APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 11, 305–311 (1997)

The Behavior of Trivalent and Pentavalent Methylarsenicals in Lake Biwa

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Seasonal changes in the distribution of arsenic species were observed in a dredged area in the southern basin of Lake Biwa, Japan. The concentrations of dimethylarsenic acid [DMAA(V)] became comparable with those of inorganic forms during a stratification period. DMAA(V) increased not only in the photic zone but also in the hypolimnion. In the photic zone, an increase in DMAA(V) was observed with the yearly maximum of water temperature. In the hypolimnion, the seasonal changes in methylarsenicals differed from those in surface waters. DMAA(V) maxima appeared seasonally under sub-anoxic conditions and developed regionally at the redox boundary and above the sediment surface. The DMAA(V) concentrations increased in the initial period of oxygen depletion and just after the disappearance of anoxia, while they diminished in the anoxic hypolimnion by midsummer. The seasonal behavior of trivalent methylarsenicals, which are readily oxidized in oxic environments, was similar to that of DMAA(V). The total arsenic concentration in the surface layer rose to a maximum in late summer. Methylarsenicals did not increase in such a way that the total arsenic concentration increased during summer.

Appl. Organometal. Chem. **11**, 305–311 (1997)
No. of Figures: 6 No. of Tables: 0 No. of Refs: 39

Keywords: arsenic speciation; seasonal change; arsenic methylation; redox conditions; freshwater; hypolimnion; biological activity

Received 20 December 1995; accepted 3 May 1996

INTRODUCTION

From the viewpoint of environmental toxicology, it is important to determine chemical forms and levels of arsenic. Arsenate [$AsO(OH)_3$; As(V)], arsenite [$As(OH)_3$; As(III)], methylarsonic acid [$CH_3AsO(OH)_2$; MMAA(V)] and dimethylarsenic acid [$(CH_3)_2AsO(OH)$; DMAA(V)] are the major arsenicals that have been found as dissolved species in natural waters.[1–4] Other organoarsenicals have not been detected and identified because of analytical limitations, although the presence of 'hidden' arsenic species, which cannot be detected with the hydride generation technique, has been reported recently.[5]

As(V) is the thermodynamically most stable state in oxic environments and the most abundant dissolved form of arsenic in natural waters.[1–4, 6, 7] Occasionally, either As(III) or methylarsenicals become the primary species with corresponding decreases in the As(V) level in eutrophic surface waters.[8–11] Many studies have revealed close relationships between photosynthetic activity and distributions of As(III) and methylarsenicals.[2, 12, 13] The reported distribution of organoarsenic compounds in natural waters suggests that methylarsenicals are present only in the surface layers. In addition, As(III) has been found to comprise a significant portion of dissolved arsenic under anaerobic conditions.[7, 8, 14]

The present study was intended to reveal the production of methylarsenic species in a lake hypolimnion during the stratification period. In the hypolimnion, the concentration of methylarsenic species varied with the redox conditions, and the changes differed from those in surface waters. The seasonal flux from the lake sediments influenced arsenic speciation in the whole water column.

In this study, the distributions of the trivalent

Contract grant sponsor: Ministry of Education, Science, Sports and Culture, Japan; Contract grant number: 07740578

CCC 0268–2605/97/040251–05 $17.50

and pentavalent methylarsenicals were observed separately. Methylarsenic(V) species are much less toxic to phytoplankton and organisms of higher trophic levels since they do not interfere with oxidative phosphorylation, in contrast with As(V).[15] On the other hand, methylarsenic(III) species, monomethylarsonous acid [$CH_3As(OH)_2$; MMAA(III)] and dimethylarsinous acid [$(CH_3)_2As(OH)$; DMAA(III)], have high toxicity because they are lipophilic and inhibit the activity of enzymic SH groups.[16–19] Although these species have been found only as $(CH_3AsO)_x$ and $(CH_3)_2As–O–As(CH_3)_2$, our experimental data suggested that they are monomeric below 10^{-8} M (unpublished data).

