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Original Article

Cadmium and arsenic bioaccumulation and bio-concentration in the endemic toothed carp *Aphanius arakensis* in salt water

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Abstract: Heavy metals are released to aquatic ecosystems from natural and anthropogenic recourses and accumulate to the body of organisms. This study aimed to assess the accumulation of As and Cd in the gill, liver, and muscle of the toothed carp *Aphanius arakensis* in salt water exposed to three concentrations of Cd and As (5, 10 and 20 mg L^{-1}) for 18 days. The specimens were collected from the Shoor River with an average weight of 1.5±0.3 g (mean±SD) and length of 3.4±0.4 cm. The findings showed that the bio-concentration factor (BCF) of Cd and As were in the following order: liver > gill > muscle, however, for 5 ppm of As the order was gill > liver > muscle. BCF in As concentrations were more than Cd concentrations. Also, the highest BCF was found at 5 ppm. The present study showed that the liver is the organ that accumulates the highest concentrations of As and Cd.

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Introduction

Fishes are suitable indicators for monitoring the land-based pollutions, particularly heavy metals which accumulate in fish tissues, being absorbed through the skin or indirectly through food (Kamrine, 2000). Heavy metals are important threat to the aquatic environments because of chemical persistent, low degradation, bioaccumulation and bio-magnification (Anbiaee et al., 2009). Among heavy metals, Cadmium is very toxic, 2-20 times more than those of others (Perez-Lopez et al., 2008). Arsenic is another hazardous heavy metal originating from fertilizers and effluents that enter to the surface and groundwater (Philip, 1978).

The heavy metals accumulate mostly in the muscle, liver and gill tissues of fishes due to their high metabolism rates (Khodabande, 2000). The liver plays an important role in accumulation of heavy metals and detoxification of wastes and its histopathological changes may be used as indicators of heavy metals (Wong et al., 2001). The gill is in direct contact with water and thus accumulates heavy metals (Oliveira-Filho et al., 2010). Bioconcentration is a process that chemicals affect the organisms and bioconcentration factor (BF) is determined by dividing concentration of heavy metals in the organism tissues to concentration of heavy metals in water (Otitoloju et al., 2009).

The genus *Aphanius* is the only representative of the Cyprinodontidae (Teleostei, Cyprinodontiformes) in Eurasia. Iran and central Anatolia show the highest diversity of *Aphanius*, and 14 extant and one fossil species are known to occur in Iran based on data derived from fish morphometry and meristics, otoliths, scales and mtDNA sequences (Coad, 2016; Teimori et al., 2014). Twelve out of 14 Iranian *Aphanius* species are endemic to this country. This genus occurs in coastal (brackish) and landlocked (freshwater to saline) water bodies in the Mediterranean and Persian Gulf basins from Iberian Peninsula as far eastwards as Iran and Pakistan (Teimori et al., 2014). *Aphanius arakensis* is a newly described species of this genus from the Namak Lake basin in Iran (Teimori et al., 2012, 2014). This species tolerates a wide range of physicochemical parameters (Coad, 2016), then it can be an appropriate model to study the effect of heavy metal pollution since it is

