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Speciation of Arsenic in Natural Waters by Solvent Extraction and Hydride Generation Atomic Absorption Spectrometry

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A new approach is described for the speciation of arsenic species including trivalent methylarsenicals in natural waters. Arsenious acid [As(III)], monomethylarsonous acid [MMAA(III)], and dimethylarsinous acid [DMAA(III)] are separated from pentavalent species by solvent extraction using diethylammonium diethyldithiocarbamate (DDDC) and determined by hydride generation atomic absorption spectrometry (HG-AAS) after cold trapping and chromatographic separation. The detection limits for the trivalent species are about 13–17 pM. The sum of concentrations of the trivalent and pentavalent species are determined directly by HG-AAS in aliquots of the same samples. This is the first report of trivalent methylarsenicals being found and measured in natural waters.

Speciation of organic forms of elements is important in understanding their interaction with the biota, mobilization, adsorption, and transport of the elements in aquatic systems. Although rigorous characterization of the organic species is ordinarily difficult, it is possible on σ-bonded organometallic compounds, which have a relatively low molecular weight and contain kinetically inert metal–carbon bonds. Arsenic is one of the elements intensively studied because of its toxicological interest, and its organic forms are dominated by σ-bonded organometallic species.[1] Arsenic-containing ribofuranosides are ubiquitous in algae,[2,3] and arsenobetaine is the predominant form in marine animals.[4,5] Arsenic-containing ribofuranosides and arsenobetaine, however, probably do not make up the bulk of the arsenic budget in natural waters, since the product excreted by algae and aquatic animal culture appear to be limited to the inorganic and methylated species.[1,6] In natural waters, monomethylarsonic acid [MMAA(V)] and dimethylarsinic acid [DMAA(V)] have been determined.[7–9] Arsenic is pentavalent in the above-mentioned species. On the other hand, inorganic trivalent arsenic [$As(OH)_3$; As(III)] is also distributed in the hydrosphere, whereas it is thermodynamically much more unstable than arsenic acid [$AsO(OH)_3$; As(V)] under aerobic conditions.[10–12]

In this context, we noted that trivalent methylarsenicals, monomethylarsonous acid [MMAA(III)] and dimethylarsinous acid [DMAA(III)], have been missed out of analysis of natural waters. MMAA(III) and DMAA(III) are produced through the reduction of MMAA(V) and DMAA(V) by hydrogen sulfide and exist for a considerable time even under aerobic conditions. The trivalent methylarsenicals are probable intermediates in the biosynthesis of organoarsenicals, where the methylation of As(V) proceeds through alternating reduction and oxidative methyl transfer.[13,14] In addition, MMAA(III) and DMAA(III) are more toxic than As(III).[15] Arsenic speciation including MMAA(III) and DMAA(III) is therefore meaningful for arsenic biogeochemistry.

The trivalent methylarsenicals cannot be determined separately from the pentavalent species with hydride generation followed by atomic absorption spectrometry (HG-AAS),[16,17] which is commonly used for the speciation of arsenic in natural waters. In the conventional method, although As(III) is separated from As(V) by selective reduction to arsine, MMAA(III) and DMAA(III) are not separated from MMAA(V) and DMAA(V), respectively, since they are simultaneously reduced to monomethylarsine and dimethylarsine. The other problem is the change in the composition of arsenic species during sample storage, because the trivalent methylarsenicals are more subject to oxidation than As(III).[18] Peterson and Carpenter have reported that even an As(III)/As(V) ratio was not stable for longer than 1–3 days after collection.[10] It is necessary, therefore, to separate the trivalent from the pentavalent species as soon as possible after sample collection.

This paper describes a new speciation method for As(V), MMAA(V), DMAA(V), As(III), MMAA(III), and DMAA(III). The trivalent species were separated and concentrated

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by solvent extraction with diethylammonium diethyldithiocarbamate (DDDC) prior to determination with HG-AAS. The sum of the concentrations of the trivalent and pentavalent species was determined in an other aliquot of the same sample, so that the concentration of the pentavalent species was obtained as the difference. Since the change in the composition of arsenic species during the storage is removed, this method provided more accurate values for As(III) than the conventional HG-AAS technique. Some results of arsenic speciation in natural waters by this method are described, which show the first distribution of MMAA(III) and DMAA(III) in aquatic systems.

