



Research Article

Bioaccumulation of Metals and Metallothionein Levels in Some Fish Species Living in Murat River

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Abstract: The aim of this study was to determine the levels of Cd, Co, Cr, Fe, Mn, Mn, Pb, Ni, Zn and hepatic metallothionein (MT) in muscle, liver, kidney and gills of *Capoeta trutta* and *Capoeta umbla* caught from the Murat River. The length and weight of the fish were measured. The physical and chemical properties of the river water were measured and compared with the tolerable limits of Turkish Standards Institute (TSE). The tissues to be analysed for heavy metals were prepared for the analyses using the wet incineration method in a closed system. The heavy metal content of the samples was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES). Differences in heavy metal accumulation of *Capoeta trutta* and *Capoeta umbla* in different tissues and different metal species were determined. The levels of Cr, Mn, Ni and Fe in the muscle tissues of *Capoeta trutta* were higher than the limits recommended for fish by the Turkish Food Codex (TGK), the Food and Agriculture Organization (FAO) and the World Health Organisation (WHO). Muscle Cr, Mn, Ni and Fe levels of *Capoeta umbla* were higher than the tolerable levels recommended by FAO and WHO. Heavy metals cannot be metabolised in tissues. They tend to accumulate. It has been observed that this accumulation varies in muscle, liver, kidney and gills according to organ structure, metabolic activity and defence mechanisms against toxic substances. MT, an indicator of tissue metal accumulation, was found to differ between *Capoeta trutta* and *Capoeta umbla* liver tissues.

Keywords: *Capoeta trutta*, *Capoeta umbla*, Heavy metal, Metallothionein, Murat River

Murat Nehri'nde Yaşayan Bazı Balık Türlerinde Metallerin Biyoakümülyasyonu ve Metallothionein Seviyeleri

Öz: Bu çalışmanın amacı, Murat Nehri'nde yaşayan *Capoeta trutta* ve *Capoeta umbla* balıklarının kas, karaciğer, böbrek ve solungaçlarındaki Cd, Co, Cr, Fe, Mn, Pb, Ni, Zn ve karaciğer metallothionein (MT) seviyelerini belirlemektir. Balıkların boy ve ağırlıkları ölçülmüştür. Nehir suyunun fiziksel ve kimyasal özellikleri ölçülmüş ve Türk Standartları Enstitüsü'nün (TSE) tolere edilebilir limitleri ile karşılaştırılmıştır. Ağır metal analizi yapılacak dokular kapalı sistem yaş yakma metodu kullanılarak analizlere hazır hale getirildi. Örneklerin ağır metal içeriği indüktif eşleşmiş plazma optik emisyon spektroskopisi (ICP-OES) ile ölçülmüştür. *Capoeta trutta* ve *Capoeta umbla*'nın farklı dokularında ve farklı metal türlerinde ağır metal birikimindeki farklılıklar belirlenmiştir. *Capoeta trutta*'nın kas dokularındaki Cr, Mn, Ni ve Fe seviyeleri, Türk Gıda Kodeksi (TGK), Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) ve Dünya Sağlık Örgütü (WHO) tarafından balıklar için önerilen limitlerden daha yüksek ölçüldü. *Capoeta umbla*'nın kas Cr, Mn, Ni ve Fe seviyeleri FAO ve WHO tarafından önerilen tolere edilebilir seviyelerden daha yüksek bulundu. Ağır metaller dokularda metabolize edilemezler. Birikme eğilimindedirler. Bu birikimin kas, karaciğer, böbrek ve solungaçlarda organ yapısı, metabolik aktivite ve toksik maddelere karşı savunma mekanizmalarına göre değiştiği gözlemlenmiştir. Dokulardaki metal birikiminin bir göstergesi olan MT seviyesi *Capoeta trutta* ve *Capoeta umbla* karaciğer dokusunda farklı bulunmuştur.

Anahtar Kelimeler: Ağır metal, *Capoeta trutta*, *Capoeta umbla*, Metallothionein, Murat Nehri

