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Full Length Research Paper

Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sublethal concentration of lead and cadmium

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Histological studies in organs like gill, liver, muscle and brain of *Cyprinus carpio communis* were made to assess tissue damage due to sublethal concentration of heavy metals lead and cadmium after 28 days of exposure. In lead treated gill, disintegration and fusion of primary lamellae, extensive vacuolization with disruption of epithelial lining was observed, whereas on sublethal exposure to cadmium, hyperplasia of branchial arch, vacuolization and congestion of blood vessels were well marked. Metal accumulation was clearly visible in treated liver with degeneration and severe necrosis. Both lead and cadmium treated fish showed marked thickening and separation of muscle bundles with severe intramuscular oedema more pronounced in sublethal treatment of cadmium. Neuronal cell degeneration, swelling of pyramidal cells, vacuolization and dystrophic changes were characteristic features observed in treated brain.

Key words: Lead, cadmium, histopathology, *Cyprinus carpio communis.*

INTRODUCTION

Heavy metal contamination of the aquatic environment has drawn increasing attention as it may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Vosyliene and Jankaite, 2006; Farombi et al., 2007). Fish readily absorb dissolved metals and may serve as indicators of the extent of pollution (Adham et al., 2002; Farkas et al., 2002; Shukla et al., 2007).

Tissue changes in test organisms exposed to a sublethal concentration of a toxicant are a functional response of organisms that provides information on the nature of the toxicant (Narayan and Singh, 1991; Mercy et al., 1996; Mathur and Gupta, 2008). The toxic effects of heavy metals have been reviewed (Rani, 2000; Adami et al., 2002; Waqar, 2006). The organisms have developed protective defense against deleterious effects of essential and non-essential heavy metals that produce degenerative changes like oxidative stress in the body (Abou EL-Naga et al., 2005).

In studies on bioaccumulation, the proportion of lead and cadmium were significantly higher in different tissues of fish (Vinodhini and Narayanan, 2008), suggesting a thorough study of their effects on tissue histology. In heavy metal pollution (Pb and Cd), organs such as the gills and liver have been identified as the storage sites (Gbem et al., 2001). However, the main sites of these heavy metal uptake and accumulation are the gills and gastrointestinal tract (Lovegroove and Eddy, 1982; Annune and Iganiwura, 1993).

Lead is a common heavy metal found in the environment and has the tendency to accumulate and undergo food-chain magnification (Vinikour et al., 1980). Cadmium is classified as the second most dangerous metal in the environment. It is biologically very reactive and therefore,

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leads to both acute and chronic poisoning (Nriagu and Pacyna, 1988). Cadmium is a major industrial pollutant particularly in areas associated with smelting of zinc and heavy rod traffic (Das et. al., 1997).

Cyprinus carpio communis is a commonly cultured and edible fish offering unique experimental advantages for the study of heavy metal toxicity due to tolerance to temperature variations, toughness and biennial breeding (Singhal, 1995). This study was an attempt to determine the response of *C. carpio communis* to sub-lethal concentrations of lead and cadmium through histopathological studies of gill, liver, muscle and brain that may provide an early warning of potential problems.

MATERIALS AND METHODS

Healthy fingerlings (size of 4 to 6 cm in length and 6 ± 0.5 g in weight) were obtained from a commercial supplier (Bharath seeds). They were supplied in an aerated polythene bag. They were fed daily on a commercially formulated feed that contained 65% crude protein and were kept in stock aquaria for acclimatization before commencement of the experiment. Toxicity tests were conducted in accordance with standard methods (APHA, 1995). Short-term toxicity or range finding test were carried out by exposing test organisms to wide range of cadmium and lead concentrations in a logarithmic ratio. Within the concentration limits ascertained, 4 to 5 narrow range concentrations at equal intervals were selected. Percentage of mortality was calculated and values were transformed into probit scale, as suggested by Finney (1971). Based on the acute toxicity tests, 96 h LC_{50} sublethal concentrations for lead and cadmium were derived.

Lead and cadmium were directly introduced into the water in sublethal concentrations of 4.3 and 1.6 ppm, respectively in the form of $Pb(NO_3)_2$ and $CdCl_2$. Introduction of the toxicant was persistent. Each treatment had three replicates while two aquaria served as controls. Conductivity of water was 12.28×10^{-4} with a pH of 7.3. At the end of the exposure period, fishes were randomly selected for histological examination.

