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An application of histological technique for monitoring health status of fish species, *Leuciscus aspius* (Linnaeus, 1758) inhabiting Aras River, Iran

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ABSTRACT

Aras River and its reservoir play an important role in fisheries and water supplies in the northwest of Iran. It is considered that the river water is polluted by various anthropogenic activities. This study was performed to determine the histopathologic effects of water pollution on a fish species, *Leuciscus aspius* inhabiting the river. In July 2015, the fish were caught in two different stations of Aras River in Poldasht, Iran. The histopathological abnormalities were detected microscopically and evaluated with quantitative analyses in three organs of the fish. Hypertrophy, filament dilatation, lamellar epithelial liftings, thickening of filament, especially curve, a decrease of the mean length, necrosis, fusion, and lifting in lamellae were observed in gills. Main histopathological abnormalities in the liver were non-homogenous parenchyma, the proliferation of hepatopancreas, congestion, and degeneration of central vein, increasing melanomacrophage aggregates, and sinusoidal dilations. The observed changes in kidney included the degenerations of renal tubules, increasing melanomacrophage aggregates, pyknotic nuclei and vacuolization in proximal and distal tubule epithelial cells, and lymphocyte infiltration in renal parenchyma. It was found that the frequencies of the histological lesions were higher in the liver in comparison with other organs. It was concluded that the increased histopathological alteration in the fish tissues may be related to the presence and elevated toxic contaminants in the river.

Keywords: *Leuciscus aspius*; Fish histopathology; Aras River; Poldasht.

INTRODUCTION

One of the most serious problems of the world is the pollution of air, soil, and water, and this pollution threatens natural resources and affects all organisms including humans. Environmental pollution is one of the most important factors that determine the future quality of life for mankind, and water pollution becomes more important every day. From the source to the use stage, water is easily polluted as it melts, flows, and overflows (Güler & Çobanoğlu 1994).

Fish absorb pollutants from the environment or food chains (Łuczyńska *et al.* 2018). Fish are suitable organisms for pollution monitoring in aquatic systems (Van Der Oost *et al.* 2003). Because fish are very sensitive to environmental changes such as pollution increases, they are reliable bioindicator organisms for assessing the general state of aquatic ecosystems (Martinez-Haro *et al.* 2015). Histological analyses of the gills, liver, and kidneys, the main organs exposed to environmental pollutants, are important biomarkers that make it possible to identify the negative environmental factors that an organism has been exposed to for a long time (Shuman *et al.* 2019). Therefore, it is very important to do histological study to determine the health status of fish populations and aquatic ecosystems (Uçar & Atamanalp 2009). The histopathological method is chosen because it displays the pollution level in the aquatic ecosystem sensitively (Camargo & Martinez 2007). One of the most significant advantages of histopathological biomarker use in environmental pollution monitoring is that it is convenient for examining specific target organs such as gills, kidneys, liver, etc. responsible for vital functions such as the respiration, secretion, accumulation, and biotransformation of xenobiotics in fish (Parikh *et al.* 2010, Naeemi *et al.* 2013).

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The main drinking water source of the northwestern provinces of Iran is Aras River. The river originates from Turkey (Aazami & Taban 2018). Aras River, which joins the Kura River in Azerbaijan after forming one part of the Turkey-Azerbaijan, Turkey-Armenia and Azerbaijan-Iran borders, has a basin of 102 thousand km² and a length of 1072 km. It is one of the biggest rivers of Caucasian and flows out of the Caspian Sea (Nasrabadi *et al.* 2010). Aras reservoir is situated 38 km of Poldasht with a capacity of 1350 million m^3 water and it is very important for biological and economic exploitation (Osalou *et al.* 2015).

Aras River's considerable potential caused the development of farming and industries in the region. Given the importance of the river, it is necessary to check and improve its water quality (Javadinejad *et al.* 2014, Aazami & Taban 2018) which has gradually been deteriorated with the increased agricultural, industrial, and domestic wastes. The increasing industrial and agricultural activities in the Aras River basin adversely affect its ecosystem (Nasehi *et al.* 2013). *Leuciscus aspius* (Linnaeus, 1758) is considered a valuable fish, and its main habitat is the lake behind the Aras Dam (Falahatkar & Tolouei Gilani 2013). In this study, the potential effects of water pollution in Aras River on *Leuciscus aspius* were determined using histopathological method.

MATERIALS AND METHODS

Study area

Poldasht is a city in West Azerbaijan Province, Iran. The city is located at the northern end of the border of Nakhchivan and the western side of Aras River. In this city, there is also a dam on Aras River. The area was chosen as a study area. Two stations were chosen based on their pollution status. Fish samples were caught from the station called the Bridge, which is located before the dam and at the entrance of the city, and from the Dam station (Fig. 1). The coordinates of the stations are as follows: the Bridge station is 2.9 km away from Poldasht. The Dam station is 30.7 km away from Poldasht. The elevations of both stations are approximately 700 m a.s.l.

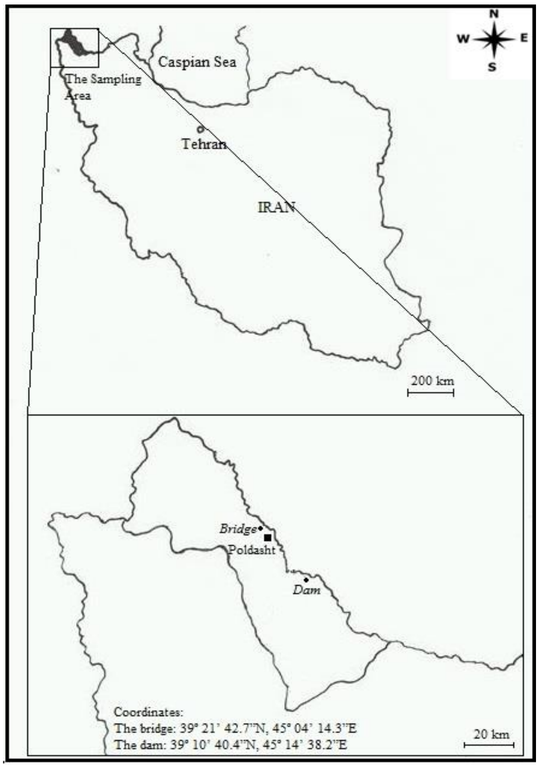

Fig. 1. Map of sampled stations (Bridge and Dam).

