

Research Article

Assessment of Heavy Metal Bioaccumulation in Some Tissues of *Leuciscus Cephalus* from Karasu River, Erzurum-Turkey

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Karasu River, the only river in the Erzurum plain, is the source of Euphrates River (Eastern Anatolia of Turkey). It is considered that artificial fertilizers, pesticides, municipal sewage, industrial and factory wastes are polluted the Karasu River. The pollution in the river also threatens the Euphrates basin. Fish live in direct contact with their immediate external environment. Therefore, fish are frequently used to determinate the aquatic pollution. In this study, we aim to determine concentrations of metals such as Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb on liver, muscle and gill tissues of *Leuciscus cephalus* (chub) which was obtained from the selected two points (Dumlu and Aşkale) on Karasu River. The heavy metals were measured by ICP-MS (Inductively Coupled Plasma–Mass Spectrometer). The concentrations of heavy metals in tissue of fish from the Aşkale site were significantly higher than those from the Dumlu site. High levels of metals were found in the gills in fish from Aşkale site. These results show that the concentrations are below the limits for fish proposed by Turkish Food Codex, FAO/WHO and EC, and safe within the limits for human consumption in the edible parts of fish species in the region.

Keywords: Heavy metals; Karasu River; Pollution; *Leuciscus cephalus*; Health risk**Introduction**

There are countless pollutants in the surface waters and sediment that are compromising the survival of the organisms, altering their physiologies or giving rise to carcinogenesis. Consequences caused by these pollutants may remain recessive for several generations or may exhibit major effects in the population. Pollution in the aquatic environment causes multiple damages in the organisms, at the level of population and ecosystem, as in organ function, reproductive performances and biological diversity [1]. In aquatic environments, particularly industrial and domestic wastes discharged without being treated, are the source of heavy metals [2]. The increasing of heavy metal contamination in aquatic systems and decreasing sediment quality may cause toxicity in freshwater ecosystems [3]. The toxicity of metals depends on the metal type and concentration, the period of exposure and other factors. Also, metals join in the food chain and are responsible for adverse effects and death in the aquatic organisms [4]. In addition to this contamination causing an adverse effect on aquatic biota, the metallic contaminants can be ingested by humans through consumption of the seafood products [5].

In some areas of the world, aquatic organisms, especially fish, are the most important sources of protein in the human diet. Fish are widely being used for evaluating the quality of the aquatic environment and as bioindicators of environmental pollution [6,7]. Fish have also been used extensively to study the physiological behavior of heavy metals in body organs [8]. The concentration of metals is a function of species, and metals accumulate more in some fish tissues than in others [9]. Different tissues of fish species have

different bioaccumulation capacities [10]. The sampling location and season, as well as diet preferences and fish size may influence the level of bioaccumulation in the same fish species [11]. Liver and gills, as metabolically active organs, are target organs for metal accumulation [12], while the accumulation in muscle tissue is lower [13]. The concentrations of metals in the organs of fish are governed primarily by the level of pollution in the water and food and so are indicative of the level of pollution in the environment [14]. Furthermore, ecological needs, gender, size and seasonal changes can affect metal accumulation in different tissues of fish [15,16].

The Karasu River, which is the only river in Erzurum plain, is the source of the famous river Euphrates. The river is also an important tributary of the Tigris River and has been polluted substantially with sewage water and effluent wastes of slaughterhouses, fat, sugar and cement factories. Hence, mass fish deaths have been occurred twice in the past 10 years [17]. The pollution in the Karasu River also threatens the Euphrates basin. *Leuciscus cephalus* (chub) is fish species belonging to the Cyprinidae family. Although the fish can be found in the upper zones of the Tigris and Euphrates rivers, they live mainly in the upper regions of Karasu River [18]. In this study, chub is used as a bioindicator for monitoring the aquatic environment because the fish is abundant at Karasu River.

