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ORIGINAL PAPER

Multivariate statistical assessment of heavy metal contamination in muscle of *Silurus glanis* L., 1758 from Kılıçkaya Reservoir in Turkey[1]

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Abstract

Heavy metals entering aquatic ecosystems from natural and anthropogenic sources are a significant water pollution problem. They accumulate in organisms living in aquatic ecosystems, and threaten human health by entering food chains. This study was carried out to assess the Cd, Cr, Cu, Mn, Ni, Pb and Zn contamination concentrations with multivariate statistical analysis in muscle tissue of the European catfish (*Silurus glanis* L., 1758) caught from Kılıçkaya Reservoir. The target tissue where heavy metals are stored and the muscle tissue, as it is the most edible part of the fish, were used in this paper. Contamination concentrations of heavy metals expressed in μg g^{-1} were determined with an atomic absorption spectrophotometer. As a result, maximum values of heavy metal concentrations in muscle tissue of *Silurus glanis* samples were measured at 0.03 μg g^{-1} for Cd, 0.37 μg g^{-1} for Cr, 3.59 μg g^{-1} for Cu, 3.32 μg g^{-1} for Mn, 0.68 μg g^{-1} for Ni, 0.43 μg g^{-1} for Pb and 23.15 μg g^{-1} for Zn. The correlation between Zn, Cd, Cr, Mn and Cu concentrations was very strong and significantly positive (r=0.818**, r=0.931**). Principal component analysis revealed two components explaining 94.50% of the total variance. The first component is dominated by Cu, Mn and Cd, while the second component is dominated by Ni and Zn. It is thought that the metals that dominate the first and second components are generally accumulated due to environmental factors and partially due to the geological structure of the region. According to the hierarchical clustering analysis results, 2 different groups were distinguished. The first group includes Cr, Pb, Ni, Cd, Cu and Mn, while the second group contains only Zn. Concentrations of Cd, Cr, Cu, Mn, Ni and Zn were below the FAO and EU recommended limits for fish, while Pb was found above the EU recommended limit. In this case, it poses a risk in terms of food safety and human health. Hence, it will be very beneficial to take the necessary measures immediately.

Keywords: European catfish, heavy metals, multivariate analyses

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INTRODUCTION

With technological developments and progressing industrialization, our natural environment is becoming increasingly polluted. Pollution with metals and other waste can originate from a wide variety of sources. Heavy metals are natural trace elements in aquatic ecosystems. However, heavy metals that knowingly or unknowingly leak into the environment due to industrial waste generation, specific geo-chemical structure, agriculture and mining are the main pollutants in aquatic ecosystems. The increasing use and widespread distribution of heavy metals threatens the health of aquatic organisms (Dirican et al. 2015). Heavy metals occupy an important place among pollutants because they are durable under environmental conditions and can easily accumulate in a food chain (Azimi et al. 2017). Heavy metals can enter the food chain directly through food and water and indirectly through the membranes of muscles (Panda et al. 2023). As a link of the biological cycle in aquatic ecosystems, fish are a major source of protein. In addition to providing easily digestible protein, fish are rich in essential amino acids, fats, minerals and fat-soluble vitamins. Fish meat is rich in long-chain polyunsaturated omega-3 fatty acids. Therefore, fish, which are the most important element of aquatic ecosystems, constitute an important component of the human diet owing to their high nutritional quality (Dirican et al. 2013). Fish are also a bio-indicator used to monitor the quality of aquatic ecosystems because they are readily available in large quantities and have the potential to accumulate heavy metals (Luczynska et al. 2022, Şirin et al. 2024). The accumulation and distribution patterns of heavy metals in fish tissues depend on the affinity of metals in fish tissues and the rates of uptake, accumulation and excretion. Accumulation also varies between species, depending on their ecology and life history, and especially their position in a food chain. Carnivorous fish species tend to accumulate higher amounts of metals than herbivorous, omnivorous or planktivorous species. However, benthic species can sometimes accumulate higher amounts of metals through sediment absorption than carnivorous species (Jovicic et al. 2023).

