Research Article

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

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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 Fırat (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 Fırat 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

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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, Ilıca 3rd–39° 57′ 10.6″ N 41° 04′ 15.2″ E, and Dumlu 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 Ilıca station was less polluted, and the Dumlu 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 = $(1 \times \Sigma I)$ + $(10 \times \Sigma II)$ + $(100 \times \Sigma III)$. 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[1], 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

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[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].

Table 1. The mean metal concentrations and physicochemical parameters of water samples (ppm), and the water quality classes of the river water according to Turkish Water Pollution Control Regulation.

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.

(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 DTC values for all tissues are given in Table 5. According to the data, the DTC values of 2015 were lower than that of 2016. The highest DTC value was obtained from the 1st station. The increase in the DTC value among stations was statistically significant (P < 0.05). The liver had the highest DTC 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 DTC 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 DTC 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

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[a,b,c]	410.4 ± 17.4[a,b,c]	400.6 ± 15.6[a,b,c]	150.6 ± 21.3[a,b]	37.3[a]	90[b]	145[c]
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[d]	50.4 ± 3.1[d]	38.4 ± 2.2[d]	36.1 ± 2.1[d]	35.7[d]	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[e]	5.9 ± 0.2[e]	4.9 ± 0.3	4.6 ± 0.2	5.9[e]	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		Station 2		Station 3		Station 4	
	2015	2016	2015	2016	2015	2016	2015	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.

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 an 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 4. Mean HSI values of *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).

Figure 2. Histological alterations in the gills of *A. mossulensis.* A; samples of 1st station, B; samples of 2nd station. Triangle: hyperplasia of the respiratory epithelium. Brown arrow: curling in the secondary lamella. Black arrow: degeneration in the secondary lamella. White arrow: vasodilatation. Red arrow: hyperplasia in the secondary lamellae. Green arrow: separation of the lamellar epithelium. Orange arrow: congestion. Yellow arrow: hypertrophy in mucous cell. Arrow head: oedema and shortening in the secondary lamellae. Circle: vacuolization. Asterisk: fusion (H & E).
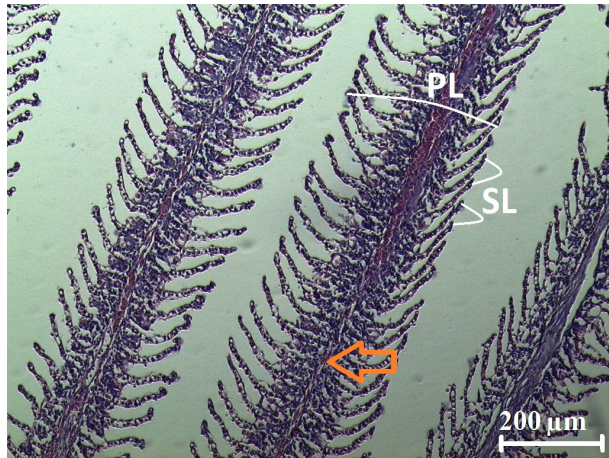

Figure 1. Normal gill filaments in the *A. mossulensis* from 4th station. PL: primer lamella, SL: seconder lamella, Arrow: central vein (H & E).

