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Seasonal control of arsenic speciation in an estuarine ecosystem

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Arsenic speciation in the Itchen estuary and Southampton Water (UK) has been shown to vary seasonally, with detectable ($>0.02\ \mu g\ As\ dm^{-3}$) dissolved arsenic(III) and methylated arsenic only being present from May to early October. This corresponds to the time period during which water temperatures exceed 12°C. For the remainder of the year, inorganic arsenic(V) was the only detectable species. At its peak, *ca* 30% of the dissolved arsenic was present as methylated forms with dimethylarsenic (DMAs) being the predominant bioarsenical. Significant quantities of monomethylarsenic (MMAs) and inorganic arsenic(III) were also present, however.

The concentrations of the bioarsenical species varied with position in the estuary and generally increased with salinity. Measurements made during the period of peak algal activity implicated the high-salinity area of the estuary as the most probable region in which the methylated arsenicals are generated. At some sites, a distinct lag was observed between the appearance of dimethylarsenic and the detection of arsenic(III) and monomethylarsenic. Chlorophyll *a* concentration proved to be a poor predictor of the appearance of reduced and methylated arsenic in the water column. Possible sources of dissolved methylated arsenic are discussed.

Keywords: Arsenic, speciation, biomethylation, estuaries, Solent, Itchen, methylation, methylarsenic, hydride generation

INTRODUCTION

The reduction and biomethylation of arsenic in marine and estuarine waters, leading to the presence of dissolved dimethylarsenic, monomethylarsenic and inorganic arsenic(III) species, is now well documented. Such processes can have a major impact in marine ecosystems; in productive estuaries such as Chesapeake Bay (USA) and the Tamar (UK), for example, between 30 and 80% of the dissolved arsenic may be present in reduced and methylated forms.[1–3] In less productive open ocean waters however, bioarsenicals normally account for less than 10% of total dissolved arsenic.[4] The nature of the organisms which are responsible for the presence of reduced and methylated arsenic species in the water column, and the biochemical mechanisms behind the methylation, are still largely unknown. Marine algae, bacteria and fungi have all been shown in culture to be capable of releasing such arsenic species,[5,6] but marine phytoplankton are believed to be the prime methylators. This is supported by the strong correlation of bioarsenical concentrations with chlorophyll *a* in oceanic depth profiles.[7,8] In culture, the degree of reduction and methylation varies markedly between phytoplankton species.[4,9–11] In general dimethylarsenic is currently believed to be the major species released into the water column by phytoplankton. Certain classes, such as coccolithophorids, produce only arsenic(III)[9] and some diatoms (e.g. *Cyclotella* spp. and *Thalassiosira pseudomona*) produce only monomethylarsenic.[1,2]

The biochemical processes underlying the methylation of arsenic by algae is still only partially understood. The uptake of arsenic has been linked with phosphate metabolism and several studies suggest that arsenic is assimilated as a consequence of poor cellular discrimination of arsenate and chemically similar phosphate.[4,12–14] Whilst there is clearly a link between these species, as in culture phosphate enrichment reduces arsenate uptake and alleviates arsenate toxicity,[15,16] at the molecular level the direct substitution appears less clear-cut. In marine algae, methylated arsenic is present as arsenoribosides[17] with

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arsenic present in a position suggesting a nitrogen substitution. This similarity with nitrogen metabolism extends to a number of other organisms which have been found to contain compounds such as arsenobetaine and arsenocholine.[18]

Field observations would also suggest that the link between phosphate levels and arsenate uptake by phytoplankton is not clear-cut. In a controlled ecosystem experiment, a field population of diatoms was shown to be capable of discriminating between phosphate and arsenate under conditions of nutrient limitation.[19] Additionally, high levels of methylated arsenic have been observed in estuaries, such as Chesapeake Bay (USA) and the Tamar (UK), where phosphate levels are greatly in excess of arsenate.[1–3] Clearly further work is required in this area.

