APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 10, 733–740 (1996)

Seasonal Changes in Methylarsenic Distribution in Tosa Bay and Uranouchi Inlet

Hiroshi Hasegawa
Department of Chemistry, Faculty of Science, Kochi University, Akebono-cho, Kochi 780, Japan

The distribution of arsenic species, including trivalent methylarsenicals, was observed in coastal seawater of Tosa Bay and Uranouchi Inlet Japan. In Tosa Bay, most arsenic was dissolved in the inorganic form throughout the year and the concentration of total dissolved arsenic was higher than that in Uranouchi Inlet. The sum of methylarsenicals found in surface waters comprised 2–25% and 10–82% of the total dissolved arsenic in Tosa Bay and Uranouchi Inlet, respectively. In Uranouchi Inlet, seasonal variations in the concentrations of arsenicals were observed both in the water column and in surface sediments. The maximum concentrations of methylarsenicals appeared during summer, and became comparable to those of inorganic arsenicals in surface water. The concentration of trivalent methylarsenicals was usually low, and their seasonal changes seemed to be independent of those of the pentavalent species. The variations in methylarsenic(V) concentration did not coincide with those of chlorophyll *a* in either Tosa Bay or Uranouchi Inlet. These results suggested that methylarsenic(V) in natural waters was produced not directly by the activity of phytoplankton but through decomposition of organic matter by bacteria.

Keywords: arsenic speciation; seasonal change; methylarsenical; seawater; sediment; biological activity

INTRODUCTION

Arsenic is one of the elements present in a variety of chemical forms in natural waters and sediments. Arsenate [$AsO(OH)_3$; As(V)] is the thermodynamically stable state in oxic waters, while arsenite [$As(OH)_3$; As(III)] is predominant when the redox potential is decreased.[1–7] Biological processes also reduce the oxidation state of arsenic in surface waters, where high As(III)/As(V) ratios diverge from those expected from thermodynamic calculations.[2,5–7]

On occasions, biological activity leads to the formation of methylarsenicals, methylarsonic acid [$CH_3AsO(OH)_2$; MMAA(V)] and dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMAA(V)], and their concentrations become comparable with those of inorganic arsenicals.[8–11] The formation of methylarsenicals is considered to be a detoxification process beneficial to phytoplankton and organisms of higher trophic levels, since these methylarsenicals do not interfere with oxidative phosphorylation and are much less toxic to organisms than As(V).[12]

It has been reported that the concentrations of As(III) and methylarsenicals is correlated with chlorophyll *a* concentrations and/or phytoplankton densities.[2,13,14] This suggests that both the reduction and the methylation of As(V) result from the activity of phytoplankton. This hypothesis, however, remains uncertain because apparent differences were observed in water column profiles and seasonal changes of As(III) and methylarsenicals.[15–17]

In this paper, we describe the seasonal changes in arsenic species in coastal seawater, and the time lag between methylarsenical production and photosynthetic activity. The discussion will be focused on the routes by which methylarsenicals find their way into the water column. In this study, we also examined the distribution of the trivalent methylarsenicals, monomethylarsonous acid [$(CH_3As(OH)_2$; MMAA(III)] and dimethylarisinous acid [$(CH_3)_2As(OH)$; DMAA(III)]. Little information regarding trivalent methylarsenicals in natural waters has been reported,[17,18] although they are considered to be intermediates in organoarsenic biosynthesis.[19,20] Our findings

CCC 0268–2605/96/090733–08

Received 5 December 1995
Accepted 19 January 1996

Our findings provide new information concerning arsenic biogeochemistry in natural waters.

EXPERIMENTAL

Study area

The locations of the sampling sites are shown in Fig. 1. Tosa Bay is off Kochi, Japan, where the Kuroshio flows through. The Uranouchi Inlet is situated on the central coast of Tosa Bay. This inlet leads to the outer sea through a shallow mouth with a depth of 2–4 m, and the central part has a depth of 10–20 m. The water column is stratified as a result of halocline and/or thermocline at a depth of 2–4 m throughout year. The exchange of water is usually limited. When a large amount of rainwater enters the inlet during the period from July to October, outer seawater with higher salinity flows into the deep layer of Uranouchi Inlet at spring tide.[21] The water upwells at the end of the inlet, and surface water flows out of the inlet. Water samples were collected monthly at eight sites in Uranouchi