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 *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 *Capoeta capoeta umbla* and *Chalcalburnus 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
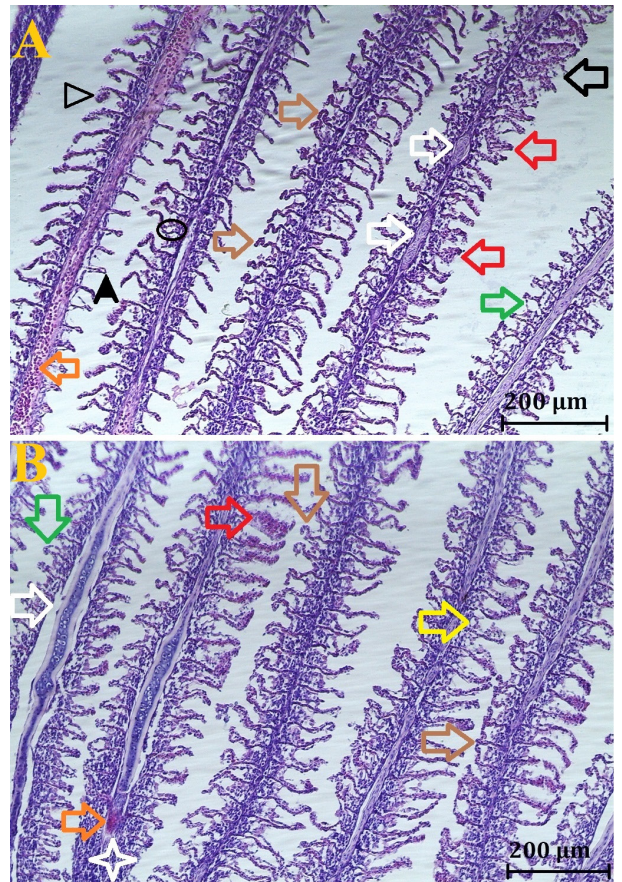
pollution is intense. In the study investigating As, Cd, Hg, and Pb levels in different fish species from the Lake Nansi, which was under the influence of industrial and mining activities, heavy metal accumulation was found to be high in the tissues of fish [20]. Similarly, high accumulation of Fe in the all organs of *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
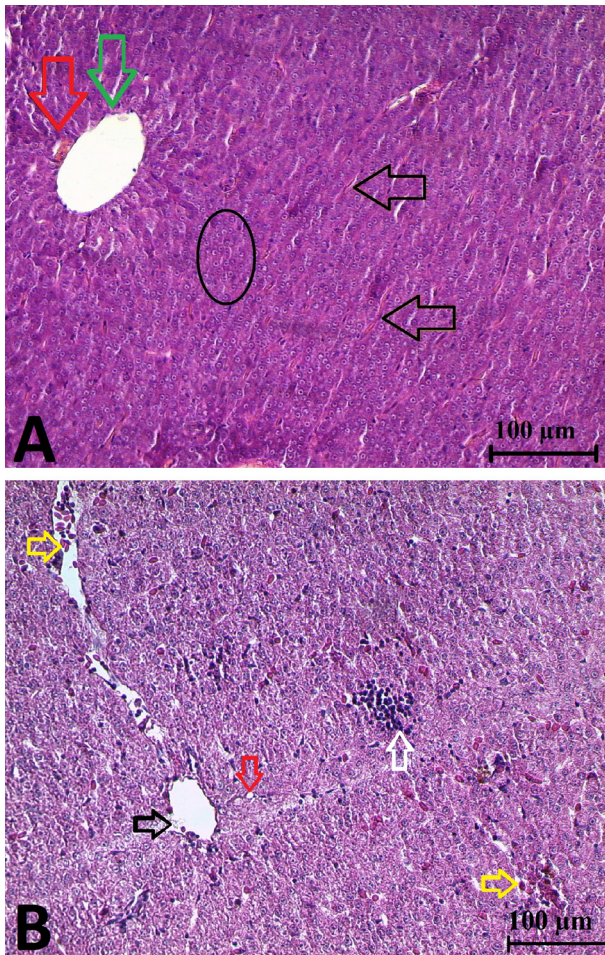
Figure 3. Structure of normal liver in the *A. mossulensis* from 4th station (A). Red arrow: melanomacrophage. Black arrows: sinusoids. Green arrow: central vein. Circle: hepatocytes (H & E). Histological alterations in the *A. mossulensis* liver tissue from 3rd station (B). Yellow arrow: congestion. Black arrow: degeneration of central vein epithelium. Red arrow: vacuolization. White arrow: infiltration in the parenchyma (H & E).
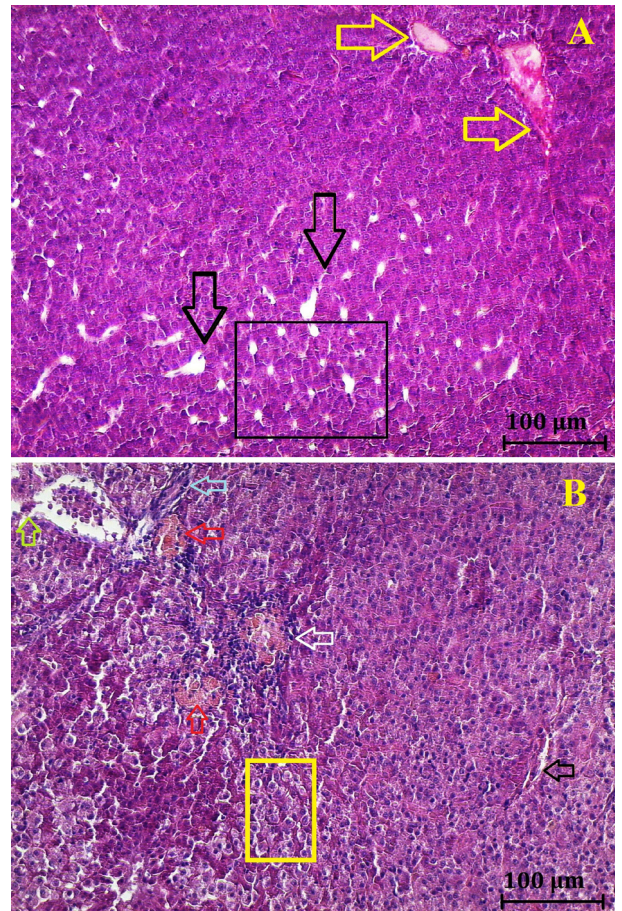

Figure 4. Histological alterations in the liver of *A. mossulensis*. A; samples of 2nd station, B; samples of 1st station. Yellow arrow: congestion. Black arrow: sinusoidal dilatation. Red arrow: melanomacrophage. White arrow: infiltration in the parenchyma. Green arrow: vascular degeneration. Blue arrow: fibrosis. Black square: vacuolization. Yellow square: degeneration in hepatocytes (H & E).