The European catfish from the Siluridae family (*Silurus glanis* L., 1758) is one of the largest fish of freshwater. It has a dorsoventrally slightly flattened body. The fish has well developed teeth in the jaws. The mouth is large, and there are 3 pairs of whisker-like barbels around it. The eyes are quite small. The dorsal fin is very small, located very close to the head. The anal fin is long. The *S. glanis* is a quality freshwater fish with very tasty meat, few bones, and a high economic value. It is a demersal fish that prefers calm waters with a soft bottom. It lives in Central and Eastern Europe and West Asian rivers and lakes (Uysal et al. 2009). The aim of this study was to determine, using multivariate analyses, the contamination with seven heavy metals in muscle tissue of *S. glanis* specimens caught from Kılıçkaya Reservoir, which is a popular source of fish for local inhabitants.

MATERIALS AND METHODS

Research area and supply of fish samples

This study was carried out in Kılıçkaya Reservoir, located in Suşehri district of Sivas province, which lies in the northeastern part of Turkey and the inland part of the Black Sea Region. Kılıçkaya Reservoir is located in the Yeşilırmak Basin, approximately 140 km northeast of the Sivas city center. Geographical coordinates of Kılıçkaya Reservoir are 40°14′0″ N, 38°11′0″ E. Kılıçkaya Reservoir is about 25 km away from Suşehri district. The basement rocks in the district consist of magmatic mass in the west and Paleozoic-aged metamorphic rocks. When metamorphic rocks are examined, gneiss, amphibolite, schist and mica-schist levels develop sequentially from the bottom, and thick marble layers appear at the top (Karagöz 2013). Suşehri Plain is covered with alluviums carried from high altitudes. For this reason, the plain is very suitable for agriculture and animal husbandry. The district, which has a high and rugged terrain, has a continental climate and steppe vegetation. The climate in this district is harsh, with long winters. The winter season is cold and very snowy. The Kılıçkaya Reservoir was created between 1980 and 1989, on the Kelkit Stream passing through the district. The most important water source that feeds Kılıçkaya Reservoir is the Kelkit Stream. The 132-meter high Kılıçkaya Dam supplies water to a power plant. The water of Kılıçkaya Reservoir is mainly used generation of electrical energy, commercial fishing, irrigation, and recreation. The surface area and maximum depth of Kılıçkaya Reservoir are 64.4 square km and 100 m, respectively. The annual average capacity of Kılıçkaya Hydroelectric Power Plant is 332 gigawatts (Dirican et al. 2015).

Sampling was carried out in two stations of Kılıçkaya Reservoir (Figure 1) in spring and summer 2021. A total of 12 fish specimens were caught with a plain extension net with a mesh size of 90x90 mm. Fish samples caught from Kılıçkaya Reservoir were brought to the laboratory by applying a cold chain. After determining the total length (cm) and weight (g) of the fish, 1.5-2 g muscle tissues were dissected with a plastic knife and stored in a deep freezer at -20°C until analysis.

Heavy metal analysis

Muscle tissue samples of *S. glanis* taken out from deep freezer were kept at room temperature to dissolve. The muscle samples were dried in an incubator at 105°C for 24 h in small heat-resistant glass tubes. After the drying process was completed, 3 mL of nitric acid were added to the samples and kept at room temperature for twenty-four hours. The samples were fully mineralized by heating them on a metal plate at a low adjustable temperature until the colored vapors disappeared. 1 mL of sulfuric acid was added to the mineralized samples. Later, samples were replenished to 50 mL with

