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Determination of heavy metals in some tissues of four fish species from the Karasu River (Erzincan, Turkey) for public consumption

by

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Abstract

The study aimed at determining the concentration of heavy metals in muscle, liver, and gill tissues of four fish species (*Acanthobrama marmid*, *Capoeta umbla*, *Capoeta trutta* and *Chondrostoma regium*) collected from five sites in the Karasu River, Erzincan, between July 2019 and January 2020. The relationships between fish size (length and weight) and metal concentrations in the tissues were also investigated using Pearson correlation analysis. Concentrations of Al, Fe, Cu, Mn and Zn were higher than those of other metals in all tissue samples from four fish species. Fe and Al concentrations were very high, while the lowest Co, Cd and Pb concentrations were determined in the muscle, liver and gill tissues. The results of Pearson correlation analysis showed that significant relationships between heavy metal concentrations and fish size (length and weight) were positive ($p < 0.01$, $p < 0.05$), except for a few cases. Furthermore, heavy metal concentrations in the edible parts (muscle) of the studied fish species did not exceed the maximum acceptable concentrations (MACs) proposed by national and international food standards and were safe within human consumption limits, except for Cr.

Key words: heavy metals, cyprinid fish, fish size, accumulation

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1. Introduction

From the perspective of all living beings in the ecosystem, environmental pollution is one of the major hazards. Of all pollutants, heavy metals are of particular concern due to their ability to accumulate in environmental elements, such as the food chain, and their non-biodegradable durability, thereby posing a significant threat to human health (Censi et al. 2006; Görür et al. 2012). Heavy metals cause inorganic contamination in the aquatic environment, thus they threaten the metabolism of organisms living there.

Of the aquatic organisms, fish in particular are an important food source for human health due to their rich content of protein, omega-3 fatty acids, vitamins and minerals. Furthermore, fish are extensively used to assess the quality of aquatic environments and as bioindicators of heavy metal pollution (Indrajith et al. 2008; Danabas et al. 2020). They are also among the species that are at the top of the aquatic food chain. Metals present in the environment are accumulated in various fish tissues and organs. Gill, liver and muscle tissues show the highest accumulation of heavy metals, respectively. Gills, a respiratory organ, can be directly affected by contaminants in water. The main organ responsible for the transformation, storage and detoxification of toxic substances is the liver. Muscle is not a target organ for bioaccumulation, but is commonly analyzed to determine concentrations of contaminants and to assess health risks as it is the main part consumed by humans (Dalzochio et al. 2017).

Fish size is an important factor in the accumulation of heavy metals, which tend to accumulate more in small fish. Heavy metal contamination varies as a function of different locations. Moreover, metal accumulation in different fish tissues can be affected by ecological requirements, sex, age, seasonal changes, reproductive cycle, swimming patterns, and feeding behavior (El-Moselhy et al. 2014; Kalkan et al. 2015).

The Karasu River is exposed to serious pollution as a result of agricultural and industrial wastewater and household runoff. For this reason, fish mortality observed in recent years is easily explainable (Sonmez et al. 2012). Tigris scraper (*Capoeta umbla* Heckel, 1843) and trout barb (*Capoeta trutta* Heckel, 1843) are economically important, while Tigris bream (*Acanthobrama marmid* Heckel, 1843) and Mesopotamian nase (*Chondrostoma regium* Heckel, 1843) are of no economic importance among fish species belonging to the Cyprinidae family in this study area. They are locally used for human nutrition. Pollution in the Karasu River is therefore a major problem for the health of aquatic animals and humans.

The objectives of this study were (i) to assess the levels of aluminum, chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead in muscle, liver and gill tissues of four fish species from the Karasu River and to assess whether the edible parts (fish muscle) are safe for human consumption; (ii) to evaluate the relationships between fish length and weight and heavy metal concentrations in sampled tissues. Furthermore, heavy metal concentrations in fish were compared with the Maximum Acceptable Concentrations (MACs) proposed by national and international food standards for human consumption.

2. Materials and methods

2.1. Site description

As one of the main tributaries of the Euphrates River, the Karasu River flows from the Dumlu Mountains in the Erzurum Plain and is located in the eastern part of Turkey. It flows through the district of Aşkale and passes through the town of Mercan in the Karasu Valley in the province of Erzincan. It forms the Euphrates River joining the Murat River near the town of Keban. Its length to the Keban Dam Lake is 460 km (Saler et al. 2015). The Karasu River, with a catchment area of 2886 km^2, flows at an elevation of 1675 m above sea level (Fig. 1).

2.2. Fish collection and analysis

Fish samples were collected from five sites located in the Karasu River. The sites were selected due to contaminants discharged into the water between July 2019 and January 2020. Four fish species (*A. marmid*, *C. umbla*, *C. trutta*, *C. regium*) were used

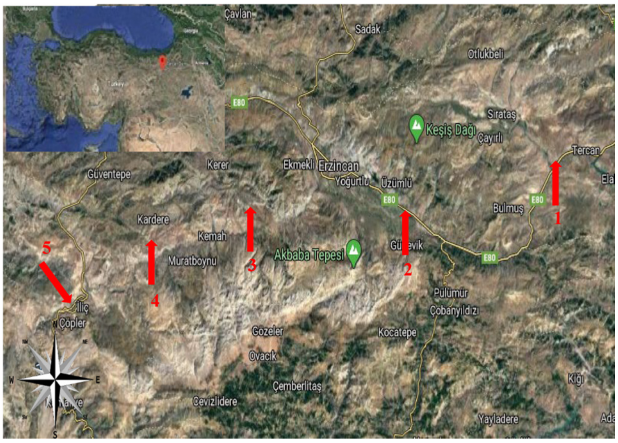

Figure 1

Location of the Karasu River and the sampling sites in the experiment. The concentrations of heavy metals were measured in the muscles, liver, and gills of fish caught by a gillnet. Gillnets with mesh sizes of 18, 28, 36 and 44 mm were used to obtain fish samples of different sizes. The collected fish were then quickly transported to the laboratory using a portable refrigerator. The total length (TL) of each fish species was measured to the nearest 0.1 cm. Individual weights (W) were determined using a digital balance with an accuracy of 0.01 g, and fish were stored at –80°C to minimize contamination. From each fish tissue (muscle, liver and gill) samples, 3–5 g samples were collected for the determination of heavy metals. Tissue samples were then dried for 24 h at 105°C in an oven; 0.5 g of each sample was placed into Teflon microwave tubes with 9 ml of concentrated nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2). Samples were mineralized at 210°C and 700 W for 20 min and at 210°C and 700 W for 15 min (Sökmen et al. 2018). The solution in a tube was then diluted to 15 ml with ultrapure water. Standard solutions for calibration graphs were prepared. Blanks were also prepared using the procedure as above, but without samples. Diluted samples and blank solutions were analyzed in triplicates using ICP-MS (Agilent 7700X) to determine aluminum, chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead levels (APHA 1985). The concentrations of metals were determined from the calibration graphs and calculated in mg kg^{-1} dry weight.

2.3. Statistical analysis

SPSS software (SPSS 17.0 for Windows) was used to perform t-test and one-way ANOVA statistical analysis, and to create graphs of data obtained during the research. Pearson correlation analysis was perform to examine the relationship between heavy metal concentrations and fish size (length and weight).

