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DETERMINATION OF HEAVY METAL LEVELS IN DIFFERENT TISSUES OF TENCH (*TINCA TINCA* L., 1758) FROM SIDDIKLI KUCUKBOGAZ DAM LAKE (KIRSEHIR), TURKEY

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ABSTRACT

Heavy metal concentrations of Cu, Fe, Mn, Zn, Cr and Al in muscle, liver, skin, intestine and gill of tench (*Tinca tinca* L., 1758) collected from Siddikli Kucukbogaz Dam Lake, in Turkey, were determined by High Resolution Continuum Source Flame Atomic Absorption Spectrometer (HR CS-FAAS) between March 2012 and February 2013. The heavy metals were accumulated at varying levels in different tissues of tench. The concentration of heavy metals in different tissues of fishes varied for Cu: 0.067-3.793, Fe: 0.460-13.22, Mn: 0.041-1.366, Zn: 0.135-3.783, Cr: nd-1.177 and Al: 0.232-2.07 $\mu\text{g g}^{-1}$ dry weight. Heavy metal levels in tissues were compared with national and international permissible limits. The values of all metals in muscles of the analyzed fish were found to be below the established limit values. Seasonal changes in metal (Cu, Fe, Mn, Zn, Cr and Al) concentration were observed in the tissues of tench, but these variations may not influence consumption advisories.

Consequently, this study suggests that various metals were present in the fish muscle tissues at different levels, but these were within the maximum residual levels permitted by Turkish standards and the WHO/FAO; thus, the fish from these areas are generally safe for human consumption

KEYWORDS:

Tinca tinca, heavy metal, fish, tissues, Kirsehir

INTRODUCTION

Water pollution with heavy metals has become a serious health concern during recent years. The aquatic environment is more susceptible to the harmful effects of heavy metal pollution because aquatic organisms are in close and extended contact with the soluble metals. Fish can be considered as

one of the most significant indicators in aquatic ecosystems for the impact of metal pollution. Fishes are often at the top of aquatic food chain in water ecosystems and they may accumulate toxic trace metals in polluted waters (Mansour and Sidky, 2002; Zhuang et al. 2013; Kayhan et al. 2015). It is well known that fish, as a regular constituent of the human diet, can represent a dangerous source of certain heavy metals (Özparlak et al. 2012).

In recent years, concentrations of heavy metal were found to have increased in lake ecosystems due to release of industrial and agricultural wastes and as a result, aquatic organisms are exposed to elevated levels of these metals (Kalay and Canil 2000; Sankar et al. 2006; Said et al. 2014). The amount of heavy metals in lake water should be within the limited values; otherwise, the accumulation of heavy metals would cause many problems to living organisms and agricultural areas. Especially high levels of heavy metals are very dangerous to freshwater ecosystems as for human if the water is being used as drinking or irrigation water. The aquatic organisms exposed to heavy metals from runoff water tend to accumulate these metals in their body, fishes being more commonly affected than other species (Güven et al. 1999; Henry et al. 2004; Mutlu et al. 2012; Miloskovic et al. 2014). The most important heavy metals from the point of view of water pollution are Zn, Cu, Pb, Cd, Hg, Ni and Cr. Some of these metals (e.g. Cu, Ni, Cr and Zn) are essential trace metals to living organisms, but become toxic at higher concentrations.

The aim of the present study is to determine the concentrations of some heavy metals in different tissues of *Tinca tinca* by using High Resolution Continuum Source Flame Atomic Absorption Spectrometer (HR CS-FAAS) from different locations of Siddikli Kucukbogaz Dam Lake. It is expected that the results of this research will assist in acquiring information about the level of toxic metals in this area.

MATERIALS AND METHODS

The Siddikli Kucukbogaz Dam is located in the borders of Kirsehir city in the central Anatolia region. It was constructed on the Kepez Ozu River and became operational in 2001. Reservoir volume is 28.500 dam³, and reservoir area is 1.65 km². The study material consisted of 27 tench (*Tinca tinca* L., 1758) specimens collected from different parts of Siddikli Kucukbogaz Dam Lake between March 2012 and February 2013 on a seasonal basis. The specimens were caught using trammel nets with mesh sizes of 8x8, 12x12, 16x16 and 22x22 mm and stored on ice until arrival at the laboratory.

