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Sub-lethal cadmium toxicity induced histopathological alterations in the gill, liver and kidney of freshwater catfish (*Heteropneustes fossilis*)

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Abstract
The present study aimed at assessing the sublethal (SL) toxicity of the heavy metal, Cadmium in the freshwater Asian Stinging Catfish, *Heteropneustes fossilis*. The SL toxicity test concentrations were selected based on the reported acute toxicity test value (44.13 mg/l). The concentrations selected were 1.11, 2.21, 4.41, 8.82 and 17.64 mg/l. At the end of the experiment (14th and 28th day), live fish samples were collected from the middle (4.41 mg/l) and last (17.64 mg/l) concentrations, sacrificed and their gill, liver and kidney were excised out and fixed in Bouins fixative for 24 hrs. Later, standard histology protocol was followed to study the histological alterations in the gill, liver and kidney of the fishes exposed to SL concentrations of Cadmium. The most common histopathological alterations in the gills of fish exposed to Cadmium were characterized by erosion of secondary lamellae, lamellar fusion, shrinkage of blood vessels, secondary lamellar damage, hyperplasia, vacuolation, epithelial lifting, and shortening of secondary lamellae. The histological changes observed in the liver of fishes exposed to Cadmium included rupture of hepatocytes, vacuolation, hemorrhage in liver sinuses, cellular necrosis, increased pycnotic nucleus, melanomacrophages. In the present work, histological changes like melanomacrophages, degenerated glomerulus, increased peritubular space, shrunken glomerulus, loss of cytoplasm, vacuolation, increased periglomerular space were observed in the kidney of the fishes treated with Cadmium. Thus the present investigation has proved that Cadmium is a potential heavy metal toxicant affecting the fishes at tissue level even at low concentrations, which might in turn affect the physiological processes in the long run.

Keywords: *Heteropneustes fossilis*, Cadmium, Sublethal, Histology, Gill, Liver, Kidney

1. Introduction
In the aquatic ecosystems, heavy metal pollution is a serious problem due to their toxicity and ability to accumulate in the aquatic organisms. Heavy metals are toxic to animals and human beings when their exposure level exceeds the tolerable limits [1]. Hence, there is a great apprehension over the impact of heavy metals on the aquatic ecosystem [2]. These heavy metals are released from domestic, industrial and several other anthropogenic activities [3]. They are harmful pollutants for the aquatic organisms by themselves or through their toxic salts that show high salinity [4]. Cadmium, a toxic heavy metal is increasingly important as an environmental hazard to both humans and wildlife [5]. Cadmium toxicity with special reference to aquatic ecosystems has been reported by several workers [6-7]. Cadmium is a ubiquitous contaminant in the aquatic environment. Exposure to cadmium is known to adversely affect fish morphology and physiology of fishes. For instance, in fishes, morphological changes in gill [8], kidney [9], liver [10-11], has been reported. It causes tissue damage, particularly in the kidney, by inducing cell death which results in renal dysfunction [12]. Histology provides direct evidence of any adverse effect on fish. Liver is a principal organ of detoxification in vertebrates particularly in fishes. Furthermore, it is the potential site for lipid deposition in these animals [13]. In addition, fish liver is considered as a good indicator of aquatic environmental pollution, as it cleans of any poisons or pollutants from the blood coming from the intestine [14].
The kidney is considered as a vital organ of the body as it helps to maintain the homeostasis. It helps to maintain volume and pH of blood and body fluids and erythropoiesis [15]. In fish, the kidney helps in electrolyte and water balance and maintenance of the stable environment. It is also an important indicator of possible pollution.

Earlier, several researchers studied the impacts of various toxicants on the histology of gill, liver and kidney of different fish species. Nevertheless, studies on histological changes in these organs of the freshwater Asian stinging catfish, *Heteropneustes fossilis* induced by Cadmium are not available. In order to fill this gap, the present study was designed to investigate the SL toxicity of Cadmium on the histology of gill, liver and kidney of *H. fossilis*.

Materials and Methods

The catfish, *Heteropneustes fossilis* with an average length of 18.36 ± 0.248 cm and weight of 38.86 ± 1.413 gm were procured from a local fish market in Tirunelveli, Tamil Nadu, India. They were acclimatized to laboratory conditions in Fiberglass Reinforced Plastic (FRP) Tanks of 500 L capacity for one month prior to exposure to Cadmium. The water was changed every day. Fishes were fed with chicken liver *ad libitum* daily.

