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Histopathological Responses of Liver and Kidney of a Freshwater Cyprinid, *Labeo rohita* to Nickel Sulphate

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Abstract.- Nickel is frequently detected as a strong contaminant in aquatic environment, exhibiting potential threat to environment and human health. The objectives of our study were to determine 96h LC_{50} values of nickel sulphate against freshwater cyprinid (*Labeo rohita*), and to determine the effects of acute doses of nickel sulphate on the histological changes of its liver and kidney. The acute toxicity test for *Labeo rohita* revealed 96h LC_{50} values of 19.21 mg/L for nickel sulphate ($NiSO_4.7H_2O$). In nickel sulphate treated group (Ni-T), the liver tissues exhibited mild cellular hypertrophy, severe fatty change, karyorrhexis, mild karyopyknosis, acute blood congestion, blood vessel sclerosis, severe irregular shaped cell with irregular shaped nuclei, and nuclear hypertrophy. In Ni-T group, the most prominent alterations investigated in the kidneys sections of *Labeo rohita* were blood congestion in blood vessel, liquefactive necrosis of first and second proximal segments, enlargement of glomerulus, irregularity in interstitial haematopoietic tissue due to tubular necrosis, glomerulus degeneration, cellular hypertrophy of renal tubule, cytoplasmic vacuolization in glomerulus, tubular necrosis and peritubular vacuolization due to narrowing of second proximal segment. These findings suggest that nickel sulphate at higher doses is highly toxic to *Labeo rohita*.

Key words: *Labeo rohita*, nickel sulfate, LC_{50} values, histopathology.

INTRODUCTION

Aquatic pollution is the most serious and emerging problem throughout the world (Stanitski, 2003). Frequent use of commercial chemicals by the chemical industries and agricultural farming ultimately become the prominent sources of aquatic pollution (McGlashan and Hughies, 2001). In addition, unregulated use of herbicides, fungicides, discharge of untreated municipal waste, sewage biochemical breakdowns, heavy monsoon rain falls and floods carrying pollutants, oil leakage and spills are other important sources of water pollution with heavy metals (Sial and Mehmood, 1999).

Pakistan has naturally flowing fresh water river system. The industrial wastes are directly dumped into the river systems without treatment. Freshwater contamination with heavy metals has been reported to be one of the most challenging issues in the country (Abid and Jamil, 2005). Consequently these heavy metals being stable and persistent in nature have the ability to get accumulated in different tissues and organs of aquatic organisms, more especially in fish. The heavy metals may indirectly enter into the human body through consumption of fish, thus causing serious health hazards, sometimes even death. Many of these metals are carcinogens and can cause genetic deformities and other fatal diseases (USEPA, 1991). World Health Organization's admissible concentration of nickel in drinking water is 0.02 mg/L. After floods of 2010, the nickel concentration in ground water in various regions of Punjab was raised to 3.66 mg/L, whereas in surface it ranged between 0 to 1.52 mg/L (Azizullah *et al.*, 2011).

A wide range of histocytological alterations in fish have been recommended as biosensors for monitoring the effects of pollution (Moore and Myers, 1994). Histopathological biosensors can be indicators of various anthropogenic pollutants and are the direct reflection of the overall health of the entire population in the ecosystem. The frequent alterations in cells and tissues in vertebrate fish are recurrently used biomarkers in many studies (Hinton *et al.*, 1985).

Keeping in view the emerging issue of aquatic pollution with nickel in Pakistan, the present study was carried out to determine the toxicological influences of nickel exposure on histopathology of

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liver and kidneys of a freshwater cyprinid fish, rohu *Labeo rohita*.

MATERIALS AND METHODS

Fish

Juveniles of rohu of both sexes, with body length of 9.32±1.54 cm and body weight of 11.46±1.25 g were used for study.

Determination of LC_{50} of nickel sulphate

To determine 96h LC_{50} values, 16 juveniles of rohu in each group were exposed to one of the seven concentrations; 4.0, 8.0, 12.0, 16.0, 20.0, 28.0, 36.0 mg/L of nickel sulphate ($NiSO_4.7H_2O$). The 96h LC_{50} values for nickel sulphate and its 95% confidence limit was determined using Probit analysis. The control mortality was corrected using Abbot (1925) formula, where necessary.

