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Research Article

Bioaccumulation and Bioconcentration of cadmium and arsenic in the organs of endemic fish toothed carp (*Aphanius sophiae*)

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Abstract

The aims of this study was to investigate the pattern of bioaccumulation and bioconcentration factor of cadmium and arsenic in livers, gills and muscles of endemic fish toothed carp (*Aphanius sophiae*) exposed to three treatments of cadmium (5, 10 and 20 mg/L) and three treatments of arsenic (5, 10 and 20 mg/L) for a period of 18 days. From Jun to August 2011, Toothed carp (*Aphanius sophiae*) with average weight (+SD) of 9.5 (± 0.3) g and average length of 7.6 (± 0.4) cm were received from Eshtehard Shoor river in Karaj city. Cadmium and arsenic was assayed using ICP-OES instrument and the results were given as mg/g wet weight. This finding showed that the accumulation patterns of cadmium and arsenic are in the following order: liver > gill > muscle. The bioconcentration factor in all concentrations of cadmium and arsenic are in the following order: liver > gill > muscle. The results indicated that the target organ for accumulation of cadmium and arsenic is liver.

Keywords: Toxicity, sub-lethal dose, fish organ, freshwater fish

INTRODUCTION

The contamination of aquatic ecosystems (e.g. lakes, rivers, streams, etc.) with heavy metals have a sever effect on aquatic organisms due to their toxicity, long persistence, bioavailability and incorporating onto the trophic chain including human being (Mansouri et al., 2012 a,b). Heavy metal pollution of aquatic environments also affects the ecological balance and threatens its biodiversity (Samanta et al., 2010). Cadmium and arsenic are widespread toxicants in the aquatic environment as a consequence of both anthropogenic and natural processes (Sobhanardakani et al., 2011; Majnoni et al., 2013). They can accumulate in various organs of aquatic animals especially in fish (Mansouri et al., 2012c).

Bioaccumulation of heavy metals can reflects the amount of toxin ingested by organism, pattern in which the metals are distributed through different organs and its extent to which the metals remained in organs (Senthil Murugan et al., 2008). Hence, the aquatic organisms such as fish is widely used as biological monitor in environmental monitoring programmes for monitoring anthropogenic heavy metal pollutants (Farkas et al., 2003). Fish as an important source of protein for human (Patterson, 2002; Majnoni et al., 2013), can absorb heavy metals from the surrounding ecosystem depending on a variety of factors including exogenous (i.e temperature, salinity, pH and seasonal changes) and endogenous (characteristic of the individuals or species as habitat, the exposure period, the concentration of the element, food preferences and metabolic rate) parameters which may change the state of toxicity of chemicals (Mansouri et al., 2011b; Ebrahimpour et al., 2010).

Fish accumulate considerable amounts of metals in their organs, especially in the livers and gills, and it is a bio-indicator of these metals for environmental pollution (Dural et al., 2006). Therefore, information of metal levels in the

organs of fish is highly important both with respect to nature management and human consumption of fish.

Liver is one of the metabolic tissues playing an important role in detoxification and storing of metals. Moreover it can be considered as a place for studying pathological affects in relation to heavy metals pollution (Tekin-Ozan and Kir, 2008). Gills are the main route for the entrance of metals into the aquatic organisms and act as a reservoir for accumulation of metals. When metal concentrations in aquatic ecosystems are high, gills are the first tissues get affected by these pollutants (Oronsaye and Brafield, 1984).

Toothed carp (*Aphanius sophiae*) is one of the endemic fish in Iran, which belongs to *Cyprinodontidae* (Helfaman et al., 2009). This fish has small size and male and female sexes are distinguishable from the appearance (Abdoli, 1999). They can live in different conditions such as salt water and fresh water. Therefore it can be used as a model for comparing the effects of metals in both environments. The objective of the present study was to investigate the pattern of bioaccumulation and bioconcentration of cadmium and arsenic in livers, gills and muscles of endemic fish Toothed carp (*Aphanius sophiae*) at laboratory condition, which has been reported only from Iran.

MATERIALS AND METHODS

In this study, a total of 175 toothed carp (*Aphanius sophiae*) 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 (35º36'31''N, 50º48'23''E) from Jun to August 2011. DO, salinity, temperature and pH of sampling stations were recorded 11.68 mgL-1, 11-12 g L-1, 12.85 ± 6.22 °C and 7-8.5, respectively.

The fish samples were transported by the polythene bags filled with the river water and oxygen to the laboratory. In the laboratory, they were introduced into pre-cleaned 35L glass aquariums filled with dechlorinated tap water and then acclimatized to the laboratory conditions for 5 days prior to the experiment.

