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RESEARCH PAPER

Determination of Heavy Metals in Tissues of *Barbus grypus* (Heckel, 1843) from Batman Dam, Turkey

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Abstract

The present study was carried out to determine concentrations of heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in liver, gill and muscle of *Barbus grypus*, which has a great economic value, in Batman Dam (Turkey). Heavy metal concentration levels in fish were investigated using inductively coupled plasma mass spectrometry (ICP-MS). The metal concentrations in the liver and gill of *Barbus grypus* were found to be higher comparing to those in the muscle. Metal concentrations in the fish tissues vary significantly ($P<0.05$). Concentrations of the metals were different in the various organs, the highest concentrations of Mn (4.414–6.101 mg kg^{-1}), Fe (111.333–69.088 mg kg^{-1}), Cu (5.201–0.437 mg kg^{-1}) and Zn (14.883–16.555 mg kg^{-1}) were found in liver and gill, while Cr (0.132 mg kg^{-1}) was the only metal that is higher in muscle tissue. Heavy metal concentrations in the muscle of the fish should not exceed the acceptable levels proposed by the Food and Agriculture Organization, World Health Organization and Turkish legislation for food sources for human consumption. Therefore, the metal levels in such environments, should be monitored periodically to avoid excessive intake of heavy metals consumed by public.

Keywords: Heavy metals**,** *Barbus grypus*, ICP- MS, Batman Dam.

Introduction

The pollution of aquatic ecosystems is one of the serious environmental problems worldwide (Ahmad *et al*., 2014). In the aquatic ecosystems, heavy metals are derived from a variety of natural and anthropogenic sources, such as geologic weathering, atmospheric deposition, agricultural activities, mining wastes, residential and industrial products (Varol, 2013). These pollutants can pose a serious threat to aquatic life due to their toxicity, long persistence, bioaccumulation and biomagnification in the food chain (Yousafzai, Chivers, Khan, Ahmad, & Siraj, 2010).

Barbus grypus (Heckel 1843) known as Şabot in public is belonging to *Cyprinidae* family. According to FAO (Food and Agriculture Organization), *Barbus grypus* (Heckel 1843) is one of the most significant fish species recorded in Iraq freshwaters, the South and Southwestern Rivers of Iran, Karoon River, Euphrates and Tigris Rivers of Turkey (Selki, Başusta, & Çiftçioğlu, 2005; Zivotofskya & Amar, 2006; Dorostghoal, Peyghan, Papan, & Khalili, 2009). To our best knowledge, there is no any study on *B. grypus*, which has a commercial and economic value, in Batman Dam although several studies on heavy metal concentration in tissues of the species in Turkey's reservoir have already been reported (Oymak, Karadede- Akın, & Doğan, 2009; Olgunoğlu & Olgunoğlu, 2011).

Both Batman Dam and hydroelectric power plant were built in 1999 on the Batman stream in order to enable irrigation and power generation as well as to prevent flood. Recently, agricultural drainage and municipal wastewater discharges from the villages and towns around the reservoirs are potential sources of pollution. In addition, substances brought by the rivers during the rainy season also contribute to it. However, there is no industrial pollution source around the reservoirs (Varol, Gokot, Bekleyen, & Şen, 2012).

The aim of the present study is to evaluate several heavy metal levels such as chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in liver, gill and muscle of *Barbus grypus* (Heckel, 1843) obtained from Batman Dam. The study provide useful information on food safety for potential consumers.

Materials and Methods

Study Area

Batman Dam, the study area, is located on the Batman River, which constitutes a natural border between Batman and Diyarbakır provinces (Figure 1). Batman Dam is far away from Batman approximately 35 km. The coordinates of Batman Dam are 38º14' 5.72" N 41º5'55.37" E, Dam's altitude is 652 m and has 49.25 km² of the lake area. The body volume of the dam is 7.181.000 m³, height from the streambed is 85.00 m and volume of the lake in normal water level is 1175 hm³.The dam, which was constructed from rocks, is one of the main tributaries of the Tigris River, was built for irrigation, flood prevention and producing electricity (Varol *et al*., 2012).