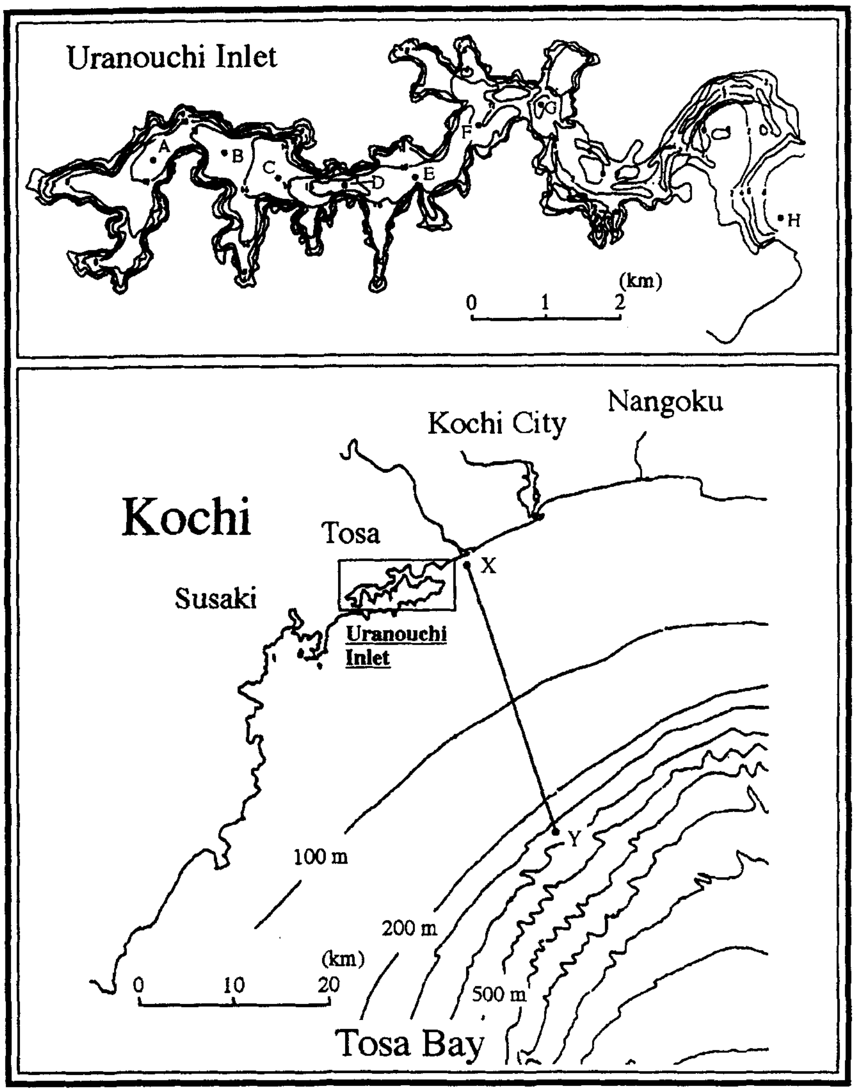

Figure 1 Study area, Uranouchi Inlet and Tosa Bay. X was at N 33° 26′ 75″ E 133° 29′ 70″; Y was at N 33° 11′ 00″ E 133° 36′ 00″.

Inlet over the sampling period from June 1993 to September 1995. Additional samples were obtained from Tosa Bay. Sampling sites in Tosa Bay were located in line from N 33° 26′ 75″ E 133° 29′ 70″ to N 33° 11′ 00″ E 133° 36′ 00″.

Methods

Water samples were collected using a 10-l Go-Flo sampler. A detailed description of the method used for arsenic analysis was reported previously.[18] For arsenic(III) analysis, unfiltered samples were used to prevent oxidation and evaporation during filtration.

