TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2021) 45: 505-516
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doi:10.3906/zoo-2105-19

Determination of oxidative, genotoxic, and histopathologic effects of metal pollution on the fish fauna inhabiting Karasu River, Turkey

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Received: 15.05.2021 • **Accepted/Published Online:** 24.10.2021 • **Final Version:** 15.11.2021

Abstract: The water quality of rivers located in areas close to major cities is deteriorating significantly due to increased pollution. The Karasu River, which passes through Erzurum city Turkey, is getting polluted day by day due to domestic and industrial wastewater. In the study, various assessments were made in three different fish species to determine the impact of possible contamination here. *Capoeta capoeta*, *Alburnus mossulensis* and *Squalius cephalus* were caught from four stations of the river. Micronucleus test was applied to find out genotoxic alterations, and plasma total oxidant status (TOS) and total antioxidant status (TAS) were measured to determine oxidative effects. Histological changes in fish brain were also assessed by histopathological alterations index (HAI). The highest serum TOS levels and the lowest TAS levels were detected in fish species inhabiting polluted stations. A high erythrocytic nuclear abnormality frequency and HAI values were observed in all species from the Aşkale stations. The abnormalities observed in the peripheral blood cells were micronucleus, binucleus, kidney-shaped nucleus, notched nucleus, lobed nucleus and bud nucleus. Histological damages like dilatation, congestion, vacuolization in Purkinje layer, infiltration, hyperemia, degenerations, and axonopathy were noticed in all fish brains. It was determined that histopathological changes were more common in fish species inhabiting the polluted stations. *S. cephalus* showed to be more sensitive to the pollution. The most important result obtained from this study is that the pollution in the river adversely affects the fish species found in the natural fauna of the river. Histopathological abnormalities, genotoxicity and increased oxidative stress detected in fish are the most important indicators of this situation.

Key words: Metal pollution, Karasu River, Cyprinidae, brain histopathology, genotoxicity, oxidative stress

1. Introduction

The ecological integrity of many rivers and streams are under constant risk due to agricultural, industrial and other anthropogenic activities (Byrne et al., 2015). Thus, vegetations in and around the rivers are also on the decrease, and aquatic biota is adversely affected by toxic substances entering rivers and streams (Topal et al., 2017). Heavy metals constitute the most significant part of these substances (Akpor et al., 2014). Heavy metals, which are at toxic levels in water, sediment and aquatic organisms, cause serious ecological damage that threatens human health along the food chain (Götze et al., 2014).

Aquatic pollution affects the fish in an extremely adverse manner. Mutation, the formation of tumors and cellular deaths are the major abnormalities that the pollution causes on the fish (Viana et al., 2017). Fish are widely used in the evaluation of the aquatic ecosystem, as their biochemical and physiological responses to environmental changes are highly sensitive (Dane and Şişman, 2015, 2017, 2020a; Turan et al., 2020). There is a range of bioindicators used for this purpose. These include the histopathologic evaluation of tissues (Bernet et al., 1999; Dane and Şişman, 2020b), biochemical analyses (Dastan et al., 2017; Oğuz et al., 2018) and the micronucleus test (Fenech, 2000). The brain plays a significant role in the physiology of the fish and has an effective role in fish toxicology. The presence of polyunsaturated fatty acid contents, low levels of enzymatic and nonenzymatic antioxidant capacity in the cerebral cortex (Perez Campo et al., 1993) make the brain more sensitive towards environmental contaminants. Therefore, the studies related to fish brain may yield benefits in the biomonitoring of contamination and provide an early warning system against contaminant exposure (Sarma et al., 2010). A great number of methods are used to identify the oxidative stress. The analyses of total antioxidant status (TAS) (Erel, 2004) and total oxidant status (TOS) reflect the biochemical changes in tissues (Erel, 2005) and the parameters are utilized to monitor the biochemical changes caused by stress factors on the fish (Dastan et al., 2017; Oğuz et al., 2018). The micronucleus test, however,

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is preferred to demonstrate the DNA damage occurring at a chromosomal level (Carrasco et al., 1990; Fenech, 2000). Of the genotoxicity tests, this test is the most important one (Arslan et al., 2015). Other erythrocytic nuclear abnormalities (ENA) are preferred simultaneously with the MN to determine the genotoxicity. ENA sets forth the disorders and cellular deaths that occur during the cell division, as well as DNA damages (Ossana et al., 2016).

There has been an increasing pollution in the Karasu River for the last ten years. It is thought that the river is polluted especially in terms of heavy metals. Fish in the river are adversely affected by this pollution. Previous studies are the most important proof of this situation (Dane and Şişman, 2015; Aydoğan et al., 2017; Dane and Şişman, 2017, Dane and Şişman, 2020a, 2020b). However, no study has yet been conducted on how this pollution affects the nervous system of fish. Therefore, the aim of this study was to evaluate the occurrence of possible histopathological changes in the brains of three different fish species (*Capoeta capoeta*, *Alburnus mossulensis* and *Squalius cephalus*) caught from four regions of the Karasu River. In addition, oxidative stress and genotoxic damage status will be evaluated and a general perspective will be tried to be obtained.

2. Material and methods

For the study, four stations were selected on the Karasu River. The selection was made based on the possible status of pollution in the river (Figure 1). The stations were determined by scanning from the upper part of the river to the lower parts where the pollution load increased. While determining the stations, attention was paid to the regions where fish species were seen. The first station was Dumlu (Station 1: 40°05'36.1"N 41°22'49.0"E). Livestock and pasture farming are carried out around this station. There is also a settlement with a population of 1413. It was rated as the least polluted station. The second station was Ilıca. There is both agricultural activity and 2 factories around the Ilıca station. In addition, the sewage water of the city is given to the river from here. The station was considered as a moderate pollution zone. (Station 2: 39°57'10.6"N 41°04'15.2"E). The third and fourth stations were Aşkale I (Station 3: 39°54'52.7"N 40°40'29.4"E) and Aşkale II (Station 4: 39°56'15.1"N 40°37'25.9"E) regions that were evaluated as very polluted. Aşkale stations are the last areas where the wastes of the whole city are mixed, and there are paint and cement factories nearby. The transport of fertilizers and pesticides used in agricultural activities to the river by surface flows and the mixing of wastewater of industrial enterprises and sewage treatment plants into the river, unfortunately, changes the nature of the river water (Sönmez et al., 2013; Aydoğan et al., 2016; Anonymous, 2016; Dane, 2018). Based on data from Dane and Şişman (2020a) on the environmental status (Karasu River water) for the period June and September 2015–2016, it is clear that metals exceeding the permissible standards. When evaluated per the quality criteria based on the classification in surface water quality regulation (YSKY, 2015), the surface waters of the Karasu River stations were reported to be extremely contaminated water of the 4th class quality in terms of the elements analyzed (Table 1).

