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Histopathological alterations in the gills of *Labeo rohita* (Hamilton-Buchanan) due to Lead toxicity

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Lead is an industrially important heavy metal used in manufacturing paints, batteries, explosives, pottery and other important products of daily life. On the other hand, it is the most damaging element for aquatic fauna especially fish as it accumulates in the body of organism and have low rate of elimination. The present paper emphasizes on the histopathological effects of different sub-lethal doses of lead nitrate on the gills of a commonly consumed freshwater fish Rohu, *Labeo rohita*. After 15, 30, 45 and 60 days of exposure, gill tissues were dissected and routine histological processes were done. No changes were seen in the gills of the control fingerlings. The histopathological changes such as cellular hypertrophy, hyperplasia, vacuolation, epithelial lifting, shortening, curling and abnormal elongation of the secondary lamellae, fusion of adjacent lamellae, telangiectasis, blood congestion, interstitial edema, necrosis, lamellar aneurysm, architectural distortion and degeneration of gills were observed in the gills of the treated fingerlings. The extent of damage of gill tissue was proportionate with the increased dosage and duration of lead nitrate exposure.

Keywords: Aquatic pollution, Bioleaching, Heavy metal, Lead nitrate, Rohu

Rapid industrialisation has substantially altered the natural quality of the environment, water bodies in particular, resulting in morphological and physiological imbalance in the bodies of terrestrial as well as aquatic fauna. The industrial effluents discharged into wetlands, rivers and streams without any prior treatment pose a serious threat to various organisms such as fishes as they come in direct contact with the pollutants. These effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids[1]. Such contaminations change the water quality to such an extent that water can no longer be considered as fit for any aquatic organism habitation. Heavy metals occur naturally in the environment and are found at varying levels in all the ground and surface waters[2,3]. Heavy metals are serious pollutants in the aquatic environment as they are accumulated by aquatic organisms[4], and cause devastating effects on the ecological balance resulting in depletion of aquatic diversity[5,6].

Some metals are essential for normal physiological and metabolic processes of the body in traces while others have no biological roles[7]. One such metal is Lead which accumulates in the body due to its low elimination rate and cause damage to aquatic life, especially fishes[8-10]. Due to rapid industrialisation and urbanisation large amount of heavy metals including Iron, Zinc, Lead and Nickel are released into the waters of Punjab[11]. Lead enters the atmosphere through production of coal, oil, iron, steel, batteries, smelters, solid wastes and tobacco smoke[12,13]. Fishes are under continuous exposure to these metals, which are eventually consumed by man causing certain physiological, biochemical, behavioural and genetic dysfunctions[14]. Therefore, it is important to determine the deleterious effects of this toxicant. The behavioural and morphological manifestations due to the effect of lead nitrate on the freshwater fish rohu, *Labeo rohita* were studied.[15] Among different organs, gills are the first target of waterborne pollutants due to their constant contact with the external environment[6,16,17]. The gills are not only participating in the gaseous exchange but also performing osmoregulation and excretion functions. The effect of different heavy metals on the gills of different fishes have been studied by different workers, but to the best of our knowledge, no sufficient data is available on the toxic effects of lead nitrate on the gills of rohu, *Labeo rohita*. Therefore, here we investigated the histopathological alterations in the

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gills of *L. rohita* on exposure to sub-lethal concentrations of lead nitrate. *Labeo rohita* (Rohu) is the most common and easily available fish in the waters of Punjab. It has high commercial value and one of the most consumed fishes of Punjab.

Materials and Methods

Reagents

Lead nitrate and concentrated nitric acid were procured from sigma E-Merck, Mumbai, India.

Collection of fish

Specimens of *Labeo rohita* (Hamilton, 1822 (syn. *Cyprinus rohita* Hamilton, 1822) were collected from local fish farm, village Nanoki, District Patiala, Punjab and acclimatized to laboratory conditions for 15 days. The water was changed after every 24 h and the fishes were fed with pelleted feed twice a day. The fish specimens selected for the experiment were ranging from 9-11 cm in length and with an average weight range 8-12 g.

Stock solution

Stock solution of lead nitrate was prepared and the toxicity tests were conducted[18]. About 0.1599 g of lead nitrate was dissolved in approximately 200 mL water and 10 mL of conc. nitric acid was added to the solution and diluted to 1000 mL with distilled water. One mL of this solution is equal to 100 µg of Lead.