The arsenic(III) species were identified and analyzed by solvent extraction and HG–AAS with cold-trapping. Reference 18 describes this speciation method for As(V), MMAA(V), DMAA(V), As(III), MMAA(III) and DMAA(III) in detail. We have confirmed that the trivalent species, MMAA(III) [$CH_3As(OH)_2$] and DMAA(III) [$(CH_3)_2As(OH)$], were quantitatively extracted with diethylammonium diethyldithiocarbamate into carbon tetrachloride, although the pentavalent species were not extracted under the same conditions. After back-extraction, separation of As(III), MMAA(III) and DMAA(III) was carried out by HG–AAS (hydride generation, liquid-nitrogen trapping, chromatographic separation and atomic absorption detection). The sum of the concentrations of the trivalent and pentavalent species was determined directly by HG–AAS in another aliquot of the same sample, so that each concentration of pentavalent species was obtained as a different calculation. We have ascertained that MMAA(III) and DMAA(III) below 10^{-8} M are monomeric in aqueous solution (unpublished data) and believe these monomeric species have chemical identity with trivalent methylarsenic in the natural environment. Precision and detection limits are shown in Table 1.

For chlorophyll *a* measurements, the samples were filtered through a Whatman GF/C glass fiber filter. Chlorophyll *a* was extracted into acetone and assessed colorimetrically.[24]

Sediment samples were collected using a core sampler, separated at 2 cm intervals with a polyethylene spoon on board, and stored in airtight polyethylene bottles. Within 3 h, the samples were dried at 110 °C in a forced-air oven, and ground in an agate mortar. One gram of the sediment was shaken for 1 h with an equal volume (0.4–1.0 ml) of 1 M sodium acetate solution (pH 8.2) to porewater. After centrifugation, the aqueous phase was filtered through 0.45 μm filters and analyzed. This fraction should contain most of the components dissolved in porewater and weakly adsorbed on clays, hydrated oxides of iron and manganese, and humic acids.[25,26] The chemical species in this aqueous phase are hereafter referred to as the exchangeable fraction. Phosphate was determined by phosphomolybdate blue spectrophotometry.[24] Iron and manganese were measured by atomic absorption spectrometry.

RESULTS AND DISCUSSION

Seasonal variation in Tosa Bay

Figure 2 shows profiles for arsenicals and chlorophyll *a* about 32 km off the coast of Kochi in Tosa Bay (depth 350 m). The water was stratified at a depth of about 90 m. In Tosa Bay, the dominant arsenic species was As(V) all year round. The As(V) profile showed a slight increase with depth, while maxima of As(III) and

Table 1. Detection limits and reproducibility in the determination of arsenic species[a]

Compd	Retention time (s)	Pentavalent Detection limit (nM)	RSD(%)	Trivalent Detection limit (nM)	RSD (%)
As	25	0.14	0.9	0.015	2.5
MMAA	60	0.18	2.5	0.017	3.9
DMAA	70	0.11	3.2	0.013	5.4

[a] Arsenic concentration: pentavalent, 10 nM; trivalent, 2 nM. Five measurements were made for each determination.

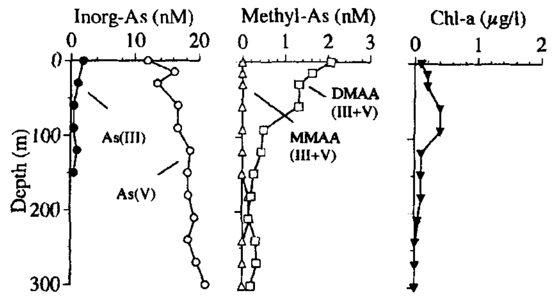

Figure 2 Vertical profiles of arsenicals and chlorophyll *a* (Chl-a) about 32 km off the coast of Kochi in Tosa Bay (Station Y; N 33° 11′ 00″ E 133° 36′ 00″) on 21 June, 1994.

DMAA(III+V) were observed in surface waters. The dimethylarsenic form was the most abundant methylated form, while other methylarsenicals (mono- and tri-methylarsenic) were scarcely detectable. The sum of methylarsenicals found in surface waters comprised 10–25% and 2–5% of total dissolved arsenic in summer and winter, respectively. The concentration of chlorophyll *a* reached a maximum at a depth of 60–90 m, below which the concentration of nutrients increased.

There was clearly a difference in the pattern of horizontal distribution between the sum of methylarsenicals and chlorophyll *a* in the surface waters of Tosa Bay. Their concentrations are plotted as a function of distance from the coast in Fig. 3. Of particular interest is that, on 21 June 1994, an increase in DMAA(III+V) concentration was observed at a distance of 16.6 km, although chlorophyll *a* rose to a peak at 7.7 km and decreased offshore from this point. On 6 September 1994, DMAA(III+V) concentration gradually increased from 1.1 nM near the coast to 1.7 nM at 32 km offshore despite the decrease in chlorophyll *a*. Both DMAA(III+V) and chlorophyll *a* were low and did not show a clear trend in winter.