Capoeta capoeta, *Alburnus mossulensis*, and *Squalius cephalus* were caught with fishing nets from the stations during the June–September 2015–2016. The permissions for the study were obtained from the relevant institutions (Atatürk University Office of Animal Experiments Local Ethics Committee, Republic of Turkey Ministry of Food Agriculture and Livestock, Ministry of Forestry and Water Affairs) before the study (No: 36643897/25.09.2013, No: 67852565/140.03.03-863, No: 72784983-488.04-63471). The live fish samples were transferred in 10 L plastic containers to the Animal Physiology and Histology Research Laboratory of Atatürk University. Before the examination, the fish were kept in 30 L aquaria with aerated river water until the assessments. A total of 244 fish were caught. The mature fish were macroscopically examined, and the weight and length of the fish were measured. The mean total length and weight, respectively, for *C. capoeta* was 20.79 ± 3.42 cm and 89.08 ± 21.32 g; for *A. mossulensis* it was 17.83 ± 3.96 cm and 44.22 ± 5.37 g; for *S. cephalus* it was 16.07 ± 2.03 cm and 48.09 ± 9.26 g.

As a specific portion of oxidants and antioxidants have a joint impact in the blood, the oxidant and antioxidant effects become much higher than those that each one causes singlehandedly. Therefore, it is reported that it would be more beneficial to measure the total oxidant status and total antioxidant status instead of separately measuring the oxidants and antioxidants, to determine the total oxidant/antioxidant balance (Erel, 2004, 2005). For these analyses, 10 of each fish species were used and blood samples were taken from the caudal fins of the fish using a heparinized syringe. The blood samples were put in the centrifuge tubes to be centrifuged for 10 min at 4 °C and 18894 × *g*. 1 mL of the plasma was analyzed in terms of TAS and TOS. The commercial TAS and TOS kits produced by the company Rel Assay Diagnostics (Gaziantep, Turkey) were used in the study. Analysis of plasma TAS and TOS levels was performed spectrophotometrically according to the method of use of these commercial kits (Erel, 2004, 2005).

Genotoxic analyses were made on the blood samples taken from the caudal fins of the fish. Two blood smear preparations from each fish species were prepared. One drop of blood was dropped on the microscope slide to make it spread with the help of a cover glass. Having been air-dried for 15 min, the preparations were fixed for 10 min

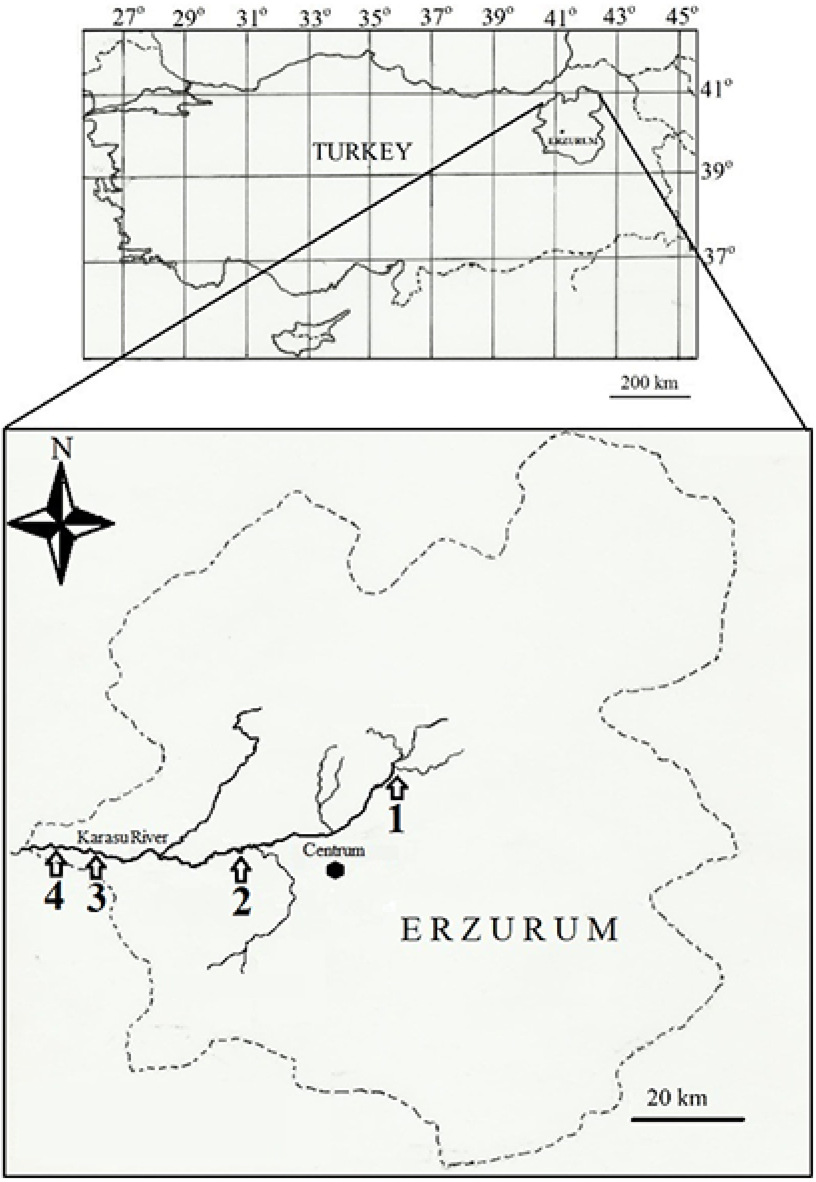

Figure 1. Sampling stations; Station 1: Dumlu, Station 2: Ilıca, Station 3: Aşkale I, Station 4: Aşkale II.

in ethyl alcohol and then stained for 20 min with 10% of the Giemsa stain. Approximately 2000 erythrocytes were analyzed in each slide at immersion microscopy (100X). Other ENA, such as MN, kidney-shaped nucleus, bud nucleus, notched nucleus and binucleus, were classified by Carrasco et al. (1990) and Fenech et al. (2003) to determine their frequencies.

