EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2023; 38(2): 81-88 • DOI: https://doi.org/10.26650/ASE20221208209

Research Article

Evaluation of Histopathological Alterations in the Liver and Kidney of Olive Barb (*Puntius sarana*, Hamilton 1822) as an Indicator of the Surma River's Pollution

Monera Zaman[1], Sarker Mohammad Ibrahim Khalil[1], Md. Zobayer Rahman[1], Arman Hossain[1], Md. Abdullah-Al Mamun[1], Md. Moshiur Rahman[1], M. M. Mahbub Alam[1]

Cite this article as: Zaman, M., Khalil S.M.I., Rahman, M.Z., Hossain, A., Mamun, M.A.A., Rahman, M., & Alam, M.M.M. (2023). Evaluation of histopathological alterations in the liver and kidney of olive barb (Puntius sarana, Hamilton 1822) as an indicator of the Surma River's pollution. *Aquatic Sciences and Engineering, 38(2), 81-88.* DOI: https://doi.org/10.26650/ASE20221208209

ABSTRACT

Water pollution poses a global hazard to aquatic biota and ecological sustainability, causing a hazard to aquatic organisms such as fish. The aim of this study was to evaluate the impact of toxic chemical contaminants on histopathological biomarkers in the liver and kidney of olive barb (*Puntius sarana*) collected from the Surma River in comparison to a control group of fish. The histological bio-monitoring assessment involved the sampling of thirty (30) table-sized fish collected from the four-sampling sites (i. Kajir Bazar, ii. Keane Bridge, iii. Shahjalal Bridge and iv. Burhanuddin Majar) of the Surma River on a monthly basis during the period of September 2019 to February 2020. Twenty (20) table-sized fish of the same species were reared in the Fish Disease Diagnosis and Pharmacology Laboratory, which has a controlled water quality system. In the present study, the river temperature was found to range from 19.7±1.57°C to 30.00±1.29°C. Dissolved oxygen of the surface water was observed to range from 4.15±0.31 to 4.82±0.67 ppm. The pH was found to range from 7.42±0.33 to 6.50±0.12. We observed several pathological changes viz., haemorrhage, lipid droplet, hypertrophy and hyperplasia, erythrocyte infiltration into blood sinusoid, pyknosis and hepatocyte degeneration in the liver tissues of *P. sarana*. The histopathological analysis of the kidney showed tubular degeneration of the distal tubule, tubular degeneration of the proximal tubule, melanomacrophage centre, glomerular shrinkage, vacuolisation, necrosis and haemorrhage. The evidence of pathological alterations in the liver and kidney of olive barb *P. sarana* appeared to be a useful biomarker to assess the impact of the toxicity of water pollution in the Surma River.

Keywords: Olive barb, *Puntius sarana*, Surma River, Environmental Pollution, Histopathology, Biomarkers

ORCID IDs of the author:
M.Z. 0000-0001-5113-0137;
S.M.I.K. 0000-0001-6925-9385;
Z.R. 0000-0001-9558-8270;
A.H. 0000-0002-7733-2527;
M.A.A.M. 0000-0002-2407-6830;
M.R. 0000-0002-8615-1672;
M.M.M.A. 0000-0002-1951-3709

[1]Laboratory Fish Disease Diagnosis and Pharmacology, Department of Fish Health Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh

Submitted: 28.11.2022

Revision Requested: 03.01.2023

Last Revision Received: 17.01.2023

Accepted: 02.02.2023

Online Published: 16.02.2023

Correspondence: Md. Abdullah-Al Mamun
E-mail: maamamun.fhm@sau.ac.bd

INTRODUCTION

Bangladesh is a territory which is bordered by around 230 rivers of different sizes and an abundance of water resources. Being one of the most densely inhabited nations in the world, these resources are constantly becoming contaminated (Hasan et al., 2019). Over the last few decades, freshwater contamination with a variety of contaminants has gained noticeable attention (Vutukuru, 2005). The Surma River's water supply has subsequently been in significant danger from anthropogenic activities and wastewater. Urban discharge in various unauthorized dumping zones, farming techniques, and tannery discharges that have been disposed of without sufficient treatment were reported to have degraded the superiority of the water (Howladar, 2013). Besides this, environmental issues have affected all types of aquatic species due to the unregulated dumping of agricultural waste into waterways (Deng et al., 2017). Industrial leakage is also a significant contributor to water pollution because they contain hazardous metal compounds which directly mix with the waterbody (Patra et al., 2007). Aquatic contamination has been reported to be responsible for the survival disturbance and incapability of regular production processes (Ogamba et al., 2016), so these effects may reduce biodiversity and alter ecosystems' constitution.

One of numerous rivers in Bangladesh, the Surma River is located in the northeast part of Sylhet city. It is considered a large river and is abundant with 51 varieties of fish species. At the same time, a majority of people rely on the Surma River basin for their daily needs (Akter et al., 2019). Currently, community waste and metro pollutants are either directly or indirectly dumped into the Surma River. Wastewater discharge has resulted in water quality deterioration over time in the Surma River (Alam et al., 2007). Pollutants discharged from the water body may be ingested by fish through their food and water and may then accumulate in large concentrations in various tissues (Mohammed, 2009).