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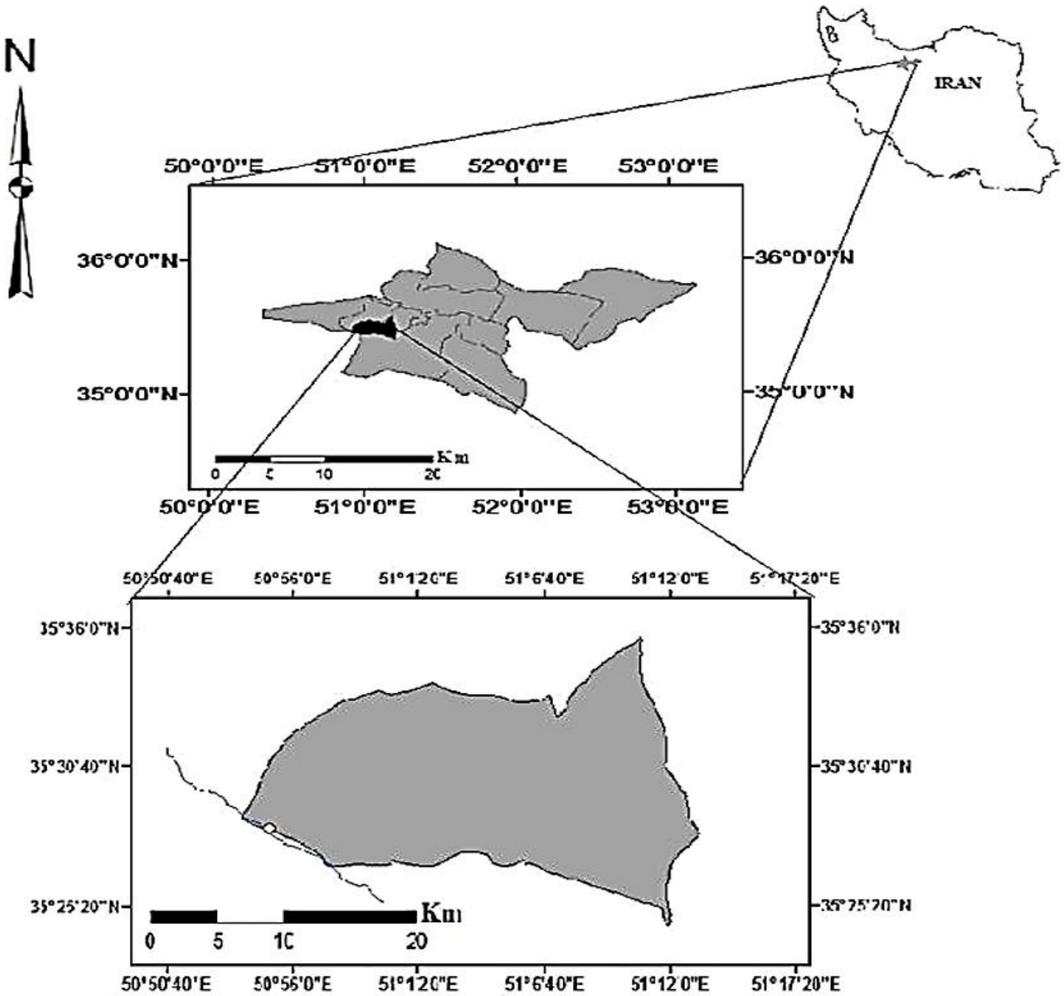

Figure 1. The sampling area (○) in the Shoor River.

found in both salt and fresh water in the Namak lake basin. Hence, this study was conducted to examine the accumulation of Cd and As in the muscle, gill and liver of *A. arakensis* and their bioconcentration factor in different concentrations of As and Cd in the laboratory condition.

Materials and Methods

In this study, a total of 375 female specimens of *A. arakensis* with an average weight of 1.5±0.3 g (mean±SD) and length of 3.4±0.4 cm were collected from the Eshtehard Shoor River (35º36'31" N, 50º48'23" E), (Alborz Province, Iran) in June 2011 (Fig. 1). Dissolved oxygen (DO), salinity, water temperature and pH were measured during the sampling, which were 11.68 mg L^{-1}, 11-12 g L^{-1}, 12.85±6.22°C and 7-8.5, respectively.

The fish samples were transported to the fisheries laboratory of University of Tehran in polythene bags. Prior to the experiment, the fish were acclimatized to the laboratory conditions for five days in pre-cleaned 30 L glass aquaria (30×30×40 cm) filled with de-chlorinated tap water. During the experiment, the fish were fed with a commercial fish food (Biomar) at a rate of 3-5% body weight twice a day. The physicochemical conditions of the aquaria were as follows: temperature, 27.5±1.2°C, pH, 7.7±0.5, $CaCO_3$ hardness, 295±18 mg L^{-1} and DO, 7.9±0.1 mg L^{-1}.

Cd and As were used as arsenic oxide (As_2O_3) and cadmium chloride ($CdCl_2$) salts (Merck, Germany). Twenty-one aquaria were filled with salt water of the sampling area and aerated throughout the experiment. The animals were randomly allocated to 21 aquaria as seven treatments each with three replicates containing 10 fish. Three aquaria received no chemical and was

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

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

the control. The other 18 aquaria received only a concentration of Cd (5, 10 and 20 mg L^{-1}) or As (5, 10 and 20 mg L^{-1}) for 18 days.