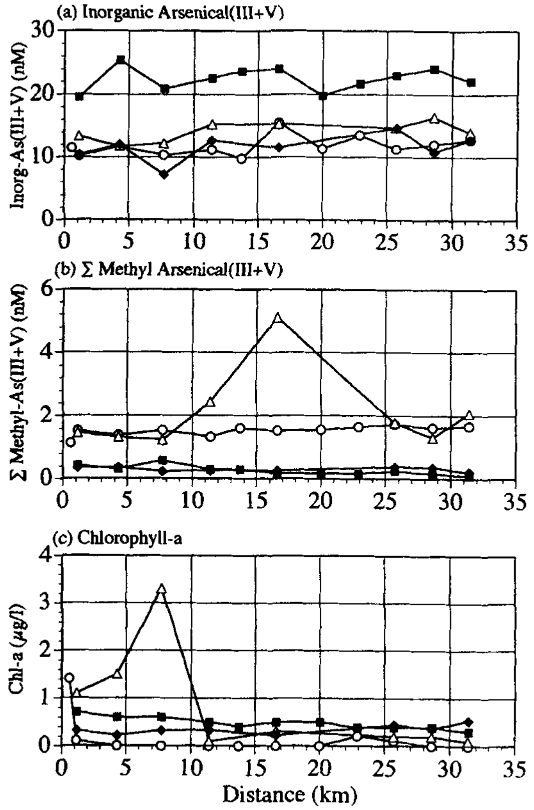

Figure 3 Distribution of arsenic(III+V) species and chlorophyll *a* in surface waters (0 m) of Tosa Bay. Concentrations are plotted as a function of distance from the coast. ◆, 26 January 1994; △, 21 June 1994; ○, 6 September 1994; ■, 26 January 1995.

Seasonal variation in Uranouchi Inlet

Figure 4 shows the seasonal changes in DMAA(III+V) distribution in Uranouchi Inlet. From January to July, the increase in DMAA(III+V) was marked in the inlet (Stations A–E). On 17 August, the maximum of DMAA(III+V) was observed at Station E. This was probably caused by seasonal inflow from Tosa Bay moving the water mass in the inlet.[21] Except for this period, DMAA(III+V) was maintained at a relatively constant concentration at Station H, which was located near the mouth

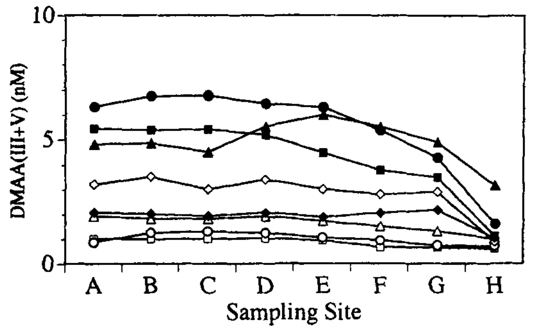

Figure 4 Seasonal changes in the DMAA(III+V) distribution in Uranouchi Inlet from January to September 1995. □, 30 January; ○, 14 March; △, 20 April; ◇, 19 May; ■, 20 June; ●, 20 July; ▲, 17 August; ◆, 12 September.

of the inlet.

Figure 5 shows seasonal variations in water temperature and in concentrations of arsenicals and chlorophyll *a* in surface waters at Station A. The increase of DMAA(V) concentration followed the rise in water temperature. The correlation coefficients of DMAA(V)/water temperature and DMAA(V)/chlorophyll *a* were 0.594 (n=223) and 0.181 (n=205), respectively. The DMAA(V) maxima reached 9.4 and 6.7 nM in the summers of 1994 and 1995, respectively. From autumn to early spring, the concentration of DMAA(V) lay within the range 0.8–2.9 nM. Similar seasonal changes in dimethylarsenicals have been reported previously in other regions.[15,17]

Isopleths of arsenic species at Station A of Uranouchi Inlet from June 1993 to September 1995 are shown in Fig. 6. As(V) was the most abundant form of arsenic in Uranouchi Inlet, except over a short period in summer. The increase in inorganic arsenic was coincident with the inflow of seawater from Tosa Bay. The concentrations of inorganic arsenic in the inlet waters were elevated with the increase in salinity (r=0.446, n=171).

