Original Article

Comparative Study of Cadmiumand Arsenic Accumulation in Toothed Carp (*Aphanius sophiae*)in Fresh and Salt Water

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ABSTRACT

Background: Anthropogenic activities release high concentrations of heavy metals into the aquatic ecosystems, which can be absorbed by the aquatic organisms. In this study, the accumulation of cadmium (Cd) and arsenic (As) was compared in liver, gill and muscle tissues of toothed carp (*Aphanius sophiae*) in fresh and salt water.

Methods: A total of 175 fish samples were collected from the Shoor River, Iran during the spring and summer of 2011. Samples were divided into two groups for salt and freshwater experiments. The individuals of each group were also divided into seven groups, a control group and the other six exposed to the concentrations of 5, 10 and 20 mg/L of arsenic and 5, 10 and 20 mg/L of cadmium. The liver, gills and muscle tissues of the specimens were dissected. The tissues were wet digested in acid and the concentrations of metals were measured using an ICP-OES instrument.

Results: The concentrations of both metals were significantly different in various organs in both fresh and salt water and it were in order of liver > gill > muscle (P <0.001). Based on t-test results, no significant difference was observed between the concentrations of As in related tissues of fish cultivated in fresh and salt water. However, Cd accumulation was significantly higher in the tissues of fish specimens cultivated in freshwater (P <0.001).

Conclusion: The bioaccumulation of Cd and As depends on organs, metals, and water condition.

Keywords: Arsenic, Cadmium, Carps, Water Pollution.

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INTRODUCTION

Contamination of aquatic ecosystems with metals is a worldwide problem. Once metals are accumulated by an aquatic organism, they can be transferred to the upper levels of the food chain [1].Metals are ecologically and biologically important due to high persistence, toxicity and accumulation in tissues. Among elements in aquatic ecosystems, Arsenic (As) and cadmium (Cd) are influential pollutants derived from agriculture and industrial activities. These metals have long been considered environmental pollutants because of their high toxicity and bioavailability at low concentrations. One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms. "Therefore, it is of great importance to know the bioaccumulation potential of a pollutant" [2].

Heavy metals can accumulate in high levels in liver, muscles, kidney and gill tissues due to their higher metabolism[3]. Liver plays an important role in absorbing and storing heavy metals and hence body detoxification. Gills are organs in direct contact with the surrounding environment; therefore, they can easily absorb pollutants such as metals [4]. *Aphanius* is a small fish and male and female sexes are distinguishable from their appearances [5]. It tolerates different aquatic conditions, for example two different populations of them live in Cheshmeh Ali Damghan (fresh water) and Eshteahard Shoor River (salt water) in Iran. Therefore, this study aimed to investigate the accumulation of arsenic and cadmium in liver, muscle and gill tissues of toothed carp (*Aphanius sophiae*) in both fresh and salt water. The results of this research could

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lead to a better understanding of the effects of pollutants on aquatic ecosystems and their organisms.

MATERIAL AND METHODS

A total of 175 toothed carp (*Aphaniussophiae*) with average weight (±SD) of 9.5 (± 0.3) g and average length of 7.6 (± 0.4) cm were collected from Eshtehard Shoor River in Karaj City from Jun to August of 2011.Sampling stations were located along one of the branches of Eshtehard Shoor River (35º36'31"N, 50º48'23"E), 1143 meters above the sea level. During the sampling season, the average dissolved oxygen concentration was 11.68 mgL^{-1}, the salinity was 11-12 g L^{-1}, mean water temperature was 12.85±6.22 °C and pH varied between 7 and 8.5.