Gill, liver, muscle and brain tissues were isolated from the control and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous bouins fixative for 8 h, routinely prepared and the sections stained with haematoxylin and eosin (HE) (Culling et al., 1985). Selected fields were photographed using Carl zeiss photomicroscope III.

RESULTS

Gills

Light microscope (LM) examination of the photomicrograph of the vertical section of the gills (Figure 1a) showed the arrangement of primary and secondary lamellar processes of which the former was thicker than the latter. The primary gill lamellae are flat leaf like structures with a central rod like supporting axis and a row of secondary gill lamellae on each side of it. The secondary lamellae (SL) were equally spaced along the columnar structures with intact cellular layer attached at their bases with the primary lamellae and free at their distal ends. The normal secondary lamellar epithelium was simple, consisting of a thin single or double sheet of epithelial cells, blood vessels and a row of pilaster cells. The region between the two adjacent secondary gill lamellae is known as interlamellar region.

In fish exposed to 4.3 ppm of $Pb(NO_3)_2$, after 28 days, the gill photomicrograph (Figure 1c) showed cyto-architectural distortion of the lamella with overlapping of the primary and secondary lamella. Considerable mucous and granulated eosinophilic cells were witnessed in their cytoplasm. Extensive vacuolization were observed with prominent disruption of epithelium. At 1.6 ppm of $CdCl_2$ (Figure 1b) for the same exposure, completely disrupted primary gill structure, marked hyperplasia of the branchial arch, pilaster cell vacuolization and congestion of blood vessels were well marked. The main response of gill epithelium was reduction in permeability.

Liver

The photomicrograph of the liver (Figure 2a) showed large polyhedral cells within the network of minute canalicules. There was irregular distribution of bile duct, blood capillaries and sinusoids that are filled with erythrocytes. Hepatocytes surrounding the central vessels appeared to be lightly arranged in a rosette pattern with 10 to 12 cells in each group. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures, known as hepatic cell cords. In fish, these structures are generally obscure.

Hepatic tissues of the fish exposed to 4.3 ppm of $Pb(NO_3)_2$ (Figure 2b) showed varied degree of hepatic cirrhosis as evidenced by vacuolization, space formation and resulting haemorrhage. Hypertrophy of hepatocytes and clumping was also evident in many places. Metal accumulation was also clearly evident in the treated tissues of *C. carpio communis*. At 1.6 ppm of $CdCl_2$ (Figure 2c) for the same exposure, acute and extensive necrosis of liver cells were observed with indistinct cell boundaries in many places and pyknotic nuclei.

Muscle

The photomicrograph of the muscle (Figure 3a) depicted the presence of normal myotomes with equally spaced muscle bundles. On exposure to sublethal concentration of lead (Figure 3b), marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Sublethal concentration of cadmium (Figure 3c) led to pronounced intramuscular oedema with minor dystrophic changes.