At the end of the experiment, the animals were anaesthetized and euthanized. A piece of their gill, liver and muscle were removed. Five specimens from each aquarium were pooled to make an appropriate weight of sample (the gill and muscle≈1 g, the liver≈0.5 g). The organ samples were digested in a mixture containing HNO_3 and $HClO_4$ (Mansouri et al., 2012c). The tissues were then weighed accurately and put into 150 mL Erlenmeyer flasks. Then, 10 mL nitric acid (65%) were added to each sample. The samples were left overnight to be digested slowly (Baramaki et al., 2012; Mansouri et al., 2012). Finally, 5 mL perchloric acid (70%) were added to each sample. Digestion was performed on a hot plate (sand bath) at 90ºC. The digested samples were diluted with 25 ml deionized water. The concentration of Cd and As were measured using ICP-OES (GBC Integra XL, Australia) and presented as mg g^{-1} wet weight (ww). All the measurements were repeated three times and the average of the values was reported along with their standard deviations.

The following equation was employed to determine the bioconcentration factor in different tissues of *A. arakensis* in different concentrations of metals.

BCF=C tissues/ C water

Where C tissues are average concentration of metals in different tissues and C water: average concentration of metals in water. The analyses of data were performed using SPSS (Version 16).

Results

Only 5% of the specimens died during the experiment. The detection limits, blanks and recoveries of the measurements of metals in the samples are presented in Table 1.

Average accumulation of As and Cd in different tissues of *A. arakensis* in salt water are shown in Figures 2. The metals were accumulated in different tissues in the following order of magnitude: liver > gill > muscle. The Figures 2 and 3 show that the highest accumulated Cd and As was found in the liver. On the other hand, the lowest accumulated Cd and As found in the muscle. The average accumulation of As and Cd in different tissues of *A. arakensis* in salt water are shown in the Figures 3 and 4. The lowest accumulation of As and Cd was observed in the control group and in the muscle, while the highest accumulation of As and Cd were found in the liver, and 10 and 20 ppm, respectively.

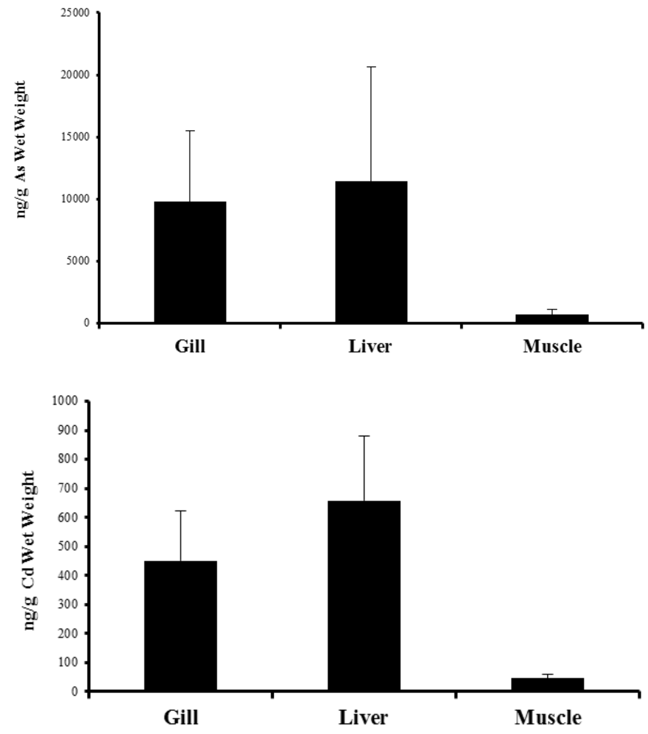

Figure 2. Mean (±SD) As and Cd average accumulation in different organs of female *Aphanius arakensis*.

Bio-concentration Factor (BCF) was calculated in different tissues of the specimens. The highest BCF of As was found in the gill and in the concentration of 5ppm. The highest BCF of Cd was found observed in the liver. The lowest BCF of Cd and As were detected in the muscle and in the concentration of 20 ppm (Fig.

