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Determination of some heavy metal levels and oxidative status in *Carassius carassius* L., 1758 from Eber Lake

A. Fatih Fidan · İ. Hakkı Ciğerci · Muhsin Konuk · İsmail Küçükkurt · Recep Aslan · Yılmaz Dündar

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Abstract In this study, the levels of some heavy metals (Al, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb and Zn) in muscle, gill, and liver of *Carassius carassius* and in the water samples from Eber Lake (Afyonkarahisar, Turkey) have been investigated. Additionally, one of the lipid peroxidation markers, malondialdehide (MDA) and glutathion (GSH) levels were investigated. All the metal analysis was performed by using ICP-AES. According to results obtained, it was observed that heavy metals were accummulated in liver in the highest degree and lowest one in the muscle tissues. MDA and GSH levels varied in the seasons but their winter levels were found to be statistically meaningful when compared with other seasons. The obtained data of metals from the study were compared with the acceptable levels of Turkish governmental regulation and they were found not to be harmful for human health. On the other hand, it was suggested that oxidative stress markers should be checked regularly in order to get important data for continuous life of aquatic organisms.

Keywords Accumulation · *Carassius carassius* · Eber Lake · Heavy metal · Oxidative status

A. F. Fidan · İ. Küçükkurt · Y. Dündar
Faculty of Veterinary Medicine,
Department of Biochemistry, Afyon Kocatepe University,
03200 Afyonkarahisar, Turkey

İ. H. Ciğerci · M. Konuk (✉)
Faculty of Science and Literature, Biology Department
ANS Campus, Afyon Kocatepe University,
Gazligol Yolu,
03200 Afyonkarahisar, Turkey
e-mail: mkonuk@aku.edu.tr

R. Aslan
Faculty of Veterinary Medicine, Department of Physiology,
Afyon Kocatepe University,
03200 Afyonkarahisar, Turkey

Introduction

Most of pollutants are discharged into the environment every day. Of these, heavy metals are regarded as one of the most serious pollutants of the aquatic environment because of their environmental persistence and tendency to be concentrated in aquatic organisms (Harte et al. 1991; Schüürmann and Markert 1998). Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial, agricultural and mining activities. As a result, aquatic animals are exposed to elevated levels of heavy metals (Kalay and Canlı 2000; Ünlü and Gümgüm 1993). They cause serious impairment in metabolic, physiological and structural systems when present in high concentrations in the milieu (Tort et al. 1987). Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain. One of the most serious results of their persistence is biological amplification through the food chain (Ünlü and Gümgüm 1993). In

the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues (Hadson 1988; Kargın 1996a). The accumulation of heavy metals in the tissues of organisms can result in chronic illnesses and cause potential damage to the population (Holcombe et al. 1976; Barlas 1999). Additionally, exogenous pesticides, heavy metals, chemical mutagenes, radiation, and various stress factors cause to increase of free radicals and oxidative stress. As a result of increasing this stress, lipid peroxidation, protein denaturation and DNA damages occur in the cells of living organism. These changes could be a great risk for the organisms' life and productivity.

The lake Eber is located in south-western Turkey and is kept as a conservation area, and has an importance for irrigation and drinking water source. Most of surface waters in western Turkey are getting polluted due to industrialization and urbanization.

In the present study, it was aimed to evaluate the pollution level of this aquatic ecosystem via determining the accumulation of heavy metals and oxidative status in fish samples of Eber Lake.

Materials and methods

Eber Lake is, a tectonic lake, situated on South east of Afyonkarahisar province (37° 40′ North–31° 12′ East) and 65 km far away from city center of this province. The lake has approximately 4 m depth and its covering area is 156 km^2. Lake water is mainly used for irrigation. Some fish species such as *Esox lucius*, *Carassius carassius* are present in the lake studied. Eber Lake is an important visiting site for several bird species (Atay et al. 2002).