Sampling fish

In this study, the *Leuciscus aspius* present in the natural fauna of Aras River was selected (Fig. 2). The fish samples were caught by fishing rod in July 2015. July was chosen as the sampling time because it is thought that pollution is very high in summer and autumn (Zhang *et al.* 2015). The necessary permission was obtained before starting the study (552/9647-22.06.2015). The fish were not differentiated in terms of sex. They were caught from each

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station in equal numbers (n = 14), and their morphometrics (including weight and length) were determined before beginning of the histopathological examinations (Table 1).

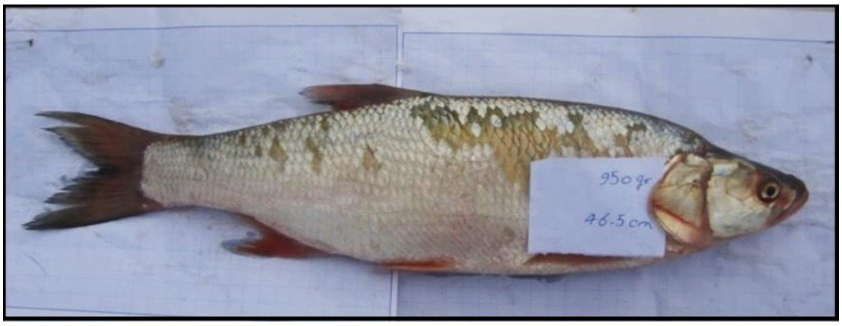

Fig. 2. Asp, *Leuciscus aspius*.

Table 1. The mean values* of length and weight of the collected fish (± SD) (n = 14).

Station	Weight (gr)	Length (cm)
Bridge	1118.25 ± 86.90	48.97 ± 12.17
Dam	1018.50 ± 93.06	46.15 ± 13.90

*No statistical difference was found between the values.

Histopathological Study

The fish were kept motionless by treating them with ether. Then, the dissection procedure was made by dissecting the fish body cavity from underneath the gill to the anus and the viscera. The gills, liver, and kidneys were then fixed in Bouin's fluid for 48 h, and brought to the laboratory. After the fixation, they were kept in buffer solution for 12 h. After dehydration, the organs were submerged in paraffin. The slides at the thickness of 5-7 µm were performed using a microtome. After staining with hematoxylin and eosin the slides were examined under a light microscope and photographed.

The existence of histological lesions in every organ was determined quantitatively with the degree of tissue change (DTC). The DTC is an observation parameter based on the prevalence of histological lesions (Dane & Şişman 2020a). The abnormalities in every organ were classified by the stage of damage for DTC calculation. These stages are as follows: Stage I: The status of tissue that functions normally, Stage II: The status of moderate impairment of tissue's normal function, and Stage III: The status of strong, irreversible damage. The fish tissue stages were determined according to Abdel-Moneim *et al.* (2012). The DTC value was calculated on each tissue of each fish using the following formula:

DTC = (1 x SI) + (10 x SII) + (100 x SIII).

where SI, SII, and SIII state the total number of abnormalities that were seen at different stages, respectively. After determining DTC values for each fish, the mean index was calculated for each station. This index was interpreted based on these values. The DTC value at the range of 0-10 is accepted as normal organ functions. The value at the range of 11-20 indicates slight damage to the organ, while 21-50 exhibits moderate damage, and 51-100 the existence of severe lesions. DTC value above 100 reveals the existence of irreversible damage in the organ (Poleksic & Mitrovic-Tutundzic 1994).

Statistical data analysis

The assessment of the results was made using SPSS ver. 20.0 software (SPSS Inc., Chicago, IL) program. The general evaluation DTC values were performed by one-way analysis of variance (ANOVA). The LSD tests were used for comparing the average DTC values obtained from each station. The difference between the stations was evaluated in terms of significance at the level of $p<0.05$.

RESULTS

The histological lesions in the gills, liver, and kidneys of *Leuciscus aspius* are given in Table 2. The comparison was made between the two stations. The histopathological abnormalities were observed in fish caught from both

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stations. The abnormality frequency was at a low level in the Bridge station samples, while a moderate level in the Dam station ones.

Gill histopathology

In some gill preparations from the Bridge fish, the filaments and lamellae were smooth, and the epithelial cells exhibited a normal appearance (Fig. 3). The increased histopathological abnormalities were observed in the fish gills of both stations especially Dam station including the thickening of filaments (Fig. 4A), a curve on lamellae (Fig. 4A), the decreased lamella length (Fig. 4A), hypertrophy of gill epithelium (Fig. 4B), filamentary dilatation (Fig. 4B), lamellar epithelial liftings (Fig. 4C, 4D), necrotic lamellae and partial fusion of some lamellae (Fig. 4D). The DTC values of the gills were determined as 45.40 ± 3.80 for the Dam and 19.76 ± 3.59 for the Bridge (Table 3). Based on these results, it was found that there was slight histopathological damage in the fish gills of Bridge station, while moderate damage in those of Dam station.

Table 2. Histopathology of gill, liver, and kidney in *Leuciscus aspius* from the two stations indicating the stages of tissue damages and frequency of occurrence (n = 14).