Over the last decades, there has been interest in determining metal contents in fresh water environment, and attention was drawn to the measurement of pollution in public food supply especially fish. Several reliable analytical methods were available for monitoring metal levels in fresh water and marine food samples, but Inductively Coupled

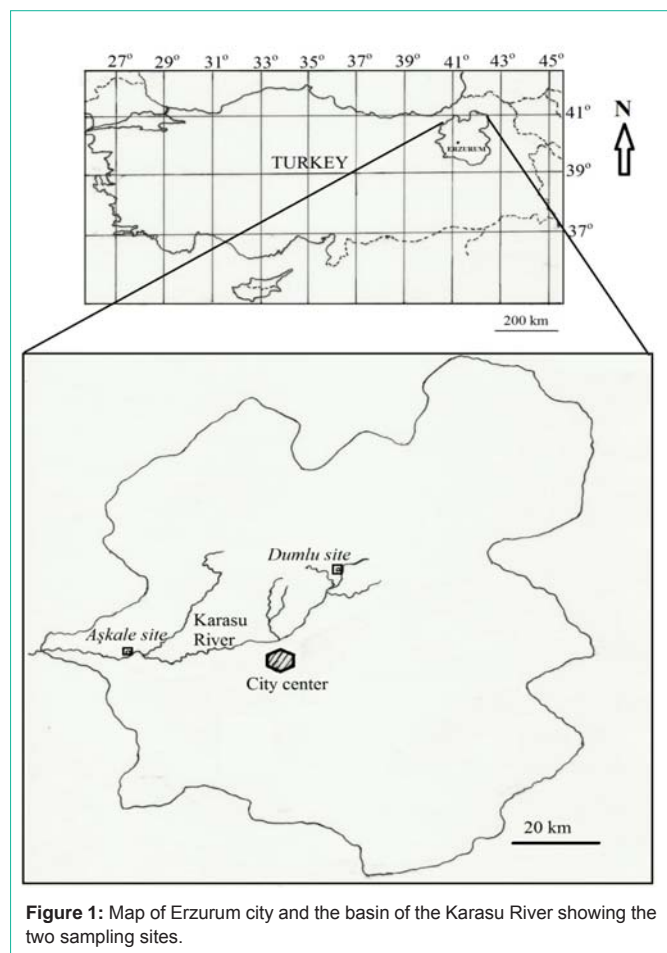


Figure 1: Map of Erzurum city and the basin of the Karasu River showing the two sampling sites.

Plasma Mass Spectrometer (ICP-MS), being the most sophisticated and reliable technique was widely used for determination and quantification of trace metals in food samples [19]. The present study was undertaken to determine the concentrations of selected metals (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb) in the surface water and liver, muscle, and gill tissues of *Leuciscus cephalus* (chub) from the selected two sites (Dumlu and Aşkale) on Karasu River.

Materials and Methods

The Karasu River is located in the Eastern Anatolia region of Turkey (Erzurum) and drains an area of approximately 1642 km² and joins the Euphrates River. At the upper section of the river, various industrial activities, like textile dyeing, cement and sugar factories release their waste into the river. Due to the extended domestic and industrial activities, various pollutants reach the river at different points and potentially change the water quality. Two sampling sites were selected in the Karasu River, with different levels of chemical impact (Figure 1). Firstly, an impacted site was Aşkale site located approximately 80 km further downstream which is a heavily contaminated tributary collecting industrial effluent. Dumlu site relatively uncontaminated location was used as control, another tributary of the Karasu River. All located inside the city provinces of Erzurum. Aşkale site at near the Aşkale district shows strong anthropogenic influence, receiving domestic, agricultural and industrial effluents. The site is 58-km outside the Erzurum city.

The coordinates of sampling Dumlu site are 40° 01' 52K, 41° 18' 49D and that of sampling Aşkale site are 38° 22' 33K, 36° 58' 26D (Figure 1).