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1. Introduction

The accumulation of heavy metals in the body is a chronic process. Their elimination half-life is decades. Heavy metals have a wide range of toxic effects such as carcinogenicity, mutagenicity and teratogenicity (Qu et al., 2012). Heavy metal pollution is an important environmental problem caused by economically developed or rapidly developing countries on a global scale (Nriagu & Pacyna, 1988; Dong et al., 2001; Wang & Stuanes, 2003). Aquatic organisms are exposed to heavy metals from many domestic, agricultural, and industrial environmental wastes (Järup, 2003; Wong & Lye, 2008). Heavy metals reach water bodies in various ways and accumulate in the body because they cannot be metabolized in the cells of living organisms in sediments and aquatic environments. The metal levels in the water are increased by events such as rivers, the atmosphere, etc. (Egemen, 1999). The fish form the top layer of the aquatic food chain (Sönmez et al., 2016). Heavy metals enter their bodies through water, gills, food, and skin. Heavy metals absorbed by the body cause toxic effects by bioaccumulating in the tissues to which they are transported by the blood (Uslu, 2007). Heavy metals, which have direct or indirect adverse effects on living organisms, should be monitored continuously in aquatic organisms because they are bioindicators in determining water pollution and revealing their effects on human health (Uslu, 2007; İsanç, 2010). Living organisms easily absorb heavy metals through the food chain and can persist in nature for a long time. The increase of heavy metal levels in tissues is known to adversely affect the metabolism of living organisms (Özan, 2016). Although some trace metals are necessary for living metabolism, even very low levels of some metals cause toxic effects (Al-Jobory & Yucel, 2019). The accumulation of heavy metals in living tissue is specific. Their solubility in water is very low. They are commonly found in nature as carbonate, sulfate, silica, and oxide minerals (Mutluay & Demirak, 1996). The excretory system cannot eliminate many heavy metals absorbed by the body. Therefore, most of these metals such as manganese (Mn), chromium (Cr), cobalt (Co), iron (Fe), zinc (Zn), cadmium (Cd), lead (Pb), and nickel (Ni) accumulate in tissues and show toxic effects (Honarmand et al., 2017). The accumulation of heavy metals in different tissues of organisms due to heavy metal exposure, which is one of the risk factors of environmental pollution, is now considered to be the cause of many diseases (Díez et al., 2009).

In this study, the aim was to determine the levels of heavy metals accumulated in muscle, liver, gill and kidney tissues of *Capoeta trutta* and *Capoeta umbla* species (n: 10 each) caught by fishermen in the Murat River in December 2019, as well as the levels of metallothionein (MT) protein in liver tissues.

2. Material and Methods

2.1. Fish and water samples collection

The fish samples of *Capoeta umbla* (Heckel, 1843) and *Capoeta trutta* (Heckel, 1843) were purchased from local fishermen fishing in the Murat River fishery of Muş province. Fish samples were transported to the laboratory in ice-protected containers. The standard, fork and total lengths of the fish samples (20 fish) brought to the laboratory were measured on a measuring board to an accuracy of ± 1 mm, their weights were measured on a Presica precision balance and muscle, liver, kidney and gill samples were taken from the fish. Water samples were collected from the same area where the fish were caught in 1000 ml glass bottles that had been previously autoclaved.

2.2. Specification of the study area

Murat River originates from the Diyadin district of Ağrı, north of Van Lake. It is formed by the confluence of branches from Aladağ and Muratbaşı Mountains (Erol, 1993). It joins the Karasu River to form the Euphrates (Figure 1).

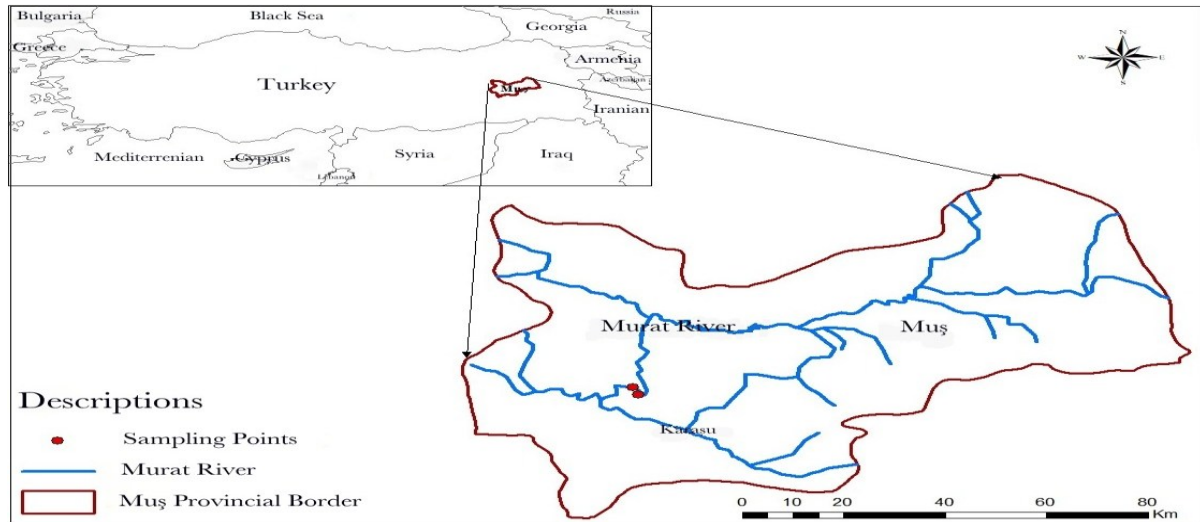


Figure 1. Location of fishing grounds in the Murat River (Muş Province).

