



Histopathological Changes in Liver, Gills and Intestine of *Labeo rohita* Inhabiting Industrial Waste Contaminated Water of River Ravi

Tayyaba Sultana,¹ Khadija Butt,¹ Salma Sultana,¹ K. A. Al-Ghanim,²
Rabia Mubashra,¹ Nouman Bashir,¹ Z. Ahmed,² Asma Ashraf¹ and Shahid Mahboob^{1,2,*}

¹Department of Zoology, Government College University, Faisalabad, Pakistan

²Department of Zoology, College of Science, King Saud University, P.O.
Box 2455, Riyadh, 11455, Kingdom of Saudi Arabia

ABSTRACT

Histopathological changes have been recorded in the gills, liver and intestinal tissue of *Labeo rohita* from River Ravi receiving industrial waste. Varying concentrations of Pb and Cu were recorded in the liver, gills and intestine of *Labeo rohita*. The pronounced histopathological changes like degeneration, necrosis and disorganization of lamellae and hyperplasia of epithelial cells was observed in the gills of the fish collected from a polluted site as compared to the fish from a control site. The liver sections of *Labeo rohita* exhibited aggregation, hypertrophy of hepatocytes, cytoplasmic degeneration, hemolysis between hepatocytes, focal necrosis and degeneration. In the intestinal tissue of *Labeo rohita* prominent histopathological changes, including edema, atrophy in the mucosal layer, hemorrhage between blood vessels, blood congestion and aggregations of inflammatory cells were observed. The polluted water has caused more histopathological alterations in *Labeo rohita*. Comparison of histopathology in each organ of *Labeo rohita* showed a same degree of tissue damage in the gills, while aggregation of hepatocytes in the liver, and atrophy of the mucosal layer in intestinal tissue of *L. rohita* were more evident.

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Authors' Contribution

TS conceived the study. KB and NB performed the experiments. SS and RM analyzed the data. AA and ZA performed statistical analysis. KB and SM wrote the manuscript. KAAG helped in manuscript preparation.

Key words

Histopathology; *Labeo rohita*; Liver; Gills; River Ravi

INTRODUCTION

Fish are relatively sensitive to changes in their surrounding environment including pollution. Fish health may reflect the status of a specific aquatic ecosystem. Early toxic effects of pollution may be evident on a cellular or tissue level before significant changes can be identified in fish behavior or external appearances (Nikalje *et al.*, 2012). Harrison *et al.* (2000) indicated that biological communities by integrating the effect of changes across a wide array of environmental factors (chemical, physical and biological) are good indicators of ecosystem health. It is not financially or technically feasible to evaluate all the organisms in the entire ecosystem at all times. Many groups of organisms have been proposed as indicators of ecosystem health and fish and other invertebrates favor no single group have received more attention, at least in freshwater systems.

Heavy metals are non-biodegradable and once discharged in water reservoirs can either be absorbed in sediment particles or accumulated in aquatic organisms. These metals can either sink in the bottom or accumulated in freshwater organism. Fish may absorb

heavy metals from the surrounding water and food, which may accumulate in various tissues in significant amounts (Mohammed, 2009). Toxic heavy metals are continuously released to the aquatic bodies because of rapid industrialization. Metals are the problem of magnitude and of ecological significance due to their high toxicology and ability to accumulate in living organisms (Javed, 2005; Hayat *et al.*, 2007). Environmental pollution in an aquatic ecosystem is usually at a low level, but chronic in nature. As fish is an important source of human nutrition, so, aquatic ecosystems polluted with heavy metals, therefore, directly threaten human nutrition and health directly (Javed, 2005; Nikalje *et al.*, 2012).

Histopathological investigations have been widely used to describe the health status of fish from polluted sites in relation to those from rather unpolluted locations; some studies, which emphasized to use histopathological indicators to assess the effects of environmental stress in fish, have included a quantitative approach (Schwaiger *et al.*, 1997; Vinodhini and Narayan, 2009). Sub-lethal exposure to environmental chemicals may result in changes in the histological structure of cells and the occurrence of pathological changes, which can significantly change the function of tissues and organs. Histopathological changes in animal tissues are powerful indicators of prior exposure to environmental stresses and are the net result of adverse biochemical and physiological changes in an organism. Histopathological

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changes allow the identification of specific target organs, cells, and organelles that have been infected by an environmental pollution.