During experiment, the fish were fed with commercial food (Biomar) in rate of 3-5% body weight twice a day and artemia naupli. Fish were maintained at 27.5 ± 6.22 °C, pH 7.7 ± 0.5; CaCO3 hardness 295 ± 18 mgL-1; and dissolved oxygen 7.9 ± 0.1 mgL-1 for at least 18 days prior to the experiments. The tap water had no detectable amount of cadmium and arsenic.

In the present study, cadmium and arsenic were used in the form of arsenic oxide (As2O3) and cadmium chloride (CdCl2) salts (Merck, Germany). Fish were divided into 7 groups of 25 each; the first group served as the control group and the others as the experimental ones (concentrations of 5, 10 and 20 mgL-1 of both Cd and As). The fish was treated for 18 days and the level of cadmium and arsenic in the aquariums were preserved during the experiment.

At the end of each exposure period, dissections for separate organs (gills, liver and muscle) were performed. Five fish were pooled in order to take one sample organ as the weight of muscles and gills of five fish was 1 g and the weight of livers was 0.5 g. The organ samples were digested in a mixture of nitric acid (HNO3) and perchloric acid (HClO4; Mansouri et al. 2012c). First, tissues were then accurately weighed and put into 150 mL Erlenmeyer flasks. Second, 10 mL nitric acid (65 %) was added to each sample. Then, samples were left overnight to be slowly digested (Baramaki et al., 2012; Ip et al., 2005). Finally, 5 mL perchloric acid (70 %) was added to each sample. Digestion was performed on a hot plate (sand bath) at 90 ºC. Afterward, the digested samples were diluted with 25 ml deionized water. Concentration of cadmium and arsenic was measured using ICP- OES (GBC Integra XL, Australia) instrument, and the cadmium and arsenic concentrations, in an organ, was presented as mg/g wet weight (ww). All the experiments were conducted in three replications and the average of the values was reported along with standard deviations. The detection limits, blanks and recoveries of the measurements of metals in the samples are presented in Table 1. The analyses of data were carried out using SPSS (Release 16). The following equation was employed to determine the bioconcentration factor in different tissues of toothed carp in different concentrations of metals.

BCF = C tissues/ C water

C tissues: average concentration of heavy metals in different tissues

C water: average concentration of heavy metals in water

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**

RESULT

Bioaccumulation of cadmium and arsenic

The accumulation mean of cadmium and arsenic in different tissues of *Aphanius sophiae* in fresh water tanks were shown in Figure 1. The results indicated that the accumulation patterns of cadmium and arsenic are in the following order: liver > gill > muscle.

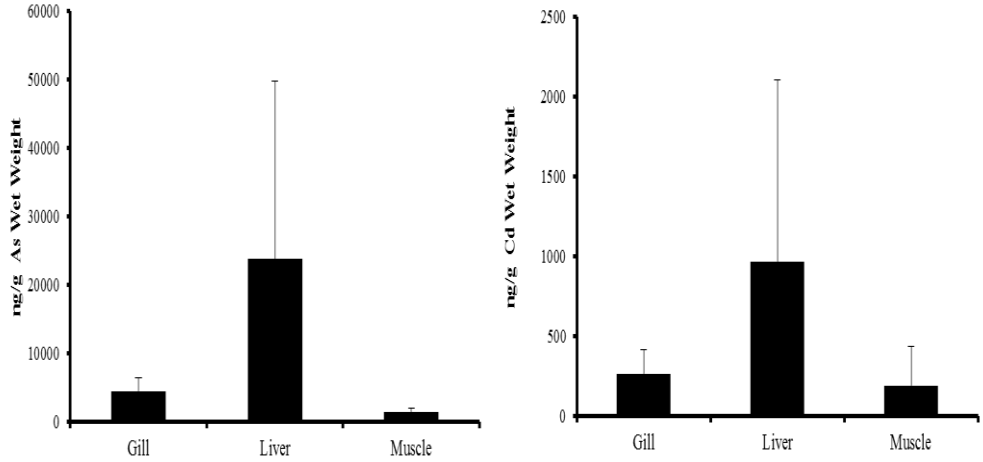

Figure 1. Average accumulation of cadmium and arsenic in different tissues of *Aphanius sophiae*

The results indicated that the highest and lowest accumulation of cadmium in the gills observed in concentrations of 10 µg/g and 20 µg/g respectively. Also, the results showed that the livers in concentration of 10 µg/g accumulated the highest of arsenic while the muscle in concentration of 20 µg/g accumulated the lowest of arsenic (Figure 2).

