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The discovery of hidden arsenic species in coastal waters

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The analysis of ultraviolet (UV)-irradiated and untreated seawater samples has shown that the dissolved arsenic content of marine waters cannot be completely determined by hydride generation–atomic absorption spectrophotometry without sample pretreatment. Irradiation of water samples obtained during a survey of arsenic species in coastal waters during the summer of 1988 gave large increases in the measured speciation. Average increases in total arsenic, monomethylarsenic and dimethylarsenic were 0.29 μg As dm^{-3} (25%), 0.03 μg As dm^{-3} (47%) and 0.12 μg As dm^{-3} (79%) respectively. Overall, an average 25% increase in the concentration of dissolved arsenic was observed following irradiation.

This additional arsenic may be derived from compounds related to algal arsenosugars or to their breakdown products. These do not readily yield volatile hydrides when treated with borohydride and are not therefore detected by the normal hydride generation technique. This has important repercussions as for many years this procedure, and other analytical procedures which are equally unlikely to respond to such compounds, have been accepted as giving a true representation of the dissolved arsenic speciation in estuarine and coastal waters. A gross underestimate may therefore have been made of biological involvement in arsenic cycling in the aquatic environment.

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INTRODUCTION

A number of simple compounds of arsenic are believed to be present in seawater: oxidized arsenic(V) (assumed to be arsenate, AsO_4^{3-}), reduced arsenic(III) (presumably arsenite, AsO_3^{3-}) and the methylarsenic species (possibly monomethyl and dimethyl arsenic oxyanions derived from $CH_3AsO(OH)_2$ and $(CH_3)_2AsOOH$ respectively). These are not however the main species in marine flora and fauna. In macroalgae,[1] crustacea,[2,3] sea squirts[4] and fish[5,6] a variety of lipid- and water-soluble arsenic compounds have been found. The first fully characterized compound was arsenobetaine $[(CH_3)_3A^+sCH_2COO^-]$.[5,7,8] Later, other compounds, such as arsenocholine $[(CH_3)_3A^+sCH_2CH_2OH]$, were also isolated and characterized[2,9,10] and the discovery of arsenosugars[1,11,12] in macroalgae revealed a possible precursor of arsenobetaine.[13]

Whilst there is no firm evidence to suggest that a significant proportion of the dissolved arsenic in marine waters is in the form of these compounds, they or their breakdown products may well be present and remain undetected by conventional procedures. Edmonds *et al.*[13] have suggested that arsenobetaine is formed from arsenosugars by decomposition, reduction and methylation, but neither arsenobetaine nor arsenosugars have as yet been found in the water column. Both acid and base hydrolyses result in the degradation of the arsenosugar 2-hydroxy-3-sulphopropyl-5-deoxy-5-(dimethylarsenoso)furanoside to dimethylarsinic acid $[(CH_3)_2AsOOH]$[1] and a similar end-product may well result from microbial activity in the water column and underlying sediments.[14]

There is little evidence at present to demonstrate the conversion of algal arsenosugars to arsenobetaine by higher organisms. Organoarsenicals in the unicellular alga *Dunaliella tertiolecta* were not metabolized to

arsenobetaine by the American lobster *Homarus americanus*, even though the native arsenic compound in the lobster is arsenobetaine.[15] The mollusc *Littorina littoralis*, feeding on *Fucus spiralis*, did not contain arsenobetaine.[16]