Tissues	Lesions	DTC stages	Bridge	Dam
Gill	Hypertrophy of gill epithelium	I	++	+++
	Filamenter dilatations	I	+	++
	Lamellar epithelial liftings	I	++	+++
	Lamella degenerations	I	+	++
	Filament thickening	II	+	+++
	Fusion	II	+	++
	Necrosis	III	0	+
Liver	Melanomacrophage aggregates	I	++	+++
	Non-homogenous parenchyma	I	+	+++
	Proliferation of hepatopancreas	I	0+	++
	Congestion of central vein	I	+	++
	Sinusoidal dilatation	I	+	+++
	Degeneration of central vein	II	+	++
Kidney	Melanomacrophage aggregates	I	+	+
	Vacuolization	I	0+	++
	Lymphocyte infiltration	I	0+	+
	Degeneration of renal tubules	II	+	++
	Pyknotic nucleus	III	0+	+

0: absent, 0+: rare, +: low frequency, ++: frequent, +++: very frequent.

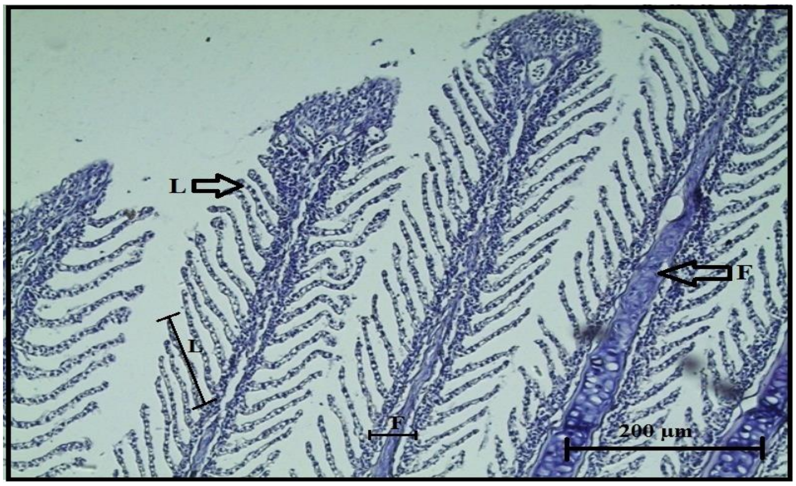

Fig. 3. Micrograph of normal gill section of the fish. The normal aspect of the gill, showing lamella (L) and filament (F), (H&E).

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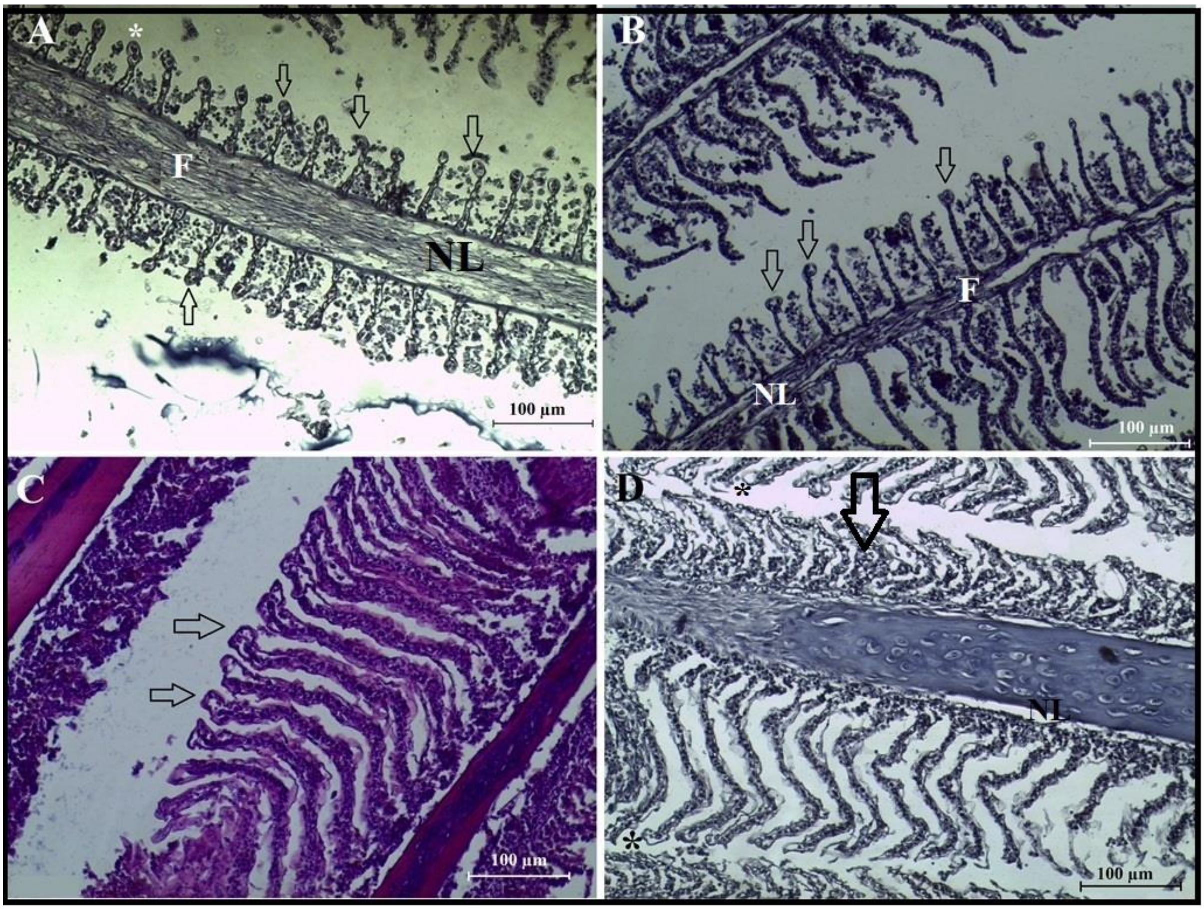

Fig. 4. Micrograph of pathologically-abnormal gill sections of the fish. A) Thickening of filament (F), the curve on the lamellae (arrows), decrease of the mean length of lamella (white asterisk) and necrotic lamellae (NL), B) Hypertrophy of gill epithelium (arrows), necrotic lamellae (NL) and filamentary dilatation (F), C) Lamellar epithelial liftings (arrows), D) Necrotic lamellae (NL), partial fusion of some lamellae (arrow) and lamellar epithelial lifting (black asterisks) (H&E).

