

Distribution of Some Heavy Metals in Muscle Tissues of *Luciobarbus xanthopterus*

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Abstract

In this study, the concentration of some heavy metals (Cu, Fe, Zn, Cr, Cd, Co and Pb) were determined in water and in the muscle of *Luciobarbus xanthopterus* fish to study the potential human risk of consumption and the relationship between the heavy metal load of fish and some of their biological aspects (weight, length and sex) and seasonal variation. Transfer Factors of heavy metals in *L. xanthopterus* were also determined. It was found that heavy metals accumulated in muscle tissue of *L. xanthopterus* were higher than that in the water. The concentration of heavy metals showed differences according to weight, length and sex of fish. The metal concentrations were determined in muscle tissue of female higher than those in male fish. The results were discussed and compared with tolerable values for heavy metals provided from the EPA, WHO, FAO and Turkish Food Codex (TFC) to determine whether this species has any risk for human consumption.

Key words: Transfer factor, heavy metal, muscle, *Luciobarbus xanthopterus*

Luciobarbus xanthopterus'un Kas Dokusunda Bazı Ağır Metallerin Dağılımı

Özet

Bu çalışmada, bazı ağır metallerin (Cu, Fe, Zn, Cr, Cd, Co and Pb) su ve *Luciobarbus xanthopterus*'un kas dokusundaki konsantrasyonları, bu balığın insanlar tarafından tüketilmesinde bir risk oluşturup oluşturmadığı ve balıkta ağır metal birikimi ile balığın bazı biyolojik özellikleri (ağırlık, uzunluk ve cinsiyet) arasındaki ilişki belirlenmiştir. Ayrıca, *L. xanthopterus*'da ağır metallerin Transfer Faktörü belirlenmiştir. *L. xanthopterus*'un kas dokusunda biriken ağır metallerin konsantrasyonu sudan daha yüksek bulunmuştur. Ağır metal birikimlerinin balığın vücut ağırlığına, uzunluğuna ve cinsiyetine bağlı olarak değişiklik gösterdiği görülmüştür. Dişi balıkların kas dokusundaki metal konsantrasyonlarının erkek balıklardan daha yüksek olduğu tespit edilmiştir. Bu türün insanlar tarafından tüketilmesinin herhangi bir risk taşıyıp taşımadığı EPA, WHO, FAO ve Türk Gıda Kodeksi tarafından önerilen kabul edilebilir değerler ile karşılaştırılarak tartışılmıştır.

Anahtar kelimeler: Transfer faktörü, Ağır metal, Kas, *Luciobarbus xanthopterus*

1. Introduction

Environmental exposure to heavy metals has varied considerably over time. Knowledge of the time trends of these exposures is important for the guidance of preventive measures. However, for almost all countries there is remarkably limited information available [1]. Metals are found naturally in the aquatic habitats or as a result of anthropogenic activities, representing an imperative factor of exposure to the aquatic animals, including fish [2, 3]. The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent

years, because they are indestructible and most of them have toxic effects on organisms. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio-accumulate in aquatic ecosystems [4]. Heavy metals represent a major group of water pollutants and concentrations of these toxicants in the aquatic ecosystems is gradually increasing because of land based human activities [5, 6]. Increases in agricultural and industrial activities in an area directly influence the quality of water. In other words, water reservoirs are collectors of all materials spread by human industrial and agricultural activities.

Heavy metals penetrate into water reservoirs via atmosphere, drainage, soil waters and soil erosion. As the concentration of heavy metals in the environment increases, the metals inevitably enter the biogeochemical cycle [7, 8]. Aquatic organisms exposed to elevated levels of heavy metals, can cause harmful effects on the health of aquatic organisms and their consumers [9].

Fishes are widely consumed because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health. Fishes are constantly exposed to heavy metals because of pollution from chemicals and contamination in waters. Fish muscle is commonly analyzed to determine contaminant concentrations and to assess the health risks because it is the main part consumed by humans. The levels of contaminants especially heavy metals in fish are of particular interest because of the potential risk to humans who consume them [10].