The sublethal toxicity test concentrations were selected based on the acute toxicity test value (44.13 mg/l) reported by Jayakumar *et al.* [16]. The concentrations selected were 1.11, 2.21, 4.41, 8.82 and 17.64 mg/l. After the acclimatization period, adult stinging catfishes were randomly selected and stocked at the rate of 10 fish per FRP tank with 140 liter water for the five experimental runs and a control. A duplicate set was also maintained simultaneously. Exposure medium was changed every 24th hr to maintain the desired concentration of Cadmium. Tissue samples were taken from the fishes exposed to the third (4.41 mg/l) and fifth (17.64 mg/l) concentrations only. At the end of the experiment (14th and 28th day), live fish samples were collected from the above-mentioned two concentrations, sacrificed and their gill, liver and kidney were excised out and fixed in Bouins fixative for 24 hrs. Later, the tissue samples were processed adopting the usual histological procedure [17] and thin sections of 5 µm was stained with haemotoxylin and eosin for microscopic observation. Also, light photomicrographs were taken. The morphological changes of the gill, liver and kidney sections noted in the experimental fish were compared with those of control fish.

Results and Discussion

Many researchers have reported the degenerative changes in selected tissues of the animals in response to pollution by various toxicants [18-19]. The histological changes in fish is a noteworthy and promising field to understand the extent to which changes in the structural organization are occurring in the organs due to environmental pollution [20].

Histopathology of Gill

The histological examination of gill sections of *H. fossilis* exposed to SL Cd concentrations of 4.41 and 17.64 mg/l for 14 and 28 days exhibited erosion of secondary lamellae, lamellar fusion, shrinkage of blood vessels, secondary lamellar damage, hyperplasia, vacuolation, epithelial lifting, and shortening of secondary lamellar (Fig. 4-7). The above cells were observed to be normal in the case of gill tissues of fishes of control fishes (Fig. 1).

Bais and Lokhande [21] observed hypertrophy of lamellar epithelium, dysfunction of gill lamellae and blood congestion in *Ophiocephalus striatus* exposed to cadmium chloride. Histopathological evaluation of *Cirrhunus mrigala*, exposed to cadmium revealed bulging of tip of gill lamellae, disturbance in the arrangement of pillar cells, shrinkage of epithelial cell and collapsed blood capillaries in the primary gill lamellae, disintegrated pillar cells, atrophy of secondary gill lamellae and cell necrosis [22]. Pantung *et al*. [23] reported histological alterations like hyperplasia of chloride cells, the breakdown of pillar cells and epithelial cells, oedema of epithelial cells in hybrid walking catfish (*Clarias macrocephalus* x *Clarias gariepinus*) exposed to cadmium in water.

In the present study, a series of alterations were noticed in the gills of fishes exposed to Cd. They occurred in the order of hypertrophy, hyperplasia of gill epithelium, mucous cell hypertrophy and proliferation, mucous hypersecretion, proliferation of eosinophilic granule cells and hyperplasia of interlamellar cells. The initial histological alterations might be due to the initial defense mechanism [24]. Gill histological alterations increased with increase in time and included necrosis of the eosinophilic granule cells, lamellar oedema, epithelial desquamation, increase in severity of lamellar hyperplasia, hyperplasia of epithelial and interlamellar cells, which are indicators of direct toxic effects of the chemicals [25].

Begum *et al.*, [26] investigated the histological changes in the gills of *Clarius batrachus* exposed to three heavy metals (Cr, Cd and Hg). In addition to the common histopathological changes in the gills, in Cd treated fishes, the interlamellar spaces were abolished but distended gill rays were the characteristics of Cr treatment. Extensive cellular and tissue damages were prominent in Hg treated fishes. Hypertrophy and hyperplasia of the lamellar epithelial cells due to Cd exposure were reported in zebrafish [27]. Mucous exudation was the general pathological symptom noted in the gills of Cd, Cr and Hg treated fishes [26]. The mucous secretion along with proliferated primary lamellar epidermis could have formed a respiratory exchange obstruction on its own [28]. Wong and Wong [29] studied morphological and biochemical changes in the gills of tilapia (*Oreochromis mossambicus*) after experimental cadmium exposure. They reported chloride cells as a prime target of cadmium toxicity, resulting into fish hypocalcemia. Similar types of gill lesions were noted in zinc-treated *H. fossilis* [30], mercury-treated *Cirrhinus mrigala* [31], and nickel-treated *Oreochromis niloticus* [32].