Experimental protocol

Two groups of freshwater cyprinid, *Labeo rohita* were randomly stocked in two separate tanks (40 fish per group) with a capacity of 300 L each. Fish juveniles of first group (Ni-T) were subjected to acute toxicity exposure to 19.21 mg/L of nickel sulphate for 96h. The fish juveniles of second group were kept untreated and designated as control (Ni-C). The physicochemical characteristics of the laboratory were: Capacity of each tank = 300 L; temperature= 25.0±1.0°C; pH= 6.5-6.8; total hardness= 65-75 mgL^{-1} (as $CaCO_3$); alkalinity= 75-80 mgL^{-1}. All experiments were carried out in semi-static systems with renewal of water every 12h with the addition of fresh solution of toxicant with same concentration to sustain the nominal concentrations of nickel sulphate. All the experimental procedure and fish handling protocols were approved by Ethical Committee of Zoology Department, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

The surviving fish juveniles after exposure to LC_{50} *viz.*, 19.21 mg/L were selected for histopathological studies according to Brungs and Mount (1978). The liver and kidneys of the fish were surgically removed from each group of fish (Ni-C and Ni-T groups) and were immediately washed in ethanol and water mixture [1:15] to remove blood traces from the selected tissues, and fixed in Bouin's fluid for 5h. Fixation was followed by dehydration in ethanol, clearing in Cedar wood oil and embedding in Paraffin wax. The sections (4-6 µm) were cut and stained with hematoxylin and eosin and examined for cellular hypertrophy of renal tubule, dilatation of glomerulus capillaries, enlargement of glomerulus, narrowing of tubular lumen, nuclear hypertrophy, hyperplastic fatty change, and anaplastic interlamellar occlusion, epitheliocapillary separation (ECS), necrosis, glomerular degeneration, blood congestion, and hemorrhage or infestation of parasites induced by toxicant.

RESULTS

The 96h LC_{50} values of nickel sulphate

The 96h LC_{50} values for nickel sulphate was found to be 19.21 mg/L, as per regression line (Fig.1). Rohu could tolerate 4.00 mg/L of nickel sulphate as no mortality was observed at this concentration. It was examined in the study that rohu could not tolerate 36.00 mg/L of nickel sulphate as 100% mortality of rohu was observed at this concentration (Fig. 1).

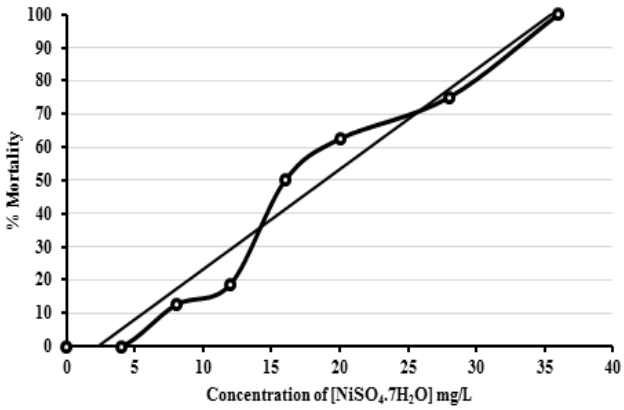

Fig. 1. Toxicity of different concentration of ($NiSO_4.7H_2O$) against *Labeo rohita* exposed for 96h.

