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Determination of some heavy metal levels in three freshwater fish in Keban Dam Lake (Turkey) for public consumption

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Introduction

Due to industrialization, a number of factories and human population have increased rapidly. As a result, the amount of waste matter such as heavy metals released to the environment has been increased. Specifically aquatic systems are more sensitive to heavy metal pollution and the gradual increase in the levels of such metals in aquatic environments, mainly due to anthropogenic sources, have become a problem of primary concern (Ashraf *et al*., 2012). The natural aquatic ecosystems may extensively be contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Kamaruzzaman *et al*., 2011).

The accumulation of toxic metals to hazardous levels in aquatic biota has become a problem of increasing concern (Olojo *et al*., 2012). Fish are considered as one of the most indicative factors, in freshwater ecosystems, for the estimation of trace metal pollution (Rashed, 2001; Yousafzai *et al*., 2010). Because they are at a high trophic level of the food web, they may accumulate large amounts of some metals from the water and often in concentrations several times higher than in the ambient water (Yousafzai *et al*., 2010).

The aim of this study was to determine the possible potential human risk of consumption of fish species, *Cyprinus carpio*, *Squalius cephalus* and *Capoeta umbla*, from Keban Dam Lake in terms of metal concentrations.

Materials and methods

Site description

Keban Dam Lake was built on the Euphrates River in the eastern part of Turkey and is the second largest artificial lake in the country. It is 845 m above sea level and has a surface area of up to 675 km^2. Its maximum depth is

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160 m and its catchment area is 64100 km^2 (Fig. 1).

Figure 1: The location of Keban Dam Lake.

Reagents and apparatus

All reagents were of analytical grade unless otherwise stated. Distilled water was used for the preparation of solutions. All the plastic and glassware were cleaned by soaking in 0.1 N nitric acid solutions overnight and then rinsed with distilled water prior to use. For digestion of muscle tissue, HNO_3 supplied by Merck was used.

The concentrations of heavy metals were determined by ICP (Perkin Elmer Optima 5300 DV).

Blank preparation

At each step of the digestion processes of the samples, acid blanks (laboratory blank) were run using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contained the same digestion reagents as the real samples with the same acid ratios but without fish samples. 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 corrections were made using its blank sample.

Fish collection and analyses

The fish samples (20 specimens for each fish species) were collected from Keban Dam Lake between September 2009 and April 2010 and they were icepacked and transported to the laboratory.

Total length and weight of each fish was measured to the nearest millimeter and gram, respectively before dissection, and then approximately 10 g of the muscle (cleaned from skin) samples were dissected from each fish. After they were individually transferred to 4ml glass vials previously washed (with 0.1 N nitric acid), dried and weighed, they were heated in an oven at 105 ºC for 24 hours and then transferred to a desiccator for a few days until constant weight was obtained. Vials were again weighed to obtain dry weight of tissues. Then samples were digested (duplicate digestion, in each case) on a hot plate by adding 2 ml pf Suprapur nitric acid (65%, Merck, Whitehouse Station, New Jersey). Digested samples were kept at room temperature for 24 hours and then diluted to 50 mL with double 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

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solutions were analyzed by ICP (Perkin Elmer Optima 5300 DV) for the determination of zinc (Zn), copper (Cu), iron (Fe) and cadmium (Cd) levels (APHA, 1985). The concentrations of metals were determined from the calibration graphs and calculated on the basis of mg kg^{-1} dry weight.

Statistical analyses

Graphpad Prisim 5.0 package programs were used to get the statistical analysis (t-test and One Way ANOVA) and graph of the data obtained during the research.

Results and discussion

Heavy metals in fish species

The concentrations of heavy metals in the muscle samples of three fish species are presented in Fig. 2. The level of metals in the muscle of selected fish follows the order of magnitude as *S. cephalus*>*C. carpio*>*C. umbla* (except Zn).

