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Assessment of Metal Concentrations in Two Cyprinid Fish Species (*Leuciscus cephalus and Tinca tinca*) Captured from Yeniçağa Lake, Turkey

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Abstract This study was performed to investigate certain major and toxic metal concentrations in muscle, gill and liver tissues of two Cyprinid species (*Leuciscus cephalus*, *Tinca tinca*). Generally liver and gill tissue exhibited higher metal concentrations than did muscle. The highest metal concentrations found in tench and chub muscle tissues were determined to be Al (59.01–108 mg kg^{-1}), Zn (45.23–57.81 mg kg^{-1}), Fe (9.23–16.03 mg kg^{-1}) and Ba (3.50–2.69 mg kg^{-1}) respectively. The level of metal accumulation is evaluated for potential risk to human health based on international standards. Zinc, lead and arsenic values of muscle tissues of the fishes were found to be above the allowed limits for human consumption.

Keywords Yeniçağa Lake · Accumulation · *Leuciscus cephalus* · *Tinca tinca*

The contamination of fresh waters with a wide range of pollutants has become a matter of great concern over the last few decades, not only the threat to the public water supplies, but also the damage caused to the aquatic life. Therefore, studies related with metals in water have been receiving increased worldwide attention, and the literature has many publications on this. The metals from natural and anthropogenic sources are continually released into aquatic ecosystems. They are a serious threat to most life forms due to their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain (Papagiannis et al. 2004). The metals may affect organisms directly by accumulating in the body or indirectly by transferring to the next trophic level of food chain. The high accumulation in these components can result in serious ecological degradations. One of the most serious consequences of their persistence is the biological amplification of metal in food chain.

Yeniçağa Lake (40°47′N, 32°02′E), with an area of 1,800 hectare is located in the Western Black Sea region of Turkey. The lake basin occurs in a depression of 7 × 2.5 km size formed after tectonic activities during the early Quaternary, and the lake is located on the North Anatolian Fault Zone. It is shallow, endorheic and eutrophic lake with a maximum depth of 5.2 m and it is one of important wetland areas in Turkey because its habitats support many bird species and plant taxa. It has been chosen as a pilot area with the aim of developing a model component for the sustainable management of peat lands' carbon storage within the framework of climate change mitigation. The lake continuously receives significant amounts of nutrient rich water from two main creeks (Kuzuviran and Deliler) due to the direct discharge of untreated domestic wastewater into the creeks. Furthermore, untreated municipal wastewater, and contaminated water with metals incoming artesian wells are the major factors increasing the pollution level in the lake. Such simultaneous activities reduced water quality and disrupted biodiversity in the lake. The metal level of the water, sediment, and plankton of the lake have been studied alongside its potential sources (water and sediment of creeks, artesian and sewage waters and soil), and these results are reported in Saygı and Atasagun (2012). According to the results of the studies, major metal which was found in the water and sediment of the lake was found

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as Al, Fe, Mn, Zn, Ba and these metals had also accumulated in plankton in high concentration (Saygı and Atasagun 2012). *Leuciscus cephalus* and *Tinca tinca* fished from Yeniçağa Lake has been an important protein source for the people living in the region over recent decades. Taking into account the metal content already found in the lake, creeks, artesian water, and plankton, an accumulation of metals in fish tissues were anticipated. Therefore, the main objective of this study was to determinate the contents of metals in the gill, muscle, and liver tissues of chub and tench from Yeniçağa Lake in order to assess accumulation level and to assess the health risk for human consumption.

