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Histopathological changes in tissues of freshwater fish Rohu, (*Labeo rohita*) exposed to TPS effluent .

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ABSTRACT

The present study was conducted to examine contamination and the hazardous effect of heavy metal from the water body nearby thermal power station (TPS) and tissues of the freshwater fish, *Labeo rohita* (Rohu). The heavy metals like As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu were observed in water body adjacent to thermal power station in varying quantities that indicates the presence of heavy metal in water body. The histological changes in fish tissues were showed various structural changes, which point out towards deleterious effect of thermal power plant effluent on the freshwater fish *L. rohita* which might be due to the state of stress caused by exposure to metals.

Keywords: : heavy metals , contamination, *L. rohita*

INTRODUCTION

The main purpose of any industrial development is to provide an opportunity for better living and an employment to the people residing the area. Though industrial development produces more employment it is also responsible for the degradation of the environment by introducing various pollutants into the atmosphere which produces air, water and land pollution. Hence now there is need to protect the environment from these harmful effects at any possible limits. In recent years the energy demand has been increased so rapidly which is being largely met by using fossil fuel. The increasing demand for energy is the one of the challenges that faces the development of the country [1].

Thermal power plants are the main source of energy production in India where the energy is produced by using coal as a fossil fuel. Coal is largely composed of organic and some inorganic components such as including trace elements which have been cited as possible cause of health and environmental effects. Due to coal combustion a significant quantity and variety of trace elements are transformed into surrounding environment by various pathways.

In natural systems even a low concentration of heavy metals and trace elements can have beneficiary or harmful effect on aquatic biota. During recent years the environment is being contaminated with wide range of pollutants that includes heavy metals, trace metals, pesticides released from various domestic, industrial and other manmade activities, which are having harmful effect on ecological balance of the recipient environment.

Heavy metal contamination has been reported in aquatic organisms [2-3] and trace metal contaminations are important due to their potential toxicity for the environment and human beings [2,4-6]. Heavy metals includes both essential and non essential elements that have a particular significance in ecotoxicology, as they are highly persistent and all have the potential to be toxic to living organisms [7].

Major pollutants released by coal based power generation include sulphur, carbon and nitrogen compounds, heavy metals and fly ash. Coal operated thermal power plant can be a source of pollution, because ash derived from burning of coal containing heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) can contaminate water, presenting a potential hazard to the environment[8].

Fly ash is a fine residue resulting from the burning of coal which is discharged into the surrounding environment either by dry or wet method. Chemically fly ash consists of Si, Al, Mg, Ca, K, Ti, and Fe in greater proportion with many trace elements such as V, Mn, Cr, Cu, Ni, As, Pb, Cd, and less quantity of various potential toxic elements. Chemical composition study of fly ash shows mostly the presence of four major elements Al, Si, Fe and Ca in the fly ash. Other metals such as K, Mg, Ba, Co, Cd, Zn, Mo, Pb etc. are present in traces. Though in the traces, compared to original coal, most of the elements are enriched in the fly ash, giving birth to the growing environmental concerns in the disposal and utilization in environment due to release of trace heavy metals.

According to Gupta *et al*.[9] the major part of fly ash is disposed off in unmanaged landfills or lagoons which lead to environmental pollution through fly ash erosion and leachate generation along with metal contamination of surface and ground water resources and hencecan transfer these contaminants into the food chain.

Singh *et al*.[10], Praharaj *et al*.[11], Suresh *et al*. [12] and Ramachandra *et al*.[13] studied leaching of trace elements in coal ashes from Bokaro Thermal Power Station, Kharagpur, Vijayawada Thermal Power Station (VTPS), Andhra Pradesh and Yellur and surrounding villages closer to a thermal power plant in Udupi district, Karnataka State. They reported that nearly every naturally occurring element is likely to be present in coal and these get entertained in the resultant coal ash.

Chakraborty and Mukherjee[14] studied the bioaccumulation of heavy metals like Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As in aquatic, terrestrial and algal species in the vicinity of thermal power station in fly ash contaminated areas in Uttar Pradesh. Studies of trace elements and the elements presents in fly ash are distributed into traction of the fly ashes based on volatilization temperature {15].

Fish are located at the end of the aquatic food chain and are the inhabitants that cannot escape from the detrimental effects of these pollutants which may accumulate metals and pass them to human beings through food, causing acute and chronic diseases [16,17,18].

