Effects of Certain Pesticides on the Histopathology of Gills of an Air Breathing Fish Channa Gachua (Bloch)

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Abstract : The present work related to effects of certain pesticides on histopathology of Gills of an air breathing teleost, Channa gachua (Bloch). exposed to lethal and sublethal concentration of electroplating industry effluent for 24, 48, 72 and 96 hrs (LC_{50} at 20%, 19.5%, 19%, 18.5% respectively). Similarly the test fish were also exposed to acute concentrations of heavy metals like Copper, Nickel and Zinc. For Copper, the LC_{50} values were found to be 180 ppm, 88 ppm, 40 ppm, and 20 ppm respectively. The LC_{50} values for Nickel were found to be 1200 ppm, 600 ppm, 300 ppm and 150 ppm respectively. The LC_{50} values for the heavy metal Zinc was found to be 630 ppm, 528 ppm, 429 ppm and 330 ppm respectively. At the end of exposure period, the fishes which survived were sacrificed gills were removed and fixed in bouins' fixative for 24 hrs. Simultaneously a control aquarium was also maintained. The results are significant from environmental pollution and human health point of view.

IndexTerms **- Histopathology, Gills, Channa gachua, Heavy metal, Environmental pollution.**

I. INTRODUCTION

Increasing human population produces a negetive impact on the resources and the availability of energy and materials. The environment gradually degrades by the processes of waste dispersal from industries and spread of crop-pests and parasites. The quality of air, water and soil deteriorates as a result of human activities.

Environmental disruption may be considered under 3 steps: 1. Insult to the environment or Mankinds misdeeds, 2. The response of the environment to the insult & 3. The consequential damage to the welfare of the society, or the environmental cost.

Examples of the insult are: the discharges of wastes into air, water and soil, production of heat, noise, the removal of natural vegetation and fauna, and physical activities such as drilling, mining, damming, pumping and dredging.

Most of the Indian rivers and freshwater streams are seriously polluted by industrial wastes or effluents which come along with waste waters of different industries such as petro-chemical complexes, fertilizer factories, oil refineries, pulp and paper, electroplating, textile, sugar and steel mills, tanneries, distileries, coal washries, synthetic material, plants for drugs, fibers, rubber, plastics, etc. The wastes of these industries and mills include heavy metals like Chromium, Copper, Zinc, Nickel, Cadmium, Mercury, Lead, etc., detergents, petroleum, acids, alkalies, phenols, carbamates, alcohol, cynide, arsenic, chlorine, and many other organic and inorganic toxicants. All these toxicants of industrial wastes are harmful and may cause death or sublethal pathology of the liver, kidney, reproductive system, respiratory system and nervous system in both vertebrate and invertebrate aquatic animals. The severity of damage depends upon the toxic potentiality of a particular compound or particle accumulated in the tissues.

Fishes are one of the main victims of aquatic pollution. Fishes exposed to industrial effluents show loss of equilibrium, increase in opercular movements, irregular movements, disclouration of body and finally death. This may be due to significant damage to the internal organs. These pollutants, especially heavy metals, find their way in the body of aquatic animals by means of gills, digestive tract & general body surface and accumulate in the different tissues of the body. Therefore it is necessary to study in details, the histopathological alterations or changes in structure produced by industrial effluent in different organs of fishes and investigate them thoroughly in order to access the extent of damage.

Channa gachua, the **dwarf snakehead**, is a species of snakehead. It is one of the dwarf snakeheads, and has a length of up to 20 cm (8 in). It gave its name to the aquarists' term dwarf snakeheads to denote the smaller *Channa* species.

II. REVIEW OF LITERATURE

Gills are the major organ for osmotic regulation, excretion and respiration in fish. They are covered with thin epithelial layer which absorb oxygen and water soluble chemicals. This epithelium also forms a barrier between the

fish's blood and the surrounding water (Santos *et al.,* 2011; Patel and Bahadur, 2010). Gills are the primary site for any histological alteration as it is directly exposed to polluted water (Rakhi *et al.,* 2013). Histopathological study on fish gill was conducted by many authors.