Histopathology of Liver

The histological examination of liver sections of *H. fossilis* exposed to SL Cd concentrations of 4.41 and 17.64 mg/l for 14 and 28 days revealed rupture of hepatocytes, vacuolation, hemorrhage in liver sinuses, cellular necrosis, increased pycnotic nucleus, melanomacrophages (Fig. 8-11). However, the histology of liver tissues of the control fishes was observed to be normal (Fig. 2).

The toxicity effect of heavy metals on fish liver has been studied by several researchers. The effects of acute and SL doses of Cd on the liver of *H. fossilis* is in conformity with other similar kind of studies. In the liver of the catfish, *Clarias batrachus* exposed to Cd, histological changes like degeneration of hepatocytes, vacuolization, congestion of hepatic tissues, subcapsular vacuolization, necrosis, indistinct cell boundaries and pyknotic nuclei were noticed [20]. Degenerative changes like hepatocellualar dissociation, necrosis and hypertrophy were detected in the freshwater fish, *Ophiocephalus striatus* exposed to cadmium chloride [21]. To a larger extent, similar histopathological changes were observed in the liver of *H. fossilis* treated with dry leaf extract, dry bark extract and dry seed extract of the plant *Madhuca indica* [33].

Lesions were observed in the liver of *Oreochromis mossambicus* exposed to copper and the sequential appearance of lesion in the order of hepatic vacuolar

degeneration, fatty degeneration and necrosis indicated a gradual increase in damage with duration and Cu concentration. The liver hyperfunction is attributed to initial liver lesion formed due to vacuolar degeneration and liver hypofunction is attributed to fatty degeneration and early stages of necrosis which could be related to the damage to cellular organelles like mitochondria [24]. The presence of macrophage aggregates in the liver is a generalized non-specific marker of environmental stress [25]. In the present study, occurrence of initial lesion in the liver during the percent study might be due to physiological changes that might have taken place in the liver tissue in the process while trying to homeostatistically regulate and detoxify the metal during continuous exposure as suggested by Naigaga [24]. The histological alterations observed in liver of *H. fossilis* is in conformity with observations made in similar work in fish [10, 23, 34].

The extent of damage caused to the fish liver histology by metal pollution was observed to depend on the duration of exposure and the concentration of the metal in the water [35]. When the common carp, *Cyprinus carpio* was exposed to copper and cadmium, histology of liver showed increased degenerative changes [36]. Similar changes were noticed in *Channa gachua* exposed to mercury chloride and copper chloride [11]; *Labeo rohita* to zinc and chromium [37, 38]; *Channa striatus* to cadmium chloride [39] and *Oreochromis niloticus* to aluminium [40].

Histopathology of Kidney

The histological examination of kidney sections of *H. fossilis* exposed to SL Cd concentrations of 4.41 and 17.64 mg/l for 14 and 28 days showed melanomacrophages, degenerated glomerulus, increased peritubular space, shrunken glomerulus, loss of cytoplasm, vacuolation, increased periglomerular space (Fig. 12-15). However, the histology of liver tissues of the control fishes was observed to be normal (Fig. 3).

Similar observations were made in the kidney of *Channa punctatus* [9], *Cirrhinus mrigala* [22], *Labeo rohita* [10], Hybrid walking catfish (*Clarias macrocephalus* x *C. gariepinus*) [24] and *Lates calcarifer* [34]. Chronic cadmium exposure in the freshwater fish *Colosoma macropomum* resulted in anomalous head kidney structure and an inflammatory process in kidney, affecting haematopoietic cell differentiation, mainly in granulocytes and perhaps affecting its functions [41].

Histological alterations like loosening, formation of cluster and lumps in haemotopoietic tissue, deshaping of uriniferous tubules, narrowing of tubular lumen, vacuolization and degeneration of the cells, increase of space in renal corpuscles and shrinkage in glomeruli were reported in *Channa punctatus* exposed to cadmium chloride [9]. Prabhahar *et al*. [22] observed ruptured tubule boundary cells, melanomacrophages, congregation of nuclei, damage of epithelial cells and coagulated mass of blood cells in *Cirrhinus mrigala* exposed to cadmium sulphate.