According to Fig. 2, Fe, Zn, Cu and Cd levels in the muscle tissue ranged from 20.01-24.33, 19.07-35.85, 1.36-2.21 and 0.010-0.370 mg kg^{-1}, respectively for *C. carpio*; 21.67-25.41, 20.22-34.39, 1.43-2.65, 0.022-0.047 mg kg^{-1}, respectively for *S. cephalus* and from 19.44-23.39, 11.03-22.52, 1.23-1.51 and 0.010-0.035 mg kg^{-1}, respectively for *C. umbla*, respectively. The order of bioaccumulation of these metals might be as a result of the fact that different metals tend to accumulate differently in the tissues of different species of fish. In this study accumulation of Zn was the highest followed by Fe and Cd. Similar results have been reported in other fish species. Begum *et al*. (2005) investigated the elemental concentration in muscle tissues of freshwater fish (*Tilapia nilotica, Cirrhina mrigala* and *Clarius batrachus*) in Dhammondi Lake. In a research on the accumulation of Zn, Cu, As, Cd, Hg, and Pb in the muscle, the order of heavy metals concentrations were found as Zn>Hg>As>Cu for *Silurus triostegus*; as Zn>Hg>As>Cu for *Aspius vorax*; as Zn>Cu>As>Pb for *C. carpio* (mirror); as Zn>Cu>As>Hg for *Carasobarbus luteus*; as Zn>Cu>As for *Capoeta trutta*; as Zn>Cu>Hg>As for *Chalcalburnus mossulensis*; as Zn>Cu>As>Pb>Hg for *Acanthobrama marmid* (Mol *et al*., 2010). In a research Fe, Mn, Cu, Zn, Cd, Cr and Pb accumulation in the organs and tissues of *C. umbla* which lives in Lake Hazar, the order of these heavy metals in relation to their concentrations in muscle tissue was found as Zn>Fe>Cu>Mn (Canpolat and Calta, 2003). Findings in these researches support the findings in the present study.

Due to the crucial role played by the essential metals (Fe, Zn and Cu) as precursors in most enzymatic activities, they are carefully regulated by the physiological mechanisms in most organisms and thus the knowledge of their concentrations in fish is important in terms of their management and for human consumption (Kamaruzzaman *et al*., 2011).

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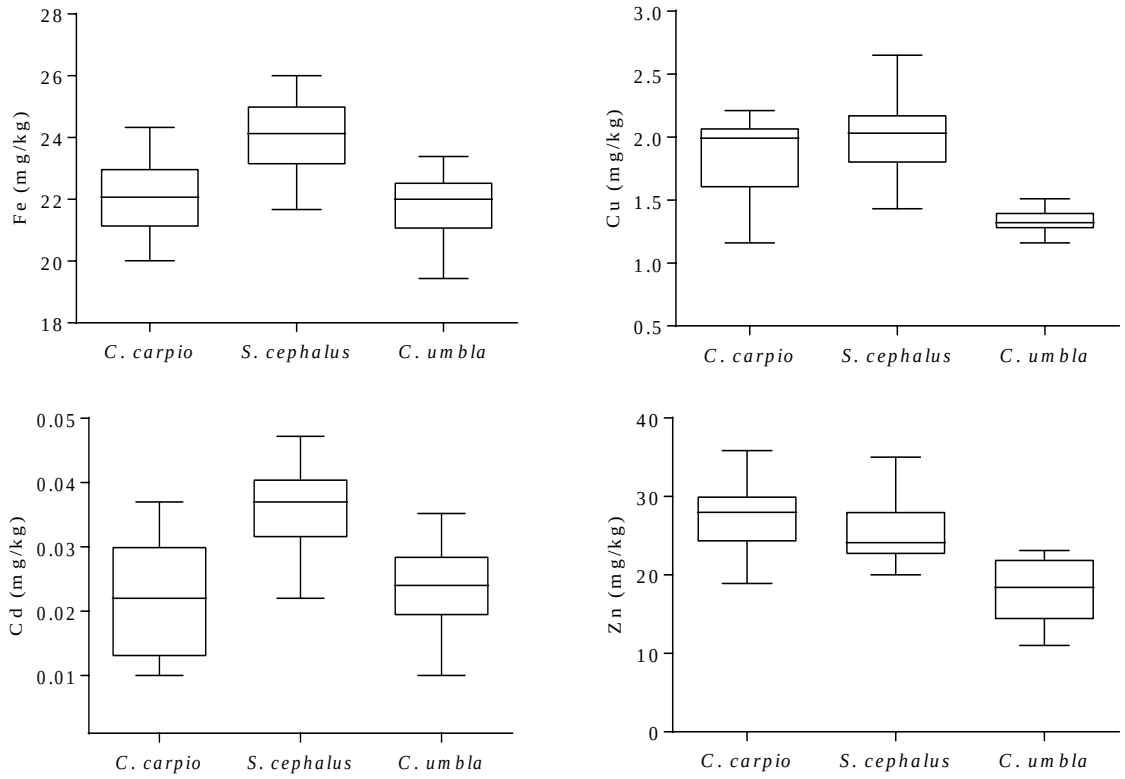

