APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 113–119 (1999)

Arsenic Speciation Including 'Hidden' Arsenic in Natural Waters

Hiroshi Hasegawa,[1]* Masakazu Matsui,[1] Shinsuke Okamura,[2] Masashi Hojo,[2] Nozomu Iwasaki[2] and Yoshiki Sohrin[3]
[1]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan
[2]Faculty of Science, Kochi University, Akebono-cho, Kochi 780, Japan
[3]Faculty of Engineering, Kanazawa University, Otateno, Kanazawa 920, Japan

Recent studies indicate the existence in natural waters of 'hidden' arsenic which had previously been undetected by the hydride generation technique. A speciation method for arsenic species has been developed in which hidden arsenic was classified into two fractions by their lability to the photochemical degradation procedure: the ultraviolet-labile fraction and the ultraviolet-resistant fraction. The ultraviolet-labile fraction was the major fraction of hidden arsenic and comprised 15–45% and 4–26% of the total arsenic in Uranouchi Inlet and Lake Biwa (Japan), respectively. The highest concentration of the ultraviolet-resistant fraction was observed in Uranouchi Inlet during the summer, in which dimethylarsinic acid increased in the water column. We discuss the hidden arsenic fraction as the key to explaining arsenic speciation in natural waters.

Keywords: arsenic; speciation; organoarsenicals; ultraviolet irradiation; microwave digestion; methylation; natural water; hydride generation

Received 27 December 1997; accepted 28 September 1998

INTRODUCTION

Chemical speciation is the determination of the individual concentrations of the various forms of an element that together make up the total concentration of that element. So far as arsenic species in natural waters are concerned, the inorganic forms (arsenate [$AsO(OH)_3$; As(V)] and arsenite [$As(OH)_3$; As(III)]) and the methylated forms (methylarsonic acid [$CH_3AsO(OH)_2$; MMAA(V)] and dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMAA(V)]) have been reported to be the main species.[1–4] The bulk of the total dissolved arsenic is inorganic species in seawater[1,2,6,7] and in fresh water,[8,9] whereas methylarsenicals are found to comprise significant amounts in the surface layers[10–17] and above the sediment surface.[16,17] The reported distributions suggest that the predominant form of methylarsenic is consistently DMAA(V), followed by MMAA(V). The existence of methylarsenic(III) species has also been demonstrated in the environment.[15–19] Several observations showed that methylarsenicals in surface waters exhibit a seasonal cycle in which the maximum concentrations of methylarsenicals appear during the summer.[13,15–17] Although there is abundant evidence regarding methylarsenicals produced biologically in natural waters,[2,20–22] apparent differences were observed in seasonal changes of phytoplankton densities and methylarsenicals.

On the other hand, other organoarsenicals make up the bulk of the arsenic stock in organisms.[23,24] Arsenosugars are ubiquitous in algae[25,26] and arsenobetaine is the predominant form in marine animals.[27,28] Arsenosugars and arsenobetaine cannot be detected with the conventional hydride generation analyses[29,30] which have been applied to natural water samples. These facts suggest the presence of additional organoarsenicals other than methylarsenicals in natural waters. Recently, two research groups have revealed fractions of organoarsenicals which were converted to hydride-reactive forms by ultraviolet irradiation[31] or alkaline digestion.[32,33] This 'hidden' or 'refractory' arsenic can be expected to offer the key to link arsenic speciation in natural waters and biological production in organisms. The information on hidden

* Correspondence to: H. Hasegawa, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan.
Contract/grant sponsor: Ministry of Education, Science, Sports and Culture, Japan; Contract/grant number: 09740557.

 CCC 0268–2605/99/020113–07 $17.50

arsenic in natural waters is limited and its identity remains unknown.

This paper describes the speciation method for hidden arsenic species using ultraviolet irradiation and microwave digestion. Arsenic determination was performed by hydride generation atomic absorption spectrometry. We used our speciation method to estimate the arsenic composition in natural waters.