According to Sahoo *et al.* (2003) both acute and subchronic exposure of aflatoxin B1 induced histopathological alterations in the gills of *Labeo rohita.* In the acute trials, treated group of fish had mild secondary lamellar epithelial hyperplasia, moderate edema of the secondary lamellae and rupture of the lamellar capillaries. There was fusion of secondary lamellae, complete loss and necrosis of secondary lamellae at some places along with inflammatory reaction. Semithin sections showed degeneration of the lamellar epithelia and damage of the capillary membrane. In the subchronic trials the lamellar epithelial hyperplasia resulting in fusion was the most predominant feature. Many secondary lamellae showed fusion up to the tip. The general organization of the lamellar epithelium was altered in both the trials. The heavy metal cadmium exposure induced severe histopathological changes in white seabass, *Lates calcarifer,* which was studied by Thophon *et al.* (2003). Scanning electron microscopic examination showed swelling of secondary lamellae, extensive aneurism with some ruptures in many secondary lamellae. They also reported swelling, enlargement, partial and complete fusion of secondary lamellae in numerous areas. Extensive edema of epithelial cells, hypertrophy and hyperplasia of epithelial were also noticed in treated fishes. Pfeiffer *et al.* (1997) performed an experiment where goldfish was exposed to carbaryl. They sacrificed the treated fishes after 96 h and observed many pathological changes. The secondary lamellae were distorted, evidence of lamellae fusion was observed, and some sloughing of surface epithelium was evident. The lamellae of treated fish were also thinner than in control fish. Strands of mucus were seen on the gill surface of carbaryl-treated fish. At higher magnification surface wrinkling and fusion of the secondary lamellae were also observed.

III. EXPERIMENTAL

In the second set of experiment, the test fish *Channa gachua* were exposed to two sub lethal concentrations of electroplating industry effluent for 30 days (chronic) prepared as mentioned in the earlier chapter of chronic toxicity. i.e., $1/5^{th}$ of 96 hrs (3.70%) and $1/10^{th}$ of 96 hrs (1.85%). Similarly for the heavy metal Chromium, the fishes were exposed for 30 days at 12 ppm ($1/5^{th}$ of 96 hrs) and at 6 ppm ($1/10^{th}$ of 96 hrs). For the heavy metal Copper, the two sublethal concentrations were found to be 4 ppm ($1/5^{th}$ of 96 hrs) and 2 ppm ($1/10^{th}$ of 96 hrs) for a period of 30 days. The fishes were also exposed to two sublethal concentrations of heavy metal Nickel at 30 ppm ($1/5^{th}$ of 96 hrs) and 15 ppm ($1/10^{th}$ of 96 hrs) for 30 days. For The heavy metal Zinc – as ZnSO4 the two sublethal concentrations were found to be 66 ppm ($1/5^{th}$ of 96 hrs) and 33 ppm ($1/10^{th}$ of 96 hrs) respectively. Simultaneously a control aquarium was also maintained. At the end of the experiment the fishes were killed and the target organs were utilized for histopathological study. All tissues were fixed in the bouins' fixative for 24 hrs and processed according to the standard procedures of routine microtechnique. Blocks were prepared in paraffin wax and sections were cut on rotary microtome to a thickness of 4-6 µ. For staining, the double staining method was followed by using hematoxylin and eosin (H & E) and mounting was done in DPX. The photographs were taken by a digital camera.

IV. RESULTS

The gills of fish exposed to Lethal concentration (LC_{50}) for 24 hrs at 20% dilution of electroplating industry effluent show marked changes like curling of secondary lamellae, swelling at the tip of secondary lamellae, fusion of secondary lamellae, degeneration of gill epithelium were the prominent changes observed. Hemorrhage in the primary lamellae is also observed. Some portions of the gill filaments are partially destroyed. (Fig 1)

The fish exposed to lethal concentration (LC_{50}) for 96 hrs at 18.5% dilution of electroplating industry effluent show marked degenerative changes in the architecture of gills like curling of secondary gill lamellae, swelling at the tip of secondary lamellae, degeneration and distortion of gill epithelium, fusion of secondary gill lamellae and vacuole formation in the gill epithelium. Some portions of the gill filaments were partially destroyed. (Fig 2)