From May to August 2012, a total number of 56 fish species (28 fish from each site) were collected. The fish were carried live to the laboratory in tanks filled with water obtained from the sites where the fish were caught. The wet weight and total body length of the fish were measured ($61,68 \pm 12,2$ - $58,15 \pm 5,8$ g; $19,25 \pm 3,0$ - $17,75 \pm 1,2$ cm). Liver muscle and gill samples were quickly removed, rinsed with distilled water, and stored at -18°C prior to analysis. We confirm that all procedures were performed in compliance with the relevant laws and institutional guidelines, and that the appropriate institutional committees have approved them.

Water samples were collected from a depth of 0.5 m below the surface into clean 1-l polyethylene bottles by means of a Nansen Sampler. The water temperature, electrical conductivity, dissolved oxygen, and pH were measured in situ. Then, 1 ml of 0.5% HNO₃ was added to acidify the water samples. The samples preservation and analyses were made following the American Public Health Association and the American Water Works Association standard methods [20].

Frozen liver, muscle and gill samples were dried using freeze dryer and homogenized. A temperature-controlled microwave heating device was used for digestion of the dried fish tissues. Sample preparation was carried out according to the procedure described by Uluozlu [21]. Approximately 0.5 g of homogenized samples were taken and placed in a Teflon digestion vessel with 3 mL of ultrapure HNO₃ and 1 mL of Hydrogen Peroxide (H₂O₂). Sealed containers were placed in a microwave oven and heated according to the digestion program. After digestion, sample solutions were cooled to room temperature then transferred quantitatively into acid cleaned 25 mL standard volumetric flasks and made up to 25 mL with double distilled deionized water and prepared under the same conditions as the calibration standards in 6% (v/v) HNO₃. Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb in fresh water fish were analyzed using ICP-MS (PerkinElmer Élan 9000 USA). Heavy metals in the fish samples were successively extracted as the acid soluble fraction, reducible fraction and oxidizable fraction. The residual fraction was determined by the mass balance between the total and the non-residual fractions. For Quality Assurance/Quality Control (QA/QC) purposes, digestion of procedure blanks, duplicates of 10% sample load. For better operating conditions the ICP-MS was adjusted to nebulizer gas flow 0.91 L/min, radio frequency 1200W, lens voltage 1.6V, cool gas 13.0 L/min, and auxiliary gas 0.70 L/min [22]. All data were tested using normality and equal variance tests. Parametric and nonparametric tests were performed to determine whether there were any significant differences in metal concentrations between the sampling sites. Statistical significance was set to a level of 5% (p<0.05). Statistical analyses were performed using the SPSS 15.0 software. Additionally, concentrations of metals in fish samples were evaluated and compared with permitted limits set by Turkish Food codex (TFC), Food and Agricultural Organization (FAO), and European Commission (EC) [23, 24, 25]. All data were expressed in microgram per gram dry weight.

Results and Discussion

The water quality of the sampling sites was evaluated by comparing

Table 1: Means and standard deviation of some metals in water from the sites ($\mu\text{g/L}$).

Metals	Dumlu site	Aşkale site
Al	192.10±22.22	287.8±51.22
Cr	0.84±0.04	0.85±0.04
Mn	18.9±1.5	24.42±1.22
Fe	1.65±0.86	1.75±0.17
Ni	37.33±10.23	88.78±15.01
Cu	0.9±0.05	0.82±0.04
Zn	0.59±0.06	0.68±0.01
As	2.90±0.22	3.28±0.62
Cd	0.03±0.01	0.04±0.01
Hg	0.02±0.01	0.04±0.01
Pb	0.25±0.01	0.56±0.02

