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HISTOPATHOLOGY CHANGES IN FRESH WATER FISH *CLARIAS BATRACHUS*(Linn.) EXPOSED TO MERCURY AND CADMIUM

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ABSTRACT

The release of heavy metals into the aquatic environment causes water pollution problems because of their toxicity, persistence and bio-accumulation .An investigation on the effect of heavy metals like Mercury and Cadmium on Fresh water fish *Clarias batrachus* carried out in the lab. Fishes weighing 10 ± 0.5 g and 10 ± 1 cm length were exposed to sublethal concentrations (derived, based on acute toxicity tests) of mercury 0.19, 0.09, 0.05 and 0.03 ppm and of cadmium chloride 0.66, 0.30, 0.17 and 0.12 ppm for 30 days. During this period fishes were fed with artificially prepared food. On the 10th, 20th, 30th day fishes were taken out, sacrificed and the tissues of gill and liver were excised out. The total autopsy was completed in less than 4 mins. The result showed that the degree of distortion of the gill, liver was proportional to the exposure period and concentration of the metals was found to be dose and time dependent.

Keywords: *Clarias batrachus,* Mercury and Cadmium Toxicity, Fish Toxicity

INTRODUCTION

More and more of our habitats are being deteriorated day by day due to increased environmental pollution by means of various anthropogenic activities. The industrial effluents that contain toxic substances like heavy metals, pesticides and other chemicals are discharged into the water bodies. Any pollutant which is discharged into water will change the surface tension, thermal properties, conductivity, density and pH value along with biodegradable (proteins, fats, pesticides, fungicides etc), and non-biodegradable pollutants. The acidic and alkaline pollutants destroy most of the invertebrates and microorganisms (Barber and Sharma, 1998). As a result the aquatic fauna and flora are adversely affected, which lead to bioaccumulation in aquatic organisms and bioconcentration in higher vertebrates (Ackerman, 2001). Eco-friendly environment is a necessary condition for the well being of human race. The degree contamination in aquatic environment is frequently assessed by comparing containment concentration in associated biota.Since bioconcentration of compounds have been determined in the environment ,it has been observed that there are many quantitative relationships between structure and biological activity of chemicals established in aquatic system. However the main sites of these heavy metal uptake and accumulation are the gills and gastrointestinal tracts. Lead is a common heavy metal found in the

environment and is derivable from urban waste waters, industrial discharges and agriculture runoff. Its inclusion in gasoline as anti-knock contribute to its occurrence in the air ,which is transported to the streams and rivers be runoffs where fish and other aquatic organism take it up and incorporate it in their body. The death of thousands of Japanese who ate fish from Minamata Bay in 1952, the sudden death of (or) 4,000 people in London in 1960 due to eating of fish and similar events indicate the necessity for ensuring a clean environment. As an indicator of environment livability, fish seems to be very important since it is affected by the living habitat (namely water), which is today polluted by effluents from industries, pesticides washed out from agricultural lands and detergents from household drains etc. This paper is aimed at determining the gills, liver, of the *Clarias batrachus to* sub lethal concentration of cadmium and mercury.

MATERIALS AND METHODS

Healthy living specimens of *C.batrachus* weighing 10 ± 0.5 gm and 10 ± 1 cm in length have been brought from Tamilnadu fishery, Poondi, Thiruvallore Dist. They were brought to the laboratory in well-aerated containers, to avoid hyperactivity, physical injuries and stress to the fish. The fishes were screened for any pathological symptoms and washedwith1% KMnO4 solution. The healthy specimen was then transferred to glass aquaria (50 x 25 x 25 cm) containing tap water. The fishes were acclimatized to the laboratory conditions for 15 - 20 days prior to experimentation. Fishes were fed with artificial pellet diet; water was replaced with clean water whenever necessary. The Physicochemical characteristics of the water were analysed as per the procedure of (APHA, 1995), American public health association. The test fish was identified to the species level. Size and weight were taken the fishes were preconditioned to unpolluted tap water for 20 Days.