Analytical Procedures and Sample Preparation for Heavy Metal Analysis

Fish samples (*Barbus grypus*) were collected from Batman Dam. During the study, twenty fish specimens were caught in July 2013. Fish samples were transported to the laboratory on ice on the same day. The number, age, size and weight of each fish species were shown in Table 1. The mean length and weight were measured which ranged from minimum and maximum value as 210.60±8.562 mm for length and 108±17.711 g for weight of *Barbus grypus*. Total fork length (mm) and weight (g) were recorded for fishes. To determine the age, six to eight scales per fish were also collected from above the lateral line and posterior to the dorsal fin area. Scales were cleared in KOH for 12 h, rinsed in distilled water and examined by using a Nikon YS100 reader for the determination of annual mean. All fish samples were kept at −30°C until analysis. Fish samples, approximately 5 g of the epaxial muscles on the dorsal surface of the entire liver and gill racers from fish samples were dissected. The samples were dried approximately at 85°C for 72 h, and then 0.1 g (d.w.) of each fish sample was weighed and transferred to microwave digestion tubes. Analysis was carried out according to the procedure described by Karadede and Ünlü (2000). To each sample tube, 7 ml 65% HNO_3 and 1 ml 30% H_2O_2 (Merck, Darmstadt, Germany) solutions were added. After digestion in the microwave oven, the tubes were cooled down to room temperature. The cooled samples were then transferred to the falcon tubes, and the solution in the tubes was diluted to 15 ml with ultra-pure water. All samples were analyzed triplicate times for Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb by Agilent 7700X inductively coupled plasma mass spectrometry (ICP-MS). The detection limits of ICP-MS were Cr; 0.036 µg L^{-1}, Mn; 0.037 µg L^{-1}, Fe; 0.125 µg L^{-1}, Co; 0.002 µg L^{-1}, Ni; 0.805 µg L^{-1}, Cu; 0.160 µg L^{-1}, Zn; 1.483 µg L^{-1}, Cd; 0.002 µg L^{-1} and Pb; 0.121 µg L^{-1}. Working standard solutions for system calibration and control of analytical accuracy were obtained by dilution of the stock solutions (1 mg L^{-1} for ICP-MS). Basic operating conditions of the instrument are described in Table 2. The accuracy of analytical procedure was checked by analyzing the standard reference materials DOLT-3 (dogfish liver, National Research Council, Canada) and DORM-2 (dogfish muscle, National Research Council, Canada). Replicate analysis of these reference materials showed good accuracy, with recovery rates for metals

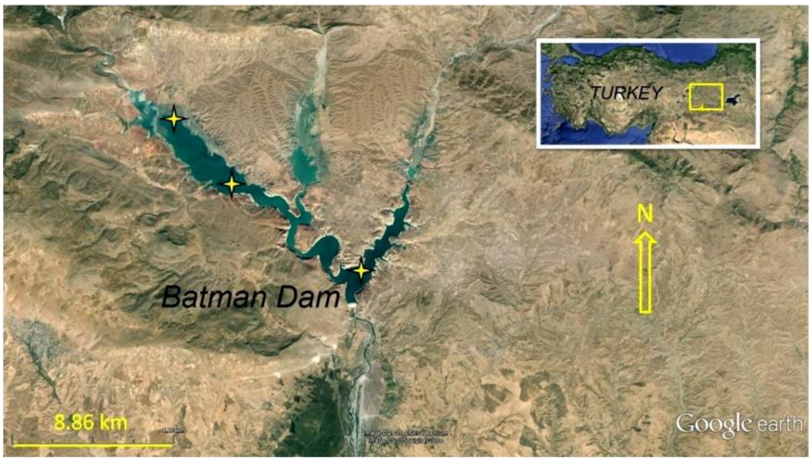

Figure 1. Map of Batman Dam (Tigris River, Turkey).

Table 1. Minumum, maximum and mean lenght, weight and age of the *Barbus grypus* in the Batman Dam

Species	N	Age (year)	Fork length (mm)	Body weight (g)
Barbus grypus	20	4-5	210.60±8.562 (198-220)	108±17.711 (82-128)

Table 2. Operating conditions for the Inductively Coupled Plasma Mass Spectrometery

Parameters	Condition
RF power (W)	1300
Plasma gas flow rate (l/min)	1.5
Auxiliary gas flow rate (l/min)	0.9
Nebulizer flow rate (l/min)	1-1.1
Sample uptake rate (ml/min)	1.8
Type of spray chamber	Scott
Type of nebulizer	Micramis
Measurement replicates	3
Analytical mass	^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Zn, ^{111}Cd, ^{208}Pb

between 92% and 110% for DORM-2, 90% and 116% for DOLT-3.

