Effects of heavy metal pollution on hepatosomatic index and vital organ histology in *Alburnus mossulensis* from Karasu River

Hatice DANE*, Turgay ŞİŞMAN

Department of Biology, Faculty of Sciences, Atatürk University, Erzurum, Turkey

Abstract: In this study, histological effects of water pollution to freshwater cyprinid, *Alburnus mossulensis*, was investigated. Water, sediment, and fish samples were taken from four stations in Karasu River (Erzurum, Turkey) between June and September in 2015–2016. In water and sediment samples, the concentrations of Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Sr, and Pb were detected. Histopathological changes in gill, liver, and kidney tissues were determined by degree of tissue change (DTC), and hepatosomatic index (HSI) was calculated. The mean concentrations of heavy metals in water and sediment samples were lower at station 4. The trend of some metal contents found in the fish was in increasing order of Zn < Cu < Cr < Pb. The observations on histopathology included cellular degeneration, congestion, dilatation, vacuolization, edema, vasodilatation, hyperplasia, hypertrophy, infiltration, melanomacrophage aggregates, glomerulopathy, fibrosis, and necrosis. It was observed that the DTC and HSI values in fish varied significantly from station to station. The results showed that the higher severity of histopathological alterations in the fish tissues was strongly correlated with agricultural and domestic activities in the area.

Key words: Water pollution, hepatosomatic index, histopathology, *Alburnus mossulensis*

1. Introduction

Microorganisms, plants, and animals, which are elements of the ecosystem, change the chemical structure of air, soil, and water. Contamination of the aquatic environment poses a threat not only to aquatic organisms but also to human and the entire ecosystem [1]. A good understanding of the factors causing pollution, which can affect the biodiversity, decrease the number of species, or even cause some of them to become extinct, plays a critical role in the protection of species. At this point, fish are often accepted as the most suitable organism to monitor the health of aquatic ecosystems [2]. Fish are considered excellent bioindicators because they are very sensitive to environmental changes such as pollution [3]. In addition, the fact that fish under stressful conditions show very similar signs to the mammalians has made them preferred especially in water pollution studies [4]. The use of biomarkers and bioindicators is often preferred in determining the effects of toxic substances, and the data obtained allows the analysis of environmental hazards, and therefore, to develop policies for the conservation of endangered species. Histopathological changes in animal tissues are also the primary bioindicators of exposure to factors causing environmental stress [5]. Especially gills, liver, and kidney of fish are the most appropriate target organs used in histopathological studies to determine the effect of pollution due to their relationship with aquatic environment. In particular, gills are considered to be a good indicator of water quality. Gills, which are in direct contact with contaminants; the liver, which plays a key role in metabolism and xenobiotic metabolism; and the kidneys, which are important for excretion and keeping the water-salt balance stable with the internal environment of the fish, are the primary indicators for aquatic pollution [6].

As known, fish (42.6%) are the largest group of vertebrate animals, and 40% of them are carps (Cyprinidae) [7]. *Alburnus mossulensis*, a species belonging to the family Cyprinidae, prefers slow flowing middle parts of rivers with clean and glossy surfaces for living. Especially, the Firat (Tigris) and Dicle (Euphrates) river systems in the Eastern and Southeastern Anatolia of Turkey are their main distribution areas [8]. The Karasu River originates from the hillside of Dumlu Mountain in Erzurum joins with the Köşk, Karagöbek, and Dumlu Streams, and reaches Keban Dam Lake on the Firat River [9]. The Karasu River is clean until it comes to the agricultural areas and is contaminated by the discharge of domestic and industrial wastes as it...
passes through agricultural areas and settlements. Fifty percent of the industrial facilities in Erzurum are located in the organized industrial zone. The wastewater of this area is connected to the sewerage of the municipality and is discharged to the Karasu River [9,10].

In this study, the Karasu River was selected as the research area. It is aimed to determine the possible heavy metal pollution in the river caused by human-induced activities by analyzing the water and sediment samples taken from the river, and to determine the effects of this pollution on the fish population of the river. For this purpose, the *Alburnus mossulensis* species, which is found in the natural fauna of the river, was caught at 4 stations in 2015-2016 and examined in histopathological terms.

2. Material and methods

2.1. Sampling stations

In the study, four different stations were selected on the Karasu River (Aşkale 1st–39° 56’ 15.1” N 40° 37’ 25.9” E, Aşkale 2nd–39° 54’ 52.7” N 40° 40’ 29.4” E, İllica 3rd–39° 57’ 10.6” N 41° 04’ 15.2” E, and Dumulu 4th–40° 05’ 36.1” N 41° 22’ 49.0” E stations). The stations were selected considering the most polluted areas where industrial and sewage wastes are heavily poured into the river and areas considered relatively less polluted than these areas. Accordingly, these points were determined considering that the Aşkale stations were polluted, the İllica station was less polluted, and the Dumulu station near the source of the river was clean.

2.2. Sample collection

In order to examine the chemical properties of the river, surface water and sediment samples were taken from the stations selected in the Karasu River. Sampling was performed in two phases; firstly, June and September 2015; secondly, June and September 2016. A total of 32 samples (16 water and 16 sediment samples) were collected. About 2 L of surface water and 1 L of sediment samples were collected from the river with the grab method [11]. After collection, the samples were acidified with nitric acid and transferred to the High Energy Spectroscopy Research Laboratory at the Physics Department of Atatürk University.

