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Biotransformation and detoxification of inorganic arsenic in a marine juvenile fish *Terapon jarbua* after waterborne and dietborne exposure

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ABSTRACT

Arsenic (As) is a major hazardous metalloid in many aquatic environments. This study quantified the biotransformation of two inorganic As species [As(III) and As(V)] in a marine juvenile grunt *Terapon jarbua* following waterborne and dietborne exposures for 10 d. The fish were fed As contaminated artificial diets at nominal concentrations of 50, 150, and 500 μg As(III) and As(V)/g (dry weight), and their transformation and growth responses were compared to those exposed to 100 μg/L waterborne As(III) and As(V). Within the 10 d exposure period, waterborne and dietborne inorganic As exposure had no significant effect on the fish growth performance. The bioaccumulation of As was very low and not proportional to the inorganic As exposure concentration. We demonstrated that both inorganic As(III) and As(V) in the dietborne and waterborne phases were rapidly biotransformed to the less toxic arsenobetaine (AsB, 89–97%). After exposure to inorganic As, *T. jarbua* developed correspondingly detoxified strategies, such as the reduction of As(V) to As(III) followed by methylation to less toxic organic forms, as well as the synthesis of metal-binding proteins such as metallothionein-like proteins. This study elucidated that As(III) and As(V) had little potential toxicity on marine fish.

1. Introduction

Arsenic (As) is the most common toxic substance in the environment, ranking first on the superfund list of hazardous substances (http://www.atsdr.cdc.gov/cercla/07list.html). It is pervasive in the marine environment as a result of natural or anthropogenic activity, such as modern industrial and agricultural activities, and mining operations. Its toxicity and accumulative behavior have been a global health problem affecting many millions of people. One identified important factor modifying the As related health effects is its biotransformation, which involves reduction reactions and methylation via one-carbon metabolism (mainly detoxification) [1].

Marine fish represent an important environmental compartment in the determination of contaminant transfer and transformation in the food web, both as consumers of As-contaminated foods and as sources themselves. However, As toxicity and bioaccumulation are not only dependent on its total content but also dependent on its speciation [2,3]. Therefore, the speciation analysis of As in marine fish attracts increasingly attention [4]. For instance, marine fish contained mostly arsenobetaine (AsB), which was less toxic, and hence As accumulated in this form presented less health hazard as compared to inorganic As [5]. Accordingly, it is necessary to investigate the biotransformation of As in marine fish, and analyze the As form finally present in the fish body. In aquatic systems, As occurs in two oxidation states: a trivalent form, arsenite [As_2O_3; As(III)] and a pentavalent form, arsenate [As_2O_5; As(V)], which are inter-converted through redox and methylation reactions. As(III) is about 60 times more toxic than As(V), and conversely, As(V) is about 70 times more toxic than methylated species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). MMA and DMA are considered only moderately toxic [6]. Moreover, fish appeared to have evolved different mechanisms for biotransformation of As to less toxic forms, which are then readily excreted [5]. For example, As(V) is first converted into As(III) and then transformed into mono-, di-, and trimethylated products [7]. Edmonds and Francesconi [8] suggested a possible scheme for the conversion of inorganic arsenate to arsenobetaine.

To date, biotransformation after As(III) or As(V) exposure has been studied in many species, including fungi [9], bacteria [10,11], algae [12,13], lichens [14,15], polychaete [16,17], and freshwater fish [18,19]. Little is known about the comparative biotransformation of different inorganic As forms in marine fish. Moreover, aquatic biota have developed several strategies to detoxify metalloids such as As, including exclusion of As from the cells [20], reduction of As(V) to As(III) followed by either excretion or

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complexation with glutathione and sequestration into vacuoles (e.g., *Saccharomyces cerevisiae* [21]), synthesis of metal-binding proteins such as metallothionein-like proteins [22], and methylation to less toxic organic forms together with excretion [23].

Therefore, the objectives of our research were to investigate and compare the speciation biotransformation of As(III) and As(V), As bioaccumulation and its subcellular distribution (detoxification) in the muscle tissues of *Terapon jarbua* following both waterborne and dietborne exposures. Growth studies were undertaken to determine the toxic effects of inorganic As waterborne and dietborne exposure on *T. jarbua*. Speciation analysis of As in fish samples could provide important information to understand the mechanisms of As biotransformation and detoxification. Therefore, it is important to link inorganic As exposure and its biotransformation in marine fish.

2. Materials and methods

2.1. Fish and experimental design

Juvenile grunts *T. jarbua* (6–9 cm in length) were obtained from a fish farm at Yung Shu Au, Hong Kong, maintained in circulating natural sand-filtered seawater (20 °C, 30‰) and fed artificial diets twice a day at about 2% of their body weight. The tanks were under a light:dark cycle of 12:12 h. Feces and uneaten food were removed twice a day. They were acclimated to the test conditions for 2 weeks prior to the beginning of exposure experiment.

T. jarbua were exposed to both waterborne and dietborne As. In the waterborne treatment, As(III) and As(V) stock solutions (as $NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$, respectively, 1 mg/mL, Sigma, USA) were spiked into a tank (containing 34 L of natural filtered seawater) at a constant nominal dissolved As concentration of 100 μg/L for As(III) and As(V). Waterborne exposure test was semi-static, and test seawater was renewed every 24 h. In the control and waterborne As treatments, the fish were fed the control artificial diet. Dietborne exposure fed artificial diets containing three doses (calculated levels) of As [As(III), and As(V)] (50, 150 and 500 μg As/g). As(III) and As(V) were added to the diet as an aqueous solution of arsenite and arsenate ($NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$, Sigma, USA), respectively, to achieve a nominal concentration of 50, 150, and 500 μg As/g diet, respectively. When the diet pellets were completely soaked with the As solution, they were dried at 60 °C for 1–2 h to constant weight. The diets were then stored at −20 °C in sealed polyethylene bags until they were used.

Before the exposure, the length and wet weight of each individual fish were measured. There were a total of 18 treatment tanks [control, one aqueous exposure, and 3 dietborne exposures for both As(III) and As(V); each treatment had two replicated tanks] with a sample number of 11 fish per tank. Fish were fed twice per day (1 h for each feeding regime) and any uneaten food was removed to prevent negligible waterborne As exposure. The acclimated fish were exposed for 10 d to waterborne and dietborne As(III) and As(V). At the end of the exposure, they were starved for approximately 24 h to allow the depuration of gut contents. The fish (n = 10–11 per treatment) from each tank were then collected and placed in a plastic bag, and seawater on the surface of whole fish body was blotted dried. They were immediately measured for standard length (SD) and wet weight (W), and then frozen at −80 °C for further analysis. The condition factor (CF, g/cm^3) was calculated as $100 \times (W/SD^3)$ with W in grams and SD in centimeters.