Liver histopathology

In the liver of some Bridge samples, it was observed that the hepatocytes were polygonal-shaped, some of them were with dual nuclei, and they were distinguished from each other with sinusoids. The blood vessels and hepatopancreas were distinguishable, but the sinusoids around the collecting channels were not apparent. It was also determined that the hepatocyte cytoplasms were granular, the nuclei were dark-colored, and most of them were settled centrally (Fig. 5). However, it was determined that there was a significant increase in the histopathology of livers in the fish of both stations, specifically the Dam region. The histopathological findings such as non-homogenous parenchyma (Fig. 6A), a proliferation of hepatopancreas (Fig. 6B), congestion of the central vein (Fig. 6C), degeneration of central vein epithelium (Fig. 6D), increasing melanomacrophage aggregates (Fig. 7A), and sinusoidal dilatations (Fig. 7B) were obtained from the fish. The DTC values of the liver were 75.48 ± 19.09 and 49.25 ±15.81 for Dam and Bridge samples, respectively (Table 3). Moderate damage was observed in the liver of the Bridge station fish, while severe damage in those of Dam station.

Table 3. DTC values* for gills, liver, and kidneys.

Station	**Gill**	**Liver**	**Kidney**
Dam	45.40 ± 3.80 ^{a}	75.48 ± 19.09^{a}	24.24 ± 4.12^{a}
Bridge	19.76 ± 3.59 ^{b}	49.25 ± 15.81^{b}	3.52 ± 0.88^{b}

*Results are mean ± SE (n = 14). Means with the different letters in the same column for each station are significantly different at *p*<0.05.

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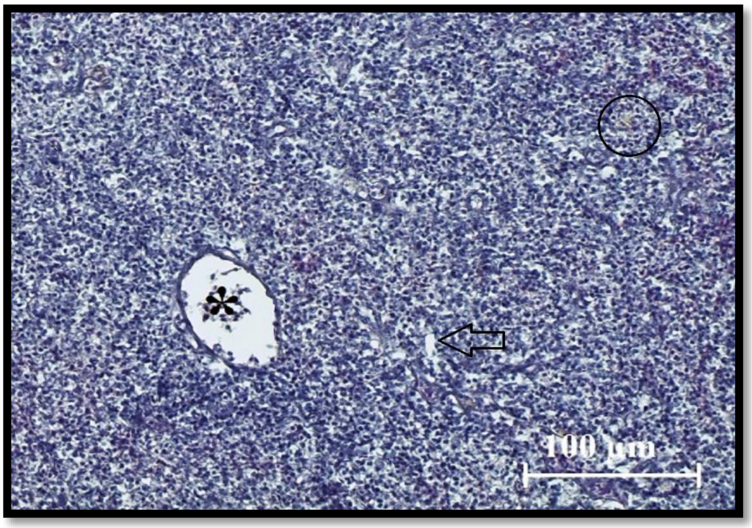

Fig. 5. Micrograph of the normal liver section of the fish. Sinusoid (arrow), central vein (*), and slight melanomacrophagic region (circle) (H&E).