2.3. Heavy metal determination

The analysis of Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Sr, and Pb elements in the water and sediment samples were carried out using EDXRF Spectrometer at the Prof. Dr. H.C. Wolf Weyrich High Energy Spectroscopy Research Laboratory at the Physics Department of Atatürk University. Moreover, some physicochemical parameters (temperature, pH, and dissolved oxygen) of the river water were measured in the field of the sampling with a portable measurement device (Multi 340i/SET, Germany).

2.4. Fish samples

The permissions required for the study were taken from the relevant institutions before the study (No:36643897/25.09.2013, No:67852565/140.03.03-863, No:72784983-488.04-63471). *A. mossulensis* species were caught with fish nets during the June–September 2015–2016 periods at the stations selected on the Karasu River. The samples were transferred to the Animal Physiology and Histology Research Laboratory of Atatürk University, alive in 10-L plastic containers. Before the examination, the fish were kept in 30-L aquariums with plenty of ventilation. In order to determine whether any external abnormalities, external parasites or injuries were present in the fish, each fish was firstly examined macroscopically, and their weight and length were measured. A total of 125 fish were caught. The total length of the fish was 15.0–19.6 cm, the fork length was 13.5–18.1 cm, and body weight was 24.0–74.8 g.

2.5. Hepatosomatic index (HSI)

Hepatosomatic index (HSI) is expressed as the ratio of liver weight to total weight, and it provides information about the health status of the fish and the quality of water. The fish were decapitated; the liver was removed, and weighed. HSI was calculated according to the following formula: HSI = [Liver weight (g) / Fish weight (g)] × 100.

2.6. Histological analyses

For histological analysis, the internal organs of decapitated fish (n = 32) were removed. Gills, liver, and kidney were fixed with 10% neutral buffered formalin (pH 7.0). The tissues were embedded in paraffin after dehydration and made transparent by passing through several steps. From the blocks of these tissues 5-µm thick sections were cut with a microtome and were stained with Hematoxylin & Eosin. The slides were examined under a digital camera-assisted Leica DM750 light microscope and photographed. The evaluation of the histological damage was quantitatively determined with the help of degree of tissue change (DTC). DTC is the observation parameter based on the frequency of damage. The tissues were classified according to the frequency of damage detected as 0: no abnormality, 1: low abnormality, 2: moderate abnormality, and 3: high abnormality [12]. Observed abnormalities were noted and classified in accordance with the stages of damage: Stage I (I): The status of tissue which functions normally; Stage II (II): The status of moderate impairment of tissue’s normal function; Stage III (III): The status of strong and irreversible damage. The DTC value was calculated on each tissue of each fish by using the following formula: DTC = (1 × ΣI) + (10 × ΣII) + (100 × ΣIII). In the formula, ΣI, ΣII, and ΣIII show the total number of abnormalities in the stages. Average DTC of each station was also calculated for each fish and then interpreted. A DTC value within the range of 0–10 is accepted as an indicator of normal organ functions.
A value within the range of 11–20 indicates slight damage to the organ, while 21–50 indicates moderate damage, and 51–100 indicates the existence of severe lesions. A DTC value above 100 was accepted as existence of irreversible damage [13].

2.7. Heavy metal analyses in liver
For heavy metal analysis, 0.5-g liver samples were taken from each fish (n = 32) and dried in an oven at 100 °C for 2 days. The samples were then weighed, and 5 mL of nitric acid was added to the samples. The mixture was burned on a hot plate at 70 °C for 2 h under fume cupboard. After the samples were homogenously burned and cooled, they were completed to 20 mL with distilled water [14]. The diluted samples were filtered through a 45-μm syringe filter. Cr, Mn, Ni, Cu, Zn, As, and Pb heavy metals of the tissues were performed by ICP-MS device in Atatürk University Eastern Anatolia Advanced Technology Research and Application Center (DAYTAM).

2.8. Statistical analyses
The general evaluation of the metal analyses and the HSI and DTC data were performed by one-way analysis of variance (ANOVA). The Duncan test was used for multiple comparisons in variance analysis. The data were interpreted by considering P < 0.05 significance level. SPSS 21.0 Software package program was used to evaluate all statistical data.

3. Results
3.1. Surface water and sediment analyses
The water and sediment samples of the Karasu River were analyzed for heavy metal content to determine the chemical properties of the environment in which the species live. It was observed that the heavy metal levels of samples taken in 2015 were lower than in 2016 (data were not shown). The mean metals levels detected in water and sediment samples taken from the 4 selected stations of Karasu River are given in Tables 1 and 2. According to the Classification of Inland Water Resources, the surface water of the 1st and 2nd stations of the river had grade IV quality, i.e. highly contaminated water elements (Table 1). The abiotic parameter values did not exceed the standard (Table 1). The sediment quality in the river was performed according to some sediment quality guidelines [15]. Cr, Cu, and As concentrations in the sediment were higher than the standard values (Table 2). As a result, it was found that the amount of metals in the sediment was higher than the amount in the water, and the most polluted station in terms of water was found to be station 2 and in terms of sediment it was station 1.

3.2. Liver heavy metal concentrations
Table 3 shows the mean concentrations of some heavy metals (Cr, Mn, Ni, Cu, Zn, As, Cd, Pb) in liver tissues of A. mossulensis in 2015 and 2016. Cr, Cu, and Pb metals at station 1 and 2, and Cu at station 3 were found to be higher than other elements in two years. Metal concentrations in 2016 increased relatively compared to 2015. According to the heavy metal concentrations in fish tissues, it was observed that the increase was at station 2 followed by stations 1, 3, and 4, respectively.

