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Biotransformation of arsenite in freshwater food-chain models†

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Bioaccumulation and biotransformation of arsenite by freshwater organisms consisting *Daphnia magna, Neocaridina denticulata* and *Tilapia mossambica* has been studied. When organisms were exposed to a medium containing arsenite, the total arsenic concentration accumulated by the organisms increased with an increase in the arsenic concentration in the medium. The order of total arsenic accumulation by freshwater organisms was *D. magna* > *N. denticulata* > *T. mossambica*. Bioaccumulation and biotransformation of arsenite in three-step laboratory food-chain models was investigated by feeding a diet of arsenite-dosed alga (*Chlorella vulgaris*) to herbivorous grazers (*D. magna* and *N. denticulata*) and then the herbivores were fed to carnivorous fish (*T. mossambica* and *Zacco platypus*). Total arsenic concentrations in the organisms decreased by an order of magnitude for each step in the food chain. Arsenite and arsenate were accumulated as the predominant arsenic species in organisms. Little methylation of arsenic in organisms occurred at each step in the food chain. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: arsenite; biotransformation; fresh water food chain; methylation

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INTRODUCTION

In recent years, the number of studies concerning the impact of arsenic on microorganisms in aquatic ecosystems has grown rapidly. Although the literature establishes arsenic accumulation and transformation in the organisms, arsenic, a toxic agent, is a priority pollutant still under investigation. It was recognized quite early that arsenic is a ubiquitous chemical distributed widely in food, water, soil, and air.[1] In the aquatic environment, arsenic can exist in several oxidation states and chemical forms.[2,3] These oxidation states and chemical forms of arsenic influence the biological availability, and physiological and toxicological effects.[4] Understanding the mechanisms of arsenic accumulation and transformation in the food chain is critically important in assessing the risk from arsenic contamination, especially in food. In this case, microalgae, as critical components of aquatic systems and food items for higher trophic level organisms, have an important role in natural food web ecosystems. When arsenic enters the aquatic environment, via natural and anthropogenic process, it is picked up first by the lowest trophic level organisms of aquatic ecosystems, namely microalgae. Algae exhibit concentrations of arsenic that are higher than those of the surrounding water. Thus, higher trophic level organisms feed on them in a food chain in the environment. At any rate, higher trophic level organisms that feed on primary producers show a level of arsenic that is higher than that of their environment.[5]

We have intensively investigated the accumulation, transformation and excretion of arsenic compounds by freshwater organisms in many food-chain routes. Previously, we reported on the transformation of arsenate in freshwater food chains consisting of an autotrophic alga (*Chlorella vulgaris*,[6–9] *Phormidium* sp.[7] and *Nostoc* sp.[10]), a zooplanktonic grazer (*Moina macrocopa*[6,7,9]), and carnivores (*Carassius carassius auratus*,[6] *Poecilia recticulata*,[7,9] *Oryzias latipes*[8] and *Cyprinus car-*

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pio[10]). It was reported that freshwater *M. macrocopa*, one of the food items for higher trophic level organisms, took up arsenic more from arsenic-containing food than from arsenic-containing water.[6–10] When the lowest trophic level organisms, *C. vulgaris*[7–9] and *Nostoc* sp.,[10] were exposed to arsenate as a source of arsenic, trimethylarsenic species (>80%) was predominant in the higher level organisms *C. auratus*[6], *O. latypes*,[8] *P. reticulata*[7,9] and *C. carpio*.[10] This study examined the bioaccumulation and biotransformation of arsenite in simple laboratory food-chain models consisting of the green alga *C. vulgaris*, the zooplanktonic grazer *Daphnia magna*, the shrimp *Neocaridina denticulata* and the carnivores *Tilapia mossambica* and *Zacco platypus*.