2.2. Total As concentrations and subcellular As distribution

The frozen fish for each treatment were thawed on ice and the dorsal muscles of the fish were carefully dissected, which were separated into two subsamples. The fresh muscles were used for the subcellular analysis, and the others were freeze dried until constant weight. The dried samples were homogenized and stored in small polyethylene plastic at −20 °C for later total As and As speciation analysis.

About 0.2–0.3 g of samples were weighted in 15 mL volumetric flasks and digested with 3 mL of concentrated HNO_3 (65%, analytical reagent grade, Fisher Scientific) in heating block at 80 °C for 24 h until clearance. After cooling, the samples were diluted to 10 mL with double deionized water (Milli-Q Millipore 18.2 M cm^{-1} resistivity). A blank digest was processed using the similar procedure. The samples were analyzed for As using inductively coupled plasma mass spectrometer (ICP-MS). The standard solution was prepared from a stock solution (National China Standard, National Institute of Metrology, China). The accuracy of our digestion method was testified by analysis of standard reference material of 2976 mussel tissue (National Institute of Standards and Technology, the National Research Council Canada, and the International Atomic Energy Agency, Marine Environment Laboratory, Monaco). The artificial diets were also digested and their As concentrations were simultaneously measured. The total As recovery rate of the 2976 mussel tissue reference material was 101.4%. The As concentrations in the muscle tissue were expressed as μg/g dry weight.

The subcellular As distribution in fish was measured using the described method [24,25]. Briefly, the fresh muscle tissues from individual fish were homogenized with a tissue homogenizer in 20 mmol L^{-1} Tris–HCl buffer spiked with 2-mercaptoethanol (5 mM) and phenylmethanesulfonyl fluoride (0.1 mM) (pH 7.4). The homogenized fish muscles were centrifuged at 1500 × g for 15 min at 4 °C, and the pellets (P1) and supernatant (S1) were obtained. Then 4 mL of 1 mol L^{-1} NaOH was spiked to the P1 to be digested at 80 °C for 10 min and centrifuged at 5000 × g for another 10 min at 4 °C to separate the metal-rich granules (P2, MRG) and the cellular debris (S2). The S1 was centrifuged at 100,000 × g for 1 h at 4 °C to separate the organelles (P3) and the cytosol (S3). Afterwards, the S3 was heated for 10 min at 80 °C, ice-cooled for 1 h, and was further centrifuged at 30,000 g for 10 min at 4 °C to obtain the heat-denaturable protein (P4, HDP) and the metallothionein-like proteins (S4, MTLPs). Afterwards, the five fractions were dried to a constant weight at 60 °C and digested in concentrated nitric acid (HNO_3, 65%). The total As concentrations were measured by ICP-MS.

2.3. Chemicals, reagents, and As speciation analysis

All solutions were prepared with double deionized water. Stock standard solutions of arsenic compounds were prepared from sodium arsenite ($NaAsO_2$) (Sigma, USA), sodium arsenate dibasic heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$) (Sigma, USA), sodium cacodylate trihydrate ($C_2H_6AsNaO_2{\cdot}3H_2O$) (Sigma, USA), disodium methylarsenate ($CH_3AsO_3Na_2{\cdot}6H_2O$) (SUPELCO, USA), arsenobetaine ($(CH_3)_3As^+CH_2COO^-$) (Fluka, Sigma). Chemicals used in HPLC mobile phases (NH_4HCO_3 and KCl) and HCl and KOH employed for hydride generations were all obtained from Guangzhou Chemical Reagent Factory (China). KBH_4 was obtained from CNW Technologies GmbH, and $K_2S_2O_8$ used in the photo-oxidation reaction was purchased from Guangzhou Chemical Reagent Factory (China). The KBH_4 solution was not filtered before use, and was prepared daily. Samples were filtered through a 0.45 μm PTFE membrane (China). The standard solutions were stored in high density polypropylene containers at 4 °C in a cold room. Analytical working standards were prepared daily by diluting the stock solutions with double deionized water prior to analysis. Certified reference materials BCR-627 Tuna Fish Tissue (Institute for Reference Materials and Measurements (IRMM), Geel, Belgium) were used in the sample analysis.

All the other samples were freeze-dried prior to the extraction procedure.

The frozen fish muscles were prepared for As speciation analysis using methanol/water (1:1, v/v) extraction as described previously [26,27]. About 0.5 g of sample was added into 50 mL centrifuge tubes to which 20 mL of 50% methanol (50% MeOH in dd H_2O) solution was added. Samples were sonicated for 30 min, then centrifuged for 10 min at 10,000 rpm and the supernatant then poured into a 100 mL beaker. This extraction process was repeated three times with the supernatant being added to the previous extract. The final extract (a combination of all three supernatants approximately 60 mL in total) was heated to 50 °C to evaporate the solvent until a volume of approximately 1 mL was reached. The concentrated samples were then diluted with ddH_2O to a volume of 10 mL. Samples were filtered through 0.45 μm syringe filters into 5 mL centrifuge tube in preparation for HPLC–UV–HG–AFS (AF-610D2 from Beifenruili Analytical Instrument Corp., Beijing, China) analysis. The extracted samples (40 μL) were injected to detect the different As species, and were analyzed within one day to prevent possible reduction of As concentrations.

Standard reference materials (SRM) were used to validate the extraction and analysis methods. BCR-627 tuna fish tissue (0.5 g) was used for AsB and DMA analyses. The BCR-627 reference material contained a certified AsB concentration of 3.90 ± 0.22 μg/g and a certified DMA concentration of 0.15 ± 0.02 μg/g, respectively. With our method, we obtained a AsB concentration of 3.69 ± 0.06 μg/g (95% recovery, $n = 6$) and a DMA concentration of 0.12 ± 0.02 μg/g (82% recovery, $n = 6$). Spikes were also used to confirm the recovery of other As species detected during speciation analysis. In our study, As(III) recoveries were 72–98%, As(V) recoveries were 71–105%, and MMA recoveries were 76–108%.

2.4. Statistical analyses

Statistical analyses were performed using SPSS version 16.0. The differences of the corresponding values between the control and exposed/treated groups were tested by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test. A probability level (p-value) of less than 0.05 was regarded as statistically significant.

3. Results

3.1. Fish growth

As concentrations in the fish diet are presented in Table 1. Based on the dietborne As concentrations, the assimilation efficiency (5.5% [28]), and the feeding rate (2% of body wet weight d^{-1}), the calculated daily dietborne As doses were 0.002 μg/g/d dw for both the control and waterborne As-exposed fish, 0.044, 0.135, 0.452, 0.054, 0.150, 0.530 μg/g/d for the As(III) 50, As(III) 150, As(III) 500, As(V) 50, As(V) 150, As(V) 500 μg/g dietborne As-exposed fish, respectively. In the waterborne exposure treatment, the calculated daily waterborne dose was 0.150 μg/g/d, comparable to the mean dose in As(III) and As(V) 150 μg/g As-exposed samples (the uptake rate constant was 1.5 L/kg/d for *T. jarbua* [28]). There was no significant difference among control, waterborne and dietborne As-exposed fish in terms of wet weight, length, or condition factor (CF), following 10 d exposure to inorganic As (Table 1). Thus, growth was not influenced by waterborne or dietborne As(III) and As(V) exposure. It may be concluded that the exposure concentration and exposure pathway were not important for fish growth. After 10 d, 6.5% mortality was received in all treatments at the end of exposure period.