Arsenic speciation in temperate marine ecosystems is dominated by the presence of strong seasonal variations which reflect climatic control of primary productivity.[20] In the River Beaulieu (UK), which is situated close to the study area reported in this paper, arsenic methylation is not observed until water temperatures exceed *ca* 12 °C and in the colder months, between October and late May, inorganic arsenic(V) is the predominant species. Such studies have illustrated the importance of taking into account temporal effects when studying the chemical processes in estuaries. This is particularly important in temperate regions where many physical, chemical and biological changes occur with the seasons.

The purpose of this study was to assess the importance of biomethylation in a commercially and biologically active estuary and to examine the dependence of this process on environmental variables which vary seasonally. An attempt has been made to link observations with indices of biological productivity such as temperature, chlorophyll *a* and bacterial cell count.

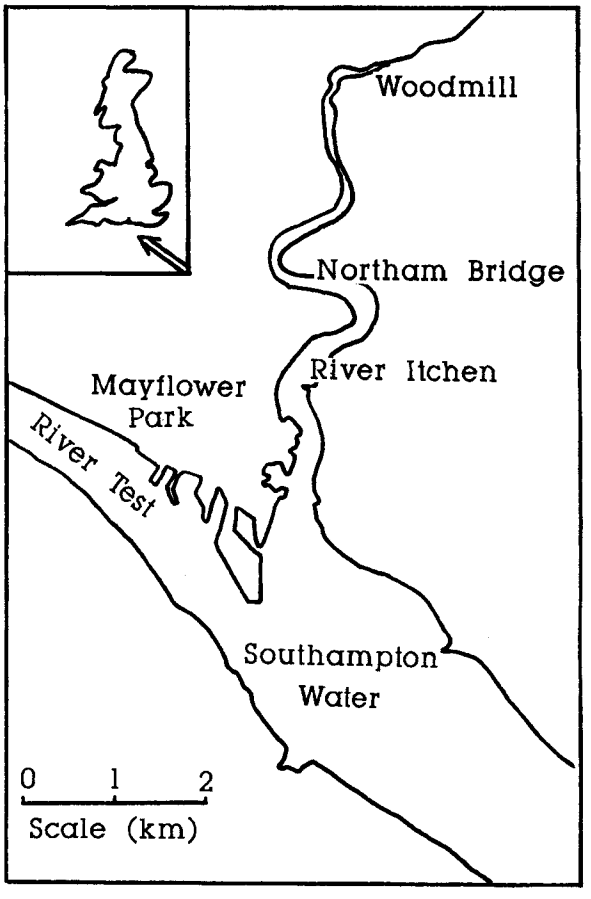

Figure 1 Map depicting the area of study.

EXPERIMENTAL

Sample collection

Surface water samples were collected regularly from three locations chosen to represent largely marine, mid-estuary and freshwater environments. These were situated within the Test/Itchen estuary system near Southampton in Southern England (Fig. 1). Water samples were collected into acid-washed polyethylene containers at approximately weekly intervals during the period March to October 1983. Additional samples were obtained from the marine station (Mayflower Park) from March to August 1984. Tidal influences were minimized by sampling as close as possible to high tide. Surface water temperature was recorded at the time of sampling.

On return to the laboratory (approx. one hour delay), the samples were filtered (Whatman GF/C) and both filters and filtrates were stored frozen (−20 °C) until required for analysis. During the 1984 sampling programme, aliquots of unfiltered water were preserved for bacterial counting by addition of glutaraldehyde solution and storage in the dark at 4 °C.

Analytical procedures

Water samples were analysed for arsenic(III), monomethylarsenic and dimethylarsenic using the hydride generation technique developed by Howard

and Arbab-Zavar.[21] The method has a detection limit of *ca* 0.02 μg As dm^{-3} for all species and a typical precision of 5% (all species) at the 1 μg As dm^{-3} level. Arsenic(III) was measured in samples obtained using the above preservation method which is now known to give levels which are approximately 10% low (S D W Comber and A G Howard, unpublished results). Such results are included as this is generally a predictable loss and does not affect the overall trends which are the subject of this paper. It must also be noted that the sodium borohydride reduction method is only selective to species that on reduction form arsine, monomethylarsine, and dimethylarsine. Some workers using similar hydride generation methods have assumed the corresponding solution species to be the oxyanions arsenite, monomethylarsonate and dimethylarsinate, but strictly speaking there is no *a priori* scientific basis for this assumption. It is probable that to some extent the species that are detected are fragments of larger, as yet unidentified, molecules. With this in view, the determined species are referred to in this paper as arsenic(III), monomethylarsenic, and dimethylarsenic.