3.3. Hepatosomatic index (HSI)
In order to determine the general health status of the species examined, the change in values of HSI, which is one of the biosomatic indices, was monitored for 2 years for each station. Mean HSI values of the species obtained for each station are given in Table 4. The highest HSI value was obtained in the 1st station in both 2015 and 2016, and the lowest value was obtained in the 4th station. Mean HSI values were found to be significantly higher in stations 1 and 2 compared to other stations in 2 years (P < 0.05). When the stations were compared, the HSI values were found to be significantly higher in the other three stations compared to the 4th station (P < 0.05).

3.4. Histopathological results
The gill tissues of the fish caught from station 4 generally exhibited a normal histological structure (Figure 1), with only minimal pathological abnormalities. Significant pathological damages were observed in the gills of fish caught from the other stations. These damages were found to be vasodilatation, congestion, hypertrophy in the mucus cell, vacuolization, separation of the lamellar epithelium, hyperplasia of the respiratory epithelium, severe degenerations in the secondary lamellae together with curling, and shortening, hyperplasia, edema, and fusion (Figure 2).

In the liver slides prepared from fish caught at the 4th station, the tissue did not deviate from the normal architecture (Figure 3A). While only mild pathologies were observed in the 4th station fish, tissue damages increased in the fish captured in other stations. Particularly severe congestions in the central vein, sinusoids, and vessels were noted (Figure 3B). Degeneration of central vein epithelium, infiltration in the parenchyma, sinusoidal dilatations, vascular degeneration, increase in the number of melanomacrophage, degeneration in hepatocytes, prominent vacuolization with the increase in fat and fibrosis were observed in the studied fish (Figures 3B and 4).

In the kidney slides prepared from fish caught at the 4th station, the renal corpuscle and the tubules forming the nephron exhibited normal histological structure

1 Official Gazette (2015). Regulation on Surface Water Quality. Number: 29327 [online]. Website https://www.resmigazete.gov.tr/eskiler/2015/04/20150415-18.htm [accessed 02 June 2015].
Figures 5A and 5B). A number of kidney histopathologies were detected in the fish obtained from the other stations at varying rates for each station. Protein accumulation in the kidneys, dilatation in the tubular lumen, significant degeneration in the tubules, parenchymal detachment, histological damage such as parenchymal vacuolization, glomerulopathy, fibrosis, and necrosis were detected (Figures 5C and 5D). No difference was observed between 2015 and 2016 in terms of the variety of pathological damages. The DT values for all tissues are given in Table 5. According to the data, the DT values of 2015 were lower than that of 2016. The highest DT value was obtained from the 1st station. The increase in the DT value among stations was statistically significant (P < 0.05). The liver had the highest DT values. In terms of gills, fish of the 4th station were found to function normally, while fish of the 2nd and 3rd stations had moderate damage, and fish of the 1st station had severe damage. According to the liver DT values, it was found that the liver of the 4th station fish worked normally, the liver of the 3rd station fish had moderate damage, and the liver of the 1st and 2nd stations fish had severe pathological damages. According to the kidney DT value, the kidneys of the fish from the 4th station were found to function normally. Only slight damage was detected in the kidneys of 3rd station fish in 2015 and moderate damage was detected in 2016. Severe damage was detected only in 2016 in the kidney of the 2nd station fish. In the 1st station, it was found that there was severe pathological damage in the species.

**4. Discussion**

According to the results of the chemical analysis of the water and sediment samples of the Karasu River, the most polluted station in terms of water was found to be the 2nd station, and the most polluted station in terms of sediment was the 1st station. The data show that the selected stations of the river are contaminated in different amounts. This situation is undoubtedly a natural result of the stations being closer to the sources of pollution. The surface water samples of the 1st and 2nd stations of the Karasu River were of grade IV quality, i.e. very contaminated water. This situation clearly shows the level of pollution of the points where the river leaves the city center of Erzurum. In previous studies conducted in the research area, similar results were recorded [16,17]. In one of these studies, it was reported that the amount of heavy elements in the water

| Heavy metals | Station 1 | Station 2 | Station 3 | Station 4 | TS III | TS IV | Classis of the samples |
|--------------|-----------|-----------|-----------|-----------|-------|-------|------------------------|
| Ti           | 206.5 ± 15.5’ | 192.1 ± 14.6’ | 120.5 ± 9.8 | 113.1 ± 7.5 | -     | -     | -                      |
| V            | 158.9 ± 12.5’ | 180.7 ± 11.8’ | 111.9 ± 6.6 | 108.5 ± 5.5 | -     | -     | -                      |
| Cr           | 13.1 ± 1.8’   | 12.1 ± 2.1’   | 6.7 ± 1.2   | 8.55 ± 1.5  | 0.2   | < 0.2 | IV                     |
| Mn           | 7.7 ± 1.4’    | 9.3 ± 1.5’    | 5.7 ± 1.3   | 5.5 ± 1.1   | 3.0   | > 3.0 | IV                     |
| Fe           | 7.8 ± 1.6’    | 8.4 ± 1.3’    | 5.5 ± 1.1’  | 4.1 ± 0.9   | 5.0   | > 5.0 | IV                     |
| Co           | 1.2 ± 0.1’    | 1.26 ± 0.2’   | 0.8 ± 0.1   | 0.7 ± 0.1   | 0.2   | > 0.2 | IV                     |
| Ni           | 1.01 ± 0.1’   | 1.17 ± 0.1’   | 0.74 ± 0.1  | 0.8 ± 0.1   | 0.2   | > 0.2 | IV                     |
| Cu           | 0.82 ± 0.1’   | 0.88 ± 0.1’   | 0.57 ± 0.1  | 0.51 ± 0.1  | 0.2   | > 0.2 | IV                     |
| Zn           | 0.61 ± 0.1’   | 0.66 ± 0.1’   | 0.46 ± 0.1  | 0.42 ± 0.1  | 2     | > 2   | -                      |
| As           | 1.46 ± 0.2’   | 1.55 ± 0.2’   | 1.27 ± 0.1  | 1.14 ± 0.1  | 0.1   | > 0.1 | IV                     |
| Se           | 0.35 ± 0.05’  | 0.24 ± 0.01’  | 0.20 ± 0.01’| 0.11 ± 0.01 | 0.02  | > 0.02| IV                     |
| Br           | 0.23 ± 0.01’  | 0.20 ± 0.01’  | 0.13 ± 0.01 | 0.10 ± 0.01 | -     | -     | -                      |
| Sr           | 0.20 ± 0.01’  | 0.34 ± 0.02’  | 0.14 ± 0.01 | 0.10 ± 0.01 | -     | -     | -                      |
| Pb           | 2.96 ± 0.6’   | 2.55 ± 0.5’   | 0.69 ± 0.1’ | 0.29 ± 0.01 | 0.05  | > 0.05| IV                     |