Table 1
Wet weight, standard length, condition factor in the marine fish *T. jarbua* after 10 d exposure to waterborne or dietborne inorganic As ($n = 20$–22). Values are mean ± SD. No significant ($p > 0.05$) treatment effect on any of the biological factors was observed.

Treatment	Control	As(III) 50 μg/g	As(III) 150 μg/g	As(III) 500 μg/g	As(V) 50 μg/g	As(V) 150 μg/g	As(V) 500 μg/g	Waterborne As(III) 100 μg/L	Waterborne As(V) 100 μg/L
As concentration in fish diet (μg/g dw) 0 d	1.4 ± 0.04	40.4 ± 1.5	123.1 ± 4.6	411.1 ± 3.2	49.3 ± 0.6	136.6 ± 2.3	481.4 ± 2.8	1.4 ± 0.04	1.4 ± 0.04
Wet weight (g)	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797	7.535 ± 2.797
Length (cm)	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8	6.6 ± 0.8
Condition factor	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248	2.506 ± 0.248
10 d									
Wet weight (g)	8.319 ± 3.005	7.930 ± 2.606	6.886 ± 2.476	6.883 ± 1.194	5.786 ± 0.795	5.731 ± 2.153	6.152 ± 0.727	7.116 ± 1.537	7.568 ± 1.496
Length (cm)	7.1 ± 1.1	6.9 ± 0.7	6.7 ± 0.6	6.8 ± 0.7	6.2 ± 0.4	6.3 ± 0.8	6.5 ± 0.3	6.8 ± 0.4	6.8 ± 0.5
Condition factor	2.292 ± 0.206	2.250 ± 0.239	2.235 ± 0.222	2.199 ± 0.182	2.401 ± 0.146	2.212 ± 0.167	2.192 ± 0.179	2.257 ± 0.227	2.401 ± 0.222

Table 2
As speciation concentrations (μg/g, dry weight) in the muscle of marine fish *T. jarbua* after waterborne or dietborne inorganic As exposure for 10 d. Data shown are mean ± SD (n = 20–22). AsB, arsenobetaine; As(III), arsenite; DMA, dimethylarsinate; MMA, monomethylarsonate; As(V), arsenate.

Treatments	As speciation concentrations (μg/g)				
	AsB	As(III)	DMA	MMA	As(V)
Control	1.625 ± 0.524[a]	0.071 ± 0.034[ab]	0.018 ± 0.007[a]	n.d.	n.d.
As(III) 50 μg/g	2.482 ± 0.883[a]	0.102 ± 0.051[b]	0.043 ± 0.025[abc]	n.d.	0.093 ± 0.024
As(III) 150 μg/g	2.152 ± 0.954[a]	0.207 ± 0.006[c]	0.027 ± 0.011[ab]	0.012 ± 0.007	0.021 ± 0.014
As(III) 500 μg/g	2.016 ± 0.185[a]	0.162 ± 0.019[c]	0.035 ± 0.014[abc]	n.d.	0.037 ± 0.028
As(V) 50 μg/g	2.017 ± 0.460[a]	0.025 ± 0.011[a]	0.027 ± 0.009[ab]	n.d.	0.034 ± 0.014
As(V) 150 μg/g	2.659 ± 1.169[a]	0.031 ± 0.009[a]	0.066 ± 0.043[c]	n.d.	0.075 ± 0.047
As(V) 500 μg/g	2.260 ± 0.650[a]	0.096 ± 0.014[b]	0.052 ± 0.021[bc]	n.d.	n.d.
Waterborne As(III) 100 μg/L	1.842 ± 0.141[a]	0.043 ± 0.016[a]	0.019 ± 0.003[a]	n.d.	n.d.
Waterborne As(V) 100 μg/L	1.778 ± 0.236[a]	0.064 ± 0.011[ab]	0.027 ± 0.006[ab]	n.d.	n.d.

Different letters show significant difference between treatments.

3.2. As bioaccumulation and subcellular distribution

There were significant differences in As bioaccumulation between the control and the dietborne exposure, whereas no significant difference was observed between the control and the waterborne exposure after 10 d exposure (Fig. 1). Dietborne uptake dominated the As accumulation during 10 d exposure. Total As concentrations accumulated by *T. jarbua* were not proportional to the As dosage [50,150, and 500 μg/g As(III) and As(V) in artificial diets, and 100 μg/L As(III) and As(V) in seawater] after 10 d of exposure. Moreover, the bioavailability of As was very low.

Fig. 2 shows the distribution of As in the five subcellular fractions (organelles, MRG, cellular debris, HDP, MTLP) in all the treatments of waterborne or dietborne exposure. After 10 d exposure, MTLP was the major binding site for As in the fish muscle (36.0–46.4% after the dietborne exposure, and 36.2–46.7% after waterborne exposure). Cellular debris was another important fraction (31.5–45.4% after dietborne exposure, and 35.2–37.0% after waterborne exposure). By comparison, only a small fraction of As was bound with MRG (6.1–14.5%), organelles (4.5–12.8%), and HDP (3.5–5.0%) after exposure. The As subcellular distribution varied little between the two different exposure pathways.

3.3. Biotransformation of As

Table 2 and Fig. 3 present the concentrations of various As species and their percentages in the fish muscles. *T. jarbua* exhibited a predominance of AsB (89–97%), while DMA, As(III), and As(V) were only about 1.0–2.3%, 1.1–8.6%, and 0.9–3.4%, respectively. These data strongly demonstrated that *T. jarbua* had a very high ability to biotransform the inorganic As into organic forms. As(III) was detectable in all treatments, while As(V) was only detected in the dietborne exposure treatments. DMA was noteworthy a minor component in the fish muscle. Furthermore, MMA and one unknown As compound (possibly arsenocholine) were found. Therefore, dietborne exposure typically included As(III), As(V), DMA, MMA, and AsB, whereas waterborne exposure only included As(III), DMA, and AsB. A clear but not significant increase in AsB concentration was noticed in the fish muscle tissues during both waterborne and dietborne exposure as compared to the control. The exposure concentration did not affect the AsB concentrations. As(III) concentrations exhibited significant increases after dietborne As(III) exposure (150 and 500 μg/g), but were below the detection after As(V) exposure. In addition, As(III) tissue concentrations increased significantly when the dietborne As(V) concentration increased from 50 μg/g to 500 μg/g. In contrast, As(V) tissue concentration was not influenced by the dietborne As(V) concentration, suggesting that As(V) may have been reduced into As(III). Interestingly, DMA levels increased significantly upon exposure to dietborne As(V) (150 μg/g, and 500 μg/g) for 10 d. MMA levels were the lowest and only detectable for the As(III) 150 μg/g treatment.