Total inorganic arsenic was not routinely determined during this study but is typically *ca* 0.7–1.0 μg As dm^{-3} at the two saline sites and 0.2 μg As dm^{-3} at the freshwater site.

Particulate chlorophyll *a* levels were determined after extraction of filters with 90% (v/v) aqueous acetone according to the method described by Stickland and Parsons.[22] Bacterial populations were estimated by epifluorescence microscopy after straining with acridine orange.[23] Sample salinities were determined using the Mohr chlorinity titration method.[24]

RESULTS

General observations

Data obtained in this study are summarized in Figs 2–5. During the winter months the concentrations of dissolved bioarsenicals in the water column never exceeded the system detection limit. The appearance of dissolved bioarsenicals has been linked with the water temperature rising to above *ca* 12°C.[20] Such behaviour was observed in the current study and confirms the results of an earlier study of the Beaulieu estuary.[25]

As with the nearby Beaulieu estuary, strong temporal

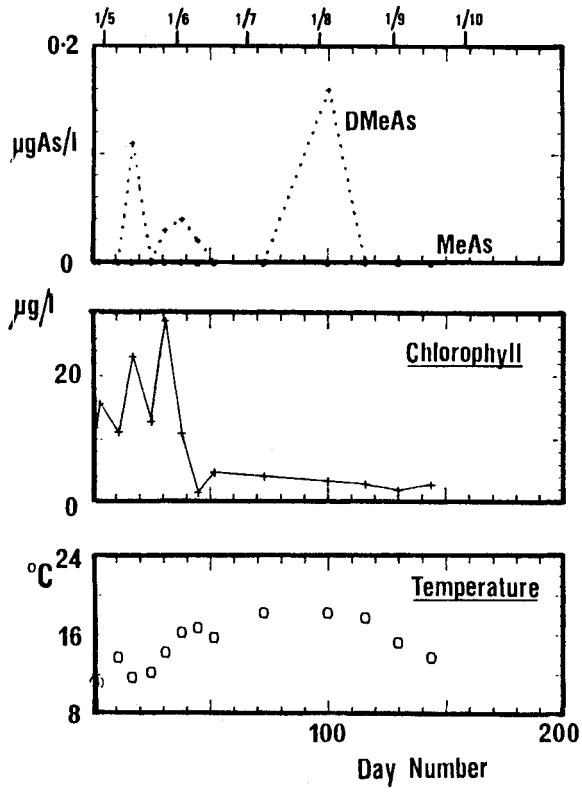

Figure 2 Profile of monitored variables: Woodmill 1983. MeAs, methylarsenic; DMeAs, dimethylarsenic.

variations in arsenic speciation were observed at all sites. Whilst each bioarsenical profile presented individual features, in general bioarsenical concentrations increased in the order: Mayflower > Northam > Woodmill. This is the trend broadly followed by salinity, the concentration of dissolved inorganic arsenic and chlorophyll *a*. Dimethylarsenic was the most abundant of the bioarsenicals and was detected at all sites; monomethylarsenic and arsenic(III), on the other hand, were only detectable at the two saline sites (Northam Bridge, Mayflower Park).