the results of some metal concentrations. Regarding total metal concentrations, higher values were recorded in the Aşkale site than in the Dumlu site water course for all the elements analyzed (Table 1). Zn, Al, Co, Pb, and As concentrations surpassed the benchmark values for surface waters provided by SWECO databases [26]. Water samples from the Karasu River showed different concentrations and accumulation levels of metallic contaminants. The concentration of Al was highest and Hg lowest in water samples. In all cases, these concentrations were higher than those recommended for drinking and irrigation purposes. As seen in Table 1, the metal concentrations in water were higher than those recommended by SWECO for surface waters [26]. Metal concentrations in the muscle, liver and gills of *Leuciscus cephalus* are presented tables 2-3, which include mean concentrations with associated standard deviations results from other studies and guidelines, and background levels. Each individual metal displays a wide variation in concentration, as reflected by the large standard deviation values. Due to variations within each element, mean concentration might be significantly affected via the extremely high or low values. In Table 2, mean element concentrations for *Leuciscus cephalus* tissue samples are presented. The Kruskal-Wallis test (a nonparametric test) revealed significant differences between sites with regard to element levels in liver for Mn, Fe, Ni, Cu, Cd, and Pb ($p<0.05$), in muscle for Cr, Fe, As, Cd, and Pb ($p<0.05$), and gills for Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb ($p<0.05$). For the liver, this test showed that Aşkale site fish had significantly higher levels of Mn, Fe, Ni, Cu, Cd, and Pb, compared to Dumlu site fish. For muscle, this test showed that Aşkale site chub had statistically different levels of Cr, Fe, As, Cd, and Pb, compared to Dumlu site. Aşkale site fish had a statistically higher level of all tested elements in gills than Dumlu site fish. The element analyses showed that fish from Aşkale site had statistically high levels of all tested elements. There was metal accumulation in the gills compared to muscle and liver tissues. We also found that Zn had the highest metal concentration in gills of Aşkale site fish ($35.9\pm0.18 \mu\text{g/g}$).

Statistically, significance analysis was performed between Dumlu and Aşkale site (Table 2). In general, the concentration differences two sites in tissues of fish were small. The subtle differences of concentration in gill, liver and muscle were reflected in the relatively small difference in the pollution ratios of two sites. In addition, the results show that the metal accumulation in tissues of *Leuciscus*

Table 2: Concentration of metals (in microgram per gram) in tissues of *Leuciscus cephalus* from Karasu River.

Metals ($\mu\text{g/g}$)	Tissues	Dumlu site	Aşkale site
Al	Liver	6.73±0.07 ^{A, a}	6.26±0.063 ^{B, a}
	Muscle	0.74±0.19 ^{B, a}	0.73±0.025 ^{C, a}
	Gill	0.03±0.01 ^{C, b}	14.83±0.47 ^{A, a}
Cr	Liver	0.06±0.01 ^{A, a}	0.05±0.01 ^{A, a}
	Muscle	0.01±0.00 ^{B, b}	0.04±0.01 ^{A, a}
	Gill	0.02±0.00 ^{B, b}	0.07±0.01 ^{A, a}
Mn	Liver	0.25±0.01 ^{B, b}	0.4±0.01 ^{B, a}
	Muscle	0.38±0.01 ^{B, a}	0.3±0.01 ^{B, a}
	Gill	1.32±0.02 ^{A, b}	11.33±0.14 ^{A, a}
Fe	Liver	19.47±0.31 ^{A, b}	21.15±0.12 ^{B, a}
	Muscle	3.08±0.05 ^{B, b}	4.03±0.04 ^{C, a}
	Gill	8.02±0.1 ^{B, b}	31.68±0.26 ^{A, a}
Ni	Liver	0.03±0.01 ^{A, b}	0.06±0.01 ^{B, a}
	Muscle	0.05±0.01 ^{A, a}	0.04±0.01 ^{B, a}
	Gill	0.06±0.02 ^{A, b}	0.44±0.04 ^{A, a}
Cu	Liver	1.3±0.03 ^{A, b}	2.76±0.08 ^{A, c}
	Muscle	0.12±0.01^{B, a}	0.14±0.03 ^{B, a}
	Gill	0.11±0.01^{B, b}	0.52±0.03 ^{B, a}
Zn	Liver	4.54±0.1^{B, a}	3.43±0.03^{B, a}
	Muscle	3.30±0.06^{B, a}	2.45±0.09 ^{B, a}
	Gill	11.47±0.3^{A, b}	35.9±0.18 ^{A, a}
As	Liver	0.03±0.01^{A, a}	0.03±0.01 ^{B, a}
	Muscle	0	0.03±0.00^B
	Gill	0.01±0.00^{A, b}	0.23±0.04 ^{A, a}
Cd	Liver	0	0.002±0.00 ^A
	Muscle	0	0.002±0.00 ^A
	Gill	0	0.003±0.00 ^A
Hg	Liver	0.03±0.00^{B, a}	0.04±0.00 ^{B, a}
	Muscle	0.03±0.00^{B, a}	0.02±0.00 ^{B, a}
	Gill	0.3±0.01^{A, b}	0.68±0.01 ^{A, a}
Pb	Liver	0	0.001±0.00 ^C
	Muscle	0	0.01±0.00 ^B
	Gill	0	0.5±0.02 ^A