MATERIALS AND METHODS

Reagents

All chemicals used were reagent grade and deionized water was used for all dilutions. Glass- and plastic-ware were cleaned by soaking in an ultrasonic bath (Branson 1200) with a cleaning solution followed by a water rinse. Plankton tissue (CRM 414) and cod muscle (CRM 422) standard reference materials were purchased from the Community Bureau of Reference, Commission of the European Communities, Brussels. Arsenic standards were freshly prepared by serial dilution from stock solutions (1000 ppm of elemental arsenic) of the following compounds: trivalent sodium arsenite [$NaAsO_2$, As(III)], pentavalent sodium arsenate [$Na_2HAsO_4{\cdot}7H_2O$, As(V)], and dimethylarsinic acid [$(CH_3)_2AsO(OH)$] were commercial products of Wako Pure Chemical Industries, Ltd, Japan; methylarsonic acid [$(CH_3)AsO(OH)_2$], arsenobetaine [$(CH_3)_3As\text{-}CH_2COOH$] were obtained from Trichemical Laboratory, Japan. Other solutions [10 M hydrochloric acid, 0.125 N Tris–HCl buffer pH 6.05, 10% oxalic acid pH 1, 10% (w/v) sodium borohydride ($NaBH_4$) in 0.1% (w/v) NaOH and 2 M NaOH] were obtained from Wako Pure Chemical Industries, Ltd, Japan, and were freshly made before use.

Culture of organisms

The freshwater organisms used throughout these experiments were cultured and fed under the following general conditions.

Freshwater green microalga *C. vulgaris*

Ten 5 dm^3 flasks of *C. vulgaris* were cultured in modified Detmer (MD) 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)] containing 50 $\mu g\,cm^{-3}$ of As(III) (calculated as elemental arsenic using $NaAsO_2$) under sterile conditions for 100 h. They were inoculated with 6 mg of cells (on a dry weight basis) obtained from the stock algal culture in the log growth phase. Constant aeration (2 $dm^3\,min^{-1}$) and continuous illumination of approximately 4000 lux around the flask for 24 h per day were provided at 25–30 °C. Algae from all flasks were mixed prior to centrifugation to ensure homogeneity of cells. Suspended algal cells were collected by centrifugation, washed with distilled-deionized water and separated by centrifuging (3000 *g*, 10 min). The washing procedure was repeated at least twice. The washed cells were dried at 60 °C for 24 h to constant mass and ground to powder. Samples of algae were analyzed for total arsenic and arsenic species.

Freshwater zooplanktonic grazer *D. magna*

Stock culture of *D. magna* was obtained from Dr M. Kobayashi (Faculty of Science, Niigata University, Japan). *D. magna* (0.5–2.0 mm in length size) were maintained continuously as laboratory cultures at 21 ± 1 °C in dilute MD medium (one part of medium, nine part of distilled water) with pH ranging from 7.6 to 7.8. The medium was renewed twice each week and *D. magna* were fed daily with an arsenic-free dried powder diet of alga *C. vulgaris*.

Freshwater herbivorous shrimp *N. denticulata*

The shrimp *N. denticulata* (20–40 mm in total length) was collected with a hand net in the area of Nagata River, Kagoshima City, and taken to the laboratory in plastic bags. The shrimp species were identified by Dr H. Suzuki (Faculty of Fisheries, Kagoshima University, Japan). In the laboratory, shrimps were fed daily with a commercial basic diet in aerated dilute MD medium. The acclimatization temperature was between 21 and 22 °C. Before the

food-chain experiment, shrimps were fed with the dried powder of arsenic-free *C. vulgaris* for 5 days. In the food-chain experiment, the shrimps were fed with the dried powder of the arsenic-containing *C. vulgaris* and *D. magna* that had accumulated arsenic via *C. vulgaris*.

Freshwater carnivorous fish *T. mossambica*

Stock culture of *T. mossambica* was obtained from Dr S. Koshio (Faculty of Fisheries, Kagoshima University, Japan). *T. mossambica* (50–80 mm in total length) were maintained continuously as laboratory cultures at 21 ± 2 °C in dilute MD medium with the pH ranging from 7.6 to 7.8. The medium was renewed each day and *T. mossambica* were fed daily with a commercial basic diet. Before the food-chain experiment, *T. mossambica* were fed with the dried powder of arsenic-free *C. vulgaris* for 5 days. In the food-chain experiment, *T. mossambica* were fed with *N. denticulata* that had accumulated arsenic via *C. vulgaris*.