EXPERIMENTAL

Sample collection and pretreatment

We collected surface water samples from two euphotic regions: the southern basin of Lake Biwa (35° 06′ N, 135° 56′ E) and the deep part of Uranouchi Inlet (33° 25′ N, 133° 21′ E), Japan. Geographical data of the sampling sites are described in detail elsewhere.[15,34]

The sampling site of Lake Biwa is in a dredged area (depth *ca* 12 m) in the southern basin, with an average depth of 3.5 m. Thermal stratification occurs from April to September, and the dissolved oxygen in the bottom layer is extinguished during the summer. Uranouchi Inlet is situated on the central coast of Tosa Bay. The water column is stratified as a result of halocline and/or thermocline at a depth of 2–4 m throughout the year. This inlet leads to the outer sea through a shallow mouth with a depth of 2–4 m, and the deep part has a depth of 10–20 m. The salinity in Uranouchi Inlet is at the same levels as the outer sea (26–34%), since there is no river from which water flows into Uranouchi Inlet. The mean density of phytoplankton was 2.5×10^{-9} and 5.7×10^{-9} cells/m^3 at the sampling site of Lake Biwa and Uranouchi Inlet, respectively. In both regions, the concentration of arsenicals follows an annual cycle whereby the maximum concentrations of methylarsenicals appear with corresponding decreases in the As(V) level during the summer.[15,16]

The samples were filtered with 0.45 μm filters (Millipore) immediately upon collection. Both filtered and unfiltered samples were acidified to pH 2 by the addition of 1 M hydrogenchloric acid, and stored in acid-washed polypropylene bottles at 5 °C in darkness.

Reagents

Stock solutions (10^{-2} M) for the identification and quantification of arsenic compounds were prepared by dissolving the corresponding sodium salts [$CH_3AsO_3Na_2$ prepared by Quick's method,[35] and $NaAsO_2$,Na_2HAsO_4 and $(CH_3)_2AsO_2Na$; Nacalai Tesque], trimethylarsine oxide [$(CH_3)_3AsO$ made by Grignard synthesis] and arsenobetaine [$(CH_3)_3As^+CH_2CO_2^-$; Trichemical Laboratory Inc.] in 0.1 M sodium hydroxide. These stock solutions were standardized by using atomic absorption spectrometry (Hitachi 180-70) and inductively coupled plasma atomic emission spectrometry (ICP–AES; Japan Jarrel Ash ICAP-500) after decomposition to As(V). They were diluted to the desired concentrations just before use. For the microwave digestion, the digestion reagent was made by dissolving potassium persulphate (Kanto Chemical) in 0.15 M sodium hydroxide (Merck). Sodium borohydride (Kanto Chemical) was used for hydride generation. A 3% (w/v) sodium borohydride solution, stabilized in 10^{-2} M sodium hydroxide solution, was prepared daily. Artificial seawater was prepared according to Lyman and Fleming[36] and distilled water was used throughout. Other reagents were of analytical reagent grade or better.

Arsenic analysis

Inorganic and methylarsenicals

Analysis for inorganic and methylarsenicals was performed by modifications of the hydride generation method (CT–HG–AAS), using a apparatus and materials similar to those described in previous papers.[19] In this technique, arsenic species were reduced to the corresponding arsines with sodium borohydride, trapped in a U-tube with liquid nitrogen, and sequentially evolved into an electrically heated quartz T-tube which was mounted in the atomic absorption spectrometer. The detection limits were 0.13–0.17 nM and the reproducibility was 3–6% for inorganic and methylarsenicals for a sample size of 50 ml.