Al: aluminum; Cr: chromium; Mn: manganese; Fe: iron; Ni: nickel; Cu: copper; Zn: zinc; As: arsenic; Cd: cadmium; Hg: mercury; Pb: lead.

Vertically letters "A, B and C" show differences among tissues for the same site fish ($p<0.05$). Horizontally, letters "a and b" show differences among sites for the same tissues ($p<0.05$).

cephalus indicates resembling variation between two sites, therefore, we conclude that there is no heavy metal adding source in Dumlu site.

In Table 3, maximum metal concentrations for *Leuciscus cephalus* two tissue samples and Maximum Acceptable Concentrations (MAC) in some guidelines are presented. The levels of Fe, Ni, Cr, Mn, As, Cu, Hg, Zn, Cd, and Pb did not exceed the MAC prescribed by the National Regulation of Turkey (Turkish Food Codex, 2002) in any of the muscle and liver samples. Furthermore, the levels of Ni, Cr,

Table 3: Maximum metal concentrations in the liver and muscle of *Leuciscus cephalus* from Aşkale site and guidelines ($\mu\text{g/g}$).

	Fe	Ni	Cr	Mn	As	Cu	Hg	Zn	Cd	Pb
This study										
Liver	21.15	0.06	0.05	0.4	0.03	2.76	0.04	3.43	0.002	0.001
Muscle	4.03	0.04	0.04	0.3	0.03	0.14	0.02	2.45	0.002	0.01
Turkish Guidelines ^a	50	-	-	20	1.0	20	0.5	50	0.1	0.1
Tolerance level in fish ^b	-	0.4	0.15	2.5	-	30	-	30	0.5	0.5
European Commission ^c	-	-	-	-	-	-	-	-	0.05	0.3

^a Turkish Food Codex (2002)

^b FAO/WHO (1989)

^c EC (2006)

(-) means that no values were presented in the standards.

Mn, Cu, Zn, Cd and Pb did not exceed the MAC prescribed by TFC, FAO/WHO and by the EC. (Table 3). Fish have high protein content, low saturated fat and also contain omega fatty acids. However, fish accumulate contaminants from aquatic environment and therefore, they have been extensively used in pollution monitoring systems of the aquatic environment [27]. Previous studies have shown that target organs such as gonad, gill and muscle have a tendency to accumulate heavy metals at higher rates in many species of fish from different geographical areas [7,16]. Metals accumulate in the tissues of fish and thus metals determined in the tissues of fish can reflect the past exposure [28]. Levels of Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb in liver, gills and muscle of *Leuciscus cephalus* caught in the Karasu River are presented in the current study. Accumulation trend of metals in *Leuciscus cephalus* is mostly in gills.

