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Transformation of arsenic compounds in a freshwater food chain

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The transport and transformation of arsenic were investigated in the food chain *Chlorella* and *Phormidium* sp. (autotrophic freshwater algae)–*Moina* sp. (zooplanktonic grazer)–*Poecilia* sp. (carnivorous guppy). The algae were grown for seven days in a modified Detmer medium containing 100 mg dm^{-3} arsenic as Na_2HAsO_4. The algae (*Chlorella* 0.64 mg g^{-1} dry mass; *Phormidium* 2.9 mg g^{-1} dry mass) were fed to *Moina* for seven days. *Moina* acquired arsenic concentrations of 76 μg g^{-1} (from *Chlorella*) and 111 μg g^{-1} (from *Phormidium*). The guppy feeding on *Moina* (with arsenic from *Chlorella*) had the lowest arsenic concentrations (5.6 μg g^{-1}) among these organisms.

Whereas the arsenic in the algae was almost all in inorganic form, 85% of the arsenic in the guppy was in di- and tri-methylated form. The higher percentages of methylated arsenic at the higher trophic levels could be the result of preferential uptake or retention of methylarsenic compounds or of methylation of arsenic compounds by the higher organisms.

Keywords: Inorganic, methylated arsenic compounds; freshwater foodchain, *Chlorella* sp., *Phormidium* sp., *Moina* sp., *Piecilia* sp.

INTRODUCTION

In recent years we have investigated the accumulation, methylation and excretion of arsenic compounds by freshwater algae that had been isolated from arsenic-polluted environments.[1–5] The transformation of arsenic compounds by organisms in a marine food chain was studied by numerous researchers.[6–9] However, only a few experiments have been conducted in freshwater systems. Previously[10] we reported on the transformation of arsenic compounds in the freshwater food chain consisting of an autotrophic alga (*Chlorella vulgaris*), a zooplanktonic grazer (*Moina macrocopa*), and a carnivorous goldfish (*Carassius carassius auratus*). This paper presents the results obtained with a similar food chain but with the carnivorous guppy (*Poecilia recticulata*) as the organism at the highest trophic level.

EXPERIMENTAL

Culture of organisms

A suspension (4 cm^3, 6 mg dry mass) of *Chlorella vulgaris* Beijerinck var. *vulgaris* or of *Phormidium* sp. (4 cm^3, 6 mg dry mass) was placed in a modified Detmer medium[1] (4 dm^3) containing 100 mg dm^{-3} arsenic as Na_2HAsO_4. The culture was kept at 25–30°C under constant aeration (2 dm^3 min^{-1}) and illumination (4000 lux) for seven days. The cells were then harvested by centrifugation and rinsed twice with the arsenic-free Detmer medium.

Two hundred and fifty *Moina macrocopa* (1.25 mg dry mass) in 1 dm^3 aerated diluted modified Detmer (100 cm^3 medium, 900 cm^3 distilled water) were fed for seven days with *Chlorella* sp. or *Phormidium* sp. (about 18 mg dry mass per day: total 126 mg) that had been grown in the arsenic-containing medium. The control group received arsenic-free bread yeast (Super camellia, dry yeast, Nissin Seifun Co., Japan). *Moina* sp. which had multiplied about ten-fold during seven days was collected with a plankton net and rinsed with distilled water. A part of the *Moina* fed for seven days was analysed for arsenic. The rest was fed continuously for another seven days with the algae in the same way.

Four *Poecilia reticulata* (1.5 cm long and 10 mg dry mass) in the aerated diluted Detmer medium was fed for seven days with the arsenic-containing *Moina* sp. (about 0.125 mg dry mass per *Poecilia* sp. per day). The control group received 'Tetrafin', a basic diet for goldfish (manufactured in

Table 1 Concentration of total arsenic and methylated arsenic compounds in the organisms of the food chain consisting of *Chlorella* sp. or *Phormidium* sp., *Moina* sp. and *Poecilia* sp.