3. Results

3.1. Differences in the ability to accumulate metals in fish tissues

The content of heavy metals in fish varied according to their tissue. Different tissues showed different capacity to accumulate heavy metals. Aluminum, chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead levels were determined in all analyzed samples. Concentrations of Al, Fe, Cu, Mn and Zn were higher compared to other metals in all tissue samples from four fish species. Fe and Al concentrations were, in particular, very high in muscle, liver and gills of the studied species.

Table 1 shows size ranges and relationships between weight (W) and total length (TL) of the sampled fish caught in the Karasu River. Thirty five individuals of *A. marmid* with a length of 11–20.9 cm and a weight of 20.0–82.0 g, 63 individuals of *C. umbla* with a length of 14.4–42.1 cm and a weight of 27.0–539.0 g, 28 individuals of *C. trutta* with a length of 11–34.2 cm and a weight of 12.0–344.0 g and 49 individuals of *C. regium* with a length of 11–24.2 cm and a weight of 10.0–99.0 g were used in the study.

Table 1

Size ranges and relationships between weight (W) and total length (TL) of the sampled fish caught in the Karasu River

Fish	n	TL ranges	W ranges	[a]Equation	*R* value
A.marmid	35	11–20.9	20.0–82.0	$y = 0.0312\ x^{2.5535}$	0.9115
C. umbla	63	14.4–42.1	27.0–539.0	$y = 0.0272\ x^{2.6627}$	0.9476
C. trutta	28	11–34.2	12.0–344.0	$y = 0.0076\ x^{3.0490}$	0.9764
C. regium	49	11–24.2	10.0–99.0	$y = 0.0439\ x^{2.4025}$	0.8893

[a]y is the total fish weight (g) and x is the total fish length (cm)

Box plots (median, first and third percentiles, minimum and maximum values) for aluminum, chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and lead levels in the muscle, liver, and gills of *A. marmid*, *C. umbla*, *C. trutta,* and *C. regium* are presented in Fig. 2, Fig. 3 and Fig. 4, respectively, based on dry weight (mg kg^{-1}). The content of Al, Cr, Mn, and Ni in tissues of *A. marmid* follows the following order: gill > muscle > liver; Fe, Co, and Pb – gill > liver > muscle; Cu, Zn, and Cd – liver > gill > muscle, respectively. The content of Cr, Mn, Ni, Zn, and Pb in *C. umbla* tissues follows the following order: gill > liver > muscle; Al, Cu, and Cd – liver > muscle > gill; Fe and Co – liver > gill > muscle, respectively. The content of Cr, Fe, Cu, Zn, Cd, and Pb in tissues of *C. trutta* follows the following order: liver > gill > muscle; Al, Mn, Co, and Ni follow the order: gill > liver > muscle, respectively. The content of Al, Mn, Co, Zn, and Pb in *C. regium* tissues follows the following order: gill > liver > muscle; Cr, Fe, Ni and Cu – liver > gill > muscle; Zn – gill > liver > muscle, respectively.

Concentrations of heavy metals (mean, standard deviation, minimum and maximum values) in different tissues of four fish species from the Karasu River (mg kg^{-1}) are presented in Table 2. In *A. marmid* muscle tissues, the highest concentration was determined for

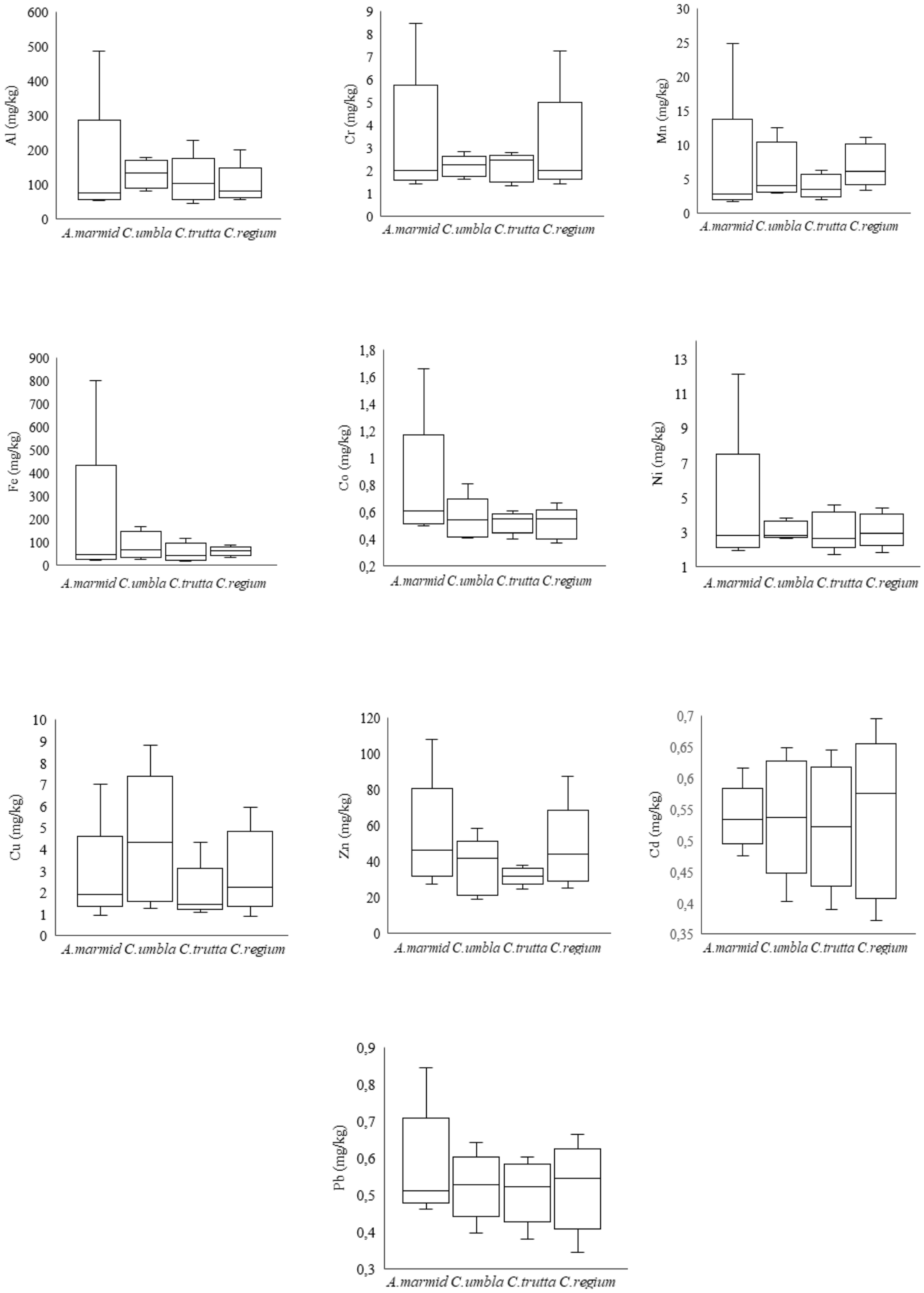

Figure 2

Comparison of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb concentrations in muscle of four freshwater fish species from the Karasu River, Erzincan