Statistical Analysis

Statistical comparison among means of more than two groups was performed by one-way ANOVA. When ANOVA was significant, the Duncan's Multiple Range Test was employed for the comparison. Differences were considered significant at $P<0.05$. Statistical analysis of data was carried out using SPSS 12.0 statistical package programs. The concentration levels of metal concentrations for fish tissues were expressed as mg kg^{-1} wet weight (ww). All values were expressed as means ± standard deviation (SD).

Results

The mean concentration (mg kg^{-1} wet weight) of heavy metals in the liver, gill and muscle tissues of *Barbus grypus* are summarized in Figure 2 and Table 3. Cr, Mn, Fe, Cu and Zn were detected in all the evaluated fish tissues, while Ni was undetectable level in all the evaluated types of tissues. Co, Cd and Pb were undetectable levels in muscle tissue.

Concentrations of heavy metals detected in the liver, gill and muscle tissues of fish were found to be different. Heavy metal concentrations were higher in the liver and gill, while it was lower in the muscle. As shown in Table 3, the highest concentrations of Fe, Co, Cu and Cd were in the liver, whereas Mn, Zn and Pb were higher in the gills and Cr in muscle tissues. Particularly, Fe and Zn concentrations among heavy metals were determined to be very high in all the evaluated tissue samples (Figure 2). Significant differences between Mn, Fe and Zn levels in fish tissues were determined ($P<0.05$).

Distribution patterns of metal concentrations in the liver tissue of *Barbus grypus* follow the order from the highest to the lowest: Fe, Zn, Cu, Mn, Cd, Pb, Co, Cr, Ni; in gill tissue Fe, Zn, Mn, Cu, Pb, Cr, Co, Cd, Ni; in muscle tissue Zn, Fe, Mn, Cu, Cr. The order of heavy metal levels of Fe, Co, Cu and Cd from the highest to the lowest in tissues follow as liver, gill, muscle. However, the order of heavy metal levels of Mn, Zn and Pb from the highest to the lowest in tissues follow as gill, liver, muscle, whereas for Cr follow the order: as muscle, gill, liver.

Discussion

The heavy metals are the most important forms of pollution and they may accumulate in the tissues of fish which are often at the top of the aquatic food chain (Rodriguez, Jimenez-Capdeville, & Giordano, 2003; Yılmaz, Özdemir, Demirak, & Tuna, 2007). We studied the concentration levels of some heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) in various tissues of *Barbus grypu*s from Batman Dam. The results confirm the differences of heavy metal concentration in the examined tissues. The highest concentrations were found in the liver and gill, while the lowest concentrations of detected metals were in the muscle. Especially, liver and gill tissues, rather than the other organs of fish, have been frequently recommended as an indicator of water and environmental pollution (Karadede, Oymak, & Ünlü, 2004). In our study, among heavy metals detected at highest concentrations in the liver and gill were Fe, Zn, Mn and Cu. Liver is capable of accumulate huge amount of contaminants from the external environment and also plays an important role in storage, redistribution, detoxification and transformation of pollutants (Evans, Dodoo, & Hanson, 1993; Liang, Cheung, & Wong, 1999). The high metal concentration in liver can be explained by the fact that the liver is the metal detoxification place where metal connector proteins like metallothionein is synthesized and deposits more metal compared to other tissues (Hogstrand & Haux, 1991; Unlu, Akba, Sevim, & Gümgüm, 1996; Kalay & Erdem, 1995). The levels of Fe and Zn in all the evaluated tissues of *Barbus grypus* were higher than the levels of Cu and Mn. Iron is also essential for the synthesis of hemoglobin and involved in numerous enzymatic reactions linked to oxido-reduction (catalyze, cytochrome oxidase, peroxidase). However, the low metal concentrations in muscle tissue can be explained with the fact that it is not an active organ in detoxification mechanism (Karadede *et al*., 2004; Bajc, Gacnik, Jencic, & Doganoc, 2005; Alhas,