Complex organoarsenicals may in fact be present in the water column but remain undetected due to the use of unsuitable analytical techniques. There are few methods which can be employed for the measurement of arsenic speciation in unpolluted waters as the levels of total arsenic are normally below 2 $\mu g\,dm^{-3}$. Since the speciation of arsenic in seawater by hydride generation – atomic spectroscopy (HG AS) was first reported some 15 years ago,[17,18] the emission and absorption variants of the technique have become widely accepted as giving a fair representation of the concentration of arsenic in the sample. However, this technique will only detect species that are, or will form, a volatile arsenic derivative; compounds such as arsenobetaine, arsenocholine and dimethylarsenosugars do not do so and hence do not show up. Only after pretreatment can these compounds be detected using a hydride generation technique. Arsenobetaine is converted by hot aqueous sodium hydroxide to trimethylarsine oxide and this can then be reduced by sodium borohydride to trimethylarsine, which is volatile and can be detected using the hydride generation system. Arsenocholine, however, cannot be detected using the same technique. Many of the arsenic compounds extracted from macroalgae do not produce volatile methylated products with sodium borohydride unless first digested with alkali. Both dimethylarsine $[(CH_3)_2AsH]$ and trimethylarsine $[(CH_3)_3As]$ are then detected, suggesting that the dimethylarsine may be formed from the decomposition of dimethyl(ribosyl)arsine oxide.[19] Cullen and Dodd[20] used ultraviolet radiation (wavelength unstated) to photo-oxidize arsenic compounds to inorganic arsenate, successfully decomposing arsenobetaine, arsenocholine and quaternary arsonium ions $[(CH_3)_4As^+]$ to arsenate. Whilst this method reveals arsenic-containing compounds, potentially significant speciation information is lost.

This paper outlines a method by which arsenic-containing material which is not detected by conventional hydride generation – atomic absorption spectroscopy (HG AA), can be broken down to detectable arsines which retain methylation information. Thus arsenic that had previously been undetected by HG AA can now be readily analysed without being completely decomposed, giving valuable speciation data and strong indications of the structure of the original compound. This paper describes the analytical method and compares results from a preliminary seasonal sampling survey performed using both the conventional and the new techniques.

EXPERIMENTAL

Sample collection and pretreatment

Samples were collected from April to November 1988 as a part of a long-term monitoring programme to observe the seasonality of arsenic speciation at Netley in Southampton Water and Calshot in the Solent, UK. The first of these is a sheltered estuarine site whilst the Calshot station is in a more open coastal environment. Water samples were filtered immediately after collection (Whatman GF/C) and then both irradiated and untreated subsamples were analysed using HG AA.

Sample irradiation

Water samples were placed in sealed quartz tubes (1 cm × 6 cm) mounted in a semicircular array 15 cm from a 200 W short-arc mercury lamp (Wotan). Cooling during the irradiation was effected by an electric fan. Under these conditions arsenite is completely oxidized to arsenate but monomethylarsonic acid and dimethylarsinic acid show no evidence of either further methylation or demethylation.

Arsenic analysis

The hydride generation method (HG AA) used for this work is described in previous papers.[21,22] A peristaltic pump is used to mix the sample with equal volumes of hydrochloric acid (1 $mol\,dm^{-3}$) and then sodium tetrahydroborate(III) ($NaBH_4$) (2% w/v). The resulting arsines are carried into a custom-built gas/liquid separator by nitrogen carrier gas. The gas stream is dried by sodium hydroxide pellets and trapped on hydrofluoric acid etched glass beads (*ca* 40-mesh) in a U-tube submersed in liquid nitrogen (~196°C). On removal of the liquid nitrogen, the trap warms slowly to room temperature resulting in the sequential elution of arsines in the order of their volatility (arsine followed by momethyl-, dimethyl- and then trimethyl-

arsine) into an electrically heated quartz T-tube in the lightpath of a Baird A5100 atomic absorption spectrometer.

RESULTS

Test irradiation was performed for time periods ranging from 0 to 12 h, using water taken at the Netley site from a depth of one metre (1 m). This demonstrated that at least 4 h were required for maximum conversion (Table 1). No increase in total dimethylarsenic species (DMAs) was observed when the sample was acidified by the addition of 10 μL of concentrated hydrochloric acid to 10 cm^3 of sample prior to irradiation.

During the warmer months of the year (April to October), monomethylarsenic (MMAs), dimethylarsenic (DMAs) and arsenic(III), as well as arsenic(V), are present in the water column.[23] The simple methylated species appear at the end of April and reach a maximum in June, before concentations slowly decrease to very low levels in November (<0.05 μg As dm^{-3}).