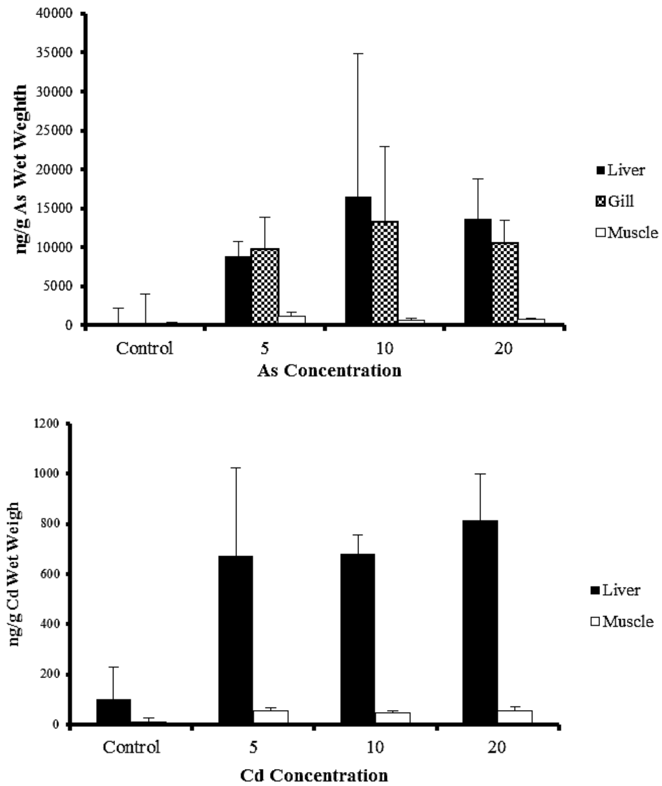

Figure 3. Mean (±SD) As and Cd accumulation in different organs of female *Aphanius arakensis* in concentrations of 5, 10, 20 ppm and control group.

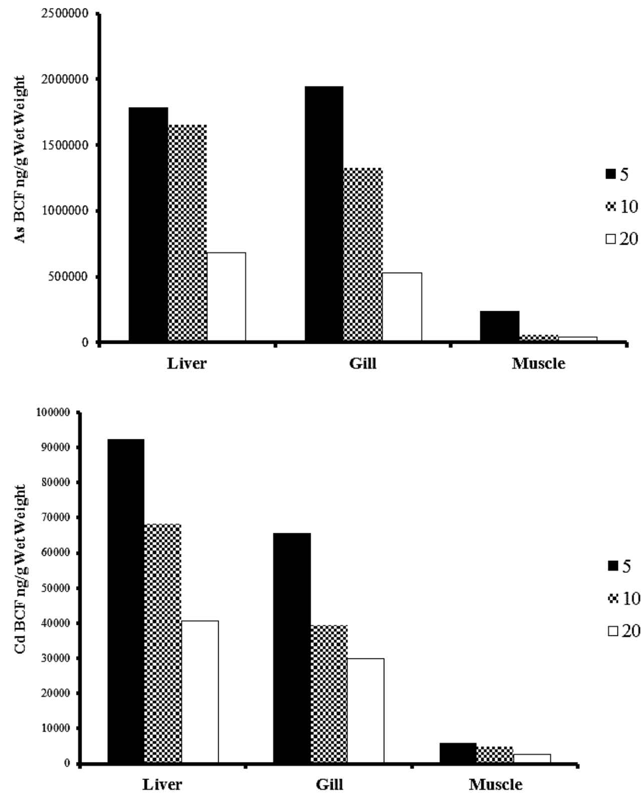

Figure 4. BCF of As and Cd in different tissues (ng g^{-1} wet weight).

4). BCF of As was more than that of Cd. The maximum BCF of As and Cd were found at the concentration of 5 ppm (5 ppm>10 ppm>20 ppm).