In 1983, bioarsenicals were first detected at the mid-estuary site (Northam Bridge) on 28 April when the water temperature was 12.5°C and then 14 days later at the other sites. In the following year, however, climatic conditions were significantly different and the appearance of bioarsenicals was 6 weeks later (18 June) by which time the water temperature had reached 22°C. Table 1 summarizes the appearance dates and the maximum concentrations attained at each site during the study. At the Northam site and Mayflower

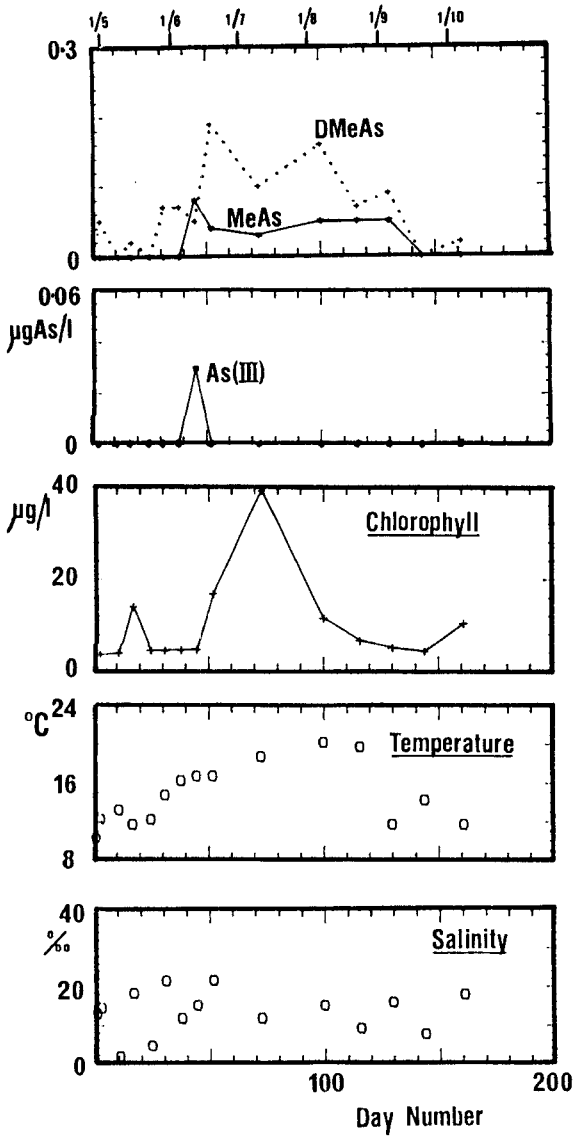

Figure 3 Profile of monitored variables: Northam 1983. Abbreviations as Fig. 2.

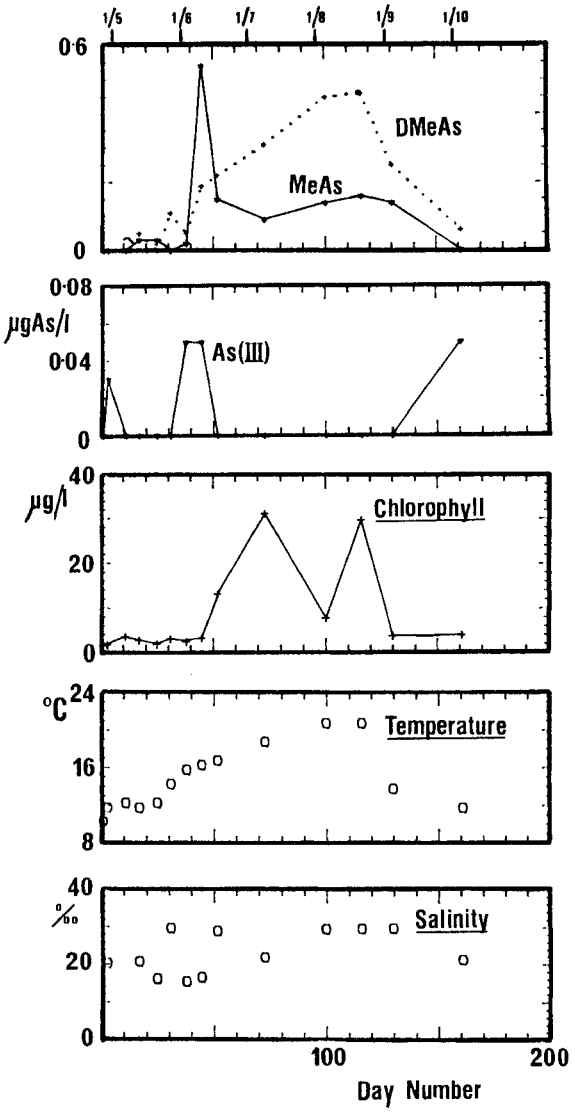

Figure 4 Profile of monitored variables: Mayflower 1983. Abbreviations as Fig. 2.

during 1984, there was a distinct lag between the initial detection of dimethylarsenic and the appearance of monomethylarsenic and arsenic(III) (Figs 3 and 5). Bioarsenical species remained detectable at the two sites until early autumn, when they rapidly disappeared from the water column and were not again measurable until the following spring.