EXPERIMENTAL SECTION

Reagents. Standard solutions of 10^{-3} M MMAA(III) and DMAA(III) were made by dissolving the corresponding bromides (Alfa) in 0.1 M sodium hydroxide under a nitrogen atmosphere. MMAA(III) and DMAA(III) were dissolved as hydroxides by alkaline hydrolysis. The standard solutions were stored in sealed glass tubes to avoid oxidation. A standard solution of 10^{-2} M arsenobetaine was prepared by dissolving appropriate amounts of $(CH_3)_3As^+CH_2CO_2^-$ (Tri Chemical Laboratory Inc.) in distilled water. For other arsenic stock solutions (10^{-2} M), the sodium salts [$CH_3AsO_3Na_2$ was prepared by Quick's method,[19] and $NaAsO_2$, Na_2HAsO_4, and $(CH_3)_2AsO_2Na$ were obtained from Nacalai Tesque] were dissolved in distilled water. They were diluted to the required concentrations daily before use. The organoarsenical solutions were standardized by the HG-AAS method after decomposition to As(V). The standards were stable at least 6 months.

A 3% $NaBH_4$ solution was prepared by dissolving a sodium borohydride tablet (Nacalai Tesque) in 10^{-2} M sodium hydroxide just before use. Super-high-purity grade sodium hydroxide (Merck) and 35% hydrochloric acid (arsenic free: <0.0000005%, Nacalai Tesque) were used. Artificial seawater was prepared according to Fleming.[20] Other reagents were of analytical reagent grade, and distilled water was used throughout. No contamination of arsenic from the reagents was ascertained.

Safety Note. The arsenic compounds described in this paper can be severe toxins and should be handled with extreme care. Avoid inhaling arsenic bromide and arsines.

Apparatus. The apparatus for HG-AAS is shown in Figure 1. It consisted of a reaction vessel, a Teflon four-way valve, a U-trap, and a quartz cuvette. The vessel and tubing (6 mm i.d.) were made by Pyrex glass. These parts were connected on the straight with Teflon joints. The reaction vessel had a volume of 150 mL and three ports. The top port was stoppered with a glass cap using a ground glass joint. The glass cap had a helium gas inlet made of a 6 mm i.d. glass tube with a ball filter. The sample was placed in the vessel from a side-arm port. This port was fitted with a natural rubber septum, from which the $NaBH_4$ solution was injected through a 1.9 mm i.d. Teflon tube using a peristaltic pump. The other side-arm port was the gas outlet. The U-trap was 40 cm in length. A gas chromatographic packing [Chromosorb W AW-DWCS 60/80 mesh OV-3 (15%)] was packed into three-fifths of the U-tube and stopped with glass wool. The U-trap was wound

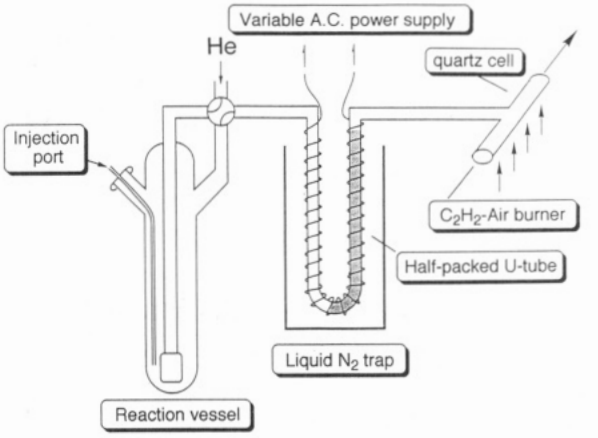

Figure 1. Schematic of hydride generation atomic absorption spectrometry with cold trapping (HG-AAS).

with Nichrome wire for heating. The length of tubing between the U-tube and the quartz cell was about 27 cm.

To prevent the irreversible adsorption of arsines, the internal surfaces of the glassware other than the reaction vessel were deactivated with silylation reagents. The glass tubes were initially filled with 15% hydrofluoric acid for 30 min and washed with water. After drying at 250 °C, the tubes were filled with 5% dimethyl dichlorosilane in toluene and maintained at 80 °C for 1 h. They were cooled, washed with ethanol, and dried at 100 °C. Silanization was repeated each time deterioration of the column performance was observed. The column was heated in a stream of helium (flow rate 0.3 L/min) for 1 h daily before use.