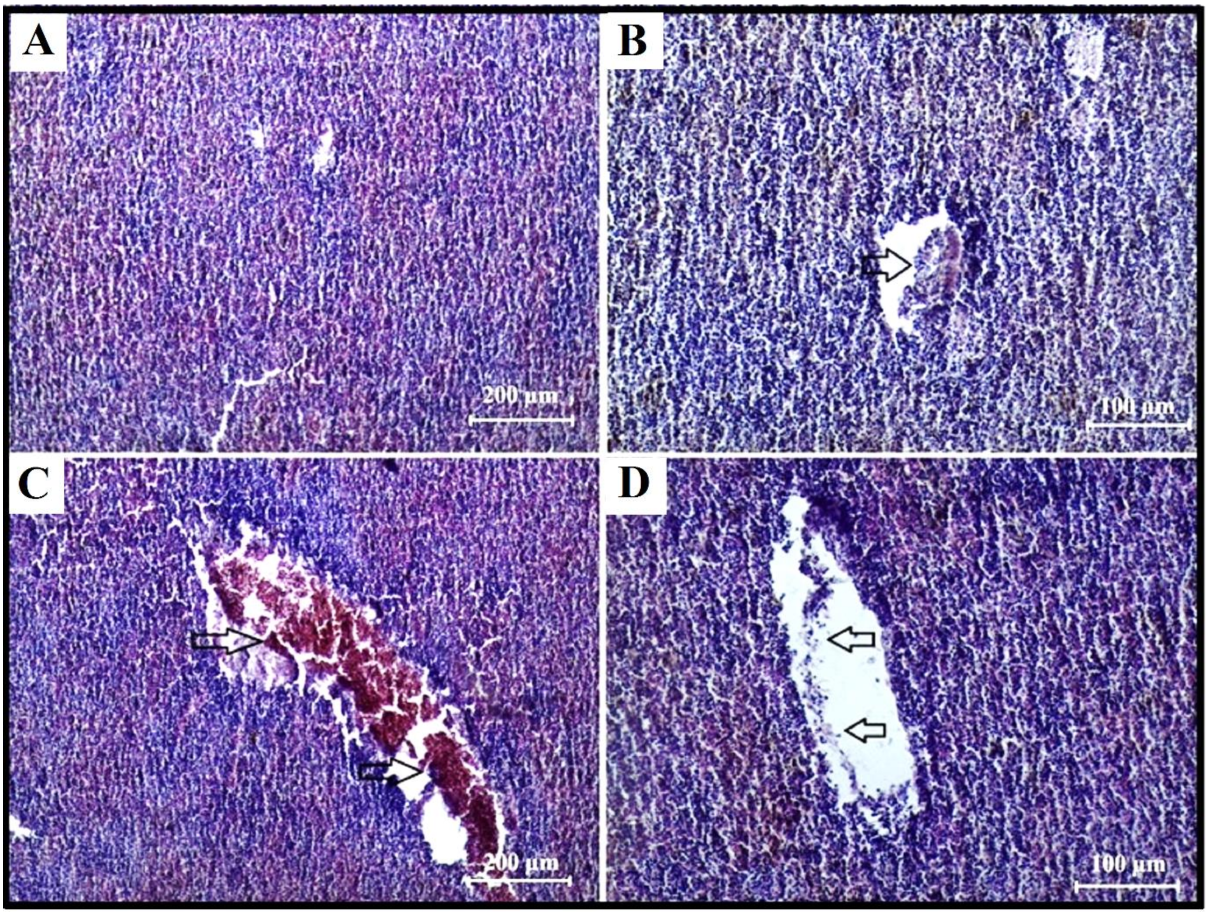

Fig. 6. Micrograph of the pathologically abnormal liver of the fish. A) Non-homogenous parenchyma tissue (colored dark and light hepatocytes), B) Proliferation of the hepatopancreas (arrow), C) Congestion at the central vein (arrows) D) Degeneration at central vein epithelium (arrows), (H&E).

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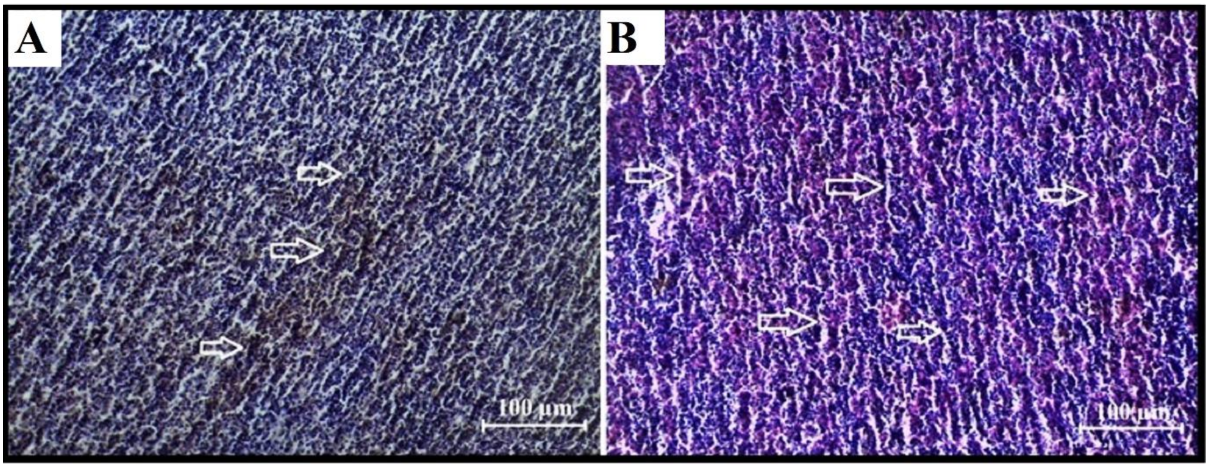

Fig. 7. Micrograph of the pathologically abnormal liver of the fish. A) Increasing melanomacrophage aggregates (arrows), B) Sinusoidal dilatations (arrows) (H&E).

Kidney pathologies

In the kidney of some Bridge fish, it was detected that the renal parenchyma was normal, and Bowman capsule and lumens of distal and proximal tubules were regular (Fig. 8). However, an increase was observed in the histopathological abnormalities in the fish kidneys. A large variety of pathological findings such as the degenerations of renal tubules (Fig. 9A), increasing melanomacrophage aggregates (Fig. 9B), pyknotic nuclei and vacuolization in proximal and distal tubule epithelium cells (Fig. 9C), and lymphocytes infiltration in renal parenchyma (Fig. 9D) were obtained from the Bridge and especially the Dam fish. The DTC values of the kidney were 24.24 ± 4.12 for the Dam and 3.52 ± 0.88 for the Bridge fish (Table 3). Based on these results, the kidneys of the Bridge fish operated normally, but the kidneys of the Dam region fish had moderate damage.

