APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 8, 325–333 (1994)

Biomethylation and Biotransformation of Arsenic in a Freshwater Food Chain: Green Alga (*Chlorella vulgaris*)→Shrimp (*Neocaridina denticulata*)→Killifish (*Oryzias latipes*)

Takayoshi Kuroiwa, Akira Ohki, Kensuke Naka and Shigeru Maeda
Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890, Japan

Tolerance, bioaccumulation, biotransformation and excretion of arsenic compounds by the freshwater shrimp (*Neocaridina denticulata*) and the killifish (*Oryzias latipes*) (collected from the natural environment) were investigated. Tolerances (LC_{50}) of the shrimp against disodium arsenate [abbreviated as As(V)], methylarsonic acid (MAA), dimethylarsinic acid (DMAA), and arsenobetaine (AB) were 1.5, 10, 40, and 150μg As ml^{-1}, respectively.

***N. denticulata* accumulated arsenic from an aqueous phase containing 1 μg As ml^{-1} of As(V), 10 μg As ml^{-1} of MAA, 30 μg As ml^{-1} of DMAA or 150 μg As ml^{-1} of AB, and biotransformed and excreted part of these species. Both methylation and demethylation of the arsenicals were observed *in vivo*. When living *N. denticulata* accumulating arsenic was transferred into an arsenic-free medium, a part of the accumulated arsenic was excreted. The concentration of methylated arsenicals relative to total arsenic was higher in the excrement than in the organism.**

Total arsenic accumulation in each species via food in the food chain

Green algae (*Chlorella vulgaris*)
→shrimp (*N.denticulata*)
→killifish (*O. latipes*)

decreased by one order of magnitude or more, and the concentration of methylated arsenic relative to total arsenic accumulated increased successively with elevation in the trophic level. Only trace amounts of monomethylarsenic species were detected in the shrimp and fish tested. Dimethylarsenic species in alga and shrimp, and trimethylarsenic species in killifish, were the predominant methylated arsenic species, respectively.

Keywords: Arsenic, methylation, transformation, freshwater food chain, green alga, shrimp, killifish

INTRODUCTION

A number of books and review articles discuss arsenic and its transformation in the environment. Particular mention should be made of one book, *Arsenic*.[1] The third Spurenelement-Symposium resulted in a useful volume, *Arsen*,[2] as did a symposium sponsored by the Chemical Manufacturers Association and the National Bureau of Standards, *Arsenic: Industrial, Biomedical, Environmental Perspectives*,[3] and the First Arsenic symposium sponsored by the Japanese Arsenic Scientists Society (JASS), *Arsenic: Chemistry, Metabolism and Toxicity*.[4] The JASS symposia resulted in successive volumes as special issues of *Applied Organometallic Chemistry*.[5–7]

Fowler has edited a book devoted to the biological and environmental effects of arsenic.[8] Other books and journals contain chapters of interest[9] and some useful reviews have appeared.[10–14]

Arsenic compounds in the marine environment are described in many books and reviews as shown above, but those in the freshwater environment are described in only a few.

There seems to be a significant difference in level and chemical forms of arsenic between terrestrial and marine organisms. Terrestrial organisms rarely contain more than 1 μg As g^{-1} (dry weight), whereas arsenic contents of marine organisms range from several micrograms per gram to more than 100 μg As g^{-1}.[15] The source of arsenic in marine ecosystems is, of course, inorganic

CCC 0268–2605/94/040325–09

Received 17 January 1994
Accepted 22 March 1994

As(V) and sodium arsenite [abbreviated as As(III)] dissolved in seawater; the concentration is generally constant at around a few micrograms of arsenic per liter in all the sea areas. Arsenic concentrations in unpolluted rivers, ponds and lakes are also of the parts-per-billion ($1/10^9$; ppb) order, but the concentrations are, however, greatly affected by the circumstances.

The transformation of arsenic compounds by organisms in a marine food chain has been studied by many researchers.[16–20] However, only a few experiments have been conducted in freshwater systems. We reported the transformation of inorganic arsenic compounds in freshwater food chains starting from autotrophs (microalgae, *Chlorella vulgaris*,[21–23] and *Phormidium* sp[22]) through grazers (zooplankton, *Moina macrocopa*,[21–23] and herbivorous shrimp, *Neocaridina denticulata*[23]) to carnivores (goldfish, *Carassius carassius auratus*,[21] and guppy, *Poecilia reticulata*[22,23]). These experimental results showed that the total arsenic concentration in each species decreased by one order of magnitude and the relative concentration of methylated arsenicals to total arsenic, on the contrary, increased successively with an elevation in the trophic levels.

This paper presents the tolerance, accumulations, transformation and excretion of As(V), MAA, DMAA and AB dissolved in the water phase by a shrimp (*Neocaridina denticulata*). Bioaccumulation of As(V) by the green alga (*Chlorella vulgaris*) and biotransformation via a three-step freshwater food chain (from the autotroph, *C. vulgaris*, through the herbivorous shrimp, *N. denticulata*, to the carnivorous killifish, *Oryzias latipes*) were investigated. The latter two organisms were collected from the natural environment at Kagoshima, Japan. This experimental three stage food chain is closer to the natural freshwater ecosystem than those previously reported.[21–23]

MATERIALS AND METHODS

Culture of organisms

The organisms tested were cultured or fed under the following general conditions.