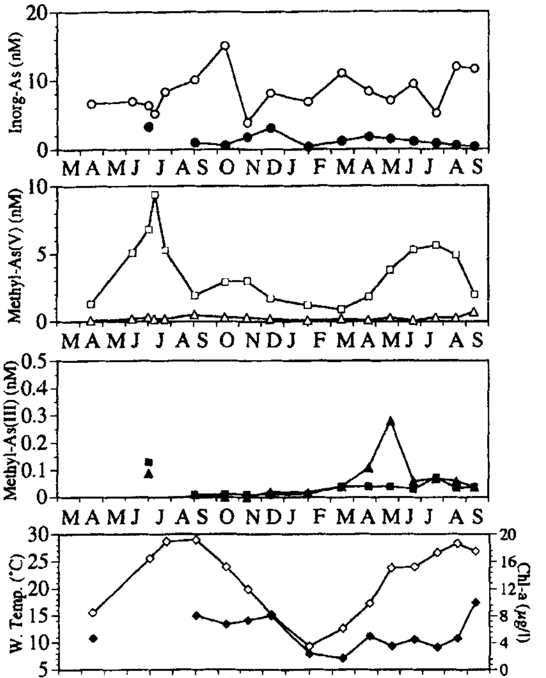

Figure 5 Seasonal variations in average concentrations of dissolved arsenic species and chlorophyll *a*, and water temperature at Station A of Uranouchi Inlet. The values are averaged at 0–4 m. ○, As(III+V); □, DMAA(III+V); △, MMAA(III+V); ●, As(III); ■, DMAA(III); ▲, MMAA(III); ◆, chlorophyll *a*; ◇, water temperature.

At Station A, the sum of methylarsenicals comprised 10–82% of total dissolved arsenic. DMAA(V) concentrations accounted for 70–98% of the sum of methylarsenicals. The highest concentration of DMAA(V) was observed in July, but no specific phytoplankton could be linked to the maximum of DMAA(V) in Uranouchi Inlet. MMAA(V) maxima appeared below the halocline (<4 m) before and after the occurrence of the yearly maximum of DMAA(V). MMAA(V) and DMAA(V) showed differences in both seasonal and vertical distributions. It is of interest that traces of trimethylarsenic(III+V) forms were observed at Station A. The levels and forms of the trimethylarsenics remain to be clarified by further study. Concentrations of the trivalent methylarsenic forms, MMAA(III) and DMAA(III), were low compared with those of the pentavalent species in most waters. However, MMAA(III) showed a marked increase to 0.05–0.60 nM from March to May 1995, when MMAA(III) was more abundant than MMAA(V). The maximum concentration of DMAA(III) was less than one-tenth of that of MMAA(III), and the sum of MMAA(III) and DMAA(III) lay within 18% of the sum of the methylarsenicals.

Behavior of arsenic in the sediment of Uranouchi Inlet

Figure 7 shows the seasonal variations in the exchangeable fractions of arsenicals, phosphate, and manganese in surface sediments (0–2 cm) at Station F. The water contents in the sediments were nearly constant between 35% and 50%. The bottom water and surface sediment at Station F were oxic, and the exchangeable fractions of iron and mono- and tri-methylarsenicals were below the limits of detection throughout the year. DMAA(III+V) increased from spring to summer. The variations in DMAA(III+V) closely paralleled those of phosphate and manganese. In marked contrast, As(III+V) concentration decreased as the manganese concentration increased. This inverse relation between manganese and As(III+V) was also observed in vertical profiles of the exchangeable fractions at Station F.