Preparation of Stock solution

Mercury and Cadmium Chloride were of reagent grade. Concentrated stock solutions were prepared by weighing correct amount of the salts and dissolved in water (1g/l). Aliquot volume of calculated stock solution to yield the desired concentration was added to the tank to give the exact required concentration of the heavy metal.In the present study 1/10, 1/20, and 1/30 of the 96h LC_{50} were selected as sub lethal concentration and the fishes were exposed to each concentration for a period of 10, 20 and 30 days. A control batch corresponding to each test group was simultaneously maintained. The experiments were repeated five times and concentrations supplied daily to maintain a constant toxic media. Fishes were taken out, blotted dry with soft absorbent paper and dissected to remove liver and gill tissue. The organ was preserved in labeled sample bottles containing formal saline, sectioned and slide preparations were made for histological investigation under the microscope.

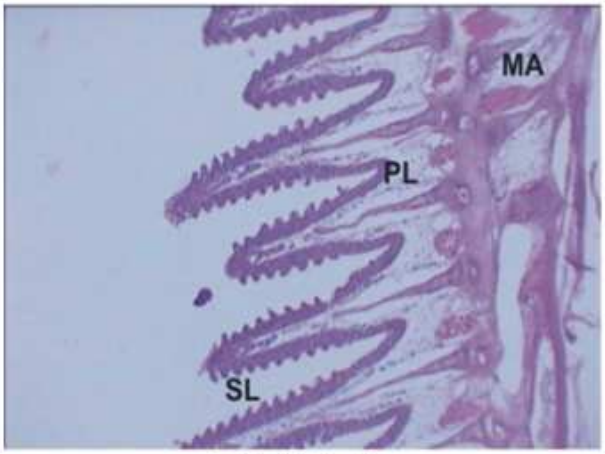
RESULT

Histological Study

Effect of Mercury on Gill

The histopathological studies indicated that the gill of *C.batrachus* were treated by sub lethal concentrations of Mercury. After 10 days (Plate 1A), minimum changes were observed. Not much changes were observed in Primary lamellae but the secondary lamellae were fused together to most of their length with the terminal 1/3 portion free (Plate 1B). The infused secondary lamellae were thinner compared to their controls. The epithelial cells were seen in between the fused secondary lamellae. In some case secondary lamellae appeared to be curved.In 20 days treated fish, pronounced changes like distortion of secondary lamellae, hypertrophy of gill filament and hyperplasia of epithelial surface, detachment of epithelium from underlying pillar system, the fusion of secondary lamellae resulting is a reduction in the surface area of the epithelium was noticed (Plate 2B).In fish treated upto 30 days, the changes observed in the gill of *C. Batrachus* were severe erosion and degeneration of gill epithelium and aggregation of blood corpuscles. Fusion of the Boundary of the secondary lamellae increased with exposure periods (Plate 3B).

Plate 1
Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Gill - 10 days



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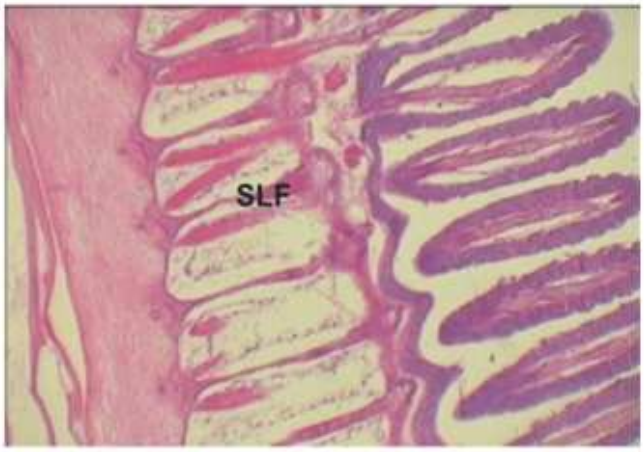
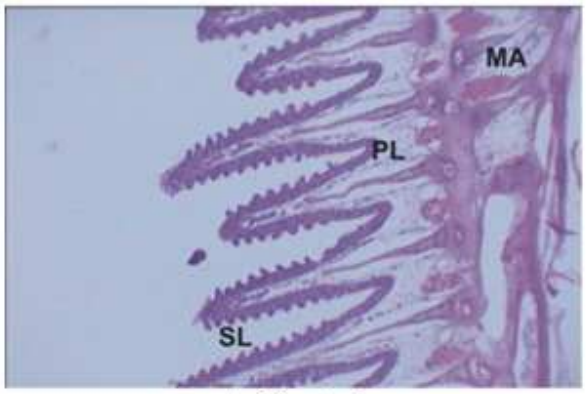