Organism	Source of As	Concentration of As in organism ($\mu g\ As\ g^{-1}$)				
		Total	Inorganic	Mono-CH_3	Di-CH_3	Tri-CH_3
Chlorella sp.	Water, 100 mg dm^{-3} as Na_2HAsO_4	640 (100)	605 (94.5)[a]	tr[b]	35.0 (5.5)	tr
Phormidium sp.	Water, 100 mg dm^{-3}	2900 (100)	2890 (99.7)	tr	5.2 (0.18)	3.4 (0.12)
Moina sp.(1)	Water, 1 mg dm^{-3}	4.7 (100)	2.1 (45)	tr	2.6 (55)	tr
Moina sp.(2)	*Chlorella*	75.6 (100)	66.2 (87.6)	tr	9.4 (12.4)	tr
Moina sp.(3)	*Phormidium*	111 (100)	83.3 (75)	9.3 (8.4)	18.4 (16.6)	tr
Poecilia sp.(1)	Water, As-free	3.3 (100)	1.9 (58)	0.1 (2.9)	1.0 (30)	0.3 (9.1)
Poecilia sp.(2)	Water, 0.5 mg dm^{-3} as Na_2HAsO_4	6.8 (100)	5.0 (72.6)	0.6 (9.3)	0.1 (1.9)	1.1 (16.2)
Poecilia sp.(3)	Water, 1.0 mg dm^{-3} as Na_2HAsO_4	6.9 (100)	5.8 (84.1)	0.1 (1.4)	0.2 (2.9)	0.8 (11.6)
Poecilia sp.(4)	Water, 10 mg dm^{-3} as Na_2HAsO_4	40.0 (100)	30.6 (76.5)	5.9 (14.8)	0.7 (1.7)	2.8 (7.0)
Poecilia sp.(5)	*Moina* (2) fed with *Chlorella*	5.6 (100)	0.9 (15)	tr	0.1 (3)	4.6 (82)
Poecilia sp.(6)	*Moina* (1) grown in water/As-free food	3.7 (100)	0.5 (14)	tr	tr	3.2 (86)

[a] Numbers in parentheses are percentages for arsenic compounds relative to total arsenic.
[b] Detected but below detection limits (1 ng As: corresponding to 0.005 $\mu g\ g^{-1}$ when 20 mg of sample was used).

West Germany). Four *Poecilia* sp. were collected with a plankton net and rinsed with distilled water.

Determination of total arsenic and methylated arsenic compounds

For the determination of total arsenic, the dry cells (10–20 mg) were mixed with 50% $Mg(NO_3)_2$ (2 cm^3); the mixture was dried and mineralized by heating at 550°C for 6 h. The mineralized samples were dissolved with 10 mol dm^{-3} HCl (10 cm^3) 40% KI (1 cm^3) was added, the solution was extracted twice with $CHCl_3$ (5 cm^3) and the $CHCl_3$ phase was then back-extracted with water (2 cm^3). Total arsenic was determined in the water phase by graphite furnace atomic absorption spectroscopy. For the determination of methylated arsenic compounds, the dry cells (*ca* 10 mg) were digested with 5 cm^3 of 2 mol dm^{-3} NaOH at 90–95°C for 3 h, using an aluminium heating block. Methylated arsenic compounds in the digest were reduced with sodium borohydride ($NaBH_4$) to the arsine compounds. The arsine gases were frozen out in a liquid-nitrogen U-trap. The arsines successively borne out of the trap upon warming the U-trap were passed through a quartz tube atomizer and determined on an atomic absorption spectrometer.