2.3. Sample preparation

The wet tissues of fish muscle, liver, gill, and kidney were weighed on a precision balance (1 g) and transferred to glass tubes. Three milliliters of 65% HNO₃ and 36.5% HCl solutions, in a ratio of 1:1, were added to these tissues and kept at room temperature for one hour. The volume of the mixture was then increased to 5 ml with 65% HNO₃ and the mixture was kept in an oven at 95°C for 2 hours. Remove the tubes from the oven and add 2 ml of distilled water at room temperature. Then 3 ml of 30% H₂O₂ was added and the mixtures were kept in an oven at 95°C for 2 hours. The tubes were brought to room temperature, diluted again with 2 ml of pure water, filtered through watmann No. 41 filter paper, and the filtrate centrifuged at 2000-3000 rpm for 10 minutes in plastic centrifuge tubes. After centrifugation, the liquid portion remaining at the top was transferred to plastic tubes and the final volume of TritonX-100 mixture prepared at the rate of 1 % was added to 10 ml. The mixtures in 10 ml plastic tubes were made ready for ICP-OES analysis (Demirezen & Aksoy, 2005).

2.4. Preparation of tissue metallothionein

The liver tissue samples were weighed 0.6 g on a precision balance at room temperature (n:10). Tissues were homogenized in an ultrasonic homogenizer by adding 6 ml of cold phosphate buffer (PBS, pH 7.4). The mixture was then transferred to plastic tubes and centrifuged at 9500 rpm for 30 minutes at 4°C. The supernatant of the tissue mixture was transferred to 1 ml Eppendorf tubes. The analysis of MT protein in the liver was performed according to the test protocol of the MT ELISA kit (fish) (Bioassay Technology Laboratory, China Cat. No. E0005Fi).

2.5. Statistical analysis

Tissue heavy metal concentrations were expressed as mean \pm standard deviation ($X \pm SEM$). Fish tissue heavy metal levels were compared according to TGK, WHO, and FAO limits.

3. Results

3.1. Length and weight data of *Capoeta trutta* and *Capoeta umbla* in Murat River

The length and weight values of the study material fish in the Murat River basin of Muş region are given in Table 1.

Table 1. The mean length (cm) and weight (g) of the fish used in the study (n:10)

	<i>Capoeta trutta</i> X±SEM	<i>Capoeta umbla</i> X±SEM
Length (cm)	31.00±0.577	30.40±0.521
Weight (g)	377.00±16.869	355.00±20.303

X ± SEM Arithmetic mean ± standard error

3.2. Physical and chemical characteristics of Murat River water

The water colour was measured as cloudy, pH 8.32, electrical conductivity 483 siemens/cm, ammonium nitrogen 0.05 mg/L, nitrite 0.16 mg/L, nitrate 1 mg/L, dissolved oxygen 13.17 mg/L, chemical oxygen demand 32.16 ml, temperature 13.17°C, faecal coliform count 4.0×10^0 cfu/100ml according to Table 2 [YSI Pro Plus multimeter (pH, temperature, electrical conductivity, dissolved oxygen), HACH 2100 Q (turbidity), HACH LANGE DR 5000 spectrophotometer (nitrite, nitrate, ammonium), Sartorius membrane filter paper (fecal coliform bacteria count)].

Table 2. Murat River water physical and chemical properties

pH	8.32
Color	Cloudy
Electrical conductivity	483 siemens/cm
Suspended solids	49 mg/L
Ammonium nitrogen	0.05 mg/L
Nitrite	0.16 mg/L
Nitrate	1 mg/L
Chemical oxygen demand	32.16 mL
Fecal coliform bacteria count	4.0×10^0 cfu/100 mL
Dissolved oxygen	13.17 mg/L
Temperature	12.8 °C

3.3. National and international limit values for some metals in fish muscle tissue

Table 3 presents the accepted heavy metal levels in fish muscle tissue (FAO, 1983; WHO, 1989; TGK, 2011).

Table 3. Limit values accepted by national-international standards for some metals in fish muscle tissue (mg kg⁻¹ ww)

Heavy metals (mg/kg ⁻¹ ww)	TGK	FAO	WHO
Cd	0.05	0.05	0.5
Co	1.0	-	-
Cr	0.5	1.0	0.05
Mn	1.0	-	0.4
Ni	0.5	-	0.07
Pb	0.30	0.5	0.5
Zn	30	30	40
Fe	0.7	-	0.30

3.4. Heavy metals contents in Murat River waters

The heavy metal content of the river water collected from the fishing locations of the study material fish from the Murat River (Muş) is presented in Table 4.

Table 4. Heavy metals contents in the water of the Murat River (ICP-OES)

Heavy metals	X ± SEM (µg /L)
Cd	6 ± 0.001
Co	52 ± 0.024
Mn	23 ± 0.005
Ni	26 ± 0.009
Pb	18 ± 0.008
Zn	12 ± 0.001
Fe	18 ± 0.012

X ± SEM Arithmetic mean ± standard error

3.5. Tolerable concentrations of heavy metals in inland waters and seas

According to the TSE, acceptable concentrations of heavy metals in inland waters and seas are given in Table 5.