The light pass of the quartz cuvette was 6 mm i.d. and 140 mm in length. The cuvette was mounted on the acetylene–air burner of a Hitachi Model 180–80 atomic absorption spectrometer and heated by a flame. An arsenic hollow cathode lamp (Hamamatsu Photonics) was operated at 10 mA. All measurements were performed using the arsenic resonance line at 193.7 nm, with a slit width of 2.6 nm.

Procedure. (A) Extraction for Separation of Arsenicals. A 9-mL sample of aqueous solution, in which the pH was adjusted to the desired value using Britton-Robinson buffers, and 10 mL of carbon tetrachloride containing 10^{-2} M DDDC were placed in a 30-mL centrifuge tube. Immediately after 1 mL of an arsenic standard solution (10^{-3} M) was added, the tube was shaken for 15 min at 25 ± 0.1 °C, using a Taitec Bioshaker BR-30L. After centrifugation, the organic and aqueous phases were separated. The arsenicals in the organic phase were back-extracted with an equal portion of 0.1 M sodium hydroxide. The arsenical concentrations in both phases were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Japan Jarrel Ash ICAP-500. The sensitivity of ICP-AES was highly dependent on the valence of arsenic species: the sensitivity for trivalent arsenic was usually much higher than that for the pentavalent species. At pH 9 (Britton-Robinson buffer), the sensitivity for MMAA(III) and DMAA(III) was 1.4 and 2.1 times that for pentavalent species, respectively. The difference in the sensitivity was less than 5% between As(III) and As(V). The sensitivity difference is due to the trivalent arsenic species being highly volatile and being introduced to the plasma more effectively than the pentavalent species.[21] Valency change of

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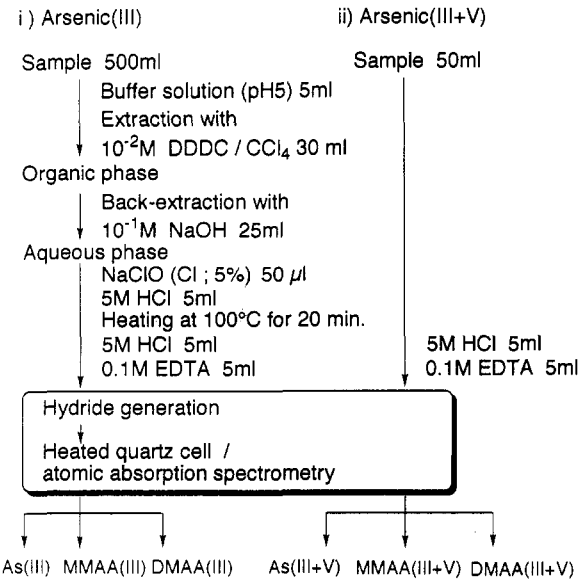

Figure 2. Flow chart of the analytical procedure for the speciation of arsenicals.

arsenic species may occur in sample solution before the measurement by ICP-AES. For accurate determination, the arsenic concentrations were determined, after the trivalent species were oxidized to pentavalent by mixing 50 μL of 30% hydrogen peroxide with 5 mL of the sample solution.

(B) Speciation of Arsenic in Natural Waters. A flow chart of the analytical method is shown in Figure 2. Arsenic(III) and arsenic(III+V) species were determined in separate aliquots.

In the analysis of arsenic(III), 500 mL of a sample was placed in a 1-L Teflon separatory funnel, and 30 mL of 10^{-2} M DDDC in carbon tetrachloride was added. Thereafter, 10 mL of acetic acid–sodium acetate buffer was added to adjust the pH of the aqueous phase to 5. The funnel was vigorously shaken for 15 min. The organic phase was quantitatively transferred to a 100-mL separatery funnel and back-extracted with 30 mL of 0.1 M sodium hydroxide for 15 min. In addition to arsenic species, some DDDC and its degradation products were extracted into the sodium hydroxide. To remove these compounds, 50 ?L of sodium hypochlorite (available chlorine 5%) and 5 mL of 5 M hydrochloric acid were added, and the solution was heated at 100 °C for 25 min in a Teflon beaker. After cooling to room temperature, 5 mL of 0.1 M EDTA and 5 M hydrochloric acid were added. The sample was diluted to 50 mL with water and introduced to the reaction vessel.