During the study, (December 2004–October 2005) seasonal fish samples (*C. carassius* L., 1758) were collected from the same station at Eber Lake (Fig. 1) with cast nets, in early morning with the assistance of local fisherman and transferred to the laboratory of Biology Department, Afyon Kocatepe University. After measuring their weight and total length, 2–5 g of muscle tissue were taken out. The liver and gills of the

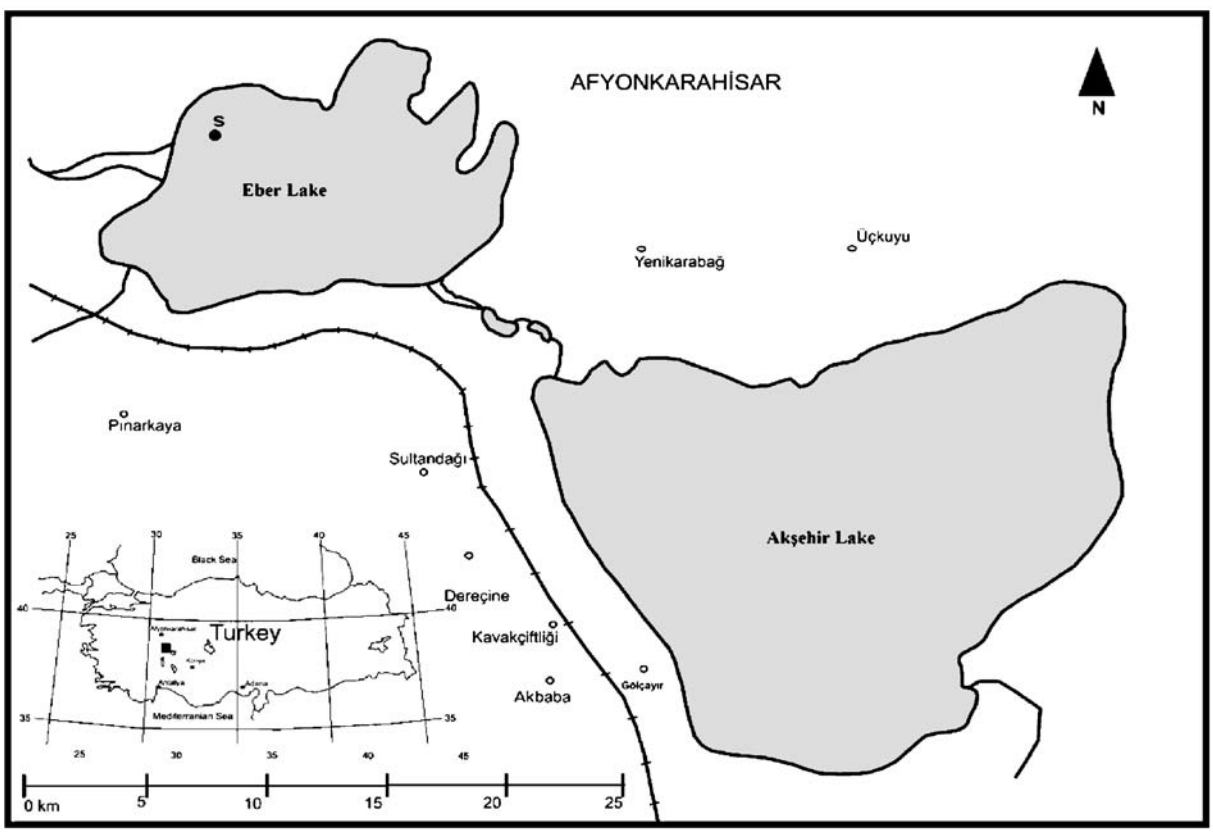

Fig. 1 Map of Eber Lake (S: Collection Station)

specimens were also weighed in order to determine their fresh weights. Accumulation levels of Al, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb and Zn were determined both in water and the muscle, liver, and gills tissues of *C. carassius*. For determination of the heavy metals by use of ICP, the methods of Soltanpour et al. (1995) and Konuk et al. (2007) were followed. All the analyses were performed by using Varian-Vista AXCCD Simultaneous ICP-AES apparatus, and the metal concentrations were calculated as microgram in per gram of fresh weight (μg g^{-1} w.wt.).

Blood samples, 2–4 ml, were taken directly from heart of the fish with heparined injection needles as they were alive. Both MDA and GSH levels were determined in the fresh blood samples. MDA levels were determined by using the method of Draper and Hadley (1990). GSH levels were determined by the method of Beutler et al. (1963) colorimetrically as protein free sulphydryl content using 5,5-dithiobis-2-nitrobenzoic acid (DTNB). These experiments were carried out by using Shimadzu UV 1601 spectrophotometer.

The obtained data were analysed statistically by using SPSS 10.0 for Windows software. Their averages and standard deviations (±SD) were also given. For the statistical comparison of the data, ANOVA and Duncan tests were applied and $p<0.05$ was accepted as statistically meaningful value.