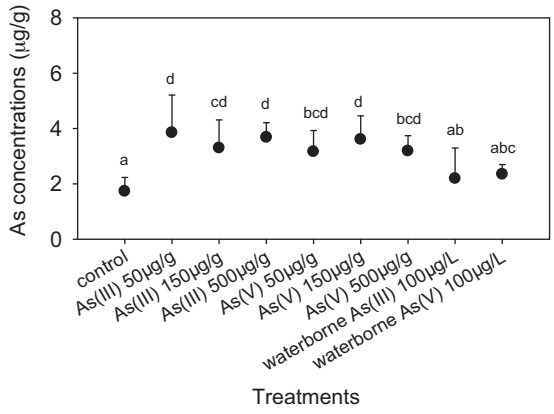

Fig. 1. Total As concentrations (μg/g, dry weight) in the muscles of marine fish *T. jarbua* after waterborne or dietborne inorganic As exposure for 10 d. Data are mean ± SD (n = 20–22). Different letters represent significant difference between treatments (p < 0.05).

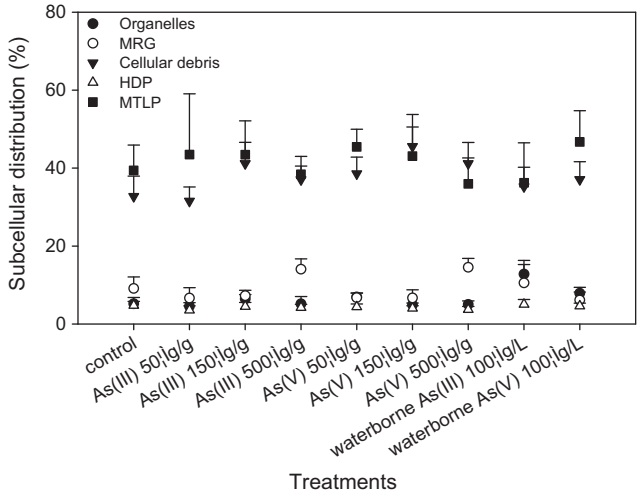

Fig. 2. Subcellular As distribution (%) in the muscles of marine fish *T. jarbua* after waterborne or dietborne As exposure for 10 d. Data are mean ± SD (n = 20–22). MRG, metal-rich granules; HDP, heat-denaturable protein; MTLP, metallothionein-like proteins.

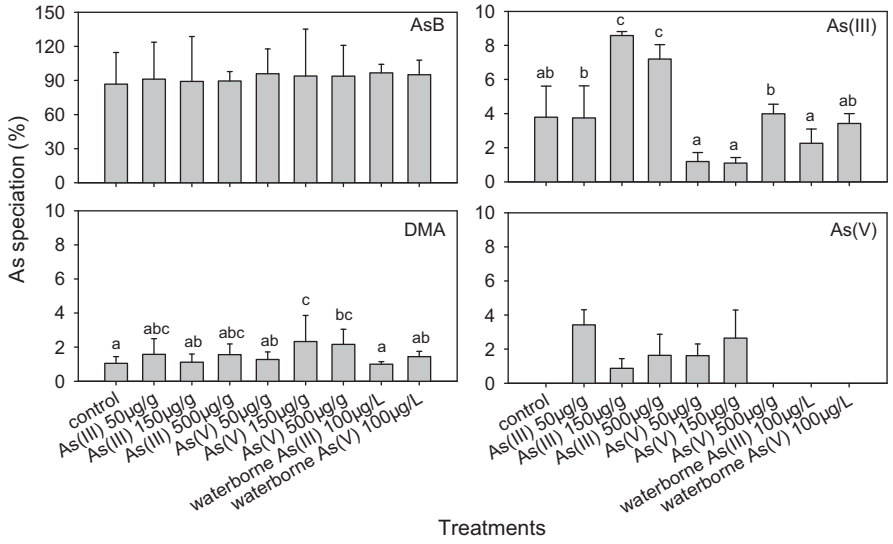

Fig. 3. As speciation distribution (%) in the muscle of marine fish *T. jarbua* after waterborne or dietborne inorganic As exposure for 10 d. Data are mean ± SD (n = 20–22). AsB, arsenobetaine; As(III), arsenite; DMA, dimethylarsinate; As(V), arsenate. Different letters represent significant difference between treatments (p < 0.05).

4. Discussion

4.1. Fish growth

There was no significant effect on wet weight, length, and condition factor (an integration of standard length and wet weight, see above) of fish following waterborne exposure to As(III) and As(V) at 100 μg/L. These results were similar to previous studies, e.g., minnow juveniles exposed to As(V) in Lake Superior water for 30 d [29]. Survival and growth of the minnows were comparable to the control levels at the two lowest As(V) concentrations tested (100 and 530 μg/L), but were lower at the two highest tested concentrations (1500–14,000 μg/L). At an exposed concentration of 1.5 mg As(V)/L, the growth rate of rainbow trout was not affected over 11 weeks of exposure period, and the whole body As concentration was 15-fold greater than our measurements [30]. Rankin and Dixon [31] pointed out that freshwater fish could immediately reduce their feeding in response to both waterborne and dietborne As exposure. There was no effect on growth in rainbow trout exposed to 0.76 mg As(III)/L (a 7.6-fold higher concentration than that measured in the present study) for 121 d.

Similarly, dietborne exposure to inorganic As for 10 d also showed no significant difference in wet weight, length, or condition factor, thus growth was not liable to dietborne As(III) and As(V) treatment. Several possibilities might explain such lack of influence for all the treatments. First, the exposure time (10 d) may be too short and there was a rather high degree of variability within each treatment group. Second, the food pellets were taken into the mouth but subsequently rejected as a result of feeding refusal and chemoreceptory detection of As in the feed. These results were consistent with previous study of no effect on growth and food consumption of juvenile rainbow trout exposed at 8 μg/g and 12 μg/g of As in the diet [32]. However, Hansen et al. [33] found reduced growth of rainbow trout fed polychaete diets of *Lumbriculus variegatus* that had been exposed to sediments collected from metal-contaminated sediments from the Clark Fork River Basin (MT, USA) and from an uncontaminated reference stream. Boyle et al. [34] showed that zebrafish *Danio rerio* fed natural As contaminated polychaete *Nereis diversicolor* had reduced reproductive output although no significant growth reduction was observed. More recently, Erickson et al. [35] found that growth reduction in fish occurred at similar concentrations of total arsenic in the oligochaete diet whether the oligochaetes had been exposed to arsenite or arsenate.