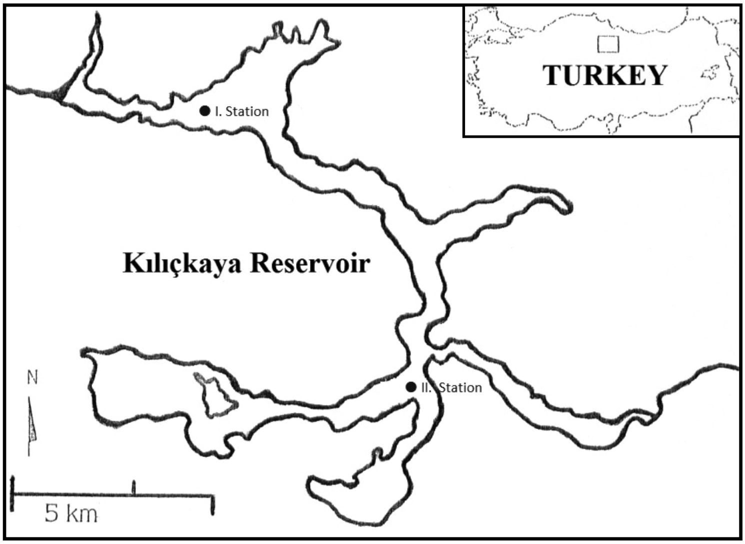

Fig. 1. Sampled stations in Kılıçkaya Reservoir

purified water and 1 or 2 drops of nitric acid were added to each sample. The samples were prepared for analysis by filtering through a 0.45 µm nitrocellulose filter. Similarly, a blank sample was prepared. Then, the samples were added 3 mL of nitric acid and kept at room temp. for 24 h (Dirican et al. 2015). Standard solutions for calibration graphs were prepared from stock solutions. All chemicals used for experiments and analyses were of analytical grades. Stock solutions of 1000 mg L^1 Cd (II), Cr (II), Cu (II), Mn (II), Ni (II), Pb (II) and Zn (II) were supplied by Merck Company and used without further purification. Standard solutions for each metal ion were prepared from stock solutions in 25 ml flasks. A calibration graph for each batch of experiments was re-constructed by using the standard solutions. Cd, Cr, Cu, Mn, Ni, Pb and Zn levels in the prepared solutions were detected in an Avanta model atomic absorption spectrophotometer at wavelengths specific for the metals (228.802 nm for Cd, 428.9 nm for Cr, 327.393 nm for Cu, 279.500 nm for Mn, 231.604 nm for Ni, 217.000 nm for Pb and 213.900 nm for Zn), and the results were given as µg g^{-1} wet weight.

Statistical analysis

The data set obtained was analyzed in the SPSS software, version 22. Descriptive statistics, Pearson correlation analysis, principal component analysis, factor analysis and cluster analysis were used within the scope of statistical analyses.

RESULTS AND DISCUSSION

A total of 12 *S. glanis* were examined during the paper. The range, minimum, maximum, mean, standard deviation, skewness and kurtosis values of the total length and weight of *S. glanis* specimens from Kılıçkaya Reservoir were showed in Table 1. The total lengths of *S. glanis* individuals in the sample size varied between 55.40-99.20 cm, and the average total length was calculated as 75.16±13.735 cm. The skewness of the total length distribution is 0.390. The kurtosis of the total length distribution is -0.790 and below zero. In the overall sample, the weight of the individuals varied between 1060 and 3460 g, and the average weight was found to be 2056.67±757.632 g. The skewness of the weight distribution is 0.759. The kurtosis of the weight distribution is -0.140 and again below zero (Table 1).

Table 1

Descriptive statistics for total length and weight of *S. glanis*

	N	Range	Min	Max	Mean	SD	Skewness	Kurtosis
Length (cm)	12	43.80	55.40	99.20	75.16	13.735	0.390	-0.790
Weight (g)	12	2400	1060	3460	2056.67	757.632	0.759	-0.140

N – number of observations, Min – minimum, Max – maximum, SD – standard deviation in the table

Differences were observed in the amounts of Cd, Cr, Cu, Mn, Ni, Pb and Zn accumulated in muscle tissue of *S. glanis*. Descriptive statistics results of Cd, Cr, Cu, Mn, Ni, Pb and Zn amounts detected in muscle tissues of the European catfish samples caught from Kılıçkaya Reservoir are given in Table 2. Mean values in muscle tissue samples of *S. glanis* from Kılıçkaya Reservoir were Cd 0.011 μg g^{-1}, Cr 0.218 μg g^{-1}, Cu 1.149 μg g^{-1}, Mn 1.022 μg g^{-1}, Ni 0.184 μg g^{-1}, Pb 0.155 μg g^{-1} and Zn 8.483 μg g^{-1} (Table 2). The decreasing order of mean values of heavy metal contents was determined as Zn>Cu>Mn>Cr>Ni>Pb>Cd. This ranking shows that Zn and Cu accumulate at higher levels in muscle tissue, while Pb and Cd accumulate at lower levels. There were studies reporting that Zn and Cu accumulate the most in muscle tissue of *S. glanis* (Andreji et al. 2006, Ettefaghdoost, Noveirian 2020). Similarly, there are studies reporting the least accumulation of Pb and Cd in muscle tissue of *S. glanis* (Mazej et al. 2010, Jovicic et al. 2015). The skewness values of Cd, Cr, Cu, Mn, Ni, Pb and Zn distributions are 0.640, 0.468, 1.295, 1.266, 1.129, 0.363 and 0.940, respectively. The kurtosis values of Cd, Cr, Pb distributions are -1.009, -1.091, -1.268 and below zero, respectively, while the kurtosis values of Cu, Mn, Ni, Zn distributions are 2.487, 1.019, 0.158, 0.066 and above zero (Table 2).