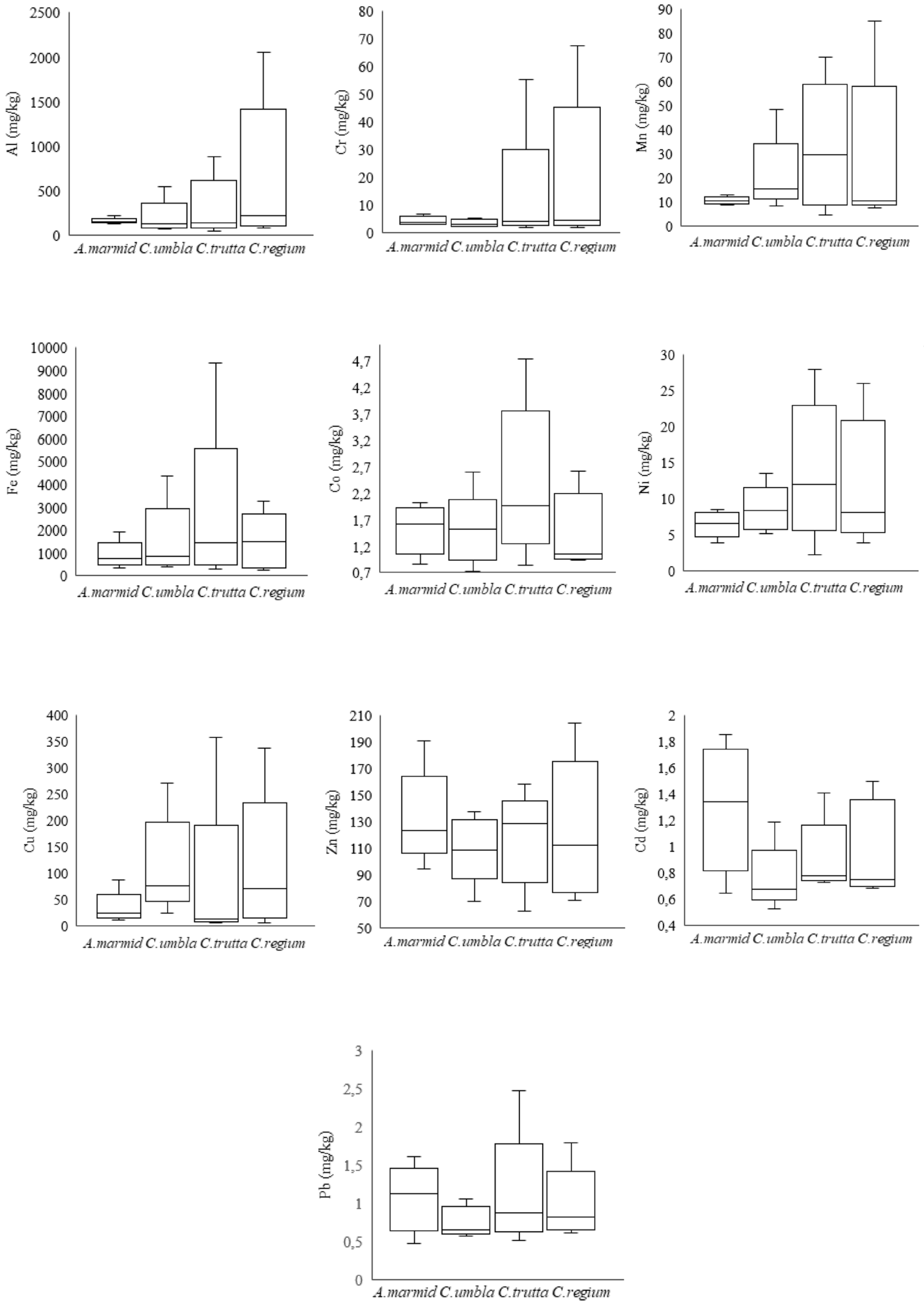

Figure 3

Comparison of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb concentrations in liver of four freshwater fish species from the Karasu River, Erzincan

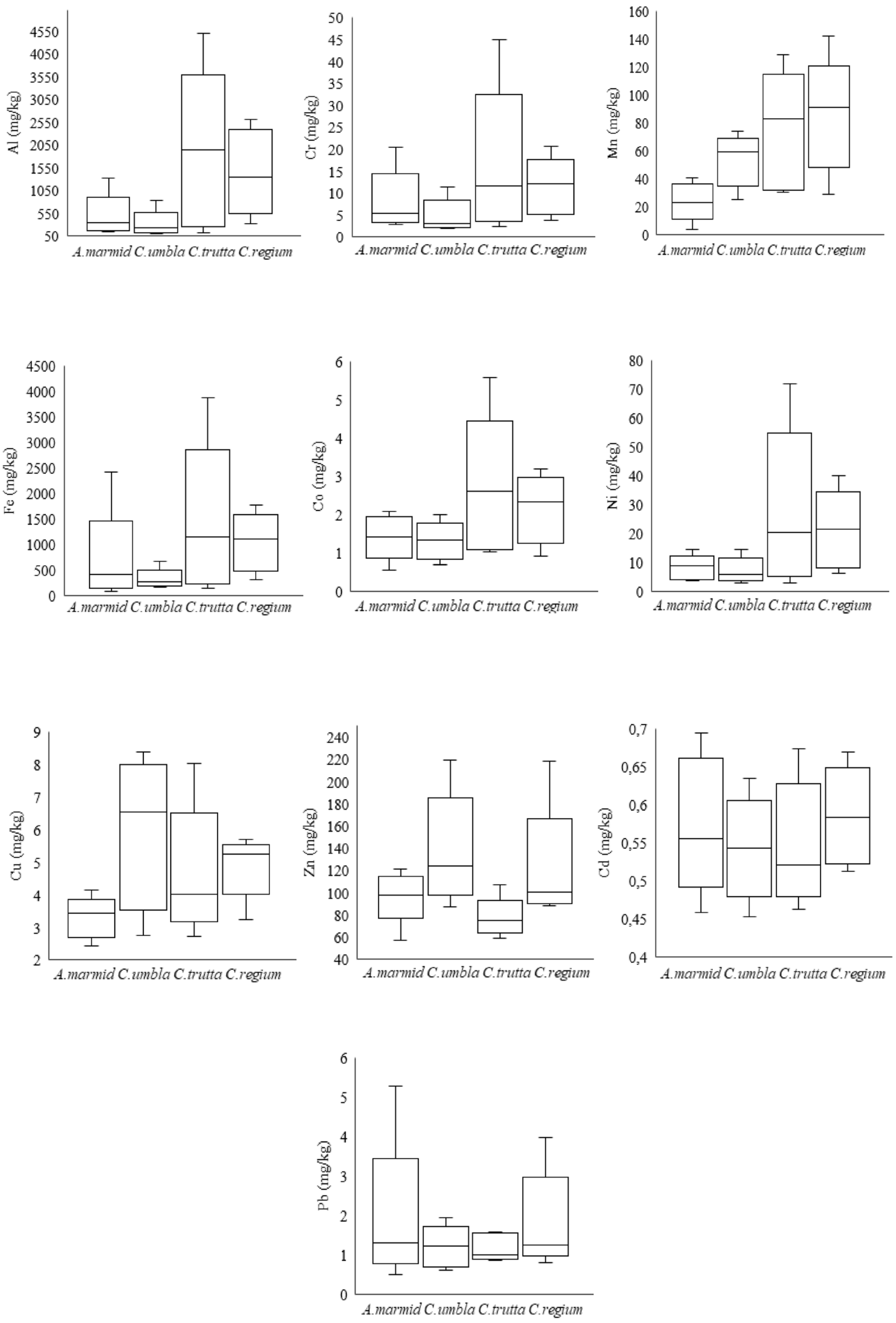

Figure 4

Comparison of Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb concentrations in gills of four freshwater fish species from the Karasu River, Erzincan