As a result of aquatic pollution, fish have become vulnerable to water contamination because their morphology is highly dependent on the quality of the surrounding water (Islam et al., 2020). Fish can absorb or accumulate toxic chemicals either from the surroundings or indirectly via other living things such as tiny fish, aquatic invertebrates and underwater vegetation (Polat et al., 2016). Heavy metal (Chromium) contamination has been reported in the Surma River, but no extensive research has been conducted yet (Islam et al., 2019). Effluents of all kinds have negative impacts on metabolism and behavioural propagation, as well as the potential to cause clinical distortions in the cells and tissues of different parts (Nikalje et al., 2012). Numerous harmful xenobiotics, which induce biochemical and histological changes in fish, are often found in water bodies (Reddy, 2012).

Among the diverse array of fish in Bangladesh, *Puntius sarana* (Hamilton, 1822), commonly known as the 'olive barb' and belonging to the group Cyprinidae, is an indigenous fish which is reported to be critically endangered and found at a stage of vulnerability in neighbouring India (Mikherjee et al., 2002). *P. sarana* is a very well-known small indigenous species (SIS), however, aquatic pollution is increasing day by day and generating a lot of complexities in the cell, tissue and other major organs of this small species (Mahabub et al., 2008). To assess the effect of contamination, histological analysis is a widely supported approach (among a number of identifiable approaches) to distinguish between the healthy and unhealthy status of an animal (Vinodhini & Narayan, 2009).

Fish liver is a major organ because it is crucial for the metabolic and biochemical processes involved in consuming food and nutrition storage (Ostaszewska et al., 2016). Variations in fish liver tissue serve as a reliable indicator of the species' overall health (Nunes et al., 2013). Liver is utilized to assess water quality because it is generally accepted in biomarker research and because its histopathological abnormalities might be linked to water contaminants (Marcon et al., 2016). On the other hand, another primary organ responsible for maintaining the equilibrium of bodily fluids in vertebrate creatures is the kidney, considered another vital organ of the fish body (Ojeda et al., 2003). Various hazardous substances disturb the kidney, which impairs its functioning and temporarily or permanently disturbs homeostasis (Miller et al., 2002). Fish have already been found to experience a variety of histological alterations (cardiovascular, degenerating, advanced changes) with a lot of inflammatory changes due to exposure to numerous environmental stresses (Guardiola et al., 2014; Rodrigues et al., 2017).

Histological alterations determine the environmental stressors that can cause organ-specific response in animals (Madureira et al., 2012; Rašković et al., 2013; Rodrigues et al., 2017). Several species were reported as the grand receiver of river water contamination, which led to histological alterations in various organs like the gill and liver of sneep *(Chondrostoma nasus)* and chub *(Leuciscus cephalus)* (Triebskorn et al., 2008), the ovary of freshwater *Channa punctatus* (Magar and Bias, 2013) and the liver and kidney of *Mystus cavasius* (Karim et al., 2022).

Considering the above aspects, the aim of the present study was to assess Surma River pollution through histopathological alterations that took place in the kidney and liver of *P. sarana,* relative to control fish that were reared in laboratory conditions. Also, we anticipated that fish from polluted river sites would exhibit more abnormalities than control fish.

MATERIALS AND METHODS

Fish collection

The Surma River in the Sylhet region is continuously polluted with a large number of toxic chemicals from a variety of industries and sewage wastes, which rapidly reduces the water quality parameters necessary for aquatic flora and fauna. *P. sarana* were harvested from i. Kajir Bazar, ii. Keane Bridge, iii. Shahjalal Bridge, and iv. Burhanuddin Majar (Figure-1). After collection, fish were then immediately transported in an isothermal box (equipped with a refrigeration system) to the Laboratory of Fish Disease Diagnosis and Pharmacology in the Department of Fish Health Management at Sylhet Agricultural University, Bangladesh. The whole experiment was conducted for 6 months (180 days), from

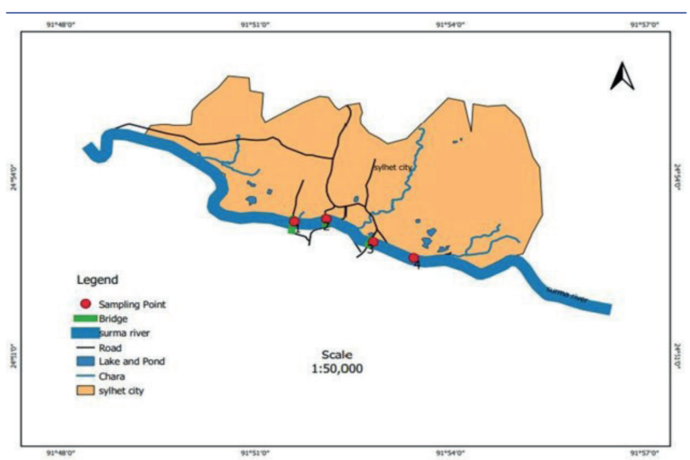

Figure 1. Map showing the sampling sites on the Surma River. (1. Kajir Bazar, 2. Keane Bridge, 3. Shahjalal Bridge and 4. Burhanuddin Majar)..

September 2019 to February 2020. Thirty (30) fish samples were randomly collected on a monthly basis from four sampling sites. For the control study, fish specimens (fingerlings) were collected from a local fish farm (Sreemangal Fish Hatchery) in the Sylhet region. Control fish were fed with commercial food twice per day.

Water sampling

Water samples were collected from the different pre-selected points of the river. The samples were collected at approximately 30 cm depth from the top of the river, and care was taken so that no floating film or organic material could enter the bottle. The samples were analysed in the Fish Disease Diagnosis and Pharmacology Laboratory, under the Department of Fish Health Management. The Digital Multimeter (YSI Professional plus multiparameter water quality meter, USA) recorded various water quality parameters.