Ultraviolet irradiation

Ultraviolet photolytic decomposition was accomplished by use of a 400 W high-pressure mercury lamp (Sigemi, AHH-400s) in a three-chamber reaction vessel constructed from quartz. Samples were acidified to pH 2 by the addition of 1 M hydrochloric acid and introduced into the outer chamber, capped with natural rubber septa. They were irradiated by a 400 W high-pressure mercury lamp mounted in the centre chamber, with stirring. A Pyrex filter was attached, if necessary, in the

centre chamber of the vessel. This filter cut off the light under a wavelength of 280 nm. During the irradiation, cooling water at 25 °C was circulated into the middle chamber from a constant-temperature bath. Aliquots were taken at selected time intervals. Analysis of the digestates for arsenic was performed by CT–HG–AAS as described above.

Total arsenic

Sample solution (20 ml) and the digestion reagent (10 ml) were introduced into a Teflon Beaker loosely closed with a Teflon watchglass. Six samples were placed symmetrically in the microwave oven, and digested at 500 W for 10 min. Following a short cool-down period, 10-ml portions of 5 M hydrochloric acid were added and the beakers were set on a hotplate at 100 °C for 25 min in order to decompose excess persulphate and remove chlorine. After cooling to room temperature, the total arsenic concentration was measured as As(V) by CT–HG–AAS.

RESULTS AND DISCUSSION

Photolysis of arsenicals in natural waters

Figure 1 shows typical photoproduction of inorganic and methylarsenic species in ultraviolet-irradiated samples. The water samples were collected from surface waters in Uranouchi Inlet on 30 April, 1997. Initially, both filtered and unfiltered samples contained only inorganic arsenic (9.3 nM and 11.5 nM, respectively), and the methylarsenic concentration was below detection limits. Inorganic and dimethylarsenic concentrations rapidly increased immediately after irradiation, and attained equilibrium in 1–3 h. The lake waters as well as other Uranouchi waters also showed similar speciation changes to those described above, although they varied as to their increments in arsenic concentration. Our results coincide with a previous study in this respect: Howard and Comber showed that irradiation of coastal seawater from a short-arc mercury lamp gave large increases in the measured arsenic concentration.[31]

Samples were adjusted to 0.01 M hydrochloric acid solutions to maintain the pH condition during the irradiation. All samples remained within the pH range 2.00–2.10, even after 48 h of irradiation. Howard and Comber reported that no increase in dimethylarsenic occurred in acidified samples

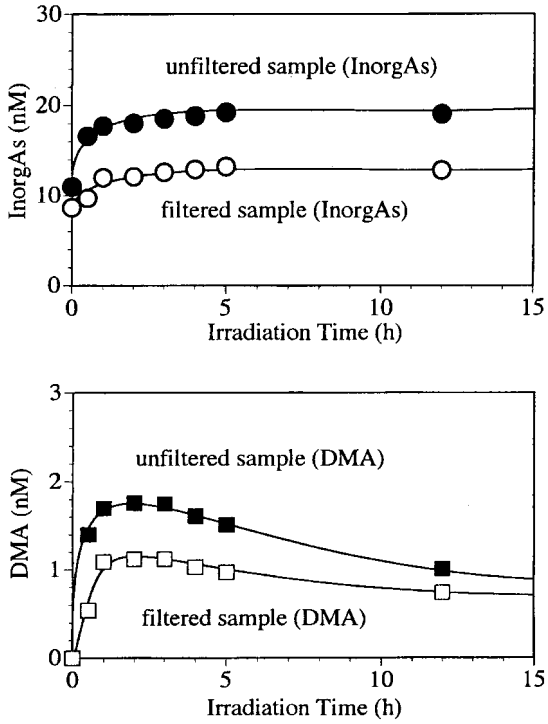

Figure 1 Effect of irradiation time with a 400 W high-pressure mercury lamp on arsenic speciation. Samples were collected from surface waters (depth 0 m) of the deep part of Uranouchi Inlet, on 30 April 1997. ○, Inorganic arsenic in filtered samples; ●, inorganic arsenic in unfiltered samples; □, dimethylarsenic in filtered samples; ■, dimethylarsenic in unfiltered samples. Monomethylarsenic was below the detection limit by CT–HG–AAS.

