Bull Environ Contam Toxicol (2012) 89:445–449
DOI 10.1007/s00128-012-0714-8

Analysis of Arsenic Bioaccumulation in Different Organs of the Nutritionally Important Catfish, *Clarias batrachus* (L.) Exposed to the Trivalent Arsenic Salt, Sodium Arsenite

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Received: 17 December 2011 / Accepted: 19 June 2012 / Published online: 6 July 2012

Abstract Pattern of arsenic bioaccumulation in six organ systems (blood, brain, gills, liver, muscles and skin) of *Clarias batrachus* was analysed following exposure to sublethal (1 mg L^{-1}; 5 % of 96 h LC_{50} value) concentration of sodium arsenite. After 60 days of treatment the liver accumulated highest concentration (9.711 ± 0.138 μg g^{-1} dry wt of tissue.) of arsenic followed by gills (6.156 ± 0.154) > blood (6.070 ± 0.043) > muscles (5.756 ± 0.123) > skin (5.606 ± 0.140) > brain (2.350 ± 0.205). The bioaccumulations of arsenic in all the tissues were time dependant and increased with exposure period. Although the exposed fish loaded with arsenic did not die after prolonged treatment (60 days), the amount of arsenic accumulated made them unsuitable for human consumption. Due to depletion of the proteineous components of their muscles, the body mass of the exposed fish decreased without corresponding decrease in their length. This made the fish lean and thin. These proteineous moieties of the muscles and other tissue systems of the stressed fish were mobilized for breakdown to generate additional requirement of energy to combat the arsenic toxicity.

Keywords Arsenic bioaccumulation · *Clarias batrachus* · Sodium arsenite · Toxicity

One of the major causes of aquatic pollution is due to contamination by toxic metals. As a result animals living in polluted waters show increasing trends of accumulation (Sorenson 1976; Larsson et al. 1985; Pazhanisamy et al. 2007) of these non-degradable toxicants. The contamination by metals has so much deteriorated the wet lands that there is a universal urge to estimate toxic metal levels in the aquatic ecosystem including its biota. Attention was also drawn to find out contamination level of public food stuffs particularly in fishes. For this, the aquatic environments need regular monitoring for metal contamination of their water, sediment and fauna. Heavy metals measured in tissues of aquatic animals reflect the past exposures because they accumulate in tissues and organs of the animals. Analysis of tissue accumulation of the toxic metals can also be a reasonable measurement for public health standards and for animals' well being. Amongst the different harmful metals, stress of arsenic has become a recent concern of great importance in various areas of the Indian subcontinent. Although the important reasons for elevated level of arsenic concentration of ground as well as surface waters are anthropogenic activities due to industrial and agricultural development, microbial activities have further aggravated the arsenic contamination. Extensive exploitation of arsenic contaminated ground waters for human use has increased the arsenic level of the surface water. Pandey et al. (2002) reported that inorganic arsenic is more mobile under anaerobic condition of ground water leading to increased chances of arsenic contamination into surface water. Arsenic pollution of the aquatic ecosystems also occurs due to weathering of arsenic containing natural rocks and volcanic eruptions as well as industrialization including petroleum refining, ceramic manufacturing, geothermal power plants, non-ferrous smelting, gold-mine tailings, fly ash leaching and, extensive use of arsenic containing pesticides and wood preservatives.

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The fish fauna inhabiting in the wetlands of these areas get easily contaminated with this toxic metalloid. The knowledge of the pattern of arsenic distribution in different tissue could illustrate its order of preference for these organ systems.

Consumption of the contaminated fishes might also cause its biotransfer as well as bioconcentration. Hence the purpose of this study has been to analyze the concentration of arsenic accumulation in certain important organ systems of the nutritionally important catfish *Clarias batrachus* exposed to a trivalent arsenic salt (sodium arsenite). Work related to arsenic accumulation in fishes is mainly related to their liver and muscles (Pazhanisamy et al. 2007). Recently Kumar and Banerjee (2012) analyzed the negative toxic impact of sodium arsenite on certain nutritionally important biomolecules of *C. batrachus*.