RESULTS AND DISCUSSION

Chlorella sp. and *Phormidium* sp., freshwater unicellular autotrophic algae, were grown for seven days in the modified Detmer medium containing 100 mg dm^{-3} of arsenic as Na_2HAsO_4. After seven days the cultures had reached the stationary phase. The cells were harvested by centrifugation and washed free of the arsenic-containing medium with arsenic-free medium. *Phormidium* sp. (2.9 mg g^{-1} As) had accumulated arsenic to a concentration approximately five times higher than *Chlorella* sp. (0.64 mg g^{-1} As). Most of the arsenic in both algal species was present as inorganic arsenic ($\geq$95%). The remaining arsenic was in the form of dimethylated arsenic (5%) in *Chlorella* sp. and in the form of trimethylated (0.1%) and dimethylated (0.2%) arsenic in *Phormidium* sp. Monomethylated arsenic was detected only in traces (Table 1). Under these conditions the algae had converted only a small fraction of the accumulated inorganic arsenic into methylated arsenic compounds. Similar results had been obtained earlier under somewhat different experimental conditions.[3,5]

These arsenic-rich algal cells were fed to *Moina* sp. for seven days. *Moina* sp. that fed on *Chlorella* had an arsenic concentration of

76 $\mu g\ g^{-1}$ (dry mass), whereas *Moina* sp. feasting on *Phormidium* sp. reached 111 $\mu g\ g^{-1}$. Although the food with higher arsenic concentration caused a higher arsenic level in *Moina* sp., the concentration ratio, As in *Phormidium*/As in *Chlorella*, of 5 was reduced to 1.5 in *Moina* sp. Most of the arsenic in *Moina* sp. was in the inorganic form (88% with *Chlorella* sp., 75% with *Phormidium* sp. as food), an observation that was also made with the algae. Dimethylated arsenic accounted for 12% of the arsenic in the *Chlorella*-fed and for 17% in the *Phormidium*-fed *Moina*. Monomethylated arsenic was found only in the *Phormidium*-fed *Moina*.[3] When *Moina* sp. was kept in water with 1 $mg\ dm^{-3}$ of arsenic as Na_2HAsO_4 and fed with arsenic-free bread yeast, *Moina* (1) had only 5 $\mu g\ g^{-1}$ (dry mass) of arsenic approximately equally divided between inorganic and dimethylated arsenic. These data indicate clearly that *Moina* took up more arsenic from the arsenic-containing food that from the arsenic-containing water. The percentage of arsenic in the methylated form in *Moina* sp. was considerably higher than in the algae or the water. *Moina* sp. could have preferentially taken up or retained methylasrsenic compounds or could have methylated inorganic arsenic. The fact that *Moina* sp. grown in water with 1 $mg\ dm^{-3}$ of arsenic as Na_2HAsO_4 had half of its arsenic in dimethylated form favours the methylation hypothesis.

After *Poecilia* sp. (a guppy) had lived for seven days on *Moina* sp. that had obtained its arsenic from *Chlorella* sp., the arsenic concentration in the guppy was only 5.6 $\mu g\ g^{-1}$ (dry mass). Most of the arsenic (82%) was in trimethylated form. A guppy fed with *Moina* that were grown in a medium with 1 $mg\ dm^{-3}$ arsenic in the form of Na_2HAsO_4 and had received arsenic-free bread yeast had a low arsenic concentration (3.7 $\mu g\ g^{-1}$) with most of the arsenic (86%) again in the trimethylated form. Because trimethyalted arsenic was present in *Moina* sp. only in traces, the guppy appears to be able to methylate mono- and di-methylated arsenic and perhaps inorganic arsenic when delivered as part of the food.