In Pakistan, rivers are not adequately monitored in order to ensure healthy and safe supply of fish to consumers. The present study was planned to study the effect of heavy metals on histological changes in liver, gills and intestine of *Labeo rohita* from the Bangla Shah Mohammad, River Ravi and Fish Seed Hatchery (control site).

MATERIALS AND METHODS

Labeo rohita (Rahu) were selected for the experimental purpose because of their commercial importance and preference of the consumers. Ten fishes were collected from the River Ravi, which is reportedly heavily contaminated (Javed, 2005) and control fish samples were collected from Fish hatchery, Satiana Road, Faisalabad., Pakistan. Fish were collected from the river Ravi from a location, Bangla Shah Muhammad (BMS), Tehsil Kamalia and District Toba Tek Singh. This reservoir emptying into the Chenab River south of Ahmadpur Sial after a course of about 450 miles (725 km). It lies between longitude 71°48' to 75°20' east and latitude 30° to 32° 51" north. The specimens were, kept in polythene bags and transported to the Department of Zoology, GC University, Faisalabad for further experimental work.

Detection of heavy metals from water samples

Water samples collected for detection of level of Pb, Cu and Ni from the same sampling sites from where fish specimens were collected. Water samples were collected in the clean water sample bottles. The water samples were preserved in 55% HNO₃ and stored at 4°C in the refrigerator. Standard methods as described by the AOAC (1995) and Mahboob *et al.* (2014) were followed, for the determination of these metals on an Atomic Absorption Spectrophotometer (Aurora A-11200).

Histopathological studies

Fish specimens were brought into the laboratory; they were washed with tap water and dried. Weight and length parameters were recorded. The body weights and lengths of specimens were recorded (Table I). Fishes were dissected to obtain the gills, liver and intestine tissue samples. Tissue specimens were further processed for studying histopathological alterations by following the method of Bernet (1999). The tissue specimens were cut into slices about 3-5 µm thick and was processed independently. The tissue was fixed in 10% formalin for

15 min and then tissues were shifted to the fixative (10% formalin) for 24 h.

Table I.- Weight and length parameters (Mean±SEM) of *Labeo rohita* collected from control and polluted sites.

Sampling sites	Weight (g) (n-10)	Fork length (cm) (n-10)	Total length (cm) (n-10)
FH	500.6±1.31	32.23±0.04	37.11±0.05
RR	500.9±1.22	27.38±0.03	33.4±0.03

FH, Fish Hatchery; RR, River Ravi

For light microscopy, Microtome (Reichert-Jung 800) was used to cut tissue sections. Tissue sections were cut 3-5 microns with a steel blade fixed microtome. The best sections were obtained from paraffin blocks that were cool and moist. The sections were floated on a water bath at 42°C to 46°C for stretching. The stretched ribbon was placed on a slide containing Glycerin and Albumin in equal parts. Slides were identified with diamond markers, were dried in an oven at 30-90°C, and were dipped in xylene. Slides were prepared by using Mayer's hematoxylin for 2-3 minutes. These slides were studied for histopathological alterations in each organ and compared to the normal histological slides.

RESULTS AND DISCUSSION

The Pb concentrations in the water samples fluctuated between 0.34-4.88 mg/L and 1.25±0.05 to 4.41±0.17 mg/l from the control and polluted sampling sites, respectively. The differences among all the three polluted sites for the Pb toxicity were statistically significant ($P<0.05$). The copper concentrations in the control and polluted sites water samples was fluctuating between a maximum of 0.27-4.95 mg/L and 1.14±0.01 to 4.88±0.02 mg/L (Table I). The differences among all the three polluted sites for the copper toxicity were statistically significant ($P<0.05$). The concentration of Ni in the water samples collected from control and polluted sites was varied as 0.37-1.27 mg/L and 0.32±0.018 to 1.22±0.006 mg/ L, respectively (Table I). The differences among all the three polluted sites for the nickel toxicity were statistically significant ($P<0.05$).