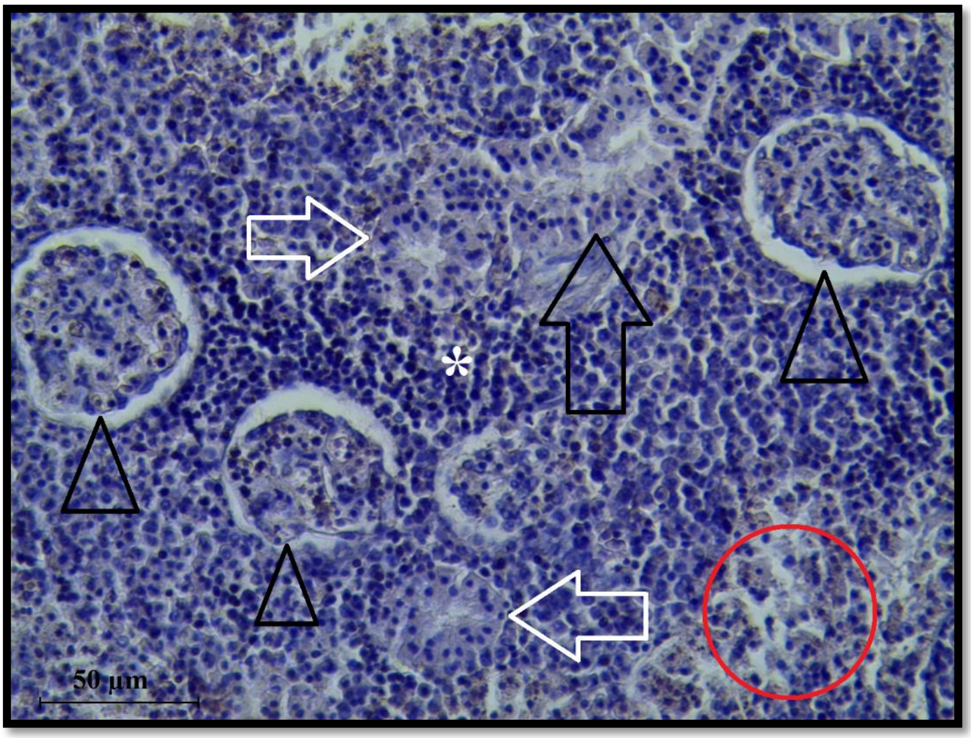

Fig. 8. Micrograph of the normal kidney section of the fish. Normal renal corpuscle showing the glomerulus and the Bowman's space well defined (arrowhead), proximal tubule (white arrow), renal parenchyma around the tubules (*), distal tubule (black arrow) and melanomacrophage aggregates (red circle) (H&E).

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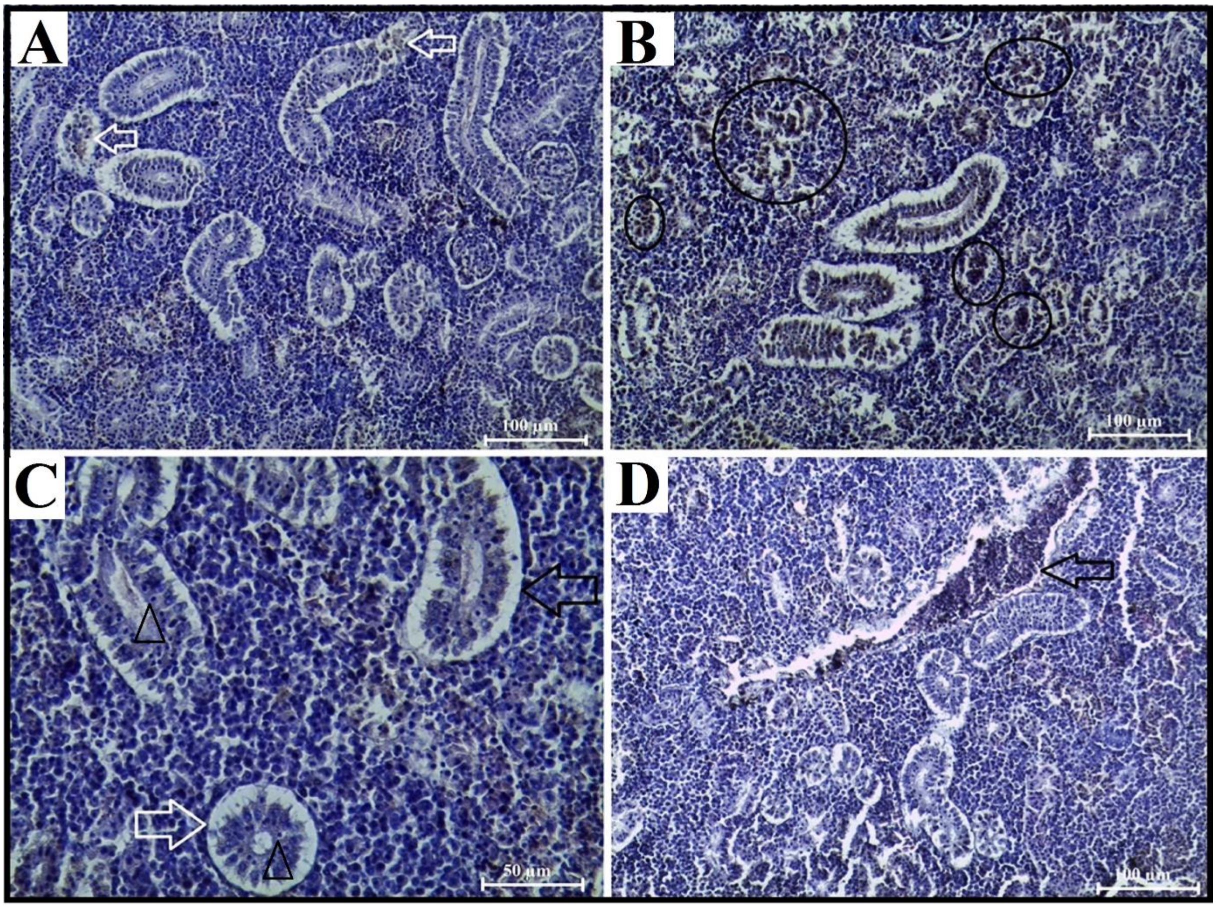

Fig. 9. Micrograph of pathologically abnormal kidneys of the fish. A) Degenerated cytoplasm of some cells of the renal tubules (arrows), B) Increasing melanomacrophage aggregates (circles), C) pyknotic nuclei (arrowheads) and vacuolization in epithelial cells of proximal (white arrow) and distal (black arrow) tubules, D) Lymphocyte infiltration (arrow) (H&E).

Finally, it was concluded that the Dam station may be polluted and the Bridge station may be less polluted. The damage ratio was determined to be significantly higher in the liver compared to the gill and kidneys in the fish, and the kidney was the organ with the lowest damage ratio.