When two guppies had been kept in an arsenic-free medium and had been fed for seven days with 'Tetrafin', a goldfish diet, arsenic was present (3.3 $\mu g\ g^{-1}$) in the guppy. The arsenic in this guppy came from the goldfish diet, which had a total arsenic concentration of 9.0 $\mu g\ g^{-1}$, and inorganic, di- and tri-methylated arsenic concentrations of 6.2, 0.3 and 2.5 $\mu g\ g^{-1}$, respectively. Guppies kept on the same diet in a medium with 0.5–10 $mg\ dm^{-3}$ arsenic as Na_2HAsO_4 survived and had arsenic concentrations of 6.8–40 $\mu g\ g^{-1}$ (dry mass). The guppies did not survive in a medium with 15 $mg\ dm^{-3}$ of arsenic as Na_2HAsO_4. Thus, guppies are not as arsenic-tolerant as goldfish (*Carassius* sp.) that survived in media with less than 25 $mg\ dm^{-3}$ of arsenic.[10] As shown in Table 1, the total arsenic concentrations in guppies increased with an increase in arsenic concentration in the medium [*Poecilia* (2), (3) and (4)]. Most of the arsenic (73–84%) was present in inorganic form. These results suggest that the guppy is able to methylate the inorganic arsenic taken up from water but to a small degree.

In the food chain *Chlorella* sp.–*Moina* sp. (2)–*Poecilia* sp. (5) the total arsenic concentration in the organisms decreased (640–75–5.6 $\mu g\ g^{-1}$) by an order of magnitude for each step up in the food chain. Whereas in the algae most of the arsenic was in the inorganic form, the guppies had most of the arsenic in the trimetlylated form. The masses of arsenic compounds both in *Chlorella* sp. that was fed to *Moina* and in the *Moina* sp. are shown in Table 2. Two hundred and fifty *Moina* (1.25 mg) were fed for seven days with the arsenic-containing *Chlorella* (126 mg dry mass), the *Moina* cells multiplied to 2500 *Moina* (12.5 mg) during the seven days' feeding. Table 2

Table 2 Arsenic mass balances in *Chlorella–Moina* food chain

Organism	Dry mass (mg)	Arsenic in organisms (μg)				
		Total	Inorganic	Mono-CH_3	Di-CH_3	Tri-CH_3
Chlorella sp. (fed for 7 days to *Moina* sp.)	126	80.6 (100)[a]	76.2 (94.5)	tr[b]	4.4 (5.5)	tr
2500 *Moina* with *Chlorella* sp.)	12.5	0.95 (100)	0.83 (87.6)	tr	0.12 (12.4)	tr

[a,b] As shown in Table 1.

Table 3 Arsenic mass balances in *Moina–Poecilia* food chain

Organism	Dry mass (mg)	Arsenic in organisms (ng)				
		Total	Inorganic	Mono-CH_3	Di-CH_3	Tri-CH_3
175 *Moina* sp. (fed for 7 days) to *Poecilia* sp.)	0.875	66.2 (100)[a]	57.9 (87.6)	tr[b]	8.2 (12.4)	tr
Poecilia	10	56 (100)	9 (15)	tr	1 (3)	46 (82)

[a,b] As shown in Table 1.

shows that only 0.95 μg As (1.2% of food −As) was present in *Moina* sp., the other (79.65 μg: 98.8%) was excreted. Although both the inorganic and dimethylated arsenic compounds were excreted by *Moina* sp., the excretion ratio of inorganic arsenic of 98.9% (75.37 μg in 76.2 μg As) was a little reduced to 97.3% (4.28 μg in 4.4 μg As) in dimethylated arsenic. Table 3 shows the mass balances in the *Moina–Poecilia* food chain. Four *Poecilia* sp. (10 mg dry mass) were fed for seven days with *Moina* (0.875 mg dry mass) per *Poecilia*. *Poecilia* sp. took up 56 ng arsenic from *Moina* sp. containing 66.2 ng arsenic and excreted 10.2 ng arsenic. Although dimethylated arsenic was present in *Moina* at only 8.2 ng 12.4%), the sum of di- and tri-methylated arsenic present was 47 ng (85%) in the guppy. These data clearly indicate that *Poecilia* sp. have an activiy to methylate inorganic and dimethylated arsenic compounds to di- and tri-methylated arsenic compounds, respectively.

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