The aim of this study is to determine heavy metal concentrations in muscle of *Luciobarbus xanthopterus* (Heckel, 1843) caught from Karakaya Dam Lake (Turkey) to determine the possible potential human risk of consumption and to investigate the relationships between seasonal variation and some biological aspects of fish and metal concentrations in the muscle and Transfer Factor.

2. Material and Methods

Karakaya Dam Lake (Figure 1) is the third largest dam lake on the River Euphrates (in respect to the surface area of lake) right after Keban Dam Lake and is situated 166 km downstream of Keban Dam [11].

At each step of the digestion processes of the samples acid blanks (laboratory blank) were done using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contain the same digestion reagents as the real samples with the same acid ratios but without fish sample. After digestion, acid blanks were treated as samples and diluted with the same factor. They were analyzed by ICP before real samples and their values were subtracted to check the equipment to read only the exact values of heavy metals in real samples.

Each set of digested samples had its own acid blank and was corrected by using its blank sample.

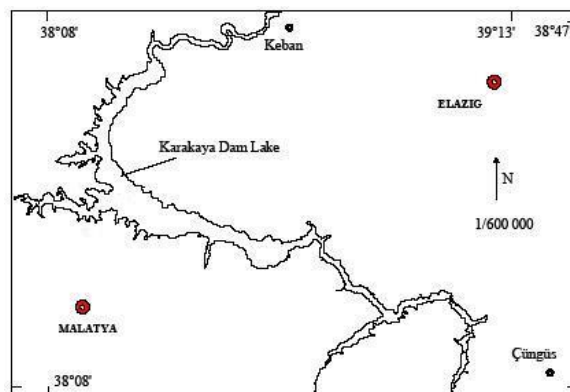


Figure 1. Study area in the Karakaya Dam Lake, Malatya, Turkey [12]

The concentrations of metals were measured in the muscles of fish collected by gill net in the open water of Karakaya Dam Lake. Captured fish were placed in plastic bags and immediately transported to the laboratory in a freezer bag with ice. Total length and weight of each fish was measured to the nearest millimeter and gram before dissection, and then approximately 5g muscle (cleaned from skin) samples were dissected from a total of 60 *L. xanthopterus* muscle samples were individually transferred to 4 ml glass vials previously washed (with 0.1 N nitric acid), dried, and weighed and then they were dried in an oven for 24 hours at 105 °C and kept in a desiccator for a few days until constant weight was obtained. Vials were again weighed to obtain dry weight of tissues, and then samples were digested (duplicate digestion, in each case) on a hot plate by adding 2 ml suprapure nitric acid (65%, Merck, Whitehouse Station, New Jersey). The solution was evaporated on hot plate. Digestion was continued until the liquor was clear. Digested samples were kept at room temperature for 24 hours and then diluted to 50 mL with deionized distilled water. Standard solutions for calibration graphs were prepared. Blanks were also prepared using the procedure as above, but without the samples. Diluted samples and blank solutions were analyzed by ICP (Perkin Elmer Optima 5300 DV) for determination of zinc (Zn), copper (Cu), iron

(Fe), cobalt (Co), cadmium (Cd), chromium (Cr) and lead (Pb) levels [13].

Microsoft Office Excel 2010 and SPSS 12.0 package programs were used to get the statistical analysis of the data (t-test and One Way ANOVA Duncan) obtained during the research.

The Transfer Factor (TF) (accumulation factor) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water. It gives an indication about the accumulation efficiency for any particular pollutant in any fish organ [14] TF was calculated according to Aboul Ezz and Abdel-Razek [15] using the following equation:

$$TF = M_{\text{tissue}} / M_{\text{water}}$$

If the TF is greater than 1.0 then bioaccumulation for metals occurs by fish species.

3. Results and Discussion

In the water samples, the concentration of heavy metals, Cu, Fe and Zn were 0.05, 0.08 and 0.11 mg L⁻¹ respectively. Zn content was the highest and that of Fe was the lowest in water. The order of heavy metal accumulation in water was Zn > Fe > Cu.