During the period of peak biological activity in 1983, when the temperature had stabilized at between 19 and 21°C, dimethylarsenic and monomethylarsenic exhibited conservative behaviour with salinity (Fig. 6), indicating that the source of methylated arsenicals is within the region of the estuary mouth. The absence of methylated arsenicals below 6‰ on this plot would point towards possible removal of these species in the low-salinity region of the estuary.

Significant correlations were observed between environmental variables at the Northam and Mayflower sites (Table 2). Of particular importance are those between dimethylarsenic, temperature and chlorophyll *a*. Care must however be taken in the interpretation of the correlation data due to covariance of many of

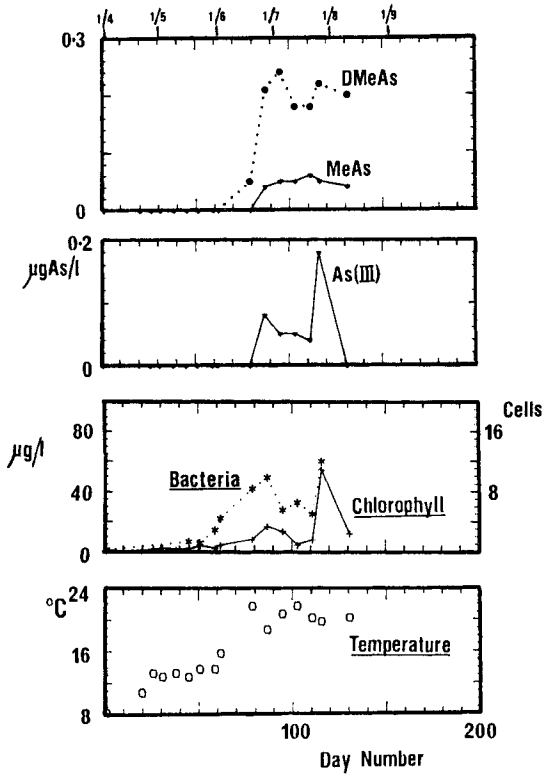

Figure 5 Profile of monitored variables: Mayflower 1984. Abbreivations as Fig. 2.

the variables (e.g. temperature and chlorophyll *a*). Significant correlations should not be taken to imply causality.

Woodmill

At Woodmill, the Itchen is a fast-flowing chalk river and this is the most riverine site studied. Dimethylarsenic, the only bioarsenical detected here, occurred in concert with the main burst of primary productivity in May and early June and once again in early August (Fig. 2). During the rest of the sampling period, bioarsenicals were not detectable and chlorophyll *a* concentrations were comparatively low (below 5 $\mu g\ dm^{-3}$).

Northam

The mid-estuarine nature of this site was reflected in salinity variations of between 5 to 25‰. Dimethylarsenic was the first detectable bioarsenical occurring spasmodically during late April and early May and then consistently from mid-May onwards (Fig. 3). Monomethylarsenic did not appear until early June and arsenic(III) was detected on only one occasion (0.08 $\mu g\ dm^{-3}$ on 9 June). Both dimethylarsenic and monomethylarsenic were present over the period June to September. There were few significant correlations