Materials and Methods

In total, 80 samples of two Cyprinid species (*L. cephalus* and *T. tinca*) were collected from Yeniçağa Lake during four consecutive seasons from March 2008 to February 2009 with fishnets. Sampling of fishes was performed in April, July, November 2008 and February 2009. Arithmetic mean values for length and weight determined for both species as follows; for *L. cephalus*: 275 mm, 294 g and for *T. tinca* 298 mm, 361 g respectively. Fish samples were transported to the laboratory in a thermos flask with ice on the same day. The samples were washed with deionized water, weighted, packed in polyethylene bags and stored at −20°C prior to analyses. De-ionized water (18.2 MΩcm) from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all aqueous solutions. All used mineral acids were of the highest quality (Suprapure, Merck). All the plastic and glassware was cleaned by soaking overnight in a 10 % nitric acid solution and then rinsed with deionized water. Approximately 5 g of the epaxial muscle on the dorsal surface of the fish, the entire liver and 2–3 gill racers from each sample were dissected and washed with distilled water. The samples were digested with HNO_3 and $HClO_4$ (2:1 v/v) on a hot plate at 80°C. The digests were left to cool at room temperature and diluted with 5 mL distilled water (Dybern 1983). The metal analyses of fish samples (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Cd, Sn, Ba, Pb) were carried out using Inductively Coupled Plasma-Mass Atomic Emission Spectrophotometry (ICP-MS Thermo Elemental X7). The metals were measured in Hacettepe University LA-ICP-MS laboratory. One-way ANOVA and/or Post Hoc LSD test was applied to compare the metal concentrations of different tissues at the level of 0.05 (Fig. 1).

