

Determination of Metal (Cu, Zn, Se, Cr and Cd) Levels in Tissues of the Cyprinid Fish, *Capoeta trutta* (Heckel, 1843) from Different Regions of Keban Dam Lake (Euphrates-Turkey)

Durali Danabas · Mesut Ural

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Abstract The aim of this study was to evaluate Copper (Cu), Zinc (Zn), Selenium (Se), Chromium (Cr) and Cadmium (Cd) concentrations in liver, muscle, gills and kidney tissues of *Capoeta trutta* collected from four sites of Keban Dam Lake, Turkey. The highest heavy metal level in all tissues was for Zn, while Cd was the lowest. The lowest heavy metal levels were generally found at Station 4 (Agin) for all tissues ($p < 0.05$). There were statistically significant differences among stations for Cu, Zn, Cr and Se in gills; for Cu, Zn, Se and Cr in liver and kidney; and Cu, Zn and Cr in muscle ($p < 0.05$). Cadmium concentrations did not differ between sites for any of the tissues. Turkish Food Codex sets the maximum limits of Zn, Cu and Cd as, 50, 20 and 0.05 mg kg⁻¹ dry weight, respectively, in the muscle of fish used for human consumption. In our study, the level of Zn was higher than Turkish permissible limits only at Station 3 (Guluskur). Cadmium levels were much higher than permissible limits at three stations [S1 (Pertek), S2 (Kockale) and S3], whereas Cu levels were within permissible limits in all stations.

Keywords Heavy metal · *Capoeta trutta* · Accumulation · Muscle · Keban Dam Lake

Contamination of waters with heavy metals is a recognized environmental problem (Staniskiene et al. 2006). There are numerous sources of metal contamination/pollution, including mining, agricultural and forestry activities, waste

disposal, industrial discharges, and fuel combustion (Kennicutt et al. 1992; Olsson 1998).

Some metals are known to be toxic, even at low concentrations, including arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), etc., (Masters 1997; Le et al. 2009). Others, such as copper (Cu) and cobalt (Co), are known to be essential elements and play important roles in biological metabolism at very low concentrations (Le et al. 2009), and either an excess or deficit can disturb biochemical functions in both humans and animals (Danabas et al. 2011; Gulec et al. 2011).

Metals differ from other toxic substances in that they are totally non-degradable, which means they are virtually indestructible in the environment (Masters 1997). Thus, their content has steadily increased in soils and subsequently accumulated in plants, animals, and even in humans (Che et al. 2006).

Pollutants enter fish through five main routes: via food or non-food particles, gills, oral consumption of water and the skin. Upon absorption, the pollutant is carried in the blood stream to either a storage point or to the liver for transformation and/or storage. Pollutants transformed in the liver, may be stored there, excreted in the bile, transported to other excretory organs such as gills or kidneys for elimination, transported to and stored in fat tissue (Heath 1991; Nussev et al. 2000; Al-Kahtani 2009; Danabas et al. 2011).

Copper and zinc (Zn) are needed in trace amounts for carrying out vital functions and for growth in animals (Cousins 1985). Also, Cu is used in producing alloys, as a chemical catalyst, and as a raw material in dyes and algicides (Torres et al. 1987). Discharge of Cu from these sources without any treatment increases its concentration in aquatic environments, increasing the potential for toxic action in aquatic organisms (Stagg and Shuttleworth 1982; Luran and McDonald 1985; Atabeyoglu and Atamanalp

D. Danabas (✉) · M. Ural
Fisheries Faculty, Tunceli University, 62000 Tunceli, Turkey
e-mail: dalid07@gmail.com

2010). Selenium (Se) is an essential trace element that exists in a variety of inorganic and organic forms in nature. The predominant dietary forms are selenocysteine and selenomethionine. Among its biologic functions, Se is a component of glutathione peroxidase, which is part of the antioxidant defense against free radicals. Selenium deficiency results in a decline in tissue Se-dependent glutathione peroxidase activity (Yoshizawa et al. 2003). Levels of Se were analyzed because Se offers some protection against mercury (Hg) exposure (Lemire et al. 2010; Burger and Gochfeld 2011). Many heavy metals are highly toxic. For example, along with Hg and Pb, Cd is one of the “big three” heavy metal poisons. Cadmium occurs as a constituent of lead and zinc ores, from which it can be extracted as a byproduct. Cadmium is used to electroplate metals to prevent corrosion, as a pigment, as a constituent of alkali storage batteries, and in the manufacture of some plastics (Manahan 2003).