(about 0.011 M hydrochloric acid) during ultraviolet irradiation.[31] Under our conditions, such an inhibition effect was not observed below at least 0.1 M. It seems reasonable to suppose that this difference was due to the wavelength and intensity of the light. In Fig. 1, dimethylarsenic gradually decreased beyond 4 h of irradiation by the high-pressure mercury light, although no decrease was observed in Howard's experiment. Brockbank *et al.* reported that methylarsenic was demethylated by irradiation by 254 nm ultraviolet light without a digestion reagent.[37]

Microwave digestion for the determination of total arsenic

The total arsenic bulk concentration in natural waters was determined by microwave digestion with added potassium persulphate combined with

CT–HG–AAS. Organoarsenicals are decomposed into As(V) by persulphate, and microwave irradiation speeds the oxidative decomposition by its rapid heating ability. Bright *et al.* have demonstrated the relative efficacy of decomposition of microwave digestion and other decomposition methods in pore water.[38] Le *et al.* provide a detailed discussion of factors that influence the decomposition of organoarsenicals using microwave digestion.[39] While microwave irradiation with persulphate is often used in the decomposition of an organic matrix, additions are necessary for speciation measurement by CT–HG–AAS. When the solution was acidified with hydrochloric acid for hydride generation, chlorine was slowly formed by the persulphate remaining in room temperature. This chlorine interfered with the measurement of arsenic species by CT–HG–AAS, since it oxidized arsines in the U-tube. To remove the remaining persulphate, the solution was heated at 100 °C after the acidification with hydrochloric acid. The digestion reagent was completely decomposed, and the chlorine formed was eliminated by vaporization.

The recovery results obtained by the microwave digestion method are presented in Table 1. Microwave heating was applied for 10 min to each sample adjusted to 0–0.10 M potassium persulphate in 0.15 M sodium hydroxide. This demonstrates that complete digestion was achieved in the range of 0.03–0.10 M potassium persulphate. If the persulphate concentration was higher than 0.10 M, the increased viscosity of the sample solution made the arsenic measurement by CT–HG–AAS difficult. Figure 2 shows the conversions of arsenic species upon decomposition of DMAA(V) and arsenobetaine in distilled water by the microwave digestion method. These compounds were demethylated step by step, and quantitatively converted into the end product, As(V), over 6 min. When 300 pmol portions of MMAA(V), DMAA(V) and arsenobetaine were spiked into 20 ml portions of Uranouchi Inlet waters, at least 8 min of irradiation was required for the quantitative decomposition of the orgnoarsenicals. We therefore decided that the sample solution should be adjusted to 0.05 M potassium persulphate in 0.15 M sodium hydroxide and irradiated for 10 min. The blank value was negligible compared with the detection limit of CT–HG–AAS. Relative standard deviations were

Table 1 Effect of initial potassium persulphate concentration on the recovery of arsenic compounds by microwave digestion

	Arsenate concentration (nM)		
$K_2S_2O_8$ (M)	DMAA(V)[a]	Sea water[b]	Fresh water[b]
0	0	12.3	7.0
0.005	2.2	13.9	7.4
0.010	4.8	14.7	8.5
0.025	6.0	16.0	8.5
0.030	6.1	18.0	8.7
0.040	6.1	18.0	8.9
0.050	6.0	18.7	8.7
0.060	5.8	19.1	8.9
0.075	5.9	18.3	9.0
0.100	6.1	18.7	8.8

[a] 6.0 nM dimethylarsonic acid in distilled water.
[b] Samples were collected from surface waters (depth 0 m) of the southern basin of Lake Biwa on 24 April 1997, and the deep part of Uranouchi Inlet on 22 October 1996.