| Physicochemical parameters |
|-----------------------------|
| Temperature (°C)            | 25 ± 5.5’ | 25 ± 5.7’ | 23 ± 4.8’ | 21 ± 4.1 | 30   | > 30 | -                |
| pH                          | 8.06 ± 1.4 | 8.03 ± 1.2 | 7.74 ± 1.6 | 7.81 ± 1.5 | 8.5  | 9.0  | -                |
| Dissolved oxygen (mg/L)     | 4.31 ± 0.8’ | 5.60 ± 1.1’ | 6.32 ± 1.5 | 7.62 ± 1.0 | 3    | < 3  | -                |

Values are expressed as mean ± standard errors. Asterisk shows statistical difference compared to reference station (Station 4). TS III; Turkish Standard III means the surface water are slightly polluted. TS IV; Turkish Standard IV means the surface water are polluted.
and sediment samples from the rivers and the surrounding wetlands differed among stations, depending on the proximity to the industrial resources, and the intensity of the motor vehicle traffic. Similarly, some metals were reported to be above acceptable limits according to water quality classification [16]. In another study, it was also reported that the levels of Cd, Al, As, Pb, and Mn metals in the surface water of Aşkale station, collected in 2013, were above the Turkish Standards Institute (TSE) standards [17]. In our study, it was observed that the amount of all other metals in sediment was higher than the amount in water except Ti and V. This is because, as Saeed and Shaker [18] reported, sediment is a kind of reservoir for all pollutants and all organic matter originating from the ecosystem.

Table 2. The mean metal concentrations in sediment samples (ppm-mg/kg) and the status of sediment quality according to standards.

| Heavy metals | Station 1      | Station 2      | Station 3      | Station 4      | TEL* | PEL* | ERM* |
|--------------|----------------|----------------|----------------|----------------|------|------|------|
| Ti           | 1.45 ± 0.5     | 1.1 ± 0.1      | 1.2 ± 0.2      | 0.9 ± 0.1      |      |      |      |
| V            | 86.2 ± 7.4     | 91.5 ± 4.8     | 68.1 ± 2.1     | 55.4 ± 7.2     |      |      |      |
| Cr           | 452.6 ± 21.3    | 410.4 ± 17.4   | 400.6 ± 15.6   | 150.6 ± 21.3   | 37.3 | 90  | 145  |
| Mn           | 34.9 ± 6.5     | 26.2 ± 4.3     | 18.2 ± 3.6     | 22.6 ± 6.4     |      |      |      |
| Fe           | 330.3 ± 18.6   | 141.3 ± 15.5   | 138.1 ± 19.3   | 130.9 ± 11.2   |      |      |      |
| Co           | 18.5 ± 2.1     | 16.2 ± 3.8     | 13.6 ± 2.1     | 11.5 ± 0.7     |      |      |      |
| Ni           | 12.7 ± 2.2     | 8.8 ± 1.1      | 5.4 ± 0.3      | 1.3 ± 0.1      | 18   | 36   | 50   |
| Cu           | 90.8 ± 6.1     | 50.4 ± 3.1     | 38.4 ± 2.2     | 36.1 ± 2.1     | 35.7 | 197  | 390  |
| Zn           | 4.1 ± 0.4      | 3.3 ± 0.3      | 3.1 ± 0.2      | 2.7 ± 0.1      | 123  | 315  | 270  |
| As           | 6.2 ± 0.7      | 5.9 ± 0.2      | 4.9 ± 0.3      | 4.6 ± 0.2      | 5.9  | 17   | 85   |
| Se           | 11.6 ± 1.1     | 10.1 ± 0.1     | 8.5 ± 1.1      | 10.4 ± 0.7     |      |      |      |
| Br           | 1.2 ± 0.1      | 0.9 ± 0.1      | 0.7 ± 0.1      | 0.8 ± 0.1      |      |      |      |
| Sr           | 4.9 ± 0.6      | 5.6 ± 0.6      | 3.1 ± 0.3      | 3.4 ± 0.1      |      |      |      |
| Pb           | 13.3 ± 1.1     | 11.1 ± 0.7     | 9.6 ± 0.8      | 9.1 ± 0.5      | 35   | 91.3 | 110  |

Every superscript with “a, d, and e” for heavy metal concentrations mean the concentration exceeds the TEL value; every superscript with “b” for heavy metal concentrations mean the concentration exceeds the PEL value; every superscript with “c” for heavy metal concentrations mean the concentration exceeds the ERM value. *TEL; threshold effect level, *PEL; probable effects level, *ERM; effect range median.