Fifteen fish from each station were used for histological analysis. The fish were dissected after anesthesia with MS 222 (Sigma Aldrich). Brain samples were fixed in 10% neutral buffered formalin. The fixed tissues were processed routinely using standard histological techniques, embedded in paraffin, cut with microtome at 5 µm, and stained with hematoxylin and Eosin (Gautier, 2011). Slides were examined under a digital camera assisted Leica DM750 light microscope and photographed. The 10 sections of tissue from each fish were examined. Twenty fields per section of tissue were observed. The evaluation of the histological damage detected in the brains was quantitatively determined by histopathological alterations index (HAI), an observation parameter based on the frequency of damages. For HAI calculation (modified from Poleksic and Mitrovic-Tutundzic, 1994) the alterations in the organ were classified in progressive stages of damage to the tissue: stage I: tissue reconstruction is possible; stage II: more severe alterations; stage III: tissue restoration no

Table 1. Current data of the mean metal concentrations and physicochemical parameters of water samples of the stations (mg/L) (Dane and Şişman, 2020a), and the water quality classes of the river water according to (YSKY, 2015), and maximum acceptable limits of metal for river waters according to United Nations Economic Commission for Europe (UNECE, 1994).

Heavy metals	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**TS IV**	**UNECE 1994**
Cr	8.55 ± 1.5^{a,b}	6.7 ± 1.2^{a,b}	12.1 ± 2.1^{a,b,*}	13.1 ± 1.8^{a,b,*}	>0.2	0.016
Mn	5.5 ± 1.1^{a}	5.7 ± 1.3^{a}	9.3 ± 1.5^{a,*}	7.7 ± 1.4^{a,*}	>3.0	
Fe	4.1 ± 0.9	5.5 ± 1.1^{a,*}	8.4 ± 1.3^{a,*}	7.8 ± 1.6^{a,*}	>5.0	
Co	0.7 ± 0.1^{a}	0.8 ± 0.1	1.26 ± 0.2^{a,*}	1.2 ± 0.1^{a,*}	>0.2	
Ni	0.8 ± 0.1^{a}	0.74 ± 0.1^{a}	1.17 ± 0.1^{a}	1.01 ± 0.1^{a}	>0.2	1.400
Cu	0.51 ± 0.1^{a,b}	0.57 ± 0.1^{a,b}	0.88 ± 0.1^{a,b,*}	0.82 ± 0.1^{a,b,*}	>0.2	0.018
Zn	0.42 ± 0.1^{b}	0.46 ± 0.1^{b}	0.66 ± 0.1^{b}	0.61 ± 0.1^{b}	>2	0.120
As	1.14 ± 0.1^{a,b}	1.27 ± 0.1^{a,b}	1.55 ± 0.2^{a,b,*}	1.46 ± 0.2^{a,b,*}	>0.1	0.360
Se	0.11 ± 0.01^{a}	0.20 ± 0.01^{a,*}	0.24 ± 0.01^{a*}	0.35 ± 0.05^{a,*}	>0.02	
Pb	0.29 ± 0.01^{a,b}	0.69 ± 0.1^{a,b,*}	2.55 ± 0.5^{a,b,*}	2.96 ± 0.6^{a,b,*}	>0.05	0.082
Physicochemical parameters						
Temperature (°C)	21 ± 4.1	23 ± 4.8*	25 ± 5.7*	25 ± 5.5*	>30	
pH	7.81 ± 1.5	7.74 ± 1.6	8.03 ± 1.2	8.06 ± 1.4	9.0	5.3
Dissolved oxygen (mg/L)	7.62 ± 1.0	6.32 ± 1.5	5.60 ± 1.1*	4.31 ± 0.8*	<3	6.0-3.0

Values are expressed as mean ± standard errors. Asterisk shows statistical difference compared to Station 1. TS IV; Turkish Standard IV means the surface water are polluted. The letter "a" indicates that metal concentration exceeds the TS IV value, and the letter "b" indicates that metal concentration exceeds the UNECE limit.

longer possible. The value of HAI was calculated for each fish by the formula: HAI = (1 × ΣI) + (10 × ΣII) + (100 × ΣIII) where I, II and III correspond to the number of alteration stages I, II and III respectively. HAI values of 0 to 10 indicate normal organ functioning; values from 11 to 20 indicate slight damage in the organ; from 21 to 50 indicate moderate damage in the organ; from 50 to 100 indicate severe damage and values above 100 indicate irreparable damages in the organ (Poleksic and Mitrovic-Tutundzic, 1994).

The TAS, TOS, ENA and HAI values were expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed to evaluate the stations (Dane and Şişman, 2020a, 2020b). Duncan test was applied for multiple comparisons in equal variances assumed analyses. The data were interpreted by considering $p < 0.05$ significance level. SPSS 21.0 Software package was used to evaluate all statistical data.

3. Results

Mean heavy metal levels and physicochemical parameters of the surface waters of the stations where samples were taken in the Karasu River are given in Table 1 (Dane and Şişman, 2020a). The permitted limit values in physicochemical parameters were not exceeded. Accordingly, the metal (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br and Pb) concentrations in the 3rd and 4th stations were higher than the reference station. It was determined that the station with the lowest pollution load in terms of all metal ions (except Cr and Ni compared to Station 2) was the Station 1, and the station with the highest one was the Station 3. This result confirmed the station selection according to the presence of pollution.

The changes in TAS and TOS levels in the blood tissues of fish from the stations are shown in Table 2. The level of TAS in fish tissues decreased significantly in the 3rd and 4th stations compared to the 1st station fish ($p < 0.05$). The highest TAS value in the *C. capoeta* species was observed in Station 1, and the lowest TAS value was observed in Station 4. The same case was observed for the *A. mossulensis* species. The highest TAS value in *S. cephalus* was observed in Station 1, and the lowest TAS value in Station 3. When the station-based TOS values of the species were analyzed, TOS levels in the tissues of the 3rd and 4th stations fish were significantly increased compared to those in the 1st station ($p < 0.05$). The highest TOS value for *C. capoeta* and *A. mossulensis* was observed at Station 4 and for *S. cephalus* at Station 3.

