

Determination of heavy metals in two barbs, *Barbus grypus* and *Barbus xanthopterus* in Karoon and Dez Rivers, Khoozestan, Iran

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Abstract Cadmium, lead, nickel and mercury were contamination in gill, liver and muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers. Significant variations in metal values were evaluated using student's t test at $p < 0.05$. In *B. grypus* high levels of cadmium, lead, nickel and mercury were measured in gill (1.49, 2.25, 1.02 and 0.89 mg kg⁻¹ dw) except for mercury (1.06 mg kg⁻¹ dw) in *B. grypus* in Dez River was high in liver. In *B. xanthopterus* high levels of cadmium, lead, nickel and mercury were measured in gill (2.17, 2.91 and 1.43 mg kg⁻¹ dw) except for mercury (1.42 mg kg⁻¹ dw) high levels were measured in liver.

Keywords Heavy metals · *Barbs* · Karoon · Dez

Karoon-Dez River basin, (10°48'–30°52'E, 20°30'–05°34'N), with an area of 6,700 square kilometers, is located in southern part of Iran. This river system supplies the water demands of 16 cities, several villages, thousands hectares of agricultural lands and several hydropower plants. Karoon River is the largest river in Iran and has an area of 60,500 Km² and an average annual discharge of 18,700 million m³, also Dez River is one of the watery river in Iran and has an area of 21,100 Km² and an average annual discharge of 7,396 million m³. They play an important role in

water and fish supply which have great economic values. *Barbus grypus* and *Barbus xanthopterus* have high market value and are the main fish products in Karoon and Dez Rivers. In fresh water systems, fish is one of aquatic products humans consume, and also provide a good indicator of trace element pollution (Rashed 2001). In the last decades, industry and agriculture near the Karoon and Dez Rivers have been rapidly developed and human activities have increased. Under certain environmental conditions, heavy metals may be accumulated to a toxic concentration, and cause ecological damage (Freedman 1989). Heavy metals were of particular concern due to their toxicity and ability to be bio accumulated in aquatic ecosystems (Miller et al. 2002), as well as persistence in the natural environment. Heavy metals like cadmium, lead, mercury and nickel are among the most metallic pollutants. Nickel is essential metal since its play an important role in biological systems, whereas cadmium, lead and mercury are non-essential metals, as they are toxic, even in trace amounts (Fernandes et al. 2008). Heavy metals tend to accumulate in advanced organisms through bio-magnification effects in the food chain. Thus they can enter into human body and accumulate in the human tissues to pose chronic toxicity. Chronic assimilation of heavy metals is a known cause of cancer (Nabawi et al. 1987). Being the complexity of heavy metal bioaccumulation of fishes, it was important to study the heavy metal accumulation in different commercial fishes such as *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers for the food safely. The main objective of this study was to determinate the contents of heavy metals (Cd, Pb, Ni and Hg) in the gill, liver and muscle of *B. grypus* and *B. xanthopterus* caught from downstream of Karoon and Dez Rivers in order to assess fish quality and to assess the health risk for humans. This could help us understand the enrichment behavior of heavy metals in down stream Karoon and

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Dez Rivers and emphasize the need to discard the most polluted tissues of the fish.