Results

During the study 40 *C. carassius* samples were studied. Their weights were between 93.4 and 255.2 g and lengths were between 176 and 255 mm. Al, Cd, Mn, Ni, Pb and Zn levels of muscle tissue did not differ in the seasons. Co, Cr, Cu, Fe and Li levels were found to be statistically significant ($p<0.05$). It was observed that in spring, Co and Li were at their highest levels and Cr in autumn. Meanwhile, Fe and Cu were at their lowest levels in summer (Table 1).

There were no meaningful seasonal variations of Al, Cd, and Co in liver. However Cr, Cu, Fe, Li, Mn, Ni, Pb and Zn levels showed statistically significant variations in liver. Winter and spring levels of Cr differed from summer and autumn levels, and these differences were found to be statistically significant ($p<0.05$). Cu levels were lower in autumn and winter seasons than those in spring and summer seasons ($p<0.05$). Fe levels were the highest in winter and lowest in spring and these were statistically significant ($p<0.05$). Li levels were low in spring rather than summer, autumn and winter level ($p<0.05$). Mn was detected in only spring and summer seasons. Spring Ni level was very low when compared with other seasons. Pb level was the highest in summer. Zn was at its highest level in spring and its lowest level in summer ($p<0.05$, Table 2).

In the gill tissues, although Cd, Co, Fe, Li, Pb and Zn level variations were not statistically significant, Al, Cr, Cu, Mn and Ni levels changed meaningfully. Al level was the highest in autumn season and lowest in spring. Autumn level of Cr was significantly higher than that of other seasons ($p<0.05$). The levels of Cu were significantly higher in both autumn and winter than the other seasons. Mn levels were found to be higher in winter than the other seasons. Ni was the remarkably lower in summer ($p<0.05$; Table 3).

Table 1 The mean heavy metals concentrations (μg g^{-1} fresh weight) in the muscle tissue of fish studied during the seasons

Heavy metal	Winter (December) $\bar{x} \pm SD$	Spring (March) $\bar{x} \pm SD$	Summer (July) $\bar{x} \pm SD$	Autumn (October) $\bar{x} \pm SD$
Al	0.88±0.12	3.20±1.81	0.22±0.06	0.66±0.15
Cd	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Co	0.00 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.02 ± 0.00^{b}
Cr	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.10 ± 0.01^{b}
Cu	0.75 ± 0.08^{b}	0.60 ± 0.07^{ab}	0.33 ± 0.09^{a}	0.51 ± 0.11^{ab}
Fe	6.98 ± 1.12^{a}	$5.41\pm.75^{b}$	2.44 ± 0.68^{b}	5.23 ± 1.18^{b}
Li	0.07 ± 0.00^{a}	0.10 ± 0.00^{b}	0.07 ± 0.00^{a}	0.08 ± 0.00^{a}
Mn	0.00±0.00	0.17±0.17	0.00±0.00	0.00±0.00
Ni	0.12±0.03	0.06±0.02	0.05±0.02	0.07±0.02
Pb	0.00±0.00	0.02±0.02	0.00±0.00	0.00±0.00
Zn	9.70±0.70	11.30±1.82	6.73±1.85	10.74±1.62

Different letters (a, b) in the same line indicate the statistically significant difference ($p<0.05$)

Table 2 The mean heavy metals concentrations (μg g^{-1} fresh weight) in liver tissues of the fish studied

Heavy metal	Winter (December) $\bar{x} \pm SD$	Spring (March) $\bar{x} \pm SD$	Summer (July) $\bar{x} \pm SD$	Autumn (October) $\bar{x} \pm SD$
Al	7.19±1.08	1.44±0.13	13.66±11.45	2.03±0.48
Cd	0.06±0.01	0.03±0.00	0.09±0.04	0.06±0.01
Co	0.21±0.08	0.14±0.10	0.05±0.02	0.12±0.06
Cr	0.36 ± 0.06^{b}	0.14 ± 0.01^{a}	0.18 ± 0.07^{ab}	0.32 ± 0.07^{ab}
Cu	1.03 ± 0.09^{a}	3.34 ± 0.98^{b}	1.90 ± 0.95^{ab}	1.16 ± 0.27^{a}
Fe	436.20 ± 79.82^{c}	57.39 ± 12.20^{a}	117.79 ± 46.33^{ab}	268.58 ± 49.82^{b}
Li	0.50 ± 0.05^{b}	0.15 ± 0.00^{a}	0.57 ± 0.17^{ab}	0.34 ± 0.03^{ab}
Mn	0.00 ± 0.00^{b}	2.56 ± 0.46^{a}	0.02 ± 0.02^{ab}	0.00 ± 0.00^{b}
Ni	0.74 ± 0.13^{a}	0.08 ± 0.01^{b}	1.05 ± 0.72^{a}	0.28 ± 0.06^{a}
Pb	0.00 ± 0.00^{a}	0.02 ± 0.02^{a}	1.30 ± 0.69^{b}	0.26 ± 0.26^{a}
Zn	19.88 ± 1.15^{ab}	24.27 ± 3.26^{b}	9.57 ± 3.77^{a}	22.88 ± 7.14^{ab}