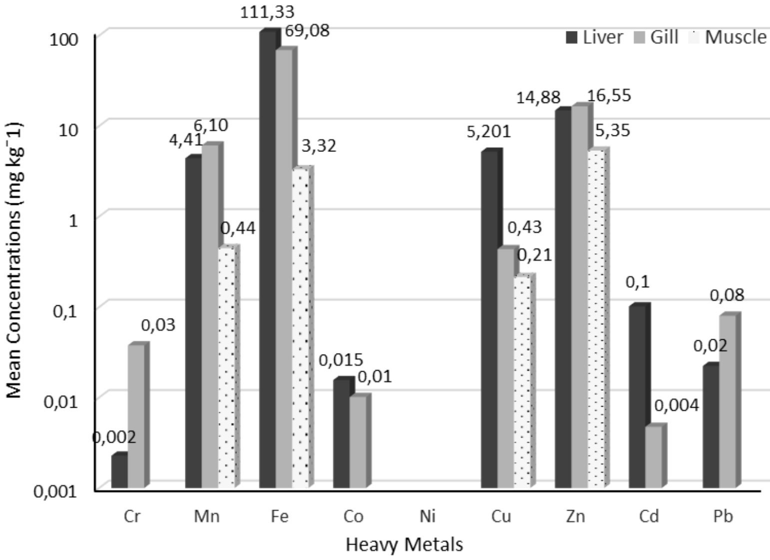

Figure 2. Heavy metal concentrations (mg kg^{-1} wet wt) in tissues of *Barbus grypus* from Batman Dam.

Table 3. The heavy metal concentrations (mg kg^{-1} wet wt.) in tissues of *Barbus grypus* from Batman Dam

Metals	Liver	Gill	Muscle
Cr	0.002±0.001^{a}	0.038±0.037^{a}	0.132±0.134^{b}
Mn	4.414±1.326^{b}	6.101±0.745^{c}	0.448±0.077^{a}
Fe	111.333±3.574^{c}	69.088±14.562^{b}	3.322±1.220^{a}
Co	0.016±0.001b	0.010±0.007^{a}	ND
Ni	ND	ND	ND
Cu	5.201±0.999^{b}	0.437±0.050^{a}	0.215±0.015^{a}
Zn	14.883±4.448^{b}	16.555±0.510^{c}	5.354±0.442^{a}
Cd	0.102±0.008^{b}	0.005±0.001^{a}	ND
Pb	0.022±0.002^{a}	0.080±0.043^{b}	ND

Mean values and ± standard deviation.
ND: Shows that the resullts were lower than the limit of detection of ICP-MS. Values with different letters are significantly different at P<0.05 level.
a,b,c - Significant differences of the means between the investigated tissues.

Oymak, & Akın, 2009). The results also showed that Mn, Zn and Pb levels were higher in fish gills. The previous studies also demonstrated that fish gills mainly contain high metal concentrations due to its large surface area, direct contact with the external environment and short diffusion distance between water and blood (Kalay & Erdem, 1995; Kuşatan & Cicik, 2004).

The results presented in Table 4 indicates the comparison of the heavy metal levels in tissues of *Barbus grypus* in Batman Dam with those in the tissues of *Barbus grypus* obtained from other locations. The mean heavy metal concentrations in the muscle tissues of *Barbus grypus* in the present study were found to be lower than those reported for *Barbus grypus* and *Barbus xanthopterus* in the Karoon and Dez Rivers in Iran (Mohammadi, Sary, & Khodadadi, 2011), for the *Barbus grypus* and *Tor grypus* in the Atatürk Dam Lake, Turkey (Oymak *et al*., 2009; Olgunoğlu & Olgunoğlu, 2011), for the *Barbus grypus*, *Barbus luteus* and *Barbus sharpeyi* in the Shadegan wetland in Iran (Alhashemi, Sekhavatjou, Kiabi, & Karbassi, 2012), and for the *Barbus capito* in the Seyhan River, Turkey (Canlı, Ay, & Kalay, 1998).