Materials and Methods

The concentration of heavy metals cadmium, lead, nickel and mercury were measured in the muscle, gills and liver of *B. grypus* and *B. xanthopterus* caught by gillnet from downstream of Karoon (Shekariyeh3 village) and Dez (Ali abad village) Rivers of Khoozestan in summer 2009. The number of samples were 48 *B. grypus* and 48 *B. xanthopterus* in each river. After capture, fish were placed in plastic bags and transported to the laboratory in freezer bags with ice. The mean length and weight were measured which ranged from minimum and maximum value as 287.5 ± 425 mm for length and 223.8 ± 585 g for weight of *B. grypus* and 248.5 ± 377.5 mm for length and 173 ± 561.3 g for weight of *B. Xanthopterus* in Karoon and Dez Rivers. Total body length (mm) and weight (g) were recorded for fishes. After biometry, fish were immediately frozen at -20°C . All samples were cut into pieces and labeled, and then all sampling procedures were carried out according to internationally recognized guidelines (UNEP 1991). Fish samples for heavy metals were put onto a dissection tray and thawed at room temperature. They were dissected using stainless steel scalpels and Teflon forceps using a laminar flow bench. In parallel gill, liver and a part of the muscle (dorsal muscle without skin) were removed and transferred in polypropylene vials. Subsequently, samples were put into an oven to dry at 90°C and reached constant weights in the oven. Before acid digestion, a porcelain mortar was employed 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). Heavy metals analysis: Cd, Ni and 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 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 using SPSS 17 software. Paired samples t 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 heavy metals (mg kg^{-1} dry weight) in gill, liver and muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers are summarized in Tables 1 and 2. Cd, Pb, Ni and Hg were detected in all samples. Pb level was higher than the other metals ($p < 0.05$). Distribution patterns of metal concentrations in the gill, liver and muscle of *B. grypus* in Karoon River follows the sequence: $\text{Pb} > \text{Cd} > \text{Hg} > \text{Ni}$, in the muscle and liver of *B. grypus* in Dez River and in the *B. xanthopterus* in Karoon and Dez Rivers follows the sequence: $\text{Pb} > \text{Cd} > \text{Hg} > \text{Ni}$ and in the gill of *B. grypus* in Dez River and in the *B. xanthopterus* in Karoon and Dez Rivers follows the sequence: $\text{Pb} > \text{Cd} > \text{Ni} > \text{Hg}$ respectively. The distribution patterns of Cd, Pb, Ni and Hg in tissues of *B. grypus* in Karoon River follows the order: $\text{gill} > \text{liver} > \text{muscle}$. The distribution patterns of Cd, Pb, Ni in tissues of *B. grypus* in Dez River and in the *B. xanthopterus* in Karoon and Dez Rivers follows the order: $\text{gill} > \text{liver} > \text{muscle}$; Hg level in the *B. grypus* in Dez River and *B. xanthopterus* in Karoon River follow the order: $\text{liver} > \text{gill} > \text{muscle}$ and Hg level in the tissues of *B. xanthopterus* in Dez River follow the order: $\text{liver} > \text{muscle} > \text{gill}$. Heavy metal concentrations in the gill, liver and muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers were higher in the gill and liver, when compared with muscle except for Hg level that in the gill, liver and muscle of *B. xanthopterus* in Dez River follow the order: $\text{liver} > \text{muscle} > \text{gill}$. In this study the results showed that the highest concentrations of heavy metals were found in the gill and liver ($p < 0.05$), while the lowest concentrations were found in the muscle, except for *B. xanthopterus* in Dez River, that the lowest concentration of Hg was found in the gill. Similar results were also reported for a number of other fish species (Karadede-Akia and Ünlü 2007), these results showed that the muscle was not an active tissue regarding the accumulation of metals. The metal concentration in muscle is important for the edible parts of the fish. Fish generally accumulate contaminants from aquatic environments, have been largely used in food safety studies. The mean concentrations of heavy metals (Cd, Pb, Ni and Hg) in the muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers were higher than the maximum permitted concentrations proposed by WHO (WHO 1985, 1994; Czarnecki 1985) and the concentrations of heavy metals have significant differences ($p < 0.05$) in muscle of *B. grypus* and *B. xanthopterus* with WHO standard in Karoon and Dez Rivers (Tables 3, 4). Water pollution leads to fish contaminated with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers (Arain et al. 2008). Different heavy metal levels were observed in the gill, liver and muscle of

B. grypus and *B. xanthopterus* in Karoon and Dez Rivers ($p < 0.05$) (Tables 1, 2). Heavy metal concentrations varied significantly depending upon the type of fish tissues and locations. The concentrations of Cd, Pb, Ni and Hg in the gill, liver and muscle of *B. grypus* in Dez River were higher than those observed in Karoon River ($p < 0.05$), except for Pb level that in *B. grypus* in Karoon river was higher than in *B. grypus* in Dez River ($p < 0.05$) and differed significantly in each tissue between Karoon and Dez Rivers ($p < 0.05$) but non-significantly for Hg in the gill of *B. grypus* between two rivers (Table 1). The concentrations of Cd, Pb, Ni and Hg in various tissues of *B. xanthopterus* in Karoon River was higher than those observed in *B. xanthopterus* in Dez River ($p < 0.05$) and differed significantly in each tissue of *B. xanthopterus* between Karoon and Dez Rivers ($p < 0.05$), (Table 2). The highest levels of Cd, Pb and Ni were found in the gill of two barbs in two rivers and Hg level in the liver was higher than other tissues except for *B. grypus* in Karoon River. The highest level of Hg in the liver has been corroborated by Kennedy (2003). Havelkova et al. (2008) reported that the target organ for Hg accumulation in fish from heavily contaminated localities was the liver. The results of our study was similar to the above study. The liver has the ability to accumulate large quantities of pollutants from the external environment and also plays an important role in storage, redistribution, detoxification and transformation of