EXPERIMENTAL

Study area

Lake Biwa is the largest lake in Japan and is geographically divided into two parts, the mesotrophic northern and the eutrophic southern basins (Fig. 1). Station S3 is in a dredged area (depth *ca.* 12 m; area 250 000 m^2) in the southern basin. Although the southern basin, with an average depth of 3.5 m, is not thermally stratified throughout the year, the dredged area becomes stratified from April to September, allowing dissolved oxygen extinction to occur during the summer.[20]

Field investigations were carried out over two periods, from February to October 1993, and from April to December 1994. The climate in 1993 was very different from that in 1994. The summer was unusually cool and rainy in 1993, while there was an unusually hot and dry summer in 1994.[21]

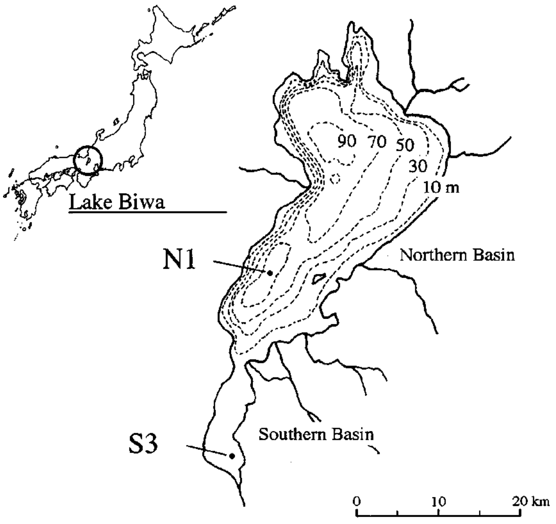

Figure 1 Study area, Lake Biwa.

Methods

The speciation method for arsenic was described previously.[22] Arsenic(III) species in sample water (500 ml) were extracted on-board with diethylammonium diethyldithiocarbamate into carbon tetrachloride and stored until analysis. After back-extraction, the aqueous phase was analyzed using hydride generation followed by atomic absorption spectrometry (HG–AAS).[23, 24] For arsenic(III + V) analysis, other samples (50 ml) were filtered with 0.45 μm filters (Millipore), acidified to pH 2 by addition of 1 M HCl and stored in a refrigerator. The concentration of arsenic(III + V) was measured directly by HG–AAS, and the concentration of arsenic(V) was calculated from the difference between arsenic(III + V) and arsenic(III) concentrations. Minimum detectable concentrations were 0.013–0.017 nM for arsenic(III) and 0.11–0.18 nM for arsenic(V).

A modified Winkler titration was used for dissolved oxygen.[25] Sulfide was determined with a spectrophotometric method based on formation of Methylene Blue.[26] Iron and manganese were determined by inductively coupled plasma atomic emission spectrometry. Chlorophyll **a** was assessed colorimetrically after acetone extraction from samples collected on glass fiber filters (Whatman GF/C).[27]

RESULTS AND DISCUSSION

Results

Figures 2 and 3 show the seasonal variations in the concentrations of dissolved methylarsenic species at Station S3 in 1993–1994. In Lake Biwa, the dominant methylarsenical was DMA(V), which comprised 64–99% of the total methylarsenic species. Concentrations of MMAA(V) and DMAA(V) were less than 0.67 nM and 10.1 nM, and those of MMAA(III) and DMAA(III) were no more than 0.16 nM and 0.19 nM, respectively. The methylarsenic(III) species concentration was one or two orders of