Capoeta trutta is an economically important fish species as a component of a significant commercial fishery in Keban Dam Lake. There is no published information available on heavy metal levels in fish from this lake. This research was organized to especially compare the information of multiply research stations and to monitoring the pollutant levels in this dam lake. The aim of this work was to evaluate Cu, Zn, Se, Chromium (Cr), and Cd concentrations in liver, muscle, gills and kidney tissues of *C. trutta* collected from four sites of Keban Dam Lake, Turkey.

Materials and Methods

Fish, *Capoeta trutta* (Heckel, 1843) were collected by gill nets with various mesh sizes at four sampling stations in Keban Dam Lake, Turkey (Fig. 1). Fifteen fish were collected at each station. The fish were immediately anaesthetized with 0.7 g l^{-1} benzocaine in ethyl alcohol (Sardella et al. 2004). The anesthetic resulted in deep sedation with loss of swimming action and partial loss of equilibrium as previously observed (Altun and Danabas 2006). They were then transferred to the laboratory under cold conditions, measured for length and weight, and dissected. Live weight (W) and total length (TL) averages of fish caught from stations were respectively, in S1, $376.20 \pm 28.39 \text{ g}$ and $33.18 \pm 1.36 \text{ cm}$; in S2, $230.44 \pm 10.72 \text{ g}$ and $28.27 \pm 0.41 \text{ cm}$; in S3, $273.23 \pm 18.97 \text{ g}$ and $30.32 \pm 0.78 \text{ cm}$, and in S4, $400.24 \pm 35.72 \text{ g}$ and $32.91 \pm 0.89 \text{ cm}$.

These sampling stations [Pertek (S1), Kockale (S2), Guluskur (S3) and Agin (S4)] were chosen according to some properties. For example; S1 and S2 have domestic and agricultural waste discharges from two different cities (Elazig and Tunceli) and S3 has a discharge from a chrome factory without purification. Station 4 was chosen as a station with no known close discharges of importance.

Samples of gill, liver, kidney and muscle tissue were removed with stainless-steel utensils, followed by drying for 24 h at 105°C . Dried tissue subsamples (0.3 g) were placed into Pyrex vessels in a microwave digestion unit