Freshwater carnivorous fish *Z. platypus*

The fish *Z. platypus* (30–50 mm in total length) were collected with a hand net from the Nagata River in Kagoshima City. In the laboratory, *Z. platypus* were fed daily with a commercial basic diet in aerated dilute MD medium. The acclimatization temperature was between 21 and 22 °C. Before the food-chain experiment, *Z. platypus* were fed with the dried powder of arsenic-free *C. vulgaris* for 5 days. In the food-chain experiment, *Z. platypus* were fed with *N. denticulata* and *D. magna* that had accumulated arsenic via *C. vulgaris*.

Determination of total and methylated arsenic compounds

The organism cells were harvested, washed with distilled-deionized water, and separated. The washing procedure was repeated at least twice. The washed cells were dried at 60 °C for 24 h to constant mass.

For the determination of total arsenic in the cells, the dried organisms (*ca* 5 mg) were mineralized in the presence of 50% magnesium nitrate (2 cm^3) at 60 °C for 12 h and then at 550 °C for 6 h in a furnace. The resulting ash was dissolved in 10 M hydrochloric acid (10 cm^3) and 40% aqueous potassium iodide solution (1 cm^3) was added. The solution was extracted twice with chloroform (5 cm^3 each), the chloroform phase was back-extracted with 0.02% aqueous magnesium nitrate solution (2 cm^3), and the aqueous phase was analyzed for arsenic with an atomic absorption (AA) spectrometer (Japan Jarrel Ash AA-890) with a flameless atomizer (FLA-1000).

Inorganic and methylated arsenic compounds in the dry cell were determined by hydride generation atomic absorption spectrophotometry (HG–AAS) after digestion with 2 M NaOH (5 ml) at 90–95 °C for 3 h in an aluminum heating block. The digest was treated with 5 cm^3 of 4% $NaBH_4$ in 0.1 M NaOH at pH 6.2 buffer solution (0.125 N Tris–HCl) to hydrogenate arsenite to arsine.[11,12] Arsenate and the methylated arsenic compounds were hydrogenated with 5 cm^3 of 10% $NaBH_4$ in 0.1 M NaOH at pH 1 buffer solution (10% oxalic acid). The arsines generated were cooled with liquid nitrogen and were collected in a U-trap. Upon warming the U-trap, the arsines volatilized in the sequence of their boiling points [bp: AsH_3 −55 °C, CH_3AsH_2 2 °C, $(CH_3)_2AsH$ 35.6 °C (747 mmHg), $(CH_3)_3As$ 52 °C (736 mmHg)], passed through a quartz-tube atomizer and were determined with an AA spectrometer (Nippon Jarrell Ash, AA-890). Triplicate analyses were performed for each sample. The absolute detection limits for total and speciation arsenic in a single injection were 0.5 ng and 5 ng respectively. The coefficients of variation for total and speciation arsenic were below 5%. The measured concentration of total arsenic in standard cod muscle, RM-422 (certified as 21.1 ± 0.5 μg of arsenic per gram dry weight), was 24.1 ± 0.6 μg of arsenic per gram dry weight and the total arsenic in plankton, RM-414 (certified as 6.82 ± 0.28 μg of arsenic per gram dry weight), was 7.1 ± 0.2 μg of arsenic per gram dry weight.