When fish are exposed to elevated metal levels in an aquatic environment, they can absorb the bioavailable metals directly from the environment via the gills and skin or through the ingestion of contaminated water and food. Metals in the fish are then transported by the bloodstream which brings it into contact with the various organs and tissues [29]. Fish can regulate metal concentrations to a certain extent after which bioaccumulation will take place [30]. Therefore ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal [31].

Mean concentrations in the liver, muscle and gills of *Leuciscus cephalus* were as follows: Fe> Al> Zn> Cu> Mn> Ni> Cr> Hg> As> Cd> Pb; Fe> Zn> Al> Mn> Cu> Ni> Cr> As> Hg> Pb> Cd; Zn> Fe> Al> Mn> Hg> Cu> Pb> Ni> As> Cr> Cd, respectively. In the study, zinc had the highest concentrations in the gill (35.9 $\mu\text{g/L}$), followed by iron (31.68 and 21.15 $\mu\text{g/L}$), aluminum (14.83 $\mu\text{g/L}$) and manganese (11.33 $\mu\text{g/L}$). Concentrations of metals detected in the tissues samples showed different capacities for accumulating. The maximum metal concentrations in the tissues of *Leuciscus cephalus* was lower than the maximum permitted concentrations proposed by Turkish Food Codex [23], EC [24], and FAO/WHO [25]. The study performed by Sönmez et al. [17], is very important to compare to our findings. In the study, Cd, Cu, Fe, Ni, Pb and Zn levels were determined in the tissues (muscle, liver and gills) of *Capoeta capoeta umbla* and *Chalcalburnus mossulensis* collected from the Karasu River (Erzurum). The average metal concentrations in muscle, gill and liver of *Capoeta capoeta*

umbla decreased in the following order: Fe>Zn>Pb>Cu>Ni>Cd, whereas the average metal concentrations in muscle, gill and liver of *Chalcalburnus mossulensis* occurred in the following descending orders, respectively: Fe>Zn>Pb>Cu>Ni>Cd; Fe>Pb>Zn>Cu>Ni>Cd; Fe>Pb>Zn>Cu>Ni>Cd. The Fe concentration was detected to be greater than the other metals in all tissues of both fish. The highest average concentration in *Capoeta capoeta umbla* in gill tissue was determined as 225.8 $\mu\text{g g}^{-1}$ (Fe). Pb and Zn had the second high level in all tissues and all fish. As average values were considered, the two fish species demonstrated higher average values of each metal than *Leuciscus cephalus* in all tissues.

Several other studies were conducted on the subject of heavy metal levels in tissues of riverine fish. Previously obtained data showed similar results for liver and gill tissue of the fish in the studies in Saricay [12], the Nile River [32], the Tanganyika Lake [33], the Tuzla Lagoon [28], the Atatürk Dam Lake [34], the Tokat Lakes [35] and the Kasumigaura Lake [36].

The results confirm the differences of accumulation of heavy metals in the different tissues. The highest concentrations of elements were found in the gill and liver, while the lowest concentrations of detected metals were in the muscle. Many studies showed that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxify by producing metallothioneins [37]. Metal concentrations 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. Thus, high concentrations of some metals can be observed in gills. The concentrations of metals of the gill reflect the concentrations of metals in the waters where the fish live; whereas, the concentrations in liver represent storage of metals [38].

Conclusion

The study was conducted to determine the metal concentration of the *Leuciscus cephalus* in Karasu River, an important branch of the Tigris River. The results of this study showed similarities with some previously published studies, and our findings were compatible with national and international standards. All results were well below the limits for fish proposed by Turkish Food Codex [23], EC [24], and FAO/WHO [25]. Metal accumulations in muscles were lower than those measured in the gills and liver. According to our results, the examined fish were not associated with enhanced metal content in their muscle and were safe within the limits for human consumption.

Although, levels of heavy metals are not high, a potential danger may emerge in the future depending on the domestic waste waters, agricultural and industrial activities in this region. Metal accumulation varied among the stations and revealed the pollution level of the river. Mean metal concentrations were found to be higher in Aşkale site than in Dumlu site.

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