Materials and Methods

The freshwater catfish *C. batrachus* (Linn.) (15 ± 1 cm in length, weighing 45 ± 5 g) were purchased from a single population from the Chaukaghat fish market at Varanasi. The fish were acclimated in tap water (dissolved O_2 6.3 mg L^{-1}, pH 7.2 water hardness 23.2 mg L^{-1} and room temperature 28 ± 3°C) (without chlorine contamination) in large plastic aquaria for 21 days under laboratory conditions. They were fed *ad libitum* with fresh minced goat liver on every alternate day. Waters were renewed after every 24 h with routine cleaning of the aquaria.

The 96 h LC_{50} of sodium arsenite for *C. batrachus* were calculated following Trimmed–Spearman–Karber Method and it was found to be 20 ppm (Fig. 1). For 96 h LC_{50} value detection we have followed the details given by Banerjee (1993).

Twenty groups of ten fish each were exposed separately to a sublethal concentration (1 mg L^{-1}; 5 % of 96 h LC_{50} value) of sodium arsenite (Batch No G270707 Loba Chemie Pvt. Ltd. Mumbai, minimum assay 98.5 %–102 %) in the plastic aquaria containing 10 L of the arsenic solution for 60 days. Side by side twenty control groups of ten fish each were retained in 10 L of plain tap water (without having the arsenic salt) under identical laboratory conditions. As per 24 h bio assay norms, both the solutions were renewed after every 24 h. During the entire period of the experiment, six fish from exposed groups and two from control troughs were found dead. They were discarded.

For the analysis of the arsenic bioaccumulation, nine fish from each of exposed as well as control groups were cold anesthetized and sacrificed by spinal dislocation after the expiry of 0 day, 10, 30, 45, and 60 days of experimentation for the collection of required tissue samples. Before sacrifice the weight and length of the fish were also measured after the various exposure periods (Table 1).

Entire brain, liver, gills and small quantities of muscle, blood and skin of experimental as well as control groups were dissected and weighed. To minimize trace element contamination, all the laboratory glasswares were soaked in 15 % nitric acid for 24 h and rinsed repeatedly in deionized water prior to use. Each of these samples was transferred separately into a 25 mL volumetric flask. 1–2 mL of deionized water was added to wet the sample followed by addition of 5 mL of analytical grade mixture of acids—H_2SO_4:HNO_3:$HClO_4$ at the ratio of 1:6:1 to the digestion container. The samples were kept overnight in an oven at 105°C for completion of digestion. Estimation of arsenic was carried out by atomic absorption spectrophotometer (Elico ASS, M-173) using arsenic (99 %) as standard. In cases where results were outside the acceptable limits for this salt, the samples were re-analysed. Care was taken to see that more than 95 % of the analyses of standard reference material were within the detectable limit of the instrument. Arsenic accumulation in the desired tissues of *C. batrachus* was estimated and the results were expressed as μg g^{-1} dry wt of tissue.

The data obtained from the experiments were shown as mean ± SEM and the results were evaluated using Student *t* test. The levels of significance of metal accumulation in

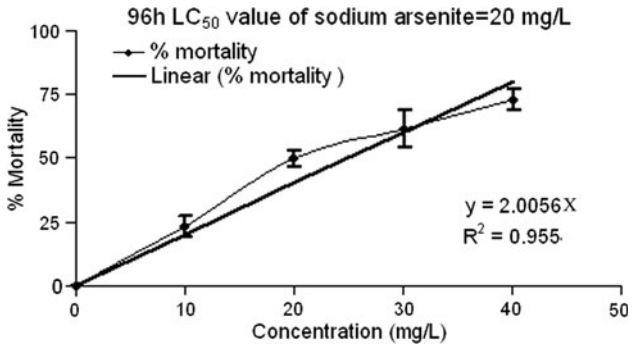

Fig. 1 Graphical presentation of 96 h LC_{50} value of sodium arsenite for catfish, *C. batrachus* (L.)