The average concentrations of the metals detected in fish muscles and the values of TF are summarized in Table 1. It was found that, the concentrations of metals in muscle are much higher than in the water. The TF from water to fish in case of *L. xanthopterus* was in the order of Fe (434.63) > Zn (230.73) > Cu (17.20). Iron was the greatest metal accumulated by *L. xanthopterus* from water, while the TF of Cu was the lowest.

Table 1. The TF from water in *L. xanthopterus*

Heavy metal concentrations:	Cu	Fe	Zn
in water (mg L ⁻¹)	0.05	0.08	0.11
in the fish muscle (mg kg ⁻¹)	0.86	34.77	25.38
Transfer Factor (TF)	17.20	434.63	230.73

In this study, Zn, Cu, Fe, Co, Cd, Cr and Pb levels in the muscle of *L. xanthopterus* from Karakaya Dam Lake were investigated, but Co, Cd, Cr and Pb could not been determined. In the present study, the effect of the seasons on the

accumulation of heavy metals in muscle tissue of *L. xanthopterus* was determined (Figure 2).

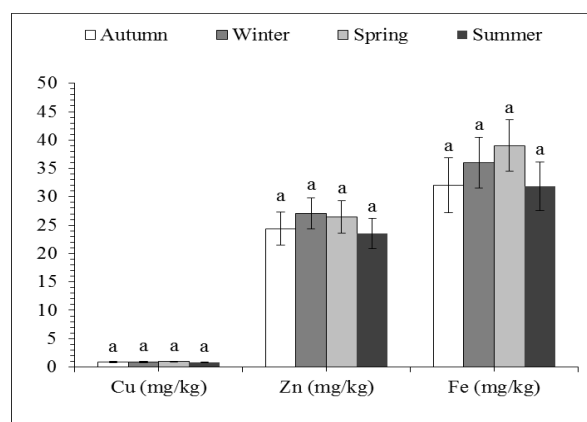


Figure 2. Trace metal levels in the muscle of *L. xanthopterus* in relation to seasons (a: Between same letters are not statistically important (P>0.05)).

In general, the concentration of the tested metals was observed to be higher in winter and spring, while being lower in autumn and summer. The highest heavy metal level in a fish was determined in winter as 1.88 mg kg⁻¹ for Cu, 69.16 mg kg⁻¹ for Fe and 49.12 mg kg⁻¹ for Zn. However, the differences amongst seasons for all heavy metals detected were not statistically significant (P>0.05).

Positive relationship between heavy metal accumulation in muscle and fish size was observed. The best relationship was found between fish weight and Cu level (R²=0.96), followed by fish weight and Fe level (R²=0.93), fish weight and Zn level (R²=0.93) (Figure 3). The strongest relationship was found between the fish length and Cu level (R²=0.54). This was followed by the Fe level (R²=0.50), Zn level (R²=0.49) (Figure 3). The lowest heavy metal levels were observed in a fish that was 248 mm in length and 186 g in weight (Cu=0.30 mg kg⁻¹; Fe=12.04 mg kg⁻¹; Zn=10.49 mg kg⁻¹). While the highest levels were observed in a fish that was 556 mm in length and 1520 g in weight (Cu=1.88 mg kg⁻¹; Fe=69.16 mg kg⁻¹; Zn=49.12 mg kg⁻¹). The heavy metal levels in muscle tissue showed a rather similar pattern in relation to fish length and fish weight.

The effect of fish sex on the level of the detected metals was also examined. The concentrations of all metals analyzed in muscle

tissue (except Zn) of male fish were found to be higher than those of female fish, but the differences were not statistically significant ($P>0.05$).

The relationship between fish ages and heavy metal levels in muscle tissue was also determined (Figure 4). The effect of fish age on heavy metal accumulation showed a similar pattern to those of fish weight and length. Generally the level of all metals analyzed

increased with fish age. Very strong relationships between fish age and heavy metal levels were found ($R^2=0.95$ for Cu and Zn, $R^2=0.94$ for Fe). According to the age groups, the differences between the accumulation of heavy metals were not statistically significant in age groups II with III and in age groups V with VI ($P>0.05$).