Whether the obtained data are suitable for a factor analysis or not can be explained by the Kaiser-Meyer-Olkin (KMO) and Bartlett's tests. The Bartlett's test and KMO test results were given in Table 3. Since the heavy metals Cr and Pb do not create a difference of at least 0.10 between the two

Table 2

Descriptive statistics of Cd, Cr, Cu, Mn, Ni, Pb and Zn values in *S. glanis* muscle tissue

Varia-bles	N	Range	Min	Max	Mean	SD	Variance	Skewness	Kurtosis
Cd	12	0.03	0.00	0.03	0.011	0.012	0.000	0.640	-1.009
Cr	12	0.26	0.11	0.37	0.218	0.091	0.008	0.468	-1.091
Cu	12	3.59	0.00	3.59	1.149	0.992	0.983	1.295	2.487
Mn	12	3.29	0.03	3.32	1.022	1.053	1.109	1.266	1.019
Ni	12	0.68	0.00	0.68	0.184	0.236	0.056	1.129	0.158
Pb	12	0.43	0.00	0.43	0.155	0.154	0.024	0.363	-1.268
Zn	12	22.87	0.28	23.15	8.483	7.395	50.972	0.940	0.066

N – number of observations, Min – minimum, Max – maximum, SD – standard deviation in the table

Table 3

Findings regarding the compliance of the data set for a factor analysis

Kaiser-Meyer-Olkin measure of sampling adequacy		0.698
Bartlett's test of sphericity	Approximately Chi-Square	54.754
	Df	10
	Sig.	0.000

factors, they were excluded from the factor analysis, and the analyses were continued. According to the results of the explanatory factor analysis made after removing the heavy metals Cr and Pb from the model, the fact that the KMO value is 0.698 and the Bartlett's test yielded meaningful results indicates that the sample size is sufficient and the data set can be submitted to a factor analysis.

The principal components analysis findings are given in the slope line graph in Figure 2. According to Figure 2, it can be observed that the slope does not change much after the 2nd main component. This shows that

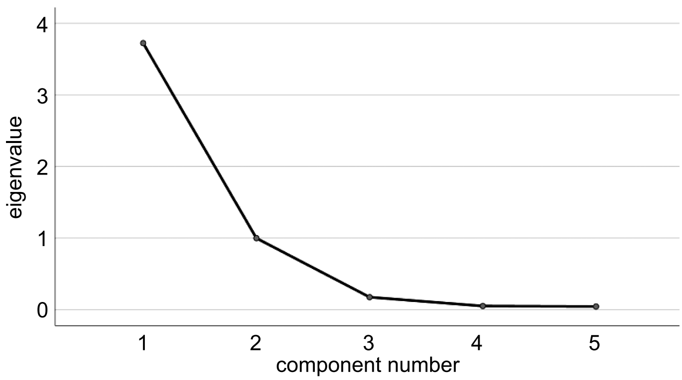

Fig. 2. Eigenvalue and factor scree plot

the data be explained in a 2-factor structure, overlapping with the results of the principal component analysis.

The factors affecting the variances determined in the principal components analysis are given in Table 4. The data set used in the research can be reduced to a 2-factor structure with an eigenvalue greater than 1. The first factor in the model explains 53.859% of the total variance, and the second factor explains 40.645%.