Different letters (a, b, c) in the same line indicate the statistically significant difference ($p<0.05$)

MDA levels have also varied during the seasons and they were found to be statistically meaningful ($p<0.05$). Its highest level was obtained in winter and the lowest levels were in spring and summer seasons. The highest GSH level was observed to be in winter season and it was statistically significant ($p<0.05$; Table 4).

Discussion and conclusion

Fish are one of the important nutrition sources for mankind and the markers of pollution where they live (sea, lake or stream). For this reason they should be monitored for controls of both ecosystem and food quality.

The concentration of heavy metals in fish is related to several factors, such as food habits and foraging behavior of the organism, trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the milieu, bio-magnification and/or bio-diminishing of a particular metal, food availability, metallothioneins and other metal detoxifying proteins in the body of the animal, temperature, transport of metal across the membrane and the metabolic rate of the animal, physical and chemical properties of the water and the seasonal changes in the taxonomic composition of different trophic levels affecting the concentration and accumulation of heavy metal in the body of fish (Shah and Altındağ 2005).

Different heavy metals are accumulated in various tissues in fish (Belinsky et al. 1996; Olsson 1998). Even this might change regarding to the species (Kargın and Erdem 1991). Usually, heavy metals accumulate in metabolically active organs in nonlethal levels (Ünlü et al. 1995).

In the literature, the amount of heavy metals bioaccumulants in tissues may vary depending on length and weight of samples (Barghigiani and Ranieri

Table 3 The mean heavy metals concentrations (μg g^{-1} fresh weight) in the gill tissues of fish studied

Heavy metals	Winter (December) $\bar{x} \pm SD$	Spring (March) $\bar{x} \pm SD$	Summer (July) $\bar{x} \pm SD$	Autumn (October) $\bar{x} \pm SD$
Al	6.42 ± 2.33^{a}	2.42 ± 0.44^{ab}	16.64 ± 7.68^{ab}	36.8 ± 20.99^{b}
Cd	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Co	0.03±0.01	0.07±0.01	0.02±0.01	0.09±0.08
Cr	0.28 ± 0.01^{a}	0.21 ± 0.01^{a}	0.18 ± 0.03^{a}	0.49 ± 0.09^{b}
Cu	0.71 ± 0.04^{b}	0.62 ± 0.04^{ab}	0.47 ± 0.10^{a}	0.78 ± 0.08^{b}
Fe	42.67±6.63	44.44±2.83	35.17±9.00	48.05±13.04
Li	0.22±0.00	0.27±0.02	0.27±0.04	0.30±0.021
Mn	9.17 ± 1.01^{a}	6.22 ± 0.63^{b}	5.10 ± 1.3^{b}	5.73 ± 0.58^{b}
Ni	0.27 ± 0.05^{ab}	0.45 ± 0.09^{b}	0.07 ± 0.01^{a}	0.31 ± 0.08^{b}
Pb	0.05±0.02	0.02±0.01	0.05±0.03	0.08±0.04
Zn	74.70±6.13	63.33±6.65	63±12.09	58.61±4.85

Different letters (a, b) in the same line indicate the statistically significant difference ($p<0.05$)

Table 4 MDA and GSH levels in all samples analysed

	Winter (December) $\bar{x} \pm SD$	Spring (March) $\bar{x} \pm SD$	Summer (July) $\bar{x} \pm SD$	Autumn (October) $\bar{x} \pm SD$
MDA (nmol/ml)	23.28 ± 2.42^{a}	12.51 ± 4.44^{b}	11.0 ± 3.51^{b}	14.98 ± 2.61^{ab}
GSH (mg/dl)	100.4 ± 17.70^{a}	59.48 ± 6.93^{b}	79.23 ± 15.81^{b}	83.29 ± 16.62^{b}

Different letters (a, b) in the same line indicate the statistically significant difference ($p<0.05$)

1992; Zyadah 1999). Therefore, the samples were chosen to be around the same weight and length.