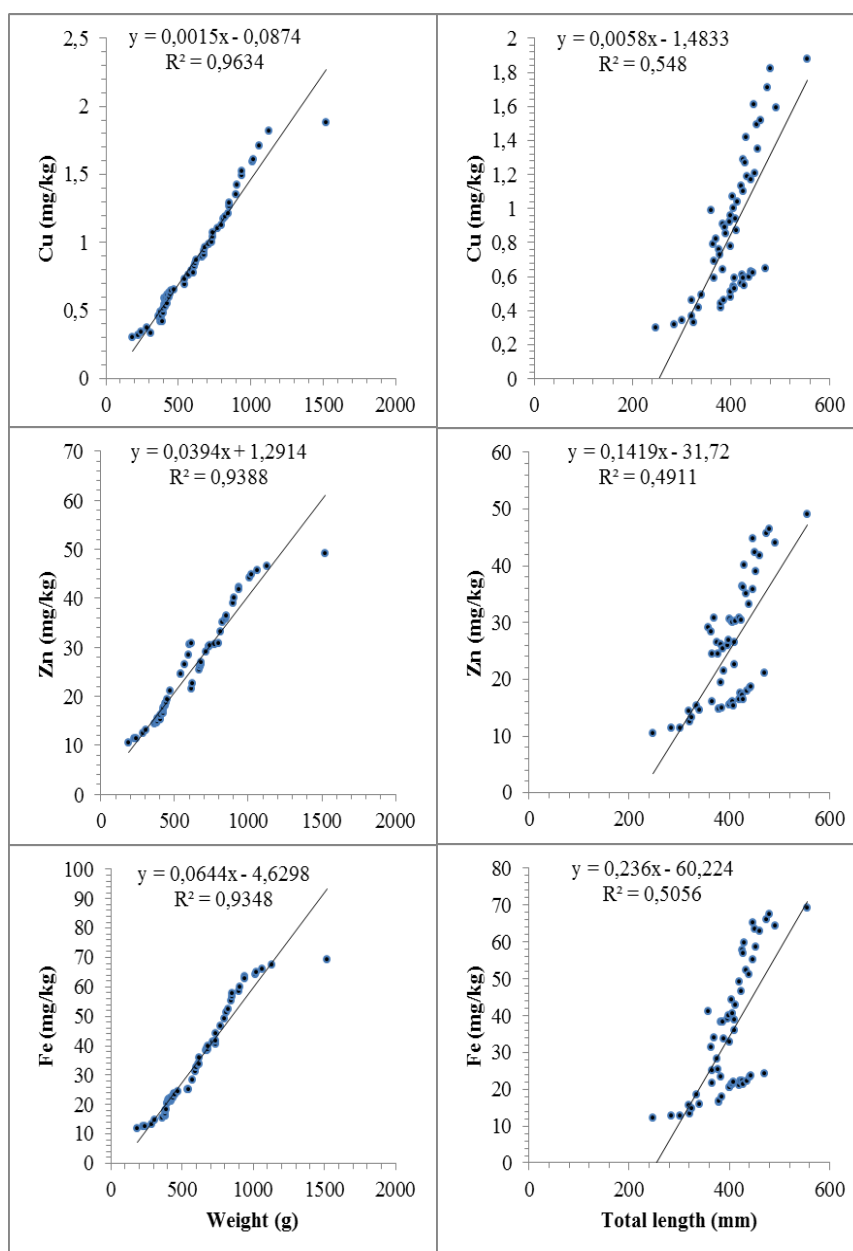


Figure 3. Trace metal levels in the muscle of *L. xanthopterus* in relation to fish weight and fish length.