Table 2

Concentrations of heavy metals (mean, standart deviation, minimum and maximum values; mg kg^{-1}) in different tissues of four fish species from the Karasu River

Heavy Metals	Tissue	*A. marmid*	*C. umbla*	*C. trutta*	*C. regium*
Al	Muscle	129.99 ± 1.12 (52.65–487.52)	130.63 ± 1.35 (80.33–177.15)	108.58 ± 1.35 (44.05–227.77)	95.12 ± 1.34 (56.49–201.15)
	Liver	156.77 ±1.98 (132.26–218.16)	184.12 ± 1.52 (73.59–542.38)	285.92 ± 1.15 (45.34–881.38)	597.37 ± 1.17 (79.79–2051.97)
	Gill	447.52 ± 1.36 (143.50–1324.99)	284.98 ± 1.00 (102.14–834.00)	1809.94 ± 1.05 (117.61–4495.28)	1450.17 ± 1.06 (322.96–2601.41)
Cr	Muscle	3.08 ± 1.64 (1.43–8.47)	2.18 ± 1.22 (1.63–2.85)	2.15 ± 1.14 (1.36–2.79)	2.84 ± 1.01 (1.41–7.25)
	Liver	4.41 ± 1.19 (3.09–6.83)	3.47 ± 1.49 (2.17–5.24)	11.04 ± 0.79 (1.74–55.28)	18.13 ± 1.27 (2.04–67.34)
	Gill	7.49 ± 1.05 (2.80–20.44)	4.45 ± 0.99 (2.01–11.43)	15.49 ± 0.99 (2.27–44.86)	11.15 ± 0.73 (3.74–20.76)
Mn	Muscle	5.65 ± 1.30 (1.74–24.83)	6.13 ± 1.22 (2.88–12.58)	3.88 ± 0.71 (1.98–6.30)	6.98 ± 0.82 (3.43–11.18)
	Liver	10.65 ± 1.24 (8.68–13.01)	20.11 ± 1.02 (8.54–48.48)	32.21 ± 0.97 (4.49–70.01)	26.34 ± 1.01 (7.67–85.28)
	Gill	24.22 ± 0.94 (3.68–40.86)	53.64 ± 0.98 (25.63–74.13)	73.17 ± 1.41 (30.66–129.29)	85.21 ± 1.22 (29.13–142.59)
Fe	Muscle	150.92 ± 1.38 (21.12–800.40)	86.05 ± 0.93 (25.58–168.14)	55.96 ± 0.94 (16.88–117.90)	62.02 ± 0.84 (34.44–89.42)
	Liver	887.05 ± 1.12 (330.71–1890.51)	1395.39 ± 0.72 (364.09–4376.27)	2299.52 ± 0.85 (265.97–9314.15)	1445.84 ± 0.82 (235.29–3232.52)
	Gill	620.34 ± 089 (93.23–2413.61)	315.25 ± 1.11 (165.85–681.08)	1360.95 ± 0.83 (152.01–3872.51)	1045.53 ± 0.97 (315.18–1778.37)
Co	Muscle	0.74 ±1.07 (0.49–1.66)	0.54 ± 0.50 (0.41–0.81)	0.53 ± 0.85 (0.40–0.61)	0.51 ± 0.93 (0.37–0.67)
	Liver	1.52 ± 0.99 (0.86–2.02)	1.47 ± 0.85 (0.73–2.58)	2.34 ± 0.89 (0.84–4.74)	1.44 ± 0.85 (0.94–2.61)
	Gill	1.44 ± 0.77 (0.55–2.10)	1.30 ± 1.07 (0.71–1.99)	2.59 ± 0.95 (1.02–5.59)	2.17 ± 1.14 (0.92–3.19)
Ni	Muscle	3.89 ± 1.60 (1.93–12.09)	3.10 ± 1.49 (2.66–3.79)	3.06 ± 1.61 (1.69–4.56)	3.14 ± 1.41 (1.85–4.38)
	Liver	6.53 ± 1.59 (3.96–8.45)	8.40 ± 1.19 (5.15–13.47)	13.68 ± 1.42 (2.21–27.97)	11.77 ± 1.47 (3.85–26.02)
	Gill	8.10 ± 1.14 (3.95–14.63)	7.06 ± 1.31 (3.17–14.50)	26.72 ± 0.87 (3.00–72.04)	20.71 ± 0.83 (6.21–40.15)
Cu	Muscle	2.54 ± 1.27 (0.91–6.99)	4.30 ± 0.80 (1.26–8.81)	1.90 ± 1.05 (1.06–4.32)	2.87 ± 1.38 (0.90–5.93)
	Liver	31.49 ± 0.93 (12.40–86.87)	108.35 ± 0.67 (23.73–271.28)	63.74 ± 0.87 (6.18–358.00)	103.28 ± 1.04 (5.95–338.15)
	Gill	3.29 ± 1.15 (2.42–4.13)	5.93 ± 0.94 (2.74–8.39)	4.57 ± 0.79 (2.72–8.04)	4.93 ± 1.01 (3.23–5.70)
Zn	Muscle	51.48 ± 0.71 (27.25–107.99)	36.39 ± 0.66 (18.85–58.36)	32.01 ± 0.79 (24.48–37.86)	45.77 ± 0.39 (24.97–87.29)
	Liver	131.43 ± 0.86 (94.35–190.74)	111.10 ± 1.58 (70.04–137.79)	118.28 ± 1.02 (62.41–158.26)	120.96 ± 0.94 (70.67–204.07)
	Gill	98.27 ± 0.77 (57.28–120.97)	135.66 ± 0.79 (86.82–219.12)	76.12 ± 0.83 (59.02–107.28)	117.16 ± 0.73 (88.43–218.23)
Cd	Muscle	0.54 ± 0.39 (0.47–0.62)	0.54 ±0.22 (0.40–0.65)	0.52 ± 0.31 (0.39–0.64)	0.54 ± 0.23 (0.37–0.70)
	Liver	1.29 ± 0.74 (0.64–1.85)	0.75 ± 0.79 (0.53–1.19)	0.90 ± 0.72 (0.73–1.41)	0.97 ± 0.75 (0.68–1.50)
	Gill	0.57 ± 0.30 (0.46–0.69)	0.54 ± 0.30 (0.45–0.64)	0.55 ± 0.38 (0.46–0.67)	0.58 ± 0.52 (0.51–0.67)
Pb	Muscle	0.57 ± 0.35 (0.46–0.84)	0.53 ± 0.32 (0.40–0.64)	0.51 ± 0.45 (0.38–0.60)	0.52 ± 0.44 (0.35–0.66)
	Liver	1.06 ± 0.41 (0.47–1.62)	0.75 ± 0.60 (0.57–1.05)	1.08 ± 0.70 (0.51–2.47)	0.96 ± 0.44 (0.62–1.79)
	Gill	1.79 ± 0.70 (0.50–5.29)	1.19 ± 0.80 (0.61–1.94)	1.19 ± 0.78 (0.88–1.59)	1.77 ± 0.83 (0.80–3.98)

[a] Metal concentrations in tissues collected from individuals of different fish species were statistically compared using one-way ANOVA. All comparisons were statistically significant at $p < 0.05$.