The histology alterations were compared in the gills, liver and intestine of *Labeo rohita* from control and polluted site. The degree of pathological changes in the gills, liver and intestine tissue were categorized into three stages viz., Stage I (common and less severe), Stage II (more severe as compared to the first stage) and Stage III (most harmful and irreparable). The degree of severity of

Table II.- Degree of severity for histological alterations in *Labeo rohita*.

	Stages		
	I	II	III
Gill	Fusion of lamellae Basal hyperplasia Distal hyperplasia Disorganization of lamellae Epithelial lifting	Dilation of blood vessels within gill arch Dilation and rupture of marginal filaments Blood congestion within filaments	Degeneration and necrosis of lamellae
Liver	Cytoplasmic degeneration Aggregation of hepatocytes Hypertrophy of hepatocytes	Haemolysis between hepatocytes Pyknosis of nucleus Thrombus formation	Focal necrosis and degeneration
Intestine	Atrophy in mucosal layer Aggregation of inflammatory cells Edema in intestinal mucosal layer	Blood congestion Hemorrhage between blood vessels Dilation within blood vessels	Severe degeneration and necrosis

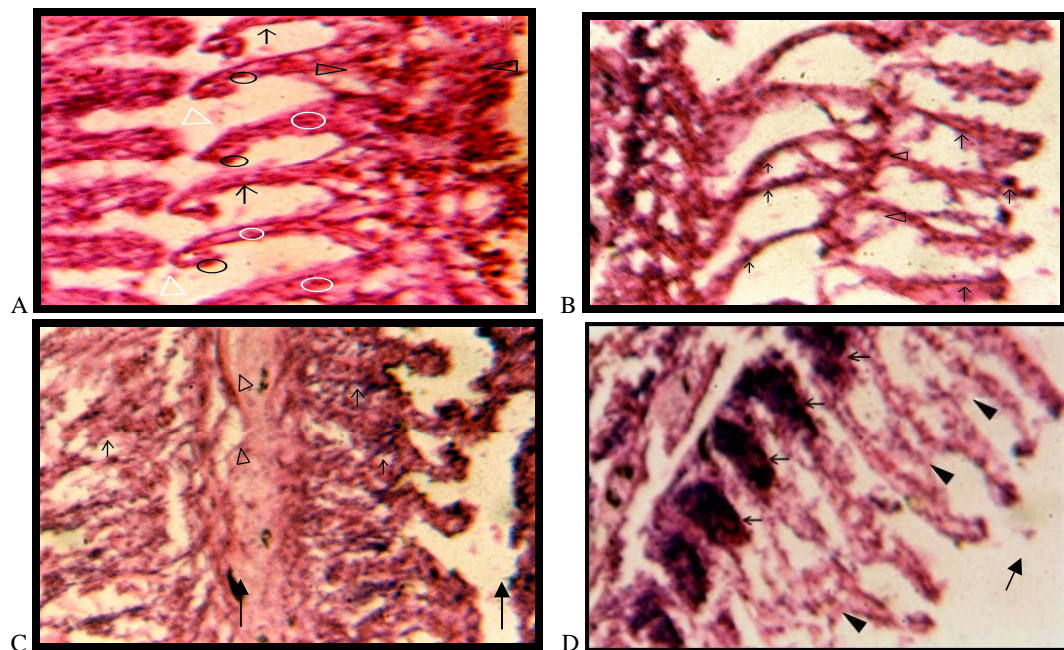


Fig. 1. Histological structure of gill of *Labeo rohita*. A, control showing gill lamellae (↑), the gill arch (Δ), water channels (Δ), pillar cells (●) and epithelial cells (○). B-D shows architecture of gill tissue in polluted samples of *Labeo rohita*. B shows fusion of three lamellae (Δ) and basal and distal hyperplasia (↑) are visible. C shows severe degeneration and necrosis within primary lamellae (↑) and dilation in blood vessels within gill arch (Δ) are quite evident. D shows architecture of gill tissue in polluted samples of *Labeo rohita*. Blood congestion within primary gill filaments (↑) and epithelium lifting (▲). Stain: hematoxylin-eosin., 400X.

histopathological variations in *Labeo rohita* is presented in Tables II.