4.2. As bioaccumulation and subcellular distribution

Dietborne exposure significantly elevated the accumulated As body levels in fish whereas aqueous exposure resulted in no significant difference in body As levels as compared to the control. Moreover, the As bioaccumulation was very low, and was not proportional with the inorganic As exposure concentrations. In a recent study, Zhang et al. [28] showed that the dissolved uptake of As increased linearly over a range of dissolved concentrations from 0.5 to 50 μg/L, with an uptake rate constant of 0.0015 L/g/d. The assimilation efficiencies (AEs) of dietborne As were only 3.1–7.4%. Dietborne uptake represented a dominant pathway for As accumulation by the fish, but overall the bioaccumulation of As in *T. jarbua* was rather low given the element's low k_u, AE, and relatively high k_e. Erickson et al. [19] reported a greater toxicity of dietborne exposure when juvenile rainbow trout and prey were exposed to the same waterborne arsenate concentration, emphasizing the need to address the dietborne exposure in As risk assessment. Most marine animals including seaweeds, gastropods, shrimps, and other invertebrates have only a limited ability to accumulate arsenate from seawater [36–38]. The bioaccumulation of dietborne arsenic in the tissues of rainbow trout peaked at 14 months and then decreased in adults, which also suggested a low As bioavailability [39]. Consistently, no significant increase in arsenic concentration in common shrimp *Crangon crangon* [40] and blue mussel *Mytilus edulis* [41] was found when the animals were exposed to arsenate at concentration as high as 100 mg As/L.

Rankin and Dixon [31] compared the acute and chronic toxicity of arsenite to rainbow trout exposed in flow-through aquaria for 121 d at concentrations of <20 (control), 760, 2480, and 9640 μg/L. Significant increases in tissue As only occurred in the two highest treatments. Suhendrayatna et al. [42] measured the bioaccumulation of As in freshwater *Tilapia mossambica* following exposure in 8 L of dilute medium containing 5, 10, and 15 mg As(III)/L or 0.1, 5, and 10 mg As(V)/L under static conditions. The accumulation of As by *T. mossambica* was proportional to the concentration of arsenicals in water, similar to the previous study on Japanese medaka, *Oryzias latipes* [43]. Rainbow trout and other fish species

had extremely high acute 96-h LC50 values (10,800–100,000/μg/L), indicating that fish were very tolerant to As [44–47].

For dietborne exposure, different concentrations of As in the diets had no effect on As bioaccumulation. Our recent study found that the calculated As AEs were very low (2.5–4.3% at different As concentrations in the fish diets, 0.05–100 μg/g), suggesting that the dietborne AEs were independent of the As(V) concentrations in the artificial diets [28]. Consistently, Pedlar and Klaverkamp [48] reported that when the adult lake whitefish (*Coregonus clupeaformis*) were fed As contaminated diets at nominal concentrations of 0, 1, 10, and 100 μg As/g food (dry weight) for 10, 30, and 64 d, As concentrations in fish muscle did not increase.

The As subcellular distribution followed the orders of MTLP > cellular debris > MRG > organelles > HDP after waterborne and dietborne exposure. These data were consistent with our recent study that MTLP was the major binding site for As in the clams and fish, and cellular debris was another important fraction after dietborne As exposure. By contrast, only a small fraction of As was bound with organelles, HDP, and MRG [28]. Since a large proportion of As (mainly AsB) was distributed in the MTLP fraction, As may have been detoxified. In earlier study, arsenic contamination induced metallothioneins in liver particularly in freshwater teleosts *Channa punctatus* [49]. Chowdhury et al. [22] also reported the dominance of As in MTLP fraction, indicating that metallothioneins may protect against As toxicity at the cellular levels. And several studies have shown that cellular debris and MRG were non-bioavailable to the higher trophic levels [50,51]. Cockell [52] reported that with continued exposure to dietborne As, epithelial cells must undergo an adaptation in order to allow them to regenerate. Such adaptation may occur by increased metabolic transformation of As to a less toxic form, reduction of net accumulation by decreasing uptake or increasing excretion of As, binding of As to proteins such as MT, or storage of As in intracellular granules.

4.3. Biotransformation of As speciation

The recovery of different As speciation as compared to the total As content in fish muscle was approximately 61–110%. In previous study, the extraction efficiencies (calculated as the summation of different As species divided by the total As concentrations) were low (10–64%, mean 36%) for total arsenic and different arsenic species in a range of freshwater samples (sediment, water, algae, plants, sponge, mussels, frog and fish species) [53]. In clams (*Meretrix lusoria*) collected from southwestern Taiwan, the recovery of different As species was 61% of the total As content [54]. The quality and usefulness of the data from speciation analysis can often be compromised by the low recovery of arsenic species. Such low recovery was probably due to different analytical techniques used for total As, inorganic As, and organic As measurements. Indeed, some As species were very close to the detection limits in some samples.

As in the control and treatments groups was predominated by organic forms, mostly AsB (89–97%), followed by DMA, As(III), As(V), and MMA, with very low concentrations of methylated and inorganic forms. These data were in accordance with As accumulation data in marine organisms [55]. In previous studies, intestinal uptake and efficient transfer of AsB from blood to muscle have been reported in marine yelloweye mullet (*Aldrichetta forsteri*) [56] and in freshwater and seawater-adapted Atlantic salmon (*Salmo salar*) [57]. Amlund et al. [58] similarly found that after three months of dietborne AsB exposure, AsB was the major arsenic species in Atlantic salmon (*S. salar* L.) and Atlantic cod (*Gadus morhua* L.) muscle, representing more than 99% of total arsenic presented. The less toxic AsB often constituted more than 95% of all As compounds found in marine fish [59]. Ciardullo et al. [60] showed that AsB was the dominant As compound in muscle tissues of freshwater fish, but several other arsenicals including As(III), As(V), MMA, DMA, trimethylarsine oxide (TMAO), and three unknown As compounds were also present. Shah et al. [61] reported that As(III) and As(V) in freshwater fish muscles tissues were in the range of 17.1–31.9% of the total arsenic measured. In the present study, we also found that levels of AsB did not significantly change compared to the control values after waterborne and dietborne exposure. However, in Mediterranean polychaete *Sabella spallanzanii*, there was a marked increase in AsB following exposure to As(V), and DMA and TMA had a progressively lower accumulation [62].

Interesting variance of inorganic As speciation in fish muscle after waterborne or dietborne exposure was observed in the present study. Over the 10 d exposure period, the percentage of total As as As(III) in the fish muscle was higher after As(III) dietborne exposure (3.8–8.6%) than after As(V) dietborne exposure (1.1–4.0%) and As(III) and As(V) waterborne exposure (2.3–3.4%). And a faster transformation of As(V) into As(III) was found both in the waterborne and dietborne exposures. The biotransformation may be attributed to the reduction of As(V) into As(III), accompanying by a prompt import of As(V) and export of As(III). Mrak et al. [14] found that the lichen *Hypogymnia physodes* transformed As(V) into As(III) and excreted this reduced As form. Moreover, no As(V) was detected after inorganic As waterborne exposure, which was inconsistent with the recent fungi study, in which only As(V) was found in the cells of *Trichoderma asperellum* SM-12F1 after cultivation of 10 d [9].