Autotrophic green alga (*Chlorella vulgaris*)
Living algal cells (6 mg in dry base) of *C. vulgaris*, which was isolated from an arsenic-polluted environment,[24] was placed in a 1-litre modified Detmer medium [KNO_3 1.0 g, $CaCl_2$ 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, NaCl 0.1 g, K_2HPO_4 0.25 g, $FeSO_4 \cdot 7H_2O$ 0.02 g, H_3BO_3 2.86 mg, $MnCl_2 \cdot 4H_2O$ 1.81 mg, $ZnSO_4 \cdot 7H_2O$ 0.22 mg, $CuSO_4 \cdot 5H_2O$ 0.08 mg, Na_2MoO_4 0.021 mg, pure water 1 litre, pH 8; abbreviated as MD medium] containing a set amount of As(V) (calculated as elemental arsenic using $Na_2HAsO_4 \cdot 7H_2O$). The culture was kept at 25–30 °C under constant aeration (2 l min^{-1}) and illumination (4000 lx, 12 h day^{-1}) for the set number of days (about one week). The cells were harvested by centrifugation (3000*g*, 10 min), washed by mixing with distilled–deionized water and separated by centrifugation, and this procedure was repeated twice or more. The washed wet cells were dried at 60 °C for 24 h and then at 105 °C until they gave a constant weight.

Optical density (at 640 nm) of the living cell suspension was found to be proportional to the cell weight concentration, so that growth of the cell (g dry weight cell/litre medium) was obtained by determining the optical density of the culture.

Herbivorous shrimp
A shrimp (*Neocaridina denticulata*, about 1.5 cm in length) was collected from a natural stream in Kagoshima Prefecture.[23] The shrimp was fed with a basic diet (Tetrafin, manufactured in Germany) in aerated dilute modified Detmer medium (1 part of the medium, 49 parts of distilled water). The shrimp was harvested by a net, washed with pure water and dried at 60 °C to constant weight. It was fed with the dried powder of arsenic-free *C. vulgaris* for five days before the food-chain experiment.

In the food-chain experiment, the shrimp was fed with the dried powder of the arsenic-containing *C. vulgaris*, the shrimp thereby accumulating arsenic.

Carnivorous killifish
A killifish (*Oryzias latipes*, wild-type, about 2.5 cm in length) was collected from a natural stream in Kagoshima Prefecture. The killifish was fed with Tetrafin in aerated dilute modified Detmer medium (1 part of medium, 49 parts of distilled water). The killifish was harvested, washed with pure water and dried at 60 °C to constant weight. It was fed with the dried powder of arsenic-free *C. vulgaris* for five days before the food-chain experiment.

In the food-chain experiment, the killifish was fed with the dried powder of the *N. denticulata* which had accumulated arsenic via *C. vulgaris*.

Determination of total and methylated arsenic compounds

For the determination of total arsenic in organisms, the dried organisms (10–20 mg) were mineralized in the presence of magnesium nitrate, the ash was dissolved in 10 mol l^{-1} hydrochloric acid (10 ml) with 40% aqueous potassium iodide solution (1 ml), the solution was extracted with chloroform (5 ml), the chloroform phase was back-extracted with 0.02% aqueous magnesium nitrate solution (2 ml), and the aqueous phase was analyzed by graphite furnace atomic absorption spectrometry (GF AA). Disodium arsenate [$Na_2HAsO_4 \cdot 7H_2O$; As(V)] was used as an authentic sample for total and non-methylated arsenic species.

For the determination of methylated arsenic compounds, the dried organism (*ca* 10 mg) was digested with 2 mol l^{-1} NaOH (5 ml) at 90–95 °C for 3 h using an aluminum heating block (hot base digestion). Methylated arsenic compounds in the digest were reduced with 20% $NaBH_4$ in 0.1 mol l^{-1} NaOH (5 ml) to the corresponding arsine compounds. The arsines generated were at once frozen out in a liquid-nitrogen U-trap. Upon warming the U-trap, the arsines were borne out of it successively, passed through a quartz tube atomizer and determined chromatographically using an atomic absorption spectrometer on the basis of the difference in the boiling points of the arsines [b.p. AsH_3 −55°C, CH_3AsH_2 2°C, $(CH_3)_2AsH$ 35–6°C (747 mmHg), $(CH_3)_3As$ 52°C (736 mmHg)].

MAA, DMAA and AB were used as authentic samples for the generation of monomethylarsenic (MMA), dimethylarsenic (DMA) and trimethylarsenic (TMA) compounds, respectively. These three methylated compounds (MAA, DMAA and AB) were degraded into monomethyl-, dimethyl- and trimethyl-arsine oxides upon hot base digestion, and then hydrided to monomethyl-, dimethyl- and trimethyl-arsines, respectively, on treatment with borohydride.[25] Non-methylated arsenic (abbreviated as IA) concentration was calculated as total arsenic minus the sum of methylated arsenic:

$$[IA = \text{Total As} - (MMA + DMA + TMA)].$$

The concentrations of all arsenic compounds are expressed units of μg As g^{-1}.