Animal treatment

LC_{50} of lead nitrate for 96 h was calculated by Probit analysis[19] and found to be 34.20 mg/L[20]. Five groups containing 10 healthy fingerlings in each were selected and introduced into the plastic tubs containing four sublethal concentrations of lead nitrate on the basis of $1/3^{rd}$, $1/5^{th}$, $1/7^{th}$ and $1/10^{th}$ of LC_{50} value. i.e, 11.4, 6.84, 4.88 and 3.42 mg/L, respectively. One group was kept as a control in which no solution was added.

Histopathological study

At the end of 15, 30, 45 and 60 days of exposure, one fish from each tank was sacrificed and dissected to remove the gill tissues. The gills were washed in saline and immediately fixed in Bouin's fixative for 24 h. Then, the gill tissues were dehydrated with graded series of alcohol and embedded in paraffin wax for further process of section cutting and staining with Haematoxylin-Eosin.

Results

Light microscopic study of the saggital section of the gill of the fish in control showed a normal morphological structure with each gill arch supporting a series of gill filaments arranged in two rows. Each filament had a series of leaf like structures called secondary gill lamellae (Fig. 1). The respiratory lining of secondary gill lamellae had a thin epithelial layer

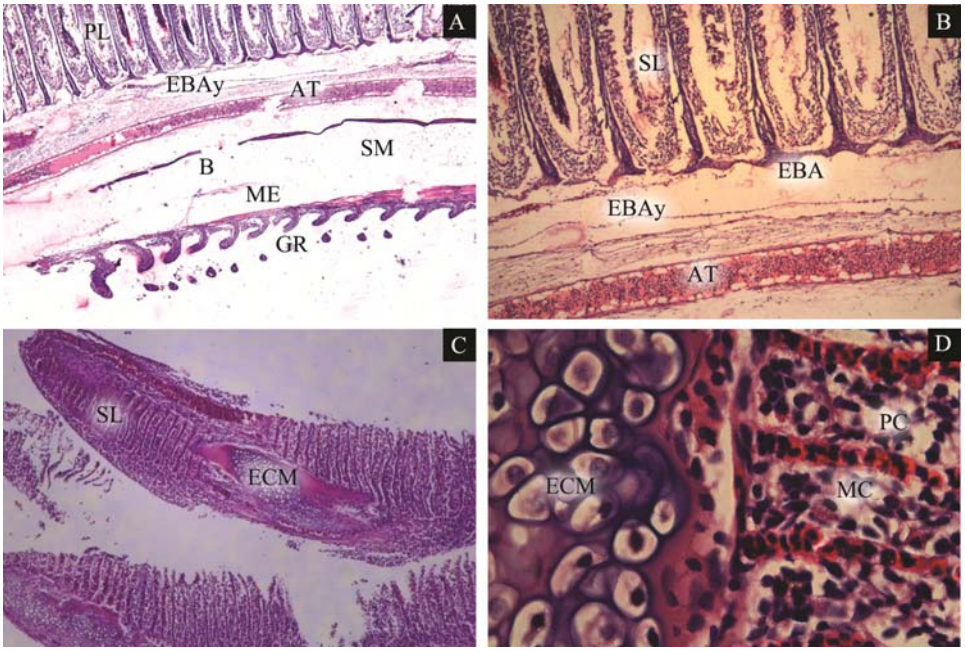

Fig. 1 — Structure of gills in control group. X400. [PL= Primary Lamella, SL= Secondary Lamella, EBA= Efferent Branchial Arteriole, EBAy= Efferent Branchial Artery, AT= Adipose Tissue, B= Bone, SM= Sub-Mucosa, ME= Mucosal Epithelium, GR= Gill Raker, C= Chondrocytes, ECM= Extra Cartilaginous Matrix, L= Lacuna, PC= Pillar Cells, EC= Epithelial Cells, MC= Mucous Cells]

which rests on a basement membrane covering the pillar cells and blood channel system. The primary gill lamellae were rounded at the apices while secondary gill lamellae were interspaced. The secondary lamellae consist of mucous cells and covered by thin epithelial cells.

