation by bacteria will release cellular DMA into the porewater system. However, in this study samples from the seaward end contained more MMA than DMA, which would require a demethylation process to convert the

Table 3 The concentrations of arsenic species at five sites in the Tamar Estuary during four surveys

Sample site	Date of survey	Concentration ($\mu g\,dm^{-3}$)		
		Total inorganic arsenic	MMA	DMA
Calstock	March	15	0.18	0.26
	April	22	0.17	0.19
	May	18	0.20	0.49
	June	19	0.21	0.17
	Mean ± S.D.	18.5 ± 2.9	0.19 ± 0.02	0.28 ± 0.15
Halton Quay	March	62	0.23	0.25
	April	23	0.18	0.15
	May	17	0.14	0.42
	June	13	0.17	0.23
	Mean ± S.D.	28.8 ± 22.5	0.18 ± 0.04	0.26 ± 0.11
Cargreen	March	8	0.29	0.45
	April	20	0.16	0.12
	May	16	0.04	0.32
	June	24	0.31	0.26
	Mean ± S.D.	17.0 ± 6.8	0.20 ± 0.13	0.29 ± 0.14
Riverside	March	42	0.70	0.15
	April	25	0.48	0.26
	May	15	0.20	0.17
	June	11	0.27	0.29
	Mean ± S.D.	23.3 ± 13.8	0.41 ± 0.23	0.22 ± 0.07
St John's Lake	March	23	0.53	0.19
	April	14	0.17	0.29
	May	14	0.52	0.35
	June	5	0.12	0.18
	Mean ± S.D.	14.0 ± 7.3	0.34 ± 0.22	0.25 ± 0.08

Table 4 Sediment characteristics at the sampling sites

Site	Arsenic sediment concentration ($\mu g\,g^{-1}$ dry weight)		Mean inorganic porewater arsenic ($\mu g\,dm^{-3}$)	Mean sediment carbon content (%)	Mean sulphide ($mg\,g^{-1}$)
	Total arsenic	Acetic-acid available arsenic			
Calstock	77.9	8.8	18.5	4.5	0.78
Halton Quay	63.7	6.8	28.8	4.1	1.60
Cargreen	43.7	8.9	17.0	3.8	2.20
Riverside	41.9	4.6	23.3	3.2	0.30
St John's Lake	35.2	4.7	14.0	4.3	2.60

plant-derived DMA to MMA. Although such processes have been observed with several species of soil bacteria,[27] there is no conclusive evidence for demethylation in aquatic systems.[28] Furthermore, the data in Table 3 show that significant quantities of MMA and DMA are present in the porewaters in March when planktonic activity and macro-algal growth are at a low level. Both these facts suggest that the contribution of methylated arsenic to the porewaters

from extraneous sources, such as biological tissue, is small and the MMA and DMA concentrations are derived mainly from an *in situ* methylation process. Given the data in Table 4, this process would appear to be independent of sediment arsenic concentration.

CONCLUSIONS

A detailed examination of the speciation and concentrations of arsenic in sediment porewaters of the Tamar Estuary has revealed that both MMA and DMA species are present. Their combined contribution is between 1.5 and 4% of the total dissolved arsenic. The results reported here strongly suggest that *in situ* methylation of inorganic arsenic can occur in the porewaters of estuarine sediments. This contrasts with the reports of other workers[16,17] and with the suggestion that methylated arsenic compounds in porewaters are derived from the decomposition of the tissue of marine phytoplankton and macroalgae.[28] It is clear from this study that further investigations are required at other locations using similar methodology to confirm the general importance of *in situ* methylation processes.

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