Table 4

Amounts of explanation of variance of main components

C	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	T	%V	C%	T	%V	C%	T	%V	C%
1	3.725	74.499	74.499	3.725	74.499	74.499	2.693	53.859	53.859
2	1.000	20.005	94.504	1.000	20.005	94.504	2.032	40.645	94.504

C – component, T – total, %V – % of variance, C% – cumulative % in the table

Table 5 shows the factor loadings obtained by the varimax rotation method with Kaiser normalization. For Table 4, principal component analysis loads >0.75 are shown in bold. The relationships between heavy metals can be easily seen in Table 5. As expected, two factors that make up 94.50% of the total variance were obtained. Cd, Cu and Mn are dominant in factor 1, while Ni and Zn are dominant in factor 2.

Table 5

Rotated component matrix for data of *S. glanis* some heavy metals

Variables	Factor 1	Factor 2
Cd	0.794**	0.518*
Cu	0.971**	-0.006
Mn	0.877**	0.439
Ni	0.078	0.986**
Zn	0.587*	0.773**

** Strong (F>0.75), * moderate (0.50<F<0.75)

Correlations between total length, weight and seven heavy metal variables of *S. glanis* samples captured from Kılıçkaya Reservoir were evaluated using Pearson correlation analysis (Table 6). A very strong positive correlation was determined between the total length and weight (r=0.961**) of the *S. glanis* specimens. The Cd concentrations in muscle of *S. glanis* was similar to the values of Dirican (2017) in Çamlıgöze Dam Lake and higher than the reported by Jovicic et al. (2015) in Danube River. In this paper, Cd contamination ranged from 0.00 to 0.03 μg g^{-1} (mean 0.011 μg g^{-1}). All values are below the maximum allowable limit of 0.05 μg g^{-1} for Cd recommended by FAO (1983) and EU (2006). According to the data of correlation analysis, while a very

Table 6

Pearson's correlation matrix for total length, weight and seven heavy metal variables

Variables	Length	Weight	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Length	1								
Weight	0.961**	1							
Cd	0.797**	0.884**	1						
Cr	0.936**	0.905**	0.725**	1					
Cu	0.737**	0.805**	0.710**	0.642*	1				
Mn	0.821**	0.914**	0.931**	0.737**	0.821**	1			
Ni	0.646*	0.596*	0.555	0.566	0.095	0.497	1		
Pb	0.642*	0.713**	0.743**	0.625*	0.505	0.737**	0.550	1	
Zn	0.948**	0.938**	0.818**	0.918**	0.589*	0.833**	0.797**	0.695*	1

** $p<0.01$ (2-tailed), * $p<0.05$ (2-tailed)

strong statistically significant positive correlation was observed between Cd and Mn (0.931**) and Cd and Zn (0.818**) in muscle tissues of *S. glanis*, Cd and Cr (0.725**), Cd and Cu (0.710**) and Cd and a strong statistically significant positive correlation was found between Pb (0.743**). On the contrary, no statistically significant correlation was found between the Cd and Ni concentrations in muscle tissues of *S. glanis*.

The mean Cr concentration in muscle of *S. glanis* was similar to those of Dikanovic et al. (2016) in Meduvrsje Reservoir and lower than the concentrations reported by Küpeli et al. (2014) in the Sakarya River. In this paper, the minimum and maximum Cr concentrations in the muscle tissues of the European catfish from Kılıçkaya Reservoir were found to be 0.11 μg g^{-1} and 0.37 μg g^{-1}, respectively. The Cr values in this paper were found to be below the upper limit value of 1 μg g^{-1} for fish recommended by FAO (1983). While there is a very strong and positive correlation between Cr and Zn (0.918**) in the muscle of *S. glanis*, there is a strong statistically significant positive correlation between Cr and Cu (0.642*), Cr and Mn (0.737**) and Cr and Pb (0.625*). In contrast, there is no statistically significant correlation between Cr and Ni.

The mean Cu concentration in the muscle tissue of *S. glanis* was similar to the mean values given by Mendil, Uluözlü (2007) in fish from Ataköy Dam Lake and higher than the concentrations reported by Dikanovic et al. (2016) in Meduvrsje Reservoir, Dirican (2017) in Çamlıgöze Dam Lake and Jovicic et al. (2023) in the Danube River. Cu was observed to achieve the highest accumulation in *S. glanis* samples in muscle tissue with 3.59 μg g^{-1}. This value was found below the maximum limit of 10 μg g^{-1} in fish recommended by FAO (1983) for Cu. While a very strong positive correlation was found between Cu and Mn (0.821**), a moderate positive significant correlation was observed between Cu and Zn (0.589*). On the contrary, no statistically significant correlation was observed between Cu and Ni, Cu and Pb.