Fe (150.92 ± 1.38 mg kg^{-1}), while the lowest for Cd (0.54 ± 0.39 mg kg^{-1}). Mean concentrations of Al, Zn, Mn, Ni, Cr, Cu, Co and Pb were 129.99 ± 1.12, 51.48 ± 0.71, 5.65 ± 1.30, 3.89 ± 1.60, 3.08 ± 1.64, 2.54 ± 1.27, 0.74 ± 1.07 and 0.57 ± 0.35 mg kg^{-1}, respectively. For *C. umbla*, mean heavy metal concentrations were as follows: 130.63 ± 1.35 mg kg^{-1} for Al, 2.18 ± 1.22 mg kg^{-1} for Cr, 6.13 ± 1.22 mg kg^{-1} for Mn, 86.05 ± 0.93 mg kg^{-1} for Fe, 0.54 ± 0.50 mg kg^{-1} for Co, 3.10 ± 1.49 mg kg^{-1} for Ni, 4.30 ± 0.80 mg kg^{-1} for Cu, 36.39 ± 0.66 mg kg^{-1} for Zn, 0.54 ±0.22 mg kg^{-1} for Cd and 0.53 ± 0.32 mg kg^{-1} for Pb. In *C. trutta*, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb mean concentrations were determined as 108.58 ± 1.35, 2.15 ± 1.14, 3.88 ± 0.71, 55.96 ± 0.94, 0.53 ± 0.85, 3.06 ± 1.61, 1.90 ± 1.05, 32.01 ± 0.79, 0.52 ± 0.31 and 0.51 ± 0.45 mg kg^{-1}, respectively. In *C. regium*, these values were: 95.12 ± 1.34, 2.84 ± 1.01, 6.98 ± 0.82, 62.02 ± 0.84, 0.51 ± 0.93, 3.14 ± 1.41, 2.87 ± 1.38, 45.77 ± 0.39, 0.54 ± 0.23 and 0.52 ± 0.44 mg kg^{-1}, respectively. Mean concentrations of the analyzed metals in the muscles of *A. marmid*, *C. umbla*, *C. trutta* and *C. regium* were determined as follows: Fe > Al > Zn > Mn > Ni > Cr > Cu > Co > Pb > Cd; Al > Fe > Zn > Mn > Cu > Ni > Cr > Co = Cd > Pb; Al > Fe > Zn > Mn > Ni > Cr > Cu > Co > Cd > Pb; Al > Fe > Zn > Mn > Ni > Cu > Cr > Cd > Pb > Co, respectively (Table 2). The correlations between fish muscle tissues and heavy metal concentrations were statistically significant ($p < 0.05$).

The highest concentration of Fe (2299.52 ± 0.85 mg kg^{-1}) in liver tissues was found in *C. trutta* and the lowest one in *A. marmid* (887.05 ± 1.12 mg kg^{-1}). Al concentration was the second highest metal concentration among the studied fish species, with *C. regium* having the higest concentration (597.37 ± 1.17 mg kg^{-1}) and *A. marmid* having the lowest concentration (156.77 ± 1.98 mg kg^{-1}). Mean concentrations of the analyzed metals in livers of *A. marmid*, *C. umbla*, *C. trutta* and *C. regium* ranked as follows: Fe > Al > Zn > Cu > Mn > Ni > Cr > Co > Cd > Pb; Fe > Al > Zn > Cu > Mn > Ni > Cr > Co > Cd = Pb; Fe > Al > Zn > Cu > Mn > Ni > Cr > Co > Pb > Cd; Fe > Al > Zn> Cu > Mn > Cr > Ni > Co > Cd > Pb, respectively. Zn values ranged between 111.10 ± 1.58 mg kg^{-1} (in *C. umbla*) and 131.43 ± 0.86 mg kg^{-1} (in *A. marmid*). On the other hand, Cu values ranged between 31.49 ± 0.93 mg kg^{-1} (in *A. marmid*) and 108.35 ± 0.67 mg kg^{-1} (in *C. umbla*). The lowest Mn and Ni levels were determined in *A. marmid* (10.65 ± 1.24 and 6.53 ± 1.59), while the lowest Co level was determined in *C. regium* (1.44 ± 0.85 mg kg^{-1}). The highest values of Mn, Ni and Co were determined in *C. trutta* (32.21 ± 0.97, 13.68 ± 1.42 and 2.34 ± 0.89 mg kg^{-1}, respectively). Cr values ranged between 3.47 ± 1.49 mg kg^{-1} (in *C. umbla*) and 18.13 ± 1.27 mg kg^{-1} (in *C. regium*). Of all the analyzed metals, the lowest concentration was found for Cd and Pb, i.e. 0.75 ± 0.79 and 0.75 ± 0.60 mg kg^{-1} (in *C. umbla*), respectively (Table 2). The correlations between fish liver tissues and heavy metal concentrations were statistically significant ($p < 0.05$).

In gill tissues, the highest concentrations were determined for Fe and Al, ranging from 315.25 ± 1.11 and 284.98 ± 1.00 mg kg^{-1} (in *C. umbla*) to 1360.95 ± 0.83 and 1809.94 ± 1.05 mg kg^{-1} (in *C. trutta*), followed by Zn ranging from 76.12 ± 0.83 mg kg^{-1} (in *C. trutta*) to 135.66 ± 0.79 mg kg^{-1} (in *C. umbla*), Mn ranging from 24.22 ± 0.94 mg kg^{-1} (in *A. marmid*) to 85.21 ± 1.22 mg kg^{-1} (in *C. regium*), Ni and Cr ranging from 7.06 ± 1.31 and 4.45 ± 0.99 mg kg^{-1} (in *C. umbla*) to 26.72 ± 0.87 and 15.49 ± 0.99 mg kg^{-1} (in *C. trutta*), Cu from 3.29 ± 1.15 mg kg^{-1} (in *A. marmid*) to 5.93 ± 0.94 mg kg^{-1} (in *C. umbla*). The lowest Co, Pb and Cd levels were determined in gills of *C. umbla* (1.30 ± 1.07, 1.19 ± 0.80 and 0.54 ± 0.30 mg kg^{-1}, respectively), while the highest Co level was determined in *C. trutta* (2.59 ± 0.95 mg kg^{-1}), the highest Pb level in *A. marmid* (1.79 ± 0.70 mg kg^{-1}), and the highest Cd level in *C. regium* (0.58 ± 0.52 mg kg^{-1}; Table 2). Mean concentrations of the analyzed metals in gills of *A. marmid*, *C. umbla*, *C. trutta* and *C. regium* ranked as follows: Fe > Al > Zn > Mn > Ni > Cr > Cu > Pb > Co > Cd; Fe > Al > Zn > Mn > Ni > Cu > Cr > Co > Pb > Cd; Al > Fe > Zn > Mn > Ni > Cr > Cu > Co > Pb > Cd; Al > Fe > Zn > Mn > Ni > Cr > Cu > Co > Pb > Cd, respectively. In general, heavy metal concentrations in the muscle, liver and gills of *A. marmid*, *C. umbla*, *C. trutta* and *C. regium* were high compared with other metals: Al > Fe > Mn > Zn; Al > Fe > Cu > Zn; Al > Fe > Mn > Zn. A statistically significant correlation was found between fish gill tissues and heavy metal concentrations ($p < 0.05$).