Brain

Histological observations of the brain (Figure 4) showed

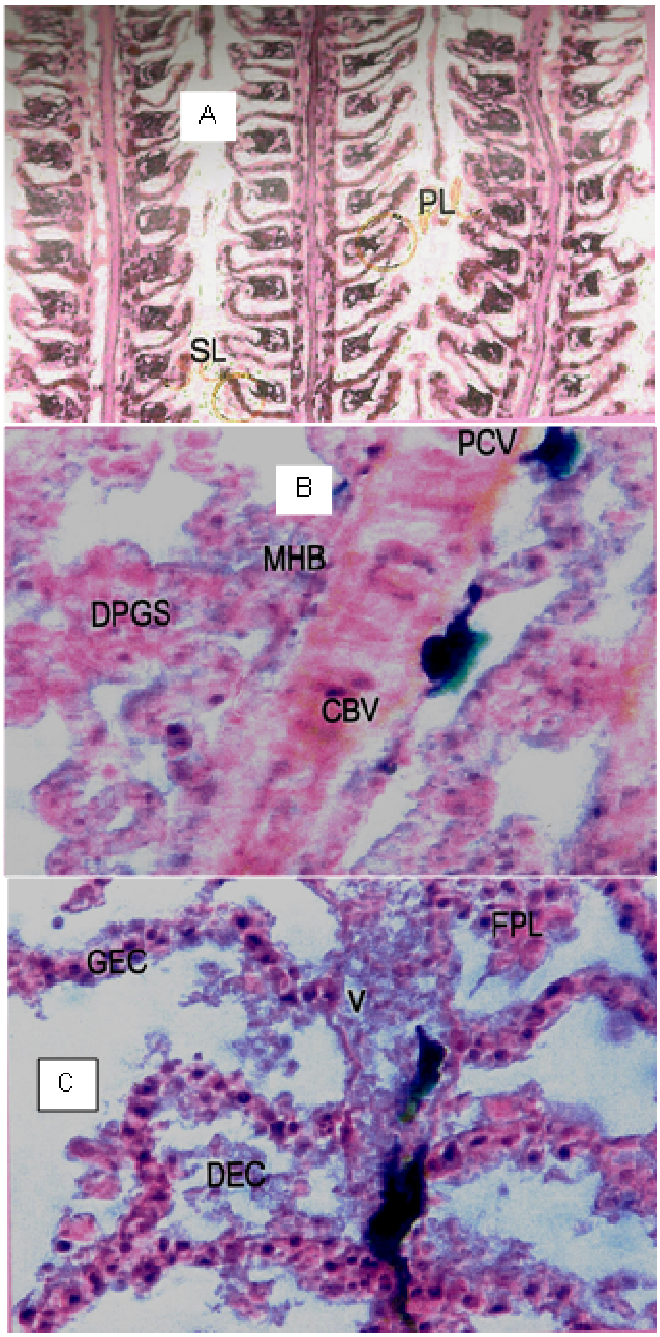

Figure 1. (a) Photomicrograph of gill (Control) of *C. carpio communis* (H and E) showing PL, primary lamellae; SL, secondary lamellae. (b) Photomicrograph of gill (Cadmium treated) of *C. carpio communis* (H and E) showing PCV, pilaster cell vacuolization; MHB, marked hyperplasia of branchial arch; DPGS, disruption of primary gill structure; CBV, congestion of blood vessels. (c) Photomicrograph of gill (Lead treated) of *C. carpio communis* (H and E) showing V, vacuolization; FPL, fusion of primary lamellae; GEC, granulated eosinophilic cells; DEC, disruption of epithelial cells.