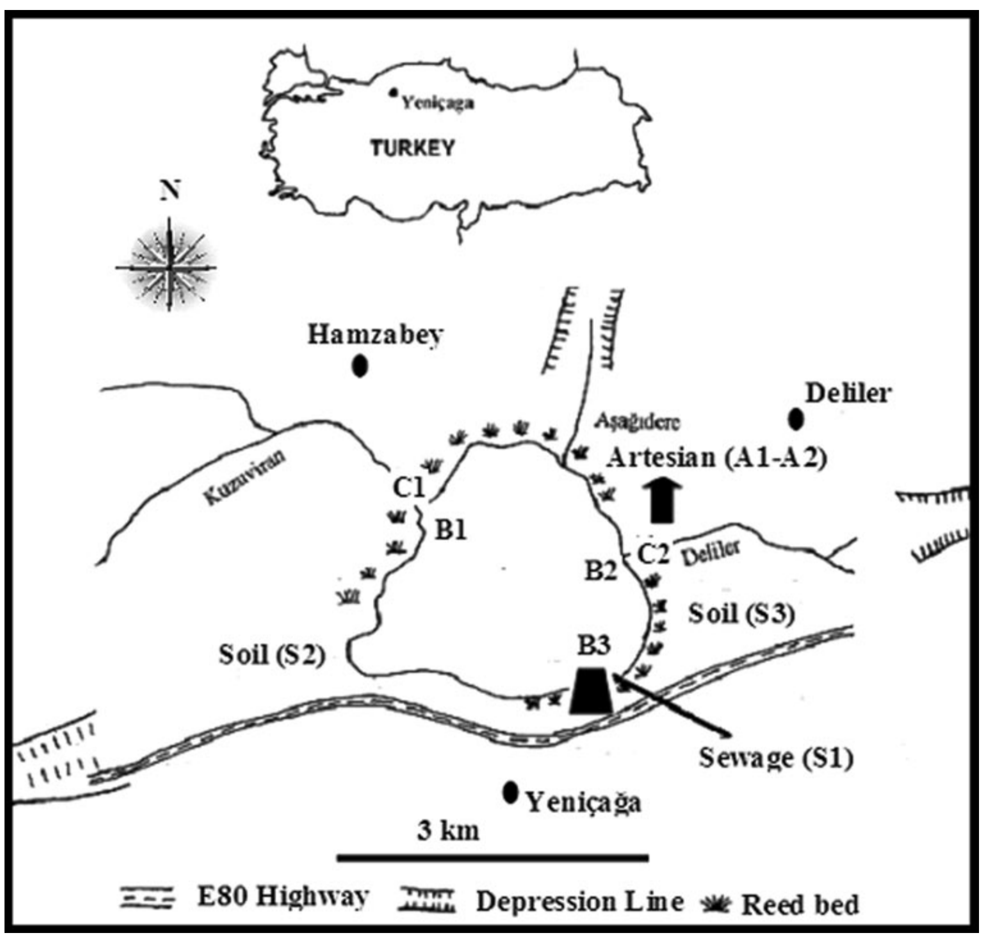

Fig. 1 Map of Yeniçağa Lake, Turkey

Results and Discussion

All metal concentrations were determined on a wet weight basis. The average concentration (mg kg^{-1} wet wt.) of the metals found in the muscle, liver and gill tissues of *L. cephalus* and *T. tinca* are summarized including a statistical analysis in Tables 1 and 2. The distribution patterns of Al, Cu, As, Mo, Pb in tissues of both species was liver > gill > muscle, Mn, Fe, Co, Ni, Ba level in tissues of both species follow the order: gill > liver > muscle. The order of Cr and Zn accumulation in the tissues was determined for *T. tinca* as liver > gill > muscle, whereas for *L. cephalus* the order was gill > liver > muscle. In the study, molybdenum accumulation in *L. cephalus* was found to be liver > muscle > gill. As shown on Tables 1, 2 with the exception Cd and Sn, all the other metals have been accumulated in all three tissue that are studied in both species. However, Cd was absent in gill of tench and in the muscle of chub, as well as Sn being absent in the gill of chub. The metals were accumulated at varying levels and were clearly distinguishable in the different tissues of both species in Yeniçağa Lake. The highest concentrations were found in the liver and gills while the lowest concentration was detected in the muscles. Relatively high concentrations of metals in the liver and gill have been reported for a number of fish species. This case were also found in *L. cephalus* and *Lepomis gibbosus* from Sarıçay (Yılmaz et al. 2007), in *Silurus triostegus* and *Mastacembelus simack* from Tigris River (Karadede and Ünlü 2007), in *T. tinca* from Beyşehir Lake (Tekin-Özan 2008).

In this study the metals that have been found in liver of *T. tinca* from higher concentration to lower concentration

Table 1 Mean concentration (mg kg^{-1} wet wt.) of metals in the tissue of *T. tinca* collected from Yeniçağa Lake and statistical analyses results

	Liver	Gill	Muscle	FAO	TFC	CAC
Al	528.56 ± 152.9[a]	82.38 ± 18.4[b]	59.06 ± 13.37[bc]			
Cr	1.03 ± 0.37[a]	0.78 ± 0.29[a]	0.16 ± 0.08[b]	2		
Mn	10.32 ± 3.23[a]	41.52 ± 14.43[b]	2.55 ± 0.78[c]	50		
Fe	84.10 ± 28.9[a]	99.21 ± 22.9[a]	9.23 ± 4.08[b]		50	
Co	0.19 ± 0.0.9[a]	0.33 ± 0.12[ab]	0.05 ± 0.02[c]			
Ni	1.52 ± 0.40[a]	1.99 ± 0.55[b]	0.34 ± 0.10[c]	10		
Cu	5.21 ± 1.78[a]	1.63 ± 0.30[b]	1.42 ± 0.25[b]	10	20	
Zn	116.04 ± 35.8[a]	86.71 ± 22.8[b]	45.53 ± 9.10[c]	40		
As	1.78 ± 0.85[a]	1.28 ± 0.57[b]	0.26 ± 0.11[c]	0.1	1	
Mo	1.42 ± 0.66[a]	0.25 ± 0.15[b]	0.18 ± 0.05[b]			
Cd	0.02 ± 0.01[a]	ND	0.01 ± 0.01[a]	0.05	0.05	
Sn	0.06 ± 0.02[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]			
Ba	6.93 ± 2.10[a]	83.73 ± 32.3[b]	3.50 ± 2.19[a]			
Pb	3.05 ± 0.79[a]	1.15 ± 0.27[b]	0.68 ± 0.19[c]		0.2	0.2

The same letter in the row shows no statistically difference ($p = 0.05$)

FAO (1983), Turkish Food Codex (2002), Codex Alimentarius Codex (2002)

Standards are given as mg/kg wet weigth basis in fish tissue

Table 2 Mean concentration (mg kg^{-1} wet wt.) of metals in the tissue of *L. cephalus* collected from Yeniçağa Lake and statistical analyses results