Table 5. Acceptable concentrations of heavy metals in inland waters and seas (TSE, 2005)

Heavy metals	Waters and seas tolerable level (µg/L)
Cd	0.01
Co	1.0
Cr	0.1
Mn	1.0
Ni	0.3
Pb	0.1
Zn	0.003
Fe	0.7

3.6. The heavy metal concentrations in the muscle, gill, kidney, and liver tissues of *Capoeta umbla* and *Capoeta trutta*

Table 6. shows that Cd was highest in the kidney tissue of *Capoeta umbla*. This was followed by liver, gill, and muscle tissues (kidney > liver > gill > muscle). Co was highest in kidney tissues, followed by muscle, liver, and gill tissues (kidney > muscle > liver > gill). Cr was highest in muscle tissue, followed by kidney, liver, and gill tissues (muscle > kidney > liver > gill). Mn was highest in gill tissue. This was followed by kidney, liver, and muscle tissues (gill > kidney > liver > muscle). Ni was mainly measured in muscle tissues, followed by kidney, gill, and liver tissues (muscle > kidney > gill > liver). The measurement of Pb was mainly in the liver. This was followed by kidney, muscle, and gill tissues (liver > kidney > muscle > gill > liver). Zn was highest in gill followed by kidney, liver, and muscle tissues (gill > kidney > liver > muscle). Fe was measured mainly in kidney tissue. This was followed by liver, muscle, and gill tissues (kidney > liver > muscle > gill). The heavy metal concentrations of Cd, Co, Cr, Mn, Ni, Pb, Zn, and Fe in the muscle, gill, kidney, and liver tissues of *Capoeta umbla* are listed in Table 6.

According to Table 7, the Cd concentration was measured at a high level in the kidney tissue of *Capoeta trutta*. This was followed by liver, muscle, and gill tissues (kidney > liver > gill > muscle). Co was highest in kidney tissues, followed by muscle, gill, and liver tissues (kidney > muscle > gill > liver). Cr was highest in kidney tissues, followed by muscle, liver, and gill tissues (kidney > muscle > liver > gill). Mn was highest in gill tissue, followed by kidney, liver, and muscle (gill > kidney > liver > muscle). Ni was measured mainly in renal tissue, followed by liver, muscle, and gill tissue (renal>liver>muscle>gill). Pb was most abundant in muscle tissues, followed by gill, kidney, and liver tissues (muscle > gill > kidney >

liver). Zn was mainly measured in kidney tissues, followed by gill, liver, and muscle tissues (kidney > gill > liver > muscle > gill > liver > muscle). Fe was mainly detected in renal tissue, followed by liver, gill, and muscle tissue (renal>liver>gill>muscle). The heavy metal levels measured for Cd, Co, Cr, Mn Ni, Pb, Zn, and Fe in muscle, gill, kidney, and liver tissues of *Capoeta trutta* species are presented in Table 7.

Table 6. Heavy metal concentrations of Cd, Co, Cr, Mn, Ni, Pb, Zn and Fe in muscle, gill, kidney and liver tissues of *Capoeta umbla*

Concentration of heavy metals in <i>Capoeta umbla</i> (mg/kg ^{w.w.})				
Heavy metals	Muscle	Gill	Kidney	Liver
Cd	0.011 ± 0.001	0.010 ± 0.001	0.662 ± 0.074	0.024 ± 0.003
Co	0.049 ± 0.009	0.090 ± 0.008	0.411 ± 0.017	0.037 ± 0.005
Cr	0.669 ± 0.040	0.487 ± 0.063	0.676 ± 0.088	0.465 ± 0.039
Mn	3.944 ± 0.302	10.887 ± 0.662	4.452 ± 0.410	3.156 ± 0.392
Ni	1.347 ± 0.060	0.894 ± 0.031	1.043 ± 0.061	0.847 ± 0.039
Pb	0.300 ± 0.051	0.739 ± 0.081	0.249 ± 0.035	0.581 ± 0.011
Zn	7.789 ± 0.352	29.545 ± 1.122	25.156 ± 1.749	16.215 ± 0.828
Fe	2.447 ± 0.756	2.211 ± 0.073	7.093 ± 0.339	3.148 ± 0.137

X ± SEM Arithmetic mean ± standard error, (n:10).

Table 7. Heavy metal concentrations of Cd, Co, Cr, Mn, Ni, Pb, Zn and Fe in muscle, gill, kidney, and liver tissues of *Capoeta trutta*

The concentration of heavy metal in <i>Capoeta trutta</i> (mg/kg ^{ww})				
Heavy metals	Muscle	Gill	Kidney	Liver
Cd	0.012 ± 0.004	0.019 ± 0.003	1.056 ± 0.136	0.077 ± 0.009
Co	0.069 ± 0.005	0.091 ± 0.007	0.165 ± 0.011	0.065 ± 0.010
Cr	0.655 ± 0.043	0.616 ± 0.058	1.424 ± 0.041	0.536 ± 0.043
Mn	4.549 ± 0.583	12.987 ± 0.628	11.205 ± 0.308	3.645 ± 0.356
Ni	1.046 ± 0.044	1.081 ± 0.057	2.220 ± 0.621	1.402 ± 0.026
Pb	0.142 ± 0.043	1.007 ± 0.231	0.402 ± 0.014	0.501 ± 0.023
Zn	8.257 ± 0.498	16.303 ± 0.862	23.641 ± 1.964	14.894 ± 0.765
Fe	2.406 ± 0.192	2.672 ± 0.049	9.529 ± 0.434	3.302 ± 0.196

X ± SEM Arithmetic mean ± standard error, (n:10).