B. Mercury

Plate 2

Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Gill - 20 days



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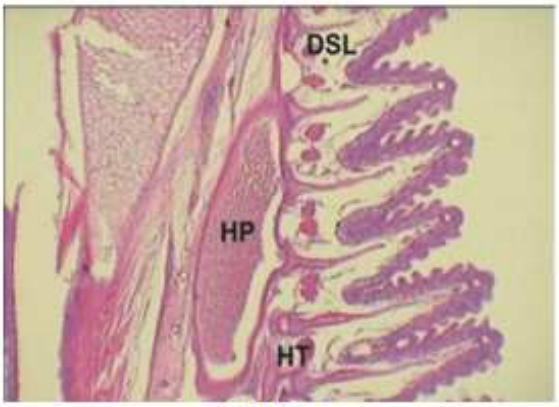
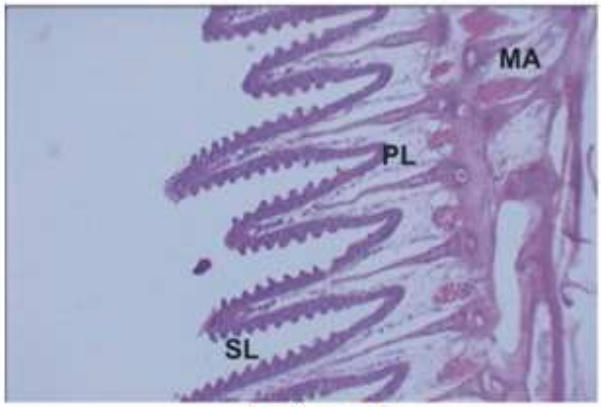


B. Mercury

Plate 3

Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Gill - 30 days



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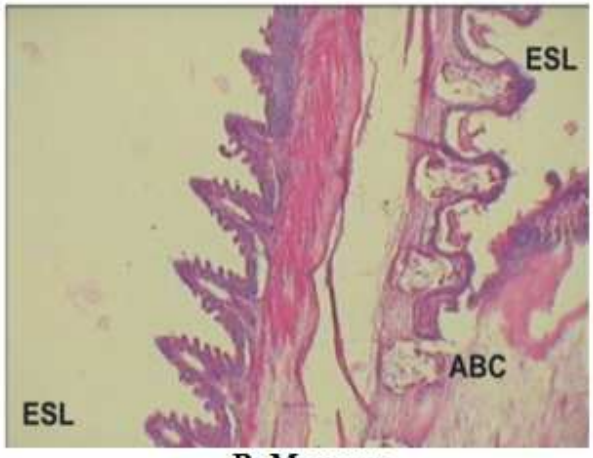