While the average Mn concentration in muscle tissue of *S. glanis* was lower than the mean values reported by Mendil, Uluözlü (2007) in fish from Ataköy Dam Lake, they are higher than the concentrations reported by Dikanovic et al. (2016) in Meduvrsje Reservoir and Küpeli (2014) in the Sakarya River. Mn contamination varied between 0.03 μg g^{-1} and 3.32 μg g^{-1} in muscle tissue (Table 1). No national or international standard limit has been proposed for Mn. Very strong and positive correlations were determined between Mn and Zn (0.833^{**}), Mn and Cd (0.931^{**}) and Mn and Cu (0.821^{**}), while Mn and Pb (0.737**) and Mn and Cr (0.737^{**}) showed strong and positive correlations. On the contrary, no statistically significant correlation was found between Mn and Ni.

While the average Ni concentration in muscle tissue of *S. glanis* was lower than the mean values given by Mendil, Uluözlü (2007) in fish from Ataköy Dam Lake, they are higher than the concentrations reported by Jovicic (2015) in the Danube River, Küpeli et al. (2014) in the Sakarya River and Khanipour et al. (2018) in Anzali Wetland. The highest concentration of Ni in the European catfish in this study was determined to be 0.68 μg g^{-1}. The values obtained for Ni were found below the 10 μg g^{-1} limit value given by FAO (1983). While a very strong positive correlation was observed between Ni and Zn (0.797^{**}), no statistically significant correlation was found between Ni and the other analyzed heavy metals.

The mean Pb concentration was similar to the mean values determined by Mendil, Uluözlü (2007) in fish from Ataköy Dam Lake and Dikanovic et al. (2016) in Meduvrsje Reservoir and higher than the concentrations reported by Jovicic et al. (2015) in the Danube River and Küpeli (2014) in the Sakarya River. The highest Pb in the muscle tissues of *S. glanis* determined in this study was 0.43 μg g^{-1}. While this value is below the maximum allowable level recommended by FAO (1983), which is 0.50 μg g^{-1} for Pb in edible part of freshwater fish, it is above the maximum level recommended by the EU (2006) of 0.30 μg g^{-1} for Pb in muscle meat of fish. Pb is used in many industries around the world. It is one of the most toxic heavy metals known in relation to environmental pollution. Pb is not vital for any aquatic organism. Pb can cause hazardous environmental effects such as metal toxicity in aquatic animals. However, people can be exposed to lead through the food chain (Kayhan 2019). While strong and positive correlations were observed between Pb and Zn (0.695*), Pb and Cd (0.743**), Pb and Cr (0.625*), Pb and Mn (0.737**) in the muscle of *S. glanis*, there was no statistically significant correlation between Pb and Cu, Pb and Ni in Kılıçkaya Reservoir.

The mean Zn concentration in muscle of *S. glanis* was similar to the mean value determined by Lenhardt et al. (2012) in fish from the Danube River, and lower than the concentrations reported by Dikanovic et al. (2016) in the Meduvrsje Reservoir and Küpeli et al. (2014) in the Sakarya River. These reported differences may be largely due to the environmental levels of heavy metals in these aquatic ecosystems and the different geological characteristics of the regions. Zn in muscle of *S. glanis* from Kılıçkaya Reservoir was measured 0.28 μg g^{-1} at the lowest, and 23.15 μg g^{-1} at the highest The findings obtained in *S. glanis* muscle for Zn were below the 50 μg g^{-1} maximum value recommended for fish and fish products by FAO (1983). Statistically significant positive correlations were found between Zn and the other 6 heavy metals. A very strong positive correlation was observed between Zn and Cr (0.918**), Zn and Mn (0.833**), Zn and Cd (0.818**), while a moderate positive correlation was found between Zn and Cu (0.589*).