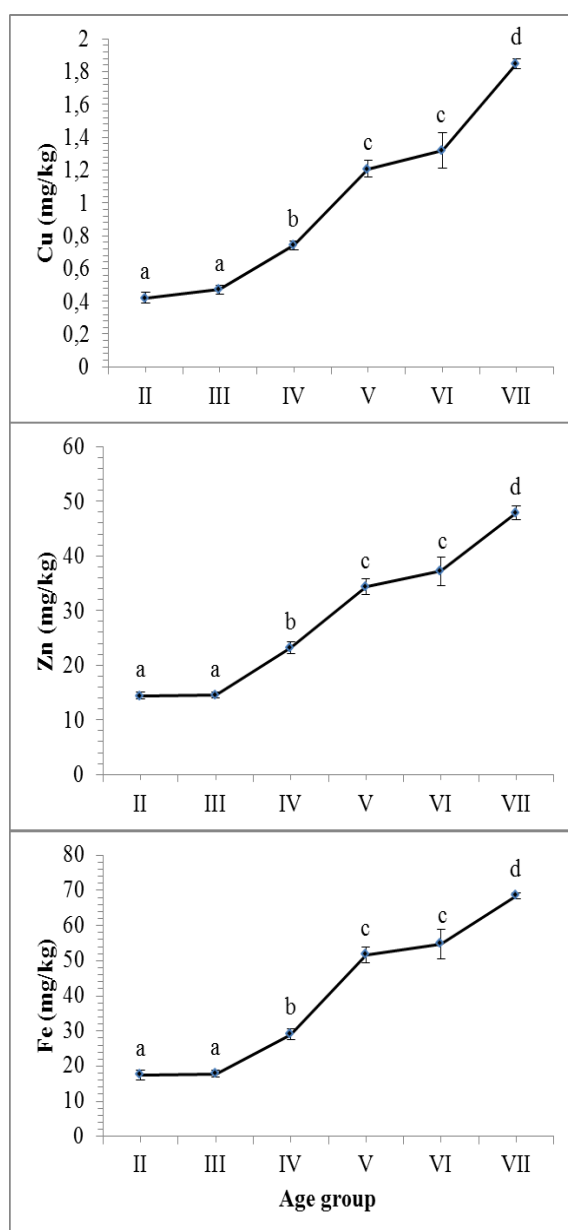


Figure 4. Trace metal levels in the muscle of *L. xanthopterus* in relation to fish age (abcd: Between same letters are not statistically important ($P > 0.05$)).

Contamination of aquatic ecosystems with heavy metals has seriously increased worldwide attention [16]. By comparing measured concentrations of metals with water quality standards, it was found that all metal concentrations were lowest than the permissible limits (Table 2).

In this study, the highest average heavy metal levels were determined in spring for Cu and Fe, and in winter for Zn. The reason of this

increase could be due to an increase of the physiological activity of fish during this season caused primarily by the increasing water temperature.

Table 2. Heavy metal concentration in the water and acceptable values suggested by USEPA (2002) [17]

	Cu	Fe	Zn
Heavy metal concentrations (mg L ⁻¹)	0.005	0.08	0.11
Permissible limits (mg L ⁻¹)			
MC	0.013	-	0.12
CC	0.009	1	0.12

MC: Maximum concentrations; CC: Continuously concentrations

Mansour and Sidky [8] investigated the accumulation of Zn, Cu, Cd, Pb and Sn in some species of fish caught in Fayoum Governorate (*Tilapia* sp., *Mugil* sp. and *Solea* sp.). They found out that the level of the heavy metal accumulation changes according to the seasons.

The heavy metal accumulation level according to the seasons is ordered as summer>autumn>winter>spring. Obasohan and Eguavoen [18] reported that seasons, pollution level of the station, and the biological activities of the living being affects the concentration of heavy metals in the tissues. Canpolat and Calta [19] found out that seasons affect the accumulation of heavy metals in the organs and tissues. They also found that the concentration of the same heavy metal in the same organ and tissue also changes according to the seasons. Riba et al. [20] made a research to investigate the Fe, Mn, Zn, Cd, Pb and Cu accumulation in *Liza ramada* which lives in Guadalquivir Estuary near the Cadiz gulf which is under the effect of Aznalcollar mine plant and they found that in relation to the type of the metal seasonal metal accumulation also has variations (while the highest concentration level of Zn and Cd in the tissues is found to be in summer and winter, Cu and Pb has the lowest concentration level in winter.). In this study the concentration level of metals in the muscle of *L. xanthopterus* from Karakaya Dam Lake was found to be higher in spring compared to other seasons. Zyadah [21] recorded the higher values of heavy metals in winter and spring and he also noted that this increase was due to the decrease in wastewater