Table 3 demonstrates the abnormalities identified in the erythrocytes of the species obtained from the stations. There was a variation in the proportion of the abnormalities in the erythrocytes of fish taken from the

Table 2. The mean TAS (mmol Trolox eq/L) and TOS (µmol H_2O_2 eq/L) values of the three fish species (N = 10) (± SD).

Fish species	Oxidative stress parameters	Station 1	Station 2	Station 3	Station 4
C. capoeta	TAS	0.86 ± 0.06^{a}	0.68 ± 0.02^{b}	0.52 ± 0.07^{c}	0.50 ± 0.08^{c}
	TOS	4.26 ± 1.18^{d}	20.57 ± 0.43^{c}	26.89 ± 1.85^{b}	40.63 ± 5.05^{a}
A. mossulensis	TAS	0.85 ± 0.07^{a}	0.53 ± 0.05^{b}	0.30 ± 0.07^{c}	0.21 ± 0.01^{d}
	TOS	9.02 ± 1.14^{d}	26.56 ± 1.22^{c}	41.87 ± 1.03^{b}	55.69 ± 2.84^{a}
S. cephalus	TAS	0.78 ± 0.04^{a}	0.59 ± 0.01^{b}	0.16 ± 0.03^{d}	0.29 ± 0.01^{c}
	TOS	19.60 ± 1.16^{d}	25.62 ± 1.11^{c}	78.64 ± 4.91^{a}	47.75 ± 3.72^{b}

Differences between the averages indicated by the different letters in the same line are statistically significant ($p < 0.05$).

Table 3. The mean frequencies of nuclear abnormalities in the fish species (‰ mean ± SD).

C. capoeta							
Stations	Micronucleus	Kidney shaped nucleus	Binucleated nucleus	Notched nucleus	Lobed nucleus	Bud nucleus	Total
1	0.86 ± 0.03^{d}	0.60 ± 0.08^{d}	0.42 ± 0.04^{d}	1.97 ± 0.33^{c}	0.49 ± 0.04^{d}	0.74 ± 0.02^{d}	5.08 ± 0.57
2	3.68 ± 0.33^{c}	2.55 ± 0.39^{c}	0.57 ± 0.01^{c}	1.86 ± 0.03^{d}	0.96 ± 0.03^{c}	1.30 ± 0.05^{c}	10.92 ± 1.15
3	6.96 ± 0.61^{a}	4.22 ± 0.43^{a}	2.34 ± 0.03^{b}	6.45 ± 0.16^{a}	3.20 ± 0.09^{b}	4.79 ± 0.72^{a}	27.96 ± 1.80
4	5.53 ± 0.35^{b}	3.10 ± 0.31^{b}	2.43 ± 0.04^{a}	4.90 ± 0.36^{b}	3.65 ± 0.15^{a}	3.02 ± 0.53^{b}	22.63 ± 1.20
A. mossulensis							
Stations	Micronucleus	Kidney shaped nucleus	Binucleated nucleus	Notched nucleus	Lobed nucleus	Bud nucleus	Total
1	1.85 ± 0.55^{d}	0.59 ± 0.37^{d}	0.48 ± 0.37^{d}	0.74 ± 0.33^{d}	0.36 ± 0.06^{d}	1.75 ± 0.20^{d}	5.77 ± 0.66
2	4.16 ± 0.81^{c}	2.17 ± 0.51^{c}	1.62 ± 0.32^{c}	4.16 ± 0.15^{c}	2.38 ± 0.86^{c}	2.59 ± 0.25^{c}	17.08 ± 1.07
3	7.50 ± 0.44^{b}	3.12 ± 0.19^{b}	3.01 ± 0.18^{a}	7.80 ± 1.64^{a}	4.62 ± 0.17^{b}	3.51 ± 0.56^{b}	29.56 ± 2.19
4	9.82 ± 1.61^{a}	4.76 ± 0.37^{a}	2.54 ± 0.31^{b}	4.43 ± 0.40^{b}	6.05 ± 0.94^{a}	5.17 ± 0.34^{a}	32.77 ± 2.43
S. cephalus							
Stations	Micronucleus	Kidney shaped nucleus	Binucleated nucleus	Notched nucleus	Lobed nucleus	Bud nucleus	Total
1	1.61 ± 0.25^{d}	0.82 ± 0.11^{d}	0.89 ± 0.06^{d}	1.35 ± 0.06^{d}	1.41 ± 0.08^{d}	0.94 ± 0.04^{d}	7.02 ± 0.33
2	3.96 ± 0.63^{c}	1.83 ± 0.03^{c}	2.25 ± 0.03^{b}	3.14 ± 0.68^{c}	3.06 ± 0.63^{c}	3.37 ± 0.01^{c}	17.61 ± 0.77
3	13.54 ± 1.94^{a}	7.19 ± 1.15^{a}	4.93 ± 0.09^{a}	6.51 ± 0.76^{a}	4.63 ± 0.05^{b}	5.26 ± 0.03^{b}	42.06 ± 3.34
4	6.46 ± 1.43^{b}	3.75 ± 0.71^{b}	2.20 ± 0.04^{c}	5.35 ± 0.63^{b}	5.07 ± 0.77^{a}	5.50 ± 0.12^{a}	28.33 ± 1.51

Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$).

four stations. These abnormalities are in the form of micronucleus, binucleus, kidney-shaped nucleus, notched nucleus, lobbed nucleus and bud nucleus (Figure 2). Such variation among the stations analyzed was significantly different ($p < 0.05$). The micronucleus formation in all of the fish was at a higher frequency than other anomalies. The total mean frequency of abnormalities was the highest in Station 3 in *C. capoeta* and *S. cephalus* and in Station 4 in *A. mossulensis*.