RESULTS AND DISCUSSION

Arsenic accumulation and transformation in freshwater organisms after exposure to arsenite

Freshwater organisms *D. magna, N. denticulata* and *T. mossambica* were exposed for 7 days to dilute MD media (2 dm^3) containing arsenite (As = 0.05, 0.5 and 1 μg cm^{-3} for *D. magna*, 0.1, 0.5 μg cm^{-3} and 1.5 μg cm^{-3} for *N. denticulata*, 0.1, 1 and 5 μg cm^{-3} for *T. mossambica*) with continuous

Table 1 Concentration of arsenic species accumulated in organisms through a food chain consisting of *C. vulgaris, D. magna, N. denticulata, T. mossambica* and *Z. platypus*[a]

Organism	Accumulation route	Arsenic in organisms [μg As g^{-1} dry wt] (%)					
		Total	As(III)	As(V)	MMA	DMA	TMA
C. vulgaris (a)	Water, 50 μg cm^{-3} As(III)	529	48 (9)	440 (83)	11 (2)	30 (6)	—
D. magna (b)	Water, 0.05 μg cm^{-3} As(III)	90	65 (72)	23 (26)	0.3 (0.3)	1.5 (1.7)	—
D. magna (c)	Water, 0.5 μg cm^{-3} As(III)	97	73 (75.2)	23 (23.7)	0.1 (0.1)	0.9 (1)	—
D. magna (d)	Water, 1 μg cm^{-3} As(III)	176	110 (62)	64 (36.7)	0.1 (0.1)	2.1 (1.2)	—
D. magna (e)	*C. vulgaris* (a)	209	92 (44)	117 (56)	tr	tr	—
N. denticulata (f)	Water, 0.1 μg cm^{-3} As(III)	5	2.3 (46)	1.1 (22)	—	1.6 (32)	—
N. denticulata (g)	Water, 0.5 μg cm^{-3} As(III)	9.4	4.5 (48)	3.2 (34)	—	1.7 (18)	—
N. denticulata (h)	Water, 1.5 μg cm^{-3} As(III)	11	4.1 (38)	6.1 (55)	—	0.8 (7)	—
N. denticulata (i)	*C. vulgaris* (a)	32	3 (9)	29 (91)	tr	tr	—
N. denticulata (j)	*D. magna* (e)	12.5	2.2 (17.6)	10.1 (80.8)	—	0.2 (1.6)	—
T. mossambica (k)	Water, 0.1 μg cm^{-3} As(III)	2.7	0.6 (23)	0.2 (7)	tr	tr	1.9 (70)
T. mossambica (l)	Water, 1 μg cm^{-3} As(III)	3.2	0.6 (19)	0.3 (9)	—	—	2.3 (72)
T. mossambica (m)	Water, 5 μg cm^{-3} As(III)	20.6	8.1 (39)	7.4 (36)	1.1	1.6 (8)	2.4 (12)
T. mossambica (n)	*N. denticulata* (i)	26.6	11 (41)	15 (56)	—	—	0.6 (3)
Z. platypus (o)	*D. magna* (e)	2.2	1.4 (64)	0.8 (36)	tr	tr	tr
Z. platypus (p)	*N. denticulata* (i)	1.6	1 (63)	0.6 (37)	tr	tr	tr

[a] Organisms cultured in MD medium (1:10) for 7 days. MMA, monomethylarsenic compounds; DMA, dimethylarsenic compounds; TMA, trimethylarsenic compounds; tr, trace amount detected; —, not detected.

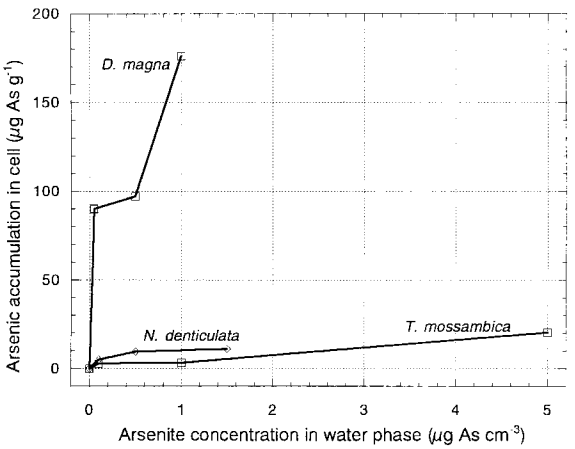

Figure 1 Total arsenic accumulation in several freshwater organisms after being exposed to arsenite for 7 days.

aeration. They were fed with a dried powder of arsenic-free *C. vulgaris*. Control organisms were maintained parallel in an arsenic-free medium for the same period. The arsenic-dosed organisms were harvested, washed with pure water, dried at 60 °C to constant weight, ground into powder and analyzed for total and methylated arsenic. The total arsenic concentrations and arsenic species accumulated in the three tissues of organisms are presented in Table 1.