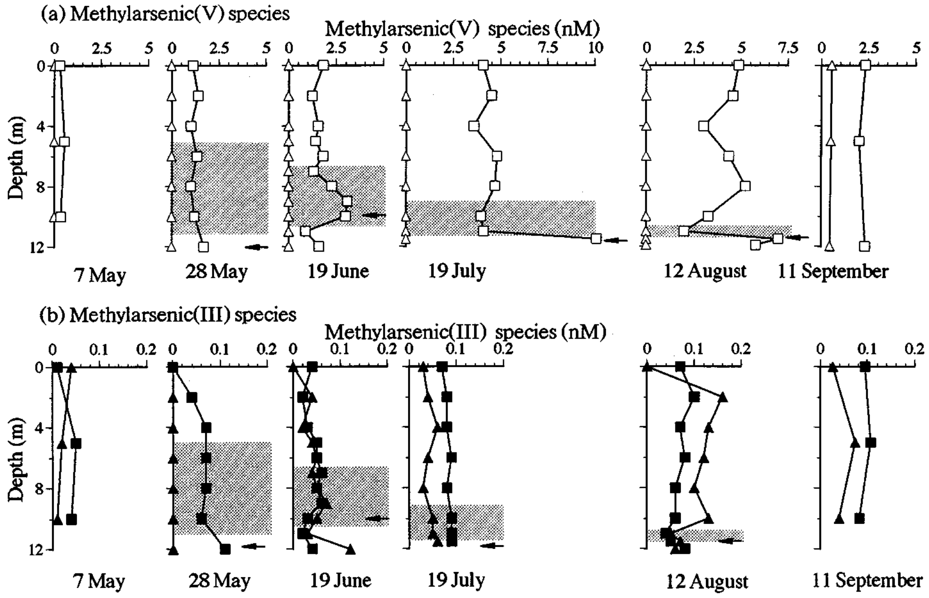

Figure 2 Vertical profiles of methylarsenicals in the southern basin of Lake Biwa (Station S3) from May to September 1993. Shading in the figures represents the thermocline. The arrows indicate the oxic/anoxic boundaries. (a) □, DMAA(V); △, MMAA(V); (b) ■, DMAA(III); ▲, MMAA(III).

magnitude lower than that of methylarsenic(V) species in each sample. High DMAA(V) concentrations appeared in and below the photic zone. In the photic zone, DMAA(V) concentrations remained at high levels of 1.4–4.9 nM during the stratification period. Below the photic zone, the maximum DMAA(V) concentration developed at depths of 6–9 m and 11–12 m, and became higher than that in surface waters from July to August 1993. In 1994, DMAA(V) maxima were

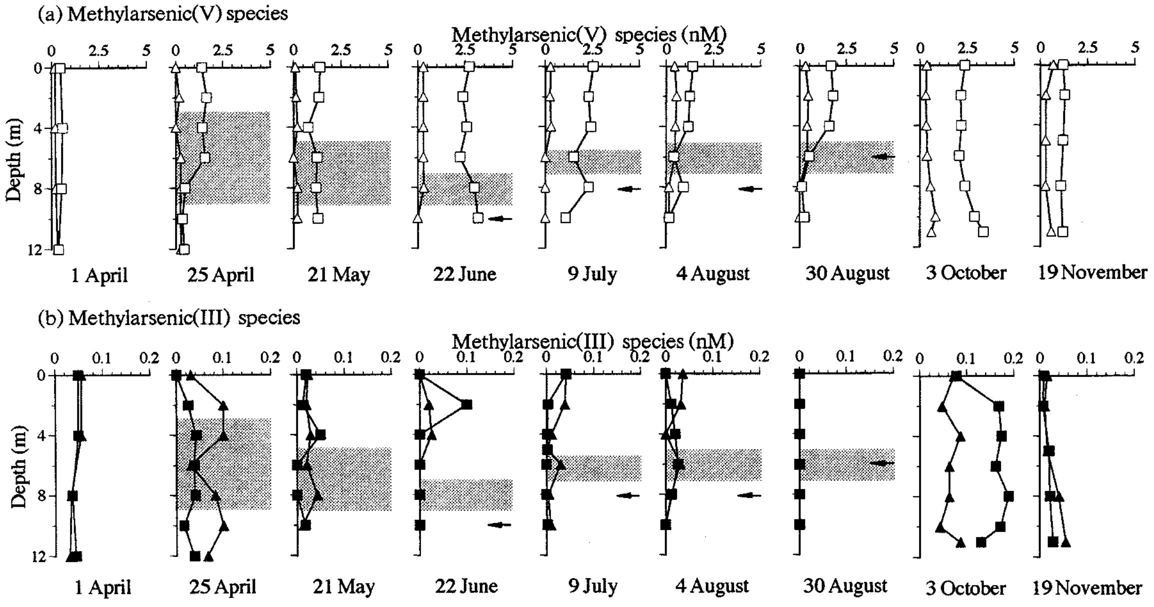

Figure 3 Vertical profiles of methylarsenicals in the southern basin of Lake Biwa (Station S3) from April to November 1994. Shading in the figures represents the thermocline. The arrows indicate the oxic/anoxic boundaries. (a) □, DMAA(V); △, MMAA(V); (b) ■, DMAA(III); ▲, MMAA(III).