Figure 1A showed the normal architecture of gill tissue in the control specimen of *Labeo rohita*. Figure 1B illustrated the fusion of three lamellae, restricting the passage of air. Hyperplasia in epithelial cells was also

observed. Both basal and distal hyperplasia was observed in polluted fish samples. The degeneration and necrosis were also observed within the gill filaments along with a dilation of the gill arch in which it expands as compared to its normal condition as shown in Figure 1C. The most common diseased condition, i.e., blood congestion within

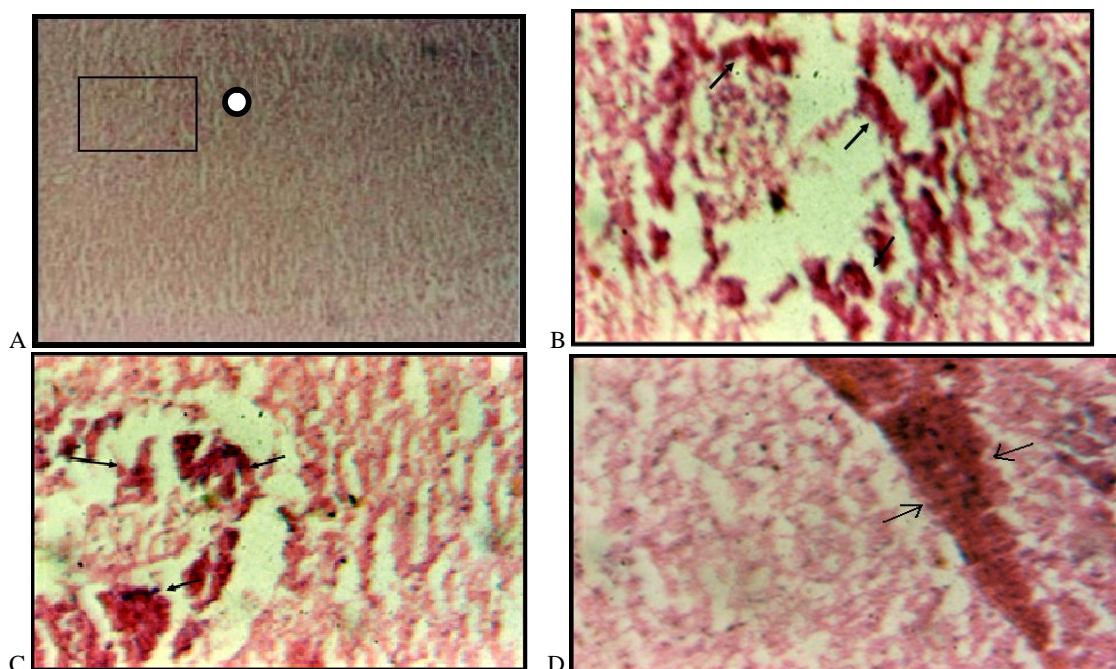


Fig. 2. Histological structure of liver of *Labeo rohita*. A is control liver showing hepatocyte's cytoplasm and nucleus. B shows architecture of liver tissue of *Labeo rohita* from polluted water in a focal area of necrosis and degeneration (↓) C shows haemolysis between the hepatocytes (↑) and D shows thrombus formation within blood vessels (↑). Stain: hematoxylin-eosin, 400X.

gill filaments and the epithelium lifting which occurs when the epithelium layer of the gills separates from the gill lamellae shown in Figure 1D was also observed.

The liver tissue from the control sample of *L. rohita* showed the normal histology of liver (Fig. 2A) showed the normal hepatocyte's cytoplasm and the nucleus. When liver tissue samples of *Labeo rohita* of polluted area were observed, which showed marked histological alterations as compared to control. Figure 2B illustrated the focal area of necrosis, which occurred due to the localized death of liver cells. Usually, a cell releases hemoglobin due to lysis in case of injury; it causes haemolysis among the hepatocytes as shown in Figure 2C. The thrombus formation within blood vessels that might occur due to the platelet aggregation (Fig. 2D).

