Bull Environ Contam Toxicol (2012) 89:78–81
DOI 10.1007/s00128-012-0604-0

Determination of Lead, Mercury and Cadmium in Wild and Farmed *Barbus sharpeyi* from Shadegan Wetland and Azadegan Aquaculture Site, South of Iran

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Received: 18 January 2012 / Accepted: 10 March 2012 / Published online: 27 April 2012

Abstract Lead, mercury and cadmium concentrations were measured in muscle, liver and gill in wild and farmed *Barbus sharpeyi* from Shadegan Wetland (SW) and Azadegan Aquaculture Site (AAS). Significant variation in metal values were evaluated in Students' tests at $p > 0.05$. Results showed: In *B. sharpeyi* high levels of cadmium, lead, and mercury were measured in gill (0.34, 0.68, and 0.06 mg kg^{-1} dw). The concentration of metals was not significantly different ($p \geq 0.05$) in the muscle between SW (Cd, 0.24; Pb, 0.49 and Hg, 0.04) and AAS (Cd, 0.23; Pb, 0.49 and Hg, 0.04). Lead concentration was higher than cadmium and mercury in different organs ($p > 0.05$). Cadmium, mercury and lead in different tissues of SW were higher than AAS and there was no significant difference between them ($p \geq 0.05$). Metal levels in different tissues were higher than WHO standard.

Keywords Metals · *Barbus sharpeyi* · Shadegan Wetland · Azadegan Aquaculture Site

Shadegan Wetland (SW) (48°17′–48°50′E, 30°17′–30°58′N) is one of the most important wetland ecosystem which is located in the southern of Iran. It is the largest wetland of Iran covering about 537,700 ha also; Azadegan Aquaculture Site (AAS) (48°39′E, 31°1′N) with an area of 12,040 ha is the major aquaculture center in the southern of Iran. In aquatic ecosystems, metals receive considerable attention in relation to their accumulation and toxicity in biota and fish. In fish, the toxic effects of metals may influence physiological functions, individual growth rates, reproduction and mortality (Farag et al. 1995). Metals like cadmium, lead and mercury are among the most metallic pollutants. These are non-essential metals due to their toxicity, even in tracing amounts (Fernandes et al. 2008). Metals enter fish bodies through body surface, gill or the digestive tract (Pourang 1995). Food may also be an important source of metals accumulation in fishes (Clearwater et al. 2000), and potentially lead to bio magnification and increase of pollutants in food chain. Fishes are located at the top of aquatic food chain and may absorb large amounts of metals from the water. Metal bioaccumulation is largely attributed to differences in uptake and depuration period of various metals in different fish species (Tawari-Fufeyin and Ekaye 2007). *B. sharpeyi* has high market value and is the main fish product in SW. In fresh water systems, fish is one of the aquatic products humans consume, and is also a good indicator of elemental pollution (Rashed 2001). In Iran, despite extensive water resources, especially in the southern coast and extensive use of seafood, little research has been so far done in the field of measuring metals in different organs of fishes in the Khouzestan Province. According to undesirable long term effects of metals on health and the possibility of entering these pollutants into the life cycle, the main objective of this study is to determine the rate of metals (Cd, Pb, Hg) in the gill, liver and muscle of *B. sharpeyi* in SW and AAS in order to assess the fish quality and its effects on humans health. This can help us to understand the enrichment behavior of metals in SW and AAS, and emphasize the need to discard the most polluted tissues of the fish.