The absolute detection limits for total and methylated arsenic in the single injection were 0.5 ng and 5 ng, respectively. Coefficients of variation for the total and methylated arsenic were below 5% and 10% respectively.

RESULTS AND DISCUSSION

Accumulation of arsenic from the water phase by shrimp (*N. denticulata*)

Five shrimps were each fed for seven days with the dried powder of arsenic-free *C. vulgaris* in diluted MD (1:50) media (500 ml) containing As(V) (0.1, 0.5, 1, 1.5 and 2 μg As ml^{-1}), MAA (1, 5, 10 and 12 μg As ml^{-1}), DMAA (1, 10, 20, 30, 40 and 50 μg As ml^{-1}), and AB (1, 100, 150 and 200 μg As ml^{-1}).

No shrimp could survive in media containing arsenic higher than 2 μg As ml^{-1} for As(V), 12 μg As ml^{-1} for MAA, 50 μg As ml^{-1} for DMAA, and 200 μg As ml^{-1} for AB. Tolerances (LC_{50}) of the shrimp against As(V), MAA, DMAA, and AB were 1.5, 10, 40, and 150 μg As ml^{-1}, respectively. LC_{50} is defined here as the arsenic concentration at which two or three of the five shrimps did not survive after 5–7 days' exposure. This result means that the toxicity of the arsenicals for *N. denticulata* decreases with an increase in the number of methyl groups bonded to arsenic. Similar results were reported for experimental animals.[26]

The arsenic-dosed shrimps were harvested, washed with pure water, dried at 60 °C to constant weight, ground into powder and analyzed for total and methylated arsenics. Experimental results for total arsenic and relative proportion of arsenic species accumulated in the shrimps are illustrated in Figs 1 and 2, respectively. Data for the experiment on As(V) accumulation (Fig. 1a) are quoted from our previous paper.[23]

Experimental results in Fig. 1 show that total arsenic concentration accumulated by *N. denticulata* nearly always increased with an increase in arsenic concentration in water for the four arsenic species, and that the higher the methylation of arsenicals administered to *N. denticulata*, the higher was the total arsenic concentration accumulated in the organism. These results imply that highly methylated and less toxic arsenic species