Histopathological responses

Liver

In Ni-C group, the liver exhibited normal

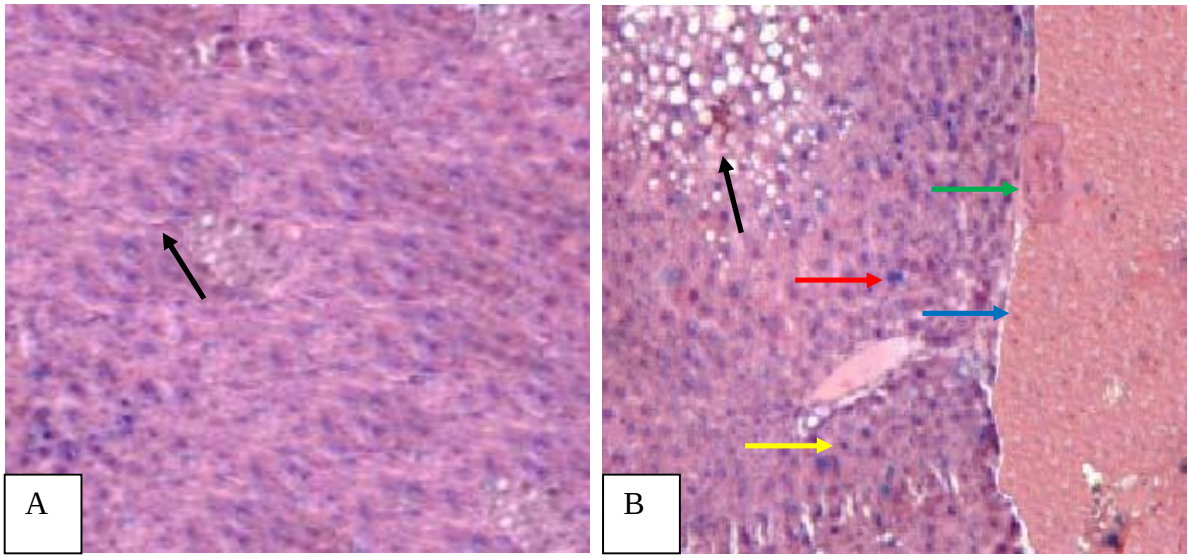

Fig. 2. Histological structure of control (A) and nickel sulphate treated (B) fish liver. Control, indicating normal hepatocytes, Black arrow indicates for Kupffer's cells. Fatty change (Single black arrow). Karyorrhexis (Red arrow); Karyopyknosis in hepatocytes (Yellow arrow); acute blood congestion (Blue arrow); sclerosis (Green arrow); and vascular dilation are visible in nickel sulphate treated liver. H & E staining, Magnification: 400X.

histology with homogeneous mass of hepatocytes having central vein and sinusoids, all were in normal appearance. The hepatocytes contained centrally located large prominent nuclei, identical in all aspects (Fig.2A). However, in Ni-T group, the liver tissues exhibited mild cellular hypertrophy, no cellular rupture, severe fatty change, karyorrhexis, mild karyopyknosis, acute blood congestion and blood vessel sclerosis, severe irregular shaped cell with irregular shaped nuclei and mild nuclear hypertrophy. Hepatocytes were compactly arranged but with a strong cytoplasmic vacuolization with increased basophilia. Hepatocellular necrosis with parenchymal vacuolization, breakdown of cell boundaries, swelling and degeneration of the endothelial lining cells leading to the damage of central veins were also observed in Ni-T group (Figs. 2, 3).

Kidney

In Ni-C group, the kidneys sections exhibited normal histology with prominent mass of glomerulus, first proximal segment (PI), second proximal segment (PII) having interstitial hematopoietic tissue, all were in normal appearance (Fig. 4A).

In Ni-T group, the most prominent alterations investigated in the kidneys sections of *Labeo rohita* were blood congestion in blood vessel, liquefactive necrosis of first and second proximal segments, enlargement of glomerulus, irregularity in interstitial haematopoietic tissue due to tubular necrosis, glomerulus degeneration, cellular hypertrophy of renal tubule, cytoplasmic vacuolization in glomerulus, tubular necrosis and peritubular vacuolization due to narrowing of second proximal segment (Fig. 4B).

DISCUSSION

Nickel tolerance

The application of 96h LC_{50} values of a toxicant had gained an international acceptance among piscine toxicologists and are generally the most highly rated tests for estimating the short-term poisoning potential effects (acute toxicity) of a toxicant material to aquatic life (Brungs and Mount, 1978). The 96h LC_{50} values of nickel sulphate (333.6 mg/L) for major carp, *Heteropneustes fossilis* was reported by Choudhary and Jha (2009), which did not exhibit similarities with our findings because several factors including experimental conditions,