The results of Pearson's correlation coefficient (R) and levels of significance (p) for the relationships between fish size (length and weight) and heavy metal concentrations in the tissue of four fish species caught in the Karasu River were summarized in Table 3. In *A. marmid*, significant positive correlations were found between fish length and Cd ($p < 0.01$), Zn and Pb ($p < 0.05$) levels in muscles; Al and Cu ($p < 0.05$), Cr, Mn, Fe, Co, Ni, Zn, Cd and Pb ($p < 0.01$) levels in the liver; Al and Cr ($p < 0.05$), Mn, Co, Ni, Cu, Zn and Cd ($p < 0.01$) levels in gills. Significant positive correlations were found between fish weight and all heavy metals ($p < 0.01$, $p < 0.05$). Correlations between the concentrations of Al, Cr, Mn, Fe, Co, Ni, and Cu in muscles and fish length were not statistically significant ($p > 0.05$). Correlations between the concentrations of Fe and Pb in gills and fish length were also not statistically significant ($p > 0.05$) for *A. marmid*.

Table 3

Pearson correlation coefficient (R) and levels of significance (*p*) for the relationships between heavy metal concentrations in tissues of four fish species caught in the Karasu River and fish size (length and weight)

Tissue	Fishes		Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
A. marmid												
Muscle	Length	R value	0.634	0.748	0.608	0.613	0.702	0.648	0.717	0.840	0.950	0.786
		p value	^{a}NS	NS	NS	NS	NS	NS	NS	^{b}*	^{c}**	*
	Weight	R value	0.798	0.893	0.774	0.783	0.857	0.803	0.848	0.933	0.968	0.915
		p value	*	**	*	*	*	*	*	**	**	**
Liver	Length	R value	0.777	0.905	0.964	0.879	0.980	0.989	0.778	0.883	0.992	0.988
		p value	*	**	**	**	**	**	*	**	**	**
	Weight	R value	0.899	0.984	0.964	0.971	0.912	0.947	0.916	0.957	0.965	0.975
		p value	**	**	**	**	**	**	**	**	**	**
Gill	Length	R value	0.795	0.813	0.965	0.713	0.990	0.935	0.989	0.899	0.975	0.750
		p value	*	*	**	NS	**	**	**	**	**	NS
	Weight	R value	0.922	0.940	0.937	0.859	0.943	0.980	0.963	0.827	0.974	0.877
		p value	**	**	**	*	**	**	**	*	**	**
C. umbla												
Muscle	Length	R value	0.974	0.990	0.873	0.958	0.931	0.897	0.972	0.981	0.968	0.969
		p value	**	**	*	**	**	**	**	**	**	**
	Weight	R value	0.946	0.967	0.939	0.981	0.986	0.938	0.990	0.965	0.932	0.932
		p value	**	**	**	**	**	**	**	**	**	**
Liver	Length	R value	0.792	0.925	0.814	0.826	0.940	0.972	0.855	0.935	0.830	0.851
		p value	*	**	*	*	**	**	*	**	*	*
	Weight	R value	0.908	0.954	0.917	0.928	0.962	00.991	0.936	0.883	0.918	0.899
		p value	**	**	**	**	**	**	**	**	**	**
Gill	Length	R value	0.827	0.836	0.975	0.882	0.982	0.937	0.969	0.941	0.970	0.967
		p value	*	*	**	**	**	**	**	**	**	**
	Weight	R value	0.930	00.939	0.895	0.967	0.976	0.994	0.921	0.991	0.960	0.971
		p value	**	**	**	**	**	**	**	**	**	**
C. trutta												
Muscle	Length	R value	0.849	0.949	0.904	0.875	0.994	0.919	0.716	0.951	0.957	0.991
		p value	*	**	**	**	**	**	NS	**	**	**
	Weight	R value	0.932	0.949	0.933	0.927	0.961	0.928	0.833	0.952	0.969	0.967
		p value	**	**	**	**	**	**	*	**	**	**
Liver	Length	R value	0.777	0.568	0.903	0.683	0.880	0.928	0.571	0.989	0.717	0.756
		p value	*	NS	**	NS	**	**	NS	**	NS	*
	Weight	R value	0.880	0.702	0.949	0.805	0.944	0.965	0.711	0.957	0.838	0.864
		p value	**	NS	**	*	**	**	NS	**	*	*
Gill	Length	R value	0.875	0.810	0.885	0.832	0.850	0.848	0.829	0.868	0.889	0.824
		p value	**	*	**	*	*	*	*	*	**	*
	Weight	R value	0.948	0.913	0.940	0.928	0.940	0.922	0.915	0.937	0.942	0.848
		p value	**	**	**	**	**	**	**	**	**	*
C. regium												
Muscle	Length	R value	0.826	0.774	0.954	0.991	0.973	0.979	0.949	0.909	0.961	0.990
		p value	*	*	**	**	**	**	**	**	**	**
	Weight	R value	0.867	0.821	0.973	0.986	0.961	0.978	0.980	0.935	0.932	0.969
		p value	*	*	**	**	**	**	**	**	**	**
Liver	Length	R value	0.817	0.802	0.799	0.923	0.850	0.900	0.868	0.937	0.878	0.856
		p value	*	*	*	**	*	**	*	**	**	*
	Weight	R value	0.868	0.858	0.856	0.930	0.902	0.936	0.905	0.958	0.931	0.895
		p value	*	*	*	**	**	**	**	**	**	**
Gill	Length	R value	0.963	0.974	0.985	0.984	0.983	0.954	0.932	0.758	0.950	0.857
		p value	**	**	**	**	**	**	**	*	**	*
	Weight	R value	0.976	0.977	0.964	0.985	0.970	0.963	0.860	0.806	0.955	0.899
		p value	**	**	**	**	**	**	*	*	**	**

^{a}NS, not significant, $p > 0.05$; * ^{b}Significant at $p < 0.05$; ** ^{c}Significant at $p < 0.01$

Positive correlations were found between all heavy metal concentrations in *C. trutta* muscle tissue and fish length (except for Cu at $p > 0.05$). Significant positive correlations were found between fish weight and all heavy metals ($p < 0.01$, $p < 0.05$). Significant positive correlations were also found between fish length and some heavy metals in the liver tissue (Al and Pb at $p < 0.05$; Mn, Co, Ni, and Zn at $p < 0.01$). On the other hand, the correlations between fish length and other heavy metals in *C. trutta* (Cr, Fe, Cu and Cd) were not statistically significant ($p > 0.05$). Similarly, significant positive correlations were found between fish weight and all heavy metals (except for Cr and Cu at $p > 0.05$). The correlations between the concentrations of heavy metals in the gill tissue and fish size (length and weight) were statistically significantly positive ($p < 0.01$, $p < 0.05$) for *C. trutta*. Positive correlations between fish size and all metals were determined for all tissues of *C. umbla* and *C. regium* ($p < 0.01$, $p < 0.05$; Table 3).