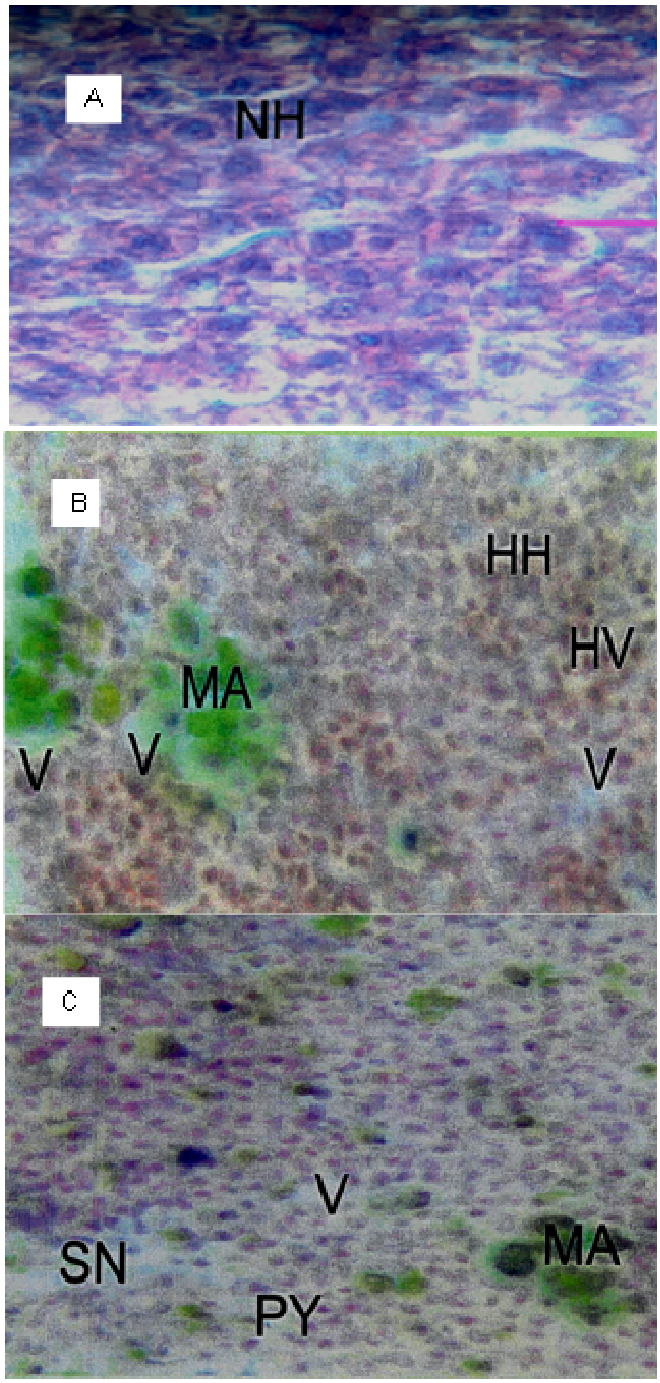

Figure 2. (a) Photomicrograph of liver (Control) of *C. carpio communis* (H and E x330) showing; NH, normal hepatocytes; (b) Photomicrograph of liver (Lead treated) of *C. carpio communis* (H and E) showing; HH, hypertrophy of hepatocytes; V, vacuolization; MA, metal accumulation. (c) Photomicrograph of liver (Cadmium treated) of *C. carpio communis* (H and E) showing; PY, pyknotic nuclei; SN, severe necrosis; V, vacuolization; MA, metal accumulation.

the presence of neuronal cells, pyramidal cells and nissl substances. Both cadmium and lead treatments at sublethal concentration effected neuronal cell degeneration, swelling of pyramidal cells, loss of nissl substances,