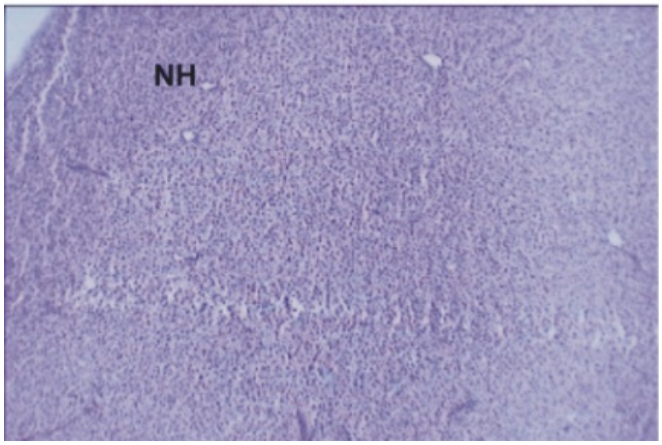
B. Mercury

Effect of Mercury on Liver

In *Clarias batrachus* treated for 10, 20, 30 days, large area of liver tissue showed disrupted hepatocytes with loss of normal palisade arrangement. After 10 days of exposure, degeneration of blood vessels, vacuolization and hypertrophy were observed (Plate 4A, B).The hepatocytes appeared swollen after treatment for 20days (Plate 5A, B). The liver showed acute inflammation including rupturing of hepatocytes and increased accumulation of pyknotic nuclei, necrosis, vacuolization of tissue and degeneration of blood vessels. At the end of 30 days exposure, the liver tissue had lost their cytoplasmic density and appeared opaque in many areas. Degeneration of hepatocytes showing distinct vacuoles, necrosis with sinusoidal lesions were the maximum alterations observed (Plate 5C).

Plate 4

Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Liver - 10 Days



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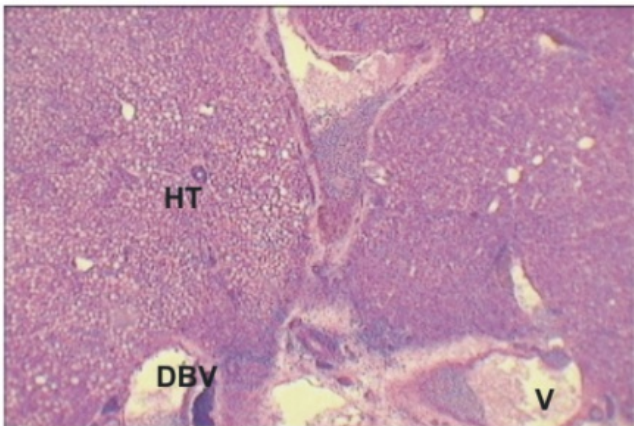
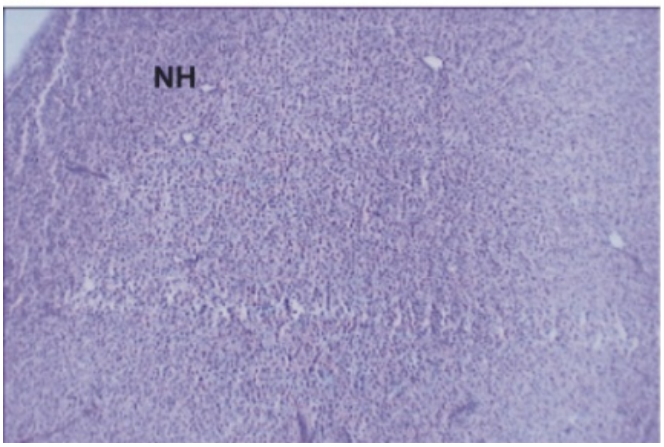


B. Mercury

Plate 5

Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Liver - 20 Days



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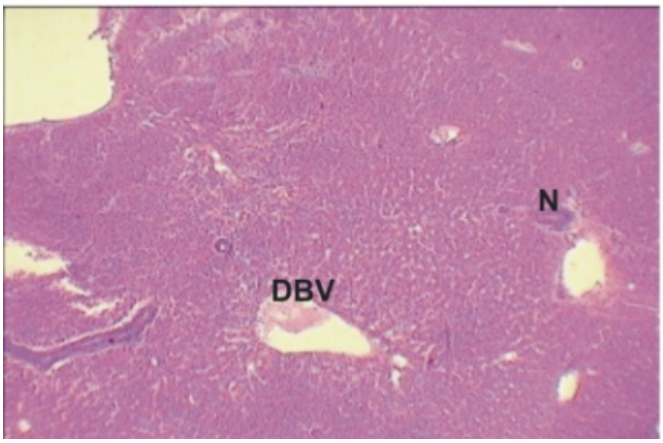
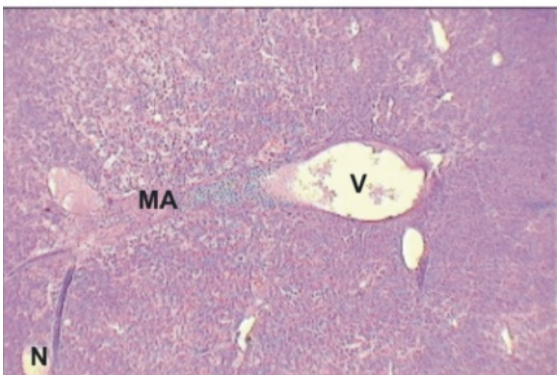