Histological study of the gills in the untreated fish showed an intact structural organisation of the lamellae (Fig. 1). Lead nitrate treatment to fish resulted in several forms of general histological changes, such as cellular hypertrophy, hyperplasia, vacuolation of cells, epithelial lifting, shortening, curling and abnormal elongation of the secondary lamellae, fusion of adjacent lamellae, telangiectasis, blood congestion, interstitial edema, necrosis, lamellar aneurysm, architectural distortion and degeneration of gills (Fig. 2-5). It was observed that the histopathological alterations in the gills of a fish, *Labeo rohita* increased with the increase in the quantity of dose of lead nitrate and with the increase in the number of days of exposure to it (Table- 1).

Discussion

The state of Punjab has three internationally important wetlands i.e., Harike, Kanjali and Ropar. These wetlands besides attracting lot of migratory birds during the winter season every year, attracts other fauna also. However, untreated industrial effluents with heavy metals are discharged into these natural aquatic ecosystems and that poses a serious threat to the valuable aquatic fauna. This aquatic fauna includes many species which comes under threatened category of IUCN like critically endangered, endangered, vulnerable and near threatened. In this context, it is necessary to have consistent knowledge on the extent of the contamination of these water bodies. Here, we measured the lead toxicity in common rohu by observing the histopathological alterations in the gills of exposed fishes for duration of 15-60 days.

The present study has shown that lead nitrate exposure resulted in several histological alterations in the gills of *Labeo rohita* and further, the degree of