In the analysis performed on the brain tissues of most of the fish taken from Station 1, it was observed that the pia mater and the underlying molecular, granular and pyramidal layers were in normal structure. The gray matter containing neuron nuclei, and the white matter containing neuroglia and myelinated axon were easily distinguished in the slides. It was also observed that the cerebral and cerebellum cortex histology and neuronal structures were normal, pyramidal or granular neuron boundaries were

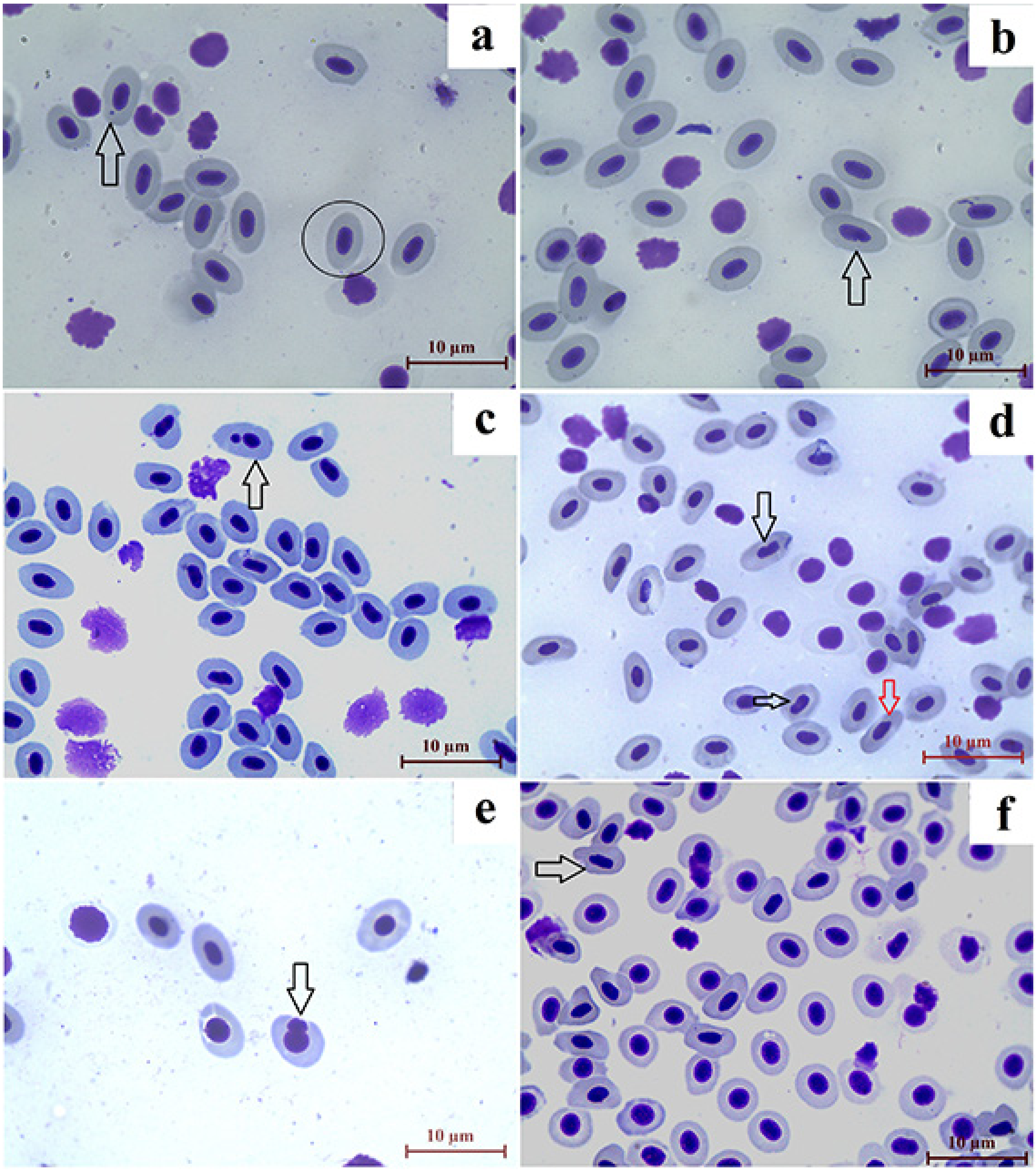

Figure 2. The erythrocytic nuclear abnormalities determined in the three fish species A) micronucleus (arrow) in *C. capoeta*, B) notched nucleus (arrow) in *C. capoeta*, C) binucleated nucleus (arrow) in *A. mossulensis*, D) kidney shaped nucleus (black arrows) and bud nucleus (red arrow) in *A. mossulensis*, E) lobed nucleus (arrow) in *S. cephalus*, F) Bud nucleus (arrow) in *S. cephalus* Giemsa.

smooth. In addition, neuron dendrites and axons, only the upper sides of which are visible, nuclei of the neuroglia cells stained dark with H & E, as well as the blood vessels, were evaluated as normal (Figure 3). Besides, various histological alterations were observed in the nerve tissue with increased intensity, especially in 3rd and 4th stations. Vascular dilatation, congestion, degeneration, vacuolization and infiltration in the Purkinje layer were the most important pathological damages found in the *C. capoeta* (Figure 4). A separation from the granular layer, hyperemia and infiltration in the cerebellum, separation in pia mater, congestion and damages in veins, degeneration and axonopathy in periventricular layer, as well as dilatation and vacuolization in the cortex, were observed in *A. mossulensis* (Figure 5). In the *S. cephalus* species, hyperemia and vascular dilatation were observed in the

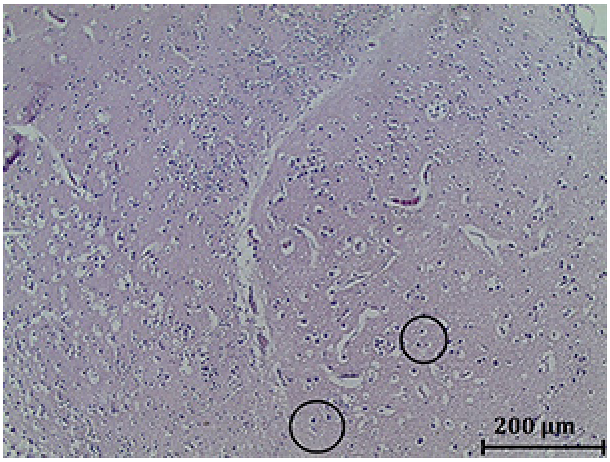

Figure 3. Pyramidal neurons (circles) in normal fish brain section (*A. mossulensis*) H & E.

cerebellum, in addition to degeneration in the white matter, separation in between the granular and molecular layers in the cerebellum, expanded blood vessels, degeneration in the granular layer, and vacuolization in the cortex (Figure 6). There was no difference in histopathological damage types among fish in all three species, and there was a difference in damage frequencies from Station 1 to Station 4. The mean HAI values of the brain tissue of the fish are showed in Table 4. Upon the analysis of the data, it was observed the *S. cephalus* species had the highest HAI value among the species except Station 4. The increase in the HAI values among the stations was statistically significant ($p < 0.05$). HAI values of *C. capoeta* showed that the function of the organ was normal in fish taken from all stations except the 3rd station. HAI values for *A. mossulensis* showed that the organ of the 3rd and 4th station fish was slightly damaged. In *S. cephalus*, the HAI values showed that brain lesions cause slight tissue damage in all station fish except the Station 1.