from agricultural activities during these seasons. In a research about the Zn, Cu, As, Cd, Hg, Pb accumulation in the muscle, the order of heavy metals concentrations were found Zn>Hg>As>Cu for *Silurus triostegus*; Zn>Hg>As>Cu for *Aspius vorax*; Zn>Cu>As>Pb for *Cyprinus carpio* (mirror); Zn>Cu>As>Hg for *Carasobarbus luteus*; Zn>Cu>As for *Capoeta trutta*; Zn>Cu>Hg>As for *Chalcalburnus mossulensis*; Zn>Cu>As>Pb>Hg for *Acanthobrama marmid* [22]. Distribution patterns of heavy metal concentrations in the muscle of *Tor grypus* were found as; Fe>Zn>Pb>Cu>Mn>Cr>Ni>Co by Oymak et al. [23]. Findings in these researches support the findings in this study.

Table 3 shows the concentration levels of heavy metals in some fish species examined by some researches and heavy metal concentration determined in the muscle of *L. xanthopterus* in this study. As it can be seen in Table 1, the rate of heavy metal concentration determined by Canpolat and Calta [24]; Onger et al. [25] in some fish species is higher when compared to

the rate found in this study. However, rates determined by Yaduma and Huphrey [26] and by Kanayochukwu et al. [27] and by Canpolat [28] are lower than the rates determined in this study. Canlı et al. [30] determined the Cd, Cr, Cu, Ni ve Pb levels in the muscle, gill and liver tissues of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* caught in Seyhan River. They determined that the heavy metal levels change highly amongst different stations and they found out that the highest concentration level is measured in a station which is thought to be contaminated by hospital wastes. Gungum et al. [31] examined the accumulation of some heavy metals in *Cyprinion macrostomus* and *Garra rufa* living in Dicle River and found that accumulation level in *Cyprinion macrostomus* is higher than the accumulation level in *Garra rufa*. These results clearly show that accumulation levels of heavy metals in their tissues and the organs change according to the habitat and species of the fish.

Table 3. Some heavy metal concentrations (mg kg⁻¹) determined in the muscle tissue of *L. xanthopterus* and some fish species

	Cu	Fe	Zn	Cr	Cd
<u>The present study</u>					
<i>L. xanthopterus</i>	0.86±0.05	34.77±2.26	25.38±1.14	nd	nd
Canpolat and Calta [24]					
<i>Capoeta umbla</i>	3.51	18.13	46.59	nd	nd
Calta and Canpolat [29]					
<i>Acanthobrama marmid</i>	3.18	9.31	13.28		
<i>Cyprinus carpio</i>	2.83	19.02	27.87		
<i>Chondrostoma regium</i>	3.13	22.51	38.66		
Canpolat [28]					
<i>Capoeta umbla</i>	1,35	19,96	13.28	nd	nd
Yaduma and Humphrey [26]					
<i>Clarias anguillaris</i>	0.63	0.75	1.24	9.01	
<i>Heterotis niloticus</i>	0.68	1.50	9.96	-	
Kanayochukwu et al. [27]					
<i>Oreochromis niloticus</i>		0.80	1.41	0.01	<0.0.1
<i>Tilapia zilli</i>		1.97	1.04	0.40	0.03
<i>Serathrodon niloticus</i>		2.84	3.80	0.40	0.14
<i>Clarias gariepinus</i>		18.01	10.80	0.80	0.20
<i>Ethmaliosa timbriata</i>		6.20	1.85	0.42	0.10
Onger et al., [25]					
<i>Lates niloticus</i>	3.56	51.40	38.30		0.22
<i>Oreochromis niloticus</i>	2.73	53.10	39.40		0.25
<i>Ostrineobola arentia</i>	6.94	154.50	284.50		0.29

The concentration rates of Cu, Fe and Zn elements determined in *L. xanthopterus* muscle tissues differs according to the weight and length and this shows that heavy metal accumulation level changes according to the weight and length. Similarly, Zyadah [21] reported that the organs tend to accumulate high concentrations of heavy metals with the increase of fish size. When the accumulation levels of Cu, Fe and Zn between female and male *L. xanthopterus* are compared. Cu and Fe elements are found in higher levels in muscle tissues of male *L. xanthopterus*.