Histopathology

The histopathology protocol adopted the following procedure (Sultana et al., 2016). Desired organ liver and kidney tissues were fixed with 10% neutral buffer formalin (40% formaldehyde) and kept for 2-3 days for proper fixation. After, fixation tissue slices were tagged and subjected to a dehydration process in alcohol with a chronological process. After dehydration, tissues were cleared in a Xylene solution. The embedding process was done in paraffin wax overnight at 58°C in the oven. Excess bubbles were removed and kept in refrigerated conditions for solidification. Tissues were sectioned with 5μm size with a microtome. Soon after that, all sections of tissues were placed in an oven at 37°C overnight. Later, tissues were stained with Haematoxylin and Eosin (counter-stains) dye. All prepared sections were mounted with Canada balsam and coverslip used for permanent preservation and kept for one night. The final histological slides were observed and photographed with a microscope (Optika camera; model B9, Italy) at 10X and 40X magnification. All histopathological changes were observed by comparing with the control and updated literature.

RESULTS

Water quality assessment

Several major water quality constraints, such as temperature (˚C), dissolved oxygen and pH, were measured from different sampling sites. All gathered data is calculated and expressed in their mean value ± SD (Table 1). During the experiment, water quality parameters were observed at different times. The average mini-

Table 1. Monthly fluctuation of water temperature, pH and dissolved oxygen of the Surma River during the study period.

Months	Kajir Bazar			Keane Bridge			Shahjalal Bridge			Burhanuddin Majar		
	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)	DO_2 (mg/ml)	pH	Temp. (°C)
Sep	4.65±0.62	7.42±0.33	30.00±1.29	4.58±0.59	7.40±0.31	31.02±1.25	4.66±0.62	7.44±0.25	30.45±1.20	4.50±0.25	7.45±0.35	31.65±1.27
Oct	4.82±0.67	6.92±0.49	29.50±1.25	4.84±0.68	6.90±0.45	30.60±1.27	5.00±0.48	6.76±0.33	29.92±1.12	4.88±0.65	6.85±0.35	30.56±1.25
Nov	4.41±0.56	6.70± 0.28	27.25±1.25	4.39±0.5	6.72± 0.25	28.19±1.23	4.65±0.5	6.86± 0.28	28.85±1.25	4.58±0.34	6.75± 0.25	28.50±1.33
Dec	4.25±0.35	6.65±0.31	21.50±1.29	4.23±0.37	6.69±0.35	21.67±1.26	4.34±0.42	6.7±0.33	20.54±1.28	4.21±0.25	6.63±0.29	21.76±1.35
Jan	4.15±0.31	6.50±0.12	19.70±1.57	4.10±0.34	6.48±0.15	18.85±1.66	4.26±0.30	6.55±0.18	19.55±1.45	4.15±0.35	6.52±0.19	18.53±1.54
Feb	4.15±0.52	7.12±0.22	22.50±1.57	4.18±0.52	7.18±0.19	21.88±1.55	4.21±0.48	7.12±0.26	21.07±1.28	4.18±0.45	7.14±0.26	21.67±1.55

mum water temperature recorded from four different sampling sites was in January (19.70±1.57 ˚C), while the maximum value was found in September (30.00±1.29 ˚C). The minimum value (4.15±0.31 mg/l) of dissolved oxygen was recorded at Kajir Bazar in January, while the maximum value (4.82±0.67 mg/l) was recorded at Keane Bridge in October. The pH value was observed to be higher in September (7.42±0.33) and found to be lowest in January (6.50±0.12).

Histopathology of Kidney

After observing kidney tissues, no alterations were identified in the control fish (Fig. 2a). On the other hand, several alterations were observed in the wild fish tissues, such as tubular degeneration of distal tubule and melanomacrophage centres (Fig. 2b). Vacuolation, necrosis, haemorrhage, tubular degeneration of the proximal tubule (Fig. 2c, 2d), degeneration of the distal tubule and degeneration of the proximal tubule showed severe damage, while vacuolation, glomerular shrinkage, and haemorrhage were found with moderate levels and mild necrosis was found in the tissue of the kidney from collected wild fish (Table 2).

Histopathology of Liver

The liver of *P. sarana* did not show any histological alterations in the control group fish (Fig 3a). At the same time, liver tissue from wild fish was found with severe histological alterations, including nuclear degeneration, cytoplasmic degeneration, cytoplasmic vacuolisation, melanomacrophage centres, erythrocyte infiltration into blood sinusoid, sinusoid dilation and hepatocyte degeneration (Figs. 3c, 3d, 3e). Melanomacrophage centre, haemorrhage, and hepatocyte degeneration indicated higher severity; on the other hand, nuclear alterations and pyknosis were found to be moderate, and minor hyperplasia was observed (Table 3).