4. Discussion

Heavy metal contamination in the rivers is quite a serious problem, which is exponentially increasing all around the world. Wastewaters from industrial, agricultural and mining activities lead to this heavy metal contamination (Joshi, 2011). It is known that the activities tend to increase every day and that the quality of aquatic systems deteriorates due to these metals (Ashraf et al., 2012). A significant amount of unprocessed or inadequately treated wastewater from sewage, industry and agriculture is discharged into the Karasu River (Anonymous, 2016). The data obtained from the current study show that the stations selected from the river were polluted at different proportions. This situation indubitably is a natural consequence of the proximity to the sources of contamination. It is estimated that the light pollution in Dumlu station, which is the first place where the selected fish species started to be detected, is the result

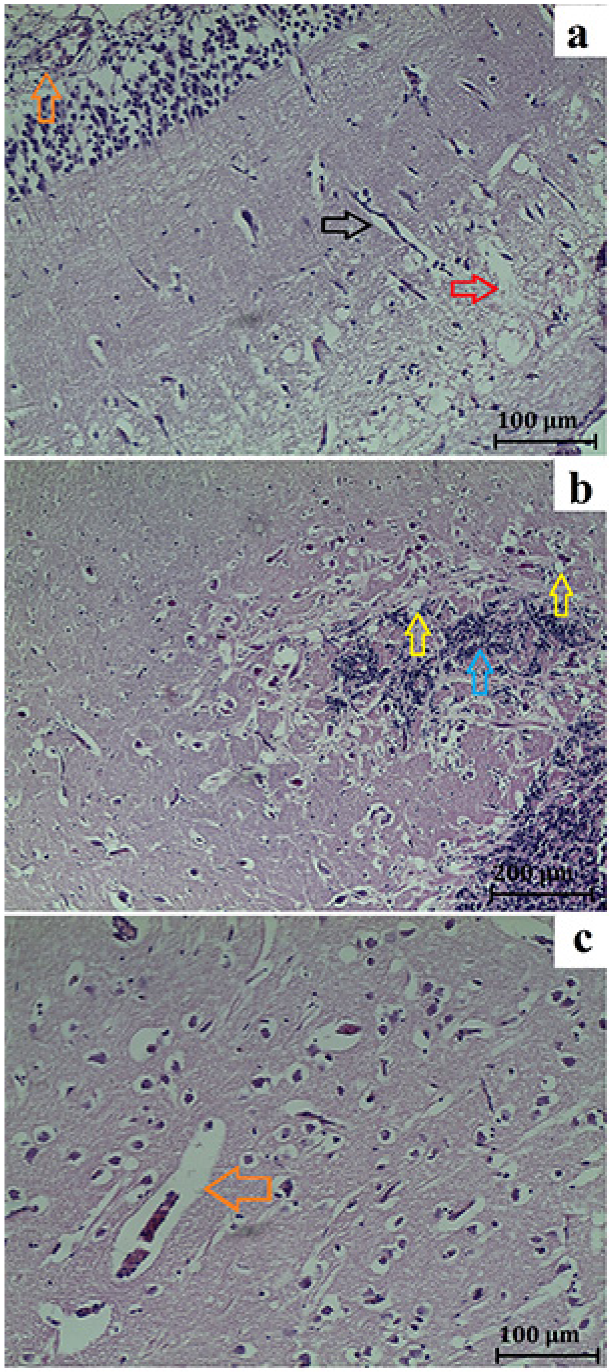

Figure 4. Pathological damages in brain of *C. capoeta* A) vascular dilatation (orange arrow), degeneration (red arrow) and dilatation (black arrow), B) vacuolization (yellow arrows) and infiltration (blue arrow) in the Purkinje layer, C) vascular dilatation with congestion (orange arrow) H & E.