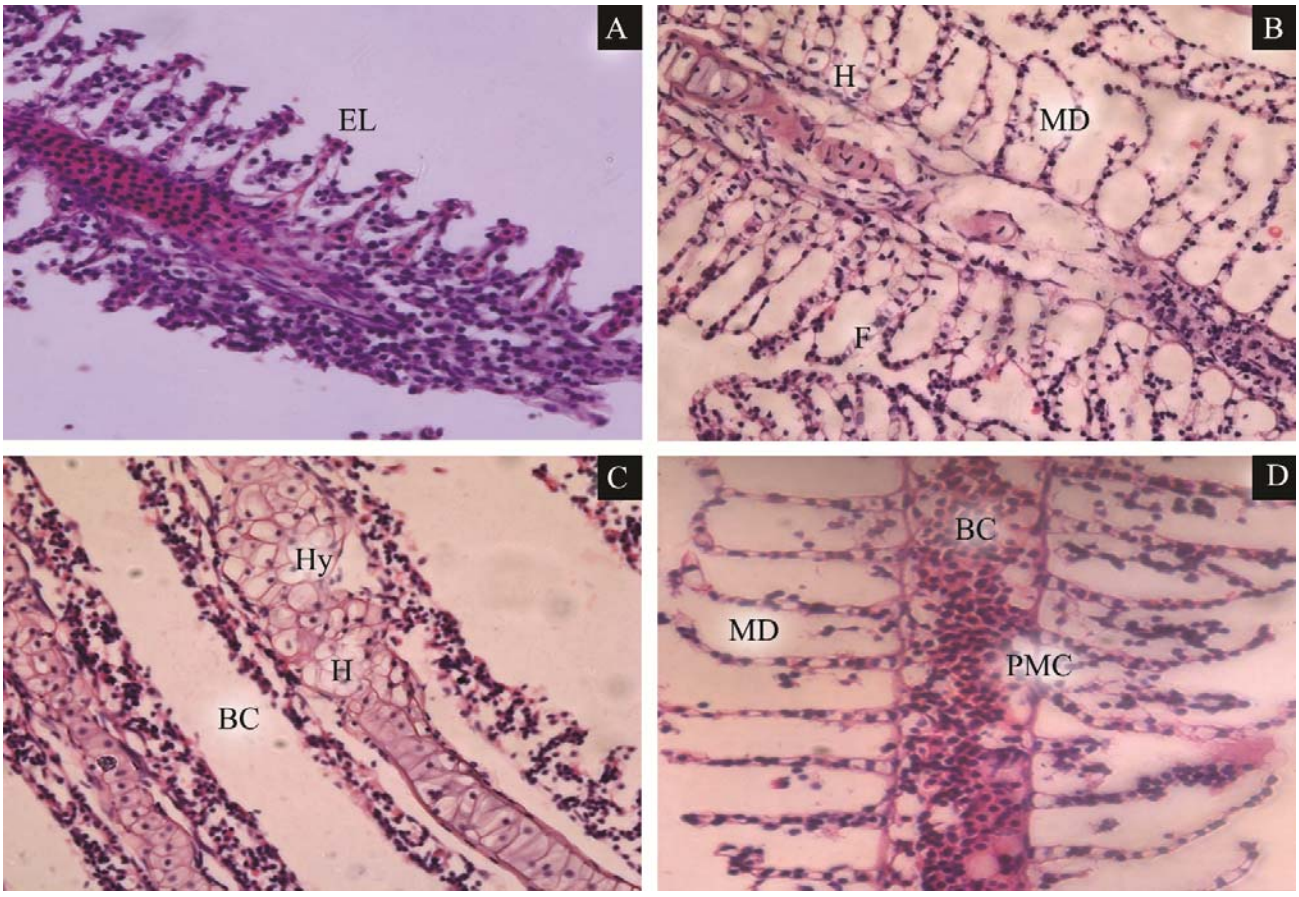

Fig. 2 — Structure of gills exposed to lead nitrate for 15 days. X400. (A) 3.42;, (B) 4.88; (C) 6.84; and (D) 11.4 mg/L. [BC= Blood congestion, MD= Marginal Dilation, EL= Epithelial Lifting, H= Hypertrophy, Hy= Hyperplasia, PMC= Proliferation of mucus cells]

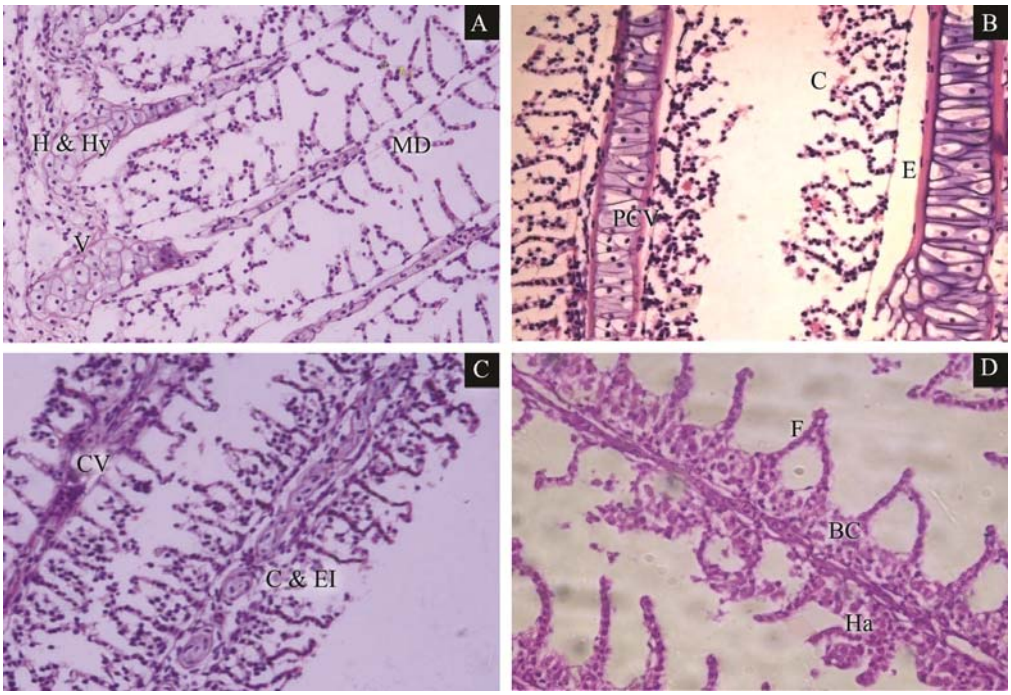

Fig. 3 — Structure of gills exposed to lead nitrate for 30 days. X400. (A) 3.42;, (B) 4.88; (C) 6.84; and (D) 11.4 mg/L. [Ha= Haemorrhage, BC= Blood Congestion, El= Elongation of Secondary Lamellae, C= Curling of Secondary Lamellae, E= Edema, Hy= Hyperplasia, H= Hypertrophy, F= Fusion of Secondary Lamellae, PCV= Pilaster cell vacuolation, V= Vacuolation, MD= Marginal Dilation]