Figure 2: Comparison of Fe, Cu, Cd and Zn concentration in muscle of three freshwater fish species from Keban Dam Lake.

Relationship between heavy metal concentrations and body sizes

An increasing trend in iron, zinc, copper and cadmium values was observed with increasing fish length and weight (Figs. 3,4). A positive relationship between heavy metal accumulation in muscle and fish size was observed. The relationships were found significant between fish weight and heavy metal levels (p<0.05). The heavy metal levels in muscle tissue showed a rather similar pattern in relation to fish length.

Some studies on the heavy metals accumulation in fish show that accumulation levels may change according to the weight of the fish. It is generally accepted that trace element accumulation in living organisms controlled by specific uptake, detoxification and elimination mechanisms, depends significantly also on the size-specific metabolic rate of organisms (Schuhmacher *et al*., 1992; Canpolat and Calta, 2003; Filipovic and Raspor, 2003).

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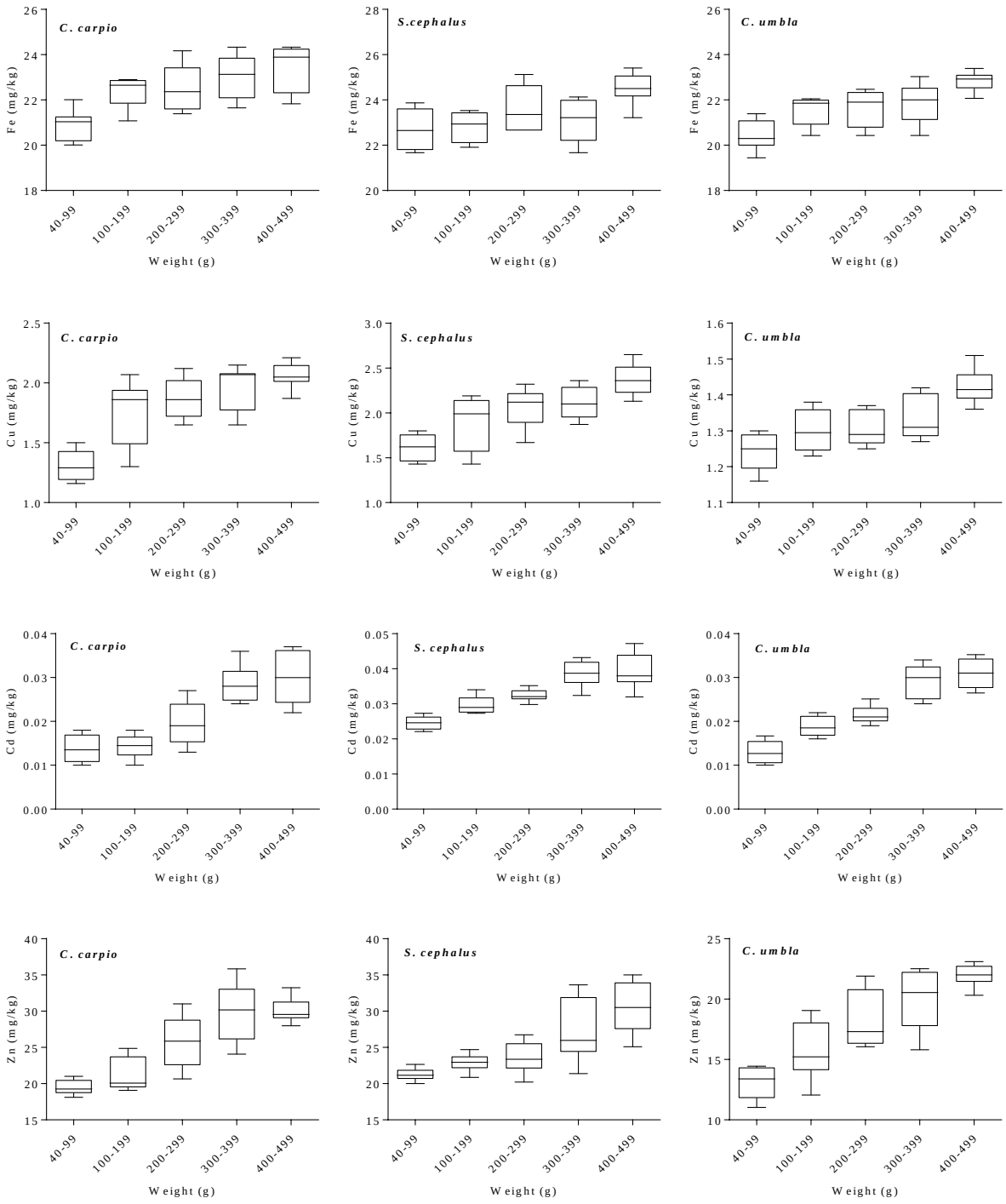