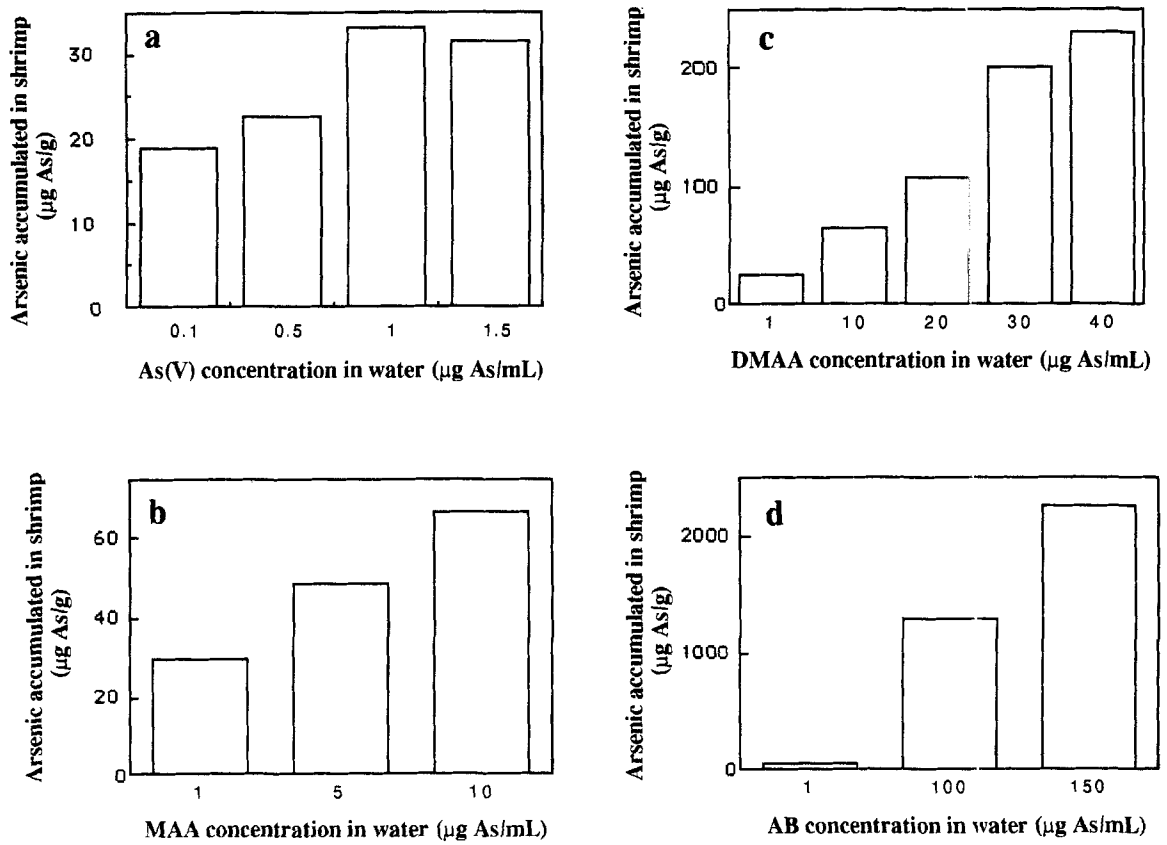


Figure 1 Total arsenic concentration accumulated by shrimp (*N. denticulata*) from the water phase containing (a) disodium arsenate [As(V)], (b) methylarsonic acid (MAA), (c) dimethylarsinic acid (DMAA) and (d) arsenobetaine (AB).

pass more easily through the membrane of the digestive organ of *N. denticulata* and are more accumulated by the cell tissues than less methylated and higher-toxicity arsenicals.

When *N. denticulata* was exposed to As(V) (Fig. 2a), the relative proportion of non-methylated arsenicals (IA) was within 70–90%: about 10–30% of arsenic accumulated was biomethylated. The predominant methylated arsenicals were dimethyl (DMA) and trimethyl (TMA) arsenic compounds. When *N. denticulata* was exposed to MAA (Fig. 2b), 10–20% of the accumulated MAA was further methylated to DMA and 20–40% of that was demethylated to IA. When exposed to DMAA (Fig. 2c), no methylation occurred, but 10–60% of accumulated DMA was demethylated to IA. When exposed to 1 μg As ml^{-1} of AB (Fig. 2d), about 40% of accumulated AB was demethylated to DMA and 20% of it was demethylated to IA. When exposed to AB concentrations higher than 100 μg As ml^{-1}, however, little demethylation occurred.

These experimental results show that *N. denticulata* both methylated and demethylated arsenic accumulated from the water phase, and dimethyl and trimethyl arsenic species were the preferable forms for the shrimp.

Excretion of arsenic by shrimp (*N. denticulata*)

Eight shrimps were each fed for seven days with the dried powder of arsenic-free *C. vulgaris* in four diluted MD (1:50) media (500 ml) containing arsenic species: As(V), 1 μg As ml^{-1}; MAA, 10 μg As ml^{-1}; DMAA, 30 μg As ml^{-1}; and AB, 150 μg As ml^{-1}.

The arsenic-dosed shrimps were harvested and rinsed with pure water, and four shrimps were dried at 60 °C to constant weight, ground into powder and analyzed for total and methylated arsenic species. The remaining four living shrimps were transferred together into an arsenic-free