The fishes exposed to sub lethal concentration ($1/5^{th}$ of 96 hrs) at 3.70% dilution of electroplating industry effluent for a chronic period of 30 days show marked pathological changes in the gills like curling of secondary lamellae, swelling at the tip of secondary lamellae, degeneration of gill epithelium and proliferation of gill epithelium. (Fig 3)

Fishes exposed to 1.85% dilution ($1/10^{th}$ of 96 hrs) of electroplating industry effluent for a chronic period of 30 days, exhibited marked pathological changes in the gills showing curling of secondary lamellae, swelling of secondary lamellae, degeneration of gill epithelium, hemorrhage at primary lamellae. The mucous cells were found swollen with degeneration of chloride cells. (Fig 4)

Fishes exposed to the heavy metal Copper (Cu) as $CuSO_4$ recorded similar degenerative changes in the gills at 24 hrs (180 ppm) like fusion of secondary lamellae, curling of primary lamellae, curling of secondary lamellae with degeneration and necrosis in gill epithelium were observed. Some portions of the gill filaments were partially destroyed. (Fig 5)

Fishes exposed to the heavy metal Copper (Cu) as $CuSO_4$ recorded similar degenerative changes in the gills at 96 hrs (20 ppm). Changes like curling of secondary lamellae, curling of primary lamellae, swelling at the tip of secondary lamellae, hemorrhage and necrosis in gill epithelium were observed. (Fig 6)

Fishes exposed to the heavy metal Copper (Cu) as $CuSO_4$ for a chronic period of 30 days at the sublethal concentration 4 ppm (1/5th of 96 hrs) recorded degenerative changes in the gills like curling of secondary lamellae, swelling of secondary lamellae, fusion of secondary lamellae and degeneration, hemorrhage and necrosis in gill epithelium were observed. (Fig 7)

Fishes exposed to the heavy metal Copper (Cu) as $CuSO_4$ for a chronic period of 30 days at the sublethal concentration 2 ppm ($1/10^{th}$ of 96 hrs) recorded degenerative changes in the gills like curling of secondary lamellae, swelling at the tip of secondary lamellae, fusion of secondary lamellae, degeneration of primary lamellae with degeneration and necrosis in gill epithelium were observed. Some portions of the gill filaments were partially destroyed. (Fig 8)

Fishes exposed to the heavy metal Nickel (Ni) as $NiSO_4$ recorded degenerative changes in the gills at 24 hrs (1200 ppm) like fusion of secondary lamellae, curling of secondary lamellae with degeneration and necrosis in gill epithelium were observed. Some portions of the gill filaments were partially destroyed. (Fig 9)

Fishes exposed to the heavy metal Nickel (Ni) as $NiSO_4$ for 96 hrs at 150 ppm recorded degenerative changes in the gills like curling of secondary lamellae, swelling at the tip of secondary lamellae, degeneration, hemorrhage and necrosis in gill epithelium were observed. (Fig 10)

Fishes exposed to the heavy metal Nickel (Ni) as $NiSO_4$ for a chronic period of 30 days at $1/5^{th}$ of 96 hrs (30 ppm) recorded degenerative changes in the gills like curling of secondary lamellae, fusion of secondary lamellae, swelling at the tip of secondary lamellae with degeneration and necrosis in gill epithelium were observed. (Fig 11)

Fishes exposed to the heavy metal Nickel (Ni) as $NiSO_4$ for a chronic period of 30 days at $1/10^{th}$ of 96 hrs (15 ppm) recorded degenerative changes in the gills like curling of secondary lamellae, swelling at the tip of secondary lamellae with degeneration and necrosis in gill epithelium were observed. (Fig 12)

Fishes exposed to the heavy metal Zinc (Zn) as $ZnSO_4$ for a period of 24 hrs at 630 ppm recorded degenerative changes in the gills like fusion of secondary lamellae, curling of secondary lamellae, degeneration of primary lamellae with degeneration and necrosis in gill epithelium were observed. Some portions of the gill filaments were partially destroyed. (Fig 13)

Fishes exposed to the heavy metal Zinc (Zn) as $ZnSO_4$ for a period of 96 hrs at 330 ppm recorded degenerative changes in the gills like fusion of secondary lamellae, curling of secondary lamellae, swelling at the tip of secondary lamellae with proliferation and necrosis in gill epithelium were observed. (Fig 14)