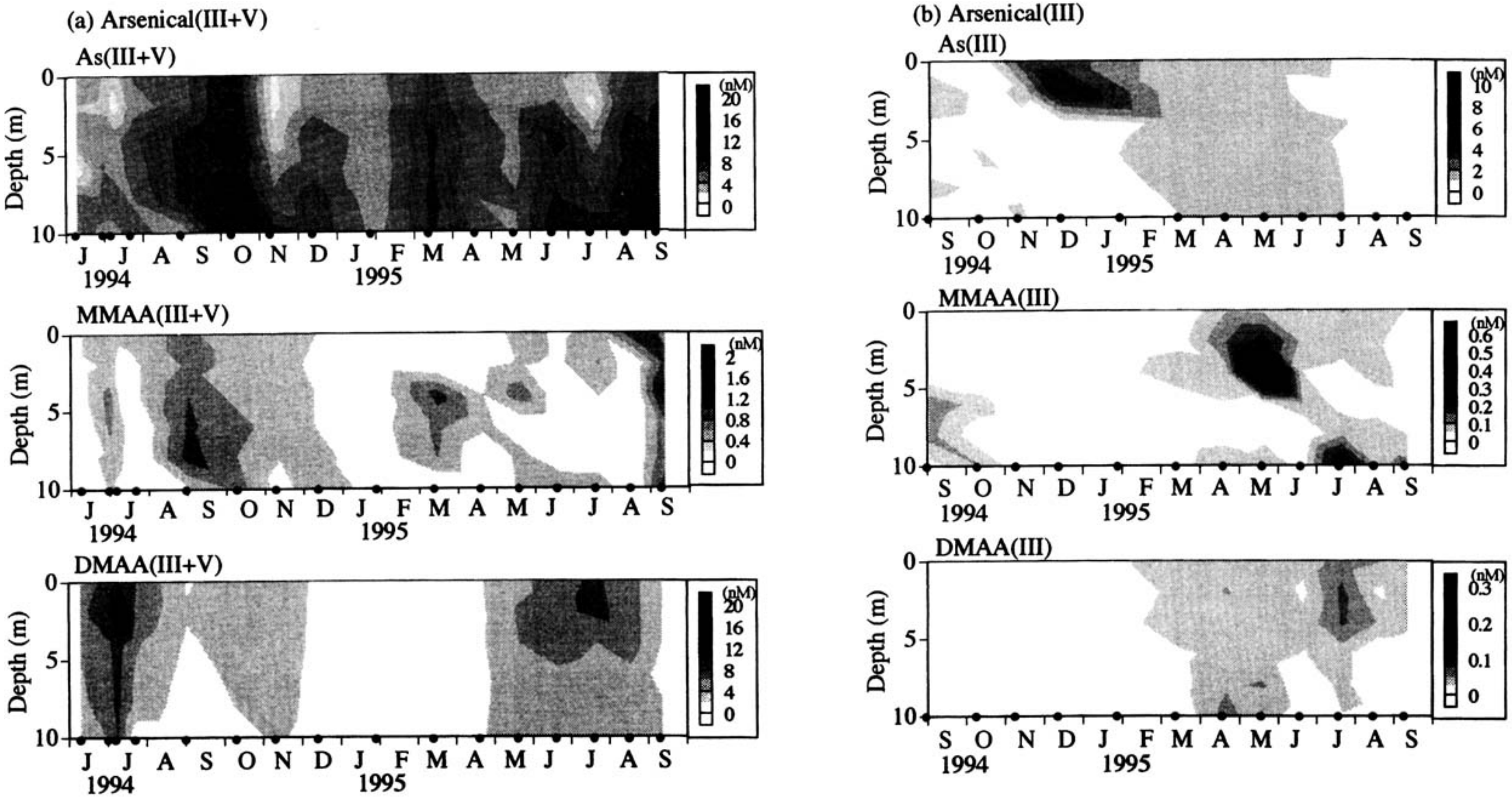

Figure 6 Isopleths of arsenic species at Station A of Uranouchi Inlet. Sampling depths were 0, 2, 4, 6, 8, and 10 m. ●, Sampling dates. (a) Arsenic(III+V) species from June 1994 to September 1995; (b) arsenic(III) species from September 1994 to September 1995.

Origin of methylarsenicals in Tosa Bay and Uranouchi Inlet

Tosa Bay and Uranouchi Inlet were obviously different in their compositions of arsenic species. In Tosa Bay, As(V) was the predominant form of arsenic throughout the year. The concentration of total dissolved arsenic in Tosa Bay was higher than that in Uranouchi Inlet. On the other hand, high concentrations of As(III) and methylarsenicals were observed in Uranouchi Inlet (Figs 4–6). These results were caused by differences in the standing crop of phytoplankton. The concentration of chlorophyll *a* in Uranouchi Inlet was about ten times that in Tosa Bay because of the abundant supply of nutrients. Thus, both assimilation of As(V) and metabolic formation of organoarsenicals were likely to be very large in the inlet.