Interestingly, DMA was the only species that increased throughout the As(V) 150 μg/g and 500 μg/g dietborne exposures, but was very low after As(III) dietborne exposure and inorganic As waterborne exposure. MMA was detected only after As(III) 150 μg/g dietborne exposure. Although MMA is an intermediate species in the biotransformation pathway from As(V) to DMA, it was not detected in the As(V)-exposed fish. The occurrence of MMA pointed to demethylation as an important mechanism of the biotransformation. Cullen et al. [23] investigated arsenic metabolism in two microorganisms (*Apiotrichum humicola* and *Scopulariopsis brevicaulis*) by exposing them to different arsenicals [As(V), As(III), MMA, and DMA]. In their study, MMA was metabolized faster than the other intermediates. Possibly, in *T. jarbua*, the transformation of MMA into DMA also occurred faster than the other transformations, and therefore the intermediate metabolite MMA showed no detectable increase following As(V) dietborne exposure and inorganic As waterborne exposure. These two methylated compounds (DMA and MMA) could thus represent the initial steps toward detoxification of inorganic As. A few studies also described the biotransformation of inorganic As to methylated forms such as MMA and DMA as the classical metabolic pathway [63].

Various mechanisms may be involved in the reduction of As(V) to As(III) once the fish ingested either As(III) or As(V) [64]. As(III)-thiol complexation occurred in S-rich proteins, and glutathione As(III)-thiol complexes are methylated to organoarsenic compounds (e.g., AsB) [65]. Organoarsenic compounds may be excreted through mucus production. Living organisms are able to activate biotransformation processes to convert As(V) into As(III) or into methylated forms. Such process has been documented in fungi, bacteria, algae, plants, animals, as well as in lichens [14]. Our results suggested that As in the fish body was ingested, transformed, and excreted by the marine fish. It has been well known that fish can convert the toxic inorganic arsenic in their bodies into less toxic methylated forms. For example, the carnivorous killifish can accumulate arsenic directly from water and partially biomethylate it [66]. Erickson et al. [19] also found that As(V) absorbed by oligochaetes was largely reduced to As(III), but there was no measurable conversion to organoarsenic species. Bears et al. [5] showed that chronic low-level arsenic exposure interfered with the ability of biomethylation and excretion of toxicant. Shah et al.

[67] indicated that methylation changed with the exposure and periods of high metabolism. Prior arsenic exposure prevented the stress-induced increase in stress-responsive *LDH-B* mRNA levels and enzyme activity observed in fish that had not been exposed to arsenic. At present, there is little study on the inorganic As transformation in marine fish.

5. Conclusion

This study examined the toxicological effects, bioaccumulation and biotransformation of inorganic As in marine fish *T. jarbua* exposed to waterborne and dietborne As(III) and As(V), at environmentally relevant concentrations. Growth was not influenced by waterborne or dietborne As(III) and As(V) exposure. Dietborne exposure was the main pathway of As bioaccumulation. Toxic inorganic As(V) was reduced to As(III), and As was mostly transformed into less toxic AsB in fish muscle. MTLP was the main binding site in *T. jarbua* muscle. It can be concluded that at least two types of As detoxification functioned in marine fish, including the As(V) reduction to As(III) followed by methylation to less toxic organic forms, and the induction of metal-binding proteins such as MTLP. Knowledge about the uptake and methylation of As in aquatic biota is important for estimating human health risk. It is becoming increasingly evident that methylation of As is critical in controlling its biological fate and effect [68]. However, each As form has different physicochemical property and bioavailability. Understanding and predicting the biotransformation of As in marine fish are thus of great values in assessing the impacts of As pollution. It will be interesting to study the toxicity of As over a longer exposure period.