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Materials and Methods

The concentration of metals cadmium, lead and mercury were measured in the muscle, gills and liver of *B. sharpeyi* caught by gillnet from SW (Darolkhovien village) and AAS in Khouzestan, summer 2010. Thirty six fishes caught in each region, were immediately packed in plastic freezer bags and transported to the laboratory. Total body length (cm), fork length (cm) and weight (g) were measured for fishes. The mean length, fork length and weight were ranging from maximum and minimum value as 34.9 ± 3.1 cm for length and 32.3 ± 3.6 cm for fork length and 530 ± 46 g for weight of *B. sharpeyi* in SW, and 36.1 ± 3.4 cm for length and 32 ± 3.6 cm for fork length and 700 ± 60 g for weight of *B. sharpeyi* in AAS. After biometry, fishes were immediately frozen at −20°C. Based on International recognized guidelines of sampling procedures, all samples were cut into pieces and labeled (UNEP 1991). Fish samples were put on to a dissection tray to be thawed at room temperature. They were dissected by stainless steel scalpels and Teflon forceps on a laminar flow bench. In parallel, gill, liver and a part of the muscle (dorsal muscle without skin) were removed and transferred to polypropylene vials. Subsequently, samples were put into an oven to dry at 90°C and reach constant weights in the oven. Before acid digestion, a porcelain mortar used to grind and homogenize the dry tissue samples. Aliquots of approximately 1 g dried gill, liver and muscle were digested in Teflon beakers for 12 h at room temperature, and then for 4 h at 100°C with 5 mL ultrapure nitric acid (65 %, Merck). Metals analysis: Cd, Pb were measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, 4100 ZL). Hg concentration was determined with a Perkin–Elmer MHS-FIAS coupled to a Perkin–Elmer 4100 ZL spectrophotometer. Results are expressed as mg/kg dry weight. The analytical procedure was checked by using reference material [MESS-1, the National Center of Canada and CRM 277, the Community Bureau of Reference, Brussels, Belgium and details were in (Meador et al. 1994)]. For each matrix, analyses of three blank samples were performed along with the samples. Quality control was assured by the analysis of reagent blank and procedural blanks. Data statistics were performed by using SPSS 18 software. Paired samples test was used to compare differences between samples. A p value less of 0.05 was considered statistically significant.

Results and Discussion

The concentrations of metals (mg kg^{-1} dry weight) in gill, liver and muscle of *B. sharpeyi* in SW and AAS have been summarized in Table 1. Cd, Pb, and Hg were detected in all samples. Pb level was higher than the other metals ($p > 0.05$). Metals based on their target organs of its metabolic activity were selected. This point causes the accumulation of metals in tissues such as liver and kidney in comparison with muscle (having low metabolism). Liver and gills are considered good indicators in terms of long-term exposure to metals in relation to the position of tissues and metabolism of metals (Filazi et al. 2003). Most of fish organs are sensitive against toxicity of metals. In this study, muscle tissues due to their important role in human nutrition and the need to ensure the safety of consumption, gill tissues due to their role in respiration and osmotic balance, and liver due to being a member that main metabolism and major injuries will endure in, were chosen (Pourang 1995). The study showed that the highest concentrations of metals are found in the gill and liver ($p > 0.05$), while the lowest concentrations are found in the muscle. The metal concentration in muscle is important for the edible parts of the fish. Fishes that generally accumulate contaminants from aquatic environments have been largely used in food safety studies. High metal concentrations in gill tissue may be due to specific physiological function of this organ in respiration and osmotic balance. While in liver, their densities are higher than muscles as they are not the primary storage location. Heavy metals are primarily stored in the liver and then transferred to the muscle. Muscles usually have the lowest levels of metals in fish (Al-Yousuf et al. 2000). Comparison of cadmium, lead and mercury in muscle of *B. sharpeyi* from SW and AAS with International standard shows that cadmium level is normal in comparison with WHO standard, Lead level is higher than WHO and the concentration of mercury is lower than WHO standard. Any change in the process of absorption and accumulation of metals in fish can be effective due to various factors such as aquatic type, element type, tissue, sex, weight and age of aquatic, food habits, fish characteristics, ecological characteristics and environmental conditions and the physical and chemical properties such as hardness of water environment, pH, temperature, nutrients and fish growth (Mohammadi et al. 2011). Different metal levels were observed in the gill, liver and muscle of *B. sharpeyi* in SW and AAS (Table 1). Metal concentrations variety significantly depends upon the type of fish tissues and locations. The concentrations of Cd, Pb and Hg in the gill, liver and muscle of *B. sharpeyi* in SW are higher than those observed in AAS but the concentration of metals was not significantly different ($p \geq 0.05$) (Table 1). The highest levels of Cd, Pb and Hg were found in the gill of *B. sharpeyi* in both regions.