The sum of methylarsenical concentrations in Tosa Bay should have been almost equivalent to that of DMAA(V), since other methylarsenicals were normally two orders of magnitude lower than DMAA(V) in oxic waters. In Tosa Bay, the distribution of DMAA(V) and chlorophyll *a* did not coincide vertically or horizontally (Figs 2, 3). DMAA(III+V) was increased in surface waters, although the standing crop of photosynthetic phytoplankton reached a maximum just above the thermocline (Fig. 2). In the seasonal data for Uranouchi Inlet (Fig. 5), the change of DMAA(V) concentration did not follow the development and decline of phytoplankton, and DMAA(V) was linearly correlated with water temperature while the change in As(III) concentration was independent of water temperature.

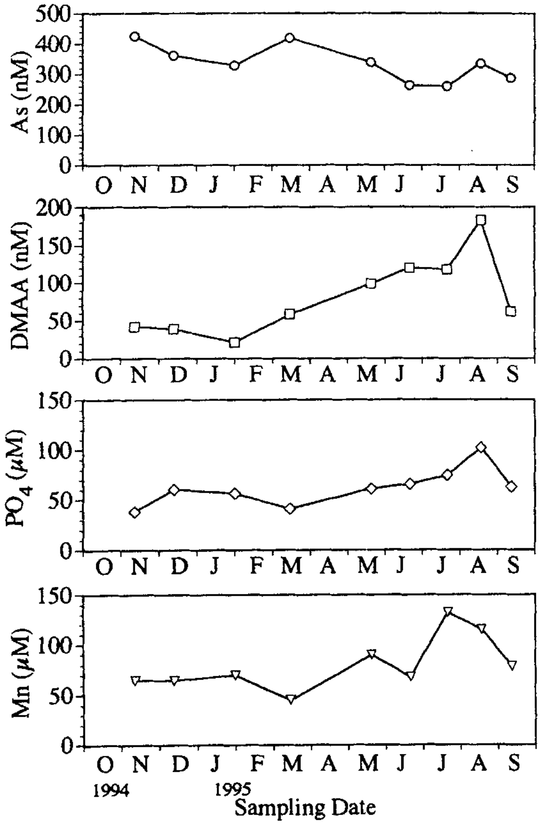

Figure 7 Seasonal variations in the exchangeable fractions of arsenicals, phosphate and manganese in surface sediments (0–2 cm) at Station F. ○, As(III+V); □, DMAA(III+V); ◇, phosphate; ▽, manganese.

These findings suggest that DMAA(V) was produced as a consequence of the bacterial decomposition of organoarsenicals such as arsenolipids and arsenosugars[27–29] in biotic particles. It is unlikely that the high concentration of DMAA(V) resulted from the long-term accumulation of DMAA(V) excreted by living phytoplankton, judging from the rapid decrease of the summer DMAA(V) maximum and little increase in methylarsenicals after several blooms during autumn and early winter. Santosa *et al.* reported the disagreement of methylarsenic and chlorophyll *a* distributions in surface waters of open oceans.[11] They suggested that the composition of arsenic species was controlled by the phytoplanktons ability to discriminate between phosphate and As(V), and that As(V) uptake by microorganisms was limited below 7 °C.

In Uranouchi Inlet, profiles of trivalent methylarsenicals changed seasonally in the water column (Figs 5, 6). Occasionally, MMAA(III) was more abundant than MMAA(V), although DMAA(III) was far lower than DMAA(V) throughout the year. The maxima of trivalent methylarsenicals were at the depths of 2–4 m and 8–10 m. Although, with respect to samples taken on the same day, the profile of trivalent methylarsenic species often resembled that of chlorophyll *a*, their seasonal variations were different. The seasonal variations in trivalent methylarsenic species also seem to be independent of those of the pentavalent species. Challenger suggested a sequential process of organoarsenic biosynthesis, in which methylation of As(V) proceeds through alternating reduction and oxidative methyl transfer and MMAA(III) and DMAA(III) are produced.[19] The observed increase in concentration of methylarsenic(III) might have been due to the seasonal blooms of phytoplankton. However, in our previous study of Lake Biwa, the profiles of methylarsenic(III) showed no significant trend between sampling periods.[17]

Arsenic species changed seasonally not only in the water column but also in the surface sediments of Uranouchi Inlet (Fig. 7). The summer increase in DMAA(III+V) in sediments at Station F can be explained by dissolution of hydrated manganese and iron oxides, degradation of organic matter, or bacterial methylation. Recently, Bright *et al.* reported that microbes in anaerobic lake sediments produced methylarsenicals containing methylarsenic(III) thiols.[30] Additional observations are needed to elucidate thoroughly the process of methylarsenic production in natural waters.