Table 3. The mean concentrations (µg/g) of heavy metals in the liver tissues of Alburnus mossulensis captured in 2015 and 2016 years (mean ± SE) (n = 32).

| Metals (µg/g) | Station 1 2015 | Station 1 2016 | Station 2 2015 | Station 2 2016 | Station 3 2015 | Station 3 2016 | Station 4 2015 | Station 4 2016 |
|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Cr           | 2.05 ± 0.40   | 2.19 ± 0.30   | 1.98 ± 0.30   | 2.59 ± 0.20   | 2.11 ± 0.10   | 2.60 ± 0.40   | 0.10 ± 0.01   | 0.13 ± 0.02   |
| Mn           | 0.26 ± 0.01   | 0.39 ± 0.03   | 0.17 ± 0.01   | 0.40 ± 0.02   | 0.12 ± 0.06   | 0.38 ± 0.03   | 0.10 ± 0.01   | 0.24 ± 0.04   |
| Ni           | 0.13 ± 0.06   | 0.22 ± 0.08   | 0.18 ± 0.02   | 0.11 ± 0.04   | 0              | 0              | 0              | 0              |
| Cu           | 2.02 ± 0.60   | 2.38 ± 0.60   | 1.96 ± 0.50   | 2.78 ± 0.50   | 2.53 ± 0.40   | 3.42 ± 0.60   | 0.02 ± 0.01   | 0.08 ± 0.01   |
| Zn           | 1.31 ± 0.40   | 1.70 ± 0.20   | 1.17 ± 0.20   | 1.90 ± 0.30   | 0.49 ± 0.10   | 0              | 0              | 0              |
| As           | 0.02 ± 0.00   | 0.04 ± 0.01   | 0.01 ± 0.00   | 0.02 ± 0.00   | 0              | 0              | 0              | 0              |
| Cd           | 0.02 ± 0.01   | 0.04 ± 0.01   | 0.09 ± 0.02   | 0.14 ± 0.04   | 0              | 0              | 0              | 0              |
| Pb           | 2.58 ± 0.60   | 3.20 ± 0.80   | 2.13 ± 0.50   | 2.49 ± 0.60   | 1.46 ± 0.30   | 2.24 ± 0.40   | 0              | 0              |

“0” indicates the undetected metal levels.
The Karasu River, which receives all the pollutants of the Karasu Basin, is resting in a pond in front of the Aşkale Cement Factory, built in 2014, and it is ensured that the pollutants in it precipitate. Despite this, when compared with the previous studies conducted in the same region [16,17], the current study shows that metal pollution in the Karasu River continues.

In the study, the mean concentrations of Cr, Mn, Ni, Cu, Zn, As, Cd, and Pb in the liver tissues of \textit{A. mossulensis} from the stations were determined. The metal levels were found to be higher in the tissues of the fish from stations 1 and 2, where the pollution load was high, compared to other stations. In a study conducted in the Karasu River, Cd, Cu, Fe, Ni, Pb, and Zn levels in the muscle, liver and gill tissues of \textit{Capoeta capoeta umbla} and \textit{Chalcalfurnius mossulensis} were reported to change according to the contamination level of the stations [19]. Due to the high industrial impact, heavy metal level is naturally high in the tissues of fish caught from areas where waste water pollution is intense. In the study investigating As, Cd, Hg, and Pb levels in different fish species from the Lake Nansi, which was under the influence of industrial and mining activities, heavy metal accumulation was found to be high in the tissues of fish [20]. Similarly, high accumulation of Fe in the all organs of \textit{Channa punctatus} from heavy-metal-polluted canal was reported by Javed et al. [21]. Ahmad et al. [22] also reported high levels of heavy metals (Cd Cr, Cu, Hg, Pb) in the gill, liver, and kidney of two fish species from the Dam Lake of Wadi Namar receiving agricultural runoff and urban wastewater.

Aquatic environmental pollution causes an increase in HSI. Therefore, HSI is seen as a good indicator for chemical water pollution and provides information on the health

### Table 4. Mean HSI values of \textit{A. mossulensis} according to the stations and years.

| Station | 2015 mean HSI (± SD) | 2016 mean HSI (± SD) |
|--------|----------------------|----------------------|
| 1.     | 1.36 ± 0.06 \(^a\)   | 1.50 ± 0.09 \(^a\)   |
| 2.     | 1.19 ± 0.04 \(^a\)   | 1.38 ± 0.03 \(^a\)   |
| 3.     | 0.83 ± 0.09 \(^b\)   | 1.07 ± 0.07 \(^b\)   |
| 4.     | 0.28 ± 0.08 \(^c\)   | 0.51 ± 0.05 \(^c\)   |

Differences between the averages indicated by the different letters in the same column are statistically significant (P < 0.05).
status of the fish [23]. In this study, significant differences between HSI and stations were determined according to the degree of pollution. Compared to station 4, higher HSI values were obtained in the other three stations. The increase in HSI was associated with the presence of pollutants in the aquatic environment, as suggested by the authors. Authman [24] reported that HSI in fish exposed to high-dose Al was higher than the control group and this situation was related to lipid accumulation in liver of the fish. The author also expressed that the presence of fatty change in histological sections supported the high HSI value. Monsefrad et al. [25] reported that some heavy and toxic metals were detected in the liver and muscle of fish caught at the Caspian Sea, and the metals increased the fish liver weight and there was a positive correlation between Cd level and HSI. In the present study, HSI values and liver histopathology findings revealed that long-term exposure to heavy metals detected in the water and sediment of the Karasu River could have negative effects on physiological functions.