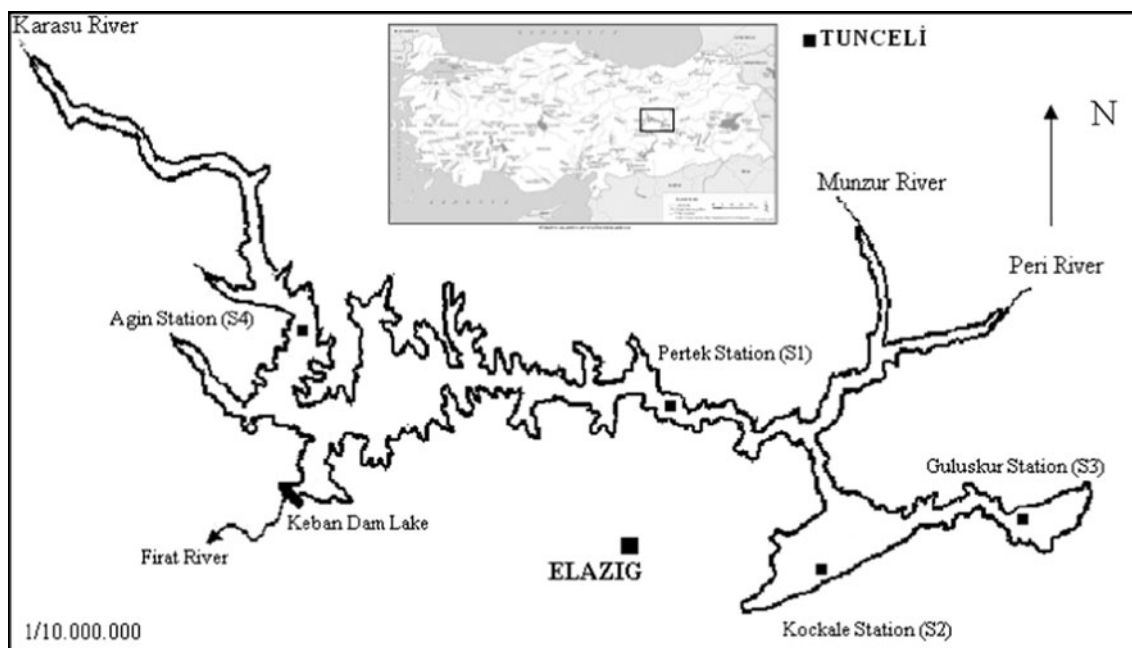


Fig. 1 The map of sampling stations on Keban Dam Lake, Turkey

Table 1 Mean (\pm SEM) water quality parameters at the four sampling stations ($n = 3$)

Water quality parameters	Stations			
	Pertek (S1)	Kockale (S2)	Guluskur (S3)	Agin (S4)
Temperature ($^{\circ}\text{C}$)	15.2 ± 0.1^a	15.5 ± 0.1	15.3 ± 0.2	15.1 ± 0.2
pH	8.0 ± 0.1	8.3 ± 0.1	8.2 ± 0.2	8.1 ± 0.3
Dissolved O_2 (mg l^{-1})	8.7 ± 0.2	7.7 ± 0.9	8.4 ± 0.3	9.2 ± 0.1
Free CO_2 (mg l^{-1})	6.2 ± 0.2	6.8 ± 0.4	7.1 ± 0.1	5.6 ± 0.4
Nitrite (mg l^{-1})	0.052 ± 0.001	0.049 ± 0.002	0.045 ± 0.004	0.013 ± 0.005
Nitrate (mg l^{-1})	17.9 ± 0.2	18.4 ± 0.1	18.1 ± 0.2	6.8 ± 0.3
Ammonia (mg l^{-1})	1.30 ± 0.2	2.0 ± 0.1	0.60 ± 0.3	0.01 ± 0.01
Phosphate (mg l^{-1})	3.48 ± 0.02	4.12 ± 0.01	2.76 ± 0.05	0.02 ± 0.001
Total hardness (mg l^{-1})	70.9 ± 0.1	74.5 ± 0.2	67.7 ± 0.1	42.9 ± 0.2

^a Means (\pm Standard error of means)

(CEM Star 5, Terra Analysis and Measurement Equipment Trade Co., Ankara, Turkey). HClO_4 : HNO_3 acids of 1:3 proportions for fish were inserted in the reactors, respectively. Samples were mineralized at 200°C for 30 min. Afterwards the samples were filtered in such a way as to make their volumes equal to 100 ml with ultra-pure distilled water. The levels of Cu, Zn, Se, Cr and Cd in samples were determined by ICP-OES (Varian 720 ES, Germany). Metal concentrations were reported as means of triplicate analyses (Astm 1985; Apha 1992; Epa 2001).

Temperature, dissolved oxygen and pH were measured by YSI professional multiparameter and the other parameters were measured by titrimetric methods (Table 1) (Apha 1992).

SPSS v15.0 statistical software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Mean metal concentrations from the stations were analyzed for differences at the $p < 0.05$ level by ANOVA followed by Duncan's multiple range test.

Results

Higher concentrations of free CO_2 , nitrite, nitrate, ammonia, phosphate and total hardness were observed at sites S1, S2 and S3, as compared to S4. However, the highest dissolved oxygen level was measured at S4.

The highest heavy metal level in all tissues was Zn, while Cd was the lowest (Table 2). The lowest heavy metal levels were generally measured at S4 (Agin) for all tissues ($p < 0.05$). There were statistically significant differences among stations for Cu, Zn, Cr and Se in gills; for Cu, Zn, Se and Cr in liver and kidney; and for Cu, Zn and Cr in muscle ($p < 0.05$). Cadmium concentrations did not differ between sites for any of the tissues. The highest levels of Cu ($16.82 \pm 3.27 \text{ mg kg}^{-1}$), Zn ($81.52 \pm 20.02 \text{ mg kg}^{-1}$), Se ($8.65 \pm 0.15 \text{ mg kg}^{-1}$), Cr ($9.53 \pm 2.67 \text{ mg kg}^{-1}$) and

Cd ($2.94 \pm 0.36 \text{ mg kg}^{-1}$) were obtained in liver, liver, kidney, gills and kidney tissues of fish caught from S1, S3, S3, S2 and S1, respectively.