Fishes exposed to the heavy metal Zinc (Zn) as $ZnSO_4$ for a chronic period of 30 days at 1/5th of 96 hrs (66 ppm) recorded degenerative changes in the gills like fusion of secondary lamellae, curling of secondary lamellae, swelling at the tip of secondary lamellae with hemorrhage and degeneration in gill epithelium were observed. (Fig 15)

Fishes exposed to the heavy metal Zinc (Zn) as $ZnSO_4$ for a chronic period of 30 days at $1/10^{th}$ of 96 hrs (33 ppm) recorded degenerative changes in the gills like fusion of secondary lamellae, curling of secondary lamellae, swelling at the tip of secondary lamellae, vacuolation in gill epithelium with degeneration of gill epithelium were observed. Some portions of the gill filaments were partially destroyed. (Fig 16)

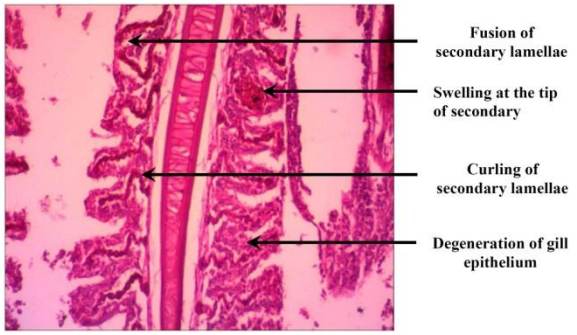

Fig 1: L. S. of Gills of *Channa gachua* after exposure to electroplating industry effluent at 20% concentration

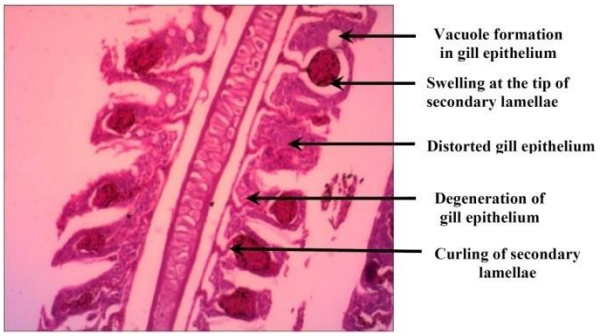

Fig 2: L. S. of Gills of *Channa gachua* after exposure to electroplating industry effluent at

for a period of 24 hrs. (H/E) (400X)

18.5% concentration for a period of 96 hrs. (H/E) (400X)

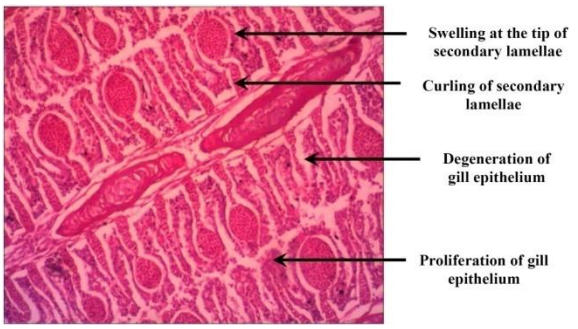

Fig 3: L. S. of Gills of *Channa gachua* after exposure to electroplating industry effluent at 1/5th of 96 hrs (3.70% concentration) for a chronic period of 30 days. (H/E) (400X)

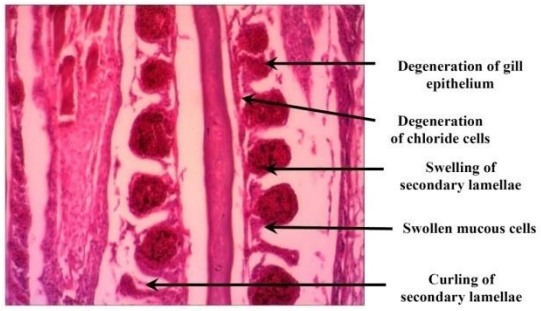

Fig 4: L. S. of Gills of *Channa gachua* after exposure to electroplating industry effluent at 1/10th of 96 hrs (1.85% concentration) for a chronic period of 30 days. (H/E) (400X)