3.7. The levels of metallothionein in liver tissues of *Capoeta trutta* and *Capoeta umbla*

In Table 8, the values of the MT measurements in the liver tissues of the species *Capoeta trutta* and *Capoeta umbla* are presented.

Table 8. Levels of metallothionein in liver tissues of *Capoeta trutta* and *Capoeta umbla*

Metallothionein levels in <i>Capoeta trutta</i> and <i>Capoeta umbla</i> liver tissue (µg/ml)	
Species	X ± SEM (Mean ± standard error)
<i>Capoeta trutta</i>	0.373 ± 0.006
<i>Capoeta umbla</i>	0.339 ± 0.019

X ± SEM Arithmetic mean ± standard error, (n:10).

4. Discussion

Water is an essential molecule for living organisms. It forms the environment in which biochemical reactions take place. Freshwater resources on Earth are very important for the health of life, agricultural activities, and the continuity of aquatic ecosystems (Noori et al., 2019). The rivers around which the densely populated areas are located are highly polluted by the residues of man-made domestic waste and harmful chemicals of industrial and agricultural origin. The various industrial accidents, especially in the regions where minerals such as gold, silver, Pb, Zn, Cu, Cd, and Mn are mined, have become one of the factors that seriously increase pollution in water resources (Atici et al., 2010; Cordeli et al., 2023). The Murat River, which has a length of 722 km, is home to various fish species (Gül et al., 2017). The species of *Capoeta umbla* and *Capoeta trutta* that are caught in this river are an important source of protein for the people who live in the region. The presence of residential areas along the course of the river is one of the main causes of human-induced water pollution (Özgür & Çalta, 2003). Heavy metals mixed into the water pose a risk to other aquatic organisms, especially fish. Fish take up heavy metals through the skin, gill respiration, and food (Heath, 2018). The accumulation of heavy metals in tissues leads to toxic effects (Canli & Atli, 2003; Akbulut & Tuncer, 2011; Lawson, 2011). The accumulation of heavy metals can vary from tissue to tissue and from organism to organism (Anan et al., 2005). They cannot be metabolized. The levels of heavy metals in fish consumed as food can cause serious health problems even if they are not in toxic concentrations (Tanır, 2021). The average length and weight of the fish used in the study are given in Table 1, the physical and chemical parameters of the Murat River water are given in Table 2, and the heavy metal concentrations are given in Table 5. Heavy metals cadmium (Cd), cobalt (Co), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn), and iron (Fe) in muscle, gill, kidney, and liver tissues of *Capoeta umbla* and *Capoeta trutta* belonging to the family Cyprinidae are given in Table 6 and Table 7. The MT levels in fish liver tissues are given in Table 8. The physical and chemical characteristics of the water of the Murat River were measured in December; water temperature was 12.8 °C, dissolved oxygen content 13.17 mg/L, and pH value 8.32 (Table 2). The pH value of Murat River water was found to be compatible with the acceptable pH values of TSE (6.5-9.2), WHO (6.5-8.8), Environmental Protection Agency (EPA 6.5-8.5), European Commission (EC 6.5-9.5) in freshwater ecosystems (Gümüş & Akköz, 2020). Temperature, pH, and dissolved oxygen are vital factors for aquatic ecosystems. Any change in these factors can negatively affect the life of organisms in aquatic ecosystems and disturb the balance of ecosystems (Güneş et al., 2019). The electrical conductivity of the Murat River water was measured to be 480 µS/cm. This value was found to be within the range of 150-500 µS/cm given in the water pollution and control protocol for aquaculture (Uslu & Türkman, 1987). Crop residues, agricultural fertilisers, domestic and industrial waste account for much suspended sediment in water (Mert et al., 2008). Suspended solids in Murat River were measured to be 49 mg/L. The level of suspended solids was found to be much higher than the 5 mg/L level accepted by TSE, and WHO (WHO, 1993; TSE, 2005). The sources of ammonia, nitrite and nitrate in the waters are from fertilisers used for agricultural purposes, mixing and decomposition of organic materials in water, domestic waste, industrial waste, etc. (Gümüş & Akköz, 2020). Ammonium nitrogen (NH₄) 0.05 mg/L, nitrite nitrogen (NO₂) 0.05 mg/L, nitrate nitrogen (NO₃) 1 mg/L were measured in Murat River. These values are lower than the criteria set by the TSE, the WHO and the EC (WHO, 1993; EC, 1998; TSE, 2005). In this study, Cd 6 µg/L, Co 52 µg/L, Ni 26 µg/L, Pb 18 µg/L were measured in the waters of Murat River. These values were found to be very high compared to I. quality waters (Cd 3 µg/L, Co 10 µg/L, Ni 20 µg/L, Pb 10 µg/L). The levels of Cd and Co were also higher than those of quality II waters (Cd 5 µg/L, Co 20 µg/L). The values of Mn 23 µg/L, Zn 12 µg/L and Fe 18 µg/L in Murat River water were higher than the values of surface water quality assessment (for Mn I. 100 µg/L, II. 500 µg/L, III. 3000 µg/L, IV. > 3000 µg/L, for Zn I. 200 µg/L, II. 500 µg/L, III. 2000 µg/L, IV. >2000 µg/L, for Fe I. 300 µg/L, II. 1000 µg/L, III. 5000 µg/L, IV. > 5000 µg/L) (Varol & Tokatlı, 2023).