Cr, Co, Cu, Zn and Pb concentrations in gill and liver of *Barbus grypus* in present study were found lower than those obtained by Alhas *et al*. (2009) in a study carried out on *Barbus xanthopterus* and *Barbus rajanorum mystaceus* in the Atatürk Dam Lake, Turkey. In addition, the present study demonstrates that heavy metal levels (Cu and Zn) at tissues of *Barbus grypus* were lower than those studied at different localities on Tigris River, on which Batman Dam is built (Gümgüm, Ünlü, Akba, Yıldız, & Namlı, 2001; Karadede & Ünlü, 2007).

According to international criterias and Turkish regulation given below, heavy metal concentrations in the muscle of *Barbus grypus* in the Batman Dam Lake were found to be lower than the acceptable limits. The permissible limits proposed by the Food and Agriculture Organization, World Health Organization and Turkish legislation were as the following maximum levels for the metals studied, above which

Table 4. Heavy metal levels (mg kg^{-1} wet wt., mean) in different tissues of *Barbus grypus* from the Batman Dam (Turkey) and other selected 1 regions (*= dry wt.)

Tissues	Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	References
	Shadegan wetland (Iran)	0.87	1.67		2.12	2.59	2.49	23.37	0.28	12.87	Alhashemi *et al.*, 2012
	Karoon and Dez Rivers (Iran)*	-	-	-	-	0.46-0.85	-	-	0.8-1.1	1.7-1.3	Mohammadi *et al.*, 2011
Muscle	Atatürk Dam Lake (Turkey)	0.22	0.45	10.94	0.15	0.16	0.56	3.98	-	1.23	Oymak *et al*., 2009
	Atatürk Dam Lake (Turkey)	-	-	-	-	-	1.084	10.848	0.03	-	Olgunoğlu and Olgunoğlu, 2011
	Batman Dam (Turkey)	0.132	0.448	3.322	-	-	0.215	5.354	-	-	Present study
	Shadegan wetland (Iran)	1.63	4.45		1.82	2.50	23.69	102.55	0.53	12.87	Alhashemi *et al.*, 2012
Gill	Karoon and Dez Rivers (Iran)*	-	-	-	-	0.80-1.02	-	-	1.2-1.4	2.2-1.7	Mohammadi *et al.*, 2011
	Atatürk Dam Lake (Turkey)	0.34	4.67	88.85	0.24	0.35	1.23	13.35	-	2.57	Oymak *et al*., 2009
	Batman Dam (Turkey)	0.038	6.101	69.088	0.010	-	0.437	16.555	0.005	0.080	Present study
	Shadegan wetland (Iran)	1.63	4.39		1.94	22.74	8.69	77.86	0.54	7.48	Alhashemi *et al.*, 2012
Liver	Karoon and Dez Rivers (Iran)*	-	-	-	-	0.62-0.95	-	-	1.0-1.3	2.1-1.5	Mohammadi *et al.*, 2011
	Atatürk Dam Lake (Turkey)	0.23	2.217	149.04	0.35	0.17	8.86	28.13	-	2.98	Oymak *et al*., 2009
	Batman Dam (Turkey)	0.002	4.414	111.33	0.016	-	5.201	14.883	0.102	0.022	Present study

consumption is not permitted: 0.1 µg/g for Cd, 5 µg/g for Cu, 50 µg/g for Zn, 50 µg/g for Cr, 0,5 µg/g for Ni, 50 µg/g for Mn, 100 µg/g for Fe and 0.5 µg/g or 1 µg/g for Pb (FAO, 1983; FAO /WHO, 1989; WHO, 1993; ITS, 2000; Dirican *et al*., 2013).

Conclusion

This study was carried out to provide information on heavy metal concentrations in *Barbus grypus* from the Batman Dam. Our results showed that the concentrations of heavy metals detected in muscles of *Barbus grypus* from the Batman Dam Lake were found to be at the levels below the FAO, WHO, ITS maximum permissible limits. Although levels of heavy metals are not high, a potential danger may emerge in the future depending on the domestic wastewaters and agricultural activities in this region. However, our opinion is that these kinds of studies should be carried out periodically in order to take precautions against possible dangers that may increase heavy metal pollution and regular supervision.

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