Heavy metals have long been recognized as serious pollutants of the aquatic ecosystem. The heavy metals are toxic to many organisms at very low concentrations. Increased discharge of heavy metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals

[19]. It had been reported that heavy metals had a negative impact on all relevant parameters and caused histopathological changes in fish. Some heavy metals are essential elements while others are non-essential [20].

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. [17,18,21]

METHODOLOGY

A. Study site:
This study was conducted at pond in the vicinity of thermal power station (TPS) located at Koradi village of Dist. Nagpur (Fig. 01).

B. Collection of water sample for heavy metal analysis:
The water samples were collected from the pond of TPS for the heavy metal analysis (Fig. 02) and were further processed as, 5 ml of concentrated HNO_3 was added to a 50 ml of water sample to digest all the organic matter and to get the clear solution. The digested and cleared water samples were filtered using Whatman filter paper and made upto original 50 ml volume and injected into Inductively Coupled Plasma Atomic Emission Spectrometer (ICP - AES) for metal estimation.

C. Sampling and collection of the fishes:
The fishes, *Labeo rohita* (Rohu) were sampled with fishing net with the help of fishermen. These fishes were scrutinized. Below aged and diseased fishes were discarded and released into pond, only healthy and about 2 year old fished were kept for experimentation in container filled with pond water.

Medium sized *Labeo rohita* of about 25-30 cm length and about 250-500 gm weight were selected for the toxicity study. The diseased and injured fish were discarded and only healthy and medium sized fishes were selected for the histological study. The fishes were captured with the help of fisherman by using fishing net, the captured fishes were scrutinized, dissected out on spot. Tissue sample for histological study tissue was collected and fixed in fixatives ex. Bouin's fixative for histology. The stored samples were brought to laboratory for further processing.

The control fish samples were collected from the artificial pond having no contamination history and where water was of good quality.

OBSERVATION:
I. Heavy Metals in Pond Water
The concentration of heavy metals in pond water from TPS was shows presence of As, Zn, Pb, Cd, Co, Ni, Mn, Fe, Cr, Al, and Cu in varying quantities. From the above data it is clear that the pond water is contaminated with heavy metals in different concentrations.

2. Histopathological Observations
The histological changes observed in all tissues of *L. rohita* in the present study indicate that the effluent caused moderate to severe alteration in liver, kidney as well as muscle architecture, which are important organs performing vital functions like detoxification, osmoregulation, acid base balance, excretion etc.

A. Histopathological Observations
T. S. Liver of *L. rohita* (Normal / Control)
The T. S. of control liver shows (Fig. 03 a,b) continuous mass of hepatic parenchymal cells arranged in cords around blood vessels. The hepatic cells are polygonal in shape and with centrally placed rounded nucleus and homogeneous cytoplasm. Hepatic cells are not arranged to form distinct lobules. Pancreatic acini of exocrine function lie embedded in between the hepatic cells surrounding the blood capillaries.

In the exposed group, the cytoplasmic damage was not so severe but the orientation of the cells was disrupted and vacuolation in the liver cells were found. The exposure also resulted in enlarged nuclei, condensation of cytoplasm and disarray of hepatic cords. Some liver cells appeared almost devoid of cytoplasmic contents. The lesions are further characterized by elongations of blood vessels, necrosis and degeneration. Widespread vacuolation within the hepatocytes and appearance of some typical globular bodies, which may be suspected as, infiltered fats. Few hepatocytes lost their polygonal shape as they were hypertrophied. The cell membranes of hepatic cells were found to be thickened. The pancreatic acini around blood capillaries were necrosed. Also sinusoids were found be degenerated resulting into bleeding in the intercellular gaps. Pancreatic acinar cells lost their identity. Degeneration of hepatic cells was seen prominently.

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The liver composed of masses of hepatocytes not organized in distinct lobules and were interrupted by sinusoids. Endothelial cells and kupper cells line the sinusoidal lumen. The blood vesicles and bile duct was randomly found throughout the hepatic parenchyma. Bile duct wall consist of a simple cuboidal epithelium with a brush border and a collagenous cover. Melanomacrophage centers are present in the hepatic parenchyma, and are usually located in the vicinity of blood vessels and bile ducts. The polyhydral hepatocytes bear spherical nuclei and moderately eosinophilic granular cytoplasm. The hepatocytes are normally multinucleated.