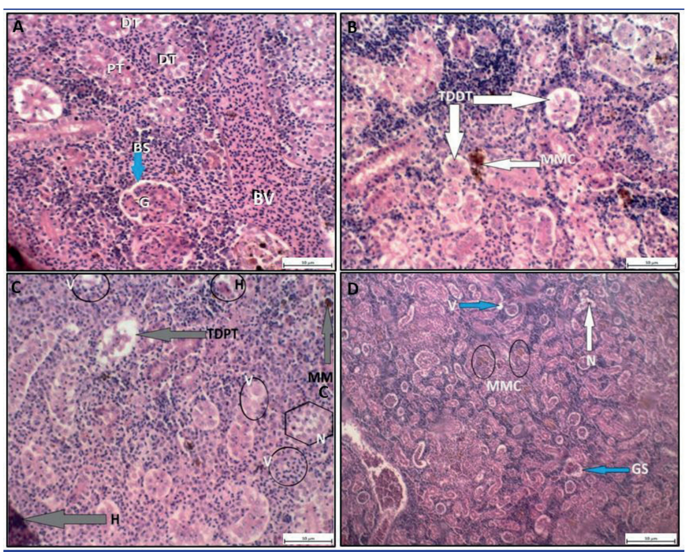

Figure 2. Photomicrograph of the kidney of *P. sarana* from control and experimental site (wild Group). Photomicrograph of kidney from **(A)** Control Group- Distal Tubule (DT), Proximal Tubule (PT), Glomerulus (G), Bowman's Space (BS); (H and E; 40X). and **(B, C, D)** from wild Group- Vacuolation (V), Necrosis (N), Haemorrhage (H), Tubular Degeneration of Distal Tubule (TDDT), Tubular Degeneration of Proximal Tubule (TDPT), Glomerular shrinkage (GS), Melanomacrophage Centres (MMC). (H and E; 10X, 40X).

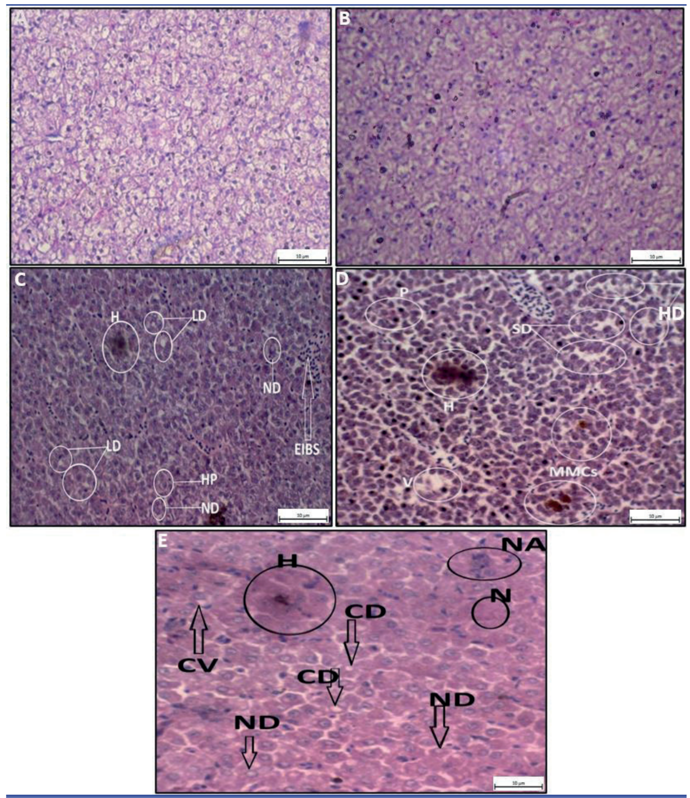

Figure 3. Photomicrograph of the liver of *P. sarana* from control and experimental site (wild Group). Photomicrograph of liver from **(A)** Control Group (H and E; 10X), **(B)** Control Group (H and E, 40X); **(C, D, E)** from wild Group (H and E; 40X). Haemorrhage (H); Lipid Droplet (LD); Necrosis (N); Pyknosis (P); Nuclear Alteration (Na); Hyperplasia (HP) and hypertrophy; Vacuole (V); Nuclear Degeneration (ND); Cytoplasmic Degeneration (CD); Cytoplasmic Vacuolisation (CV); Melanomacrophage Centres (MMC); Erythrocyte Infiltration into Blood Sinusoid (EIBS); Sinusoid Dilation (SD); Hepatocyte Degeneration (HD).

Graphical presentation of histological alteration observed in the kidney and liver of *P. sarana*

In this figure, No. of histological alteration is represented in the Y axis and observed months are represented in the X axis. Several histological alterations of the kidney and liver were observed. On the other hand, the control group of fish species represents low to no histological changes in this study period, both in the kidney and liver (Fig 4a, b).

Table 2. Histopathological alterations observed in the kidney during the experiment.

Abnormalities	Control fish	Wild fish			
		Kajir Bazar	Keane Bridge	Shah-jalal Bridge	Burha-nuddin Majar
Haemorrhage	-	++	+	+++	+
Necrosis	-	+	+++	+	++
Degeneration of Distal Tubule	-	+++	+	++	+
Vacuolation	-	++	++	+	+++
Degeneration of Proximal Tubule	-	++	+++	+	+
Glomerular shrinkage	-	++	+++	++	+

-, None (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (>50%).

Table 3. Histopathological alterations observed in the liver during the experiment.

Abnormalities	Control fish	Wild fish			
		Kajir Bazar	Keane Bridge	Shah-jalal Bridge	Burha-nuddin Majar
Hyperplasia	-	+	++	+++	+
Nuclear alter-ations	-	++	+	+	+++
Haemorrhage	-	+	+++	++	+
MMCs	-	+++	++	+	++
Hepatocyte degeneration	-	+	+++	+	+++
Pyknosis	-	+	++	++	++

-, None (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (>50%).