produced above the surface sediments in June and October. DMAA(V) was depleted in the anoxic hypolimnion during summer. The trivalent methylarsenicals, MMAA(III) and DMAA(III), were found even in aerobic surface waters. Methylarsenic(III) species were uniformly distributed throughout the water column over 1993. On the other hand, they increased throughout the water column in April and October 1994. From June to August 1994, the methylarsenic(III) species concentrations were depleted to below the detection limit in the hypolimnion.

The seasonal data on average water temperature and concentrations of As(V), As(III), MMAA(V), DMAA(V) and chlorophyll a in surface waters from April 1993 to December 1994 are presented in Fig. 4. DMAA(V) reached peaks of 4.5 nM in July 1993 and 2.6 nM in June 1994. It is likely that the DMAA(V) and As(III) production in this layer depended upon water temperature and primary production, respectively.[28] There were no significant correlations between the concentrations of any arsenic species and nutrients.

Figure 5 shows the seasonal changes in arsenicals, oxygen, sulfide, manganese and iron in the hypolimnion at Station S3. In 1993, the stratification was not well developed all year round. The DMAA(V) concentration above the sediment surface rose to a peak of 10.1 nM during the summer. In 1994, the anoxic hypolimnion occurred by early summer and was maintained until the fall (autumn) turnover. DMAA(V) increased to 2.6 nM in the initial term of oxygen depletion, and disappeared in the hypolimnion during a long period of oxygen extinction. In September 1994, just after the disappearance of anoxia, the DMAA(V) concentration again reached a maximum of 3.4 nM.

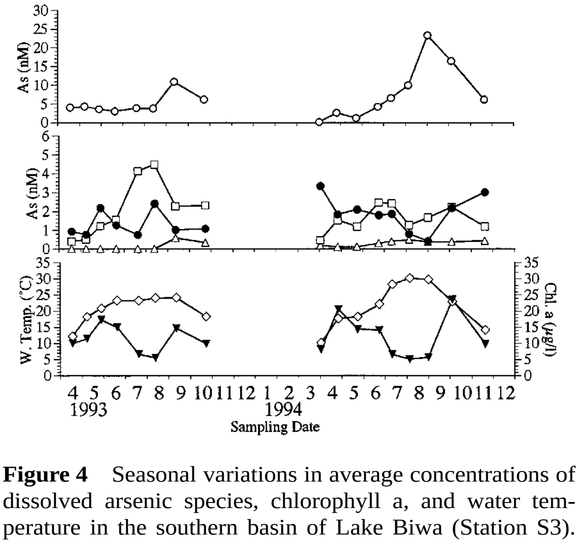

Figure 4 Seasonal variations in average concentrations of dissolved arsenic species, chlorophyll a, and water temperature in the southern basin of Lake Biwa (Station S3). Values are averaged in the epilimnion during the stratification period and throughout the water column under mixed conditions. ○, As(V); ●, As(III); □, DMAA(V); △, MMAA(V); ▼, chlorophyll a; ◇, water temperature.