T. S. Liver of TPS Effluent Exposed *L. rohita*

The T. S. liver of *Labeo rohita*, (Fig. 03 c,d) after exposure to effluent revealed swelling of hepatic nuclei, disorganization of hepatic cells with edematous hepatocytes and many cells were devoid of cytoplasmic contents. Also liver tissue became a necrotic spongy mass with degeneration of sinusoids. Most of the hepatocytes lost their cell boundaries and some of them showed indistinct cell boundaries. Hepatocytes showed disintegration and at several places the nuclei could not be seen distinctly.

T. S. of Kidney of *L. rohita* (Normal/ Control):

The T. S. of control kidney (Fig. 04 a,b)composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The glomerular tissue was closely arranged with renal tubules including distal and collecting tubules and intact interstitial cells. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent.

T. S. of Kidney of TPS Effluent Exposed *L. rohita*

The T. S. kidney alterations found shown in (Fig. 04 c,d). The most important change found in the glomerulus of *L. rohita* kidney was glomerular expansion, resulting in reduction of Bowman's space. In the tubules, the most frequent alterations were: cloudy swelling, occlusion of tubular lumen and hyaline droplet degeneration. Less frequently, regenerating tubules were seen. The section showed mild edema. The cell size was reduced and the glomerular tissues remained more or less intact, mild interstitial edema and mild damage of renal tubes was found in several areas. The hydrophobic degeneration of renal tubes in the glomerular tissue was seen. The experimental kidney sections show severe damage and disorganization of tubules. The glomerular edema and necrosis were also noticed.

T.S. of Muscles of *L. rohita* (Normal/ Control)

The T. S. of control muscles (Fig. 05 a,b) of the body consist of a double series of muscle segments, the mytomes in the region of the trunk and tail. The trunk musculature consist of successive segments the myomeres, running along each flank. The muscle fiber are oriented in anterioposterior position in each myotome and are separated from the adjacent ones by stout sheets of connective tissue; the myocommata. The myotomes are bent forward and backward and fit with the adjacent ones by the cone within the cone arrangement. In the surface view, each myomere is generally in the form of a 'W' with upper edge turned forward. Prominent block of lateral trunk muscle (myotomes) are visible as the meat of fish when it is skinned. Histologically muscle fibers have peculiar ribbon like myofibrillar bundles and rod edges of the fiber. Muscle fibers are arranged like spoks from a samall central sarcoplasmic hub.

T.S. of Muscles of TPS Effluent Exposed *L. rohita*

The histological alterations in the muscle of studied fish *L. rohita* (Fig. 05 c,d) included degeneration in muscle bundles accompanied with focal areas of necrosis. Also, vacuolar degeneration and atrophy of muscle bundles were seen.

RESULTS AND DISCUSSION

Histological Studies

The **liver** composed of masses of hepatocytes not organized in distinct lobules and were interrupted by sinusoids. Endothelial cells and kupffer cells line the sinusoidal lumen. The blood vesicles and bile duct was randomly found throughout the hepatic parenchyma. Bile duct wall consist of a simple cuboidal epithelium with a brush border and a collagenous cover. Melanomacrophage centers are present in the hepatic parenchyma, and are usually located in the vicinity of blood vessels and bile ducts. There may be accumulated antigenic bodies; they store products that are difficult to eliminate. The polyhydral hepatocytes bear spherical nuclei and moderately eosinophilic granular cytoplasm. The hepatocytes are normally multinucleated.

The organ most associated with the detoxification and biomarker process is liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water [22]. The liver of fish exposed to effluent showed vacuolar degeneration, swelling in the hepatocytes with indistinguishable cellular outline. These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all type of toxins and chemicals. It seems that there is a temporal sequence of the events that starts with vacuolization, swelling and necrosis. Rodrigues and Fanta [23]; Camargo and Martinez, [22]; and Mohamed, [24] have also reported parallel observations with pesticides in various fishes. The exposure of heavy metal caused significant escalation of metal in the liver of fish.

The liver of studied fish showed marked histopathological changes. Degeneration and necrosis of the hepatocytes may be due to the cumulative effect of metals and the increase in their concentrations in the liver. These results agreed with Authman and Abbas, [25] who stated that the liver has an important detoxicating role of endogenous waste products as well as externally derived toxins as heavy metals. Many authors have reported similar histopathological alterations in the liver of fish exposed to metals [19,26,27].