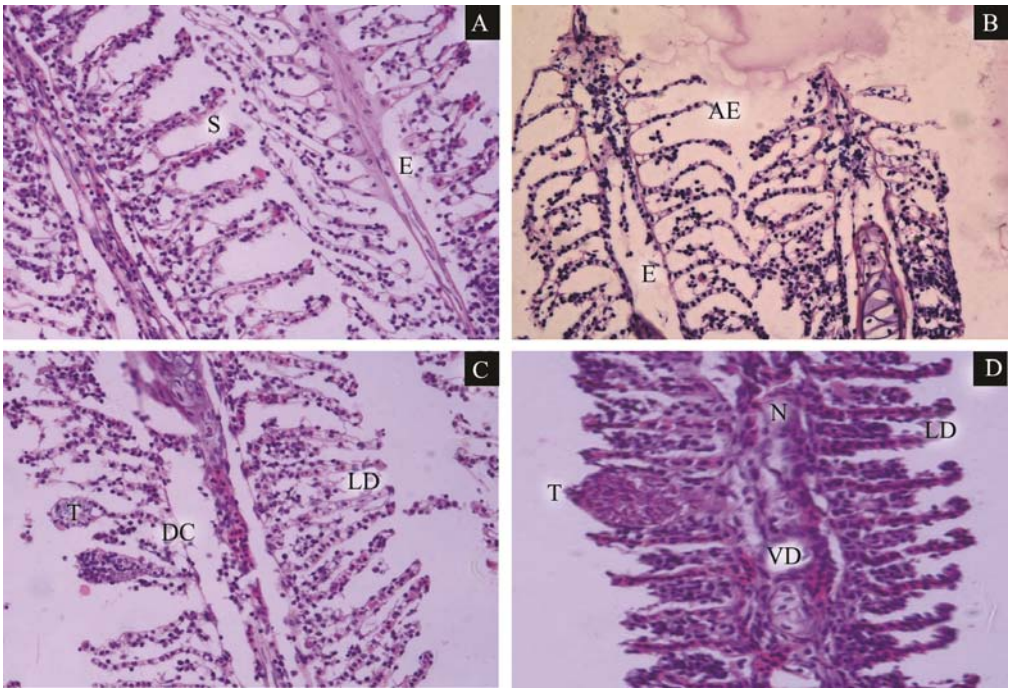

Fig. 4 — Structure of gills exposed to lead nitrate for 45 days. X400. (A) 3.42;, (B) 4.88; (C) 6.84; and (D) 11.4 mg/L. [T= Telangiectasis, LD= Lamellar Disorganisation, DC= Dilation of central venous, S= Swelling of the tip of secondary lamellae, Ha= Haemorrhage, VD= Vasodilation with blood congestion, DL= Dilation of lamellae, AE= Abnormal Elongation of secondary lamellae, E= Edema, N= Necrosis