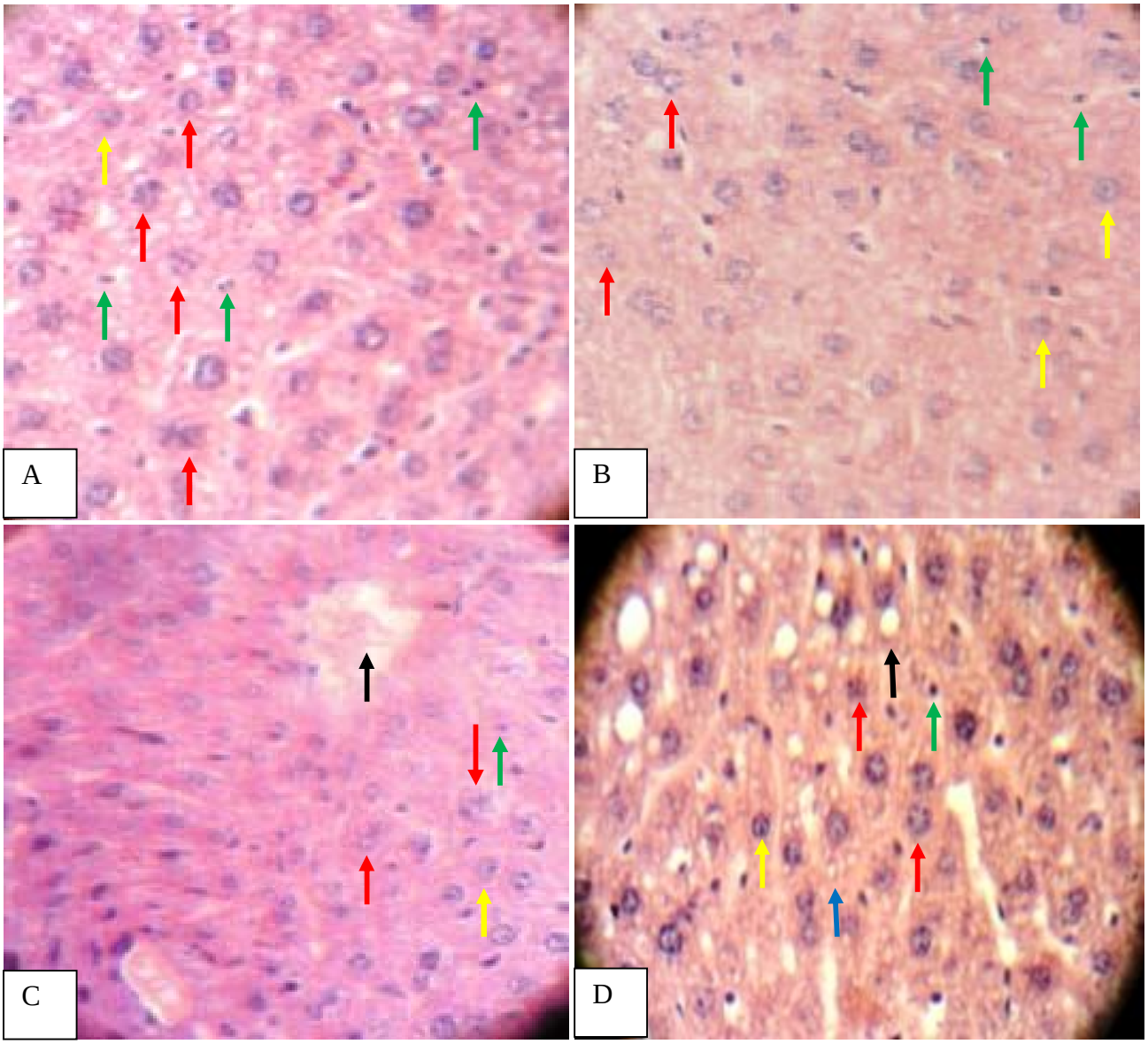

Fig. 3. Histological structure of liver of *Labeo rohita* treated with nickel sulphate; A, Normal hepatocyte (Yellow arrow), Karyorrhexis (Red arrow), Karyopyknosis (Green arrow); B, Normal hepatocyte (Yellow arrow), Karyorrhexis (Red arrow), Karyopyknosis (Green arrow). C, Normal hepatocyte (Yellow arrow), Karyorrhexis (Red arrow), Karyopyknosis (Green arrow), Cellular hypertrophy (Black arrow); D, Normal hepatocyte (Yellow arrow), Karyorrhexis (Red arrow), Karyopyknosis (Green arrow), Fatty change (Black arrow), and Nuclear vacuolization (Blue arrow). H & E staining; Magnification: all at 1000X.

chemical composition of toxicant, species tolerance and age of fish may affect the sensitivity of fish to heavy metal exposure (Abdullah *et al.*, 2007).