Figure 3: Box-Whisker dissemination diagram of Fe, Cu, Cd and Zn concentration in muscle tissues of three fish species in relation to fish weight.

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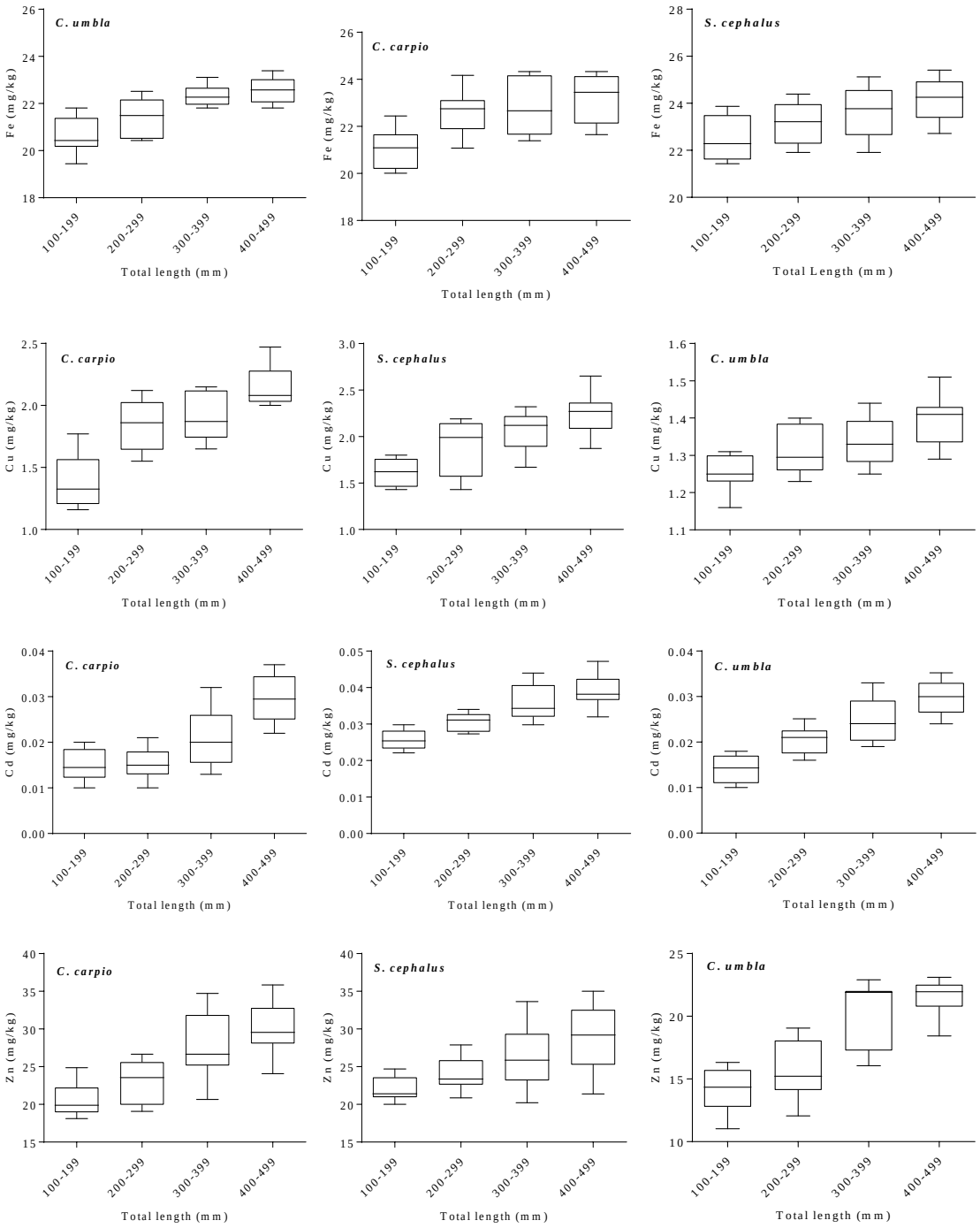

Figure 4: Box-Whisker dissemination diagram of Fe, Cu, Cd and Zn concentration in muscle tissues of three fish species in relation to total length of fish.

Relationship between heavy metal concentrations and sex

It is obvious that the concentrations of heavy metals in muscle tissue were higher in the females in comparison with those of the males (Fig. 5). All tested heavy metal levels were found to be statistically significant between sexes (p<0.05).

The present results showed that there were positive relationships between fish size (length and weight) and sex with

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metal concentrations in most cases. Generally the level of all metals analysed increased with fish size. Canpolat and Calta (2003) determined that concentration of Cu, Fe, Zn and Mn in the tissues and organs of *C. capoeta umbla* was changeable according to the weight groups. Zyadah (1999) examined the concentration of heavy metals such as Cu, Zn and Cd in the muscle, gills, 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. Heavy metal concentrations were found to be at higher levels in female specimens and it was determined that the heavy metal concentration is lower in the short length group (8-11 cm) and higher in the medium length group (11-13 cm). In their research on the concentration of some heavy metals in the muscles of *Lethrinus lentjan*, Al-Yousuf *et al*. (2000) found that Cu, Zn and Cd concentrations were higher in female fish compared to the male fish.