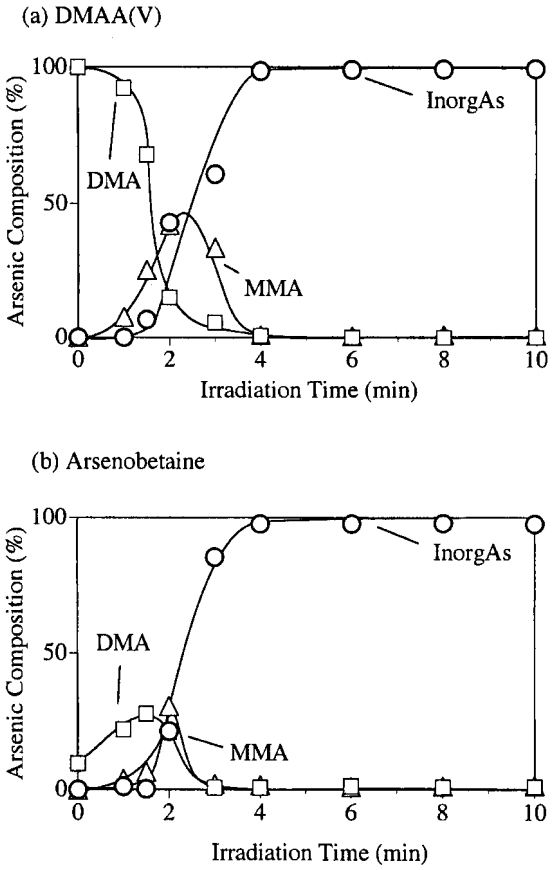

Figure 2 Effect of digestion time on the decomposition of organoarsenicals with microwave digestion, using 0.05 M potassium persulphate and 0.15 M sodium hydroxide as digestion reagents. (a) DMAA(V) in distilled water; (b) arsenobetaine in distilled water. ○, Inorganic arsenic; △, monomethylarsenic; □, dimethylarsenic.

<4% for five measurements of 1.0–10 nM DMAA(V) standard solutions. At the approximate detection limit of 0.2 nM, the standard deviations were 20% in distilled water and artificial seawater.

Arsenic speciation in natural waters

Hidden arsenic is defined as the fraction that had previously been undetected by hydride generation atomic absorption spectrometry. Our results suggest that hidden arsenic can be classified into different fractions by their lability to the photochemical degradation procedure: the ultraviolet-labile fraction and the ultraviolet-resistant fraction. We estimate the ultraviolet-labile fraction as the increment in measurable arsenic concentration before and after the 2.5 h of ultraviolet irradiation, and the ultraviolet-resistant fraction as the difference in measurable arsenic after the ultraviolet irradiation and the microwave digestion.

Figure 3 shows the measured arsenic fractions in Uranouchi Inlet and Lake Biwa. UV-InorgAs, UV-MMA and UV-DMA are the corresponding inorganic, monomethyl- and dimethyl-arsenic concentrations in the ultraviolet-labile fraction. The observed results strongly suggest that hidden arsenic exists in both seawater and in fresh water. Uranouchi Inlet clearly showed higher concentrations of hidden arsenic than Lake Biwa, in spite of the similar composition of the inorganic and methylarsenic fractions. This pattern was consistent with the higher dissolved organic carbon (DOC) of Uranouchi Inlet relative to Lake Biwa. The values of DOC were 2–4 mg C l^{-1} and <0.3 mg C l^{-1} in Uranouchi Inlet and Lake Biwa, respectively. Between filtered and unfiltered samples, the difference in hidden arsenic was significant compared with that of inorganic and methylarsenicals. It is likely that the hidden arsenic in the >0.45 μm size fraction was derived from the organoarsenicals in biological organic detritus. Hanaoka *et al.* reported that arsenobetaine was decomposed by marine microorganisms in sinking particles from the photic zone.[40] Bright *et al.* suggested that