DISCUSSION

Pollution in the aquatic environment changes the self-cleaning capacity of the aquatic ecosystem when it reaches a dangerous level at some points. Therefore, aquatic organisms were used to determine the pollution level in aquatic environments. In the current study, the histopathological effects of pollution were examined on *Leuciscus aspius* inhabiting Aras River which passes through the settlements in the Poldasht City and flows into the Caspian Sea. Unfortunately, the river passing through tens of cities and villages carries the wastes of numerous industrial plants (Nasrabadi *et al*. 2010). Therefore, the river water is potentially threatened. The fast-growing agricultural and industrial activities in Aras basin within the borders of Iran, Azerbaijan, and Armenia have adversely affected the ecosystem of the river, especially in recent years (Nasehi *et al.* 2013, Aazami & Taban 2018, Farsani *et al.* 2019).

In our study, the histopathological effects of water pollution on *Leuciscus aspius* caught from two stations (the Bridge and the Dam) on the river were determined by histological methods. In histological slides, various pathological abnormalities were determined. Although the histopathological alterations of fish tissues were discovered in both stations, their severity in those of Dam station was found to be higher than those of the Bridge station. A few studies conducted concerning water and sediment analyses provided the insight that Aras River may have been polluted. Mohebbi *et al.* (2016) analyzed the water quality of Aras Dam (5 locations) from August 2013 to May 2014 reporting poor water quality and high nutrient load mainly due to human activities. Solgi and

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Sheikhzadeh (2016) investigated the water quality of Aras River at 10 stations in June 2016 reporting that the concentrations of nitrate (297 mg L^{-1}), nitrite (0.57 mg L^{-1}), and EC (1254.3 µs7cm) were higher than the maximum concentration permitted by WHO standard, concluding that the municipal wastewater and agricultural runoff were important factors affecting the river water quality. Javadinejad *et al.* (2014) reported that the various pollutants were dischrged into the Aras River. Farsani *et al.* (2019) also reported that some heavy metals (Cd, Hg, and Pb) were above the permitted level in the water samples taken from Aras Dam Lake (39°33"82"N, 46°52'99"E). According to Nasrabadi *et al.* (2010), the concentrations of Cd, S, Sr, and Pb increased along with anthropogenic activities, emphasizing that the river may be polluted. In another study, surface water and sediment samples were taken from five different stations in Mogan Dam, analyzed in terms of the heavy metals such as Cu, Fe, Pb, Ni, Cd, and Hg reporting that Ni, Pb, and Fe exceeded the maximum permitted levels. The factories adjacent to Aras river and also domestic wastes were considered as the pollution source (Nasehi *et al.* 2013). Osalou *et al.* (2015) also analyzed the surface water physico-chemical characteristics of Aras reservoir (three sites in the river, two sites at the entrance, and two sites at the outlet of the river) reporting the maximum levels of ammonia, nitrate, nitrite, and phosphate as 0.582, 49.0, 0.24 and 4.0 mg L^{-1}, respectively, concluding that Aras reservoir belonged to eutrophic waters (Osalou *et al.* 2015). They suggested that the erosion resulting from weathering, floods, residential wastewater, and especially waste from industrial activities were the main factors responsible for deteriorating the water quality of Aras River (Fataei *et al.* 2012). These studies show that the Aras River water was increasingly polluted as a result of nearby activities. The current study claims that the fish inhabiting the river may be affected by the pollution.

It is estimated that water pollution in Aras River may negatively affect the natural fish fauna. In the literature, there is no study concerning reverine fish histopathology and few studies on this field in Iran. Salamat *et al.* (2012) performed a study related to histopathological effects of water contamination on the gills of *Acanthopagrus latus* (yellowfin bream) collected from five different sites in Musa Stream (Persian Gulf) reporting pathological abnormalities such as hyperplasia on lamellar cells, aneurysm, lamellar fusion, and epithelial lifting on the fish gills, quite compatible with the present study. Their DTC values observed on the gills (13.33 and 346.34) were partially similar to our findings (19.76 to 45.40). In another study, the adverse effects of water pollution were reported on *Vimba vimba* from the Caspian Sea and Sefidrud River, Iran and the fish gill histopathologic effects such as degenerations of filaments and lamellae, edema, curling and clubbing in lamellae, hyperplasia, hematomas, aneurism, dilatations and fusion of lamellae were oberved in both environments (Amiri *et al.* 2011). Parallel results were also obtained in studies conducted in Turkey. In a study, Dane & Şişman (2017) investigated the effects of water pollution on *Squalius cephalus* inhaniting three different regions of Karasu River in Erzurum which is located adjacent to Aras River, reporting hypertrophy and hyperplasia of the gill epithelium, vasodilatation, lamellar epithelial lifting and cartilage tissue, lamellae shortening, lamellar disorganization, blood congestion, lamellar fusion, and lamellar aneurysm. They also reported the proliferation of hepatopancreas, nonhomogeneous parenchyma, increasing melanomacrophage aggregates, sinusoidal dilatation, hepatocyte hypertrophy, congestion of central vein, blood congestion, degeneration of central vein, necrosis, pyknotic nucleus, and hepatic granuloma in the fish liver. In another study, Dane & Şişman (2020b) investigated the histopathological effects of water pollution on *Alburnus mossulensis* in the Karasu River, finding that the river surface water causes similar pathological disorders on fish gill and also in its liver. In addition, renal histopathological findings such as dilatation in the tubular lumen, significant degeneration in the tubules, parenchymal detachment, parenchymal vacuolization, glomerulopathy, fibrosis, and necrosis were observed in the fish kidneys. Most of our histopathological findings in fish gill, liver, and kidney were compatible with these abovementioned studies.