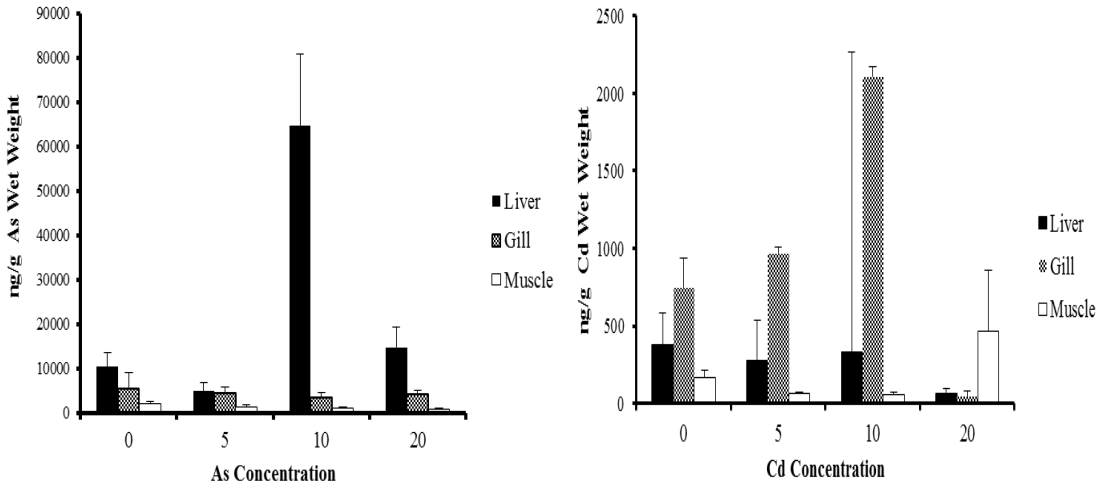

Figure 2. Average accumulation of cadmium and arsenic in different tissues of *Aphanius sophiae* in concentrations of control group, 5, 10 and 20 mg/L

Bio Concentration Factor

Cadmium and arsenic BCF calculated in different tissues of *Aphanius sophiae* are shown in Figure 3. The highest and lowest BCF of cadmium identified in 10 mg/L and 20 mg/L concentrations in the gills. While, the highest BCF of arsenic found in the liver in concentration of 10 mg/L and the lowest BCF of arsenic observed in concentration of 20 mg/L in muscle (Fig. 3). Generally, BCF in all concentrations of arsenic in the livers was more than other tissues (liver < gill < muscle). The results indicated that the BCF of cadmium in 5 µg/g and 10 µg/g concentration in the gill was more than other tissues (gill < liver < muscle).