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References

[1] M. Vahter, Mechanisms of arsenic biotransformation, Toxicology 181–182 (2002) 211–217.
[2] X.C. Le, X.F. Lu, M.S. Ma, W.R. Cullen, H.V. Aposhian, B.S. Zheng, Speciation of key arsenic metabolic intermediates in human urine, Anal. Chem. 72 (2000) 5172–5177.
[3] J.A. Caruso, B. Klaue, B. Michalke, D.M. Rocke, Group assessment: elemental speciation, Ecotoxicol. Environ. Saf. 56 (2003) 32–44.
[4] M. Leermakers, W. Baeyens, M.D. Gieter, B. Smedts, C. Meert, H.C.D. Bisschop, R. Morabito, P. Quevauviller, Toxic arsenic compounds in environmental samples: speciation and validation, Trends Anal. Chem. 25 (2006) 1–10.
[5] H. Bears, J.G. Richards, P.M. Schulte, Arsenic exposure alters hepatic arsenic species composition and stress mediated-gene expression in the common killifish (*Fundulus heteroclitus*), Aquat. Toxicol. 77 (2006) 257–266.
[6] K.F. Akter, G. Owens, D.E. Davey, R. Naidu, Arsenic speciation and toxicity in biological systems, Rev. Environ. Contam. Toxicol. 184 (2005) 97–149.
[7] D.J. Thomas, S.B. Waters, M. Styblo, Elucidating the pathway for arsenic methylation, Toxicol. Appl. Pharmacol. 198 (2004) 319–326.
[8] J.S. Edmonds, K.A. Francesconi, Transformations of arsenic in the marine environment, Experientia 43 (1987) 553–557.
[9] S. Su, X.B. Zeng, L.Y. Bai, L.F. Li, R. Duan, Arsenic biotransformation by arsenic-resistant fungi *Trichoderma asperellum* SM-12F1 Penicillium janthinellum SM-12F4, and *Fusarium oxysporum* CZ-8F1, Sci. Total Environ. 409 (2011) 5057–5062.
[10] G.S. Nagvenkar, N. Ramaiah, Arsenite tolerance and biotransformation potential in estuarine bacteria, Ecotoxicology 19 (2010) 604–613.
[11] X.-X. Yin, J. Chen, J. Qin, G.-X. Sun, B.P. Rosen, Y.G. Zhu, Biotransformation and volatilization of arsenic by three photosynthetic cyanobacteria, Plant Physiol. 156 (2011) 1631–1638.
[12] J. Qin, C.R. Lehr, C.G. Yuan, X.C. Le, T.R. McDermott, B. Rosen, Biotransformation of arsenic by a yellowstone thermoacidophilic eukaryotic alga, PNAS 106 (2009) 5213–5217.
[13] S. Miyashita, S. Fujiwara, M. Tsuzuki, T. Kaise, Rapid biotransformation of arsenate into oxo-arsenosugars by a freshwater unicellular green alga, *Chlamydomonas reinhardtii*, Biosci. Biotechnol. Biochem. 75 (2011) 522–530.
[14] T. Mrak, Z. Slejkovec, Z. Jeran, R. Jačimović, D. Kastelec, Uptake and biotransformation of arsenate in the lichen *Hypogymnia physodes* (L.) Nyl, Environ. Pollut. 151 (2008) 300–307.
[15] T. Pisani, S. Munzi, L. Paoli, M. Bačkor, S. Loppi, Physiological effects of arsenic in the lichen *Xanthoria parietina* (L.) Th. Fr, Chemosphere 82 (2011) 963–969.
[16] J. Ventura-Lima, P.B. Ramos, D. Fattorini, F. Regoli, L. Ferraz, L.M. de Carvalho, J.M. Monderrat, Accumulation biotransformation, and biochemical responses after exposure to arsenite and arsenate in the estuarine polychaete *Laeonereis acuta* (Nereididae), Environ. Sci. Pollut. Res. 18 (2011) 1270–1278.
[17] A.E. Geiszinger, W. Goessler, K.A. Francesconi, Biotransformation of arsenate to the tetramethylarsonium ion in the marine polychaetes *Nereis diversicolor* and *Nereis virens*, Environ. Sci. Technol. 36 (2002) 2905–2910.
[18] J. Ventura-Lima, D. Fattorini, F. Regoli, J.M. Monserrat, Effects of different inorganic arsenic species in Cyprinus carpio (*Cyprinidae*) tissues after short-time exposure: bioaccumulation, biotransformation and biological responses, Environ. Pollut. 157 (2009) 3479–3484.
[19] R.J. Erickson, D.R. Mount, T.L. Highland, J. Russell Hockett, C.T. Jenson, The relative importance of waterborne and dietborne arsenic exposure on survival and growth of juvenile rainbow trout, Aquat. Toxicol. 104 (2011) 108–115.
[20] A.A. Meharg, M.R. Macnair, Suppression of the high-affinity phosphate-uptake system—a mechanism of arsenate tolerance in *Holcus lanatus* L., J. Exp. Bot. 43 (1992) 519–524.
[21] B.P. Rosen, Families of arsenic transporters, Trends Microbiol. 7 (1999) 207–212.
[22] M.J. Chowdhury, B. Baldisserotto, C.M. Wood, Tissue-specific cadmium and metallothionein levels in rainbow trout chronically acclimated to waterborne or dietborne cadmium, Arch. Environ. Contam. Toxicol. 48 (2005) 381–390.
[23] W.R. Cullen, L.G. Harrison, H. Li, G. Hewitt, Bioaccumulation and excretion of arsenic compounds by a marine unicellular alga, *Polyphysa peniculus*, Appl. Organomet. Chem. 8 (1994) 313–324.
[24] W.G. Wallace, B.G. Lee, S.N. Luoma, Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM), Mar. Ecol. Prog. Ser. 249 (2003) 183–197.
[25] L. Zhang, W.-X. Wang, Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish, Limnol. Oceanogr. 51 (2006) 2008–2017.
[26] Y. Shibata, M. Morita, Characterization of organic arsenic compounds in bivalves, Appl. Organomet. Chem. 6 (1992) 343–349.
[27] V.W. Lai, A.S. Beach, W.R. Cullen, S. Ray, K.J. Reimer, Arsenic speciation in whelks, *Buccinum undatum*, Appl. Organomet. Chem. 16 (2002) 458–462.
[28] W. Zhang, L.M. Huang, W.-X. Wang, Arsenic bioaccumulation in a marine juvenile fish *Terapon jarbua*, Aquat. Toxicol. 105 (2011) 582–588.
[29] D.L. DeFoe, Arsenic(V) test results, Memorandum to RL Spehar, US Environmental, 1982.
[30] S.M. McGeachy, D.G. Dixon, Effect of temperature on the chronic toxicity of arsenate to rainbow trout (*Oncorhynchus mykiss*), Can. J. Fish. Aquat. Sci. 47 (1990) 2228–2234.
[31] M.G. Rankin, D.G. Dixon, Acute and chronic toxicity of waterborne arsenite to rainbow trout (*Oncorhynchus mykiss*), Can. J. Fish. Aquat. Sci. 51 (1994) 372–380.
[32] K.A. Cockell, J.W. Hilton, W.J. Bettger, Chronic toxicity of dietborne disodium arsenate heptahydrate to juvenile rainbow trout (*Oncorhynchus mykiss*), Arch. Environ. Contam. Toxicol. 21 (1991) 518–527.
[33] J.A. Hansen, J. Lipton, P.G. Welsh, D. Cacela, B. MacConnell, Reduced growth of rainbow trout (*Oncorhynchus mykiss*) fed a live invertebrate diet pre-exposed to metal-contaminated sediments, Environ. Toxicol. Chem. 23 (2004) 1902–1911.
[34] D. Boyle, K.V. Brix, H. Amlund, A.K. Lundebye, C. Hogstrand, N.R. Bury, Natural arsenic contaminated diets perturb reproduction in fish, Environ. Sci. Technol. 42 (2008) 5354–5360.
[35] R.J. Erickson, D.R. Mount, T.L. Highland, J. Russell Hockett, E.N. Leonard, V.R. Mattson, T.D. Dawson, K.G. Lott, Effects of copper cadmium, lead, and arsenic in a liver diet on juvenile fish growth, Can. J. Fish. Aquat. Sci. 67 (2010) 1816–1826.
[36] W.J. Langston, Availability of arsenic to estuarine and marine organisms: a field and laboratory evaluation, Mar. Biol. 80 (1984) 143–154.
[37] J. Wrench, S.W. Fowler, M.Y. Ünlü, Arsenic metabolism in a marine food chain, Mar. Pollut. Bull. 10 (1979) 18–20.
[38] J.G. Sanders, R.W. Osman, G.F. Riedel, Pathways of arsenic uptake and incorporation in estuarine phytoplankton and the filter-feeding invertebrates *Eurytemora affinis*, *Balanus improvisus* and *Crassostrea virginica*, Mar. Biol. 103 (1989) 319–325.
[39] S. Ciardullo, F. Aureli, E. Coni, E. Guandalini, F. Iosi, A. Raggi, G. Rufo, F. Cubadda, Bioaccumulation potential of dietborne arsenic cadmium, lead, mercury, and selenium in organs and tissues of rainbow trout (*Oncorhyncus mykiss*) as a function of fish growth, J. Agric. Food Chem. 56 (2008) 2442–2451.
[40] D.A. Hunter, W. Goessler, K.A. Francesconi, Uptake of arsenate trimethylarsine oxide, and arsenobetaine by the shrimp *Crangon crangon*, Mar. Biol. 131 (1998) 543–552.
[41] J. Gailer, K. Francesconi, J. Edmonds, K. Irgolic, Metabolism of arsenic compounds by the blue mussel *Mytilus edulis* after accumulation from seawater spiked with arsenic compounds, Appl. Organomet. Chem. 9 (1995) 341–355.
[42] Suhendrayatna, A. Ohki, T. Nakajima, S. Maeda, Studies on the accumulation and transformation of arsenic in freshwater organisms. II. Accumulation and transformation of arsenic compounds by *Tilapia mossambica*, Chemosphere 46 (2002) 325–331.