Approximately 0.5 g samples of tissues (muscle, gill, liver, skin, intestine) from each fish were dissected, washed with distilled water, weighed, packed in polyethylene bags and stored at -20°C until metal analyses. All tissue samples were transferred into 100 ml teflon beakers and samples (0.5 g) were digested with 8 mL of HNO₃ (65%(m/m)) in microwave oven (CEM MARS-5 Closed Vessel Microwave Digestion System) using the following microwave digestion program; pressure 200 psi, ramp time 15 min., temperature 210 °C, maximum power 450 W, hold time 10 min. The Teflon beaker was covered with a watch glass, and heated at 50-100 °C on a hot plate for 4 h, until the solution evaporated slowly to near dryness. Two milliliters of HNO₃ (65%(m/m)) and H₂O₂ (30%(m/m)) were added to the residue and the solution was evaporated again on the hot plate. After cooling, 2.5 ml of 1 N HNO₃ was added to digested residue and was transferred to 10 ml volumetric flasks, then diluted to level with deionized water (Ciftci et al. 2011). Before the analyses, the samples were filtered through a 0.45 µm syringe filter (Sartorius).

The analyses were performed by ContrAA 300 a High Resolution-Continuum Source Flame Atomic Absorption Spectrometer (HR-CS AAS) (Analytik Jena AG, Jena, Germany) equipped with a 50 mm burner head and an injection module (SFS-6). All absorption lines of an element in the spectral range of 185–900 nm can be analytically evaluated by using a Xe short-arc lamp as a continuum lamp source. The spectral background of the sample in the HR-CS FAAS is always corrected directly on the analysis line simultaneously and independently. All measurements were carried out under optimum conditions in three replicates using an injection module SFS-6 enabling the computer controlled aspiration of blanks, analytical solutions and samples (Ciftci et al. 2011; Ciftci and Er, 2013).

All solutions were prepared using ultra-pure water (specific resistance 18 MΩ cm) from a Milli-Q purification system (Millipore Corporation,

Massachusetts, USA). Standard solutions of analyses were prepared from their 1000 mg L⁻¹ stock solutions (Merck).

Results are expressed in µg g⁻¹. One-way ANOVA and Tukey test were performed to test the differences of the metal levels among the specimens (significance level p<0.05). All statistical calculations were performed with SPSS 16.0 for Windows.

RESULTS

The present study is the first for tench in Siddikli Kucukbogaz Dam Lake and supplies valuable information about metal contents in different tissues. The accumulation values of the respective metals in various tissues were as follows: Cu, liver > intestine > gill > muscle > skin; Fe, liver > gills > intestine > skin > muscle; Mn, gill > intestine > liver > skin > muscle; Zn, intestine > liver > skin > gill > muscle; Cr, intestine > liver > gill > muscle > skin and Al, gill > skin > muscle > intestine > liver.

The mean concentrations of Cu, Fe, Mn, Zn, Cr and Al in different tissues of tench, *Tinca tinca* L., 1758, were given in Table 1. A total of 27 individuals were analyzed in this study. The highest metal concentrations were found in the liver. Cu, Fe and Zn were the most abundant metals in the liver with the concentrations of 3.793, 13.220, 3.783 µg g⁻¹, respectively. The second highest metal concentrations were found in the gills. Mn and Al were the most abundant metals in the gills with the concentrations of 1.366 and 2.070 µg g⁻¹, respectively. Muscles showed lowest level of concentration throughout the study. Except Mn and Al, all metal concentrations in livers were higher than others.

The total concentrations of Al, Fe, Cu, Mn, Zn and Cr in tench were 0.882 (±0.280), 4.320 (±0.889), 0.365 (±0.086), 2.053 (±0.186), 0.344 (±0.096) and 0.895 (±0.1150 µg g⁻¹, respectively. The minimum and maximum Cr levels in tench were 0.174 µg g⁻¹ in the skin and 0.419 µg g⁻¹ in the intestine. Cr levels reported in the literature were in the range of 0.215–0.255 for muscles of fish from the Beyşehir Lake of Turkey (Altındağ and Yiğit, 2005). The lowest and highest Cu levels were 0.67 µg g⁻¹ in muscle and 3.793 µg g⁻¹ in liver. Cu levels in the literature have been reported as 0.03–0.12 µg g⁻¹ for muscle and 0.02–0.35 µg g⁻¹ for liver of fish in Tuzla Lagoon, Mediterranean region, Turkey (Dural et al. 2007), 0.05–4.29 µg g⁻¹ for muscles of fish from Atatürk Dam Lake, Turkey (Karadede and Ünlü, 2000).