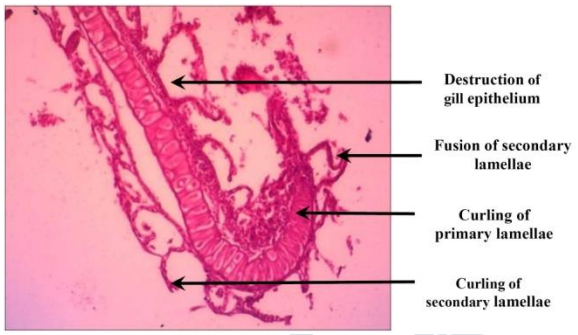

Fig 5: L.S. of Gills of *Channa gachua* after exposure to heavy metal Copper (Cu) as $CuSO_4$ at 180 ppm for a period of 24 hrs. (H/E) (400X)

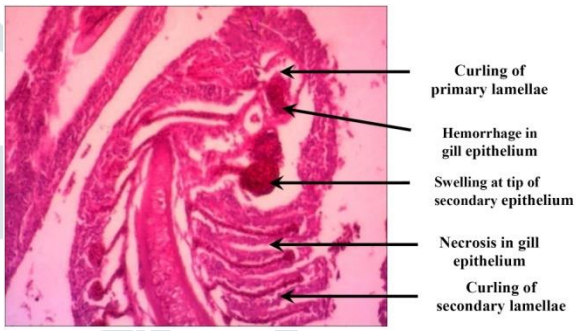

Fig 6: L.S. of Gills of *Channa gachua* after exposure to heavy metal Copper (Cu) as $CuSO_4$ at 20 ppm for a period of 96 hrs. (H/E) (400X)

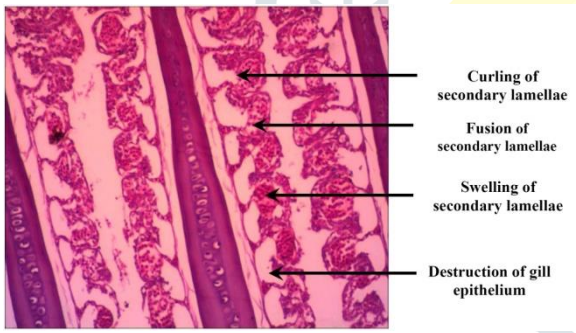

Fig 7: L.S. of Gills of *Channa gachua* after exposure to heavy metal Copper (Cu) as $CuSO_4$ at 1/5th of 96 hrs (4 ppm) for a chronic period of 30 days. (H/E) (400X)

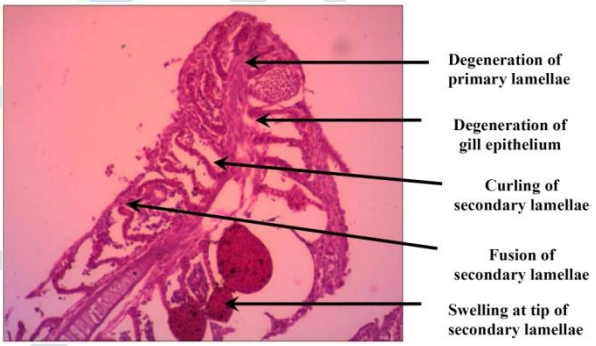

Fig 8: L.S. of Gills of *Channa gachua* after exposure to heavy metal Copper (Cu) as $CuSO_4$ at 1/10th of 96 hrs (2ppm) for a chronic period of 30 days. (H/E) (400X)

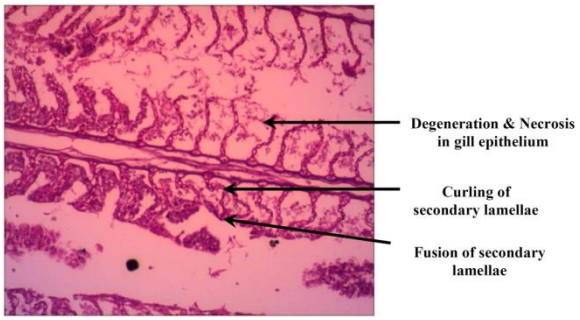

Fig 9: L.S. of Gills of *Channa gachua* after exposure to heavy metal Nickel (Ni) as $NiSO_4$ at 1200 ppm for a period of 24 hrs. (H/E) (400X)