Conclusion

Exposure of fish to various concentrations of Cadmium poses great stress on the fish and leads to severe changes in their histology. Histological changes in the catfish, *Heteropneustes fossilis* exposed to cadmium can be used a sensitive model to monitor the aquatic pollution. The present study revealed that the sublethal toxic exposure to Cadmium leads to damages in the tissues of gill, liver and kidney of stinging catfish, *Heteropneustes fossilis*, confirming the possibility of Cadmium to be a toxicant. These degenerative changes would adversely affect the physiology of respiration, digestion / detoxification and excretion and cause several adverse effects in the fishes in the long run.

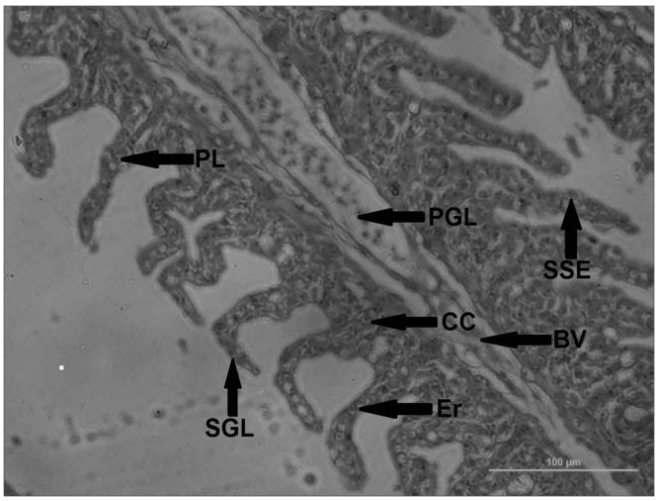

Fig 1: Photomicrograph of gill control. PL - Pilaster Cell, SGL - Secondary Gill Lamella, Er - Erythrocytes, CC - Chloride Cell, PGL - Primary Gill Lamella, BV - Blood Vessels, SSE - Simple Squamous Epithelium. (5µm thick; H&E staining; 200X)

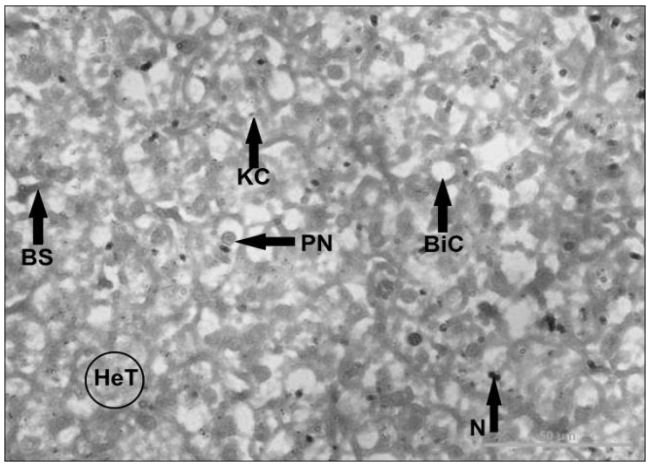

Fig 2: Photomicrograph of liver control. BS - Blood Sinusoid, HeT - Hepatic Tissue, KC - Kupffer Cell, PN - Pycnotic Nucleus, BiC - Bile Canaliculi, Nu - Nucleus.(5µm thick; H&E staining; 400X)

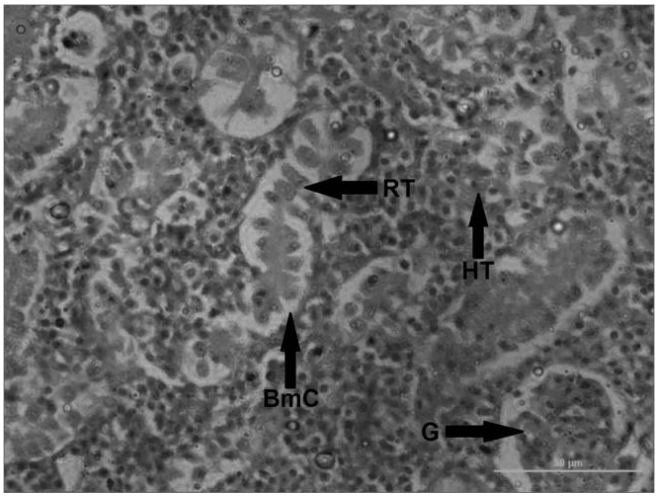

Fig 3: Photomicrograph of kidney Control. BmC - Bowman's Capsule, RT - Renal Tubules, HT - Hematopoietic Tissue, G - Glomerulus (5µm thick; H&E staining; 400X).