Determination of the concentrations of metals in fish is an important subject with respect to both nature management and human consumption of fish (Amundsen et al. 1997). Turkish Food Codex (2002) sets the maximum limits of Zn, Cu and Cd as respectively, 50, 20 and 0.05 mg kg^{-1} dry weight in the muscle of fish. The level of Zn is higher in S3 than the maximum limits. Cadmium levels were higher in three stations (S1, S2 and S3) than the limits, whereas Cu levels were within limits at all stations. Mean concentrations of Cd in muscle tissue were more than 20-fold higher than the permissible limits established in the Turkish Food Codex. The permissible Cr levels for fish muscle are 1.0 mg kg^{-1} reported by FAO (Kandemir et al. 2010). The levels of Cr are under the permissible limits in all of the stations except for S1.

Al-Kahtani (2009) studied the accumulation of metals in tilapia fish (*Oreochromis niloticus*) from Al-Khadoud Spring, Al-Hassa, Saudi Arabia. He stated that levels of metals were higher in liver than muscle and the levels in liver and muscle tissues were ranked $\text{Zn} > \text{Cu} > \text{Cd}$. The metal levels of our study are lower than his results. Karadede and Unlu (2000) studied the concentrations of some trace metals in muscle of various species from Ataturk Dam Lake (Euphrates, Turkey). They reported that levels of Cu and Zn in *A. marmid*, *C. trutta* and *C. mossulensis* were 0.81 and 8.71 mg kg^{-1} ; 1.68 and 5.32 mg kg^{-1} and 2.41 and 17.96 mg kg^{-1} , respectively. They expressed that levels of Zn and Cu were lower than the limits like those in our study.

Papagiannis et al. (2004) studied the Cu and Zn levels in freshwater fish from Pamvotis Lake (Greece). They reported that levels of Cu and Zn in *C. carpio* were 3.08 and 52.81 mg kg^{-1} , respectively. They mentioned that there are no guidelines on acceptable levels of Cu and Zn

Table 2 Mean (\pm SEM) Cu, Zn, Se, Cr and Cd contents in fish samples (mg kg^{-1} - dry weight) of Pertek (S1), Kockale (S2), Guluskur (S3) and Agin (S4) Stations on Keban Dam Lake (Elazig, Turkey)

Fish tissues	Metals	Stations			
		Pertek (S1)	Kockale (S2)	Guluskur (S3)	Agin (S4)
Gills	Cu	$4.88 \pm 0.68^{b*}$	6.82 ± 0.47^c	4.55 ± 0.56^b	0.06 ± 0.02^a
	Zn	54.0 ± 3.74^b	59.8 ± 4.68^b	60.7 ± 5.58^b	0.16 ± 0.01^a
	Se	7.85 ± 2.25^c	5.52 ± 0.49^b	6.25 ± 0.84^{bc}	0.03 ± 0.002^a
	Cr	8.06 ± 0.14^b	9.53 ± 2.67^b	1.88 ± 0.78^a	0.04 ± 0.003^a
	Cd	1.38 ± 0.12	1.68 ± 0.08	1.25 ± 0.07	ND
Liver	Cu	16.82 ± 3.27^c	7.90 ± 0.95^b	15.32 ± 4.03^c	0.15 ± 0.02^a
	Zn	42.0 ± 7.35^b	28.2 ± 3.58^{ab}	81.5 ± 20.02^c	0.2 ± 0.02^a
	Se	6.15 ± 0.95^b	4.40 ± 0.80^b	4.47 ± 0.81^b	0.03 ± 0.003^a
	Cr	7.98 ± 0.21^b	3.25 ± 1.72^a	4.17 ± 3.12^{ab}	0.04 ± 0.007^a
	Cd	1.22 ± 0.07	1.11 ± 0.03	1.42 ± 0.10	ND
Kidney	Cu	3.64 ± 1.02^b	3.05 ± 0.26^b	3.52 ± 0.66^b	0.13 ± 0.04^a
	Zn	25.7 ± 3.97^b	24.4 ± 2.07^b	47.3 ± 18.8^b	0.16 ± 0.02^a
	Se	4.60 ± 0.70^b	5.39 ± 0.58^b	8.65 ± 0.15^c	0.03 ± 0.006^a
	Cr	8.06 ± 0.10^c	2.57 ± 0.65^b	0.45 ± 0.15^a	0.04 ± 0.004^a
	Cd	2.94 ± 0.36	2.01 ± 0.09	1.86 ± 0.27	ND
Muscle	Cu	5.68 ± 1.32^c	2.50 ± 0.48^b	6.12 ± 0.80^c	0.12 ± 0.06^a
	Zn	18.6 ± 0.84^{ab}	27.9 ± 1.85^b	56.2 ± 16.15^c	0.53 ± 0.42^a
	Se	5.60 ± 0.50	6.11 ± 0.70	ND	0.46 ± 0.44
	Cr	7.66 ± 0.13^c	0.43 ± 0.09^b	0.40 ± 0.10^b	0.04 ± 0.005^a
	Cd	1.14 ± 0.04	1.16 ± 0.03	1.10 ± 0.09	ND