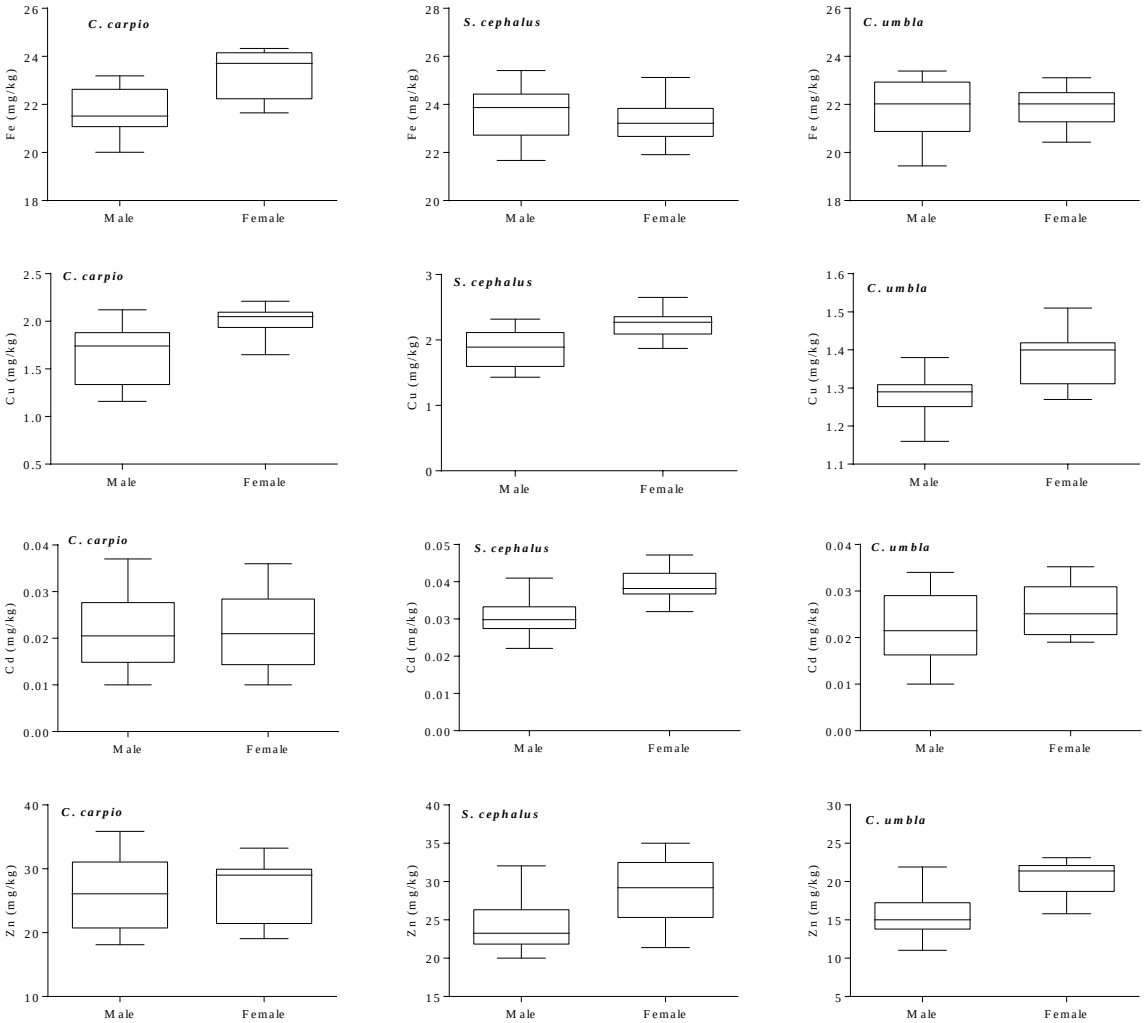

Figure 5: Box-Whisker dissemination diagram of Fe, Cu, Cd and Zn concentration in muscle tissues of three fish species in relation to sex of fish.

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Table 1 shows the concentration levels of heavy metals in some fish species examined by some researchers and heavy metal concentrations determined in the muscle of *C. umbla* in this study. As it can be seen in Table 1, heavy metal concentrations (Cu and Zn) determined by Canpolat and Calta (2001) and Calta and Canpolat (2006) in some fish species were higher when compared to the levels found in this study. However concentrations determined in other studies (Karadede-Akin, 2009; Yaduma and Humprey, 2009; Kanayochukwu *et al*., 2010; Nwani *et al*., 2010; Ambedkar and Muniyan, 2011; Canpolat, 2013) were lower than the levels determined in this study. The heavy metal accumulation levels in organisms are much higher than the level of changes in the environment and concentrations are changeable according to the type and concentration of the metal, water quality, species of the organism, season, age and nutrition type (Newman and Doubet, 1989; Biney *et al*., 1994; McCoy *et al*., 1995).