Acknowledgements I sincerely thank Dr Y. Sohrin, Dr M. Matsui, Kyoto University, and Dr M. Hojo for providing insights and suggestions that will last throughout my career. I also thank Dr N. Iwasaki, T. Sugimoto and S. Takizawa for helping with sample collection and analyses. This research was supported by a Grant-in-Aid for Encouragement of Young Scientists (07740578) 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. L. Peterson and R. Carpenter, *Mar. Chem.* **12**, 295 (1983).
4. M. O. Andreae and P. N. Froelich, *Tellus* **36B**, 101 (1984).
5. M. O. Andreae, Organoarsenic compounds in the environment. In: *Organometallic Compounds in the Environment: Principles and Reactions*, Craig, P. J. (ed), Longman, New York, 1986, pp. 198–228.
6. W. R. Cullen and K. J. Reimer, *Chem Rev.* **89**, 713 (1989).
7. G. A. Cutter, *Mar. Chem.* **40**, 65 (1992).
8. A. G. Howard, M. H. Arbab-Zavar and S. C. Apte, *Estuarine Coastal Shelf Sci.* **19**, 493 (1984).
9. L. C. D. Anderson and K. W. Bruland, *Environ. Sci. Technol.* **25**, 420 (1991).
10. G. F. Riedel, *Estuaries* **16**, 533 (1993).
11. S. J. Santosa, S. Wada and S. Tanaka, *Appl Organomet. Chem.* **8**, 273 (1994).
12. M. J. Gresser, *J. Biol. Chem.* **256**, 5981 (1981).
13. J. G. Sanders, *Mar. Chem.* **17**, 329 (1985).
14. J. G. Sanders and G. F. Riedel, *Estuaries* **16**, 521 (1993).
15. A. G. Howard, S. D. W. Comber, D. Kifle, E. E. Antai and D. A. Purdie, *Estuarine Coastal Shelf Sci* **40**, 435 (1995).
16. A. G. Howard, S. C. Apte, S. D. W. Comber and R. J. Morris, *Estuarine Coastal Shelf Sci* **27**, 427 (1988).
17. H. Hasegawa, Y. Sohrin, M. Matsui, M. Hojo and M. Kawashima, In: *Proceedings of 6th International Conference on the Conservation and Management of Lakes-Kasumigaura '95*, Vol. 2, 1995, pp 1144–1147.
18. H. Hasegawa, Y. Sohrin, M. Matsui, M. Hojo and M. Kawashima, *Anal. Chem.* **66**, 3247 (1994).
19. F. Challenger, *Chem. Rev.* **36**, 315 (1945).
20. D. J. A. Thompson, *Chem–Biol. Interactions* **88**, 89 (1993).
21. Y. Munekage and H. Kimura, *Coastal Eng. Japan* **35**, 129 (1992).
22. M. O. Andreae, *Anal. Chem.* **49**, 820 (1977).
23. R. S. Braman, D. L. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker, *Anal. Chem.* **49**, 621 (1977).
24. APHA, AWWA and WEF, *Standard Methods for the Examination of Water and Wastewater*, 18th ed., American Public Health Association, (1992).
25. A. Tessier, P. G. C. Campbell and M. Bisson, *Anal. Chem* **51**, 844 (1979).
26. W. A. Maher, *Chem. Geol.* **47**, 333 (1984).
27. J. S. Edmonds and K. A. Francesconi, *Nature (London)* **289**, 602 (1981).
28. J. S. Edmonds and K. A. Francesconi, *J. Chem. Soc., Perkin Trans. I* 2375 (1983).
29. J. S. Edmonds and K. A. Francesconi, *Experientia* **43**, 553 (1987).
30. D. A. Bright, S. Brock, W. R. Cullen, G. M. Hewitt, J. Jafaar and K. H. Reimer, *Appl. Organomet. Chem.* **8**, 415 (1994).