pollutants (Evans et al. 1993). Studies carried out with various fish species have shown that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxify by producing metallothioneins (Kargin and Erdem 1991). In *B. grypus* in Karoon River, the highest levels of Cd, Pb, Ni and Hg were found in the gill. The gills are the first organs to be exposed to resuspended sediment particles, so they can be significant sites of interaction with metal ions (Pawert et al. 1998). Gills are the uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Kamaruzzaman et al. 2010). Metal concentration in the gills could be due to the element complexing with the mucus, which is impossible to remove completely from between the lamellae, before tissue is prepared for analysis. The absorption of metals on to the gill surface, as the first target for 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. grypus* and *B. xanthopterus* in Karoon and Dez Rivers shows that the metal levels of tissues are very variable in fishes. The observed variability of heavy 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) and their habitats (Canli and Atli 2003). Concentrations of

Table 1 Concentrations of heavy metals (mg kg^{-1} dry weight) in various tissues of *Barbus grypus* in Karoon and Dez Rivers, Khoozestan, Iran

Tissues	River	Cd	Pb	Ni	Hg
Gill	Karoon	1.27 ± 0.07^a	2.25 ± 0.1708^a	0.80 ± 0.04^a	0.89 ± 0.07^a
	Dez	1.49 ± 0.01^b	1.70 ± 0.02^b	1.02 ± 0.05^b	0.94 ± 0.30^a
Liver	Karoon	1.07 ± 0.08^a	2.10 ± 0.13^a	0.62 ± 0.08^a	0.79 ± 0.08^a
	Dez	1.34 ± 0.01^b	1.55 ± 0.01^b	0.95 ± 0.06^b	1.06 ± 0.06^b
Muscle	Karoon	0.84 ± 0.08^a	1.75 ± 0.10^a	0.46 ± 0.05^a	0.73 ± 0.06^a
	Dez	1.09 ± 0.01^b	1.29 ± 0.06^b	0.85 ± 0.05^b	0.90 ± 0.03^b

Data are presented as means $\pm$ SE

^{a, b} $p < 0.05$, significantly different in each tissue between two rivers

Table 2 Concentrations of heavy metals (mg kg^{-1} dry weight) in various tissues of *Barbus xanthopterus* in Karoon and Dez Rivers, Khoozestan, Iran

Tissues	River	Cd	Pb	Ni	Hg
Gill	Karoon	2.17 ± 0.10^a	2.91 ± 0.48^a	1.43 ± 0.02^a	1.34 ± 0.10^a
	Dez	1.19 ± 0.08^b	1.40 ± 0.11^b	0.85 ± 0.03^b	0.63 ± 0.02^b
Liver	Karoon	1.91 ± 0.04^a	2.78 ± 0.47^a	1.30 ± 0.07^a	1.42 ± 0.08^a
	Dez	1.04 ± 0.07^b	1.26 ± 0.11^b	0.75 ± 0.01^b	0.95 ± 0.02^b
Muscle	Karoon	1.67 ± 0.05^a	2.37 ± 0.42^a	1.15 ± 0.08^a	1.28 ± 0.06^a
	Dez	0.79 ± 0.06^b	0.95 ± 0.10^b	0.59 ± 0.01^b	0.77 ± 0.14^b

Data are presented as means $\pm$ SE

^{a, b} $p < 0.05$, significantly different in each tissue between two rivers

Table 3 Comparison of heavy metals (mg kg⁻¹ dry weight) in muscle of *Barbus grypus* with WHO standard in Karoon and Dez Rivers, Khoozestan, Iran