The histological alterations noticed in the present study are in accordance to the chronic exposure to the different pollutants. The lesions developed in the liver might be due to the cumulative action of toxicant on blood and ultimately to other cellular structures. There seems to be a definite correlation between tissue damage and certain physiological alterations [28]. Liver is the major metabolic center and any damage to it would subsequently harm the fish, so many physiological disturbances leading to subsequent mortality of fish [29]. The hepatic lesions observed in the present investigation are in accordance to findings made by different workers during acute exposure to different pollutants. Sastry and Gupta [30]; Patil [31] have reported higher degree of liver damage in acute treatment than the chronic exposure with mercury on *Channa punctatus*. Kumari and Kumar [32] have also reported similar changes in *Channa punctatus* collected directly from highly polluted lake.

The present study revealed that the alterations noticed in the acute exposure were in line with the observations recorded in ninety days chronic exposure to eslan, mercury and ammonia on fish liver [33]. Infiltration of blood filled spaces in the liver along with disarray of cords supports the view of previous workers that heavy metals cause haemorrhage in the internal organs [31,34]. But it seems that the reported lesions are not heavy metal specific, as other workers have reported similar pathological lesions in the liver of the fish after exposure to insecticides and herbicides [28,34, 35]. The present results are well in agreement with those of Bhoraskar and Kothari [36] who reported severe damage in the liver of *Clarias batrachus* exposed to 10 mg/l zinc sulphate. Histological damage due to zinc in fish liver has also been reported by several workers [37,38]. Osman *et al.*, [39] recorded congestion and hemorrhage in the hepatic sinusoids with dilation of hepatic vessels, vacuolization and degeneration of hepatic cells with fatty changes with atrophy of pancreatic acini; in liver of the *oreochromis neloticus* exposed to the polluted water containing heavy metal salts. António *et al.*,[40] studied histopathological changes in liver of Nile tilapia, *Oreochromis niloticus* exposed to waterborne copper and observed vacuolization and necrosis of the liver parenchyma. Moreover, it was also reported by many researchers that chronic heavy metal accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and ultimately death [29,41,42]. The liver plays a key role in accumulation and detoxification of heavy metals [43]. Although, according to Roch and McCarter [44], fishes are known to possess sequestering agent (metallothionein), the bioaccumulation of these trace element in the liver tissue reaches a proportion in which the function of the liver is impeded, thus resulting in gradual degeneration of the liver cell syncytial arrangement. Thus cirrhosis, the outcome of prolonged hepatocellular injury is manifested by fibrosis of hepatic cords. Oxygen required to support the intense metabolic activity of the liver is supplied in arterial blood via the hepatic artery. In effect, necrosis of parenchyma cells had taken place [28].

Moderate histopathological and cellular lesions were observed in the liver of most examined fish with great individual variability. Extensive vacuolization was observed in many specimens. Accumulation of vacuoles resulted in the displacement of nuclei to the cell margin with pyknosis of the nuclei. The histology of heavy metal exposed liver caused a reduction in size and shape of nucleus with degenerative changes in parenchyma cells with necrosis and apoptosis. The decreased number of nucleus in the hepatic tissue was reported in copper exposed to *Oreochromis niloticus* (Figueiredo, 2007). Concentration of heavy metals create an adduct in the liver cells due to their metal chelating proteins that target the cells to release lipofuscin an end product of lipid peroxidation and pigment hemosiderin as a result of internal bleeding in the hepatic tissue of *Cyprinus carpio.* The characteristic appearance of liver fibrosis in the heavy metal exposed fish was supported by report of sunfish in Texas reservoir contaminated with selenium enriched power plant [62].

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Fig. 01: Location map of the thermal power station, Koradi, Nagpur (Maharashtra). (Source:wikimedia.org).