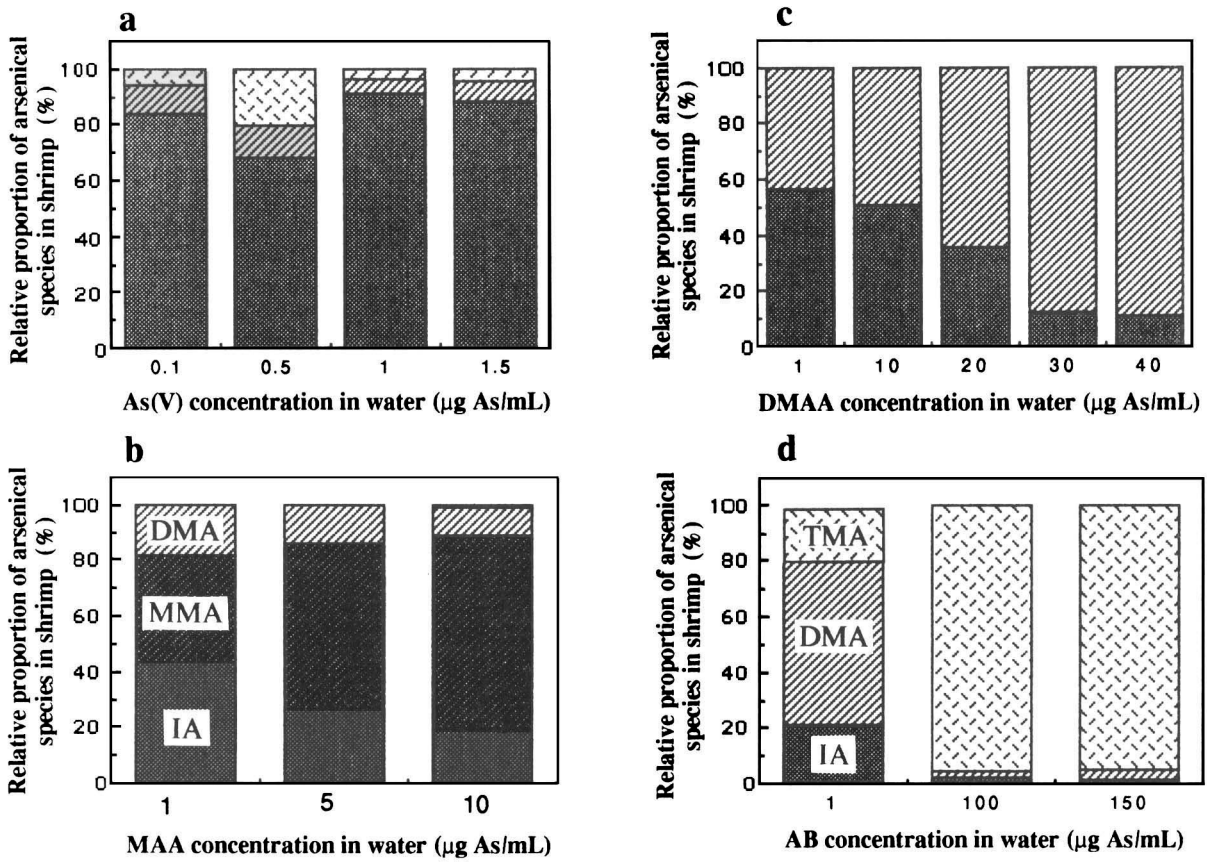


Figure 2 Relative proportion of arsenic species accumulated by shrimp (*N. denticulata*) from the water phase containing (a) disodium arsenate [As(V)], (b) methylarsonic acid (MAA), (c) dimethylarsinic acid (DMAA), and (d) arsenobetaine (AB): ▒, IA; ■, MMA; ▨, DMA; ⊠, TMA.

medium (500 ml) and fed with the dried powder of arsenic-free *C. vulgaris* for five days. The shrimps were harvested and washed with pure water, dried at 60 °C to constant weight, ground into powder and analyzed for total and methylated arsenics, and the medium into which arseni-

Table 1 Excretion of arsenic from shrimp (*N. denticulata*) which had been exposed to disodium arsenate [As(V)] for one week[a]

	Arsenic accumulated and excreted: μg As g^{-1} (%)				
As	Total	IA[b]	MMA[c]	DMA[d]	TMA[e]
Arsenic in *N. denticulata*	34.1	30.0 (88.0)	—[f]	2.7 (7.9)	1.4 (4.1)
Arsenic excreted into water	13.3	5.9 (44.3)	1.9 (14.3)	2.1 (15.8)	3.4 (25.6)

[a] *N. denticulata* was exposed to 1 μg As ml^{-1} of As(V) for one week and transferred into arsenic-free water for five days, and arsenic species excreted in the water were analyzed.
[b] IA, non-methylated arsenic species.
[c] MMA, monomethylarsenic species.
[d] DMA, dimethylarsenic species.
[e] TMA, trimethylarsenic species.
[f] Not detected.

Table 2 Excretion of arsenic from shrimp (*N. denticulata*) which had been exposed to methylarsonic acid (MAA) for one week[a]

As	Arsenic accumulated and excreted; μg As g^{-1} (%)				
	Total	IA[b]	MMA[b]	DMA[b]	TMA[b]
Arsenic in *N. denticulata*	66.5	12.8 (19.3)	46.3 (69.6)	6.8 (10.2)	0.6 (0.9)
Arsenic excreted into water	18.2	2.7 (14.9)	6.4 (35.1)	3.5 (19.3)	5.6 (30.7)

[a] *N. denticulata* was exposed to 10 μg As ml^{-1} of MAA for one week and transferred into arsenic-free water for five days, and arsenic species excreted in the water were analyzed.
[b] IA, MMA, DMA, and TMA: see Table 1.

cals were excreted from the shrimp was also analyzed for total and methylated arsenic species.

Experimental results are shown in Tables 1–4, which show that the predominant arsenic species accumulated in the shrimp were the same species originally dissolved in the aqueous phase. When arsenic-dosed shrimps were transferred into arsenic-free water, 13.3 (39%) out of 34.1 μg As g^{-1}, 18.2 (27%) out of 66.5 μg As g^{-1}, 48.6 (24%) out of 201 μg As g^{-1} and 171 (7.6%) out of 2253 μg As g^{-1} were excreted from the shrimps which had been pre-exposed to As(V), MAA, DMAA, and AB, respectively. The amount of excreted arsenic increased with an increase in the degree of methylation of the arsenic species dosed. On the other hand, the excretion ratio decreased inversely.

Comparing arsenic species in the cells with

Table 3 Excretion of arsenic from shrimp (*N. denticulata*) which had been exposed to dimethylarsinic acid (DMAA) for one week[a]

As	Arsenic accumulated and excreted; μg As g^{-1} (%)				
	Total	IA[b]	MMA[b]	DMA[b]	TMA[b]
Arsenic in *N. denticulata*	201	25 (12.4)	—[c]	115 (57.4)	61 (30.2)
Arsenic excreted into water	48.6	11.0 (22.7)	1.8 (3.7)	15.3 (31.5)	20.5 (42.1)

[a] *N. denticulata* was exposed to 30 μg As ml^{-1} of DMAA for one week and transferred into arsenic-free water for five days, and arsenic species excreted in the water were analyzed
[b] IA, MMA, DMA, and TMA: see Table 1.
[c] —, Not detected.