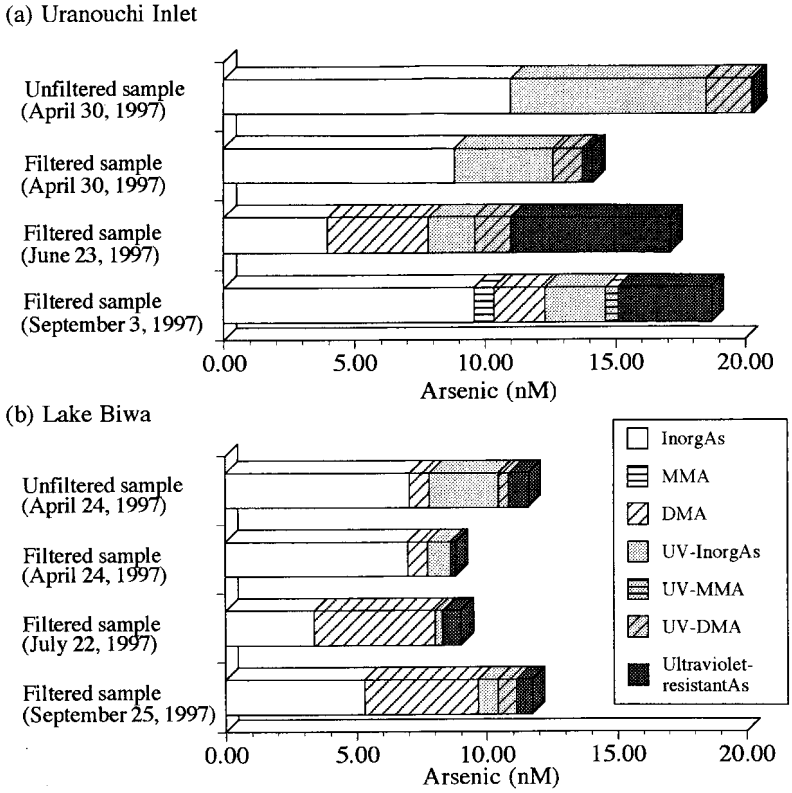

Figure 3 Mean concentration and distribution of arsenic in natural waters. Samples were collected from surface waters (depth 0 m) of (a) the deep part of Uranouchi Inlet on 30 April 1997, and (b) the southern basin of Lake Biwa on 24 April 1997.