Target organs, such as liver, gonads, kidney and gills, have a tendency to accumulate heavy metals in high values, as shown in many species of fish in different areas: in *M. cephalus* from Mediterranean Sea (Abdel-Moniem et al. 1994); in *Trachurus mediterraneus* from eastern Mediterranean waters (Yılmaz 2003; Abdel-Moniem et al. 1994); in *Mullus barbatus* and *S. aurata* from Iskenderun Gulf (Kargın 1996b; Yılmaz 2003). It is generally accepted that muscle does not accumulate the metals (Legorburu et al. 1988).

Our findings showed that they were found mostly in liver and followed by gills and muscle tissues, respectively. Cd, Co, Fe, Li, Ni and Pb accumulated mostly in liver; and Cr, Al, Mn and Zn accumulated in gills.

Çalta et al. (2002) reported that Cu, Fe, Mn, and Zn accumulated in liver of *Capoeta capoeta umbla* (Heckel 1843) from Hazar Lake, and these metals had been found very low concentrations in muscle tissue. This report gave similar results with ours.

Canlı and Kargın (1995) reported that Cu accumulation was the highest level in liver tissue of Carp. Kargın and Erdem (1992) have also reported similar results from *Tilapia nilotica* specimens.

Karadede et al. (1997) carried out a study on some heavy metal accumulations (Cu, Fe and Zn) in different (gonad, liver, muscle, gills and kidney) tissues of *Mastacembelus simack* and found that Zn accumulation was reported to be at lowest level in muscle tissue. This report was also similar to our findings. We have also other findings that gill tissue had the highest Zn concentration. Çalta et al. (2000) reported the similar results, for Cu, Fe, Mn, Zn, from *Capoeta trutta* in Keban Dam Lake. Yazkan et al. (2002) reported that Zn was the highest accumulated one among the metals (Cu, Zn, Pb, and Cd) studied on fish from Antalya gulf. Our results showed that Fe accumulation was the highest.

Cyprinus carpio L., 1758 and *Stizostedion lucioperca* L., 1758 from Seyhan Dam Lake were studied by Göksu et al. (2003) for same purpose with ours. They found that the concentration ranges of Fe, Zn and Cd in the eaten parts of these fish were similar to our findings.

Mugil cephalus and *Sparus aurata* from Iskenderun bay were studied by Yılmaz (2005). Their gonads, skin and muscle tissues were analysed in determining the heavy metal (Fe, Cu, Ni, Cr, Pb, and Zn) accumulations. This study showed that metal accumulations varied according to stations where the fish collected, fish and tissue, living depthness, and nutrition habit types.

Our findings indicate that metabolically active organs are under a great risk. Apart from these, Pb and Cd levels were in acceptable levels of Turkish official regulations (Official Gazette 1991). Especially, Al, Cd, and Pb levels are of importance. Even if they are not in toxic level, they have harmful effects in the links of food chain.

Reactive oxygen metabolites, produced by aerob organisms after oxygen consumption, reacts with cellular macromolecules and cause lipid peroxydation, protein oxidation and DNA damages. Cells have their antioxidant systems that protect themselves against to these harmful effects. Although these antioxidant levels depended on the age and gender, environmental conditions affect these systems abundantly. One of the crucial markers of free radicals is MDA. In this study its levels were also studied. According to the data obtained, MDA levels were found to be at its lowest level in summer and highest level in winter. Moreover, GSH, a natural antioxidant, levels were found to be the same as MDA due to increase and decrease of oxidative stress. MDA level increases when the natural antioxidant system insufficient. MDA is also a cancerogenic agent (Şahan et al. 2003). The increase of its level in winter could be because of the cold conditions of the fish environment, and lipid catabolism occur in cold seasons to get much more energy for their metabolisms.

GSH is an endogenous compound that protects the fish against xenobiotics. For this, it plays a key role in

detoxification, anticarcinogenesis and prevents the tissue necrosis and chemical lesions (Şahan et al. 2003). In our study, it was observed that its level increased in the winter to protect the tissues against free radicals occurring.

Although our findings showed that the heavy metal levels were within acceptable levels, it is strongly suggested that the liver and gills of the fish studied should be removed during the consumption. On the other hand, heavy metal pollution of the lake should be checked and oxidative stress of the fish controlled regularly for food safety and environmental pollutions. Otherwise, environmental pollutions can be dangerous for fish and human health.

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