Table 1 Summary of observations

	First appearance				Maximum concentration			
Station	Date	Concn of As ($\mu g\ dm^{-3}$)	Temperature (°C)	Chlorophyll *a* concn ($\mu g\ dm^{-3}$)	Date	Concn of As ($\mu g\ dm^{-3}$)	Temperature (°C)	Chlorophyll *a* concn ($\mu g\ dm^{-3}$)
Dimethylarsenic								
Woodmill 1983	12 May	0.11	12.0	23.2	12 May	0.11	12.0	23.2
Northam 1983	28 Apr.	0.05	12.5	1.8	16 Jun.	0.19	17.0	8.5
Mayflower 1983	12 May	0.05	12.0	2.8	19 Aug.	0.46	21.0	29.8
Mayflower 1984	18 Jun.	0.05	22.0	8.1	4 Jul.	0.24	21.0	13.4
Monomethylarsenic								
Woodmill 1983	–	–	–	–	–	–	–	–
Northam 1983	9 Jun.	0.08	17.0	2.4	9 Jun.	0.08	17.0	2.4
Mayflower 1983	12 May	0.03	12.0	2.8	12 May	0.03	12.0	2.8
Mayflower 1984	26 Jun.	0.04	19.0	16.7	26 Jun.	0.06	20.5	7.9
Arsenic(III)								
Woodmill 1983	–	–	–	–	–	–	–	–
Northam 1983	9 Jun.	0.03	17.0	2.4	9 Jun.	0.03	17.0	2.4
Mayflower 1983	2 Jun.	0.05	16.0	2.5	2 Jun.	0.06	16.0	2.5
Mayflower 1984	26 Jun.	0.08	19.0	16.7	25 Jul.	0.18	20.0	54.3

– = Not analysed.

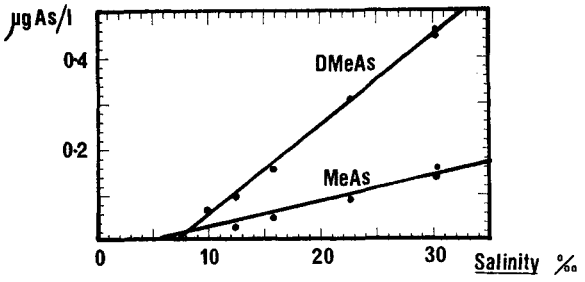

Figure 6 The salinity dependence of monomethylarsenic and dimethlarsenic at Mayflower Park and Northam during the peak methylation period in 1983.

between measured variables but this may reflect the extreme salinity variation at the site.

Mayflower

The Mayflower Park sampling site is typified by essentially saline conditions, being close to the mouth of the Itchen estuary on Southampton Water. During the 1983 study the highest chlorophyll *a* and bioarsenical concentrations were recorded at this site. In common with observations at the Northam site, methylated arsenicals were detected over the period from late May to September (Fig. 4). Dimethylarsenic was the major bioarsenical except on one occasion in early June when the monomethylarsenic concentration was very high (0.54 μg As dm^{-3}). On the same day, at the Northam site, the monomethylarsenic concentration was also higher than that of dimethylarsenic. The concentration of dimethylarsenic correlated strongly with temperature ($r = 0.917$, $P < 0.001$) and less strongly with chlorophyll *a* ($r = 0.725$, $P < 0.01$). Arsenic(III) was not a major component, being detected only in spasmodic traces.

In the following year there was a marked difference in the timing of events. The overall concentrations of bioarsenicals were similar, but the main pulse of activity occurred much later – dimethylarsenic was not present until late June, when its concentration rose sharply. Temperature, chlorophyll *a* and bioarsenical concentration, together with bacterial numbers, all rose in concert (Fig. 5). Once again, dimethylarsenic correlated strongly with temperature ($r = 0.813$, $P < 0.001$). Arsenic(III) levels were much higher than in the previous year, and correlated very strongly with chlorophyll *a* ($r = 0.951$, $P < 0.001$).