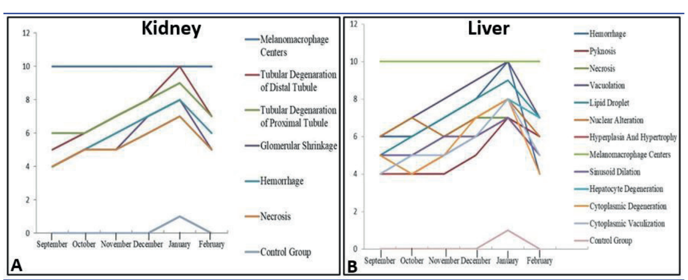

Figure 4. Histological alterations observed at different months. Alterations observed for the kidney (A) and alterations observed for the liver (B).

DISCUSSION

Water quality has been considered an important criterion for successful aquaculture practice, proper growth and good health, which is directly engaged with good water quality. Over the course of time, the Surma River has been disturbed by a huge number of pollutant-discharging industries (Islam et al., 2019). Municipal waste and clinical discharging have a harmful effect on the water quality of a water body (Iqbal et al., 2003). All the sampling sites in this experiment were chosen on the basis of water turbidity and close contact between industries and the river. In our experiment, the minimum temperature was found at the 19.70±1.57°C Kean Bridge site. Water temperature and pH values from different locations in a water body vary at different times (Sazzad et al., 2017). The minimum temperature of a water body plays a crucial role in disease outbreaks (Moniruzzaman, 2000). The lowest value of dissolved oxygen was found at the Keane Bridge and the maximum value was found at the Mendibag station. A DO level of 3mg/L or lower was reported harmful for aquatic organisms; moreover, 5mg/L is considered a preferable limit (Ahmed et al., 2005). In the present study, the pH was found to range from 6.50±0.12 to 7.42±0.33. The minimum pH value was recorded at the municipal sewage disposal site Kajir Bazar and the maximum was observed in Keane Bridge in September. pH value was at its minimum during the time of the monsoon because of heavy rainfall (Sridhar et al., 2008).

Fish liver is the final receiver of all metabolic compounds, and detoxification of all biochemical pollutants takes place in the liver. Exposure to toxic materials and lesion accumulation with other histopathology is very common in this organ (Mohamed, 2009). Wolf et al. (2015) found the liver to be the most sensitive organ damaged due to toxic chemical exposure. The present study demonstrates that the liver of control fish exhibit a normal architecture and there were no pathological abnormalities (Fig 3a, b). In this experiment, liver tissue of *P. sarana* collected from the polluted site (wild group) showed several abnormal changes, including haemorrhage, lipid droplet, necrosis, pyknosis, nuclear alteration, hyperplasia and hypertrophy, vacuole, nuclear degeneration cytoplasmic degeneration, cytoplasmic vacuolisation, melanomacrophage centres and erythrocyte infiltration into blood sinusoid (Fig 3c, d, e). Exposure to contaminated water resulted in increased vacuolisation of the hepatic cell, which is a deteriorating process (Pacheco & Santos, 2002). Reddy & Baghel, (2010, 2012) noticed limited necrosis, vacuolation in cytoplasm and nucleus and other deteriorative signs of parenchymal liver cells in experimental fish exposed to industrial effluent. However, similar histological changes in the liver of *Sarcomento splittail* were reported by (Teh et al., 2005). The *Mugill cephalus*'s liver tissues, which were taken from the contaminated Ennore estuary, exhibited histological changes such as vacuolisation in the hepatocytes, fibroblast growth, vacuole formation, granular degeneration and necrosis (Vasanthi et al., 2013). However, Pinto et al. (2010) reported that histological alterations depend on the length of the individual's exposure to the polluted source. Several histological alterations in the fish liver were also observed by Chavan & Muley, (2014) and Chai et al., (2017). Additionally, diverse complications in the liver tissues of freshwater fish gathered from various contaminated rivers and streams hampered the routine activities of fish (Ahmed et al., 2009; Karim et al., 2022), which fully corroborated our current findings conducted on the Surma River.

Fish kidney is another vital organ that plays an important role in osmoregulation and removes all unnecessary contaminants. Various studies reported that pollutant exposure causes several deformities in kidneys (Velmurugan et al., 2007). So, abnormalities in the kidney can easily predict environmental contamination that can lead to physical stress in any aquatic organisms (Ortiz et al., 2003). Several alterations, including vacuolation, necrosis, haemorrhage, tubular degeneration of distal tubule, tubular degeneration of proximal tubule, glomerular shrinkage and melanomacrophage centres resulted due to environmental contamination. In teleost, cellular hypertrophy was separated from the degeneration of tubular epithelial cells (Happ et al., 2008). Destruction of red blood cells is mainly associated with melanomacrophage centre formation (Agius & Roberts, 2003). Numerous researchers have documented the histological alterations in the kidney at the level of the glomerulus and tubule in fish exposed to pollutants such as insecticides and weedicides (Benli et al., 2008; Yenchum, 2010). Several abnormalities (renal injury, necrosis area, haematopoietic tissue, degradation of renal tubules, shrinkage of glomerulus, increased space within Bowman's capsule, distortion of glomerular capillaries, intracellular droplets, enlarged glomerulus, abnormal nucleus) have been observed in various species by many researches; the occurrence of these abnormalities depends on the sensitivity of the species and the toxic substances that are released into the environment (similar to the liver) (Capkin et al., 2006; Mahmoud et al., 2008; Samanta et al., 2016). Researchers are worried about the severity and consequences of water body contamination on a global scale (Dutta & Dalal, 2008). The cumulative impact of several hazardous contaminants, including heavy metals, industrial effluent, pesticides and fertilizer waste, could have been the cause of all the irregularities seen in the fish's liver and kidney in the proposed investigation. However, an effective study is needed to figure out the precise causes of these histopathological disorders.