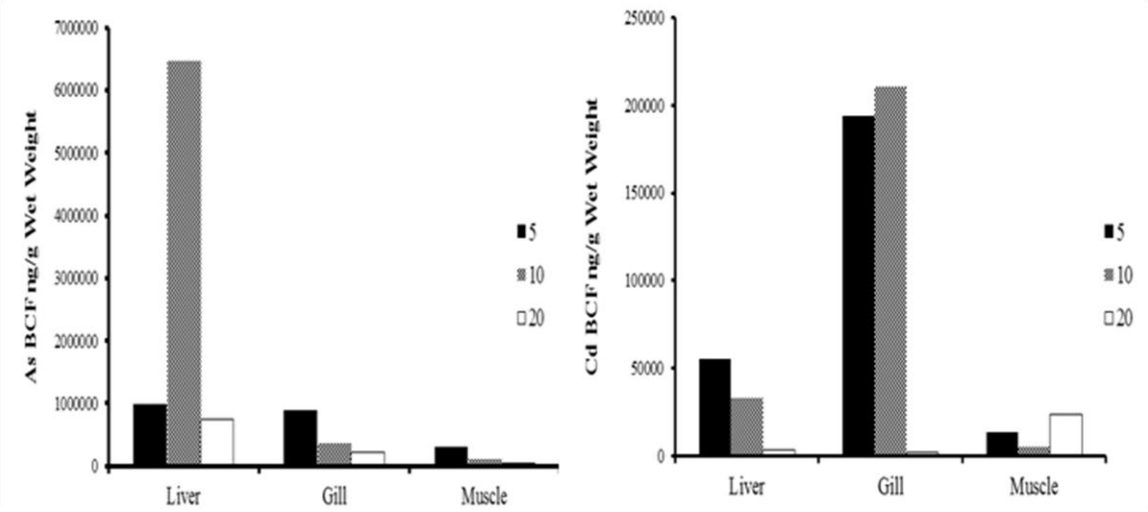

Figure 3. Cadmium and arsenic BCF in different tissues of *Aphanius sophiae*

DISCUSSION

Metals accumulation in aquatic environment suggests that fish can serve as a suitable bio-indicator for contaminating metals in aquatic environment, because they reply with great sensitivity to variations in the aquatic ecosystem (Vinodhini and Narayanan, 2008; Mansouri et al., 2012c). Figures 1 summarize the data of the mean concentration of cadmium and arsenic in the selected organs of *Aphanius sophiae*. The livers accumulated the highest levels of cadmium and arsenic (Figure 1). Next to livers, the gills accumulated the highest levels of cadmium and arsenic (Figure 1). The increased accumulation of cadmium and arsenic in the liver can possibly be attributed to the involvement of liver in detoxification and removal of toxic substances circulating in the blood stream (Asagba et al., 2008). Numerous studies shows that in many cases the liver also has an important role in contaminant storage, redistribution, detoxification or transformation and acts as an active site of pathological effects induced by contaminants (Licata et al., 2005; Norouzi et al., 2012; Baramaki et al., 2012). Consequently, the liver organs in fish are more than other organs recommended as an environmental indicator of water pollution.

The increased accumulation of cadmium and arsenic in the gills in concentration of 10 µg/g (Figure 2) could be due to the element complexing with the mucus in gills over time, which is practically impossible to ignore as it has direct contact with the surrounding water. This means that, the gills and the liver are the main sites of metallothionein (MT) production and metal retention (Asagba et al., 2008). One of the main reasons for the increased of cadmium and arsenic content in these organs is their capacity to accumulate cadmium and arsenic by induction of the metal-binding protein, MT. In general, gills receive the most exposure to contaminations in aquatic ecosystems (Oliveira-Filho et al., 2010), and are main sites for the entrance of metals that provoke lesions and gill damage (Vinodhini and Narayanan, 2008). Therefore, the concentration of metals concentrations in gills may be a reflection of their concentration in the water column (Ikem et al., 2003).

The muscle accumulated the lowest levels of cadmium and arsenic, even after 18 days of exposure (Figure 1), because this organ is not active organ in accumulating metals (Alam et al., 2002) and this organ usually have the lowest essential and nonessential metal concentration (Wen et al., 2003). This is similar to that found by Mansouri et al. (2012c) and Mansouri et al. (2011a), where the rate of bioaccumulation in muscle organ was lower than those in the livers and gills.

Heavy metals accumulation in various organs can disturb structural and functional of fish body (Jezierska and Witesk 2006).The toxicity of heavy metals in fish is as a function of free metal ion concentration controlled by the concentration of chloride in water (Erickson et al., 1996; Mclusky et al., 1986). When the chloride ion concentration increases, the concentration of free metal ions to total metal concentration reduces due to bond with chloride ions (Johnson 1988). By decreasing salinity, negative potential difference between inner and outer wall of the body increases, consequently ion transport to organs is enhanced (Karakoc 1999). 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 (Howard and Hacker 1990).

Exposure of tissues to Cd in dilute seawater enhances the metal uptake rate possibly due to calcium transport mechanisms (Zanders and Rojas, 1996). Metal accumulation in organisms depends on several factors including

ecological requirement, metabolism, severity of water pollution, food, salinity, temperature and sediments (Yilmaz, 2007).The interactions of organ type and different concentrations of Cd and As are in conformity with findings of Howard and Hacker (1990), Shuhaimi-Othman et al.(2006) and Karakoc (1999). 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).

Based on results of our study and Vinodhini and Narayanan (2008) research, in fresh water tanks BCF in all concentrations of As in the liver was more than other tissues (liver < gill < muscle) while in concentrations of 5 and 10µg/g of Cd, BCF was more in the gills (gill <liver < muscle) which is confirmed by Olivera-riberio et al.(1996) and Gbema (2001) studies. The highest level of bioaccumulation observed in liver due to trend of metals to accumulate more in the liver than other tissues .It is generally accepted that induction of large amount of low molecular weight metal-binding proteins such as metallothione happens in the liver tissue of fish. Liver can sequester and detoxify metals taken up from the environment by the binding on these proteins (Kalay and Canli, 2000).In aquatic organisms, gills are the first tissues influenced by pollutants so they can be considered as good indicator for measurement of fresh water, aquatic ecosystems and marine environments. BCF in As concentrations was more than Cd concentrations which is in contrast with Zare studies 2011.

CONCLUSION

In fresh water tanks accumulation of heavy metals in different tissues of fish in As concentrations were more than Cd concentrations therefore expected mortality in As tanks was more than Cd tanks while results was exactly reverse. Probably, mortality enhances by decreasing in salt concentration and water hardness. Generally it can be said that toxicity of heavy metals decreases by increasing water hardness therefore high levels of water hardness can be useful for aquatic organism.

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