Table 4 Excretion of arsenic from shrimp (*N. denticulata*) which had been exposed to arsenobetaine (AB) for one week[a]

As	Arsenic acumulated and excreted, μg As/g (%)				
	Total	IA[b]	MMA[b]	DMA[b]	TMA[b]
Arsenic in *N. denticulata*	2253	41 (1.8)	—[c]	82 (3.6)	2130 (94.5)
Arsenic excreted into water	171	6.8 (4.0)	23.1 (13.5)	107 (62.5)	34.1 (19.9)

[a] *N. denticulata* was exposed to 150 μg As ml^{-1} of AB for one week and transferred into arsenic-free water for five days, and arsenicals excreted in the water were analyzed.
[b] IA, MMA, DMA, and TMA: see Table 1.
[c] —, Not detected.

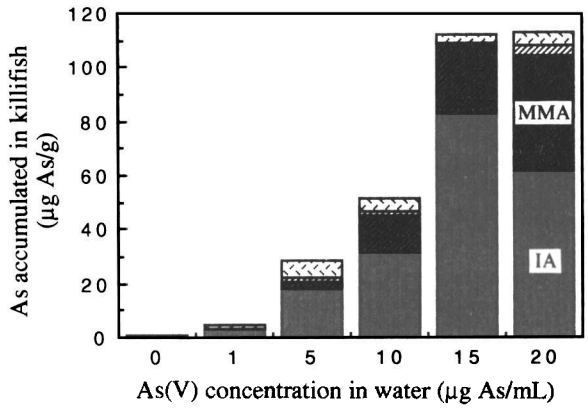


Figure 3 Accumulation and transformation of arsenic by killifish (*O. latipes*) from water containing different levels of disodium arsenate [As(V)]: ▩, IA; ■, MMA; ▨, DMA; ▧, TMA.

those in the excrement, a drastic increase in the relative proportion of methylated arsenic species in the excrement was observed in Table 1. The increase of TMA in the excrement is worthy of notice. Similar results were obtained on excretion of arsenic from *C. vulgaris*[27] and *Klebsiella oxytoca*.[28]

Table 2 shows that the relative proportion of MMA in the excrement was one-half of that in the shrimp and those of DMA and TMA in the excrement were considerably larger than those in the shrimp. In Table 3, the relative proportion of DMA in the excrement decreased; TMA, MMA and IA increased, on the contrary. In Table 4, about 80% of total arsenic excreted was demethylated and the predominant arsenic species in the excrement was DMA.

These experimental data indicate that accumulated inorganic arsenic and MAA are biomethylated up to DMA and TMA, being less toxic arsenic species, and are excreted in those forms. IA and MMA are excreted but the less toxic arsenic species (DMA and TMA) are preferentially excreted. Demethylation of arsenic species especially in the excrement was observed in this experiment, but it is not clear at the present stage of our investigation whether the demethylation is mediated by the shrimp itself or by bacterial contamination in the shrimp.

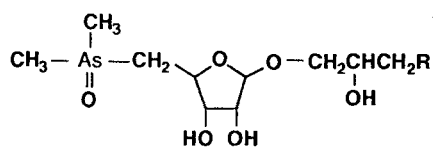
Accumulation of arsenic(V) from the water phase by killifish (*O. latipes*)

Five carnivorous killifish (*O. latipes*) were each fed with the dried powder of arsenic-free *C. vulgaris* in a diluted MD (1:50) medium (500 ml) containing 0, 1, 5, 10, 15, 20 and 25 μg As(V) ml^{-1} for seven days. The killifish did not survive in a medium containing 25 μg As(V) ml^{-1}. The tolerance (LC_{50}) of the killifish against As(V) is 20 μg As(V) ml^{-1}. Figure 3 shows experimental results on the accumulation and biomethylation of As(V) by the killifish.

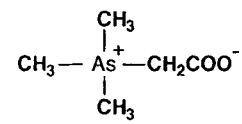
From Fig. 3, total arsenic accumulation in killifish increased with an increase in arsenic concentration in the aqueous phase in a similar manner to that in shrimp. About 20–40% of accumulated arsenic was methylated to MMA, DMA and TMA. The predominant methylated arsenic species was MMA. These experimental results show that carnivorous killifish also can take arsenic directly from the aqueous phase and partially biomethylate it. These results are essentially the same as those for shrimp, but the proportion of methylated arsenic in killifish was a little higher than in shrimp. Organisms at the higher trophic level may have a larger capacity for biomethylation.