Irradiated samples from the survey showed an average 0.29 μg As dm^{-3} increase (25%) in measurable dissolved arsenic. Earlier (April) and later in the year (November), this dropped to only *ca* 0.1 μg As dm^{-3}. Using the conventional hydride generation technique, the concentration of methylated arsenic in Netley water was below the system detection limits on 15 April 1988. Both monomethyl- and dimethyl-arsenic were however revealed by irradiation (Fig. 1). Dimethylarsenic displayed the largest increase (79% average), with concentrations at Calshot (Fig. 2) doubling in July and August from 0.15 to 0.30 μg As dm^{-3}. At Netley, where non-irradiated levels of DMAs were higher, a similar elevation of *ca* 0.15 μg As dm^{-3} was observed during the summer (e.g. 0.35 to 0.52 μg As dm^{-3} in June). In November, the increase was less than half of that during the biologically productive summer months (*ca* 0.045 μg As dm^{-3}).

Monomethylarsenic increases after irradiation were proportionally less than those of DMAs, typically 41% at Netley and 56% at Calshot. Whilst the proportional increases in inorganic arsenic after irradiation were less than those of MMAs and DMAs, in absolute terms they were significant. Maximum enhancement took place in September of 0.20 (23%) and 0.26 μg As dm^{-3} (28%) at Netley and Calshot respectively, with concentrations differing little between sites. By November there was no significant difference between the irradiated and non-irradiated samples.

DISCUSSION

The seaonal survey of irradiated and conventionally analysed samples has revealed a typical 25% underestimate of dissolved arsenic using the conventional technique, dimethylarsenic being a major contributor to this increase. Monomethylarsenic contributes less than 10% of the increase.

Whilst the possibility of photochemically induced methylation reactions cannot be completely excluded, a greater potential complication is likely to arise from

Table 1 Effect of irradiation time on arsenic speciation. Netley seawater (11 July 1988) obtained from 1 m depth

Time (h)	As_{tot}	(μg dm^{-3})	MMAs[a]	(μg dm^{-3})	DMAs[b]	(μg dm^{-3})
0	0.76	± 0.05	0.084	± 0.005	0.22	± 0.01
1	0.86	± 0.05	0.098	± 0.006	0.27	± (HCl)
1	0.98	± 0.06	0.11	± 0.007	0.23	± 0.01
2	0.99	± 0.06	0.12	± 0.007	0.35	± 0.02
3	0.95	± 0.06	0.12	± 0.007	0.345	± 0.02
4	0.98	± 0.06	0.13	± 0.008	0.36	± 0.03
8	1.00	± 0.06	0.14	± 0.008	0.42	± 0.03
12	0.93	± 0.06	0.14	± 0.008	0.395	± 0.02

[a]MMAs, monomethyl arsenic, [b]DMAs, dimethylarsenic.