Helium gas (flow rate 0.3 L/min) was bubbled into the solution for 5 min to remove oxygen. Ten milliliter of 3% $NaBH_4$ was injected using the peristaltic pump while continuously stripping arsines with helium. The arsines were collected in the U-trap that was immersed in liquid nitrogen. Five minutes later, the helium pathway was switched to flow directly into the U-trap, and its flow rate was increased to 1.4 L/min. The liquid nitrogen was removed, and the arsines were revaporized by heating the trap. The arsines reached the quartz cell in the order of boiling point and were atomized. The atomic absorption of the arsenic species was recorded as a chromatogram.

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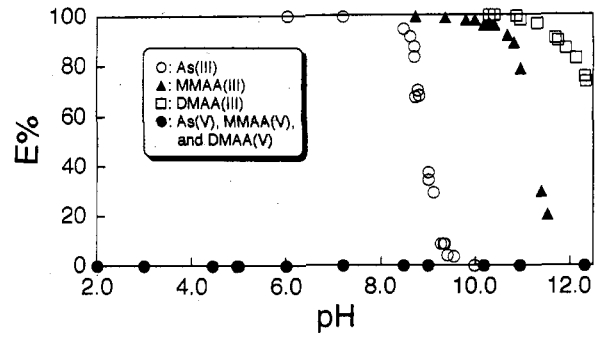

Figure 3. Effect of pH on the extraction of arsenicals with 1.0×10^{-2} M DDDC in carbon tetrachloride. The concentrations of arsenic compounds were 1.0×10^{-4} M.

To measure arsenic(III+V), 50 mL of the sample was placed in the reaction vessel, followed by the addition of 5 mL of 0.1 M EDTA and 5 mL of 5 M hydrochloric acid. The concentrations of arsenic(III+V) were determined in a manner similar to that described above, and the concentrations of arsenic(V) were calculated by the difference on those for arsenic(III+V) and arsenic(III).

(C) Determination of Total Arsenic in Natural Waters. Total arsenic concentration (T-As) was determined after the alkaline persulfate oxidation procedure, which is a modification of the method described by Lewis and Andreae et al.[22] A total of 25 mL of a sample and an equal volume of 10% $K_2S_2O_8$ containing 2 M sodium hydroxide were placed in a 100-mL Teflon digestion bomb. The oxidation proceeded at 70 °C for 2 h, and the resulting solution was diluted with water. The concentration of As(V) in this solution was determined by HG-AAS.

RESULTS AND DISCUSSION

Separation of Trivalent Arsenicals by Extraction. Figure 3 shows the effect of pH on the extraction percentage (E%) of inorganic and methylarsenicals into carbon tetrachloride with DDDC. The As–C bonds were not cleaved during the extraction procedure. The recovery was more than 95% for all the arsenic species. The E% of the arsenic species reached a constant value after 15 min of shaking.

Several sulfur-containing species were shown to reduce arsenical(V) to arsenical(III) in previous publications.[23,24] Ebdon et al. have reported that MMAA(V) and DMAA(V) were reduced with methylthioglycolate and converted into thioarsenite derivatives.[23] We found that diethyldithiocarbamate (DDC) also reduced arsenical(V) partly under an acidic condition. In 3 M H_2SO_4, log D (D; distribution constant) was −2.2, −1.6, and −1.0 for DMAA(V), MMAA(V), and As(V), respectively, and increased with increasing acid concentration. However, none of the pentavalent arsenicals were extracted at pH 2–13 (log $D < -3.5$).

The trivalent species were quantitatively extracted in the acidic and neutral pH region. The trivalent species were not oxidized in the extraction with DDDC. The E% decreased as the pH increased beyond 8 for As(III), beyond 9.5 for MMAA(III), and beyond 11 for DMAA(III). Log D of the

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DDC complexes exceeded 3.0 at pH 5. Similar results were obtained in extraction from artificial seawater.

The composition of the extracted species was estimated by slope analysis. The extracted species were mononuclear complexes and contained 3, 2, and 1 molecules of DDC for As(III), MMAA(III), and DMAA(III), respectively.