Total arsenic concentration accumulated by *D. magna, N. denticulata* and *T. mossambica* increased with an increase in arsenic concentration in water (Fig. 1). These results agreed with the data in our previous papers for the other arsenicals.[7,8,13] When compared with similar studies with arsenate, the total arsenic accumulation in *D. magna*[13] and *N. denticulata*[8] was higher when exposed to arsenate than to arsenite. These results imply that arsenate is more easily passed through the membrane of the digestive organ and more easily metabolized by *D. magna* and *N. denticulata* compared with arsenite. Furthermore, the order of total arsenic accumulation by freshwater organisms was *D. magna* > *N. denticulata* > *T. mossambica*. It seems that the activities of lower trophic level aquatic organisms on accumulating arsenite were higher than upper trophic level organisms.

The arsenic species accumulated in cells after exposure to arsenite for 7 days are shown in Table 1 [(a)–(d), (f)–(h), (k)–(m)]. When *D. magna* was exposed to arsenite, the relative concentration of non-methylated arsenical species in the cells was within 98–99%; 62–75% as arsenite and 24–37% as arsenate. When arsenite was accumulated, a quarter or more was transformed into arsenate. The predominant non-methylated arsenical species in cells was arsenite. A small amount of the inorganic arsenic accumulated was biomethylated to monomethylarsenic (0.1–0.3%) and dimethylarsenic (1.0–1.7%) species, but not to trimethylarsenic species (0%). Dimethylarsenic species were predominant among the methylated arsenicals accumulated in *D. magna*. When *N. denticulata* was exposed to arsenite, the relative concentration of non-methylated arsenical species in cells was within 68–93%; 38–48% as arsenite and 22–55% as arsenate. 7–32% of the arsenic accumulated was biomethylated to dimethylarsenic species. No monomethyl- or trimethyl-arsenic species were found in the tissue. Our previous data[6–10] reported that the arsenic accumulated was biomethylated up to trimethylarsenic species when *N. denticulata* was exposed to arsenate. The results of this study demonstrate that *N. denticulata* has the ability to methylate arsenite only up to the dimethylarsenic species. When *T. mossambica* was exposed to a medium containing arsenic of 5 $\mu g\,cm^{-3}$ of arsenite, most of the tissue arsenic concentration was present as non-methylated arsenic species within 75%; 39% of this was as arsenite and 36% as arsenate. The remaining species were in the form of monomethylarsenic (1.1%), dimethylarsenic (1.6%) and trimethylarsenic (2.4%). When exposed to arsenic of 0.1 and 1 $\mu g\,cm^{-3}$, the predominant arsenic species in cells was the trimethylarsenic species. These results show that lower trophic level organisms transform accumulated inorganic arsenic into monomethyl- and dimethyl-arsenic species, whereas upper trophic level organisms can transform accumulate inorganic arsenic as the trimethylarsenic species.

Biotransformation of arsenite in the freshwater food chain

The accumulation and transformation of arsenite in the laboratory food-chain model was investigated by feeding arsenic-dosed algal diets (*C. vulgaris*) to organisms for 7 days. *C. vulgaris* as the first-step organism was cultured in MD medium containing arsenic at 50 $\mu g\,cm^{-3}$ of arsenite for 10 days. The total arsenic accumulation in *C. vulgaris* was 529 μg of arsenic per gram dry weight, in which most of the arsenic was present as arsenate (83%). The remaining arsenic was in the form of arsenite (9%),