According to the results of the Pearson correlation analysis, 30 out of 45 correlation pairs between total height, weight, Cd, Cr, Cu, Mn, Ni, Pb and Zn levels were found to be statistically significant (p<0.01, p>0.05) – Table 6. While a very strong positive correlation was determined between total length and Cr, Mn, Zn (r=0.821, r=0.948), a strong positive correlation was determined between total length and Cd, Cu, Ni, Pb (r=0.642, r=0.797). While a very strong positive correlation was found between weight and Cd, Cr, Cu, Mn, Zn (r=0.805, r=0.914), a strong positive correlation was found between weight and Ni, Pb (r=0.596, r=0.713) – Table 5. Accordingly, statistically significant positive correlations were determined between the total length and weight of *S. glanis* and the accumulation of elements. These findings are consistent with the results of a study conducted by Tokatlı et al. (2016) on *Barbus oligolepis*, *Capoeta tinca* and *Squalis cii*. The significant positive correlations between heavy metal accumulations and total heights and weights recorded in the muscle tissues of *S. glanis* in this experiment reveal once again the risk of heavy metals in Kılıçkaya Reservoir to human health.

According to the component matrix, the factor 1 constitutes 53.859 of the cumulative variance of the data (Table 4). In the factor 1, the highest loads belong to Cu (0.971**), Mn (0.877**) and Cd (0.794**) variables, respectively. Cu, Mn and Cd have the highest positive effect. Zn (0.587*) has a moderate and positive effect (Table 5). Since fish are a good bio-accumulator, heavy metals easily accumulate in fish tissue (Sabullah et al. 2015). Natural events and processes, such as volcanic discharges, erosion of rocks, as well as anthropogenic activities, such as use of fossil fuels, generation of urban and industrial waste, are known as important sources of Cd release into the water (Alipour et al. 2021). Cd can exhibit high toxicity even at very low concentrations. Cd causes various acute and chronic effects on the environment and fish (Elbeshti et al. 2018). The presence of Cu in aquatic ecosystems occurs in various ways, for example because of mining activities, discharge of industrial and agricultural waste. Cu is an essential trace element necessary for the normal growth and metabolism of organisms. However, it can become very hazardous when found above its limit. It can cause imbalance in nature by accumulating in the aquatic ecosystem (Padrilah et al. 2018). Mn is found in most types of rocks and soils. It is an essential component of a number of enzymes and cofactors, and trace amounts are essential for many species.

Often, Mn is associated with steel and battery manufacturing and is a key ingredient in products such as fertilizers and pesticides. High Mn concentrations are commonly found in discharges from mining and other industrial processes (Harford et al. 2015). In this paper, factor 1 is dominated by Cu, Mn and Cd. These metals may have originated from natural and anthropogenic sources.

The factor 2 explains 40.645% of the total variance (Table 4). In the factor 2, the highest loads are Ni (0.986**) and Zn (0.773**), respectively. Significantly, Ni and Zn have a strong and positive effect. Cd (0.518*) has a moderate and positive effect (Table 5). Ni occurs naturally in soils and volcanic rocks. It naturally enters aquatic ecosystems through the erosion of rocks and soils and the leaching of minerals. Ni is used in a variety of industrial applications such as batteries, spark plugs, and stainless steel. In particular, it is widely used in the manufacture of Ni and Cd batteries. It also enters aquatic ecosystems with nickel-containing wastewater. Water-soluble salts of Ni are major problems of contamination in aquatic ecosystems (Isangedighi, David 2019). Zn may originate from geological rock erosion or from industrial and domestic wastewater discharges. Zn is an essential element and one of the most common heavy metal pollutants. However, it can be toxic to fish at very high concentrations. At very high concentrations, Zn adversely affects the growth and development of fish (Afshan et al. 2014). In this paper, factor 2 is dominated by Ni and Zn. The source of these metals can be natural and anthropogenic.

As it can be understood from the results of the principal component analysis, the pollution may have originated from natural and anthropogenic sources to a large extent. Also, a significant impact may come from non-point sources, such as agricultural activities. Agricultural areas around the research site constitute an important share. Therefore, to prevent heavy metal contamination, the necessary regulatory measures should be taken into account.