Table 2 Correlation between observed variables[a]

	n	*r*	Significance level (%)
Northam			
Temp vs Chl	13	0.414	
Temp vs MMAs	15	0.609	98
Temp vs DMAs	15	0.662	99
Chl vs MMAs	13	0.123	
Chl vs DMAs	13	0.415	
Chl vs Sal	13	0.173	
MMAs vs DMAs	15	0.576	95
MMAs vs Sal	15	0.206	
DMAs vs Sal	15	0.528	95
Mayflower 1983			
Temp vs Chl	14	0.725	99
Temp vs MMAs	14	0.454	
Temp vs DMAs	14	0.917	99.9
Chl vs MMAs	14	0.155	
Chl vs DMAs	14	0.714	99
Chl vs Sal	14	0.452	
MMAs vs DMAs	14	0.452	
MMAs vs Sal	12	−0.060	
DMAs vs Sal	12	0.660	98
Mayflower 1984			
Temp vs Chl	16	0.477	90
Temp vs MMAs	16	0.766	99.9
Temp vs DMAs	16	0.813	99.9
Temp vs Bact	11	0.780	99
Chl vs MMAs	16	0.568	95
Chl vs DMAs	16	0.636	99
Bact vs Chl	11	0.377	
Bact vs As(III)	11	0.796	99
Bact vs MMAs	11	0.596	90
Bact vs DMAs	11	0.731	98
MMAs vs DMAs	16	0.962	99.9
MMA vs DMAs (bloom only)	6	−0.283	
MMAs vs As(III)	15	0.751	99
MMAs vs As(III) (bloom)	5	−0.244	
DMAs vs As(III)	15	0.801	99.9
DMAs vs As(III) (bloom)	5	0.364	

[a]Abbreviations: Temp. temperature; Chl, chlorophyll *a* concentration; MMAs, monomethylarsinic compounds; DMAs, dimethylarsenic compounds; Sal, salinity; Bact, bacteria.

DISCUSSION

The results of this study have provided a detailed description of seasonal changes in the chemical form of dissolved arsenic in the aquatic water column. During the summer months, typically 30% of the total dissolved arsenic is present as bioarsenicals. Although by comparison with ocean systems these are particularly high levels,[4,7,9] they are comparable with

concentrations observed in other estuaries.[2,3] In common with other reports, dimethylarsenic was by far the most prevalent bioarsenical; monomethylarsenic and arsenic(III) levels were generally lower – even when compared with those recorded in the nearby Beaulieu estuary.[20,25] Low monomethylarsenic levels have also been found in the open oceans, where this species is typically less than 1% of total dissolved arsenic.[7,8] The dominance of monomethylarsenic on one occasion during 1983, when it was more than 50% of the total dissolved arsenic, may be the result of a specific algal bloom. Such an effect has been demonstrated in Chesapeake Bay,[2] where *Chroomonas* species are believed to be responsible for monomethylarsenic production. Aside from selective excretion by certain algal species, there is also the possibility that monomethylarsenic and arsenic(III) are degradation products of dimethylarsenic. This would be supported by the observation of a lag in the appearance of dimethylarsenic and the detection of monomethylarsenic and arsenic(III).

Previous studies[4,7,8,14,26] have suggested that the high bioarsenical concentrations, as encountered in this study, occur under conditions of very high productivity or low phosphate levels. Such conditions lead to increased arsenic metabolism by phytoplankton. The chlorophyll *a* levels measured in this study do not suggest abnormally high levels of productivity and phosphate levels are greatly in excess of those of arsenate. Phosphate levels in the study area are supplemented by wastewater from the Southampton area and typical levels in the estuary are between 0.5 and 16 μmol dm^{-3}.[27,28] Apart from on very infrequent occasions, phosphate is in 20–40-fold molar excess over arsenate. Sanders[2] has suggested that high bioarsenical concentrations in estuaries are not specifically a result of low phosphate:arsenate ratios but are due to different nutrient strategies employed by estuarine and oceanic phytoplankton. In estuaries, where phosphate levels are generally quite high, plankton has a lower nutrient affinity than its oceanic counterparts. In effect, more phosphorus and consequently more arsenic is cycled in estuaries.