Biotransformation of arsenic(V) in the food chain: alga (*C. vulgaris*)→shrimp (*N. denticulata*)→killifish (*O. latipes*)

The alga *C. vulgaris* was cultured in modified Detmer medium containing 100 μg As(V) ml^{-1} for one week; the arsenic-dosed cells were harvested, washed with distilled water and dried to constant weight. Five shrimps (*N. denticulata*: 1.5 cm long, 10 mg dry mass each) were fed for seven days with the arsenic-dosed dried alga (arsenic concentration: 472 μg As g^{-1} dry mass; about 4 mg dry mass per five shrimps a day; 28 mg total) in aerated dilute MD medium (1:50), then collected, and washed with distilled water. Five killifish (*O. latipes*: 2.5 cm long, 50 mg dry mass each) were fed for seven days with the dry powder of the arsenic-dosed shrimp (arsenic concentration: 16.9 μg As g^{-1} dry mass; about 5 mg dry



Arsenosugars (1)



Arsenobetaine (2)

Table 5 Biotransformation of arsenic in the three-step freshwater food chain: green alga (*C. vulgaris*)→shrimp (*N. denticulata*)→killifish (*O. latipes*)

Organism	Accumulation route	Arsenic in organism. μg As g^{-1} (%)				
		Total	Methylated[b]	MMA[c]	DMA[c]	TMA[c]
C. vulgaris	Water	472 (100)	57.6 (10.9)	14.2 (2.7)	43.4 (8.2)	—[d]
N. denticulata	*C. vulgaris*	16.9 (100)	6.6 (39.1)	Trace (—)	6.1 (36.1)	0.4 (3.0)
O. latipes	*N. denticulata*	0.5 (100)	0.4 (80)	Trace (—)	Trace (—)	0.4 (80)

[a] *C. vulgaris* was cultured in modified Detmer medium containing 100 μg As(V) ml^{-1} for one week, *N. denticulata* was fed for seven days with the dried powder of the arsenic-dosed *C. vulgaris*, and *O. latipes* was fed for seven days with the dried powder of the arsenic-dosed *N. denticulata*.

[b] Methylated: sum of MMA + DMA + TMA.

[c] MMA, DMA and TMA: see Table 1.

[d] —, Not detected.

mass per five killifishes a day; 35 mg total) in aerated dilute MD medium (1:50), collected, and washed with distilled water. These arsenic-dosed organisms were analyzed for total and methylated arsenic compounds. The experimental results are summarized in Table 5, and the relative proportion of arsenic species in the organisms are illustrated in Fig. 4.

Table 5 shows that total arsenic concentrations in the organisms decreased by one order of magnitude successively with an elevation in the trophic level. On the other hand, the relative proportion of methylated arsenic to total arsenic compounds increased dramatically, as shown in Fig. 4. Only trace amounts of monomethylarsenic species were detected in the shrimp and fish tested; and dimethylarsenic in alga and shrimp, and trimethylarsenic in killifish, were the predominant methylated arsenic species, respectively. Trimethylarsenic was the only methylated arsenic species detected in killifish. Similar results were reported in our previous work using the following food chains: *C. vulgaris*→*Moina macrocopa*→goldfish (*Carassius carassius auratus*),[21] *C. vulgaris*→*Moina macrocopa*→guppy (*Poecilia reticulata*)[22,23] and *Nostoc* sp.→shrimp (*N. denticulata*)→carp (*Cyprinus carpio*).[29] These results indicate that lower trophic levels of organisms have a greater ability to accumulate arsenic and higher trophic levels of organisms have a greater ability to methylate arsenic.

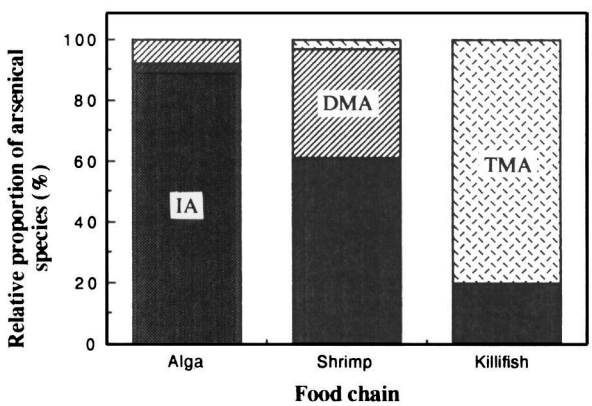


Figure 4 Relative proportion of arsenic species in the three-step freshwater food chain, alga→shrimp→killifish: ■, IA; ■, MMA; ▨, DMA; ⊡, TMA.

It is similarly observed in seawater ecosystems that arsenic bioaccumulation decreased via the food chain.[30] That is, arsenic concentrations are not biomagnified in the aquatic food chain; this is in striking contrast to other toxic heavy-metal species such as mercury, methylmercury, lead, tin, and so on.

However, the proportion of biomethylated arsenic species in the higher levels of organisms in this freshwater food chain is considerably different from those in marine plants such as kelp and marine animals. In the plants and animals in sea ecosystem,[13] the greater part (>90%) of arsenic accumulated is methylated, and the methylated arsenic compounds are almost all in dimethyl- and trimethyl-arsenic compounds, respectively. Arsenosugars (**1**) and arsenobetaine (**2**) have

been found most abundantly in marine plants and marine animals respectively.

Biomethylation of arsenic by freshwater organisms was experimentally proved as mentioned above. The original chemical structures of the arsenic compounds present in the living cells of the freshwater organisms, however, have not yet been revealed. It is necessary for a consideration of the biochemical pathway of arsenic methylation to reveal the original chemical forms of the methylated arsenic compounds in freshwater organisms. This is now under investigation.

Acknowledgement The authors are grateful to the Ministry of Education, Culture and Science, Japan, for support of this research through a Grant-in-Aid for Scientific Research on Formulation and Management of Man–Environment System (Project No. N-17B-56; 04202242).