In the study, it was examined that fingerlings of *Labeo rohita* had the potential to tolerate the concentration of 4 mg/L of nickel sulphate as no mortality rate was observed at this concentration. In contrast, the fingerlings of *Labeo rohita* could not cope with the concentration of 36 mg/L of nickel sulphate as they exhibited 100% mortality at this concentration. Based on data, it was concluded from the study that nickel had the potential to penetrate into body of candidate fish by simple diffusion via gills pore, or through drinking process and by skin absorption. This active pollutant after entry into the body had shown bioaccumulation. Bioaccumulation

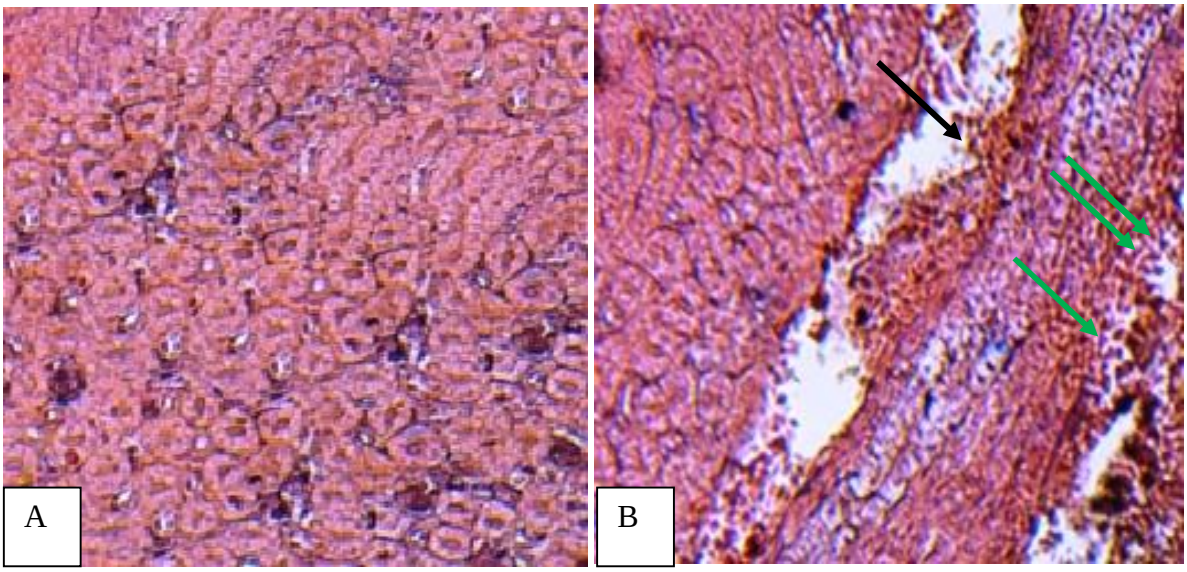

Fig. 4. Histological structure of kidney of *Labeo rohita*, A, Control, indicating normal glomerulus histology, Normal first proximal segment; and normal second proximal segment and normal interstitial haematopoietic tissue. B, *Labeo rohita*, treated with ($NiSO_4.7H_2O$). Single black arrow indicating congestion of blood vessel, Green arrow indicating liquefactive necrosis of first and second proximal segments and double green arrows showing irregularity in interstitial haematopoietic tissue due to tubular necrosis. H & E staining, Magnification 400X.

of the pollutant is followed by detrimental effects for *Labeo rohita.* It is inferred from the study that the pollutant in *Labeo rohita* may have developed affinity with life sustaining elements and exercise most of their toxic effects in them. It is also inferred from the study that nickel metal whenever present in excess amount in the medium has potential to alter the physiological events in different tissues most probably in liver and kidneys, causing the death of fish (Basa and Rani, 2003).