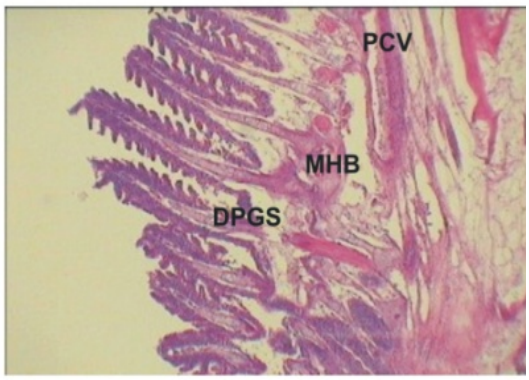
B. Mercury



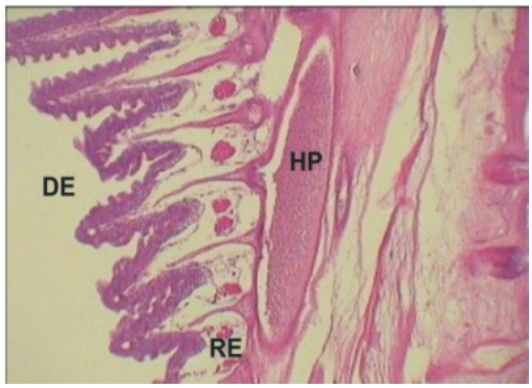
C. Cadmium

Effect of Cadmium on Gill

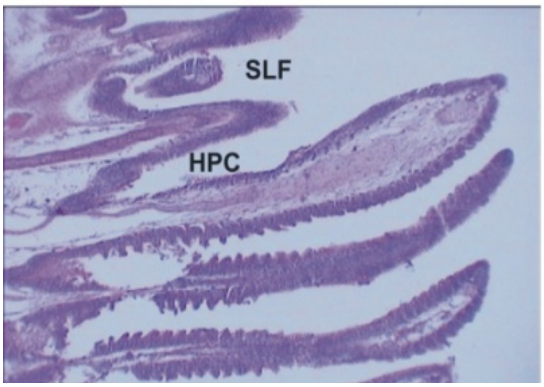
In fish exposed to sub lethal concentration of Cadmium various histopathological alterations are observed after 10 days of exposure (Plate 1C). The secondary lamellae are thin and are fused together. The shape was altered. Erosion of hypertrophy of mucus cells, epithelial cells, degeneration of pilaster cells and epidermal cells were noticed. The fusion of secondary lamellae may have been due to excessive secretion of Mucous. In 20 days treated fish, changes like distortion of epithelial cells, hypertrophy of gill filaments, hyperplasia of epithelial surface was evident. Reduction in surface are of the epithelium and detachment of epithelium from pillar system was noticed. (Plate 2C).At the end of 30 days, as the duration of treatment increased, mucous cells increased along with distortion of gill filaments and lamellar regions. Severe edema, separation of respiratory epithelium and vacuolization alterations were observed (Plate 3C).