3.2. Health risk assessment associated with fish consumption

To assess public health risk associated with the consumption of fish from the Karasu River, concentrations of heavy metals in fish were compared with the Maximum Acceptable Concentrations (MACs) for human consumption set by various organizations. The determined concentrations of heavy metals in the studied fish species from the Karasu River were below the MACs for human consumption recommended by the Turkish Food Codex (TFC), Food and Agricultural Organization (FAO), the World Health Organization (WHO), FAO/WHO and the European Commission (EC) with a few exceptions (Table 4).

4. Discussion

Heavy metals cannot be biologically reduced, and neither humans nor fish can metabolize them. Even if they do not exceed toxic concentrations in fish tissues, they can enter human bodies through fish consumption and cause severe health problems (Elbeshti et al. 2018). In this study, concentrations of selected heavy metals were determined in muscle, liver, and gill tissues of four fish species (*A. marmid*, *C. umbla*, *C. trutta,* and *C. regium*) from the Karasu River. Distribution patterns of heavy metal concentrations in the tissue of the studied fish species were as follows: gill > liver > muscle or liver > gill > muscle. In this study, heavy metal concentrations were found to be higher in the gills and liver than in the muscle tissue. Similar results related to the fact that muscle was not an active tissue in accumulating heavy metals were reported for other fish species (Canlı & Atlı 2003; Canpolat & Calta 2003; Altındağ & Yiğit 2005; Erdogrul & Erbilir 2007; Öztürk et al. 2009; Sonmez et al. 2012; Kalkan et al. 2015; Ural & Danabas 2015; Olgunoglu et al. 2015; Aydogan et al. 2017; Gunes et al. 2019; Kacar & Karadede-Akın 2019; Danabas et al. 2020). These results also indicated that the accumulation and biological demand for each metal varied between fish tissues. Many previous studies revealed that heavy metals are stored for detoxification through the production of metallothioneins, especially in metabolic organs such as the liver (Karadede et al. 2004; Demirak et al. 2006; Ploetz et al. 2007). It is one of the organs most affected by toxic substances (Dane & Şişman 2020). The concentration of metals in gills may result from their complexation with the mucus that cannot be completely removed from lamellae prior to tissue

Table 4

Heavy metal concentrations (mg kg^{-1}) in muscle tissues of four fish species from the Karasu River and Maximum Acceptable Concentrations (MACs) according to international standards

Heavy Metals	Concentrations of heavy metals (mg kg^{-1}) in different species				Maximum Acceptable Concentrations (MACs)				
	A. marmid	*C. umbla*	*C. trutta*	*C. regium*	TFC[a] 2002	FAO[b] 1983	WHO[c] 1985–1996	FAO/WHO 1989	EC[d] 2006
Al	129.99	130.63	108.58	95.12					
Cr	3.08	2.18	2.15	2.84	–		0.15	0.5/1	–
Mn	5.65	6.13	3.88	6.98	20		0.5–1	2.5	–
Fe	150.92	86.05	55.96	62.02	50		100		
Co	0.74	0.54	0.53	0.51					1
Ni	3.89	3.10	3.06	3.14	–		0.6	0.4	40
Cu	2.54	4.30	1.90	2.87	20	30	30	30	
Zn	51.48	36.39	32.01	45.77	50	30–150	100	40	
Cd	0.54	0.54	0.52	0.54	0.1	0.5	1	0.5	0.05
Pb	0.57	0.53	0.51	0.52	0.1	0.5	2.00	0.5	0.2–0.5

[a]Turkish Food Codex; [b]Food and Agriculture Organization of the United Nations; [c]World Health Organization; [d]European Commission

preparation for analysis (Başyiğit & Tekin-Özan 2013; Erdogrul & Erbilir 2007). Therefore, some metals are found in high concentrations in gills. In general, the concentration of metals in gills may reflect the concentration of metals in water. Since these are not edible parts, the accumulation of metals in gills and liver of consumed fish has no direct effect on human health (Indrajith et al. 2008).

It was found that Fe and Al were present in the tissues of the studied fish at the highest concentrations, while Co, Cd and Pb were present at the lowest concentrations. For *A. marmid*, the highest concentrations were reached by Fe in all tissues, while the lowest concentrations were determined for Cd and Pb. For *C. regium*, Fe and Al were present at the highest concentrations in all tissues, while the lowest concentrations were determined for Cd and Pb. These values were much higher than those obtained by Erdogrul and Erbilir (2007) for *A. marmid* and *C. regium* from the Sır Dam Lake. Fe values were higher than those obtained in this study, while Cd and Pb values were not determined by Karadede and Ünlü (2000) in *A. marmid* and *C. regium* from the Atatürk Dam Lake. Similarly, Fe values determined for the muscles in this study are higher than those determined for *A. marmid* and *C. regium* collected from the Keban Dam Lake (Çalta and Canpolat 2006). Kaçar and Karadede-Akın (2019) reported the highest concentrations of Fe in the tissues of *C. regium* from the Batman Dam Lake. Furthermore, metal concentrations in all tissues of *C. regium* in this study were found to be higher than those reported for *C. regium* from the Karasu River, Turkey (Aydogan et al. 2017). The concentrations of Fe and Pb in all tissues of *C. regium* specimens collected from the Seyhan River (Canlı et al. 1998) and the Atatürk Dam Lake (Sitilce samples; Fırat et al. 2018) were lower compared with those found in this study. Fe and Cd levels were higher than those measured in the Yeşilırmak River (Mendil et al. 2010). In the case of *C. umbla*, Fe and Al were the metals with the highest concentrations in all tissues, while Cd and Pb showed the lowest concentrations. Earlier research (Canpolat & Calta 2003) on the concentration of Fe, Mn, Cu, Zn, Cd, Cr and Pb in organs and tissues of *Capoeta capoeta umbla* from Lake Hazar showed that the concentrations of these heavy metals in muscles follow the following pattern: Zn > Fe > Cu > Mn. Another research (Ural et al. 2012) showed that the concentrations of Fe, Zn, Cd, and Pb in tissues of *Capoeta umbla* from the Uzunçayır Dam Lake follow the following pattern Fe > Zn > Cd > Cu. Fe and Cd levels were higher than those measured in Lake Hazar (Canpolat 2013) and the Keban Dam Lake (Cağlar et al. 2019). Similar results were obtained for tissues of *C. umbla* in studies conducted in the Tercan Dam Lake (Gunes et al. 2019) and the Karasu River (Sokmen et al. 2018). Fe values were higher, while Pb values were lower than those obtained by Sonmez et al. (2012) for *C. umbla* from the Karasu River. For *C. trutta*, the values of Fe in the muscle tissue and Pb in all tissues determined in this study are lower than those determined in samples collected from the Keban Dam Lake (Ural and Danabas 2015). Fe values in the muscle tissue were also higher than those measured in the Karakaya Dam Lake (Eroglu et al. 2016).