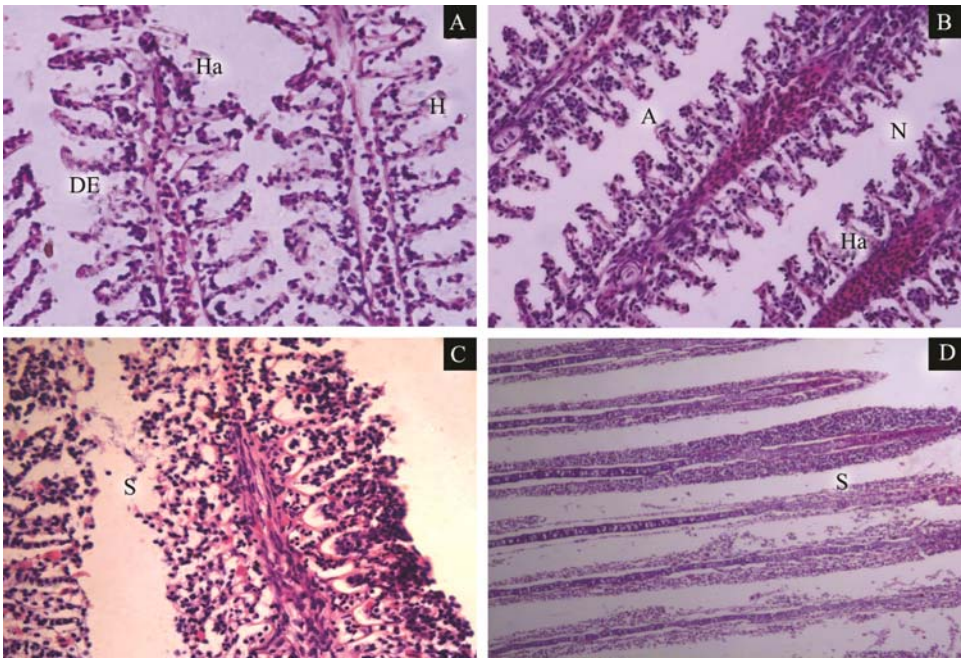

Fig. 5 — Structure of gills exposed to lead nitrate for 60 days. X400. (A) 3.42;, (B) 4.88; (C) 6.84; and (D) 11.4 mg/L. [DE= Destruction of Epithelial Cells, Ha= Haemorrhage, H= Hypertrophy, A= Aneurysm, N= Necrosis, S= Sloughing of Secondary Lamellae, BC= Blood Congestion]

Table 1 — The histopathological alterations observed in the gills of a fish, *Labeo rohita* on exposure to different doses of Lead nitrate for different number of days

No. of Days	Lead nitrate dose (mg/L)			
	3.42	4.88	6.84	11.4
15	epithelial lifting, hypertrophy of epithelial cells	marginal dilation, hypertrophy of epithelial cells, fusion of adjacent secondary lamellae	hypertrophy and hyperplasia	proliferation of mucous cells, severe blood congestion
30	vacuolation of cells, hypertrophy, hyperplasia, marginal dilation	pilaster cell vacuolation, edema, curling of secondary lamellae	dilation of central venous, elongation and curling of secondary lamellae	fusion of secondary lamellae, haemorrhage and blood congestion
45	edema, swelling of tip of secondary lamellae	abnormal elongation of secondary lamellae and edema	telangiectasis	telangiectasis, dilation of secondary lamellae, destruction of epithelial cells, necrosis
60	hypertrophy, haemorrhage and disruption of epithelial cells	aneurysm, haemorrhage and necrosis	necrosis and severe blood congestion, complete destruction of epithelial cells	sloughing and complete loss of secondary lamellae

damage increased with the increase in dose of lead nitrate administered and the number of days of exposure. Similar observations were reported due to exposure of Lead[8,21,22] and copper[23] in other freshwater fishes. Lead caused pathological alteration in the gill filaments and respiratory lamellae of *Ctenopharyngodon idella.* The gills showed different degrees of effect depending on the concentration and duration of exposure of lead nitrate, such as clubbing and fusion in secondary lamellae, hyperplasia, destruction and separation of epithelial layer, bleeding and bite tissues[24]. Similar histopathological changes caused by various heavy metals and organic toxicants in gills, liver, kidney, gonads and other organs of fishes are not uncommon[25-28].

Morphological changes are seen in some tissues of trout fishes such as swollen and degenerative epithelial layer after exposure to cadmium and lead[29]. The lesions such as hyperplasia of epithelial cells covering the secondary lamellae, edema with separation of respiratory epithelium, dilation of lamellar blood vessels as well as desquamation of

epithelial covering after long term exposure of *Oreochromis niloticus* to Lead Acetate were observed[30]. Increase in intracellular vacuolation with edematous changes was observed in the gills of *Clarias gariepinus* exposed to 0.08 mg/L Lead for nine days[26]. The copper and lead ions caused hyperplasia and fusion of lamellae in the gills of *Carassius carassius*[31] and Lamellar aneurysm in *Prochilodus*[32].