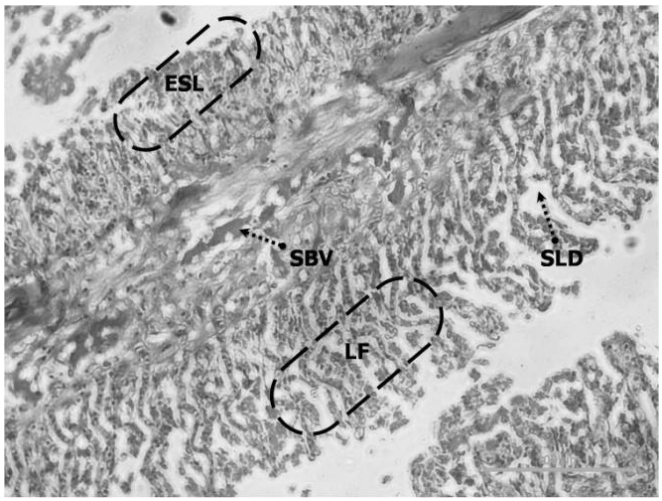

Fig 4: Photomicrograph of gill of fish exposed to Cd at 4.41 mg/l after 14th day. ESL - Erosion of Secondary Lamellae, LF - Lamellar Fusion, SBV - Shrinkage of Blood Vessels, SLD - Secondary Lamellar Damage (5µm thick; H&E staining; 200X)

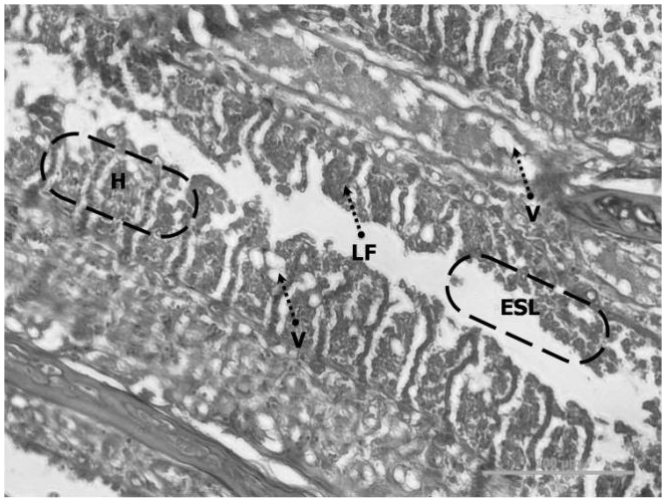

Fig 5: Photomicrograph of gill of fish exposed to Cd at 17.64 mg/l after 14th day. H - Hyperplasia, V - Vacuolation, LF - Lamellar Fusion, ESL - Erosion of Secondary Lamellae (5µm thick; H&E staining; 200X)

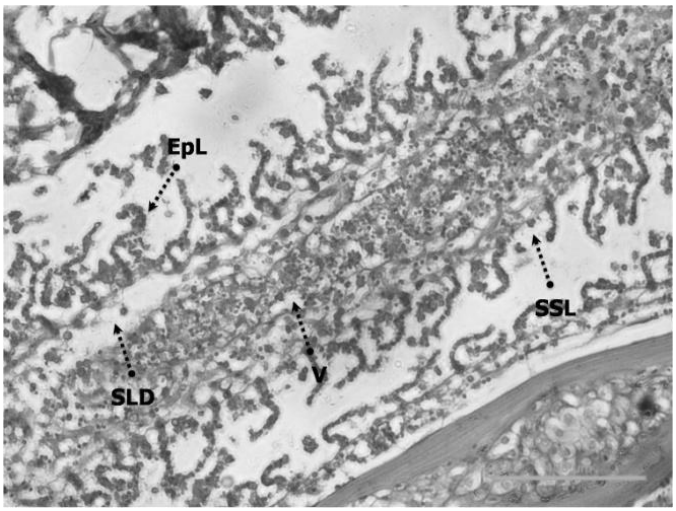

Fig 6: Photomicrograph of gill of fish exposed to Cd at 4.41 mg/l after 28th day. SLD - Secondary Lamellar Damage, EpL - Epithelial Lifting, V - Vacuolation, SSL - Shortening of Secondary Lamellae (5µm thick; H&E staining; 200X)