* Means (\pm Standard error of means) in the same row followed by different letters are significantly different according to Duncan's test ($p < 0.05$)

ND not determined

in fish suggested by EEC or FAO/WHO. Chi et al. (2007) stated that Cd and Zn levels in *C. carpio* from Taihu Lake (China) were 0.021 and 25 mg kg^{-1} , respectively. Ebrahimi and Taherianfard (2010) investigated concentrations of some heavy metals in cyprinid fish from the Kor River, Iran, and reported that the maximum concentrations of Cd, Pb, and Hg were higher than the permissible levels for human consumption, as we also did for Cd. On the other hand, Mendil and Uluzozlu (2007) reported that levels of Cu, Zn and Cr in muscle tissue were respectively, 3.6, 48.6, 1.6 mg kg^{-1} in five cyprinid species in Tokat, Turkey. Papagiannis et al. (2004) obtained generally lower results than ours. Cu levels were higher in S1 and S3, Zn in S3, and Cr in S1 than results reported by Mendil and Uluzozlu (2007).

Canpolat and Calta (2003) studied some metals in tissues and organs in *C. capoeta umbla* from the Keban Dam Lake (Turkey). They reported that the lowest levels of Fe, Zn, Mn and Cu were measured in muscle and skin, compared to other tissues and organs. Danabas et al. (2011) studied the levels of Cu in liver, muscle and gill tissues in *Capoeta trutta* (Heckel, 1843) from Munzur River (Turkey). They obtained relatively lower Cu levels in liver and higher Cu

levels in muscle and gills than our results. Staniskiene et al. (2006) studied the distribution of metals (Pb, Cd, Cu, Zn, iron (Fe), nickel (Ni), Cr and manganese (Mn)) in tissues (muscle, bone, liver, gill and intestine) of freshwater fish in Lithuania. They obtained lower levels of Cd, Cu, Zn and Cr in muscle, liver and gills than ours. Le et al. (2009) studied metals in a tropical eel (*Anguilla marmorata*) from the central part of Vietnam. They obtained higher level of Zn and lower levels of Cu and Cd in muscle than our results, whereas the Cr level was similar. Arnwine and Graf (2010) studied mercury air deposition and Se levels in Tennessee fish. They determined lower Se levels in muscle of 9 fish species than Se levels of *C. trutta* in our study except in S1. Burger and Gochfeld (2011) researched Hg and Se levels in skinless muscle tissues of 19 species of saltwater fish from New Jersey and determined lower levels of Se in all species than for the level in *C. trutta* of this study.

Determination of the concentrations of metals in fish is an important subject, both with respect to nature management and human consumption of fish (Amundsen et al. 1997). The muscle of fish is used for human consumption, and Turkish Food Codex (2002) sets the maximum limits of Zn, Cu and Cd as respectively, 50, 20 and 0.05 mg kg^{-1} dry weight in

muscle tissue. However, the maximum Cr level is 1.0 mg kg^{-1} (Kandemir et al. 2010). In our study, the level of Zn is higher in only S3 than maximum limits. Cadmium levels were higher in three stations (S1, S2 and S3) than the limits, whereas Cu levels were in limits in all stations. In addition to these, the levels of Cr were within limits in all of the stations, except S1. In conclusion, *C. trutta* caught from Keban Dam Lake, based on the higher levels of Zn in S3 and Cd in S1, S2 and S3 might be unsafe for human consumption. Care should be taken in consuming these fish, and close monitoring of heavy metal accumulation in fish from Keban Dam Lake in all seasons is recommended in view of possible health risks to consumers.

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