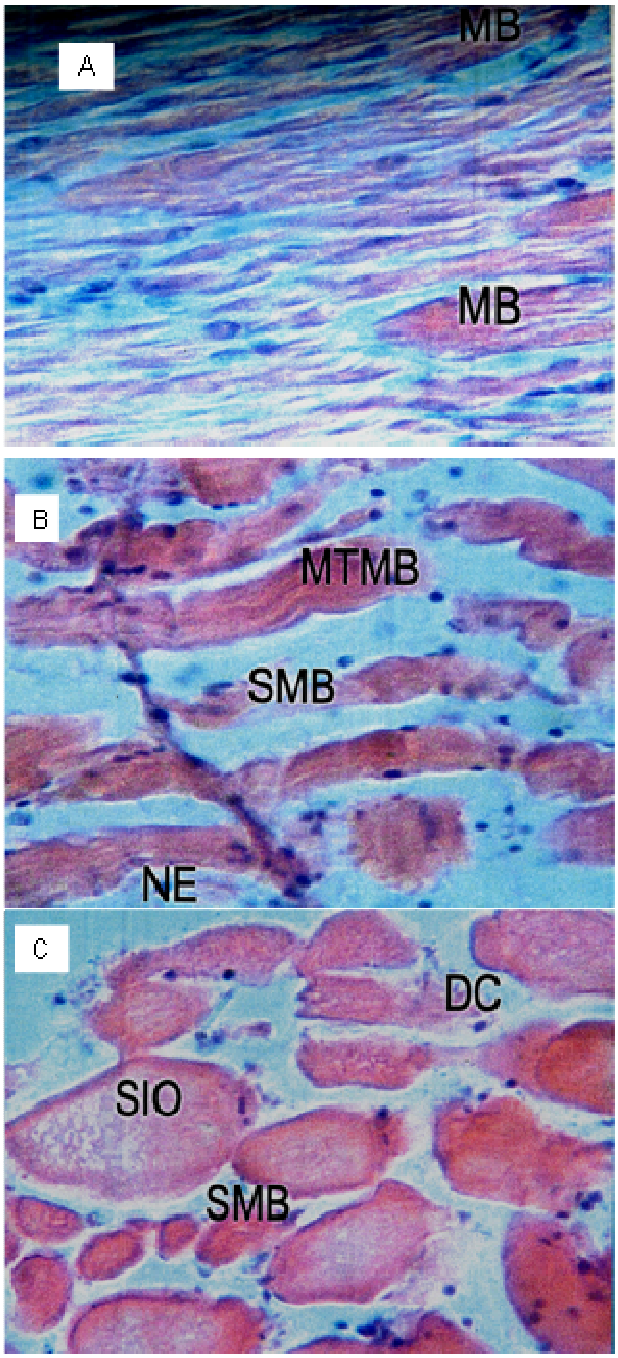

Figure 3. (a)Photomicrograph of muscle (Control) of *C. carpio communis* (H and E) showing; MB, Muscle bundles. (b) Photomicrograph of muscle (Lead treated) of *C. carpio communis* (H and E) showing; MTMB, marked thickening of muscle bundles; SMB, shortening of muscle bundles; NE, necrosis. (c) Photomicrograph of muscle (Cadmium treated) of *C. carpio communis* (H and E) showing; SIO, severe intramuscular oedema; SMB, shortening of muscle bundles; DC, dystrophic changes.

vacuolization and dystrophic changes after 28 days of exposure.