Table 1 Alterations in morphological parameters of *C. batrachus* exposed to sodium arsenite

Exposure period	Body weight (g)	Body length (cm)
Unexposed (control)	47.00 ± 0.66	15.44 ± 0.07
0 day	47.00 ± 0.66	15.44 ± 0.07
10 days	44.00 ± 0.86*	15.36 ± 0.08
30 days	40.00 ± 0.99*	15.27 ± 0.07
45 days	36.77 ± 0.79*	15.17 ± 0.79
60 days	33.55 ± 1.13*	15.05 ± 0.20

Values are shown as mean ± SEM (n = 9), the level of significance was denoted as: * ($p < 0.05$) for comparisons of arsenic exposed fishes to unexposed control fish

exposed fish were compared to 0 day exposed fishes, because the metal accumulation in all the tissues of untreated control fish were below the detectable limits. Differences were considered as statistically significant at $p < 0.05$ and $p < 0.001$.

Results and Discussion

The arsenic levels in all the six tissues of untreated control *C. batrachus* were below the detectable limits of the instrument (Table 2). Palaniappan and Vijayasundaram (2009) also noticed below detectable level of arsenic in untreated control major carp *Labeo rohita* fingerlings. However small quantities of arsenic were detected in all the organ systems of 0 day exposed fish. The concentration of arsenic in these tissues was however not identical. The liver contained maximum amount (0.133 ± 0.004) followed by gills (0.113 ± 0.002) > blood (0.107 ± 0.002) > muscles (0.106 ± 0.003) > skin (0.104 ± 0.002) > brain (0.102 ± 0.003). This finding is supported by the fact that several fish species (Sorenson 1976; Maher et al. 1999) have relatively high arsenic tolerance values. Following exposure the arsenic concentration in all the six tissues of *C. batrachus* increased progressively and after 60 days when the experiment was terminated, the amount of arsenic in liver continued to be maximum (9.711 ± 0.138) followed by gills (6.156 ± 0.154) > blood (6.070 ± 0.043) > Muscle (5.756 ± 0.123) > skin (5.606 ± 0.140) > brain (2.35 ± 0.205) (Table 2).

The rate of arsenic uptake in different tissues of exposed *C. batrachus* was almost linear in respect of exposure period (Fig. 2a–f). This study also found that the amount of arsenic uptake is also organ specific. Chen et al. (2001) found that *Tilapia* potentially regulate the concentration of metal in the tissue with time by combining the process of absorption, excretion, detoxification and storage.

The arsenic level in the liver of 0 day exposed as well as 60 days exposed fish was maximum because it is the main organ of detoxification and also synthesis of stress proteins. According to Datta et al. (2007) it is a major target organ for arsenic toxicity and acts as sensitive index of the toxicant. Maher et al. (1999) also found highest level of arsenic accumulation in the liver than any other tissue of *Mugil cephalus*. Palaniappan and Vijayasundaram (2009) noticed that after kidney the bioaccumulation pattern of arsenic in *Labeo rohita* was in order: liver > gills > muscle > brain > bone. Accumulations of substantial amount of several other heavy metals were also found in the liver of many fishes (Noel-Lambot et al. 1978). The reason for increased concentration of various metals in the liver might be because this vital organ system is the main site of synthesis of various proteins and other molecules known to have high affinities for metals forming complexes (Fernandes et al. 2008).

Apart from brain, the skin of 0 day exposed *C. batrachus* showed least amount of arsenic accumulation (Table 2). This was caused by loss of arsenic due to sloughing of excessive amount of slime secreted by the goblet cells under stressed condition which takes place instantaneously. The glycoprotein components of the slime secreted by the fish epidermis are well known for binding metals from the environment and eliminate them from the body surface. Being the boundary tissue the skin faces the contact stress of the ambient arsenic salt. Hence its epidermal cells get easily damaged due to metal contamination. These damaged cells are frequently discarded by regular sloughing from the outer layers of the epidermis (Banerjee 2007) causing reduction of its arsenic load. After liver the gills showed maximum quantity of arsenic accumulation perhaps due to the thin barrier distance between the ambient arsenic and blood in their secondary lamellae. The other reason might be the increased density of the chloride cells whose active role in ion accumulation is well established (Banerjee 2007). The higher concentration of arsenic accumulation in the brachial

Table 2 Bioaccumulation of arsenic (μg g^{-1} dry weight) in different tissues of *C. batrachus* at various stages of exposure to sodium arsenite