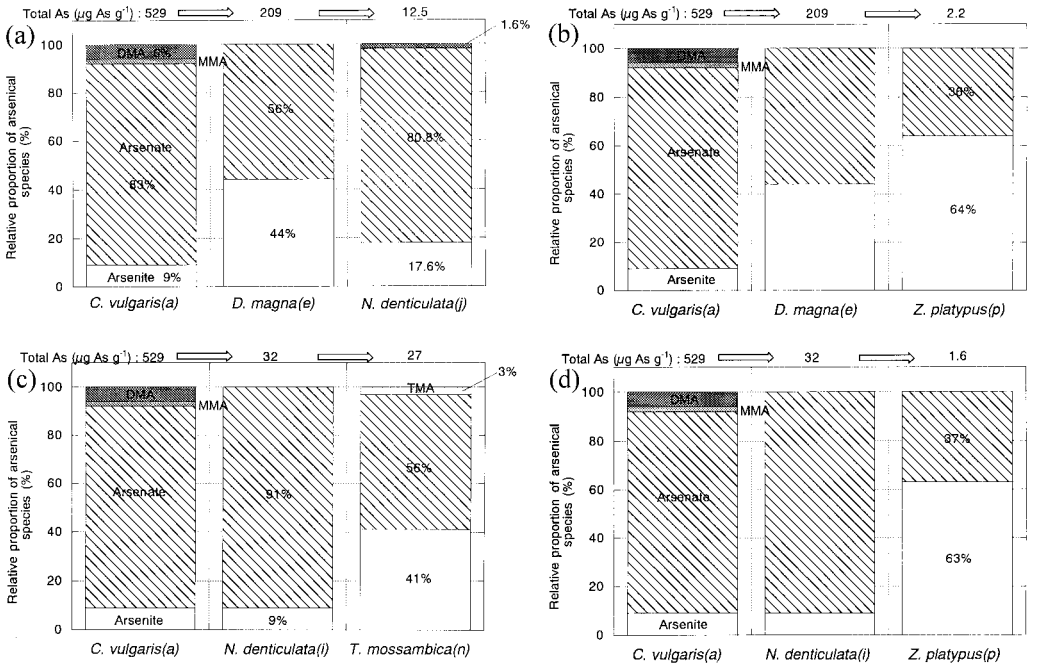

Figure 2 Relative proportion of arsenic species in the three-step freshwater food-chain models: (A) *C. vulgaris* (a) → *D. magna* (e) → *N. denticulata* (j); (B) *C. vulgaris* (a) → *D. magna* (e) → *Z. platypus* (o); (C) *C. vulgaris* (a) → *N. denticulata* (i) → *T. mossambica* (n); (D) *C. vulgaris* (a) → *N. denticulata* (i) → *Z. platypus* (p).

monomethylarsenic (2%) and dimethylarsenic species (6%). No trimethylarsenic species was found in the cell samples. The results showed that *C. vulgaris* converted only a small fraction of accumulated inorganic arsenic into methylated arsenic compounds. Similar results have been obtained in earlier experiment under similar conditions.[14]

The arsenic-dosed algae (*C. vulgaris*: 529 μg of arsenic per gram dry weight of arsenic concentration; 5 mg dry weight per day; 35 mg total) were fed to 500 zooplankton (*D. magna*: 0.5–2.0 mm long, 8 mg dry weight) for 7 days in aerated dilute MD medium, then *D. magna* was collected and washed with distilled water. Three shrimps (*N. denticulata*: 20 mm long, 28 mg dry weight) were fed for 7 days with arsenic-dosed *D. magna* (arsenic concentration: 13.8 μg of arsenic per gram dry weight; about 0.8 mg dry weight per three shrimps a day; 5.6 mg total) in aerated dilute MD medium, collected and washed with distilled water. These arsenic-dosed organisms were analyzed for total and species of arsenic compounds.

The experimental results (summarized in Table 1) show that in the food chain model, *C. vulgaris* (a) → *D. magna* (e) → *N. denticulata* (j), total arsenic concentrations in the organisms decrease (529 → 209 → 12.5 μg of arsenic per gram dry weight) by an order of magnitude for each step up in the food chain, whereas the non-methylated arsenic species are predominant in each organism; arsenite (9–44%) and arsenate (56–83%). The relative proportion of methylated arsenic compounds decreased. Fig. 2A shows the relative concentration of arsenic species accumulating via the food chain in this study. Dimethylarsenic species were predominant among the methylated arsenic compounds detected in the *N. denticulata* (1.6%); no trimethylarsenic species was found in any of the food-chain organisms. Similar results were obtained from other experimental food-chain models.