Canpolat and Calta [19] determined that concentration of Cu, Fe, Zn and Mn in the tissues and organs of *Capoeta capoeta umbla* changeable according to the weight groups. Zyadah [21] examined the concentration of heavy metals such as Cu, Zn, Cd in the muscle, gill, liver and gonads of *Tilapia zilli* caught in Lake Manzalah in Egypt and observed a relationship between the percentage of metals in the organs and length and sex of the fish. The rate of heavy metal concentration were found to be in higher levels for female species and it is determined that the heavy metal concentration is lower in the short length group (8-11 cm) and higher in medium length group (11-13 cm). In their research about the concentration of some heavy metals in the muscles of *Lethrinus lentjan*, Al-Yousuf et al. [32] found out that Cu, Zn and Cd concentration is more in female fish compared to the male fish. Findings of these researchers about the relationship between the heavy metal concentration and the weight and length groups have similarities with the findings of this study.

The observed variability of heavy metal levels in different species depends on feeding habits, ecological needs, metabolic rate, physiological condition of fish, age, size and length of the fish and their habitats [33, 34].

It is found that heavy metals are hazardous for the aquatic ecosystems especially for the cyprinid species which are nourished in deep water. As a result it is determined that these species are more contaminated when compared to the predator fish. These differences in the results may come from the features of the terrestrial environment, industrial, domestic and

agricultural facilities. Because of the contamination of water directly or indirectly by the wastes, rise of the heavy metal concentration level is inevitable [35].

The presence of metals in high levels in fish environment does not indicate a direct toxic risk to fish, if there is no significant accumulation of metals by fish tissues Kamaruzzaman et al. [36]. On the other hand, all TF from water were higher than 1.00 which means that the *C. umbla* accumulated metals from water. This result agrees with many previous studies. Canpolat [28] and Rashed [37] determined transfer factors for results indicated that only transfer factors from water for all metals were >1.00, which means that fish accumulated metals from water. Also Abdel-Baki et al. [38] calculated TF of five heavy metals from water and sediment in Tilapia fish, results indicated that fish accumulated all metals in its tissues from water. TF of metals from water in fish muscles were 41.789, 8.621, 11.923 24.714, 35.938 for Pb, Cd, Hg, Cu and Cr respectively. Saygi and Atasagun [39] determined bioaccumulation factor (BAF) values of Al (6024), Fe (1298), Cu (8600), Zn (13361) and Mo (1118) were remarkably high in all of the carp tissues investigated. In particular, Pb and As results from the muscle tissue of the carp were not within the safe limits for human consumption.

4. Conclusions

In conclusion there was a clear difference between the concentrations of heavy metals within muscle tissue of fish. However, there was no rather clear difference for some metal levels between the comparable parameters such as fish size, sex and seasons. Sometimes, smaller fish showed higher concentrations of a metal or bigger fish of another metal. Heavy metals pollution affects not only aquatic organisms, but also public health as a result of bioaccumulation in food chain. Our results show that heavy metal levels in the muscle samples taken from *L. xanthopterus* caught from Karakaya Dam Lake were under the dangerous limits given by EPA [40], WHO [41], FAO [42] and Turkish Food Codex (TFC) [43] and there is no any risk for public health by eating *L.xanthopterus* (Table 4).

Table 4. Heavy metal concentration in the muscle tissue of *L. xanthopterus* and acceptable values suggested by EPA [40], WHO [41], FAO [42] and Turkish Food Codex (TFC) [43]

	Heavy metals				
	Cu	Fe	Zn	Cr	Cd
EPA [40] (mg kg ⁻¹)	54	410	410	4.1	1.4
WHO [41] (mg/kg ⁻¹)	3	146	10-75	0.15	0.18
FAO [42] (mg/kg ⁻¹)	10.0	*	150	*	0.2
TFC [43] (mg/kg ⁻¹)	20	50	50	*	0.05
<i>L. xanthopterus</i> (mg/kg ⁻¹)	0.86	34.77	25.38	*	*

*: not detectable

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