Exposure period	Liver	Gills	Blood	Muscles	Skin	Brain
Unexposed (control)	<Detection[a] limit	<Detection limit	<Detection limit	<Detection limit	<Detection limit	<Detection limit
0 day	0.133 ± 0.004	0.113 ± 0.002	0.107 ± 0.002	0.106 ± 0.003	0.104 ± 0.002	0.102 ± 0.003
10 days	1.845 ± 0.011	1.804 ± 0.010	1.743 ± 0.025	1.42 ± 0.045	1.310 ± 0.062	0.105 ± 0.001
30 days	3.769 ± 0.095	2.856 ± 0.061	4.050 ± 0.1249	3.433 ± 0.037	3.200 ± 0.098	0.182 ± 0.003
45 days	5.938 ± 0.054	4.253 ± 0.093	5.476 ± 0.263	4.133 ± 0.023	4.030 ± 0.098	1.653 ± 0.015
60 days	9.711 ± 0.138	6.156 ± 0.154	6.070 ± 0.043	5.756 ± 0.123	5.606 ± 0.140	2.350 ± 0.205
	($p < 0.001$)	($p < 0.001$)	($p < 0.001$)	($p < 0.001$)	($p < 0.001$)	($p < 0.001$)

Values are shown as mean ± SEM (n = 9), the level of significance was denoted as: $p < 0.001$ for comparison of arsenic exposed fishes to zero (0) day exposed fishes

[a] Detection limit for arsenic of atomic absorption spectrophotometer is 0.01 μg g^{-1} dry wt of tissue