TABLE 1
The heavy metal concentrations in different tissues of Tench ($\mu\text{g g}^{-1}$)

	Mean. \pm SE (Max.-Min.)					Total
	Gill	Intestine	Skin	Muscle	Liver	
Cu	0.316 \pm 0.109 ^{a,x} (0.625-0.113)	0.444 \pm 0.107 ^{a,x} (0.592-0.133)	0.134 \pm 0.405 ^{a,x} (0.253-0.078)	0.272 \pm 0.084 ^{a,xy} (0.457-0.067)	3.243 \pm 0.332 ^{b,z} (3.793-2.281)	0.882 \pm 0.280 (3.793-0.067)
Fe	6.103 \pm 0.529 ^{b,y} (6.807-4.557)	2.842 \pm 0.700 ^{a,y} (4.800-1.797)	1.016 \pm 0.325 ^{a,y} (1.701-0.460)	0.923 \pm 0.056 ^{a,yz} (1.014-0.766)	10.711 \pm 1.112 ^{c,w} (13.220-7.807)	4.320 \pm 0.889 (13.220-0.460)
Mn	0.994 \pm 0.167 ^{b,x} (1.366-0.588)	0.384 \pm 0.137 ^{a,x} (0.677-0.114)	0.191 \pm 0.032 ^{a,xy} (0.234-0.097)	0.060 \pm 0.010 ^{a,x} (0.078-0.041)	0.196 \pm 0.034 ^{a,x} (0.293-0.140)	0.365 \pm 0.086 (1.366-0.041)
Zn	1.677 \pm 0.560 ^{a,x} (2.676-0.135)	2.619 \pm 0.399 ^{a,y} (3.661-1.725)	1.913 \pm 0.143 ^{a,z} (2.343-1.759)	1.464 \pm 0.197 ^{a,z} (1.920-0.960)	2.593 \pm 0.438 ^{a,yz} (3.783-1.696)	2.053 \pm 0.186 (3.783-0.135)
Cr	0.382 \pm 0.274 ^{a,x} (1.164-nd)	0.419 \pm 0.255 ^{a,x} (1.037-nd)	0.174 \pm 0.171 ^{a,xy} (0.688-nd)	0.339 \pm 0.195 ^{a,xy} (0.771-nd)	0.407 \pm 0.261 ^{a,xy} (1.177-0.022)	0.344 \pm 0.096 (1.177-nd)
Al	1.492 \pm 0.273 ^{a,x} (2.070-0.923)	0.683 \pm 0.175 ^{a,x} (1.037-0.236)	0.922 \pm 0.270 ^{a,xy} (1.570-0.250)	0.715 \pm 0.240 ^{a,xy} (1.387-0.280)	0.665 \pm 0.132 ^{a,xy} (1.022-0.383)	0.895 \pm 0.115 (2.070-0.236)

Horizontally, letters a, b and c show statistically significant differences ($p < 0.05$).

Vertically, letters x, y, z and w show statistically significant differences ($p < 0.05$).

nd: not detected

Analyses of the heavy metal levels in all tissues of fish samples showed that, on average, Fe is the highest and Cr is the lowest. The highest concentrations of Fe and Cu were found in the liver. A total heavy metals concentration was found in this study $\text{Fe} > \text{Zn} > \text{Al} > \text{Cu} > \text{Mn} > \text{Cr}$. In Lake Beyşehir, the accumulation orders of heavy metals were $\text{Cd} > \text{Pb} > \text{Cr} > \text{Hg}$ in the muscle and gill tissues of tench (Altındağ and Yiğit 2005). Radwan et al (1990) found higher accumulations of Pb than of Cd in the tench. Canlı et al (1998) reported that the accumulation of heavy metals in the liver and the gill tissues of different species living Seyhan River was much higher than the metals accumulated in their muscles; Küçükbay and Örün (2003) also obtained similar results that the Cu and Zn accumulation in the liver of *Cyprinus carpio* living in Karakaya Dam Lake was higher than the other tissues.