C. Cadmium



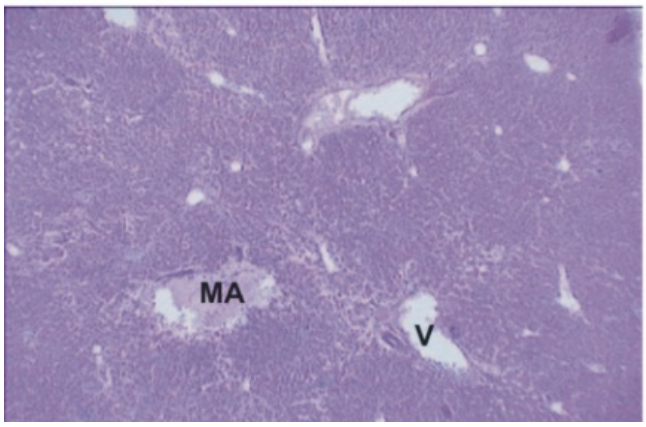
C. Cadmium



C.Cadmium

Effect of Cadmium on Liver

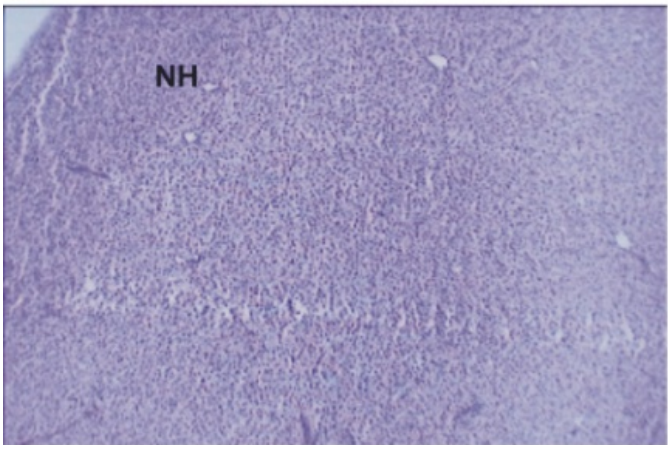
In fish at 10 days of treatment, swollen hepatocytes were noticed. Degeneration of hepatocytes, vacuolation space formation was evident, throughout the tissue (Plate 4C). At 20 days of exposure and high concentration accumulation of blood vessels, congestion of hepatic tissue were also observed (Plate 5C). After 30 days of treatment the liver was highly damaged subcapsular vacuolization, necrosis, indistinct cell boundaries in many places and pyknotic nuclei were also observed. As the duration increased, severe degradation of the liver cells or hepatocytes and hypertrophy of hepatic nuclei and clumping was evident in many places (Plate 6C)



C. Cadmium

Plate 6

Histological lesion in Clarius batrachus (Cat fish) exposed to Mercury and Cadmium, Liver - 30 Days



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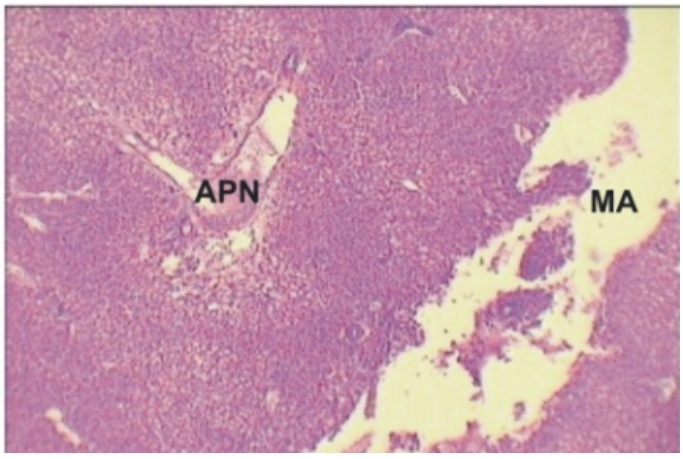
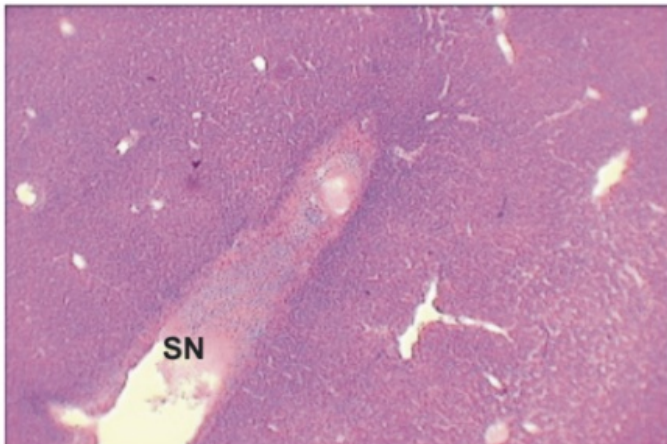