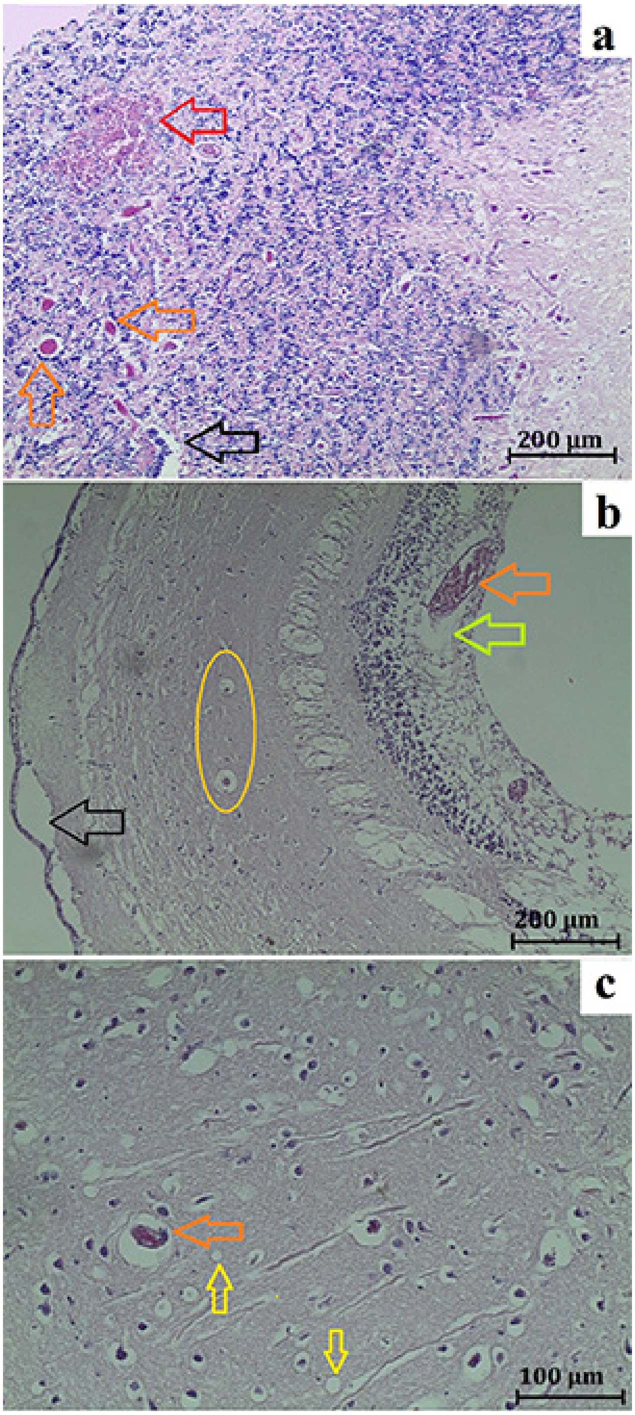

Figure 5. Pathological damages in *A. mossulensis* A) separation from the granular layer (black arrow), hyperemia (orange arrows) and infiltration (red arrow) in the cerebellum, B) separation in pia mater (black arrow), congestion and damage in vein (orange arrow), degeneration in periventricular layer (green arrow) and axonopathy (circle) C) dilatation (orange arrow) and vacuolization (yellow arrows) in the cortex H & E.

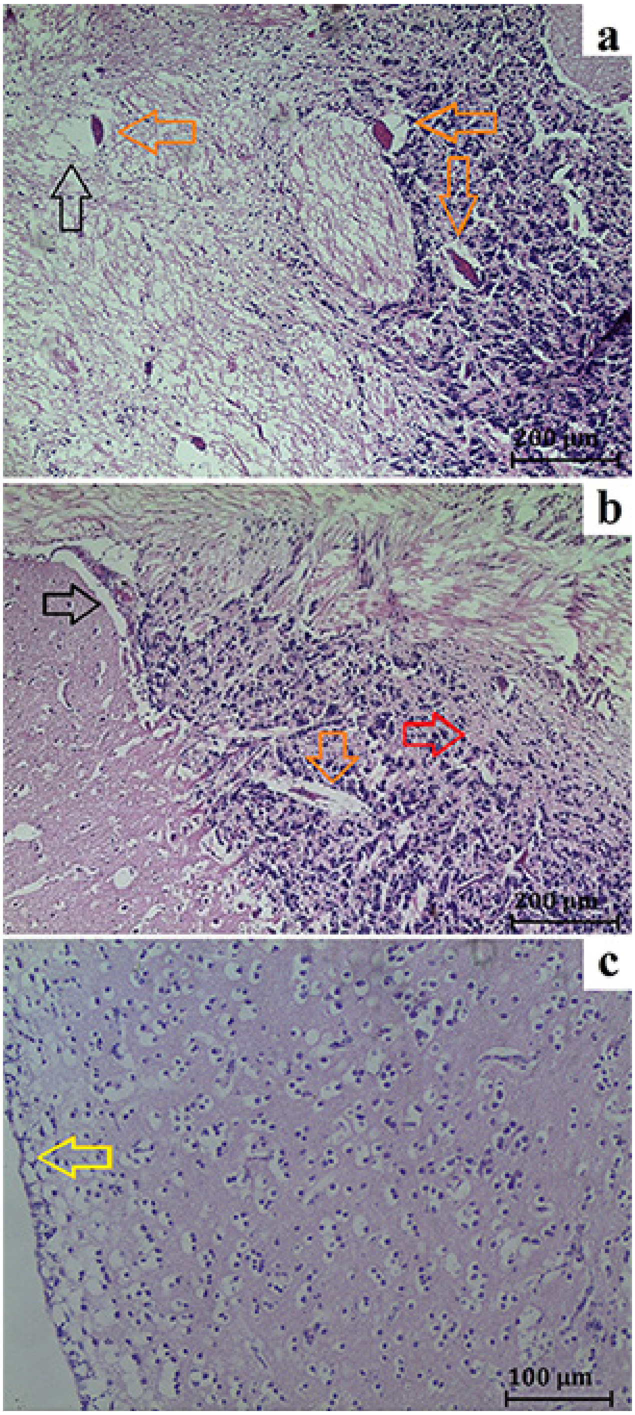

Figure 6. Pathological damages in *S. cephalus* A) hyperemia and vascular dilatation in the cerebellum (orange arrows), and degeneration in the white matter (black arrow), B) separation in between the granular and molecular layers in the cerebellum (black arrow), degenerations in expanded blood vessel (orange arrow) and the granular layer (red arrow), C) vacuolization in the cortex (yellow arrow) H & E.

Table 4. The mean HAI values of three fish species from the stations (mean ± SD).

Stations	*C. capoeta*	*A. mossulensis*	*S. cephalus*
Station 1	2.90 ± 0.10^{d}	1.60 ± 0.18^{d}	4.07 ± 0.16^{d}
Station 2	3.30 ± 0.12^{c}	9.90 ± 0.04^{c}	11.19 ± 0.18^{c}
Station 3	13.18 ± 0.13^{a}	14.30 ± 0.15^{b}	20.17 ± 0.10^{a}
Station 4	8.77 ± 0.16^{b}	17.22 ± 0.14^{a}	15.70 ± 0.26^{b}

Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$).

of the surrounding settlement and agricultural activities. The other stations carry the city's pollution load at different rates. In previous studies on the Karasu River and its tributaries, it has been reported that various pollutants from different sources pollute the river (Sönmez et al., 2013). In a study, it was reported that the quantity of heavy metals presents in the water and sediment samples, which were taken from the river and the wetlands surrounding it, differed between the stations based on the proximity to industrial sources and the density of motor vehicle traffic, and that the quantity of some metals similarly exceeded the acceptable levels of water quality classes (Aydoğan et al., 2017). In our previous study, it was reported that the levels of Cd, Al, As, Pb and Mn metals in surface water in 2013 at the Aşkale station located near our sampling site were above the standards reported by the Turkish Standards Institute 2005 (Dane and Şişman, 2017). In the period of 2015–2016, the surface waters of all the stations selected in the Karasu River were found to be fourth class irrigation water in terms of the analyzed elements (Dane and Şişman, 2020a, 2020b).