In this study, the relationship between fish size and heavy metal concentrations in their tissues was analyzed and the results were compared with previous studies carried out on freshwater fish in Turkey and other parts of the world. The results indicated a positive correlation between fish size (length and weight) and heavy metal levels in most statistically significant cases. In general, an increasing trend was observed for all heavy metals in all investigated tissues with increasing fish length and weight (Table 2).

According to Ziyadah (1999), tissues tend to accumulate high concentrations of heavy metals with increased fish size. Al-Yousuf et al. (2000) found a positive correlation between the accumulation of heavy metals in the liver, skin and muscle of *Lethrinus lentjan* and fish size. Canpolat and Catla (2003) analyzed the relationship between metal (Cu, Fe, Mn and Zn) levels and fish (*Capoeta capoeta umbla*) size in the Keban Dam Lake and suggested that there was a significant correlation between fish weight and Fe levels in the liver and gills, the Mn level in the liver, gills and skin, between fish length and Fe levels in the liver and gills, and the Mn level in the liver and gills. Aktan and Tekin-Özan (2012) found that there was a positive correlation between some heavy metals and weight and length of *Scomber japonicus*. Yi and Zhang (2012) found that there was a positive correlation between fish size and Zn, Cd, Pb in grass carp, *Coreius heterodom,* and *Cyprinus carpio,* while a negative correlation in the case of *Silurus asotus* and *Pelteobagrus fulvidraco*. Canpolat (2013) and Canpolat et al. (2014) found positive correlations between fish size and heavy metal concentrations in *Capoeta umbla* from Lake Hazar and in *Aspius vorax* from the Karakaya Dam Lake. Kasımoğlu (2014) reported a significantly positive correlation between heavy metals (Co, Cr, Cu, Fe, Mn, Ni, and Zn) and fish age, weight and total length for *Anguilla anguilla* from the Tersakan Stream. As determined for different fish species, differences in the correlations between metal concentrations and fish size could result from differences in ecological requirements, swimming behaviors, metabolic activity and feeding habits.

A positive correlation between heavy metal concentrations in muscles of *Luciobarbus esocinus* and *Squalius cephalus* from the Karakaya Dam Lake and the fish size was observed by Düsükcan et al. (2017) and Düsükcan (2018), respectively. Similarly, another study (Eroglu et al. 2016) reported that organs tend to accumulate high concentrations of heavy metals as the size of *Capoeta trutta* increased. Sokmen et al. (2018) suggested that, except for a few cases, there was a positive significant correlation between metal concentrations in the muscle tissue and fish size (length and weight) for *Capoeta umbla* in the Karasu River (Erzincan, Turkey). Another study showed that in most cases there was a positive correlation between fish size (length and weight) and sex. In general, studies on heavy metals indicated that heavy metal concentrations increased with increasing fish size (Cağlar et al. 2019). Various factors such as the metabolic rate and growth dilution of elements are known to affect the growth-dependent differences in heavy metal levels (Kasımoğlu 2014). Liu et al. (2015) found in their study that young individuals accumulate more heavy metals than older ones. However, Yi & Zhang (2012) reported that there is a positive correlation between the fish size and metal concentration in tissues.

Consistent with the literature reports, the studies listed below described negative correlations between fish length and heavy metal concentrations in their tissues. Widianarko et al. (2000) found that there was a significant decline in lead concentrations with increasing fish size (*Poecilia reticulata*), while concentrations of copper and zinc were not correlated with body weight. Canlı and Atlı (2003) suggested that Cd, Cr, Cu, Fe, Pb and Zn accumulation decreased with increasing size of some fish species sampled in the Mediterranean Sea. Furthermore, Agah et al. (2009) reported that for many metals there was a negative correlation indicating that the contamination level decreased with fish size. Metabolic activity, which is important in the accumulation of heavy metals in fish, varies in fish of different sizes (Roesijadi & Robinson 1994), and is higher in young individuals (Langston 1990). Farkas et al. (2003) observed that Cd, Cu, Pb, and Zn concentrations in fish decreased from autumn to spring, whereas Hg concentrations increased. No statistically significant correlation was found between the content of metals in fish tissues and fish length for all species ($p > 0.05$). The negative correlation between fish size and metal levels may have been due to differences in metabolic activity between younger and older fish. Smaller fish were more active and needed more oxygen to generate more energy (Canlı & Atlı 2003).

The concentrations of Mn were lower than those specified by TFC and higher than those specified by WHO and FAO/WHO. The concentrations of Fe were higher than those specified by TFC and lower than those specified by WHO (except for *A. marmid*). The concentrations of Co and Ni were lower than the levels specified by EC. Cu did not exceed the MACs recommended by TFC, FAO, WHO, and FAO/WHO. The concentrations of Zn, Cd, and Pb were clearly below the permissible limits set by WHO. *A. marmid*, *C. umbla*, *C. trutta,* and *C. regium* were contaminated by Cr, which poses a threat to public health. Consistent with the Priority List of Hazardous Substances established by the Agency for Toxic Substances and Disease Registry (ATSDR 2013), the descending order of heavy metals threatening the human health was as follows: As > Pb > Cd > Ni > Zn > Cr > Cu > Mn. These results show that heavy metal values determined in fish muscles are safe for human consumption.

5. Conclusions

Fish are at the top of the aquatic food chain. They are indicators of the water quality status and of water pollution, especially by heavy metals. In freshwater, heavy metals accumulate more in fish species (Cyprinidae) that feed on sediment (Cağlar et al. 2019). In this context, the present study was carried out to provide information on heavy metal concentrations in muscle, liver, and gill tissues of Tigris scraper, trout barb, Tigris bream, and Mesopotamian nase inhabiting the Karasu River, which are consumed by local people, and to evaluate the relationships between the size (length and weight) of the fish and the concentrations of metals in their tissues. The highest concentrations of heavy metals were found in gills or liver, while the lowest concentrations were found in muscle. Muscle was not an active tissue in the accumulation of heavy metals. The accumulation of metals varied depending on species-specific factors, including mainly feeding behavior, fish size and age. The results of Pearson correlation analysis showed that, except for a few cases, significant correlations between metal concentrations and fish size (length and weight) were positive ($p < 0.01$, $p < 0.05$). Furthermore, heavy metal concentrations recorded in the edible parts of the four fish species studied were within the permissible limits set by national or international organizations for human consumption. It was concluded that the determined concentrations of heavy metals in fish collected from the Karasu River did not pose any threat to human health. Although the levels of the analyzed metals were below the recommended limits for human consumption, a potential risk could arise for people consuming fish depending on the size of fish and the load of heavy metals in the area. People living around the river and the authorities of these areas, in particular, should be made aware of these issues, as well as informed about fertilizers and treatment of domestic wastewater.

Future studies are recommended to more comprehensively determine the relationship between the content of heavy metals in sediments and their levels in fish, taking into account the physicochemical properties of water, including heavy metal content.

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