Fig. 02: Satellite image for location of thermal power plant at Koradi, Nagpur (Source: Google earth)

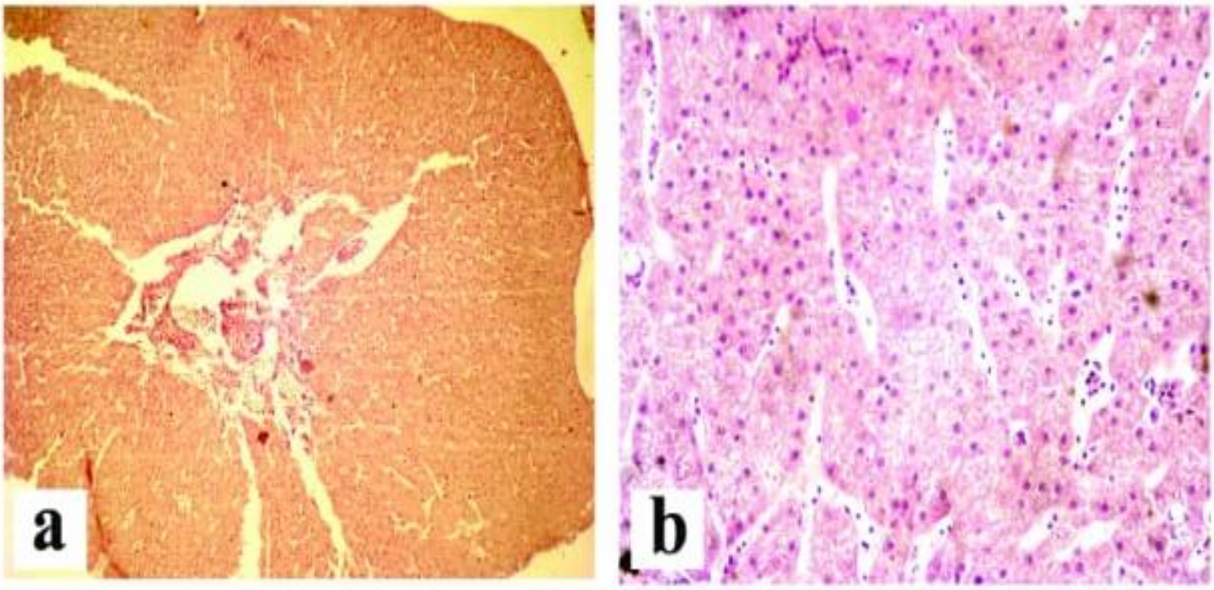

Fig. 03 (a, b): T.S. of liver of *L. rohita* (control) showing continuous mass of polygonal shaped hepatic parenchymal cells arranged in cords around blood vessels . (HE: a, 100X; b, 400X).