In recent years, there have been many studies using the Hierarchical Cluster Analysis in order to evaluate heavy metal pollution in fish (Fatima et al. 2020; Isibor et al. 2020; Partheeban et al. 2021). The Hierarchical Cluster Analysis in this paper grouped heavy metals into clusters based on similarities and differences. Metals belonging to the same cluster are likely to have come from a common source. In Figure 3 two clusters are distinguishes that clearly reflect the similarities and differences between the heavy metals studied in muscle tissue of *S. glanis*. This is in agreement with the Principal Components Analysis results. The cluster analysis results showed that Cr, Pb, Ni, Cd, Cu and Mn clustered together, while Zn formed a separate cluster, 25 units away from the others (Figure 3). This means that the Zn concentration in muscle tissue samples from *S. glanis* showed less association with the other heavy metals. The cluster analysis also showed that Zn absorption in the muscles of *S. glanis* samples from Kılıçkaya Reservoir varied significantly compared to the other metals

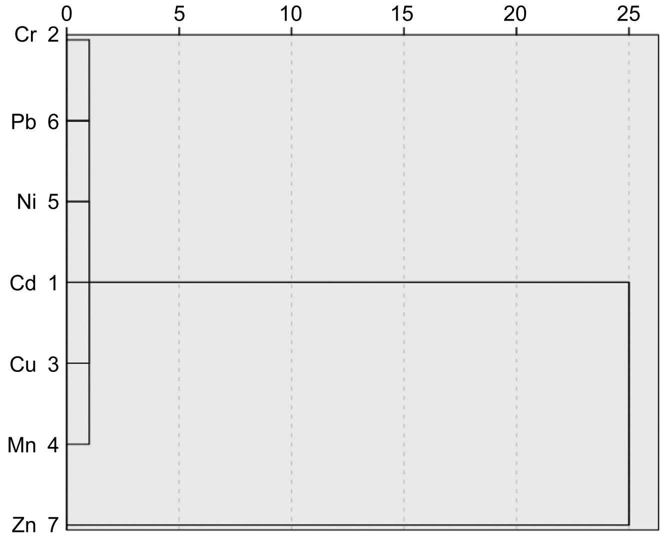

Fig. 3. Similarity dendrogram with respect to heavy metals

(Cr, Pb, Ni, Cd, Cu and Mn), among which high compatibility occurred. Generally, the cluster analysis results of *S. glanis* show similar findings with the cluster analysis results of heavy metal concentrations in tissues of *Clarias gariepinus* by Isibor et al. (2020). The tendency of heavy metals that can be transferred to humans with the consumption of *S. glanis* living in Kılıçkaya Reservoir to accumulate in the body and thus their potential to pose health risks to consumers should be taken into consideration. Additionally, the sustainability of *S. glanis* in Kılıçkaya Reservoir, an economically important species, is critical to maintaining a stable source of income for the people of the region.

CONCLUSIONS

Pollution with heavy metals is becoming a major concern due to the negative effects it causes in the world. The world is so heavily polluted with heavy metals, mostly due to man-made contamination, that the health of many animal organisms and people is adversely affected. This study revealed that the analyzed *S. glanis* samples had different concentrations of heavy metals in muscle tissues. In conclusion, it was determined that the Cr, Cd, Cu, Mn and Ni concentrations in muscle tissue of *S. glanis* from Kılıçkaya Reservoir were below the international legal limits, while the Pb concentration was above the legal limit. For this reason, it would be beneficial to increase the frequency of inspections for heavy metal residues in *S. glanis* which lives in Kılıçkaya Reservoir and is consumed by the people of the region. The fact that *S. glanis* consumption is relatively higher, especially by pregnant women and children, owing to its nutritional value, indicates that these individuals are in the risk group. *S. glanis* living in Kılıçkaya Reservoir are exposed to heavy metal contamination from many sources. Among the important dangers that play a role in heavy metal pollution of Kılıçkaya Reservoir are the natural structure of the research area, as well as agricultural and industrial developments. This situation poses a risk in terms of food safety and may adversely affect consumer health. Therefore, it is thought that necessary measures should be taken without delay for the factors polluting the reservoir in order to minimize the pollutants in Kılıçkaya Reservoir.

Author's contribution

The author confirms that the text, figures, and tables are original and that they have not been published before.

Conflicts of interest

No conflict of interest was declared by the author.

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