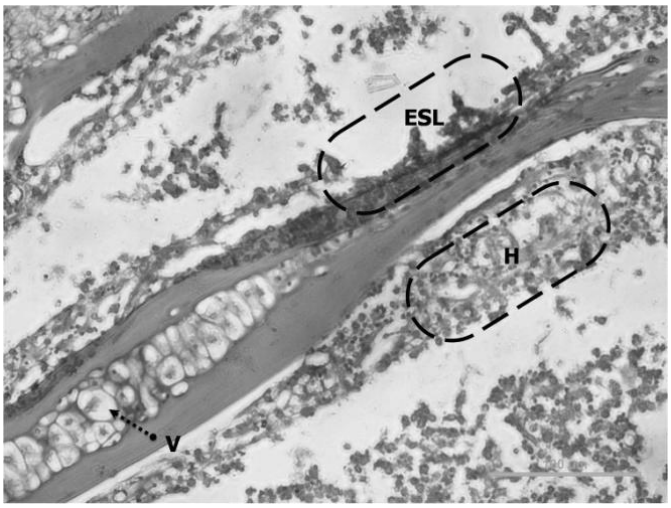

Fig 7: Photomicrograph of gill of fish exposed to Cd at 17.64 mg/l after 28th day. V - Vacuolation, ESL - Erosion of Secondary Lamellae, H - Hyperplasia (5µm thick; H&E staining; 200X)

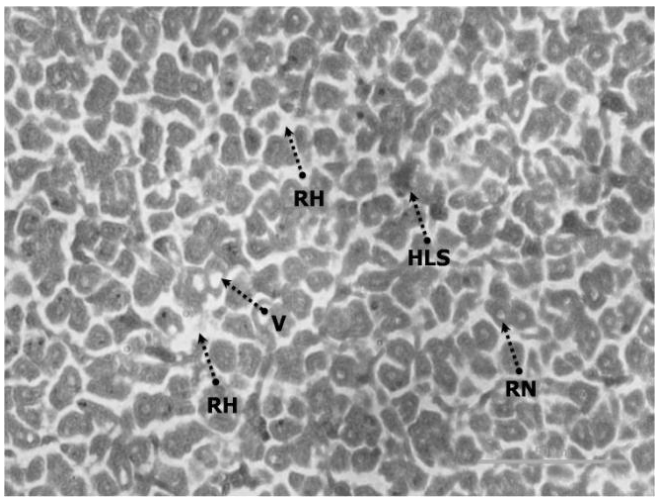

Fig 8: Photomicrograph of Liver of fish exposed to Cd at 4.41 mg/l after 14th day. RH - Rupture of Hepatocytes, V – Vacuolation, HLS - Hemorrhage in Liver Sinuses (5µm thick; H&E staining; 400X)

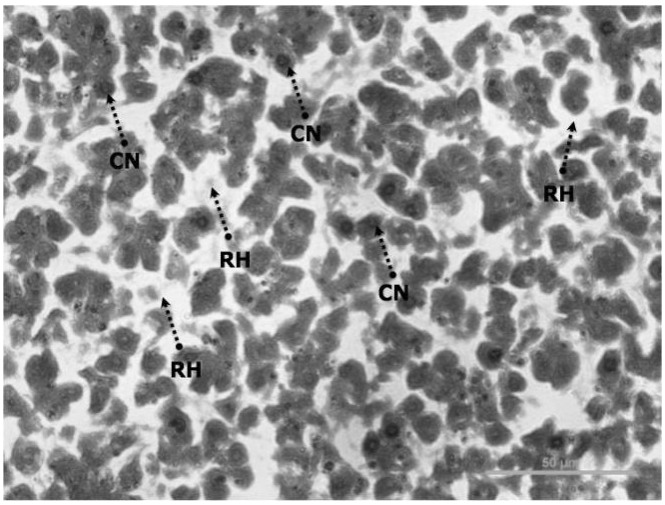

Fig 9: Photomicrograph of liver of fish exposed to Cd at 17.64 mg/l after 14th day. CN - Cellular Necrosis, RH - Rupture of Hepatocytes (5µm thick; H&E staining; 400X)