Removal of Interferences to HG-AAS. There were two kinds of major interferences to HG-AAS. The first was caused by molecular absorption of volatile compounds in the determination of trivalent arsenicals. After back-extraction, the aqueous phase contained a small amount of DDDC, its decomposition products, and carbon tetrachloride. When the solution was acidified for hydride generation, DDC decomposed into diethylamine and carbon disulfide, and the latter was further converted into hydrogen sulfide. These compounds interfered with the measurement of As(III) and DMAA(III), since hydrogen sulfide (bp −60 °C), carbon disulfide (bp 46.3 °C), and carbon tetrachloride (bp 76.7 °C) had similar chromatographic behavior to that of arsine (bp −62.5 °C) or dimethylarsine (bp 37 °C) and absorbed radiation of arsenic resonance lines. To remove this interference, the back-extracted aqueous phase was heated at 100 °C for 25 min after the addition of sodium hypochlorite and acidification with hydrochloric acid. After being heated in acidic solution, DDC was completely decomposed, and carbon tetrachloride, carbon disulfide, and hydrogen sulfide were eliminated by vaporization. Hydrochloric acid was used because other acids, such as nitric and sulfuric acids, suppressed the subsequent formation of arsines. When the trivalent arsenicals were heated in hydrochloric acid, chloride complexes were formed and vaporized. Hence, the trivalent arsenicals were oxidized to the pentavalent form with an oxidizing agent prior to the addition of hydrochloric acid. Hydrogen peroxide and sodium hypochlorite were tested as the oxidizing agent. With hydrogen peroxide, although arsenicals(III) were oxidized, the interference of DDC was not removed. It is likely that hydrogen peroxide favored the formation of a DDC dimer, that was relatively stable to heating and decomposed at the hydride generation stage. On the other hand, sodium hypochlorite exerted no effect on the removal of interfering molecules. Sodium hypochlorite was therefore used as the oxidant.

The other major interference was caused by the transition metal ions, especially iron, which were present in the samples collected from anaerobic and polluted sites. Many workers have reported the suppression of arsine evolution by transition metal ions during the reduction step of hydride generation.[16,24,25–27] To prevent this potential interference, EDTA was added to the samples before the hydride generation as suggested by Howard and Arbab-Zavar[28] and Belcher et al.[29]

Analytical Merits of Speciation Method for Arsenicals. Composite standard solutions of the pentavalent arsenicals were used to determine methylated and inorganic arsenicals by HG-AAS. The retention times were 25, 60, and 70 s for As(V), MMAA(V), and DMAA(V), respectively (Figure 4). Peak heights rather than peak areas of the atomic absorption signal were used for calibration, since the former gave more reproducible results than the latter. The calibration curves were linear up to about 40, 60, and 30 nM for As(V), MMAA-(V), and DMAA(V), respectively. The detection limits, the standard deviations for 10 nM pentavalet and 2 nM trivalent arsenicals, and the recovery for trivalent arsenicals are summarized in Table 1. The sensitivity and reproducibility were not affected by the major sea salts, and the same results were obtained by the method of standard addition against the waters collected from Lake Biwa and the Uranouchi Inlet. We also confirmed that the sensitivity and reproducibility of HG-AAS for trivalent arsenicals were equal to those for pentavalent species. Thus, arsenic(III+V) can be accurately determined using the pentavalent standards. Arsenic(III) species were also usually determined using the pentavalent standards, because they were oxidized into pentavalent species with hypochlorite prior to the hydride generation.

A 500-mL composite standard solution containing 2 nM of As(III), MMAA(III), and DMAA(III) was extracted with DDDC followed by HG-AAS. These trivalent arsenicals were concentrated 10-fold to the original by the extraction. The results in Table 1 show that As(III) and MMAA(III) were quantitatively recovered. The values of DMAA(III), though not quantitative, were reproducible with about 6% of the