Metal concentration in the gills could be due to the element complex with the mucus, which is impossible to be removed completely from the lamellae gaps, before tissue is prepared for analysis. The absorption of metals onto the

Table 1 Concentrations of metals (mg kg^{-1} dry weight) in various tissues of *B. sharpeyi* in Shadegan Wetland (SW) and Azadegan Aquaculture Site (AAS), Khoozestan, Iran

Tissues	Region	Cd	Pb	Hg
Gill	SW	0.3437 ± 0.037[a]	0.6783 ± 0.034[a]	0.0649 ± 0.002[a]
	AAS	0.3150 ± 0.022[a]	0.6460 ± 0.037[a]	0.0592 ± 0.002[a]
Liver	SW	0.2730 ± 0.022[a]	0.5777 ± 0.047[a]	0.0569 ± 0.003[a]
	AAS	0.2620 ± 0.009[a]	0.5717 ± 0.049[a]	0.0444 ± 0.005[b]
Muscle	SW	0.2377 ± 0.014[a]	0.4990 ± 0.030[a]	0.042 ± 0.001[a]
	AAS	0.2317 ± 0.005[a]	0.4903 ± 0.005[a]	0.0381 ± 0.004[a]

Data are presented as mean ± SE

[a, b] $p > 0.05$, significantly different in each tissue between two regions

Table 2 Comparison of metals (mg kg^{-1} dry weight) in muscle of *B. sharpeyi* with WHO standard in Shadegan Wetland (SW) and Azadegan Aquaculture Site (AAS), Khoozestan, Iran

Species	Region	Cd	Pb	Hg
B.sharpeyi	SW	0.2377 ± 0.014[a]	0.4990 ± 0.030[a]	0.042 ± 0.001[a]
	AAS	0.2317 ± 0.005[a]	0.4903 ± 0.005[a]	0.0381 ± 0.004[a]
WHO standard		0.2[a]	0.3[b]	0.1[c]

[a, b, c] $p > 0.05$, significantly different in muscle of (*B. sharpeyi*) in comparison with WHO (World Health Organization) standard. Reference: WHO (1985)

gill surface, as the first target of pollutants in water, could also be an important influence in the total metal levels of the gill (Heath 1987). The comparison of mean concentrations of metals in all tissues of *B. sharpeyi* in SW and AAS shows that the metal levels of tissues are very variable in fishes. The observed variability of metal levels in different species depends on feeding habits (Romeo et al. 1999), ecological needs, metabolism (Canli and Furness 1993), age, size and length of the fish (Linde et al. 1998). Concentrations of metals detected in the muscle, gill and liver samples show different capacities of accumulation. In general, the highest metal concentrations are found in the gill and liver (Table 2).

Thus, the gill and liver in fish are more often recommended as environmental indicator organs of water pollution than other fish organs (Karadede et al. 2004). Alhas et al. (2009) reported that in *B. xanthopterus* and *B. rajanorum mystaceus* in Atatürk Dam Lake, Turkey, metal concentrations in gill and liver were maximum, while these concentrations were least in muscle. Thus this result was alike our result in this research. Malik et al. (2010) have determined the concentrations of Pb, Cd, Zn, Ni, Cu, Cr and Hg in gill, liver and muscle of *Labeo rohita* and *Ctenopharyngodon idella* in lake of Bhopal, India and Mohammadi et al. (2011) determined the concentrations of Cd, Pb, Ni and Hg in gill, liver and muscle of *B. grypus* and *B. xanthopterus* from Karoon and Dez Rivers, Khouzestan, Iran; They have reported that the gill and liver showed higher metal concentrations than muscle. So, the results of our study are similar to the above studies. The results of this research have indicated that metal accumulation depends on the tissues, probably as a consequence of metabolic needs, physiochemical properties, and detoxification processes specifically for each element. In this study, mercury in comparison with other metals had the lowest concentration. The results of this study showed that Pb is the highest accumulating metal compared with other metals ($p > 0.05$). The main sources of Pb contamination are smelting works, application of waste water treatment sludges to soil, transportation, rain, hail and so on. Approximately 98 % of Pb in the atmosphere originates from the human activities (Fadrus et al. 1979). The high levels of Pb in the SW and AAS have toxic effects on fish metabolism and as a result it is important to consider the biological effects of contamination on fish health in SW and AAS. So, waste management in urban and industrial centers in Khoozestan, especially around the cities of SW and AAS, have remained very unsatisfactory to data and it can be concluded that this poses a health hazard to both aquatic life and humans a like.

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