Samples were transported into the laboratory using a tank equipped with aeration. Fish specimens were kept in a 1000-liter tank with proper aeration for 5 days in order to be adapted with the new conditions (including: average dissolved oxygen 8 mg L^{-1}, average water temperature 27.5 ± 6.22 °C and pH between 7.27 to 8.24). Samples were then divided into two groups for salt and freshwater experiments. Each group were also divided into seven groups, six groups were exposed to concentrations of 5, 10 and 20 µg/g of arsenic and 5, 10 and 20 µg/g of cadmium (12 treatments in total) and one group was the control. Metal sollutions were prepared using arsenic oxide (As_2O_3) and cadmium chloride ($CdCl_2$) (Merck, Germany). The fish were kept for 18 days, and then 15 specimens from each treatment were collected for further analysis. After biometry and sex determination, the gill, liver and muscle organs were dissected and weighed. For metal analysis in each organ, five fish specimens were considered as one sample (due to the very low weight of the organs). Therefore, three samples of liver, gill and muscle were obtained from each treatment (extracting from 15 fish specimens). Ten mL of nitric acid (65%) (Merck, Germany) was added to each sample (in a conical flask) and left overnight. Then, 3mL of hydrochloric acid (70%) (Merck, Germany) was added and the samples were heated on a hotplate until evaporation. Afterwards, 25 mL of 1% nitric acid was added to each flask and the weight of each solution was measured and recorded [6,7]. All plastic and glassware used in this study were acid washed in an acid bath (HCl 10%) for two days then rinsed three times with deionized water.

The concentrations of As and Cd in the solutions were measured by an ICP- OES (GBC Integra XL, Australia) instrument. The laboratory contamination and recoveries were controlled using blank and spiked samples, respectively. The detection limits, blanks and recoveries of the measurements of metals in the samples are presented in Table 1.

Spilt- plot design was used to investigate the effects of different environmental conditions on accumulation of Cd and As. Data normality and homogeneity were examined by Kolmogorov-Smirnov and Levene test. s One-way-ANOVA and Duncan's test were used for general and multiple comparisons (at a 99% of confidence), respectively. All statistical analyses were performed by SPSS16 software (Chicago, IL, USA). Ethical considerations in this paper were carried out.

RESULTS

Results of *t*-test (Figures 1 and 2) indicated that As accumulation in fish organs had no significant difference between fresh and salt water, while Cd accumulation in organs was significantly different between these two environments (P <0.001).

Table 1. Detection limits, blanks and recoveries of the measurements.

Detection limit (µg/g)	Recovery (Mean (%)±SD)	Blank (Mean (µg/g)±SD)	Element
0.29	96.10 ± 3.77	0.054 ± 0.006	Cd
0.19	97.50 + 1.46	0.0±0.0	As

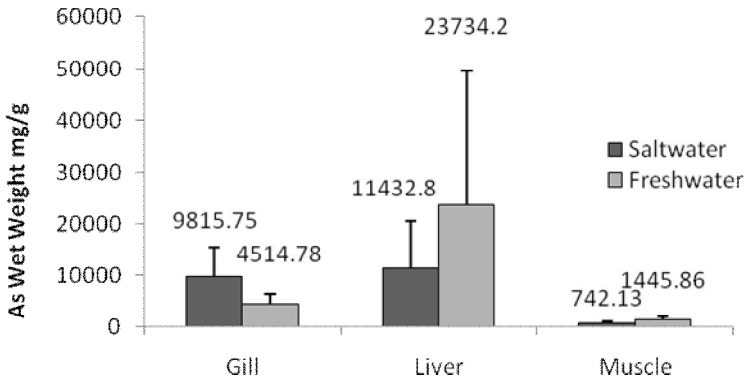

Figure 1. Comparison of As accumulation in fish (*Aphanius sophiae*) organs in salt and fresh water.

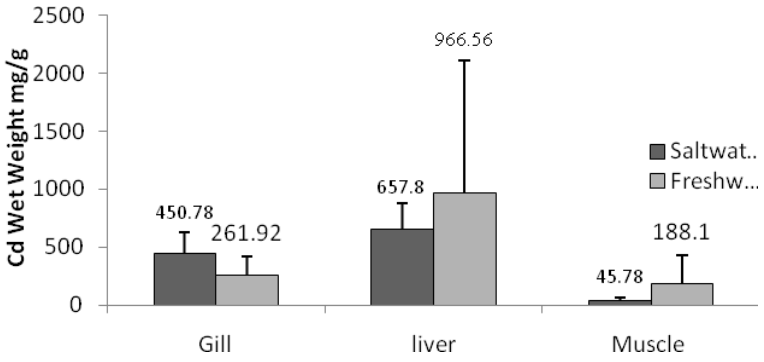

Figure 2. Comparison of Cd accumulation in fish (*Aphanius sophiae*) organs in salt and fresh water

The concentrations of both metals were significantly different in various organs in both fresh and salt water; and the concentrations were in order of liver > gills > muscles(P <0.001). Due to comparatively high accumulation of Cd and As in liver, it was identified as the target organ for Cd and As accumulation. Generally, accumulation of As in the organs were higher than Cd. Results indicated that fish mortality in fresh water was 57% (25% and 32% in As and Cd treatments, respectively). The percentage of fish mortality in salt water was 5%, observed only in As treatments. Therefore, mortality in fresh water was 11.4 times higher than in salt water.