	Liver	Gill	Muscle	FAO	TFC	CAC
Al	453.62 ± 225[a]	257.04 ± 35.8[b]	108.26 ± 10.9[c]			
Cr	0.50 ± 0.25[a]	0.65 ± 0.19[a]	0.16 ± 0.05[b]	2		
Mn	6.88 ± 3.70[a]	30.41 ± 8.23[b]	2.50 ± 0.38[c]			
Fe	49.73 ± 16.1[a]	85.38 ± 34.5[b]	16.03 ± 4.78[c]		50	
Co	0.16 ± 0.09[a]	0.33 ± 0.12[b]	0.05 ± 0.03[c]			
Ni	0.57 ± 0.25[a]	1.21 ± 0.43[b]	0.06 ± 0.01[c]	10		
Cu	3.16 ± 0.75[a]	2.04 ± 0.35[b]	1.79 ± 0.55[b]	10	20	
Zn	123.64 ± 0.64[a]	273.74 ± 125[b]	57.81 ± 21.5[c]	40		
As	2.29 ± 0.71[a]	1.86 ± 0.62[a]	0.58 ± 0.26[b]	0.1	1	
Mo	0.44 ± 0.18[a]	0.33 ± 0.12[a]	0.34 ± 0.9[a]			
Cd	0.01 ± 0.01[a]	0.01 ± 0.01[a]	ND	0.05	0.05	
Sn	0.03 ± 0.01[a]	ND	0.01 ± 0.01[a]			
Ba	5.43 ± 1.47[a]	20.70 ± 7.93[b]	2.69 ± 0.68[c]			
Pb	2.56 ± 0.67[a]	1.70 ± 0.40[ab]	0.46 ± 0.17[c]		0.2	0.2

The same letter in the row shows no statistically difference ($p = 0.05$)

* FAO (1983), Turkish Food Codex (2002), Codex Alimentarius Codex (2002)

* Standards are given as mg/kg wet weigth basis in fish tissue

are as follows: Al > Zn > Fe > Mn > Ba > Pb > Cu > As > Ni > Mo > Cr > Co > Sn > Cd. With the exception of Cr and Mo, same order has also been the case for *L. cephalus* liver. That is, there was a lot more chrome than molybdenum in the liver tissue. The metals accumulated in the gills of both species has differed, the order of metals from higher to lower concentrations was Fe > Zn > Ba > Al > Mn > Ni > Cu > As > Pb > Cr > Co > Mo > Sn in *T. tinca* and Zn > Al > Fe > Mn > Ba > As > Pb > Cu > Ni > Cr > Co = Mo > Cd in *L. cephalus*. The order of metals in the muscle of *T. tinca* have been found to be Al > Zn > Fe > Ba > Mn > Cu > Pb > Ni > As > Mo > Cr > Co > Cd = Sn and in muscle of *L. cephalus* it was Al > Zn > Fe > Ba > Mn > Cu > As > Pb > Mo > Cr > Ni > Co > Sn. In general alumina, zinc, iron, barium and manganese had highest concentrations in all tissues of the investigated species, while cadmium and tin were the elements that had lowest detected concentrations. The accumulation of the metal in the liver tissue of both species was same order. Despite some observed variability of metal orders in gill and muscle tissues of both species, considerably similar ranking were found in these tissues. This may be the result of the fact that the investigated species are of the same family (Cyprinidae) and share the same habitat. This case was also reported for some Cyprinid species by Uysal et al. (2009). On the other hand, variations of metal order of both species can depends on feeding habits (Romeoa et al. 1999), size and length of the fish (Linde et al. 1998).

In the Yeniçağa Lake, water and plankton sampling was done on a monthly basis for the metals analysis; the concentration levels of metals found in water from highest to lowest was Ba > Mn > Fe > Al > Zn > As > Cd > Pb > Ni > Cr > Co > Mo > Sn > Cu and it was Al > Fe > Zn > Mn > Ba > Pb > As > Cu > Cr > Ni > Co > Mo > Sn > Cd in plankton samples (Saygı and Atasagun 2012). In our study shows that high levels of the metals present in both water and plankton are in fact the highest metals found in *L. cephalus* and *T. tinca* tissues. The metals as Fe, Cu, Zn, and Mn are essential elements such that these metals also has function as cofactors for the activation of many enzymes, as a result they are found in high concentrations in metabolic organs such as liver (Guillaume et al. 2001). High rates accumulation of the metals in liver and gill tissue has been reported by many researchers (Čelechovska et al. 2007; Alhas et al. 2009). The liver and gill are excellent target organs for many metal accumulations. This may well be due to presence of high levels of metallothionein in the liver. The concentration of metals accumulated in the gill tissue is fairly good indicator of the concentration of metals in the water the fish live (Heath 1987). Also, studies have shown that in terms of metal accumulation muscle tissue is not as active as some others tissues (Fidan et al. 2008). In this regard, the results of our study are quite consistent with results reported in the literature. Thus, the gill results we found were very noteworthy due to the high accumulation of Al, Zn, Fe, Mn and Ba we observed. High levels of metal accumulation in the gill of both fish is quite representative of the high levels of metal concentrations found in lake water. As gills are in constant contact with water, they are also in contact with the contaminant metals. As a result high levels of metals found in Yeniçağa Lake water, as they are filtered through the fish gills, high levels of accumulation has taken in the tissue.