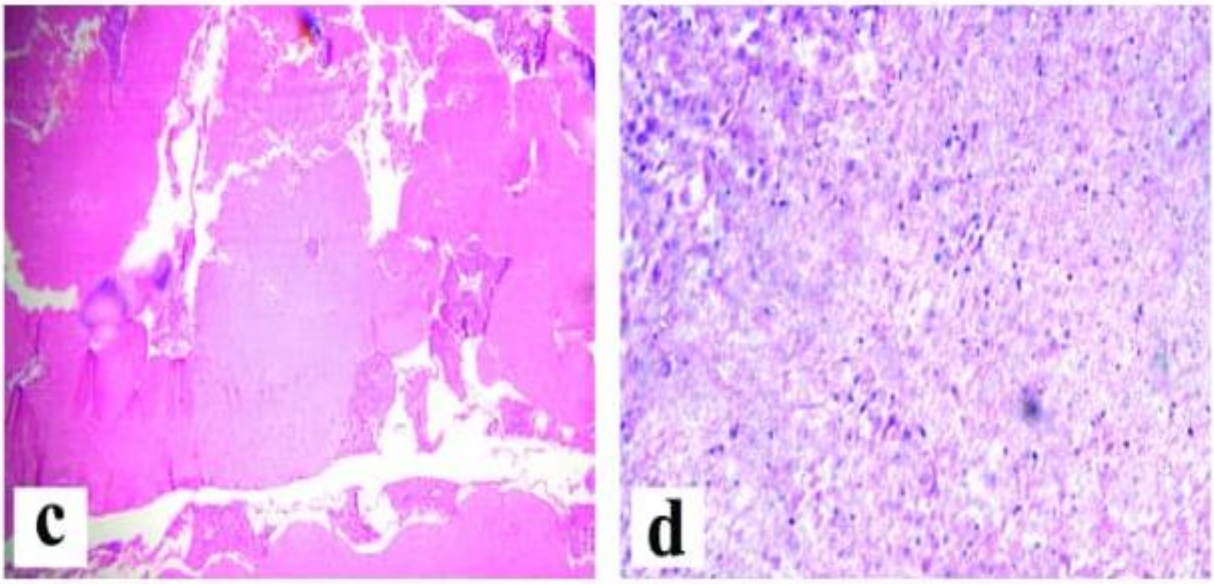

Fig. 03 (c, d): T.S. of liver of *L. rohita* (TPS effluent exposed) showing swelling of hepatic nuclei, disorganization of hepatic cells with edematous hepatocytes. (HE: a, 100X; b, 400X).

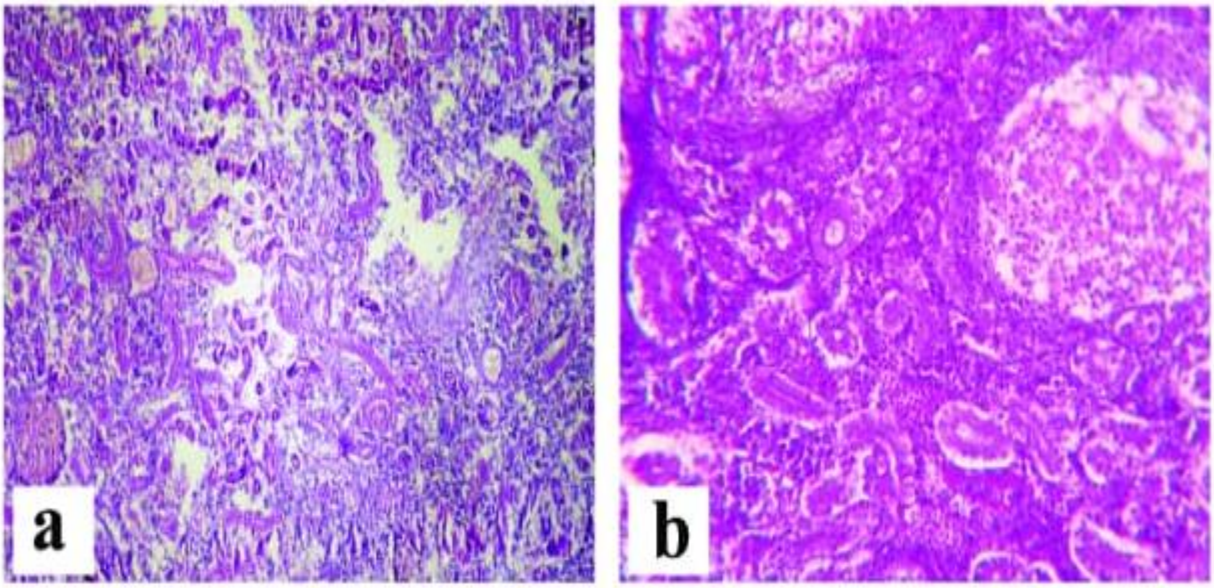

Fig. 04 (a, b): T.S. of kidney of *L. rohita* (control) showing numerous renal corpuscles with well developed glomeruli and a system of tubules. (HE: a, 100X; b, 400X).