Numerous studies conducted in the world show that waters polluted with various wastes have negative effects on the aquatic ecosystem. Fish are exposed to environmental pollutants, affecting the histological structure of fish in a long or short time. A study was conducted in which the histopathological effects of heavy metal pollution on *Coregonus peled* collected from the Lower Ob basin, Russia (Shuman *et al.* 2019). They reported various pathological abnormalities in the gill, liver, kidney, and gonads were observed in all fish samples including epithelial hyperplasia, thickening, desquamation, and degradation of lamellae in the gills; necrosis, caverns in the hepatic tissue, degeneration apoptosis of hepatocytes, and areas with connective tissue thickenings in the liver; renal tissue necrosis, hemorrhage, numerous melanomacrophage centers, fibroelastosis in the kidney (Shuman *et al.* 2019). Paulo *et al.* (2012) reported vacuolization of the cytoplasm in the melanomacrophage center, abnormal hepatocytes, eosinophil granules in the cytoplasm, cellular and nuclear hypertrophy, irregular nucleus, leukocyte infiltration, degeneration of the cytoplasm and nucleus, pycnotic and vacuolated nucleus, focal necrosis, and tumor were determined in the liver of *Poecilia vivipara* caught from five polluted and one clean points in Cachoeira River, Brazil. In another study, the effect of water pollution of the Park and Cedeteg Lakes in Southern Brazil on *Astyanax aff. fasciatus* and *Oreochromis niloticus* were examined, reporting aneurysms, hyperplasia, lamellar fusion, and neoplasia in the gills; and necrosis and leukocyte infiltration in the liver (Liebel *et al.* 2013) compatible with the present study.

The gills are the main target organ for toxicants in the aquatic system. The toxicants lead to pathological abnormalities in the gill such as hyperplasia, fusion, degradation of the lamellar epithelium, etc. (Mataqueiro *et al.* 2009). Toxicants interfere with the process of a glycoprotein for mucus formation in gills. Thus, it disrupts the negative ion charge of the gill epithelium by fusion and lamellar hyperplasia (Ikisa *et al.* 2019). Hyperplasia, hypertrophy, and epithelial lifting abnormalities in the gills prevent the entrance of the pollutant by increasing the distance between the external environment and the blood. However, this situation also leads to impaired oxygen assimilation (Fernandes & Mazon 2003). In addition, with the disruption of ionic and osmotic balance in the gills, rapid cell necrosis and even death can be observed in fish (Alazemi *et al.* 1996). In Teleostei fish, the kidney is another organ that is negatively impacted by contaminants (Thophon *et al.* 2013). Xenobiotics that come to the kidneys through the blood circulation damage the renal corpuscle and the tubules. The effects of toxic substances at the molecular and cellular levels appear in the form of degeneration and neoplastic damage in the kidneys (Pacheco & Santos 2002). The main target organ of toxicants is the liver. Hepatocytes are a biological marker of aquatic contamination. Histological changes in the liver of fish exposed to pollution are checked to assess the effects of xenobiotic compounds. For example, hydropic inflammation is a liver pathology of fish exposure to polluted sediment (Peebuaa *et al.* 2006). The presence of glycogen and lipids in hepatocyte vacuoles indicates that the liver performs normal metabolic functions. There is a balance between the production rate in hepatocytes and the rate of discharge to the systemic circulation, and the presence of vacuoles is important in this balance. The increase in the vacuoles (vacuolization) indicates that the fish is exposed to polluted water and there is metabolic damage (Ikisa *et al.* 2019). In brief, the pollutants cause damage in the fish by impairing the integrity of physiological and histological mechanisms.

CONCLUSION

This study can be considered as another investigation exhibiting that histological markers are very useful in the evaluation of aquatic system health. Notably, there may be a correlation between the increeased histological lesions and the presence of pollutants in water. In most cases, the aquatic systems contain chronic pollution. According to our results, the fish in Aras River may also be exposed to chronic pollution. The pollution has been confirmed by previous analyzes. However, it should be kept in mind that the link between histological lesions and contaminants is not certain and further studies are needed.

Authors' contribution

Mohsen Abbaszadeh performed experimental parts. Turgay Şişman purposed the research idea, analyzed histological slides, designed the study protocol, wrote the manuscript draft and prepared the final version of the paper, and supervised the whole research.

Declarations

It is confirmed that work has not been published, not under consideration for publication elsewhere, approved by all authors and if accepted, it will not be published elsewhere in the same form, in English or any other language.

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کاربرد روش بافت‌شناسی برای نظارت بر وضعیت سلامت ماهی *Leuciscus aspius* (Linnaeus 1758) ساکن رودخانه ارس، ایران

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چکیده

رودخانه ارس و مخزن آن نقش مهمی در شیلات و تأمین آب در شمال غربی ایران دارد. در نظر گرفته شده است که آب رودخانه توسط فعالیتهای مختلف انسانی آلوده می شود. این مطالعه برای تعیین اثرات هیستوپاتولوژیک آلودگی آب بر روی یک گونه ماهی، Leuciscus aspius ساکن رودخانه انجام شده است. در ژوئیه 2015 ، ماهی ها در دو منطقه مختلف در رودخانه ارس در پلدشت، ایران صید شدند. ناهنجاری‌های هیستوپاتولوژی میکروسکوپی تشخیص داده شد و با تجزیه و تحلیل کمی در سه اندام ماهی ارزیابی شد. هایپرتروفی، اتساع رشته، لیفتینگ اپیتلیال لایه‌ای، ضخیم شدن رشته، به خصوص منحنی، کاهش طول متوسط، همجوشی و بلند شدن لاملاها در آبشش‌ها مشاهده شد. ناهنجاریهای اصلی هیستوپاتولوژیک در کبد، پارانشیم غیر هموژن، تکثیر کبد، لخته، و انحطاط ورید مرکزی، افزایش دانه‌های ملانوماکروفاژ و اتساع سینوسی بود. تغییرات مشاهده شده در کلیه شامل تخریب توبول‌های کلیه، افزایش رنگدانه‌های ملانوماکروفاژ، استحاله سلول‌های پوششی لوله پروکسیمال و دیستال و نفوذ لنفوسیت‌ها در پارانشیم کلیه بود. مشخص شد که فراوانی ضایعه بافت‌شناسی بیشتر در کبد است. نتیجه‌گیری شد که افزایش هیستوپاتولوژی در ماهی ممکن است مربوط به وجود و افزایش آلاینده‌های سمی در رودخانه باشد.

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