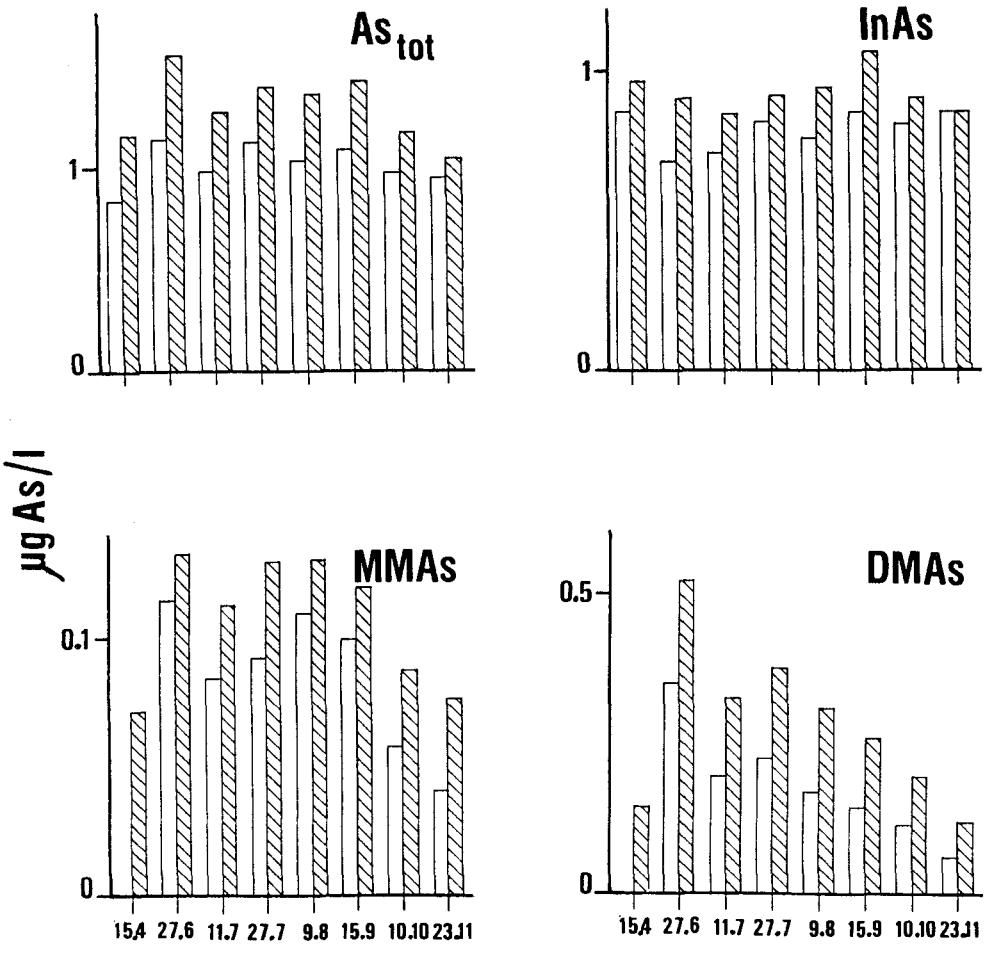

Figure 1 Arsenic speciation at the Netley Station during 1988. Hatched areas show analyses of irradiated water, open areas without pretreatment. InAs, non-methylated arsenic. As_{tot} = InAs + MMAs + DMAs.

demethylation resulting from irradiation. Complete appraisals of these complications cannot be made until pure samples of the dissolved marine organoarsenicals are available, but tests on monomethylarsonic acid and dimethylarsinic acid show no evidence of methylation or demethylation. At present it must therefore be assumed that the measured extent of methylation reflects the minimum number of methyl groups attached to the arsenic.

There are a number of possible explanations for the observed increase in the inorganic arsenic. Demethylation is a possible source but this would be expected to result in the concurrent diminution of the methylated species, which is not observed. Alternatively, some inorganic arsenic may be present as material which is not normally susceptible to borohydride reduction being, for example, bound to colloidal material or incorporated in organoarsenicals as non-methylated forms which are present in high concentrations during the summer months.

A possible explanation for the observed increase in dimethylarsenic is that this material is derived from algal arsenosugars.[1,12] In moderately open waters, the most probable source of this additional arsenic is the phytoplankton which, during the summer, can reach several thousand cells per millilitre (cm^3) of water, and make up a large percentage of the biomass. Release of dimethyl arsenoribosides or similar compounds into the water, either actively or as a result of bacterial degradation, is then a likely source of dimethylarsenic in the water column.

Edmonds and Francesconi[24] suggest that arsenoribosides are microbially decomposed, reduced and methylated to arsenobetaine in the sediments. The