CONCLUSION

In the present study, a variety of histopathological changes were caused by the high water pollution of the Surma River. This has a number of harmful consequences on aquatic creatures, particularly fish, and as a result, customers are quite concerned. This could pose a serious danger to the river's biodiversity, causing losses in the near future. The liver and kidney of fish show a number of histopathological diseases that can serve as a biomarker for investigations that are linked. Therefore, the administration and appropriate authorities should take the required steps to safeguard this river's ecosystem against all forms of contamination.

Conflict of interest: We confirm that there are no conflicts of interest among the authors or between the authors and other people, institutions or organisations.

Ethics committee approval: In the present study, fish were sacrificed according to the guidelines as described the Animal Ethics Committee of Sylhet Agricultural University (Memo: SAU/AEC/FOF/FHM-102)

Financial disclosure: We are grateful to the Sylhet Agricultural University Research System (SAURES) under Sylhet Agricultural University, Sylhet, Bangladesh and the University Grants Commission (UGC) of Bangladesh for funding this research project [Memo: SAU/SAURES/OPI/2020(3)]. The first author warmly acknowledges the financial support received from the National Science and Technology Fellowship for her MS research work.

Acknowledgements: The authors would like to express sincere gratitude to the honourable Dean of the Faculty of Fisheries at Sylhet Agricultural University for providing all kinds of essential facilities and support to conduct the present study.

REFERENCES

Agius, C., & Roberts, R. J. (2003). Melano-macrophage centres and their role in fish pathology. *Journal of fish diseases*, *26*(9), 499-509.

Ahmed, K. K. U., Ahmed, S. U., Haldar, G. C., Hossain, M. R. A., & Ahmed, T. (2005). Primary production and fish yield estimation in the Meghna River system, Bangladesh.

Akter, S., Howladar, M. F., Ahmed, Z., & Chowdhury, T. R. (2019). The rainfall and discharge trends of Surma River area in North-eastern part of Bangladesh: an approach for understanding the impacts of climatic change. *Environmental Systems Research*, *8*(1), 1-12.

Alam, J. B., Hossain, A., Khan, S. K., Banik, B. K., Islam, M. R., Muyen, Z., & Rahman, M. H. (2007). Deterioration of water quality of Surma river. *Environmental Monitoring and Assessment*, *134*(1), 233-242.

Benli, A. Ç. K., Köksal, G., & Özkul, A. (2008). Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. *Chemosphere*, *72*(9), 1355-1358.

Butchiram, M. S., Kumar, M. V., & Tilak, K. S. (2013). Studies on the histopathological changes in selected tissues of fish *Labeo rohita* exposed to phenol. *J Environ Biol*, *34*(2), 247-251.

Camargo, M. M., & Martinez, C. B. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical ichthyology*, *5*, 327-336.

Canli, M. A. Y. O., Ay, Ö., & Kalay, M. (1998). Levels of Heavy Metals (Cd, Pb, Cu, Cr and Ni) in Tissue of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma regium* from the Seyhan River, Turkey. *Turkish journal of zoology*, *22*(2), 149-158.

Capkin, E. R. O. L., Altinok, I., & Karahan, S. (2006). Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. *Chemosphere*, *64*(10), 1793-1800.

Chai, L., Chen, A., Luo, P., Zhao, H., & Wang, H. (2017). Histopathological changes and lipid metabolism in the liver of *Bufo gargarizans* tadpoles exposed to Triclosan. *Chemosphere*, *182*, 255-266.

Chavan, V. R., & Muley, D. V. (2014). Effect of heavy metals on liver and gill of fish *Cirrhinus mrigala.International Journal of Current Microbiology and Applied Sciences*, *3*(5), 277-288.

Deng, H., Chai, L., Luo, P., Zhou, M., Nover, D., & Zhao, X. (2017). Toxic effects of NH4+- Non embryonic development of *Bufo gargarizans* and *Rana chensinensis*. *Chemosphere*, *182*, 617-623.

Dutta, H. M., & Dalal, R. (2008). The effect of endosulfan on the ovary of bluegill sunfish: a histopathological study (*Lepomis macrochirus*).

Guardiola, F. A., Gónzalez-Párraga, P., Meseguer, J., Cuesta, A., & Esteban, M. A. (2014). Modulatory effects of deltamethrin-exposure on the immune status, metabolism and oxidative stress in gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology*, *36*(1), 120-129.

Haap, T., Triebskorn, R., & Köhler, H. R. (2008). Acute effects of diclofenac and DMSO to *Daphnia magna*: immobilisation and hsp70-induction. *Chemosphere*, *73*(3), 353-359.

Hayat, S., Javed, M., & Razzaq, S. (2007). Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system.*Pakistan Veterinary Journal*, *27*(1), 8.

Howladar, M. F. (2013). Coal mining impacts on water environs around the Barapukuria coal mining area, Dinajpur, Bangladesh. *Environmental Earth Sciences*, *70*(1), 215-226.

Iqbal, M., Ahmad, M., Abbas, K., & Mustafa, K. (2003). The impact of institutional credit on agricultural production in Pakistan [with comments]. *The Pakistan Development Review*, 469-485.

Islam, A., Hossain, M. M., Rahim, M. M., Hasan, M. M., Hassan, M. T., Begum, M., & Ahmed, Z. (2019). Assessment of heavy metals concentration in water and Tengra fish (*Mystus vittatus*) of Surma River in Sylhet region of Bangladesh. *Arch. Agric. Environ. Sci*, *4*, 151-156.