The intestinal tissue from the control sample of *Labeo rohita* showed the normal histology of intestine (Fig. 3A). Intestinal tissue samples of *Labeo rohita* of a polluted site depicted the most chronic condition of degeneration and necrotic changes within mucosal and sub mucosal layer (Fig. 3B). Figure 3C illustrates hemorrhage in blood vessels, which might occur when it is ruptured along with dilation of blood vessels, which forced the blood to come outside the vessel. The atrophy in the mucosa was observed in intestinal tissue of *Labeo*

rohita from polluted site, in which mucosal layer decreased in thickness as compared to normal along with the aggregation of inflammatory cells that are used in reducing inflammation from the intestine and in an increasing immunity.

Three organs of each fish specimen were selected for histopathological evaluation, which included gills, liver and intestine. The gills are important for various functions in the fish, such as respiration, osmoregulation and excretion. Gills are in contact with their and sensitive to any changes in the water quality being a primary target of the pollutants (Fernandes and Mazon, 2003; Camargo and Martinez, 2007).

In this study, the gills *Labeo rohita* showed degenerative, necrotic, proliferative changes in gill filaments and secondary lamellae, and congestion in the blood vessels of gill filaments. These findings are in agreement with Olurin *et al.* (2006), Fernandes and Mazon (2003) and Camargo and Martinez (2007). These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants through the gill surface. Alterations like proliferation in the epithelial cells, fusion of some lamellae and epithelial lifting are defense mechanisms and it caused by an increase in the distance between the

Table III.- The concentrations of heavy metals (mg/L) in polluted and control water samples.

Metals	Site 1		Site 2		Site 3	
	Polluted	Control	Polluted	Control	Polluted	Control
Lead	4.41±0.17***	3.56±0.16	3.08±0.02***	0.27±0.02	1.25±0.05***	0.16±0.01
Copper	4.88±0.02***	2.76±0.03	2.04±0.06***	0.24±0.01	1.14±0.01***	0.17±0.008
Nickel	1.22±0.006***	1.08±0.004	1.16±0.006 ^{NS}	1.15±0.012	1.21±0.014***	0.32±0.018