REFERENCES

1. Committee on Medical and Biological Effects of Environmental Pollutants, National Research Council (ed.), *Arsenic: Medical and Biological Effects of Environmental Pollutants*. pp. 1–332. National Academy of Sciences, Washington DC (1977).
2. M. Anke, H.-J. Schneider and C. Brückner (eds), *Arsen: 3rd Spurenelement-Symposium, Friedrich-Schiller-University, Jena*, 3 September 1980, pp. 1–316.
3. W. H. Lederer and R. J. Fensterheim (eds), *Arsenic: Industrial, Biomedical, Environmental Perspectives*, pp. 1–443. Van Nostrand Reinhold, New York (1983).
4. N. Ishinishi, S. Okabe and T. Kikuchi (eds), *Hiso: Kagaku, Taisha, Dokusei (Arsenic: Chemistry, Metabolism and Toxicity)*, pp. 1–157. Koseisha-Koseikaku, Tokyo (1985).
5. K. J. Irgolic, T. Kikuchi, S. Maeda and P. J. Craig (eds), *Appl. Organomet. Chem.* **2**, 283–404 (1988): special issue, Natural and industrial arsenic.
6. K. J. Irgolic, T. Kikuchi, S. Maeda and P. J. Craig (eds), *Appl. Organomet. Chem.* **4**, 181–295 (1990): special issue, Natural and industrial arsenic.
7. S. Maeda and P. J. Craig (eds), *Appl. Organomet. Chem.* **6**, 307–420 (1992): special issue, Natural and industrial arsenic.
8. B. A. Fowler (ed), *Biological and Environmental Effects of Arsenic*, pp. 1–281. Topics in Environmental Health, Vol. 6. Elsevier, Amsterdam (1983).
9. F. E. Brinckman and J. Bellama (eds), *Organometals and Organometalloids—Occurrence and Fate in the Environment*, ACS Symp. Ser. Vol. 82, pp. 1–447. American Chemical Society, Washington DC (1978).
10. D. J. H. Phillips, *Aquat. Toxicol.* **16**, 151 (1990).
11. D. J. H. Phillips and M. H. Depledge, *Mar. Environ. Res.* **17**, 1 (1985).
12. S. Maeda and T. Sakaguchi, Accumulation and detoxification of toxic metal elements by algae. In: *Introduction to Applied Phycology*, edited by I. Akatsuka, pp. 109–136. SPB Academic Publishing, The Hague, The Netherlands (1990).
13. W. R. Cullen and K. J. Reimer, *Chem. Rev.* **89**, 713 (1989).
14. S. Maeda and T. Takeshita, Kagaku-no-ryoiki **36**, 686 (1982).
15. G. Lunde, *Environ. Health Perspect.* **19**, 47 (1977).
16. J. Wrench, S. W. Fowler and M. Y. Ünlü, *Mar. Pollut. Bull.* **10**, 18 (1979).
17. M. Y. Ünlü, *Chemosphere* **5**, 269 (1979).
18. D. W. Klumpp, *Mar. Biol.* **58**, 265 (1980).
19. D. W. Klumpp and P. J. Peterson, *Mar. Biol.* **62**, 297 (1981).
20. R. V. Cooney and A. A. Benson, *Chemosphere* **9**, 335 (1980).
21. S. Maeda, R. Inoue, T. Kozono, T. Tokuda, A. Ohki and T. Takeshita, *Chemosphere* **20**, 101 (1990).
22. S. Maeda, A. Ohki, T. Tokuda and M. Ohmine, *Appl. Organomet. Chem.* **4**, 251 (1990).
23. S. Maeda, A. Ohki, K. Kusadome, T. Kuroiwa, I. Yoshifuku and K. Naka, *Appl. Organomet. Chem.* **6**, 213 (1992).
24. S. Maeda, T. Kumamoto, M. Yonemoto, S. Nakajima, T. Takeshita, S. Higashi and K. Ueno, *Sep. Sci. Technol.* **18**, 375 (1983).
25. H. Yamauchi and Y. Yamamura, *Jpn. J. Ind. Health* **21**, 47 (1979).
26. For example: T, Kaise, S. Watanabe and K. Itoh, *Chemosphere*, **14**, 1327 (1985); T. Kaise, H. Yamauchi, Y. Horiguchi, T. Tani, S. Watanabe, T. Hirayama and S. Fukui, *Appl. Organomet. Chem.* **3**, 273 (1989).
27. S. Maeda, K. Kusadome, H. Arima, A. Ohki and K. Naka, *Appl. Organomet. Chem.* **6**, 407 (1992).
28. S. Maeda, A. Ohki, K. Miyahara, S. Higashi and K. Naka, *Appl. Organomet. Chem.* **6**, 415 (1992).
28. S. Maeda, K. Mawatari, A. Ohki and K. Naka, *Appl. Organomet. Chem.* **7**, 467–476 (1994).
30. M. O. Andreae, Biotransformation of arsenic in the marine environment. In: Ref. 3, pp. 378–392.