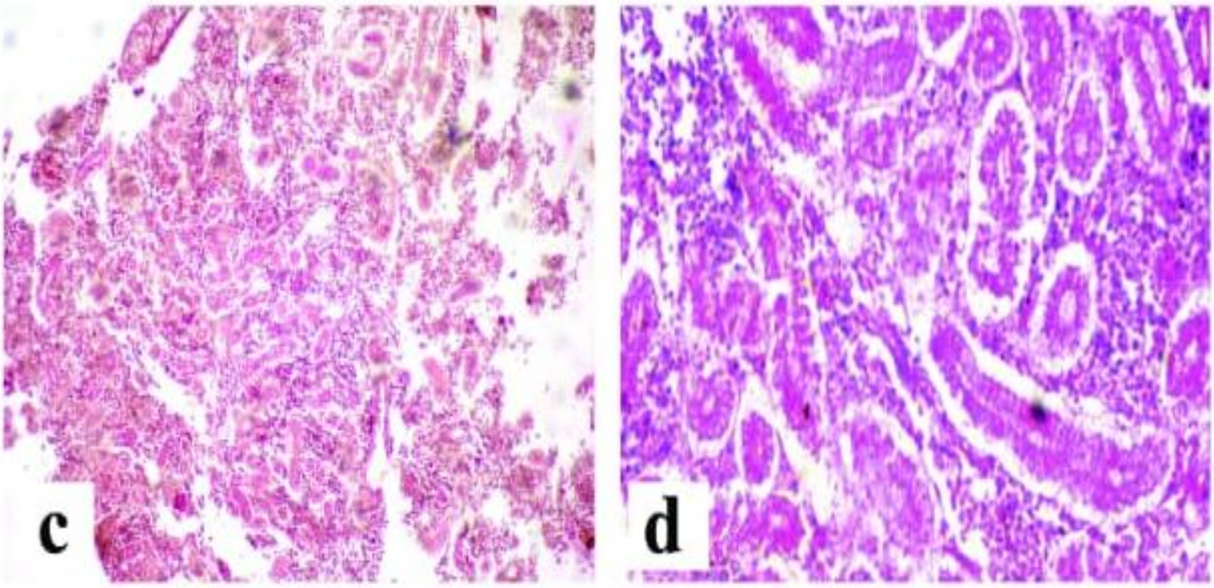

Fig. 04 (c, d): T.S. of kidney of *L. rohita* (TPS effluent exposed) showing mild interstitial edema, mild damage and disorganization of tubules along with glomerular edema. (HE: a, 100X; b, 400X).

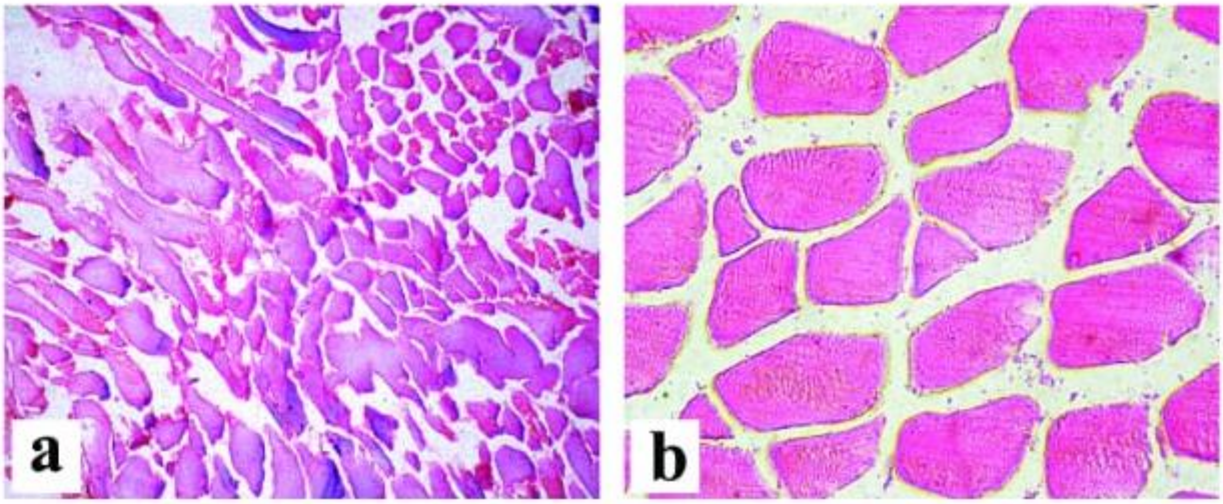

Fig. 05 (a, b): T.S. of muscle of *L. rohita* (control) showing ribbon like myofibrillar bundles and rod edges of the fiber forming sacroplasmic hub. (HE: a, 100X; b, 400X).