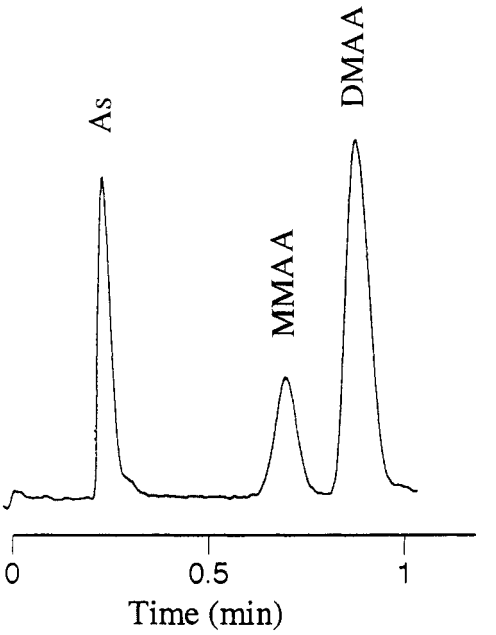

Figure 4. Typical chromatogram of arsenicals. The arsenic concentrations of As(V), MMAA(V), and DMAA(V) were 0.50, 0.25, and 0.50 nmol, respectively.

Table 1. Detection Limits, Reproducibility, and Recovery in Determination of Trivalent and Pentavalent Arsenicals[a]

		pentavalent		trivalent		
compd	retention time (s)	detection limit (nM)	rsd (%)	detection limit (nM)	rsd (%)	recovery (%)
As	25	0.14	0.9	0.015	2.5	95
MMAA	60	0.18	2.5	0.017	3.9	104
DMAA	70	0.11	3.2	0.013	5.4	87

[a] Arsenic concentration; pentavalent, 10 nM; trivalent, 2 nM. Five measurements each.

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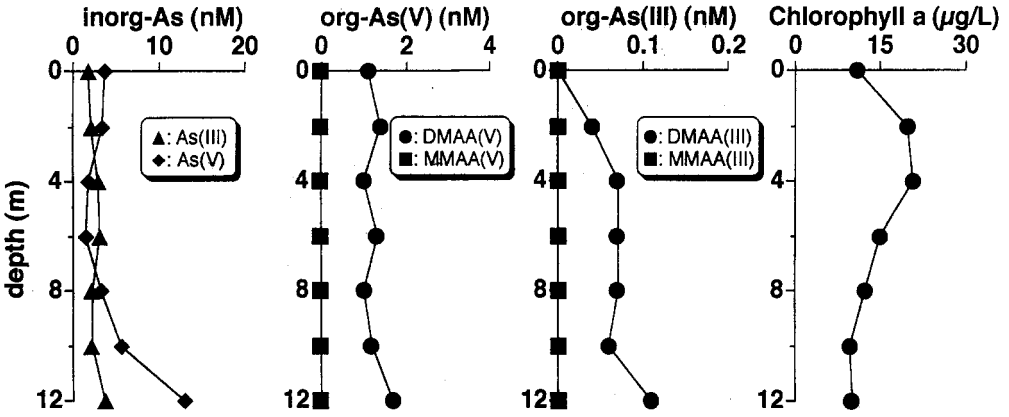

Figure 5. Distribution of arsenic species and chlorophyll *a* at the dredged area (ca. 25 ha) in the southern basin of Lake Biwa (5/28/1993).

Table 2. Recovery of Arsenic from Dimethylarsinic Acid, Monomethylarsonic Acid, Phenylarsonic Acid, and Arsenobetaine Using Alkaline Oxidation[a]

compound	recovery (%)	rsd (%)
DMAA(V)	100	5.3
MMAA(V)	96	3.6
phenylarsonic acid	104	4.9
arsenobetaine	96	5.2

[a] Each sample contained 10 nM As. Five measurements each.

Table 3. Comparison of Total Arsenic and Inorganic and Metylated Arsenic Concentrations in Natural Waters[a]

			arsenic(III+V)			
sample	depth (m)	T-As (nM)	Σ-As[b] (nM)	As (nM)	MMAA (nM)	DMAA (nM)
Biwa Lake; dredged area	0	10	9.6	5.8	nd[c]	3.8
(6/19/1992)	12	27	28	14	3.0	11
Biwa Lake; northern lake	5	7.4	7.1	4.9	0.3	1.9
(9/11/1993)	30	6.2	6.2	5.9	nd	0.3
	70	9.3	9.3	9.1	nd	0.2
Yodo River (6/26/1992)	0	24	24	24	nd	nd
Setouti Bay (5/7/1992)	0	24	24	22	nd	2.2

[a] Single measurement each. [b] The sums of As(III+V), MMAA(III+V), and DMAA(III+V). [c] Not detected (nd).

relative standard deviation. The DMAA(III) concentrations were calculated therefore as the product of the values obtained from the chromatogram and the factor derived from the recovery.