Species	River	Cd	Pb	Ni	Hg
<i>B. grypus</i>	Karoon	0.84 ± 0.08 ^a	1.75 ± 0.10 ^a	0.46 ± 0.05 ^a	0.73 ± 0.06 ^a
	Dez	1.09 ± 0.01 ^b	1.29 ± 0.06 ^b	0.85 ± 0.05 ^b	0.90 ± 0.03 ^b
WHO standard		0.2 ^c	0.3 ^c	0.3 ^c	0.1 ^c

^a, ^b, ^c $p < 0.05$, significantly different in muscle of *Barbus grypus* and *Barbus xanthopterus* with WHO (World Health Organization) standard
Reference: WHO 1985, 1994, Czarneski 1985

Table 4 Comparison of heavy metals (mg kg⁻¹ dry weight) in muscle of *Barbus xanthopterus* with WHO standard in Karoon and Dez Rivers, Khoozestan, Iran

Species	River	Cd	Pb	Ni	Hg
<i>B. xanthopterus</i>	Karoon	1.67 ± 0.05 ^a	2.37 ± 0.42 ^a	1.15 ± 0.08 ^a	1.28 ± 0.06 ^a
	Dez	0.79 ± 0.06 ^b	0.95 ± 0.10 ^b	0.59 ± 0.01 ^b	0.77 ± 0.14 ^b
WHO standard		0.2 ^c	0.3 ^c	0.3 ^c	0.1 ^c

^a, ^b, ^c $p < 0.05$, significantly different in muscle of *Barbus grypus* and *Barbus xanthopterus* with WHO (World Health Organization) standard
Reference: WHO 1985, 1994, Czarneski 1985

heavy metals detected in the muscle, gill and liver samples showed different capacities for accumulating. In general, the highest metal concentrations were found in the liver and gill. Thus, the liver and gill in fish are more often recommended as environmental indicator organs of water pollution than other fish organs (Karadede et al. 2004). The relatively high concentrations of heavy metals in liver and gill were also found in different species of fish in Tigris River and Atatürk Dam Lake (Karadede and Ünlü 2000). Alhas et al. (2009) reported that in *B. xanthopterus* and *B. rajanorum mystaceus* in Atatürk Dam Lake, Turkey, heavy 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. Oymak et al. (2009) has reported the concentrations of Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in gill, liver, muscle and kidney of *Tor grypus* in Atatürk Dam Lake, Turkey and Malik et al. (2010) 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; They reported that the liver and gill showed higher metal concentrations than muscle. So, the results of our study were similar to the above studies. Also, the mean of Ni and Pb concentrations in the muscles of *B. xanthopterus* and *B. rajanorum* in Ataturk Dam Lake of Turkey, were reported as: 0.08, 0.68; 0.04, 0.66 µg g⁻¹ respectively (Alhas et al. 2009) and in Sunderban mangrove wetland of northeast India, mean Hg concentration in the muscle, liver and gill of *L. parsia* was reported as: 0.12, 0.65 and 0.54 µg g⁻¹ respectively (Saha et al. 2006), a comparison of these concentrations with our data clearly shows higher values in two barbs in Karoon and Dez Rivers. The results from this research indicated that metal

accumulation depended on the tissues probably as a consequence of metabolic needs, physiochemical properties, and detoxification processes specific for each element. The results of this study showed that Pb was the highest accumulating metal compared with other metals ($p < 0.05$), as Pb is a microelement naturally present in trace amount in all biological materials in soil, water, plants and animals, it has no physiological function in the organism (Cibulka 1991). The main source of Pb contamination are smelting works, application of waste water treatment sludge's to soil, transportation, rain, hail and other, approximately 98% of Pb in the atmosphere originates from the human activities (Fadrus et al. 1979). So the Pb finds its way in rivers through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries etc. Pb also enters the rivers both from terrestrial sources and atmosphere and the atmospheric input of Pb aerosols can be substantial (Mittra et al. 2010). The high levels of Pb in the Karoon and Dez Rivers have toxic effects on fish metabolism, it is important to consider the biological effects of contamination on fish health in Karoon and Dez Rivers. So, Waste management in urban and industrial centers in Khoozestan, especially around the cities of Karoon and Dez Rivers, have remained very unsatisfactory to—data and it can be concluded that this poses a health hazard to both aquatic life and humans alike.

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