In the present study, there was a normal lamellar organisation in the gills of untreated fish, *Labeo rohita* (Fig. 1). On exposure to 3.42 mg/L of lead nitrate for 15 days, the lamellae showed epithelial lifting and hypertrophy of the cells (Fig. 2A). On exposure to 4.88 mg/L for same duration, it showed hypertrophy of the cells, marginal dilation and fusion of adjacent secondary lamellae (Fig. 2B). On exposure to further higher doses, 6.84 and 11.4 mg/L, hypertrophy, hyperplasia, blood congestion, marginal dilation and proliferation of mucous cells were observed (Fig. 2 C and D). It was further observed that the lesions increased with the increase in the concentration and with the duration of exposure. Hypertrophy, hyperplasia, vacuolation of the cells, marginal dilation (Fig. 3A); pilaster cell vacuolation, edema and curling of secondary lamellae (Fig. 3B); curling and elongation of secondary lamellae and intra-epithelial edema, dilation of central venous (Fig. 3C) and fusion of the adjacent secondary lamellae (Fig. 3D) were reported on exposure to different doses of lead nitrate for 30 days. Lead nitrate exposure @ 3.42 and 6.84 mg/L of for 45 days resulted in edema, abnormal elongation and swelling of the tip of secondary lamellae, (Fig. 4 A and B). Necrosis, telangiectasis and lamellar disorganisation were observed on exposure to higher dosage (4.88 and 11.4mg/L of lead nitrate (Fig. 4 C and D). Destruction of epithelial cells (Fig. 5A), aneurysm, haemorrhage and necrosis (Fig. 5B), necrosis (Fig. 5C) and sloughing of secondary lamellae (Fig. 5D) were observed when exposed for 60 days.

Fishes that inhabit polluted environment are susceptible to contaminants. These contaminants cause deleterious effect in cellular structures including epithelium and pillar cells[33]. Effect of lead were studied by many workers in different fishes as in *Clarias batrachus*[8], in *Ctenopharyngodon idellus*[24] and in trout[27], and the histopathological alterations observed were similar to the present study. The fish exposed to heavy metals revealed extensive damage in their gill architecture and this is in agreement with several earlier observations[21,26,34-37]. Aneurism was noticed with the rupture of the respiratory epithelium of the secondary lamellae and breakdown of the pillar cell system were observed in the gills of Cadmium exposed *Lates calcifer*[38]. Epithelial lifting was seen in the gills of carps (*Cyprinus carpio*) and tilapias (*Oreochromis mossambicus*) when exposed to the effluents of a waste water treatment plant[39]. Similar toxic impact of the trace element Zinc on the gills and accessory respiratory organs of *Heteropneustes fossilis* were studied[40]. Epithelial necrosis and rupture of the gill epithelium were found to be induced by the action of copper sulphate on the gill architecture of freshwater fish, *Oreochromis mossambicus*[41].

The gill alterations found in *Labeo rohita* in the present study can be classified into two types: one constitutes the lesions caused by direct effect of lead and second due to the defence response of the gill. The histological changes observed in the gills during the present study indicate that the fish were responding to the direct effect of the contaminants as much as to the secondary effects caused by stress. During the present course of work alterations like hyperplasia, hypertrophy, epithelial lifting and fusion of the adjacent secondary lamellae may be a result of defence mechanisms of the gills which serve as a barrier to the entrance of toxicants. This is in agreement with the above discussed studies. These histopathological changes may lead to a great disturbance of gas exchange and ion regulation. These may result in the reduced flow of oxygen enriched water to lamellar tissues. Lamellar telangiectasis results from rupture of pillar cells and capillaries under effect of heavy metals pollution and leads to an accumulation of erythrocytes in the distant portion of the secondary lamellae. The changes in the appearance of the secondary lamellae are due to the collapse of the pillar cell system and breakdown of the vascular integrity with the release of large quantities of blood that push the lamellar epithelium outward[42]. Epithelial oedema increases distance between the contaminant and the bloodstream while secondary lamellar fusion reduces the gill surface thereby decreasing the contact between the pollutant and gill epithelium[43]. The absence of such lesions in the control group confirms the deleterious effect of Lead exposure.

Conclusion

The present study is an effort to reveal the damages caused by heavy metals discharged from various

sources on the commercially important fish species. The results have demonstrated histopathological alterations viz. cellular hypertrophy, hyperplasia, vacuolation, epithelial lifting, shortening, curling and abnormal elongation of the secondary lamellae, fusion of adjacent lamellae, telangiectasis, blood congestion, interstitial edema, necrosis, lamellar aneurysm, architectural distortion and degeneration of gills. The extent of damage of the gill tissue was proportionate to the dose and duration of exposure of the fish to lead nitrate. The pollution in natural aquatic ecosystems is one of the main reasons for fast depletion of fish diversity. It will not only disturb the natural ecology but will also seriously affect the commercially important fish fauna.

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