***, Highly significant; NS, Non-significant.

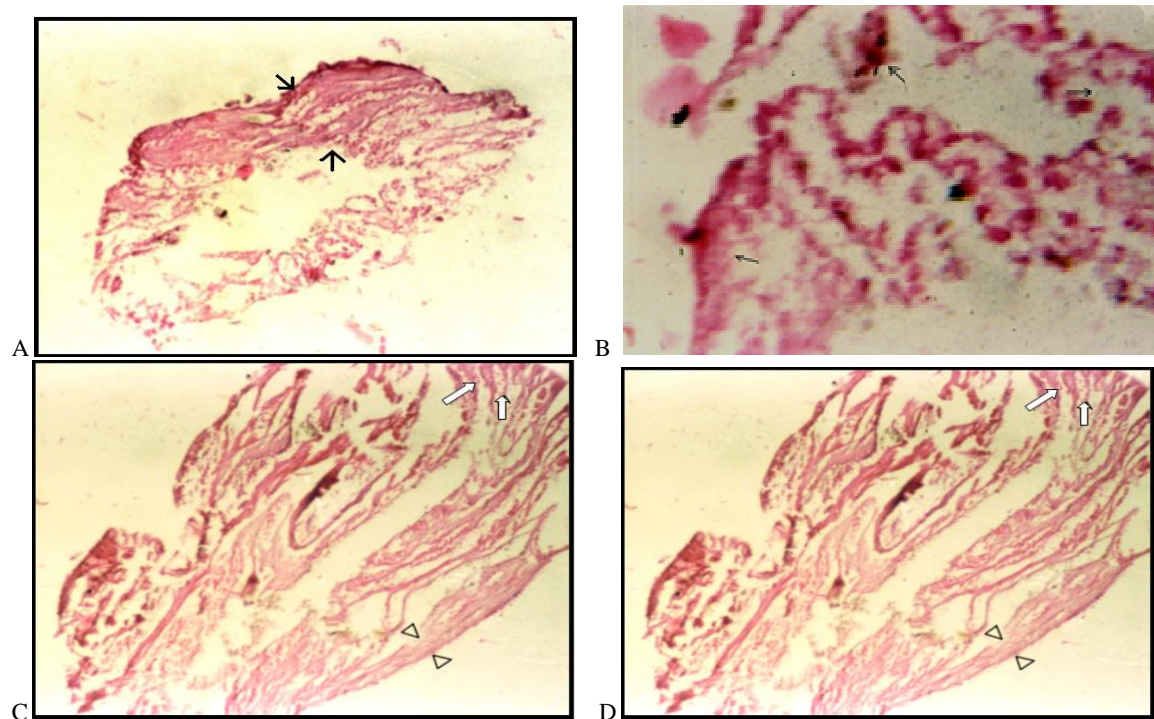


Fig. 3. Histological structure of intestine of *Labeo rohita*. A, control showing the normal histology of intestine (↑). B, shows severe degeneration and necrosis of mucosal membrane of intestine (↑). C shows hemorrhage (↑) and dilation within blood vessels within serosa of mucosa (⇈). D, atrophy in a mucosal layer (Δ) and aggregation of inflammatory cells (⇈). Stain: hematoxylin-eosin, 400X.

external environment and the blood and thus serve as a barrier to the entrance of contaminants. Because of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003). Necrosis observed in gill tissue can adversely affect the gas exchange and ionic regulation (Dutta *et al.*, 1993; Mohammed, 2009).

The fish specimens collected in the present study were from those sites that have been confirmed for heavy metal contamination (Bashir, 2010). The gill abnormalities, including hyperplasia of epithelial cells,

fusion of secondary lamellae, lifting of the lamellar epithelium, blood congestion, proliferation of epithelial cells of primary and secondary lamellae, and necrosis in the present study, have been reported as a result of industrial, domestic and agricultural wastes and heavy metals. (Camargo and Martinez, 2007; Triebkorn *et al.*, 2008; Mohammed, 2009). Blood congestion, or even an aneurysm may be resulted due to an increased blood flow inside the lamellae causing the dilation of the marginal filaments, are in agreement with Martinez *et al.* (2004).

Authman and Abbas (2007) mentioned that liver has detoxical role of endogenous waste products and externally derived toxins as heavy metals. The liver of

Labeo rohita showed marked histopathological changes, including degeneration and necrosis of the hepatocytes, vacuolar degeneration in the hepatocytes, thrombosis formation in central veins, dilation, hypertrophy and pyknosis of a nucleus in the hepatic tissues, irregular shaped nucleus and cytoplasmic vacuolation. The present findings are in agreement with Triebkorn *et al.* (2008) and Mohammed (2009). Cellular degeneration and necrosis in the liver, might be reported due to vascular dilation, intravascular haemolysis and thrombus formation in the blood vessels with subsequent stasis of blood (Mohammad, 2009). Atamanalp *et al.* (2008) reported that exposure of copper sulphate was found to induce degeneration of hepatocytes, and congestion in the blood vessels of the liver. Increased vacuolization of the hepatocytes can be described as a signal of a degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water (Pacheco and Santos, 2002).

However, uptake of metals occurs mainly through gills, but may also occur *via* intestinal epithelium. Histopathological alterations in the intestine of *Labeo rohita* included severe degenerative and necrotic changes in the intestinal mucosa and sub mucosa, atrophy in the muscularis and sub mucosa and aggregations of inflammatory cells in the mucosa and sub mucosa with edema between them. These findings are in agreement with those of Hanna *et al.* (2005), Bashir (2010), Yousafzai *et al.* (2010) and Soufy *et al.* (2007). We were of the opinion that these histopathological alterations were might be due to heavy metal contamination which had affected the gills by epithelial lifting, hyperplasia of epithelial cells and blood congestion within filaments and in liver tissue produced hemolysis between hepatocytes, cytoplasmic degeneration and necrosis. Whereas an aggregation of inflammatory cells, edema in an intestinal mucosal layer and hemorrhage between blood vessels were the main alterations observed in the intestine. The changes seemed to be more pronounced in the liver and gills rather than the intestine.

CONCLUSION

It has been concluded that histopathological evaluation is a useful biomarker to determine environmental pollution and to assess the health status of fish.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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