Bioaccumulation of metals within an organism results from interactions between physiological factors (growth, weight loss, absorption and accumulation), chemical factors (metal concentration, speciation and bioavailability) and environmental factors (temperature and food concentration) (Casas and Bacher, 2006).

Accumulation of metals in fish varies between the tissues and is influenced by the environmental conditions of the medium in which the fish live (Unlu *et al*., 2009). It is found that heavy metals are hazardous for aquatic ecosystems especially for the Cyprinidae 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 (Popek *et al*., 2003). Our results show that heavy metal levels in the muscle samples taken from *C. umbla* caught from Keban Dam Lake were under the dangerous limits given by EPA (1989) and FAO (1983) and there is no risk for public health from eating *C. umbla* (Table 2).

Table 1: Some heavy metal concentrations (mg kg^{-1}) determined in the muscle tissue of *Capoeta umbla* and some fish species

Fish species	Cu	Fe	Zn	Cd	Ref.
C. carpio	1.82±0.34	22.14±1.24	26.92±4.68	0.02±0.01	
S. cephalus	2.02±0.30	23.95±1.12	25.48±4.27	0.04±0.01	Present study
C. umbla	1.33±0.08	21.79±1.04	18.04±3.98	0.02±0.01	
C. umbla	3.51	18.13	46.59	nd	Canpolat and Calta, 2001
Acanthobrama marmid	3.18	9.31	13.28		
Cyprinus carpio	2.83	19.02	27.87		Calta and Canpolat 2006
Chondrostoma regium	3.13	22.51	38.66		
Clarias anguillaris	0.63	0.75	1.24		Yaduma and Humprey,
Heterotis niloticus	0.68	1.50	9.96		2009
Capoeta capoeta umbla	0.14	0.53	2.31	0.010	Karadede-Akin, 2009
Oreochromis niloticus		0.80	1.41	<0.0.1	
Tilapia zilli		1.97	1.04	0.03	
Serathrodon niloticus		2.84	3.80	0.14	Kanayochukwu *et al*., 2010
Clarias gariepinus		18.01	10.80	0.20	
Ethmaliosa timbriata		6.20	1.85	0.10	

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Table 1 continued:					
Chrysichthys nigrodiitatus	0.59	197.60	7.75		Nwani et al., 2010
Clarias anguillaris	0.56	186.00	14.20		
Tilapia zilli	1.33	443.20	23.30		
Mormyrus rume rume	0.74	245.50	8.60		
Mormyrusmacrophthalmus	0.64	214.60	11.20		
Mormyrus tapirus	0.89	296.80	14.50		
Mystus vittatus	0.62		0.1	0.66	Ambedkar and Muniyan, 2011
Tilapia mossambicu	1.46		0.3	0.48	
Ctenopharynodon idella	0.62		0.6	0.48	
Saurida undosquamis	1.4		1.2	0.64	
Heteropneustus fossilis	0.6		0.6	0.02	
C. umbla	1.35	19.96	13.28	nd	Canpolat, 2013

nd: not detectable

Table 2: Heavy metal concentration in the muscle tissue of *Cyprinus carpio, Squalius cephalus and Capoeta umbla* and acceptable values suggested by EPA and FAO.

	Heavy metals			
	Cu	**Fe**	**Zn**	**Cd**
EPA (1989) (mg g^{-1})	54	410	410	1.4
FAO (1983) (mg kg^{-1})	10.0	-	150	0.2
C. carpio (mg kg^{-1})	1.82±0.34	22.14±1.24	26.92±4.68	0.02±0.01
S. cephalus (mg kg^{-1})	2.02±0.30	23.95±1.12	25.48±4.27	0.04±0.01
C. umbla (mg kg^{-1})	1.33±0.08	21.79±1.04	18.04±3.98	0.02±0.01

nd: not detectable

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