Discussion

Accumulation of metals in the aquatic environment suggests that fishes can serve as a suitable bioindicator for contaminating metals in aquatic environment, because they respond to variations with great sensitivity in aquatic ecosystems (Mansouri et al., 2011). Our findings confirm differences in accumulation of heavy metals in the different tissues. Based on the results of this study, the liver identified as target organ in salt water aquaria (liver > gill > muscle) which is in agreement with Kojadinovic et al. (2007), Monsef Zadeh (2008), Naseri et al. (2005), Norouzi et al. (2012) and Mansouri et al. (2012). Similarly, many studies showed that the liver can accumulate high concentrations of heavy metals due to its key role in the metabolism of the body. Therefore, the liver is suitable indicator for the assessment of impact of environmental pollutants (Yılmaz, 2009). After the liver, the gill showed a high accumulation of Cd and As concentrations in salt water tanks. The gills are places that accumulate water ions and one of the first tissues that have direct contact with environmental pollutants. The gill is also exposed to the pollutants to a greater extent compared with other organs, causing a higher rate adsorption and accumulation of the pollutants than those of other organs. As in the liver, the gill can be considered as an indicator of pollutants in both marine and freshwater habitats (Oliveira-Filho et al., 2010).

The present study showed that accumulation of Cd and As was lower in the muscle than those of the liver and gill. This finding is similar to those of Mansouri et al. (2012) and Majnoni et al. (2013), where the rate of bioaccumulation in the muscle was lower than those of the liver and gill. Generally, tissues which have low metabolic activities accumulate high concentrations of heavy metals (Amini Ranjabr and Sotoodenia, 2005). However, the muscle is not an active tissue to accumulate heavy metals which has been confirmed in this study and by others (Tekin-Ozan and Kir, 2007;

Karadede, 2000; Wong et al., 2001). Terra et al. (2008) indicated that low concentrations of heavy metals in the muscle may be a result of a low level of binding proteins.

According to the finding of this study and other studies (Subathra and Karuppasamy, 2008; Senthil Murugan et al., 2008), the order of the magnitude of BCF for Cd and As in tissues were liver > gill > muscle but this order in 5 ppm of As was gill > liver > muscle which is similar to findings of Jayakumar and Issac Paul (2006) and Asagba et al. (2008). The liver is a place for storage, detoxification and biological transmission of heavy metals in fishes. Therefore, the highest accumulation of heavy metals is normally found in the liver (Kalay and Canli, 2000). It is shown that a large amount of metallothionein induction happens in the liver of fishes. Transmission of a large volume of water through the gills to obtain oxygen in stressful condition may increase rapidly toxic heavy metals in the gills (Karuppasamy, 2004). Hamidian et al. (2013) ignored results of this study that BCF of As was more than that of Cd. However, metabolic processes such as biological transmission, active uptake and fat metabolism affect bioaccumulation plus other factors including contamination gradients of water, temperature, salinity and interacting agents (Van Geest, 2010).

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چکیده فارسی

تغلیظ و تجمع زیستی کادمیوم و آرسنیک در گورماهی بومی *Aphanius arakensis* در آب شور

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چکیده:
فلزات از منابع طبیعی و انسانی به اکوسیستم‌های آبی منتقل می‌شوند و در بدن موجودات زنده انباشته می‌شوند. هدف از این مطالعه ارزیابی انباشت As و Cd در آبشش، کبد و عضله *Aphanius arakensis* در آب شور و در معرض سه غلظت Cd و As (۵، ۱۰ و ۲۰ میلی‌گرم در لیتر) برای ۱۸ روز بود. نمونه‌ها از رودخانه شور با میانگین وزنی ۱/۵±۰/۳ گرم (میانگین±SD) و طول ۳/۴±۰/۴ سانتی‌متر جمع‌آوری شد. یافته‌ها نشان داد که ضریب تغلیظ زیستی Cd و As به این ترتیب است: کبد> آبشش> عضله، اگرچه در غلظت ۵ ppm از As به‌ترتیب آبشش> کبد > عضله است. BCF آرسنیک بیشتر از کادمیوم بود. همچنین بیشترین میزان BCF در غلظت ۵ ppm مشاهده شد. مطالعه حاضر نشان داد که کبد اندامی است که بیشترین غلظت As و Cd را تجمع می‌دهد.
کلمات کلیدی: سمیت، فلزات سنگین، اندام‌ها، تغلیظ زیستی.