The concentrations of Cd in muscle, liver, gill, and kidney tissues of *Capoeta umbla* were observed in the order kidney > liver > muscle > gill (Table 6). These values showed that the Cd levels measured in the muscle tissue of *Capoeta umbla* were below the WHO, FAO, and TGK limits (FAO, 1983; FAO/WHO, 1989; TGK, 2011). In another study measuring heavy metal levels in the muscle tissue of fish living in water resources close to organized industrial zones, Cd accumulation was found to exceed WHO, FAO, and TGK limits (FAO, 1983; FAO/WHO, 1989; TGK, 2011). The

bioaccumulation of Cd in *Capoeta trutta* was measured in the following order: kidney > liver > gill > muscle (Table 7). Cd levels measured in edible muscle tissues of *Capoeta trutta* were found to be low compared to WHO, FAO, and TGK (FAO, 1983; FAO/WHO, 1989; TGK, 2011). The difference in tissue accumulation of Cd levels (kidney > liver > gill > muscle) has been reported to be due to the long-term effect of heavy metals at low concentrations in metabolically active tissues (Hogstrand & Haux, 1990). Cd concentrations in the muscle, liver, and gills of *Capoeta trutta* caught from Erzincan Karasu River were reported to be in the order liver > gill > muscle (Tanır, 2021). The inhibitory effects of some metal ions on gill and liver enzymes in the liver and gill of *Capoeta trutta* caught from Murat River were determined in an in vitro cell culture study (Kırıcı et al., 2013). It has been reported that industrial, agricultural, and other anthropogenic wastes are discharged into these sources in these regions (Dindaş & Emre, 2020). The accumulation of metals in the kidneys, liver, and gills of fish exposed to low concentrations of Cd has been detected in several fish species (Karaytuğ et al., 2007; Yeşilbudak & Erdem, 2014; Karayakar et al., 2022). They cannot be metabolized in the cell, so in metabolically active tissues such as the kidney, liver, gills, and spleen, they bind to proteins such as metallothioneins. The accumulation of this heavy metal in fish, even at low levels, is detrimental to their development. It has been reported that accumulation of the heavy metal Cd in the body causes damage to the nervous system, liver, kidney, and brain tissues of fish, blockage of capillaries, melting of gill lamellae, and reproductive problems (George, 1991).

The cobalt concentration in *Capoeta umbla* tissues was measured in the following order; kidney > gill > muscle > liver. In *Capoeta trutta*, the tissue concentration was measured in the order kidney > gill > muscle > liver (Table 6 and Table 7). In both fish species, Co levels in muscle tissues were measured below the Co limit (1.0 mg/kg) of the TGK, although the tissue order was parallel (TGK, 2011). While there was no significant difference in muscle Co levels in some fish in the Euphrates River, higher levels were detected in carp caught in the area where wastewater from a leather factory was discharged (Erdoğan & Erbilir, 2007). In our study, the parallels in tissue levels of Co heavy metal in both fish species may be related to exposure time, primary exposure status, histological structure, and metabolic functions of the tissues.

The tissue Cr concentrations measured in *Capoeta umbla* and *Capoeta trutta* were parallel in both fishes and were classified as kidney > muscle > gill > liver (Table 6 and Table 7). It was observed that the Cr concentration in the muscle tissue of *Capoeta trutta* exceeded the limits of WHO and FAO and that the Cr concentration in *Capoeta umbla* exceeded the acceptable limits of TGK and WHO (FAO, 1983; FAO/WHO, 1989; TGK, 2011). It has been reported that heavy metals (Cd, Cr, Pb, Cu, and As) in muscle tissues of freshwater fishes living in the Tigris River within the borders of Baghdad were above the FAO and WHO limits that would pose a risk to human health (Kacar et al., 2017). In the present study, high Cr metal accumulation in *Capoeta umbla* and *Capoeta trutta* muscle tissues may pose a risk to human health depending on the consumption levels of these fish. The parallelism of Cr levels in the tissues of both fish species may be related to the fact that both species originate from the same water body and share the same habitat and exposure processes. In a similar study, it was reported that the order of heavy metal levels in *Capoeta tinca* and *Squalius pursakensis* tissues in the gill, liver, and muscle of both fish species originated from the same water body and habitat (Varol et al., 2020). In a study conducted at Atatürk Dam, it was reported that the heavy metal levels of Fe, Pb, Cu, Cr, Zn, and Cd in gill, liver, and muscle tissues of some fish species in stations with different pollution levels in the dam were higher than those in fish tissues from areas with higher pollution levels (Firat et al., 2018). Another study reported that Cr causes ulceration even at low doses, and long-term exposure at high doses causes kidney and liver damage, damage to the nervous and circulatory systems, and carcinogenic effects (Kacar et al., 2017).