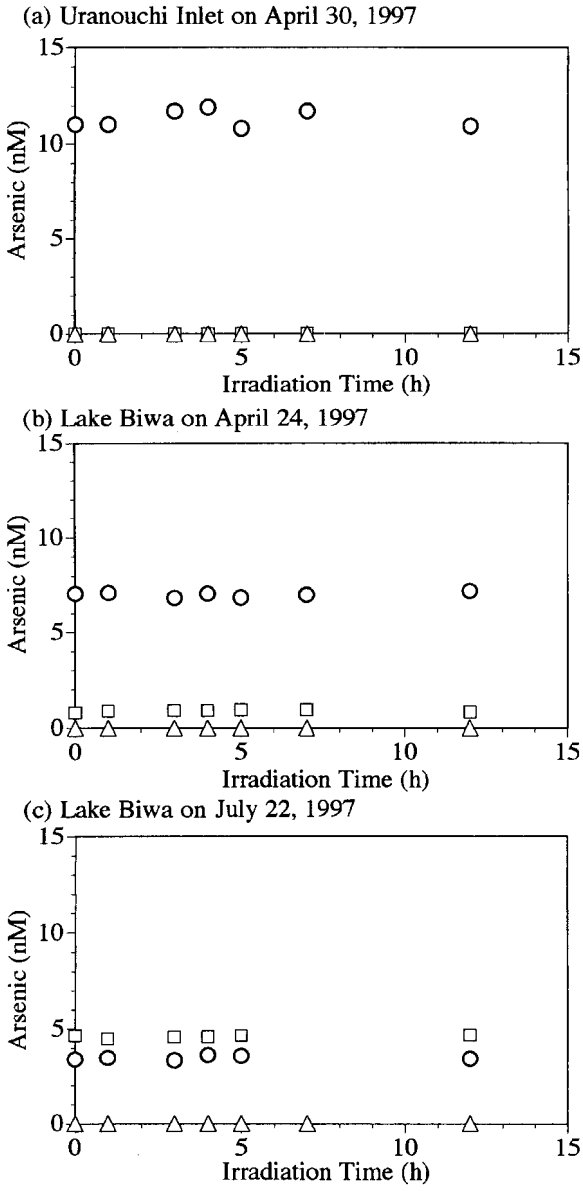

Figure 4 Effect of irradiation time with a 400 W high-pressure mercury lamp attached a Pyrex filter on arsenic speciation in unfiltered waters. The Pyrex filter cut off the light below a wavelength of 280 nm. Samples were collected from surface waters (depth 0 m) of (a) Uranouchi Inlet on 30 April 1997, (b) Lake Biwa on 24 April 1997, and (c) Lake Biwa on 22 July 1997. ○, Inorganic arsenic; △, monomethylarsenic; □, dimethylarsenic.

hidden arsenic in interstitial waters might occur in a number of forms such as arsenicals tightly adsorbed to organic matter, or complex organoarsenicals.[38]

In Fig 3, UV-InorgAs and UV-DMA were the major fractions of hidden arsenic, though little or no UV-MMA was detected. The ultraviolet-labile fractions comprised 15–45% and 4–26% of the total arsenic in Uranouchi Inlet and Lake Biwa, respectively. It was reported that 25% and 18–420% increases in the concentration of total arsenic were observed in coastal waters[31] and interstitial waters,[38] respectively, before and after ultraviolet irradiation.

On 23 June, the highest concentration of the ultraviolet-resistant fraction was observed in Uranouchi Inlet with the highest dimethylarsenic concentration and a lower concentration of the ultraviolet-labile fraction. It appears that the increased dimethylarsenic concentration was due to photodegradation of hidden arsenic by strong sunlight in early summer. However, when exposed to the filtered light above a wavelength of 280 nm from a mercury lamp, both unfiltered samples, of seawater and of fresh water, showed no significant change in the arsenic speciation (Fig. 4). It is evident that photoproduction of UV-InorgAs, UV-MMA and UV-DMA occurred in response only to the ultraviolet region of 250–280 nm when a high-pressure mercury lamp was used as an illuminator. These results indicate that photochemical degradation by sunlight rarely contributes to the production of methylarsenic compounds in natural waters. Another possibility is to assume that dimethylarsenic was produced from the ultraviolet-labile fraction by additional biological processes such as bacterial decomposition, although the evidence is not compelling. Clearly there is much to be learned from further examination of hidden arsenic in natural waters. Further analyses of other water samples are in progress.

Acknowledgements This research was supported by a Grant-in-Aid for Encouragement of Young Scientists (09740557) from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

1. M. O. Andreae, *Deep-Sea Res.* **25**, 391 (1978).
2. M. O. Andreae, *Limnol. Oceanogr.* **24**, 440 (1979).
3. M. O. Andreae, Organoarsenic compounds in the environment. In: Organometallic Compounds in the Environment: Principles Reactions, Craig, P. J. (ed.), Longman, New York, 1986, pp. 198–228.
4. W. R. Cullen and K. J. Reimer, *Chem. Rev.* **89** 713 (1989).
5. M. O. Andreae and P. N. Froelich, *Tellus* **36B**, 101 (1984).