Islam, M. R., Kunda, M., Pandit, D., & Rashid, A. H. A. (2019). Assessment of the ichthyofaunal diversity in the Juri River of Sylhet district, Bangladesh. *Archives of Agriculture and Environmental Science*, *4*(4), 488-496.

Javed, M. (2005). Heavy metal contamination of freshwater fish and bed sediments in the. *Pak J Biol Sci*, *8*(10), 1337-1341.

Kadry, S., Yacoub, A., Siliem, T., & Mabrouk, D. (2003). Injuries in kidney tissue of the mugil fish *Liza ramada* as a biomarker for environmental pollution in the Lake Manzalah. *Egyptian Journal of Aquatic Biology and Fisheries*, *7*(3), 1-24.

Kirubagaran, R., & Joy, K. P. (1988). Toxic effects of mercuric chloride, methyl mercuric chloride, and emisan 6 (an organic mercurial fungicide) on ovarian recrudescence in the catfish *Clarias batrachus* (L.). *Bulletin of environmental contamination and toxicology*, *41*(4), 902-909.

Lauriano, E. R., Calò, M., Silvestri, G., Zaccone, D., Pergolizzi, S., & Cascio, P. L. (2012). Mast cells in the intestine and gills of the sea bream, *Sparus aurata*, exposed to a polychlorinated biphenyl, PCB 126. *Acta Histochemica*, *114*(2), 166-171.

Liebel, S., Tomotake, M. E. M., & Ribeiro, C. A. O. (2013). Fish histopathology as biomarker to evaluate water quality. *Ecotoxicology and Environmental Contamination*, *8*(2), 09- 15.

Madureira TV, Rocha MJ, Cruzeiro C, Rodrigues I, Monteiro RAF, Rocha E 2012. The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal): evaluation of impacts on fish liver, by histopathology, stereology, vitellogenin and CYP1A immuno histochemistry, after sub-acute exposures of the zebrafish model. *Environ Toxicol Pharmacol*; 34:34–45.

Magar, R. S., & Bias, U. E. (2013). Histopathological impact of malathion on the ovary of the freshwater fish *Channa punctatus. International Research Journal of Environmental Sciences*, *2*(3), 59-61.

Mahmoud, S. A., Tayel, S. I., & Yacoub, A. M. (2008). Histopathological changes in kidneys of the fish Tilapia zillii and *Clarias gariepinus* under the effect of several pollutants along the river Nile. *J. of the Egyptian German Society of Zoology*, *56*, 219-246.

Marchand, M. J., Van Dyk, J. C., Pieterse, G. M., Barnhoorn, I. E. J., & Bornman, M. S. (2009). Histopathological alterations in the liver of the sharptooth catfish *Clarias gariepinus* from polluted aquatic systems in South Africa. *Environmental Toxicology: An International Journal*, *24*(2), 133-147.

Marcon, L., Lopes, D. S., Mounteer, A. H., Goulart, A. M. A., Leandro, M. V., & dos Anjos Benjamin, L. (2016). Pathological and histometric analysis of the gills of female *Hyphessobrycon eques* (Teleostei: Characidae) exposed to different concentrations of the insecticide Dimilin®. *Ecotoxicology and environmental safety*, *131*, 135-142.

Mekkawy, I. A. A., Mahmoud, U. M., Wassif, E. T., & Naguib, M. (2012). Protective roles of tomato paste and vitamin E on cadmium-induced histological and histochemical changes of liver of *Oreochromis niloticus* (Linnaeus, 1758). *Journal of Fisheries and Aquatic Science*, *7*(4), 240.

Mela, M., Randi, M. A. F., Ventura, D. F., Carvalho, C. E. V., Pelletier, E., & Ribeiro, C. O. (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus. Ecotoxicology and Environmental Safety*, *68*(3), 426-435.

Mijkherjee, M., Praharaj, A., & Das, S. (2002). Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India. *Aquaculture Asia*, *7*(2), 8-11.

Miller, G. G., Sweet, L. I., Adams, J. V., Omann, G. M., Passino-Reader, D. R., & Meier, P. G. (2002). In vitro toxicity and interactions of environmental contaminants (Arochlor 1254 and mercury) and immunomodulatory agents (lipopolysaccharide and cortisol) on thymocytes from lake trout (*Salvelinus namaycush*). *Fish & shellfish mmunology*, *13*(1), 11-26.

Mohamed, F. A. (2009). Histopathological studies on Tilapia zillii and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences*, *1*(1), 29-39.

Mohammed, F. A. S. (2009). Histopathological studies on Tilapia zilli and *Solea vulgaris* from lake Quran, Egypt. *World J. Fish Mar. Sci*, *1*, 29-39.

Moniruzzaman, M. (2000). Investigation on disease of some small indigenous freshwater fishes of Bangladesh. *Master's thesis. Department of Aquaculture, Bangladesh Agricultural University, Mymensingh*, 80.

Nunes, B., Antunes, S. C., Gomes, R., Campos, J. C., Braga, M. R., Ramos, A. S., & Correia, A. T. (2015). Acute effects of tetracycline exposure in the freshwater fish *Gambusia holbrooki*: antioxidant effects, neurotoxicity and histological alterations. *Archives of environmental contamination and toxicology*, *68*(2), 371-381.

Ojeda, J. L., Icardo, J. M., & Domezain, A. (2003). Renal corpuscle of the sturgeon kidney: An ultrastructural, chemical dissection, and lectinć binding study. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*, *272*(2), 563-573.