The metal concentration in muscle tissue is important for the edible parts of the fish. The mean concentrations of heavy metals analyzed in the muscle of tench were lower than the maximum permitted concentrations proposed by FAO (2005). In muscles, the mean metal levels in were found as 0.067-0.457 (Cu), 0.766-1.014 (Fe), 0.041-0.078

(Mn), 0.960-1.920 (Zn), nd-0.771 (Cr) and 0.280-1.387 $\mu\text{g g}^{-1}$ (Al). Metal levels for fish muscles in literature were reported as 13.4-23.7 (Fe), 0.44-0.85 (Mn), 0.42-1.87 (Cr), 0.07-0.38 (Cd), and 0.62-0.81 $\mu\text{g g}^{-1}$ (Pb) (Türkmen et al., 2009); 5.67-54.49 (Fe), 0.273-0.986 (Mn), 3.30-16.6 (Cr) and 0.002-0.029 $\mu\text{g g}^{-1}$ (Cd) (Company et al., 2010). Similar results were reported from a number of fish species which show that muscle is not an active tissue in accumulating heavy metals (Visnjic-Jeftic et al., 2010; Jarić et al., 2011; Duran et al., 2014). The levels of Al, Zn, Fe, Pb, Cu, Cr and Mn were determined in the muscle because of its importance for human consumption and also the liver and gill were analyzed since these organs tend to accumulate metals (Marcovecchio et al., 1991).

As consumption of fish is a possible source of metal accumulation in people, there is great interest in estimation of the daily intakes of heavy metals through fish consumption. The estimated daily and weekly intakes for the economically important fish species consumed by adult people in Turkey are illustrated in Table 2. These values for economically important fish examined in present study were far below the recommended values



according to Table 2 (FAO/WHO, 2004; WHO, 1993; EPA, 2008).

TABLE 2
The estimated daily and weekly intakes (EDI-EWI) of heavy metals through consumption of economically important fish species by adult people in Turkey

Metal	PTWI ^a	PTWI ^{*b}	PTDI ^c	EWI ^d (EDI) ^e	Tech, EWI ^d (EDI) ^e
Cd	7 ^a	490	70	106.40 (15.2)	–
Co	–	–	–	44.8 (6.4)	–
Cr	–	–	–	140 (20)	107.94 (15.42)
Cu	3500 ^a	245000	35000	529.2 (75.6)	63.98 (9.14)
Fe	5600 ^a	392000	56000	7499.8 (1071.4)	141.96 (20.28)
Ni	35 ^g	2450	350 ^f	827.4 (118.2)	–
Mn	980 ^g	68600	9800 ^h	95.2 (13.6)	10.92 (1.56)
Pb	25 ^a	1750	250	681.8 (97.4)	–
Zn	7000 ^a	490000	70000	2457(351)	268.8 (38.4)

* Provisional Permissible Tolerable Weekly Intake (PTWI) in lg/week/kg body weight.

Mean weekly fish consumption in Turkey is 0.14 kg per person (FAO, 2005).

^a (FAO/WHO, 2004).

^b PTWI for 70 kg adult person (lg/week/70 kg body weight).

^c PTDI, permissible tolerable daily intake (lg/day/70 kg body weight).

^d EWI, estimated weekly intake in lg/week/70 kg body weight.

^e EDI, estimated daily intake in lg/day/70 kg body weight.

^f WHO recommends a TDI (tolerable daily intake) of 5 lg/day/kg body weight, i.e. 350 lg/day for a 70-kg person (WHO, 1993).

^g Calculated for a week (lg/week/kg body weight).

^h EPA recommends a RfD (reference dose) of 0.14 mg/day/kg body weight, i.e. 9800 lg/day for a 70-kg person (EPA, 2008).

The accumulations of heavy metals were statistically assessed by One Way ANOVA and Tukey tests. For the difference of Cu between liver and other tissues; Fe between liver and other tissues, and gill and intestine, gill and skin, gill and muscle; Mn between liver and other tissues were found to be statistically significant (Table 1). The differences in the accumulation of Zn, Cr and Al in different tissues were not statistically significant. The differences in the accumulation of Cr-Mn, Cr-Al and Mn-Al in livers were found to be statistically insignificant while the changes for other metals were of statistical significance. Like this, the accumulations of between Fe and other metals in gills were statistically significant while the changes of the accumulations of other metals were found to be statistically insignificant.

DISCUSSION AND CONCLUSIONS

This study was carried out to provide information on heavy metal concentrations in tench

collected from Sidikli Kucukbogaz Dam Lake. Although, significant differences of heavy metal accumulation were determined among muscle, liver, skin and gill of tench, all results were well below the limits for fish proposed by EU, 2001 and FAO/WHO, 1987 and Turkish standards. According to these results, the examined fish were not associated with enhanced metal content in their muscle and were safe within the limits for human consumption. Moreover, our results also suggest that precautions need to be taken in order to prevent future heavy metal pollution. Otherwise, these pollutions can be dangerous for fish and human health.

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