Results from the period in 1983 when the water temperature had stabilized at between 19 and 21°C suggest a marine source for the bioarsenicals. As the levels of methylated arsenic in the surrounding saline waters of the Solent are not exceptionally high,[25] this would lead to the conclusion that the major source is predominantly at the mouth of the estuary. Unlike shallower systems such as the estuaries of the Beaulieu and Tamar, which have extensive tidal mudflats often covered by algal mats, the size, structure and underlying geology of the system under study makes it comparatively unlikely that the major source of this material is to be found in either the secretions or decay of macro-algae[4] or the interstitial waters of underlying sediments.[29–31] In addition, the absence of a significant freshwater input is confirmed by results from the Woodmill site (Fig. 2). The river Test, the other main input of freshwater into the system, is very similar in character to the Itchen and significant inputs of bioarsenicals are therefore unlikely. It would appear that arsenic methylation in this system is dominated by marine biological processes. A similar pattern has recently been demonstrated in the Tamar estuary,[3] where fairly uniform arsenate and phosphate concentrations are observed throughout the estuary. In spite of a primary productivity maximum in the brackish water zone, peak bioarsenical concentrations are found in the higher-salinity region of the estuary.

At no time during this study was biomethylation observed when water temperatures were below 12°C. This observation is in support of our previous studies of arsenic biomethylation in the UK.[20,25] Notably, during field studies of spring diatom blooms occurring at low temperature,[19,32] neither arsenate uptake was observed nor methylated species detected, even during times of phosphate limitation. Similarly, in Chesapeake Bay, Sanders[2] observed little evidence of biomethylation in winter despite algal cell densities similar to those found in the summer months when methylated arsenicals were in abundance. Such observations have led us to believe that under conditions of low water temperature (<12°C) phytoplankton turn over less arsenic or do not need to, or are unable to, excrete bioarsenicals into the water column. Alternatively, larger biomolecules may be excreted which are not broken down to compounds which respond as methylated arsenic in the analysis.

The importance of removal processes is emphasized by the rapid disappearance of methylated arsenicals from the water column during the early autumn (see also Refs 20, 25). The appearance of methylarsenicals in the water column represents a balance between the release processes and their subsequent removal by

degradative processes such as bacterial demethylation[33] or oxidation[34] and adsorption onto particulates. Little is known about these processes but based on culture experiments,[16,33] a demethylation rate of 1 ng dm^{-3} per day for an estuarine bacterial system can be assumed. This rate, however, would be insufficient to explain the disappearance of the bioarsenicals in the autumn and rapid flushing of material from the estuary into the bioarsenical-poor coastal waters is more probable.

The absence of a simple relationship between the levels of chlorophyll and bioarsenical concentration observed in the open oceans[7] supports the view that species composition is of great importance. During late June 1984, the concentrations of methylated species increased five-fold at a time when chlorophyll *a* showed little sign of a corresponding increase.

Finally, it is worth considering the fate of methylated arsenic incorporated in the cells of phytoplankton. A number of arsenic-containing lipids and water-soluble arseno-sugars have been reported in algae.[9,17] In conditions of high productivity such as estuaries, it is possible that a significant quantity of larger arsenic-containing species may be released during the decomposition of algal debris. Such forms may not be detectable by direct analysis using hydride generation techniques. These would gradually break down in the water column to detectable forms. The importance of this potential pool of arsenic, which is not detectable by available analytical techniques, and the significance of specific algal species, are aspects of marine arsenic chemistry currently being investigated in our laboratory.

CONCLUSIONS

This study confirms previous observations[3,25] that biomethylation is a process primarily restricted to the high-salinity regions of the estuary and that the presence of methylated arsenic at lower salinities is predominantly the result of the mixing of saline water (containing bioarsenicals) with river water. The results cannot be adequately interpreted on the basis of previous studies[4,7,8,14,26] which suggest phosphate limitation is the major stimulus for arsenic biomethylation.

The seasonal variations observed were complex. It would appear that dimethylarsenic is the major biogenic product in temperate estuaries with monomethylarsenic and arsenic(III) having less importance. The delay between the appearance of dimethylarsenic and the detection of the other two species suggests that these species may in fact be degradation products. Excretion of monomethylarsenic and arsenic(III) by specific algal species cannot however be eliminated.

Further work is required to elucidate the effects of successional changes in algal species, the impact of nutrient status, bacterial cycling and the nature of arsenicals released both actively into the water column and during decomposition. This work is in progress.

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