In the food chain model *C. vulgaris* (a) → *D. magna* (e) → *Z. platypus* (o), total arsenic concentration in the organisms decreased (529 → 209 → 2.2 μg of arsenic per gram dry weight) through the food chain (Fig. 2B). Most of the arsenic species accumulated in *Z. platypus* was non-methylated species; arsenite (64%) and arsenate

36%. Trace elements of methylated arsenic species were found in cells.

In the food chain model *C. vulgaris* (a) → *N. denticulata* (i) → *T. mossambica* (n), total arsenic concentration in the organisms decreased (529 → 32 → 26.6 μg of arsenic per gram dry weight) through the food chain (Fig. 2C). Most of the arsenic species accumulated in *T. mossambica* were non-methylated species; arsenite (41%) and arsenate 56%. No monomethyl- or dimethyl-arsenic species were found, and the predominant form of the methylated species was trimethylarsenic species (3%).

In the food chain model *C. vulgaris* (a) → *N. denticulata* (i) → *Z. platypus* (p), the total arsenic concentration in the organisms also decreased (529 → 32 → 1.6 μg of arsenic per gram dry weight) through the food chain (Fig. 2D). Most of the arsenic species that accumulated in *Z. platypus* were non-methylated species; arsenite (63%) and arsenate 37%. Also, trace elements of methylated arsenic species were found in cells.

These results suggest that the source of the arsenic that accumulated in *C. vulgaris* as the lowest level organism in this experiment is taken from the medium containing arsenite. Little methylation of arsenic in organisms occurred at each step in the food chain. It was found that non-methylated arsenics, arsenite and arsenate, were accumulated as the predominant arsenic species in organisms. Transformation of arsenite to arsenate occurred more easily than biomethylation of accumulated arsenic in the organisms with elevation in the trophic level. Dimethylarsenic species (2%) and trimethylarsenic species (3%) were found only in *N. denticulata* (i) and *T. mossambica* (n) respectively. Differed results were reported in our previous paper when the lowest trophic level organisms *C. vulgaris*[7–9] and *Nostoc* sp.[10] were exposed to arsenate as a source of arsenic, the trimethylarsenic species (>80%) was predominant in the highest level organisms *C. auratus*,[6] *O. latypes*,[8] *P. recticulata*[7,9] and *C. carpio*.[10] In the case of arsenate as a source of arsenic accumulation in lowest trophic level organisms, the biomethylation ratio of arsenic increased successively with elevation in the trophic level.

Further, Table 1 indicates that organisms (*D. magna* and *N. denticulata*) in the second step of the food chain accumulated more arsenic from arsenic-dosed food than from arsenic-containing water. Higher trophic level organisms feeding on primary producers were found to have a higher level of arsenic than that of the surrounding water, even in the case of an arsenite-polluted environment as well as in the case of an arsenate-polluted environment.

CONCLUSIONS

The results of this study led to the following conclusions for food-chain models starting from arsenite-dosed *C. vulgaris*.

(a) Total arsenic concentration accumulated directly from the water phase by freshwater organisms increased with increase in arsenic concentration in the medium when exposed to a medium containing arsenite.
(b) Total arsenic concentrations in the organisms decreased by an order of magnitude for each step up the freshwater food chain in models starting from arsenite-dosed *C. vulgaris*, as well as from food-chain models starting from arsenate-dosed *C. vulgaris*.
(c) Biomethylation of arsenic in organisms occurred scarcely at each step in food-chain models starting from arsenite-dosed *C. vulgaris*. The data suggest that there is a clear difference in biomethylation of arsenic through the freshwater food chain between arsenite and arsenate as sources of arsenic accumulation in the lowest trophic level organisms.

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