In this study, metal accumulation level has shown significant variation on the fish type. In terms of comparing metal levels in the species; Al, Fe, Cu, Zn, As, Mo in *L. cephalus* and Mn, Ni, Pb, Ba levels in *T. tinca* are found to be higher in the muscle tissue. Also, with the exception of arsenic and zinc, many of the metals (Al, Cr, Mn, Fe, Co, Ni, Cu, Mo, Ba, Pb) were found to be much higher levels in liver tissue of *T. tinca*. In the gill tissue of *L. cephalus* Al, Cu, Zn, As, Mo, Pb was found to have high levels of accumulation. These differences may have been due to the two species of fish having different diet (omnivorus or carnivorus) and the reason of this state can be also related with the difference in growth rates, size and length of the fishes (Linde et al. 1998; Papagiannis et al. 2004; Yılmaz et al. 2007).

In Yeniçağa Lake tench and chub fishing is an important source of income for the local fishermen. The fish caught by the fishermen are sold in local markets due to being cheap source of protein; they are consumed significantly by the local community. Therefore, the accumulation of metals in fish species is very important for human health risk assessment. Allowable/safe metal limits in fish for human consumption are decided on by Codex Alimentarius Commission (2002), Turkish Food Codex (2002) and International Standards (FAO) (1983). The permissible limits proposed by these standards and the following maximum levels for the metals studied, above which consumption is not permitted are: 0.2 mg kg^{-1} for Pb; 0.05 mg kg^{-1} for Cd, 10–20 mg kg^{-1} for Cu, 0.1 mg kg^{-1} for As, 50 mg kg^{-1} for Fe, 40 mg kg^{-1} for Zn, 50 mg kg^{-1} for Mn, 2 mg kg^{-1} for Cr, 10 mg kg^{-1} for Ni (Tables 1, 2). In this study, on *T. tinca* and *L. cephalus*, metals found in the muscle tissues are evaluated according to standards, the following results were obtained:

(a) In both fish species high levels of Al was found in the muscle tissues. Aluminum, according to Agency for Toxic Substances and Disease Registry (ATSDR), is among the most toxic components. Also fish muscle, skeletal system, nervous tissue and the respiratory system are some of the most effected organs by aluminum; recommended daily dose of aluminum is given as 1 mg/kg/day. However, we were not able to compare since there were no limits set for aluminum. (b) Zinc, lead and arsenic values we analyzed in the muscle tissue were higher than the maximum permitted concentrations proposed by the guidelines. Zinc is essential elements and is carefully regulated by physiological mechanisms in most organisms. However, it is regarded as potentially hazardous substance that can endanger human health (Yılmaz et al. 2007). Lead is a neurotoxin that causes behavioral deficits in vertebrates, a decrease in survival and growth rates, learning disabilities (Qiao et al. 2007). Arsenic is hazardous elements notified by the USFDA (1993). Therefore, we can conclude that these metals pose a threat to health after the consumption of both fish species in Yeniçağa Lake, particularly in the case of As, Zn and Pb. (c) Copper, iron, chromium, nickel levels in the muscle tissue of *T. tinca* and *L. cephalus* were found to be below the permitted limit values set by international standards for human consumption. The limit for Cd was not exceeded in the tissue of *L. cephalus* and *T. tinca* analyzed in this study. Although Mn is one of the most abundant metals in the water of Yeniçaga Lake (Saygı and Atasagun 2012), no important Mn accumulation was found in the muscle of the *L. cephalus* and *T. tinca* of the lake, and the results were below the permissible limit.

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