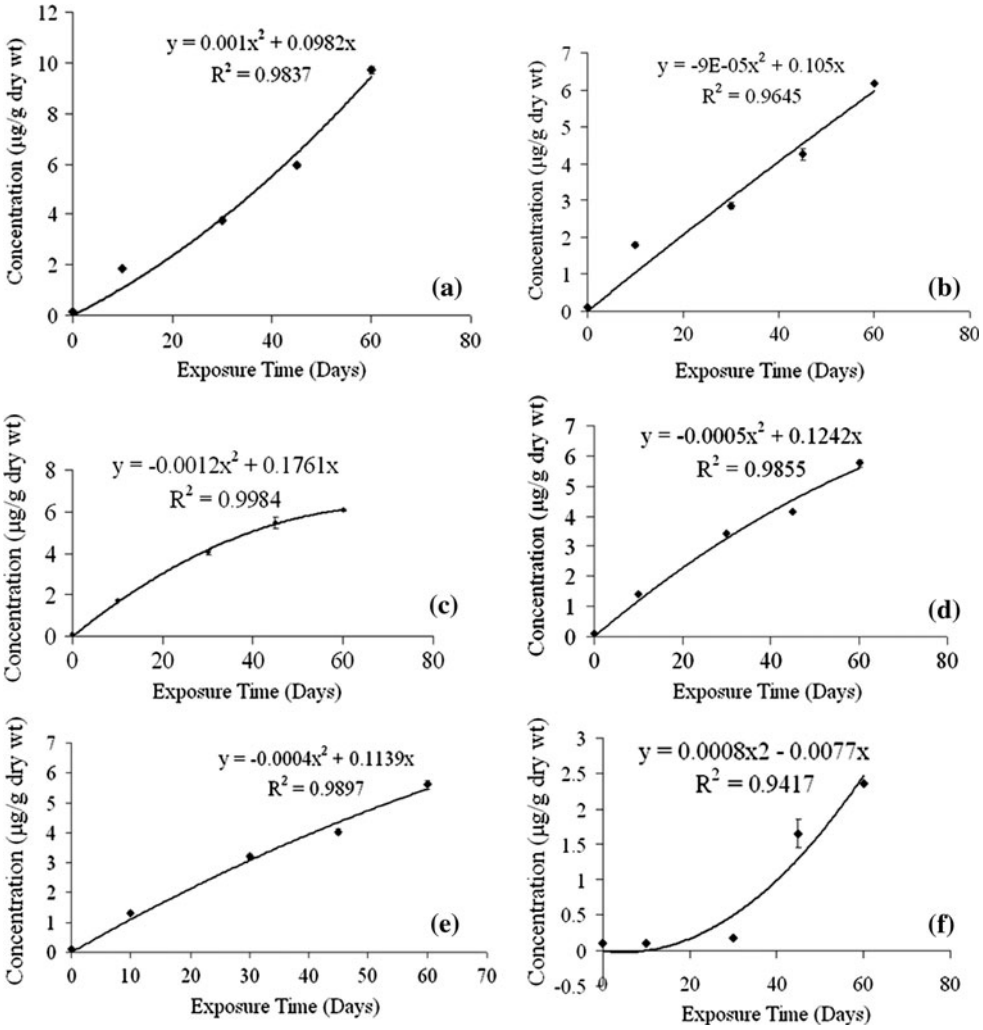

Fig. 2 Regression curve showing relationship between exposure time and arsenic accumulation in **a** liver, **b** gills, **c** blood, **d** muscles, **e** skin and **f** brain tissues respectively

tissue reflects the direct contact of the gills with arsenic contaminated water. Accumulation of large amounts of several other heavy metals in the gills of exposed fishes is well documented (Yang et al. 2007). The accumulation of arsenic in the blood after 60 days is quite high, reaching nearer to the level of the gills.

The amount of arsenic in the muscles are not so high as compared to liver, gills and blood perhaps because the muscles are comparatively less metabolically active in accumulating metals (Wagner and Boman 2003). Accumulation of lesser quantity of arsenic and other metals in the muscles of different fishes in comparison to liver and other tissues has also been reported by Maher et al. (1999). Among the tissues examined the arsenic accumulation after 60 days in the brain was least, perhaps due to relatively less blood flow in the brain. Palaniappan and Vijayasundaram (2009) also noticed least concentration of arsenic accumulation in fish brain. The reason for less arsenic accumulation in brain after 30 days is tight blood brain barrier. However increase in the arsenic level subsequently might be due to damage caused by the arsenic to this tight blood brain barrier as reported in rats by Rodriguez et al. (2001). This might also be true for arsenic contaminated fishes.

Following exposure the weight of fish also decreased and became less by 21.15 % after 60 days. At this stage the amount of arsenic accumulation was highest in all the tissues. The decrease in the body weight took place mainly due to decrease in the proteineous content of the skeletal muscles (45.42 % decrease in muscle protein was noticed by Kumar and Banerjee 2012). However there was no simultaneous decrease in the length of the fish which indicates leaning and thinning of the fish body due to loss of its muscular components which build the mass of the fish. This loss of body weight of the fish might also be due to depletion of lipids (29.83 %) and carbohydrates (51.72 %) so reported by Kumar and Banerjee (2012). The loss of these macromolecules might have taken place following their breakdown for maintaining additional energy supply to combat the toxicity of the arsenic stress (Kumar and Banerjee 2012).

Gilderhus (1966) observed reduced survival and growth of bluegills after exposure to 4,000 μg L^{-1} sodium arsenite for 16 weeks. Similar to these findings the decreased body weight exhibited by *C. batrachus* in this experiment might be due to depletion of proteins from liver and muscles as noticed by Kumar and Banerjee (2012) who found progressive and significant reduction of glycogen and protein molecules from these two vital tissue systems after 60 days of arsenic exposure. Maximum accumulations of arsenic in these two tissues were also observed after this period of exposure (Table 2). Kumar and Banerjee (2012) also estimated the lipid contents in the liver and muscles of arsenic stressed fish. The increase in the lipid moieties were however not progressive with the increased concentration of arsenic accumulation but fluctuated at different exposure period. They concluded that the increased lipoidal contents in the liver were perhaps due to fatty degeneration of this vital organ system. Although, no significant mortality of the fish occurred till the conclusion of the experiment (after 60 days of exposure), excessive deposition of arsenic in the different tissues of *C. batrachus* made them unsuitable to serve as food. Fishes living in natural waters even having low concentration of inorganic arsenic for prolonged periods might accumulate substantial amount of the toxic metal and make themselves unsuitable for human consumption. Hence regular monitoring of water as well as the fish tissue is important.

Acknowledgments The senior author thanks the University Grant Commission Government of India for providing a Junior Research Fellowship. Thanks are also due to Dr. A. S. K Sinha Professor and Incharge Sophisticated Instruments laboratory, Department of Chemical Engineering and Technology, Institute of Technology, Banaras Hindu University, Varanasi for providing the Atomic absorption spectrophotometer facilities.

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