The order of Mn bioaccumulation in the tissues of *Capoeta umbla* and *Capoeta trutta* is consistent for both fish species, with the levels found in the following order: gill > kidney > muscle > liver (Tables 6 and 7). The Mn concentrations measured in the analysed tissues of both fish species were above the tolerable limits set by the TGK and WHO (WHO, 1989; TGK, 2011). In a study on seasonal heavy metal levels (including Cr, Cd, Ni, Co, Cu, Mn, Zn) in the muscle tissue of *Capoeta umbla* from the Murat River in Bingöl, Mn was found to be highest in March (Kırıcı et al., 2013). Another study reported elevated metal levels in muscle, liver and gill tissues of *Capoeta umbla* from the Karasu River, indicating that these levels exceeded the nationally and internationally accepted limits (Sökmen et al., 2019). In our study, the Mn levels detected in the tissues of both fish species may be influenced by the

physicochemical properties of their habitat. Manganese plays a crucial role in human physiology, serving as a co-factor in the structure of several enzymes. Deficiencies in Mn can lead to skeletal and reproductive abnormalities, as well as diseases affecting the nervous and respiratory systems, such as Parkinson's disease, schizophrenia, and bronchitis. However, while Mn toxicity is rare in humans, high concentrations can pose health risks (Cucu et al., 2019).

The tissues of *Capoeta umbla* demonstrate a pattern of Ni accumulation as follows: muscle > kidney > gill > liver. In contrast, *Capoeta trutta* exhibits a different accumulation order: kidney > liver > gill > muscle (Tables 6 and 7). In this study, the Ni levels found in muscle tissue were higher than the tolerable limits established by the WHO and the TGK (WHO, 1989; TGK, 2011). The bioaccumulation of heavy metals can vary between fish species and their respective tissues, depending on the habitats in which they reside. For instance, a previous study reported tissue Ni concentrations in *Capoeta umbla* in the order of gills > muscle > liver (Sökmen et al., 2018). The results suggest that there may be differences in the sequence of nickel (Ni) accumulation compared to the current study, possibly influenced by metabolic activities within the tissues. Additionally, environmental heavy metal contamination levels, along with different ecological needs, metabolic rates, and feeding habits, can lead to varied accumulation across tissues (Allen-Gil & Martynov, 1995; Shovon & Majumdar, 2017). Another study found that heavy metal accumulation was highest in the liver, followed by gills, kidney, and muscle tissues, with muscle tissue showing the lowest level of metal binding (Mol et al., 2010; Sökmen et al., 2018). In this study, it is significant for food safety that the nickel content in muscle tissue from both fish species exceeded the acceptable limits set by the WHO and TGK (WHO, 1989; TGK, 2011).

Heavy metal accumulation of lead (Pb) in *Capoeta umbla* was found to follow the order: gill > liver > muscle > kidney (Table 6). In contrast, lead accumulation in *Capoeta trutta* was observed in the order: gill > liver > kidney > muscle (Table 7). The accumulation of heavy metals in these tissues is influenced by metabolic activity and the level of exposure. Research has shown that prolonged and high exposure to lead can cause damage to various systems in the body and have carcinogenic effects (Fujita et al., 2002). In a study that measured heavy metal levels in different tissues of *Capoeta trutta* and *Capoeta umbla* in the Karasu River (Erzincan), lead accumulation was reported in the order of gill > liver > muscle (Tanır, 2021). Additionally, heavy metals were found to cause cytotoxic effects and bioaccumulation in certain tissues of *Tilapia zillii*, which inhabit waters with varying levels of pollution. This exposure led to various chromosomal aberrations, adhesions in gill cells, and nuclear abnormalities in erythrocytes (Abass, 2024). In our study, the Pb levels in the muscle of *Capoeta trutta* were found to be similar to those in *Capoeta umbla*, but slightly lower when compared to the acceptable levels established by TGK (TGK, 2011).

In *Capoeta umbla*, zinc (Zn) tissue accumulation was observed in the following order: kidney > liver > gill > muscle (Table 6). For *Capoeta trutta*, the order was kidney > gill > liver > muscle (Table 7). In this study, muscle Zn levels in both fish species were found to be below the limits set by the TGK (TGK, 2011). A study analyzing metal accumulation in different tissues of *Capoeta trutta* and *Capoeta umbla* reported that zinc accumulation occurred in the order of gill > liver > muscle (Tanır, 2021). These results align with our findings. Notably, the highest Zn accumulation was found in the liver, while the lowest was in the muscle. Heavy metal analyses of *Clarias gariepinus*, *Channa punctatus*, and *Labeo rohita* also indicated that the highest Zn accumulation occurs in the liver, followed by the kidney, gill, and muscle (Coetzee et al., 2002; Murugan et al., 2008; Javed & Usmani, 2011 and 2013). In this study, the pattern of Zn accumulation in the metabolically active tissues liver, kidney, and gill was consistent with other studies that assessed heavy metal levels.