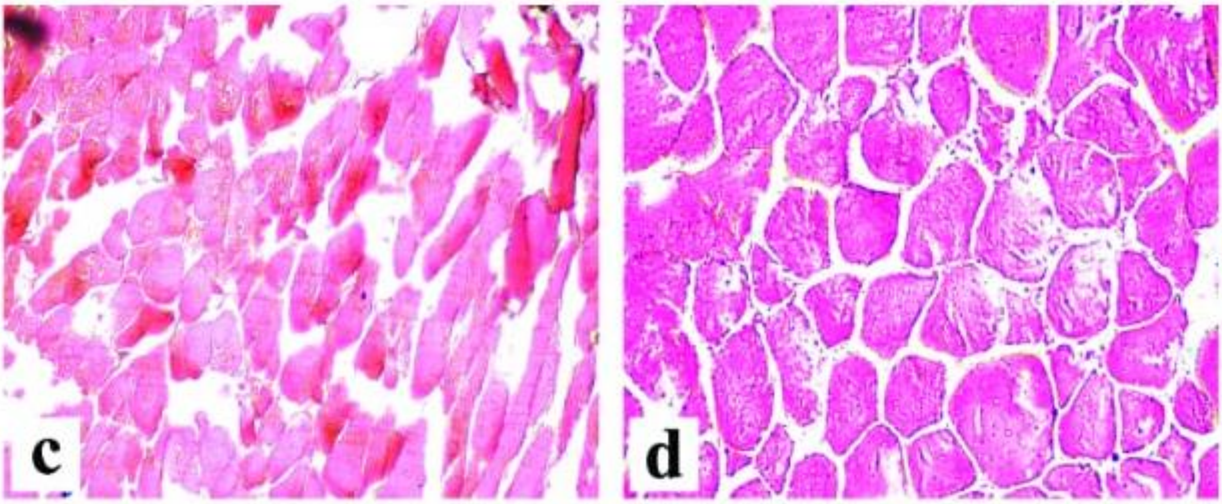

Fig. 05 (c, d): T.S. of muscle of *L. rohita* (TPS effluent exposed) showing degeneration in muscle bundles accompanied with focal areas of necrosis along with vacoular degeneration and atrophy of muscles bundles (HE: a, 100X; b, 400X).

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Concentration of heavy metals create an adduct in the liver cells due to their metal chelating proteins that target the cells to release lipofuscin an end product of lipid peroxidation and pigment hemosiderin as a result of internal bleeding in the hepatic tissue of *Cyprinus carpio.*

The liver of *Labeo rohita*, after exposure to effluent revealed swelling of hepatic nuclei, disorganization of hepatic cells with edematous hepatocytes and many cells were devoid of cytoplasmic contents. Also liver tissue became a necrotic spongy mass with degeneration of sinusoids. Most of the hepatocytes lost their cell boundaries and some of them showed indistinct cell boundaries. Hepatocytes showed disintegration and at several places the nuclei could not be seen distinctly.

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for sensitive reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoiesis [45]. The alterations found in the kidney of fish glomeruli enlargement and edema in bowman's capsules; the kidney exhibited vacuolar degeneration accompanied with hemolysis. With severe intoxicated conditions, the degenerative process leads to tissue necrosis. The necrosis of the tubules will affect the metabolic abnormalities in fish. The present results are in agreement with those observed in *C. carpio* exposed to sewage [46, 47,48].

A selective dystrophic change in kidney tubules together with hyper secretion of mucus cells in the affected region showing atrophy in the underlying tissue was observed.

In the animal kingdom fish are the most vulnerable to environmental chemicals having immunosuppressive action as because they cannot escape from their polluted ambience. Increasing attention has been paid to the immune system of fish as a bioindicator of xenobiotic stress [49,51,52] and metals have been shown to alter immune responses of fish [50,53].

Exposure to heavy metals alters the immunological competence of fish. Metals in this capacity includes Al, Cd, Cr, Cu, Pb, Mn, Ni, Sn and Zn [54]. Since healthy cellular and humoral responses are imperative for protection against diseases, mental stressors interfering with immune system alters the susceptibility of fish to infective diseases [55,56]. The kidney in fish is the major haematopoietic tissue and the head kidney is a variable source of macrophages [57,58]. It has been proposed that the immune system plays a crucial role in maintaining health which can also be a target of xenobiotics expressing immunotoxic changes. Environmental pollutants of a wide variety such as polychlorinated compounds, pesticides or heavy metals are potent immuno – modulators expressing their effect either by immunosuppression or immunoenhancement.

Separation and degeneration of muscles, atrophy of muscle bundles and focal area necrosis were an interesting observation in muscle tissue leading to vacuolar degeneration and splitting of muscle fiber were seen. The histopathological alteration in the fish muscle of both the dose are in agreement with those observation by many investigators who have studied the effect of different pollutants on fish muscle [59,60,61].

Conflicts of interest: The authors stated that no conflicts of interest.

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