Ortiz, J. B., de Canales, M. L. G., & Sarasquete, C. (2003). Histopathological changes induced by lindane (?-HCH) in various organs of fishes. *Scientia Marina*, *67*(1), 53-61.

Ostaszewska, T., Chojnacki, M., Kamaszewski, M., & Sawosz-Chwalibóg, E. (2016). Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon. *Environmental Science and Pollution Research*, *23*(2), 1621-1633.

Pacheco, M., & Santos, M. A. (2002). Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and environmental safety*, *53*(3), 331-347.

Paithane, K. T., Sonawane, D. L., Bhandare, R. Y., & More, P. R. (2012). Histophatological changes due to induced dimethoate in the liver of freshwater fish *Channa punctatus* from river Shivana, Aurangabad (MS) India. *The Ecoscan*, *1*, 213-217.

Patra, R. C., Swarup, D., Naresh, R., Kumar, P., Nandi, D., Shekhar, P., ... & Ali, S. L. (2007). Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas *Ecotoxicology and Environmental Safety*, *66*(1), 127-131.

Pinto, A. L., Varandas, S., Coimbra, A. M., Carrola, J., & Fontaínhas-Fernandes, A. (2010). Mullet and gudgeon liver histopathology and macroinvertebrate indexes and metrics upstream and downstream from a wastewater treatment plant (Febros River—Portugal). *Environmental monitoring and assessment*, *169*(1), 569-585.

Rašković, B., Jarić, I., Koko, V., Spasić, M., Dulić, Z., Marković, Z., & Poleksić, V. (2013). Histopathological indicators: a useful fish health monitoring tool in common carp (*Cyprinus carpio* Linnaeus, 1758) culture. *Open Life Sciences*, *8*(10), 975-985.

Reddy, P. B. (2012). Histopathogical studies as potential and direct biomarkers of pollution. *Trends in Life sciences*, *1*(1), 27-31.

Reddy, P. B., & Baghel, B. S. (2010). Archana Kushwah and Shehla Ishaque, Biochemical and hematological studies on effect of textile Industry effluent in *Muscus albinus*. In *Proc. ICEM* (Vol. 10, No. 2, pp. 713-719).

Reddy, P. B., & Baghel, B. S. (2012). Impact of Industrial waste water on the Chambal River and Biomarker responses in fish due to pollution at Nagda. MP India. *DAV Int. J. Sci*, *1*(1), 86-91.

Rodrigues, S., Antunes, S. C., Nunes, B., & Correia, A. T. (2017). Histological alterations in gills and liver of rainbow trout (*Oncorhynchus mykiss*) after exposure to the antibiotic oxytetracycline. *Environmental toxicology and pharmacology*, *53*, 164-176.

Rodrigues, S., Antunes, S. C., Nunes, B., & Correia, A. T. (2017). Histological alterations in gills and liver of rainbow trout (*Oncorhynchus mykiss*) after exposure to the antibiotic oxytetracycline. *Environmental toxicology and pharmacology*, *53*, 164-176.

Samanta, P., Mukherjee, A. K., Pal, S., Kole, D., & Ghosh, A. R. (2016). Toxic effects of glyphosate-based herbicide, Excel Mera 71 on gill, liver, and kidney of *Heteropneustes fossilis* under laboratory and field conditions. *Journal of Microscopy and Ultrastructure*, *4*(3), 147-155.

Sazzad, H., & Kiron, D. (2017). B. Sc. Engineering thesis, Department of Irrigation and water Management. *Faculty of Agricultural Engineering and Technology, SAU.*, 60.

Sridhar, R., Thangaradjou, T., & Kannan, L. (2008). Comparative investigation on physico- chemical properties of the coral reef and seagrass ecosystems of the Palk Bay.

Sultana, T., Butt, K., Sultana, S., Al-Ghanim, K. A., Mubashra, R., Bashir, N., ... & Mahboob, S. (2016). Histopathological changes in liver, gills and intestine of Labeo rohita inhabiting industrial waste contaminated water of river Ravi. *Pakistan Journal of Zoology*, *48*(4).

Teh, S. J., Deng, D., Werner, I., Teh, F., & Hung, S. S. (2005). Sublethal toxicity of orchard storm water runoff in Sacramento splittail (*Pogonichthys macrolepidotus*) larvae. *Marine Environmental Research*, *59*(3), 203-216.

Vasanthi, L. A., Revathi, P., Mini, J., & Munuswamy, N. (2013). Integrated use of histological and ultrastructural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in Ennore estuary, Chennai. *Chemosphere*, *91*(8), 1156-1164.

Velmurugan, B., Selvanayagam, M., Cengiz, E. I., & Unlu, E. (2007). The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. *Journal of Environmental Science and Health Part B*, *42*(2), 157-163.

Vinodhani R and Narayan M 2009. Heavy metal induced histopathological alternations in selected organs of *Cyprinus carpio* (Common carp). Int. J. Environ.; 3: 95-100.

Vutukuru, S. S. (2005). Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of environmental Research and Public health*, *2*(3), 456-462.

Wolf, J. C., Baumgartner, W. A., Blazer, V. S., Camus, A. C., Engelhardt, J. A., Fournie, J. W., ... & Wolfe, M. J. (2015). Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: a guide for investigators, authors, reviewers, and readers. *Toxicologic pathology*, *43*(3), 297-325.

Yenchum, W. (2010). Histological effects of carbofuran on guppy *Poecilia reticulata Peters* (Doctoral dissertation, Chulalongkorn University).