In aquatic and environmental toxicology, oxidative stress responses are commonly preferred to monitor the changes in internal organs (Ibrahim and Ibrahem, 2020). Oxidative stress plays a significant role in many chemical-induced cellular damages (Pan et al., 2018). In this study, it was determined that the TAS level was on the decrease as the TOS level was increasing similarly in all the three fish species, based on the contamination levels of the stations. The increase found in the TOS level, as well as the decrease in the TAS level, bring to mind that the oxidative stress and the elements of the antioxidant defense system were insufficient in the organism. Many studies report a response to oxidative stress in fish tissues due to metal pollution (Arojojoye et al., 2018; Turan et al., 2020). A number of enzymes play important role in neutralizing and or detoxifying the oxidative damage by reactive oxygen species. The depletion of antioxidants leads to oxidative stress (Al-Ghais, 2013; Javed et al., 2016). Previous studies have shown that metal exposure can either activate or inhibit antioxidant enzyme activities in fish, depending on the species (Pereira et al., 2013), metal concentration and exposure time (Simonato et al., 2016). Topal et al. (2015) reported that antioxidant responses, and histopathological damage occurring in the relation to Ni exposure could lead to altered physiological functioning in the brain, indicating that Ni is neurotoxic to fish. The oxidative stress responses given by the species to the presence of the contaminants in the water determined in the current study, can be interpreted as an indicator of the fact that these animals were under stress in their habitats.

The contaminants in aquatic environments not only affect the physiology of the organisms but also lead to genetic changes that cause mutations and cancer (Russo et al., 2004). In our study, nuclear abnormalities were found that tended to decrease from the more contaminated stations to the less polluted one based on the results of the erythrocytic nuclear abnormality analyses. Many studies have reported that the high rate of heavy metals detected in the habitats affected by domestic and industrial wastes causes significant micronucleus and other nuclear abnormalities in the erythrocytes of the fish (Ferreira et al., 2010; Marcon et al., 2010; Ribeiro et al., 2013; Arantes et al., 2016). The occurrence of micronucleated cells can be from spontaneous nature or induced by genotoxic compounds. In this study, the nuclear damages found in the tissues of the fish may be related to oxidative stress because redox active metals can exert toxicity related to oxidative stress, which can lead to DNA damage (Simonato et al., 2016). The oxidative stress produced induces secondary modifications in DNA, causing single or double strand breaks, a reduction in the level of repair (Kasprzak et al., 2003), or base oxidation (Valavanidis et al., 2006; Almeida et al., 2007). It is also claimed that the metals may lead to genotoxicity through nonspecific binding to significant biological molecules (Gabbianelli et al., 2003) or by breaking the microtubule function (Bonacker et al., 2005). In the study, the micronucleus and other nuclear abnormalities observed in the blood samples indicate genotoxic damage in the fish.

Although biochemical studies provide some opinions regarding the pathological condition of the animal, the cytoarchitectural changes produced during chemical intoxication can be monitored through histopathologic studies. These studies help assess the level of contamination in the ecosystem and provide significant advantages in determining the effects of the contaminants in various organs and organ systems of a specific organism (Lakshmaiah, 2017). In this study, we elucidated the toxic effects of metal contamination on the brain histopathology of freshwater fish in their natural environment. Among the sampled stations, the highest frequency of histopathological abnormalities was observed in samples taken from the 3rd and 4th stations. This evidence refers to these regions with possibly the worst environmental quality given the degree of histological damage observed in the samples. In addition, this situation is reinforced by the results obtained from the Karasu River regarding the water quality and showing its contaminated status (Dane and Şişman, 2020a, 2020b). The HAI values showed that the nervous system of the 1st station fish was functioning normally, while the 3rd and 4th station fish showed the presence of slight pathological damage to the organ. In many studies, significant histological damage has been reported in the tissues of teleost fish exposed to metal concentrations (Grassie et al., 2013; Ilavazhahan et al., 2017). The metal-induced vacuolization seen in this study produced results similar to changes in the brain of fish exposed to different metal concentrations (Patnaik et al., 2011; Bose et al., 2013). It has been reported that toxicant exposed tissue show vacuolation because of the excessive accumulation of fat in the cytoplasm (Velma and Tchounwou, 2010). Also, vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions (Ilavazhahan et al., 2017). Congestion of blood vessels may be due to pressure of blood exerted due to toxicants resulting in haemorrhage. In a study conducted on cerebral tissues, it was reported that the Ni exposure caused demyelination in the cerebral cortex of the rainbow trout, as well as a necrosis in the Purkinje cells (Topal et al., 2015). In an another study, it was reported that the sublethal concentrations of Pb and Cd caused degeneration in the neurons, aneurysm in the pyramidal cells, as well as dystrophic changes (Patnaik et al., 2011).

Various regions in fish brain are concerned with different kind functions. The degeneration of tissue of a region in the brain observed in this study may lead to the curtailment of the particular function in the fish. This alters the physiological functions of the fish. Al-sawafi et al. (2017) reported that Cd exposure caused severe damage to the fish brain, with degeneration of Purkinje cells and necrosis which could subsequently affect normal physiological activities. Bose et al. (2013) reported that a various pathologies such as degeneration in cerebral tissues, losses in the nissl structures, expansion, atrophy, necrosis and congestion in pyramidal cells and axon, as well as dilatation in meningeal ducts, and infiltration in mononuclear cells were observed in *Catla catla* species exposed to Cu and Fe. All these results comply with the brain pathologies determined in our study. Histopathologic damages found in the brain, which is the center where the movements and all functions of the body are controlled, showed that the metal contamination could lead to neurotoxicity in the fish.

The protection of natural resources and living species is of paramount importance for the continuity of ecological life. The data produced by this study provides an overview of the health of the ecosystem, which is very useful in risk assessment studies. This study clearly demonstrated that fish species in the river contaminated with metals suffer from oxidative stress, genotoxicity and brain tissue damage, which have an impact on resistance to environmental stressors, susceptibility to disease, and survival.

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