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 other researchers. 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. In a
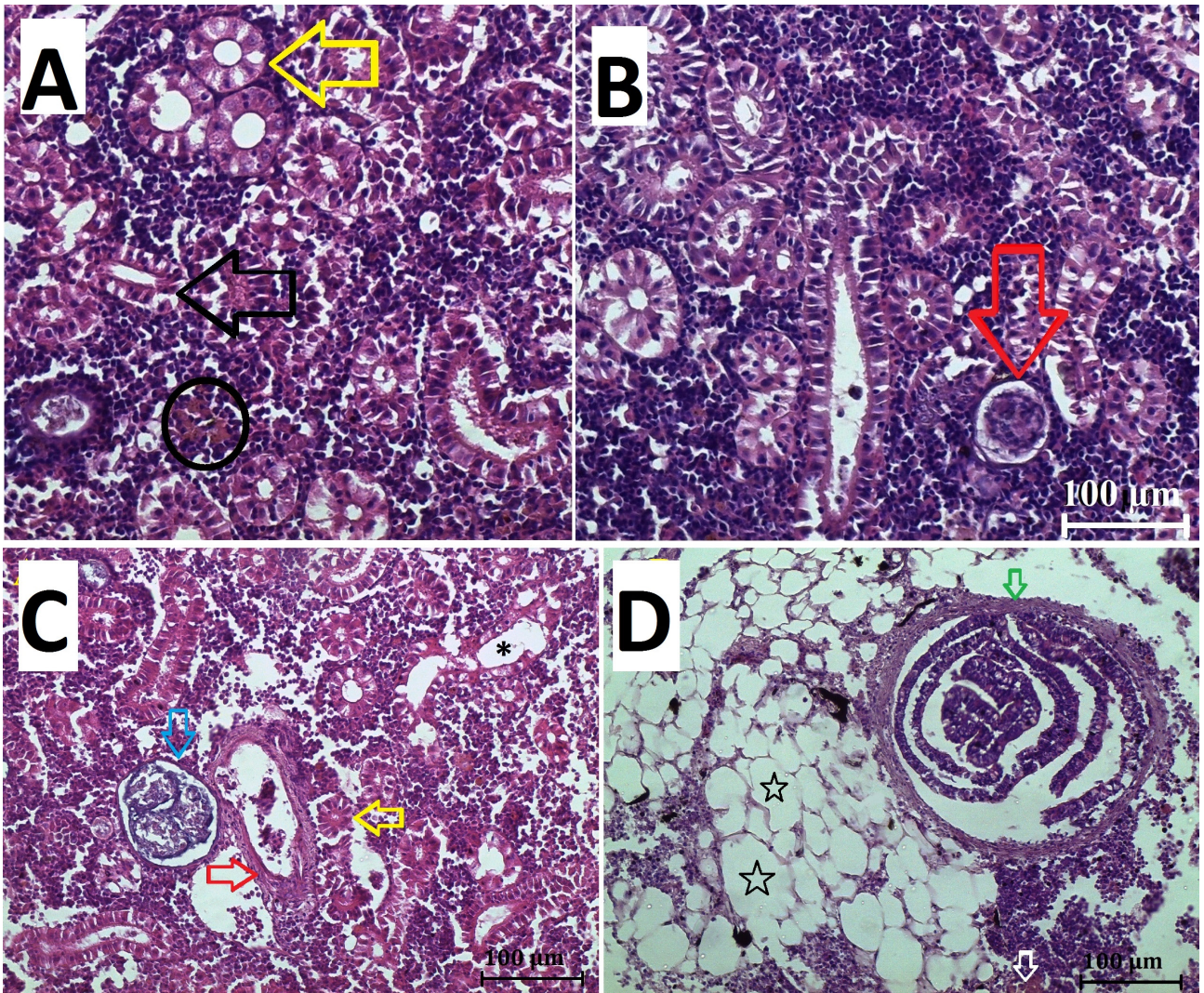
Figure 5. Structure of normal kidney in the *A. mossulensis* from 4th station (A, B). Yellow arrow: proximal tubule. Black arrow: distal tubule. Red arrow: Bowman's capsule. Circle: melanomacrophage (H & E). Histological alterations in the kidney of *A. mossulensis* C; samples of 1st station, D; samples of 2nd station. Blue arrow: protein accumulation. Yellow arrow: degeneration in the tubules and parenchymal detachment. Red arrow: fibrosis and necrosis. Green arrow: glomerulopathy. White arrow: melanomacrophage. Asterisk: dilatation in the tubular lumen. Stars: parenchymal vacuolization (H & E).

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

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.

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