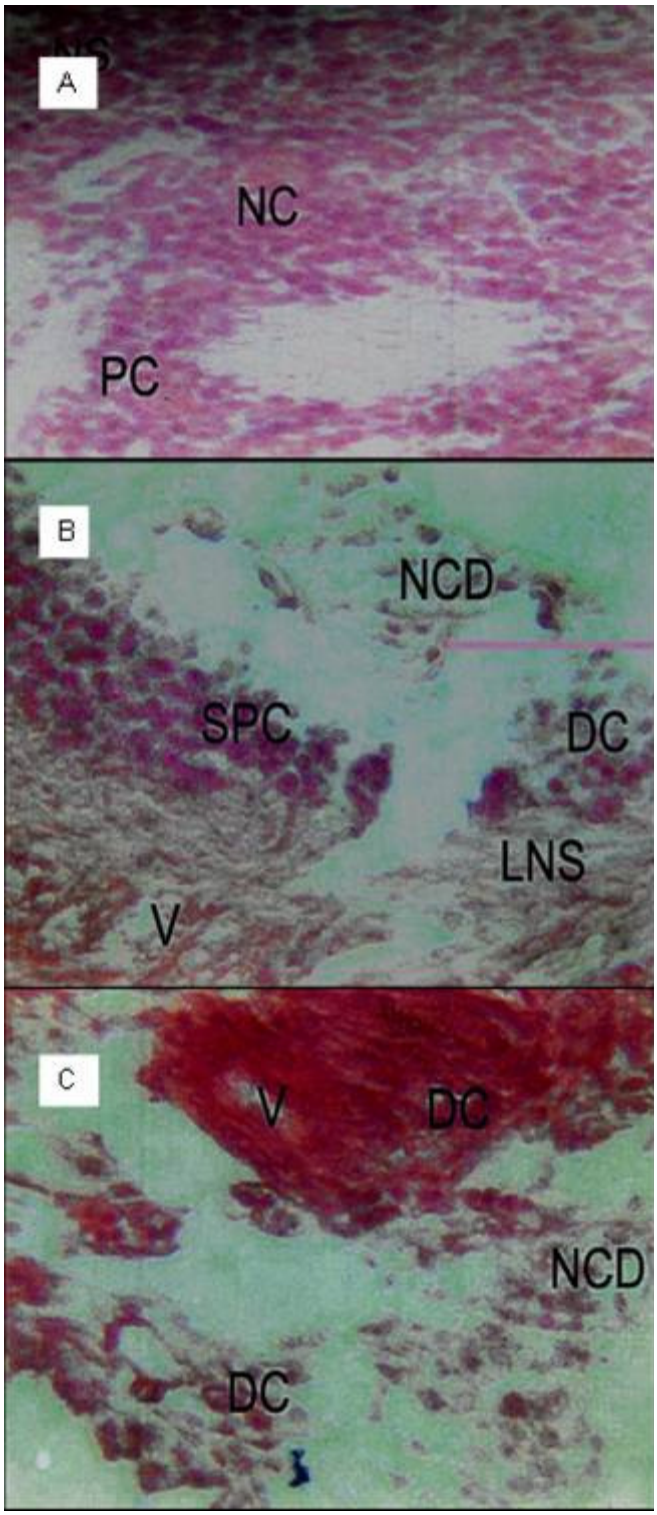

Figure 4. (a) Photomicrograph of brain (Control) of *C. carpio communis* (H and E) showing; NC, neuronal cells; NS, nissl substances; PC, pyramidal cells. (b) Photomicrograph of brain (Lead treated) of *C. carpio communis* (H and E) showing; NCD, neuronal cell degeneration; DC, dystrophic changes; SPC, swelling of pyramidal cells; LNS, loss of nissl substances; V: vacuolization. (c) Photomicrograph of brain (Cadmium treated) of *C. carpio communis* (H and E) showing; DC, dystrophic changes; NCD, neuronal cell degeneration; V, vacuolization.

DISCUSSION

Histological study of the gills showed a typical structural organization of the lamella in the untreated (Figure 1a). The treated (Figure 1b and c) however, showed progressive architectural distortion at the end of the exposure period. This corroborates with the observation of Jana and Bandopadhyaya (1987) which reported that, gill is an important tissue because of its direct contact with water and any effect or agency has to go through it to come into the fish body. The lamella epithelial lining reacts to dissolved lead creating tissue osmoregulatory imbalance. According to Cladwell (1997), the end result would be reduced flow of oxygen-enriched water to lamellar tissues and ultimately, a reduction in the fish's performance capacity. The change in physiological property was evident in the shrinkage and fusion observed at the lamella thus, suggesting that lead intake mostly occurs via the gills. In cadmium treated gill (Figure 2b and c), marked hyperplasia of branchial arch, pilaster cell vacuolization and congestion of blood vessels were well marked. The results are in parallel with the works of Kapila and Ragothaman (1999) who reported for *Boleopthalmus dumieric* exposed to sublethal concentrations of cadmium. Histopathological changes in the gill of *Labeo rohita* were reported by Vijayalakshmi and Tilak (1996) which included epithelial proliferation, congestion of blood vessels and hyperplasia. Tilak et al. (2005a) subsequently reported dropsy, vascular degeneration, cloudy swelling and necrosis in epithelial and pillar cells of the gills upon chlorpyrifos intoxication.

The high accumulation of lead and cadmium in the liver, which Gbem et al. (2001) also noted in their findings, is related to the fact that liver plays a role in accumulation and detoxification. It appears to be a general feature of the liver of intoxicated fish that the degree of structural heterogeneity is enhanced with increasing concentration of the toxicant (Hawkes, 1980). Although, according to Friberg et al. (1971), fishes are known to possess sequestering agent (metallothionein), the bioaccumulation of these trace elements in the liver tissue reaches a proportion in which the function of the liver is impeded, thus resulting in gradual degeneration of the liver and syncytial arrangement. The surface area of the liver cell is also decreased, which may be due to increase in intrabiliary fibre-connective tissue. The vacuolization observed are zones of total cell degeneration. Similar degenerating changes were observed in liver of both sexes of *C. carpio* after exposure to $HgCl_2$ at 0.1 ppm for 45 and 60 days (Masud et al., 2001, 2003).

As with gills, muscle tissue also come in close contact with pollutants dissolved in water. Hence, reactions in the ultrastructure of the muscle were spontaneous. Separation of muscle bundles was an interesting observation. Initial stimulus of lead and cadmium can induce hyperactivity and excitability in animals, leading to release of lactic acid and subsequent muscular fatigue (Das and Mukherjee, 2000).

Vacuolization in brain tissue may be the result of glycolysis leading to microsomal and mitochondrial dysfunctions. Severe necrosis of neuronal cells in the cerebrum indicating loss of nissl substances evident in this study was also supported by the studies of Loganathan et al. (2006), due to 10 ppm exposure to zinc. Meyer (1958) and Pentschow (1958) observed that, lead acts directly on the cerebral vasculatures including blood-brain barrier and causes cerebral edema.

In conclusion, metals are stored in different sites in animals depending on the metal and on the animal species. Bioaccumulation of sublethal concentrations of metals like lead and cadmium and their subsequent tissue damage led to impaired physiology and behavior of the stressed organism.

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