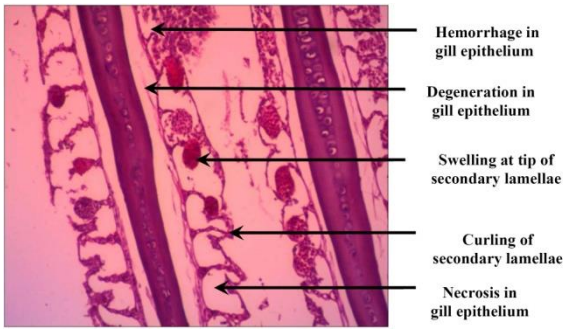

Fig 10: L.S. of Gills of *Channa gachua* after exposure to heavy metal Nickel (Ni) as $NiSO_4$ at 150 ppm for a period of 96 hrs. (H/E) (400X)

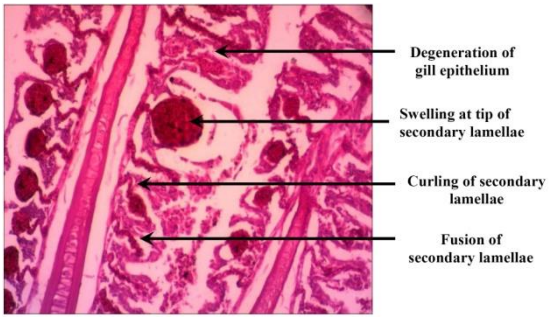

Fig 11: L.S. of Gills of *Channa gachua* after exposure to heavy metal Nickel (Ni) as $NiSO_4$ at $1/5^{th}$ of 96 hrs (30 ppm) for a chronic period of 30 days. (H/E) (400X)

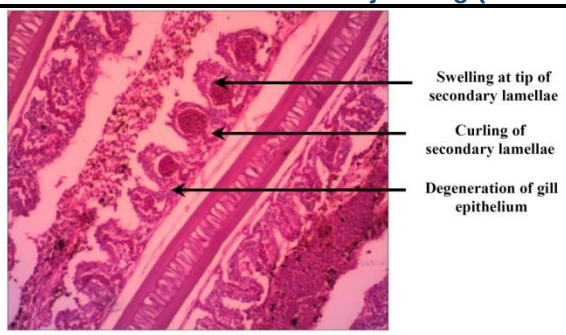

Fig 12: L.S. of Gills of *Channa gachua* after exposure to heavy metal Nickel (Ni) as $NiSO_4$ at $1/10^{th}$ of 96 hrs (15 ppm) for a chronic period of 30 days. (H/E) (400X)

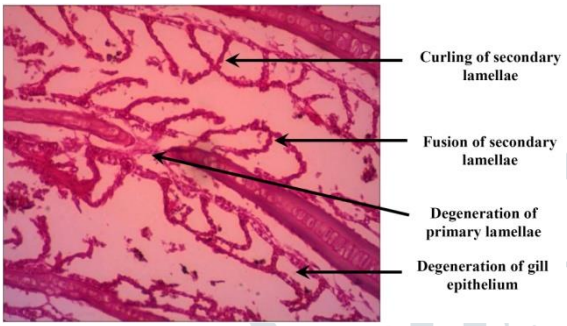

Fig 13: L.S. of Gills of *Channa gachua* after exposure to heavy metal Zinc (Zn) as $ZnSO_4$ at 630 ppm for a period of 24 hrs. (H/E) (400X)

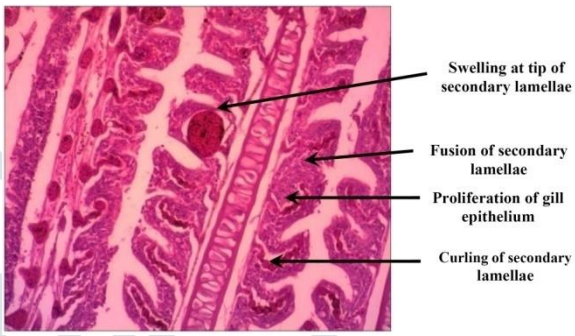

Fig 14: L.S. of Gills of *Channa gachua* after exposure to heavy metal Zinc (Zn) as $ZnSO_4$ at 330 ppm for a period of 96 hrs. (H/E) (400X)