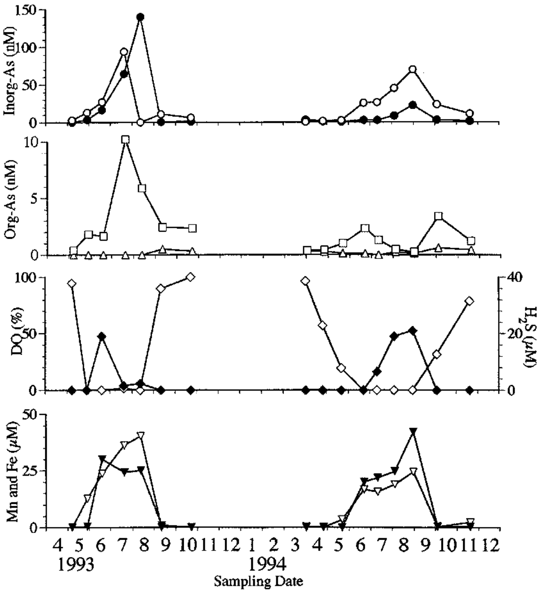

Figure 5 Seasonal variations in concentrations of arsenicals, sulfide, oxygen, manganese and iron in waters just above the sediment in the southern basin of Lake Biwa (Station S3). ○, As(V); ●, As(III); □, DMAA(III+V); △, MMAA(III+V); ◆, sulfide; ◇, oxygen; ▽, Mn; ▼, Fe. DO, dissolved oxygen.

When the surface sediment and hypolimnion of the dredged area became anoxic, inorganic arsenic concentration showed a rapid and marked increase. In August 1993, the total inorganic arsenic above the sediment surface reached 140 nM, which was ten times the surface concentration. As(V) was reduced to As(III) in the bottom layers from June to August 1993. Although the ratio of As(III)/As(V) was less than 1:1, with a hydrogen sulfide concentration of 20 μM on 19 June 1993, it was approximately 3:1 with a hydrogen sulfide concentration of 5.0 μM on 12 August 1993. The As(III) production rate constants gave values of 0.56, 1.4 and 2.9 in June, July and August 1993, respectively. They were 0.05, 0.22 and 0.48 in May, July and August 1994, respectively. The production rate (k) was estimated by $k = (C_t - C_0)/t$, where C_0 is

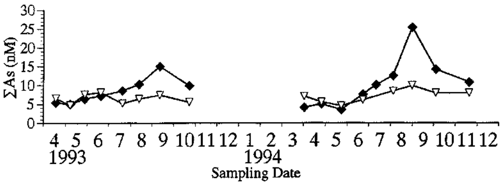

Figure 6 Seasonal variations in total arsenic concentration (ΣAs) in surface waters of Lake Biwa. ▽, The northern basin (Station N1); ◆, the southern basin (Station S3). The values of ΣAs represent the sum of As(III + V), MMAA(III + V) and DMAA(III + V).

the initial concentration and C_t is the concentration at time t. The As(V) reduction rate measured by Peterson and Carpenter gave a value of 0.074 nM/day ($[H_2S] = 26$ μM, $[As(V)] = 1.3$ nM, water temperature = 6°C, $t = 3$ days).[14] The discrepancy of the As(III) production rate constants was probably the result of differences in the experimental conditions.

Figure 6 shows seasonal data for the sum of inorganic and methylated arsenic concentrations (ΣAs) over two periods, from April to October 1993, and from April to December 1994. In July and December 1993 and in July 1994, we determined the total arsenic concentration (T-As) by an alkaline oxidation method.[22] This method converted into As(V) not only methylarsenicals but also arsenobetaine, which is the predominant form in many aquatic animals.[29, 30] The ΣAs agreed well with the T-As in the samples. While the value of ΣAs at Station N1 in the northern basin was relatively steady between 5.0 and 10 nM throughout both periods, there was a marked increase in the value of ΣAs at Station S3 (the southern basin) during summer. ΣAs rose to its peak in September, with a maximum concentration of 16 nM in 1993 and 26 nM in 1994, but showed a rapid drop in October. A similar pattern of change was observed in surface waters collected at stations over the southern basin. The increase of ΣAs in the surface water apparently followed the increase in inorganic arsenic concentration in the bottom layer at Station S3.