6. M. L. Peterson and R. Carpenter, *Mar. Chem.* **12**, 295 (1983).
7. G. A. Cutter, *Mar. Chem.* **40**, 65 (1992).
8. P. Seyler and J. M. Martin, *Environ. Sci. Technol.* **23**, 1258 (1989).
9. A. Kuhn and L. Sigg, *Limnol. Oceanogr.* **38**, 1052 (1993).
10. A. G. Howard, M. H. Arbab-Zavar and S. C. Apte, *Estuarine Coastal Shelf Sci.* **19**, 493 (1984).
11. L. C. D. Anderson and K. W. Bruland, *Environ. Sci. Technol.* **25**, 420 (1991).
12. G. F. Riedel, *Estuaries* **16**, 533 (1993).
13. A. G. Howard, S. D. W. Comber, D. Kifle, E. E. Antai and D. A. Purdie, *Estuarine Coastal Shelf Sci.* **40**, 435 (1995).
14. S. J. Santosa, S. Wada, H. Mokudai and S. Tanaka, *Appl. Organometal. Chem.* **11**, 403 (1997).
15. H. Hasegawa, *Appl. Organometal. Chem.* **10**, 733 (1996).
16. H. Hasegawa, *Appl. Organometal. Chem.* **11**, 305 (1997).
17. Y. Sohrin, M. Matsui, M. Kawashima, M. Hojo and H. Hasegawa, *Environ. Sci. Technol.*, **31**, 2712 (1997).
18. D. A. Bright, S. Brock, W. R. Cullen, G. M. Hewitt, J. Jafaar and K. J. Reimer, *Appl. Organometal. Chem.* **8**, 415 (1994).
19. H. Hasegawa, Y. Sohrin, M. Matsui, M. Hojo and M. Kawashima, *Anal. Chem.* **66**, 3247 (1994).
20. J. G. Sanders, *Mar. Chem.* **17**, 329 (1985).
21. J. G. Sanders and G. F. Riedel, *Estuaries* **16**, 521 (1993).
22. A. G. Howard, S. C. Apte, S. D. W. Comber and R. J. Morris, *Estuarine Coastal Shelf Sci.* **27**, 427 (1988).
23. K. A. Francesconi and J. S. Edmonds, *Adv. Inorg. Chem.* **44**, 147 (1997).
24. S. Maeda, Biotransformation of arsenic in the freshwater environment. In: Arsenic in the Environment, Part I: Cycling and Characterization, Nraigu, J. O. (ed.), John Wiley and Sons, New York, 1994, pp. 155–187.
25. J. S. Edmonds and K. A. Francesconi, *Nature (London)* **289**, 602 (1981).
26. J. S. Edmonds and K. A. Francesconi, *J. Chem. Soc. Perkin Trans. I* 2375 (1983).
27. J. S. Edmonds and K. A. Francesconi, *Tetrahedron Lett.* **18**, 1543 (1977).
28. J. S. Edmonds and K. A. Francesconi, *Experientia* **43**, 553 (1987).
29. M. O. Andreae, *Anal. Chem.* **49**, 820 (1977).
30. R. S. Braman, D. L. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker, *Anal. Chem.* **49**, 621 (1977).
31. A. G. Howard and S. D. W. Comber, *Appl. Organometal. Chem.* **3**, 509 (1989).
32. A. M. M. de. Bettencourt and M. O. Andreae, *Appl. Organometal. Chem.* **5**, 111 (1991).
33. A. M. de. Bettencourt, M. H. Florĕncio, M. F. N. Duarte, M. L. R. Gomes and J. F. C. Vilas Boasand, *Appl. Organometal. Chem.* **8**, 43 (1994).
34. Y. Sohrin, T. Tateishi, S. Mito, M. Matsui, H. Maeda, M. Kawashima and H. Hasegawa, *Lakes Reserv.* **2**, 77 (1997).
35. A. J. Quick and R. Adams, *J. Am. Chem. Soc.* **44**, 805 (1922).
36. J. Lyman and R. H. Fleming, *J. Mar. Res.* **3**, 134 (1940).
37. C. I. Brockbank, G. E. Batley and G. K.-C. Low, *Environ. Technol. Lett.* **9**, 1361 (1988).
38. D. A. Bright, M. Dodd and K. J. Reimer, *Sci. Total Environ.* **180**, 165 (1996).
39. X. C. Le, W. R. Cullen and K. J. Reimer, *Appl. Organometal. Chem.* **6**, 161 (1992).
40. K. Hanaoka, T. Kaise, N. Kai, Y. Kawasaki, H. Miyashita, K. Kakimoto and S. Tagawa, Appl. Organometal. Chem. **11**, 265 (1997).