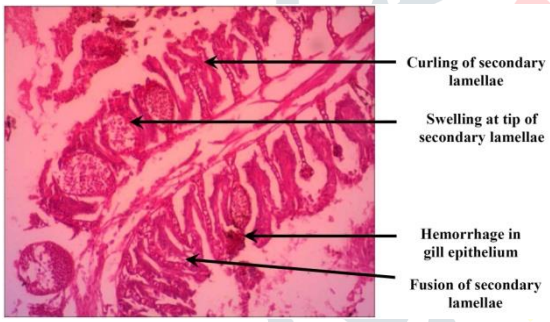

Fig 15: L.S. of Gills of *Channa gachua* after exposure to heavy metal Zinc (Zn) as $ZnSO_4$ at $1/5^{th}$ of 96 hrs (66 ppm) for a chronic period of 30 days. (H/E) (400X)

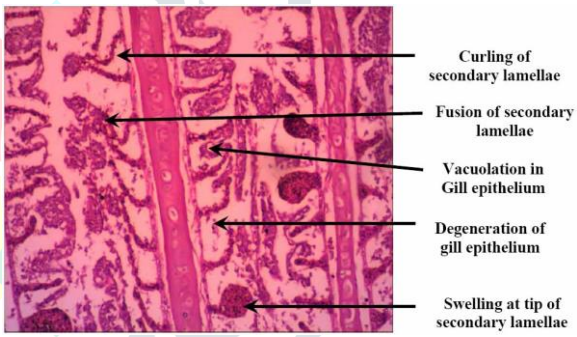

Fig 16: L.S. of Gills of *Channa gachua* after exposure to heavy metal Zinc (Zn) as $ZnSO_4$ at $1/10_{th}$ of 96 hrs (33 ppm) for a chronic period of 30 days. (H/E) (400X)

V. DISCUSSION

The fish gill is a highly specialized tissue for vital function like respiration. It offers a suitable site for studying the toxic effect of substances as they enter the fish body mainly through the gill surface. The gills have a large superficial area, the primary lamellae, through which gaseous exchange between blood and external medium takes place. Besides respiration, this organ also performs the other vital function such as osmoregulation and excretion. The chloride cells responsible for ionic exchanges are usually distributed on the secondary lamellae under condition of low ionic concentration besides transporting Na++, Cl+ and other substances. Any pathological changes in the gills cause hindrance to O_2 absorption through gill surface.

In the present investigation, histopathological changes in the gills of *Channa gachua (Bloch)* exposed to lethal and sublethal concentration of electroplating industry effluent and heavy metals Copper, Nickel and Zinc caused severe degenerative changes in the gills like hemorrhage at primary lamellae, fusion in secondary gill lamellae , swelling of secondary gill lamellae, curling of secondary lamellae, pillar, chloride and mucous cells found swollen, cell necrosis, epithelial cell hypertrophy, exudation and bulging of the tip of primary filament, disorganization and rupture in secondary lamellae. These changes might have been observed due to shifting of aerobic to anaerobic pathway under stress. The present findings are concurrent with the workers Fernandes and Mazon, 2003; Mazher and Dawood, 2004; Randi et al, 2006; Fatma and Mohammed (2008).

V. CONCLUSION

In the present study, histology of the control fishes and histopathology of experimental fishes exposed to electroplating industry effluent and 3 heavy metals - Cu, Ni and Zn are recorded. The target organs selected for study are gills. The control fishes showed the normal architecture in the target organs whereas the experimental fishes showed histopathological alterations in the target organs such as degenerative changes in the cells, necrosis, general inflammatory responses and neoplasia/hyperplasia in the target organs.

Histopathology is the study of the structure of abnormal tissue. In the present investigation, examination of tissues from organisms served to identify the cause of death and possibly the causative agent. Suitable preparations of affected, exposed tissues are observed under light and electron microscopes, for changes in the tissues. Several specific and non-specific microscopic lesions develop in the target tissues selected that are of diagnostic significance which can be studied and analysed because of the histopathological examinations.

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