Nickle-induced liver pathology

The histopathological abnormalities reported here may be attributed to direct toxic effects of nickel pollutant on hepatocytes, as liver is the site of detoxification of all types of toxins and chemicals (NRC, 1997). The fatty change in liver indicates imbalance between the rate of entry and utilization of fatty acids in metabolic processes in the hepatocytes and the rate of their release into the circulation system. The vascular dilation, intravascular hemolysis and thrombosis formation observed in the blood vessels with subsequent stasis of blood may be responsible for necrosis in liver (Mohamed, 2001). The histopathological alterations such as karyorrhexis, mild karyopyknosis, acute blood congestion *etc* observed in study were in agreement with those observed by many investigators who have studied the effects of different pollutants (Depledge and Fossi, 1994; Decaprio, 1997; Adams, 2002). Such types of histopathological modifications have also been noticed in liver of *Clarias gariepinus* by Olojo *et al*. (2005), when exposed to Pb and Cu, in *Channa punctatus* by Mishra *et al*. (2009), when fish was exposed to chromium, and in *Oreochromis niloticus* exposed to Cu by Figueiredo-Fernandes *et al*. (2007). Variations in hepatocytes nuclei shape in the present study match with the observations of *Tilapia mossambica* by Radhaiah and Jayantha (1992) when treated with fenvalerate and by Tilak *et al*. (2005) in liver of *Catla catla,* when exposed to chlorpyrifos and also exhibited equality with the findings in *Oreochromis niloticus,* kept in water borne Cu by Figueiredo-Fernandes *et al*. (2007). The fallouts of liver exposed with toxicant in the present study also match with liver of *Pomacentrus pavo*, treated with chlorpyrifos by Weisman and Miller (2006) and to that of Molly fish exposed to sodium perchlorate by Kolbas *et al*. (2009). But the results of the present study exhibited an inverse relationship with the findings of other authors, who have studied the

impacts of various water borne pollutants on fish liver (Ptashynski *et al.,* 2002; Fanta *et al.,* 2003). Atamanalp *et al.* (2008) had investigated that exposure of *Oncorhynchus mykiss* to copper sulphate was found to induce degeneration of hepatocytes and sinusoidal dilation in the liver. Kloepper-Sams *et al.* (1994) investigated an increase in liver size and fluctuation in serum biochemistry in long nose suckers exposed to bleached-kraft effluent. Similarly, Lohner *et al.* (2001) reported the effects of increased levels of metals on sunfish from a fly ash pond-receiving stream, and found that heavy metals had adverse effects on the health of fish. Higher concentrations of copper, and nickel were found in liver of effluent-exposed fish than in reference fish.

Nickle-induced kidney pathology

The kidneys are one of the foremost organs to be affected by water borne metal contaminants and are especially vital for ion reabsorption, minimize water loss and to eliminate of divalent ions (Nishimura and Imai, 1982; Thophon *et al.,* 2003). The most common alterations found in the kidney of *Labeo rohita* treated with nickel sulphate in the present study were the congestion of blood vessel; liquefactive necrosis of first and second proximal segments; and irregularity in interstitial haematopoietic tissue due to tubular necrosis. It has been informed that renal tubular necrosis affects the renal metabolic activities and promotes metabolic abnormalities in fish (Yokote, 1982). The results of the present study are in agreement with the findings observed in *Cyprinus carpio* by sewage exposure (Kakuta and Murachi, 1997). *Prochilodus lineatus* exposed with trichlorfon (Veiga *et al.,* 2002) and *Lates calcarifer* exposed by cadmium (Thophon *et al.,* 2003). Velmurugan *et al.* (2007) observed renal tubular necrosis, hypertrophy in renal tubular epithelial cells, mild narrowing of the tubular lumen, chronic expansion of space within the Bowman's capsules and slight contraction in the glomerular portions in the kidney of *Cirrhinus mrigala* exposed to fenvalerate. While Camargo and Martinez (2007) found severe cloudy swelling and degeneration in the renal tubular epithelium in the kidney of *Prochilodus lineatus* caged in Cambe stream, Brazil, heavy polluted by domestic, agricultural, and industrial wastes.

CONCLUSIONS

The higher concentrations of nickel in aquatic environment in Pakistan is a potential source of environmental pollution. It causes serious abnormalities in the liver and kidney of *Labeo rohita*. Since this fish is also a staple food in Pakistan it can be a source of human health hazard.

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