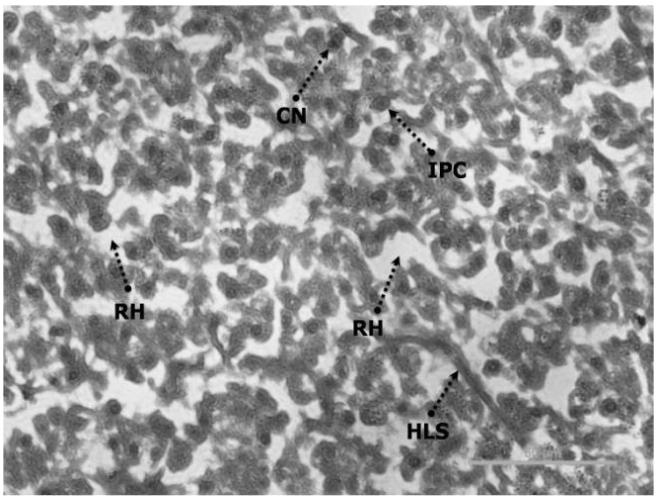

Fig 10: Photomicrograph of liver of fish exposed to Cd at 4.41 mg/l after 28th day. RH - Rupture of Hepatocytes, CN - Cellular Necrosis, IPN - Increased Pycnotic Nucleus, HLS - Hemorrhage in Liver Sinuses (5µm thick; H&E staining; 400X)

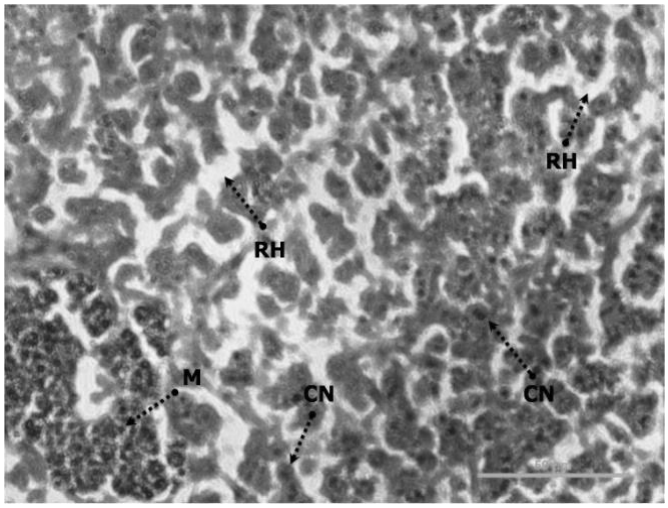

Fig 11: Photomicrograph of liver of fish exposed to Cd at 17.64 mg/l after 28th day. M - Melanomacrophages, RH - Rupture of Hepatocytes, CN - Cellular Necrosis (5µm thick; H&E staining; 400X)

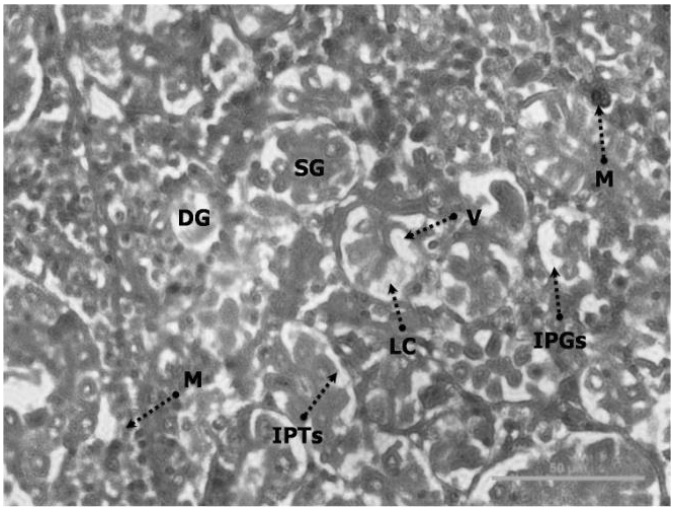

Fig 12: Photomicrograph of kidney of fish exposed to Cd at 4.41 mg/l after 14th day. M - Melanomacrophages, DG - Degenerated Glomerulus, IPTs - Increased Peritubular Space, SG - Shrunken Glomerulus, LC - Loss of Cytoplasm, V - Vacuolation, IPGs - Increased Periglomerular Space (5µm thick; H&E staining; 400X)