B. Mercury



C. Cadmium

DISCUSSION

The histological changes on 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. Singh *et al.*(1990) studied environmental pollution and its effects on aquatic animals.Heavy metal toxicant leads to many pathological changes in different tissues of fish and has been reported for *Labeo rohita* exposed to mercurichloride and *Chana punctatus* exposed to phenyl mercuri acetase (Karuppasamy, 2000). Srivastava *et al*. (1982) have observed the hisotopathological changes and accumulation potential in the fish tissues under chromium stress. Cadmium exposure induces the appearance of granular deposits in the liver, atrophy of the proximal renal tubules, and increases in chloride cell turnover at the gills (Pratap and wendelaar Bonga, 1993).Gill hyperplassia, necrosis of intestinal mucosa, fat infiltration of liver parenchyma cells, destruction tubus were observed due to ammonia poisoning in the intestinal tract, kidney and gills of juvenils *Sparus auratus* (Zaki *et al.,* 1987).Gill is an important tissue because of its direct contact with water and any effect of agency has to go through it to come into the fish body (Jana and Bandopadhyaya, 1987).It has been suggested that waterborne agencies damage fish gills, presumably by causing breakdown by the gas-exchange mechanisms with the consequent tissue hypoxic conditions (Natarajan, 1985a).The microphotographs of gill of *C. batrachus* have been observed. In control fish, the structure of gills is similar to that of other fresh water cat fish as described by Laurent,(1989) where as the gills of the experimental fish exposed to cadmium and mercury (10, 20 and 30 days) show active secretion of mucous. More specifically, in the mercury treated gill the epithelial layer was disrupted. In few regions, disintegration and fusion of primary lamella were observed. In cadmium treated gill, marked hyperplasia of the branchialarch, pilaster cell vacoulizaton and congestion of blood vessels were well marked. Similar results have also been observed by Skidmore,(1972) in rainbow trout when exposed to zinc sulphate. Histological experimentations of the gills of rainbow trout by Lloyd, (1965) when exposed to zinc, lead and copper showed separation of the epithelium from the gill lamellae and cells sloughed off into the spaces between the filaments. These changes are in agreement with the present observation when the fish is intoxicated with the heavy metals mercury and cadmium. The results are also in arrangement with the works of Kapilamanoj and Ragothaman, (1999) who have reported for *Boleopthalmus dumieric* exposed to sublethal concentration of cadmium.In the present study vacuolization, space formation and resulting haemmorhage, hypertrophy of hepatocytes and clumping were observed in mercury treated tissues and extensive necrosis, pyknotic nuclei in cadmium treated liver.Kabir and Begum (1978) reported cytoplasmic degeneration, pyknotic nuclei in liver tissues, vacoulation in hepatic cells and rupture of blood vessels; degenerate hepatic cells and necrotic nucleic when *Heteropneustes fossils* was exposed to *C. punctatus* to a sublethal concentration of endocrine and observed hypertrophy of hepatic cells and liver cord dis rray, vacoulation of cytoplasm and necrosis, ruputre of hepatic cell membrance and necrotic centrolbular area.These types of histological alterations were also noticed in the live tissue of *Labeo rohita* exposed to tannery effluent (Rana and Sudhir, 1999). The liver tissues of *L. rohita* showed histological changes due to aflatoxin given to fish intraperitoneally (Sahoo *et al.,* 2001).Conclusively, metals are stored in different sites in animals depending on the metals and on the animal species. To check the continual introduction of these metals in to the food chain, a more cautious application of insecticides and pesticides should be employed and effluents from industries must be treated before disposal.

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