In this study, the pathological abnormalities (lamellar degenerations, hyperplasia, edema, vacuolization, vasodilatation, congestion, fusion, hypertrophy) detected in the gill tissues of the fish showed that metal contamination caused significant damage. Many heavy metals, especially Hg, Pb, Cu, Zn, and As, are toxic to fish tissues and organs. Their high affinity to amino acids, enzymes, proteins, and neurological receptors (tendency to bind) is influential in the formation of toxic effects.
study investigating the relationship between water quality and gill pathology in Portugal, histopathological damages (edema, fusion in lamellae, necrosis, vasodilatation) in fish were associated with the higher concentrations of heavy metals, in particular Zn and Pb, at the polluted point of the river [26]. In another study, Javed et al. [21] reported that the waste water of thermal power plants contained heavy metals and that this wastewater affected the physicochemical properties of water, and noted that metals caused necrosis, rupture of the epithelium, lamellar fusion, hyperplasia, and lymphocyte infiltration in the gills of *Channa punctatus*. Since histopathological changes occurring in the gills, which are the first target organs for pollutants, can generate potential biomarkers, many investigators have studied gill damage [27,28]. In this study, the lesions detected in fish caught from heavily contaminated areas showed that the fish were stressed in their habitats.

Fish liver is an important organ that functions both in anabolism and catabolism, thus controlling many vital functions. The liver, whose one of the functions is to remove contaminants from the blood, is reported to be a good indicator for the pollution in the aquatic environment [29]. Because the liver is the main organ of the detoxification process, it is one of the organs that is most affected by toxic substances. In this context, it was observed that the histological damages (infiltration, melanomacrophage aggregations, sinusoidal dilatation, vacuolization,
Table 5. DTC values calculated for each tissue of the samples collected different stations and years.

| Stations | Gill 2015 | Gill 2016 | Liver 2015 | Liver 2016 | Kidney 2015 | Kidney 2016 |
|----------|-----------|-----------|------------|------------|-------------|-------------|
| 1.       | 75.66 ± 0.23a | 78.79 ± 0.20a | 83.11 ± 0.10a | 87.94 ± 0.35a | 56.20 ± 0.27a | 66.95 ± 0.21a |
| 2.       | 43.6 ± 0.21b | 51.99 ± 0.11b | 75.12 ± 0.20b | 81.27 ± 0.32b | 37.83 ± 0.29b | 54.04 ± 0.23b |
| 3.       | 22.62 ± 0.16c | 33.40 ± 0.26c | 35.98 ± 0.25c | 45.91 ± 0.10c | 13.25 ± 0.18c | 28.86 ± 0.20c |
| 4.       | 9.90 ± 0.04d | 8.69 ± 0.10d | 8.57 ± 0.32d | 6.05 ± 0.15d | 8.11 ± 0.34d | 9.30 ± 0.42d |

Results are mean ± SE. Means with a different letter in the same column for each station are significantly different at P < 0.05.

congestion, vascular and cellular degenerations, fibrosis) detected in the liver of the fish were good biomarkers indicating the pollution of the river and toxicity. Indeed, the increase in melanomacrophage occurs in toxic cases, and indicates heavy metal concentrations [30]. Congestion is an increase in blood flow to the hepatic tissue and is an indication of the detoxification mechanism, and therefore, congestion is considered as an indication of the stress that occurs in fish with the presence of xenobiotic chemicals [31]. The present study revealed a correlation between metal contamination and liver pathologies. Similarly, various researchers have reported several pathologies in the livers of different fish species in connection with heavy metal contamination in different rivers [32,33]. In a study, Cd exposure was reported to damage the characteristic structure of the liver and cause infiltration in erythrocytes, hemorrhage, vacuolization in hepatocytes, and infiltration in leukocytes and sinusoids [34].

The kidney ensures the excretion of excess water entering the body through gills in freshwater fish especially. Therefore, toxic substances in the water directly cause damage to the kidneys. Metals cause various pathologies by inhibiting Phase-I and Phase-II reactions [35] in the kidney, and the kidney is damaged when exposed to metals for a long time [36]. In this study, it is not a coincidence that the histopathology of the kidneys (dilatation and degeneration in the tubule, vacuolization, glomerulopathy, protein accumulation, fibrosis, necrosis) increased in the most polluted areas of the river with water and sediment analysis findings. Jafarizadeh et al. [37] reported, edema, necrosis, urolith, hemorrhage, and degeneration in the kidneys depending on the water quality in fish (Hypophthalmichthys molitrix) obtained from four different points in Iran. It is obvious that heavy metals detected in high concentrations in the aquatic environment cause pathologies leading to serious deterioration of metabolic and physiological systems in fish. In the kidneys of the fish caged in the river where the waste water from sewage treatment plants are discharged, inflammation and necrosis in hematopoietic tissue, dilatation and protein residues in proximal and distal tubules, macrophage infiltration, necrosis, with hypertrophy in the tubular cells, vacuolization, nuclear swelling, necrotic glomerulus, and dilatation in capillaries were reported [38]. In another study where fish were caught from 3 sampling points in the Tapee River, dilatation in the Bowman’s capsule and the glomerulus, and necrosis in some tubule cells were reported [39]. At this point, it is understood that the renal pathologies detected in the present research reflect the pollution in the habitats of the fish as biomarkers.

The findings of our study conducted on the Karasu River for 2 years clearly show that the metal pollution in the river continues and the fish here are affected. Although A. mossulensis are histologically affected, they still survive despite the pollution load of the river. However, if pollution continues this way, it is obvious that it will be difficult for these species to survive. Conservation of our natural resources and species living here is essential for the continuity of ecological life.