[43] Suhendrayatna, A. Ohki, S. Maeda, Studies on the accumulation and transformation of arsenic in freshwater organisms. I. Accumulation and transformation of arsenic compounds by the Japanese Medaka, *Oryzias latipes*, Chemosphere 46 (2001) 319–324.
[44] J.G. Hale, Toxicity of metal mining wastes, Bull. Environ. Contam. Toxicol. 17 (1977) 66–73.
[45] A. Inglis, E.L. Davis, Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish, Tech. Pap. Bur. Sport Fish Wildl. U.S. Dept. Interior 67 (1972).
[46] R.D. Cardwell, D.G. Foreman, T.R. Payne, D.J. Wilbur, Acute toxicity of selected toxicants to six species of fish, Ecol. Res. Ser. EPA-600/3-76-008. U.S. Environ. Prot. Agency 125 (1976).
[47] E.M.B. Sorenson, Toxicity and accumulation of arsenic in green sunfish, *Lepomis cyanellus* exposed to arsenate in water, Bull. Environ. Contam. Toxicol. 15 (1976) 756–761.
[48] R.M. Pedlar, J.F. Klaverkamp, Accumulation and distribution of dietborne arsenic in lake whitefish (*Coregonus clupeaformis*), Aquat. Toxicol. 57 (2002) 153–166.
[49] S. Roy, S. Bhattacharya, Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of *Channa punctatus*, Ecotoxicol. Environ. Saf. 65 (2006) 218–229.
[50] J.A. Nott, A. Nicolaidou, Bioreduction of zinc and manganese along a molluscan food-chain, Comp. Biochem. Physiol. A 104 (1993) 235–238.
[51] W.G. Wallace, G.R. Lopez, Bioavailability of biologically sequestered cadmium and the implications of metal detoxification, Mar. Ecol. Prog. Ser. 147 (1997) 149–157.
[52] K.A. Cockell, Chronic toxicity of dietborne arsenic to rainbow trout, *Oncorhynchus mykiss*, Ph.D. Thesis, University of Guelph, Guelph, Ontario, 1990, p. 187.
[53] R. Schaeffer, K.A. Francesconi, N. Kienzl, C. Soeroes, P. Fodor, L. Váradi, R. Raml, W. Goessler, D. Kuehnelt, Arsenic speciation in freshwater organisms from the river Danube in Hungary, Talanta 69 (2006) 856–865.
[54] C.-W. Liu, C.-P. Liang, K.-H. Lin, C.-S. Jang, S.-W. Wang, Y.-K. Huang, Y.-M. Hsueh, Bioaccumulation of arsenic compounds in aquacultural clams (*Meretrix lusoria*) and assessment of potential carcinogenic risks to human health by ingestion, Chemosphere 69 (2007) 128–134.
[55] D. Fattorini, A. Notti, F. Regoli, Characterization of arsenic content in marine organisms from temperate tropical, and polar environments, Chem. Ecol. 22 (2006) 405–414.
[56] K.A. Francesconi, J.S. Edmonds, R.V. Stick, Accumulation of arsenic in yelloweyed mullet (*Aldrichetta forsteri*) following oral administration of organoarsenic compounds, Sci. Total Environ. 79 (1989) 59–67.
[57] H. Amlund, M.H.G. Berntssen, Arsenobetaine in Atlantic salmon (*Salmo salar* L.): influence of seawater adaptation, Comp. Biochem. Physiol. 138C (2004) 507–514.
[58] H. Amlund, K. Francesconi, C. Bethune, A.-K. Lundebye, M. Berntssen, Accumulation and elimination of dietborne arsenobetaine in two species of fish, Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.), Environ. Toxicol. Chem. 25 (2006) 1787–1794.
[59] K.A. Francesconi, J.S. Edmonds, Arsenic and marine organisms, Adv. Inorg. Chem. 44 (1997) 147–189.
[60] S. Ciardullo, F. Aureli, A. Raggi, F. Cubadda, Arsenic speciation in freshwater fish: focus on extraction and mass balance, Talanta 81 (2010) 213–221.
[61] A.Q. Shah, T.G. Kazi, J.A. Baig, H.I. Afridi, G.A. Kandhro, M.B. Arain, S.K. Wadhwa, N.F. Kolachi, Determination of inorganic arsenic species (As^{3+} and As^{5+}) in muscle tissues of fish species by electrothermal atomic absorption spectrometry (ETAAS), Food Chem. 119 (2010) 840–844.
[62] A. Notti, D. Fattorini, E.M. Razzetti, F. Regoli, Bioaccumulation and biotransformation of arsenic in the Mediterranean polychaete *Sabella Spallanzanii*: experimental observations, Environ. Toxicol. Chem. 26 (2007) 1186–1191.
[63] D.J. Thomas, Molecular process in cellular arsenic metabolism, Toxicol. Appl. Pharmacol. 222 (2007) 365–393.
[64] K.J. Irgolic, Arsenic in the environment, in: A.V. Xavier (Ed.), Frontiers in Bioinorganic Chemistry: International Conference on Bioinorganic Chemistry., VCH, Weinheim, German; Deerfield Beach, FL, 1986.
[65] C.J. Langdon, A.A. Meharg, J. Feldmann, T. Balgar, J. Charnock, M. Farquhar, T.G. Piearce, K.T. Semple, J. Cotter-Howells, Arsenic-speciation in arsenate-resistant and non-resistant populations of the earthworm, *Lumbricus rubellus*, J. Environ. Monit. 4 (2002) 603–608.
[66] T. Kuroiwa, A. Ohki, K. Naka, S. Maeda, Biomethylation and biotransformation of arsenic in a freshwater food chain: green alga (*Chlorella vulgaris*) → shrimp (*Neocaridina denticulata*) → killifish (*Oryzias latipes*), Appl. Organomet. Chem. 8 (1994) 325–333.
[67] A.Q. Shah, T.G. Kazi, M.B. Arain, M.K. Jamali, H.I. Afridi, N. Jalbani, J.A. Baig, G.A. Kandhro, Accumulation of arsenic in different fresh water fish species-potential contribution to high arsenic intakes, Food Chem. 112 (2009) 520–524.
[68] D.J. Thomas, M. Styblo, S. Lin, The cellular metabolism and systemic toxicity of arsenic, Toxicol. Appl. Pharmacol. 176 (2001) 127–144.