The results of Spilt-plot design showed significant relationship between salinity, type of organs and different concentrations of As ($F(9.72) = 13.132$, P<0.0001). An increase in salinity enhanced As accumulation in treatments of 5 and 20µg/g, but not in the treatments of 0 and 10µg/g As. Accumulation of As decreased in liver and muscle by increasing salinity, while in gill it increased. No significant relationship was found between type of organ, different treatments of Cd and salinity. Unlike in the concentrations of 0, 5 and 10 µg/g, Cd accumulation increased by increasing salinity in concentration of 20 µg/g. By increasing salinity, Cd concentrations in muscle and liver decreased while, in gills increased. The highest Cd and As accumulations were observed in liver and in 10µg/g treatment, however no difference was observed in other organs or other treatments.

DISCUSSION

The order of Cd and As accumulation in both environments was: liver > gill > muscle. This order was similar to the findings of other researchers in some fresh and salt-water fish [8-11]. Although Ekeanyanwu*et al.* [12] and Turkmen and Ciminli [13] determined gill as target organ of Cd in fresh water fish (Gill > liver > muscle).

The five main routs of pollutant uptake in fish are gills, food or non-food particles, water passing through the mouth, and absorption via the skin. Then the pollutants are carried by blood vessels into the liver for storage or transmission

to gall, gill or muscle, for further storage or excretion [14]. Liver tends to accumulate high concentrations of metals and has a key role in metabolism. It is also a place for accumulation of metals, biological transmission and detoxification in fish [15-17]. Therefore, liver is a proper indicator of environmental pollution [16].

Gills can absorb metals directly from water or indirectly from food. Metals can be accumulated in gills after formation of metal complexes, which is the product of the reaction of metals and mucus [17, 16]. Gill is the first organ exposed to water pollutants and can be considered as an indicator of metal pollution. Metal uptake in different organs varies depending upon their metabolic activities [18]. The lowest concentrations of As and Cd observed in muscles was due to lower metabolic activity of this organ[15, 19, 20]. It might also be due to low level of binding proteins in muscle tissues [21].

Researchers have reported different interactions between salinity and metal uptake. Dutton andFisher stated that accumulation of As and Cd in gill was enhanced by increasing salinity[22]. However, a reverse relationship between salinity and As and Cd accumulation in muscle and liver, was reported by Karakoc [23], Zanders and Rojas [24].

Heavy metals accumulation in various organs can disturb structural and functional integrity of fish organs [25]. The toxicity of heavy metals in fish is a function of free metal ion concentration controlled by the concentration of chloride in water [26]. When chloride ion concentration increases, the concentration of free metal ions to total metal concentration reduces due to bonds with chloride ions[27]. By decreasing salinity, negative potential difference between inner and outer walls of the cells increases, consequently ion transport to organs is enhanced [23]. Decrease in salinity and increase in Cd concentration and temperature enhance cadmium binding protein (CdBP) levels. Accumulation of CdBP is not only affected by Cd concentration, but also by environmental factors [28].

Exposure of tissues to Cd in dilute seawater enhances metal uptake rate possibly due to calcium transport mechanisms [24]. Metal accumulation in organisms depends on several factors including ecological requiremenst, metabolism, severity of water pollution, food, salinity, temperature and sediments [16]. The interactions of organ type and different concentrations of Cd and As in our study were in conformity with findings of Howard and Hacker [28], Shuhaimi-Othman et al. [29] and Karakoc [23]. Generally, accumulation of heavy metals depends on metal concentration, exposure time, environmental conditions (water temperature, pH, hardness and salinity) and inherent factors (fish age and nutrition habitats).

CONCLUSION

The accumulation of Cd and As in *A. sophiae* was observed to be rapid and bioaccumulation of Cd and As was higher in liver than gill and muscle. The accumulation of Cd and As in *A. sophiae* depend on the organ, water condition (fresh and salt water), and kind of metal.

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