Discussion

In Lake Biwa, DMAA(V) was the predominant form of methylarsenic, and was produced not only in the photic zone but also in the hypolimnion. In the photic zone, the concentrations of DMAA(V) correlated linearly with water temperature ($r = 0.56$, $n = 88$).[28] The seasonal changes in DMAA(V) were different from those of As(III), which was associated with chlorophyll a. Similar relationships between DMAA(V) and water temperature have been reported previously in surface freshwater and seawater in the temperate zone.[11, 31]

In the hypolimnion, DMAA(V) was distributed at the oxic/anoxic boundary and just above the sediment (Figs 2, 3). Their seasonal changes differed from those in surface waters, and the DMAA(V) concentration was not proportional to the water temperature. In 1994, the seasonal variations of trivalent and pentavalent methylarsenic species suggest that methylarsenical production occurred in the sub-anoxic hypolimnion, and that methylarsenic species were decomposed under anoxic conditions. In 1993, the anoxic layer was not well developed in the dredged area of Lake Biwa because of the unusually cool summer. The DMAA(V) maxima in the hypolimnion developoed continuously, and methylarsenic(III) species concentrations remained relatively high throughout the water column until the fall (autumn) turnover. Methylarsenicals in the hypolimnion did not directly result from the *in situ* activity of photosynthesis, since the transparency was at 1.0–2.4 m and chlorophyll a was almost absent below the depth of 6 m at Station S3. Many workers have suggested that detritus may be a source of methylarsenicals in the interstitial waters.[32, 33] In the anoxic hypolimnion, the detritus on the surface of the lake sediment was consumed by anaerobic respiration of bacteria. It is likely that the methylarsenic species observed in the sub-anoxic layer were degradation products or/and the results of *in situ* bacterial methylation, and that they were decomposed to inorganic arsenic under anoxic conditions by facultative and obligate anaerobes. Bright *et al.* reported that microbes isolated from anaerobic lake sediments produced methylarsenicals containing methylarsenic(III) thiols.[34] Experimental studies *in vitro* demonstrated that arsenobetaine contained in organisms degraded to inorganic forms via methylarsenical intermediates.[35, 36]

A significant increase in inorganic arsenic was correlated with the dissolution of manganese and iron on the surface of sediment (Fig. 5). Takamatsu *et al.* suggested that, in Lake Biwa, hydrous manganese oxides, which are positively charged because of the adsorption of divalent cations, could play a significant role in the accumulation of arsenic in sediment.[37] At times when the Mn(IV) and Fe(III) oxides in sediment are reduced and dissolved under anaerobic conditions, the release of significant quantities of adsorbed arsenic to deep waters has been observed.[9, 20] This process was not clearly observed in the northern basin of Lake Biwa.[28] As(III) was produced in response to the redox changes in the lake waters. The ratio of As(III)/As(V), however, was not correlated with the concentration of hydrogen sulfide. Our results are in agreement with those of other studies of the conversion of As(V) to As(III) in sulfidic freshwater, where As(V) was also present.[38, 39] The dissolved arsenic species released from the sediment were not only As(III) but also As(V). The ratio As(III)/As(V) was controlled kinetically rather than thermodynamically in Lake Biwa.

Arsenic released from the lake sediment also influenced the total arsenic concentration in surface waters (Fig. 6). The total arsenic concentration in the surface layer rose to a maximum in late summer. The maximum value of ΣAs at Station S3 in 1994 was about twice that in 1993, which was due to extensive development of anoxia. Although areas other than that at Station S3 were not stratified, arsenic should be released with the reduction of the ferromanganese oxides in the sediment in the whole lake.[37] It is considered that benthic organisms were active and the anoxic layer well developed in the lake sediment with the increase in temperature in 1994. Moreover, a mass of algae withered around the southern basin in 1994 because of the unusual decline of the water level (-123 cm). This caused local anoxic conditions in various places and enhanced the reductive release of arsenic. A similar change in the phosphate concentration was also observed.[21] It is very likely that phosphate, a chemical analogue of As(V), was concurrently released with arsenic under anoxic conditions. Methylarsenicals did not increase in such a way that the total arsenic concentration increased during the summer in Lake Biwa.

Acknowledgements I sincerely thank Dr Y. Sohrin, Dr M. Matsui, Kyoto University, Dr M. Kawashima, Shiga University, and Dr M. Hojo for providing insight and suggestions that will last throughout my career. 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.

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