Conflict of interest
The authors declare that they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References
1. Matsumoto ST, Mantovani MS, Malagutti MIA, Dias AL, Fonseca IC et al. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticus and chromosome aberrations in onion root tips. Genetics and Molecular Biology 2006; 29 (1): 148-158. doi: 10.1590/S1415-47572006000100028
2. Van der Oost R, Beyer J, Vermeulen NP. Fish bioaccumulation and biomarkers in environmental risk assessment. Environmental Toxicology and Pharmacology 2003; 13 (2): 57-149. doi: 10.1016/s1382-6689(02)00126-6
3. López-López E, Sedeño-Díaz JE. Biological indicators of water quality: The role of fish and macroinvertebrates as indicators of water quality. Environmental Indicators 2015; 643-661. doi: 10.1007/978-94-017-9499-2_37

4. Kelly SA, Havrilla CM, Brady TC, Abrama KH, Levin ED. Oxidative stress in toxicology established mammalian and emerging piscine model system. Environmental Health Perspectives 1998; 106 (7): 375-384. doi: 10.1289/ehp.98106375

5. Teh SJ, Adams SM, Hinton DE. Histopathological biomarkers in freshwater fish populations exposed to different types of contaminant stress. Aquatic Toxicology 1997; 37: 51-70. doi: 10.1016/S0166-445X(96)00808-9

6. Hibiya T. An atlas of fish histology. Normal and Pathological Features. Tokyo, Japan; 1982: 147.

7. Çelikkale S. Balık Biyolojisi. Karadeniz Teknik Üniversitesi Sürmene Deniz Bilimleri ve Teknolojisi Yüksek Okulu 1991; 1-387 (in Turkish).

8. Geldiay R, Balk S. Türkiye Tatlısu Balıkları. Ege Üniversitesi Su Ürünleri Fakültesi Yayınları No: 46 VI. Baskı Bornova, İzmir, 2009; 644 (in Turkish).

9. Sönmez AY, Hisar O, Yañık T. Determination of heavy metal pollution in Karasu River and classification of water quality. Atatürk Üniversitesi Ziraat Fakültesi Dergisi 2012; 43 (1): 69-77 (in Turkish with an abstract in English).

10. Sönmez AY, Haşıloğlu S, Hisar O, Arslan Mehan HN, Kaya H. Fuzzy logic evaluation of water quality classification for heavy metal pollution in Karasu Stream, Turkey. Ekoloji 2013; 22 (87): 43-50 (in Turkish with an abstract in English). doi: 10.5053/ekoloji.2013.876

11. Shanbehzadeh S, Dastjerdi MV, Hassanzadeh A, Kiyanizadeh M. Concentration of metals in fish tissues from polluted wetland environments, Saudi Arabia. Chemosphere 2012; 88 (8): 1028-1035. doi: 10.1016/j.chemosphere.2012.04.001

12. Abdel-Moneim AM, Al-Kahtani MA, Elmenshawy OM. Histopathological biomarkers in gills and liver of Oreochromis niloticus from polluted wetland environments, Saudi Arabia. Ecotoxicology and Environmental Safety 2005; 61 (3): 287-312. doi: 10.1016/j.ecoenv.2004.09.003

13. Poleksic V, Mitrovic- Tutundzic V. Fish gills as a monitor of sublethal and chronic effects of pollution. In: Müller R and Lloyd R (Editors). Sublethal and Chronic Effects of Pollutants on Freshwater Fish, Cambridge Univ. Press, Cambridge, 1994. pp. 339-352.

14. Hoyle I, Shaw BJ, Handy RD. Dietary copper exposure in the African walking catfish, Clarias gariepinus: Transient osmoregulatory disturbances and oxidative stress. Aquatic Toxicology 2007; 83: 62-72. doi: 10.1016/j.aquatox.2007.03.014

15. MacDonald DD, Ingersoll CG, Berger TA. Development and evaluation of consensus based sediment quality guidelines for freshwater ecosystems. Archives of Environmental Contamination and Toxicology 2000; 39: 20-31. doi: 10.1007/s002440010075

16. Aydoğan Z, İncekara Ü, Şişman T, Gürol A. Monitoring chemical contamination levels in Karasu River based on the use of Enochrus spp.(Hydrophilidae) and Chondrostoma regium (Cyprinidae). The Turkish Journal of Occupational/ Environmental Medicine and Safety 2017; 1 (3): 486-492.

17. Dane H, Şişman T. A histopathological study on the freshwater fish species chub (Squalius cephalus) in the Karasu River, Turkey. Turkish Journal of Zoology 2017; 41 (1): 1-11. doi:10.3906/zoo-1509-21

18. Saeed SM, Shaker IM. Assessment of heavy metals pollution in water and sediment and their effect on Oreochromis niloticus in the Northern Delta Lakes, Egypt. 8. International Symposium on Tilapia in Aquaculture; 2008. pp. 475-490.