In this study, iron (Fe) levels were measured in different tissues of the fish species *Capoeta umbla* and *Capoeta trutta*. The findings indicated that Fe accumulation in *Capoeta umbla* followed the order of kidney > liver > muscle > gill, while in *Capoeta trutta*, the order was kidney > liver > gill > muscle (Table 6 and Table 7). Notably, the Fe levels in the muscle tissue of both species exceeded the limits set by the WHO and the TGK (WHO, 1989; TGK, 2011). A separate study measuring heavy metal levels in the muscle tissue of *Capoeta trutta* from Karakaya Dam Lake reported that the concentrations of Fe and zinc (Zn) were higher in fish muscle compared to the surrounding water. Additionally, the study found that heavy metal levels varied based on the fish's weight, length, and sex (Eroğlu & Düşükcan, 2016). In our research, Fe bioaccumulation was highest in the liver and lowest in muscle for *Capoeta trutta*, whereas for *Capoeta umbla*, it was again higher in the liver but lower in the gills. Previous studies investigating heavy metal levels in various fish species, such as *Clarias gariepinus*,

Tinca tinca, and *Labeo rohita*, have similarly shown that Fe concentrations were higher in liver tissue and lower in muscle tissue (Tekin-Özan & Kir, 2005; Osman & Kloas, 2010; Javed & Usmani, 2011). The high concentrations of Fe measured in the liver tissue of both *Capoeta trutta* and *Capoeta umbla* may be attributed to the presence of iron-containing enzymes and the structural and functional characteristics of the liver.

The content of metallothionein was measured to be 0.373 ± 0.006 µg/ml in the liver tissue of *Capoeta trutta*, while it was 0.339 ± 0.019 µg/ml in the liver of *Capoeta umbla* (Table 8). Metallothionein (MT) is commonly used as an early warning indicator in the biomonitoring of metal exposure in aquatic organisms. However, significant variations in MT concentrations have been reported across different studies (Mijošek et al., 2019). The levels of MT can vary based on the type of cell exposed, the properties of the metal, and the concentration of the metal involved in detoxification processes (Kägi, 1991; Vallee, 1995). The determination of MT protein levels in the liver is linked to the metabolic activity of this organ (Van et al., 2003). Metals such as copper, manganese, iron, and zinc, which are integral to many metalloproteins, play crucial roles in various processes within the body's defense system, as they act as cofactors in enzymes (Çiftçi et al., 2021). In living organisms, cells produce metallothionein proteins to protect themselves from heavy metal exposure by binding to these metals and facilitating their removal from the body (Bayhan et al., 2016). In a study measuring liver metallothionein levels in sea bass and grey mullet, MT levels were found to be 109.44 mg/kg in sea bass liver and 115.62 mg/kg in grey mullet liver. However, these values were reported to be below acceptable limits (Küçük, 2018). In this study, the MT levels measured in the liver tissues of *Capoeta trutta* and *Capoeta umbla* were lower in comparison to similar studies.

5. Conclusion

It was observed that the accumulation of heavy metals (Cd, Co, Cr, Mn, Ni, Pb, Zn, Fe) in muscle, gill, kidney, liver and muscle tissues of *Capoeta umbla* and *Capoeta trutta*, which belong to the family Cyprinidae and are consumed by the public and have economic value within the borders of Muş region, varied from tissue to tissue and according to the type of heavy metal. The levels of Cr, Mn, Ni, and Fe in the muscle tissues of *Capoeta umbla* and *Capoeta trutta* were found to be above the TKG, FAO, and WHO limits. In this study, heavy metal concentrations were found in the order liver-kidney-gill. The liver, kidney, and gill are metabolically active tissues. In these tissues, MT proteins reduce the destructive effects of heavy metals by binding to metals through their detoxifying functions. MT levels were measured to be higher in *Capoeta trutta* liver tissue compared to *Capoeta umbla* liver MT levels. In this study, it was observed that heavy metal accumulation in fish species varies from tissue to tissue and according to the type of metal. Although they belong to the same family and share the same habitat, the heavy metal levels in the tissues of the two fish species measured in this study were different. This may be due to differences in feeding habits or growth and developmental characteristics of the fish.

Author contributions

All authors contributed to the conception and design of the study. The first draft of the manuscript was written by Necati Özok, and all of Hülya Yıldırım commented on earlier versions of the manuscript. All authors read and approved the final version of the manuscript. Fish and water sample collection, sample preparation for ICP-OES, tissue metallothionein preparation and analysis were performed by Hülya Yıldırım.

Disclosure statement

No potential conflicts of interest were declared by the author(s).

Ethical Statement:

Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee's decision dated 28.11.2019 and numbered 2019/11

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