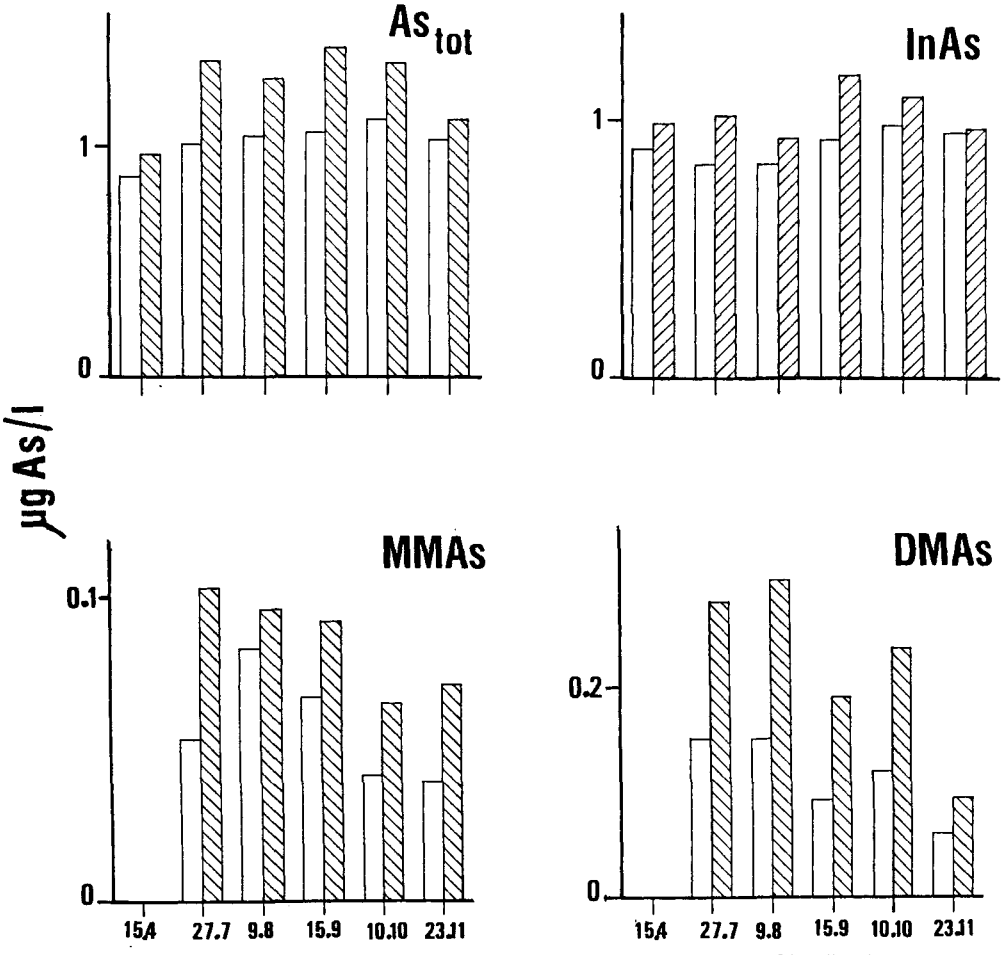

Figure 2 Arsenic speciation at the Calshot Station during 1988. Hatched areas show analyses of irradiated water, open areas without pretreatment. InAs, non-methylated arsenic. As_{tot} = InAs + MMAs + DMAs.

procedure employed in this work produces trimethylarsine from arsenobetaine but no trimethylarsine was evident in the analysis; it therefore appears that the 'hidden' arsenic species in the water column is not arsenobetaine. A more likely hypothesis is that, if the 'hidden' dimethylarsenic entities are in fact derived from arsenoribosides and their breakdown products, they are constantly being released into the water by phytoplankton during the summer months. These are then utilized by marine organisms as a source of energy, resulting in the release of dimethylarsenic. As winter approaches the biological activity diminishes and the ribosides are either diluted to below detection limits or broken down by microbial activity.

It is reasonable to expect that 'hidden' arsenic would be found where the other methylated arsenic species are also present – typically waters of high biological activity, such as estuaries and coastal water, and hence a source for this increase in total arsenic. By the same argument, it is unlikely that such an increase would be as evident in areas of relatively low productivity, such as much of the world's oceans. The ultraviolet (UV)-liberated methylated arsenic shows a seasonality which is similar to that observed previously for methylated arsenic,[23,25] appearing early in the year, reaching a plateau around late June (0.17 μg As dm^{-3}) before dropping to *ca* 0.05 μg As dm^{-3} in late November. MMAs produced by UV irradiation remain fairly constant throughout the sampling period except at Netley (1 m depth) where it peaks at a maximum of 0.05 μg As dm^{-3} in July.

Hydride generation–atomic absorption and emission methods are generally considered to be the most suitable techniques, in terms of sensitivity and

speciation capability, for the study the very low concentrations of arsenic species in natural waters. As a consequence, these techniques have been widely used for the analysis of arsenic; many models, flux calculations and impact assessments have been based on data obtained by the technique. Whilst the conventional methods now appear to have limitations, they are probably still the most powerful techniques available for arsenic speciation in unpolluted waters. Similar criticisms could well be raised on all such speciation methods. It may well turn out that many of the previously employed methods for the analysis of total arsenic have been incapable of dealing with all the species which are present in natural waters.

In conclusion, it has been shown that under conditions of high biological activity organoarsenicals are formed which cannot be determined by conventional HG AS methods. This can lead to a significant underestimation of the concentration, with a large percentage of this increase being from dimethylarsenic.

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