Pentavalent arsenicals at a concentration of 10 nM in the sample did not affect the determination of trivalent species. The results were not affected by the presence of 10^{-2} M hydrogen sulfide, which is a principal reductant in anaerobic natural waters.[10] Moreover, major sea salts did not affect the results, and the recoveries of arsenicals from natural waters were the same. Extraction with DDDC is, therefore, satisfactory to separate trivalent from pentavalent arsenicals in natural waters.

Total Arsenic in Natural Waters. To determine T-As, organoarsenicals were converted into the inorganic form [As(V)] by the alkaline oxidation. The oxidation was examined using ubiquitous organoarsenicals, such as MMAA(V), DMAA(V), phenylarsonic acid,, and arsenobetaine. The results are shown in Table 2. The recovery meant that the percentage of the arsenicals measured as As(V) by HG-AAS was within the range of 96–104%. The cleavage of the As–C bonds in MMAA(V), DMAA(V), and phenylarsonic acid was also confirmed in D_2O by the disappearance of peaks for the methyl and phenyl groups on ^{1}H-NMR spectra. While more complex organic forms, such as arsenic-containing ribofuranosides, are dominant in vivo[2,3] and might be present in natural waters, Crecelius reported that they were not stable in alkaline solutions and converted into DMAA(V).[30] Thus, such arsenic compounds should be measured as As(V) by this means.

Table 3 shows T-As, As(III+V), MMAA(III+V), and DMAA(III+V) concentrations in natural waters. As(III+V), MMAA(III+V), and DMAA(III+V) were determined by HG-AAS. The T-As values agreed closely with the sums of inorganic and methylated arsenic concentrations in each sample. These results were similar to those of most previous studies.[31,32] Recently, Bettencourt and Andreae have reported that species undetectable by HG-AAS constitute a significant fraction of the total arsenicals and that it is also refractory to alkaline digestion.[33] We are studying these unusual refractory species at the present.

Arsenic Speciations in Natural Waters. Water samples were analyzed using the method described here. Samples were collected with Tygon tubing (Norton) and a pump that prevented gas exchange between the sample and the atmosphere and that enabled closely spaced sampling of less than 1-m intervals. The samples for arsenic(III) analysis were stored as carbon tetrachloride solutions after on-board extraction, and those for arsenic(III+V) analysis were acidified with 10^{-2} M hydrochloric acid. Arsenic species were determined within 1–2 days of collection, during which period of time losses of the methylarsenicals were less than 6%. Trivalent species were detected in various samples (Table 4). Figure 5 presents a vertical distribution of the arsenicals found in a dredged area in the southern basin of Lake Biwa in May 1993. The behavior of As(III) and As(V) was similar to that previously reported by several investigators.[7,10,34,35] An increase in the As(III) concentration was connected with the photosynthetic process in the euphotic zone and with the

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Table 4. Speciation of Arsenic Compounds in Water Samples[a]

sample	depth (m)	pentavalent As (nM)	pentavalent MMAA (nM)	pentavalent DMAA (nM)	trivalent As (nM)	trivalent MMAA (nM)	trivalent DMAA (nM)
Lake Biwa	0	3.8	nd[b]	4.9	1.5	nd	0.077
(8/12/1993)	2	4.5	nd	4.6	1.2	0.14	0.076
Uranouchi Bay	0	5.2	0.11	2.2	1.7	0.13	0.10
(9/13/1993)	2	6.7	0.08	2.3	1.6	0.036	0.063
Tosa Bay	0	7.5	0.12	1.5	0.75	0.067	0.024
(9/20/1993)	30	8.8	0.16	1.1	0.73	0.065	0.034

[a] Single measurement each. [b] Not detected (nd).

reductive dissolution of manganese and ferric oxides under anoxic conditions. The distribution of trivalent methylarsenicals mostly followed that of As(III). An extensive study of arsenic speciation is now underway, and precise data and biogeochemical discussion will be reported in forthcoming papers.

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