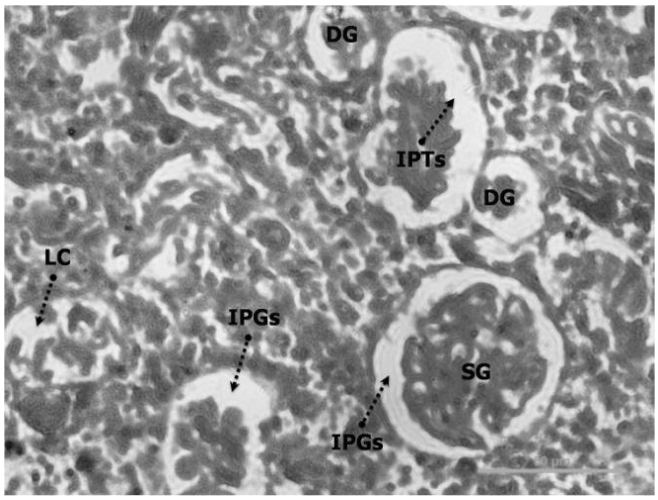

Fig 13: Photomicrograph of kidney of fish exposed to Cd at 17.64 mg/l after 14th day. LC - Loss of Cytoplasm, IPGs - Increased Periglomerular Space, SG - Shrunken Glomerulus, DG - Degenerated Glomerulus, IPTs - Increased Peritubular Space (5µm thick; H&E staining; 400X)

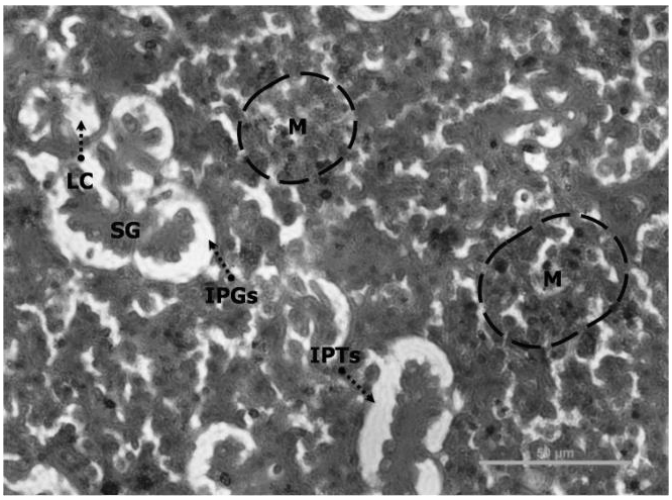

Fig 14: Photomicrograph of kidney of fish exposed to Cd at 4.41 mg/l after 28th day. LC - Loss of Cytoplasm, SG - Shrunken Glomerulus, IPGs - Increased Periglomerular Space, IPTs - Increased Peritubular Space, M - Melanomacrophages (5µm thick; H&E staining; 400X)

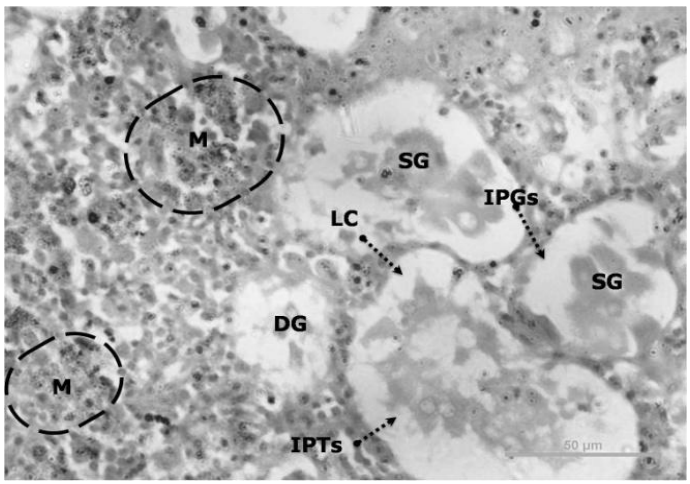

Fig 15: Photomicrograph of kidney of fish exposed to Cd at 17.64 mg/l after 28th day. M - Melanomacrophages, DG - Degenerated Glomerulus, IPTs - Increased Peritubular Space, LC - Loss of Cytoplasm, SG - Shrunken Glomerulus, IPGs - Increased Periglomerular Space (5µm thick; H&E staining; 400X)

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