19. Sönmez AY, Yağanoğlu AM, Arslan G, Hisar O. Metals in two species of fish in Karasu River. Bulletin of Environmental Contamination and Toxicology 2012; 89: 1190-1195. doi: 10.1007/s00128-012-0841-2

20. Pengfei L, Zhang J, Xie H, Liu C, Liang S, Ren Y, Wang W. Heavy metal bioaccumulation and health hazard assessment for three fish species from Nansi Lake, China. Bulletin Environmental Contamination and Toxicology 2015; 94 (4): 431-436. doi: 10.1007/s00128-015-1475-y

21. Javed M, Usmani N, Ahmad I, Ahmad M. Studies on the oxidative stress and gill histopathology in Channa punctatus of the canal receiving heavy metal-loaded effluent of Kasimpur Thermal Power Plant. Environmental Monitoring and Assessment 2015; 187 (1): 4179. doi: 10.1007/s10661-014-4179-6

22. Ahmad Z, Al-Ghanim KA, Al-Balawi HFA, Al-Misned F, Mahboob S, Suliman EM. Accumulations of heavy metals in the fish Oreochromis niloticus, and Poecilia latipinna and their concentration in water and sediment of Dam Lake of Wadi Namar, Saudi Arabia. Journal of Environmental Biology 2015; 36 (1): 295-299.

23. Pyle GG, Rajotte JW, Couture P. Effects of industrial metals on wild fish populations along a metal contamination gradient. Ecotoxicology and Environmental Safety 2005; 61 (3): 287-312. doi: 10.1016/j.ecoenv.2004.09.003

24. Authman MMN. Environmental and experimental studies of aluminium toxicity on the liver of Oreochromis niloticus (Linnaeus, 1758) fish. Life Science Journal 2011; 8 (4): 764-776.

25. Monsef F, Imanpour Namin J, Heidary S. Concentration of heavy and toxic metals Cu, Zn, Cd, Pb and Hg in liver and muscles of Rutilus frisii kutum during spawning season with respect to growth parameters. Iranian Journal of Fisheries Sciences 2012; 11 (4): 825-839.

26. Fonseca AR, Sanches Fernandes LF, Fontainhas-Fernandes A, Monteiro SM, Pacheco FAL. From catchment to fish: Impact of anthropogenic pressures on gill histopathology. Science of the Total Environment 2016; 550: 972-986. doi: 10.1016/j.scitotenv.2016.01.199
27. Neves MP, Amorim JPA, Delariva RL. Influence of land use on the health of a detritivorous fish (Ancistrus mullerae) endemic to the Iguassu ecoregion: relationship between agricultural land use and severe histopathological alterations. Environmental Science and Pollution Research, 2018; 25 (12): 11670-11682. doi: 10.1007/s11356-018-1283-0

28. Nimet J, Amorim JPA, Delariva RL. Histopathological alterations in Astyanax bifasciatus (Teleostei: Characidae) correlated with land uses of surroundings of streams. Neotropical Ichthyology 2018 16 (1): e170129. doi: 10.1590/1982-0224-20170129

29. Saleh HH. Fish liver as indicator of aquatic environmental pollution. Bulletin of the Institute of Oceanography and Fisheries A.R.E. 1982; 8 (1): 69-79.

30. Poleksic V, Lenhardt M, Jaric I, Djordjevic D, Gacic Z et al. Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (Acipenser ruthenus Linnaeus, 1758). Environmental Toxicology and Chemistry 2010; 29 (3): 515-521. doi: 10.1002/etc.82

31. Rezende KFO, Santos RM, Borges JCS, Salvo LM, Silva JRMC. Histopathological and genotoxic effects of pollution on Nile Tilapia (Oreochromis niloticus, Linnaeus, 1758) in the billings reservoir (Brazil). Toxicology Mechanisms and Methods 2014; 24 (6): 404-411. doi: 10.3109/15376516.2014.925020

32. Stentiford GD, Massoud MS, Al-Mudhhi S, Al-Sarawi MA, Al-Enezi M et al. Histopathological survey of contaminant related biological effects in species of fish and shellfish collected from Kuwait Bay, Arabian Gulf. Marine Environmental Research 2014; 98: 60-67. doi: 10.1016/j.marenvres.2014.03.005

33. Feist SW, Stentiford GD, Kent ML, Ribeiro Santos A, Lorance P. Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. Marine Environmental Research 2015; 106: 42-50. doi: 10.1016/j.marenvres.2015.02.004

34. Younis EM, Abdel-Warith AA, Al-Asgha NA, Ebaid H, Mubarak M. Histological changes in the liver and intestine of Nile Tilapia, Oreochromis niloticus, exposed to sublethal concentrations of cadmium. Pakistan Journal of Zoology 2013; 45 (3): 833-841.

35. Förblin L, Haux C, Karlsson-Norr gren L, Runn P, Larsson A. Biotransformation enzyme activities and histopathology in rainbow trout, Salmo gairdneri, treated with cadmium. Aquatic Toxicology 1986; 8 (1): 51-64.

36. Spry DJ, Wiener JG. Metal bioavailability and toxicity to fish in low-alkalinity lakes a critical review. Environmental Pollution 1991; 71: 243-304. doi: 10.1016/0269-7491(91)90034-t

37. Jafarizadeh M, Peyghan R, Mohammadian B. The reports of lesions in kidney and intestine of apparently normal cultured silver carp (Hypophtalmictys molitrix). Advances in Bioscience and Biotechnology 2012; 3 (2): 115-120. doi:10.4236/abb.2012.32017

38. Vincze K, Scheil V, Kuch B, Köhler HR, Triebskorn R. Impact of wastewater on fish health: a case study at the Neckar River (Southern Germany) using biomarkers in caged Brown trout as assessment tools. Environmental Science and Pollution Research 2015; 22 (15): 11822-11839. doi: 10.1007/s11356-015-4398-6

39. Senarat S, Kettratad J, Poolprasert P, Yenchum W, Jiraungkoorskul W. Histopathological finding of liver and kidney tissues of the yellow mystus, Hemibagrus filamentus (Fang and Chaux, 1949), from the Tapee River, Thailand. Songklanakarin Journal Science Technology 2015; 37 (1): 1-5.