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Lead Nitrate Toxicity in the Suprabranchial Chamber and Air Sac of an Air Breathing Cat Fish *Heteropneustes fossilis*(Bloch)

Sachchida Nand Mishra
Deptt. Of Zoology, M. K. College, Laheriasarai, Darbhanga

Abstract: ***Suprabranchial chamber and air sac is a vital accessory respiratory organ help the air breathing fish in ambient oxygen consumption which is one of the primary metabolic need for the survival of the the fish. Heteropneustes fossilis is an air breathing cat fish also capable to use atmospheric oxygen through their suprabranchial chamber and air sac which is known as its accessory respiratory organ. Lead nitrate, as a prominent heavy metal, when discharged directly in to water body as industrial waste, adversely effect in the capacity of oxygen consumtion of the fish by inducing toxic effect in the respiratory organs, gills, suprabranchial chamber and air sac of accessory respiratory organ. This study provide a background for evaluating the possible danger for human health resulting for consumption of the heavy metals toxicity affected fish. The toxic effect of Lead nitrate appear to be two types, one that occurs on a short term exposure to the toxicant evidenced by fish death and the other of chronic or long term effect detected by the constant changes in the normal biological system of the test fish.***
Keywords: Lead Nitrate, Suprabranchial Chamber, Air sac.

I. INTRODUCTION

The air bteathing cat fish *Heteropneustes fossilis (Saccobranchus fossilis*),commonly called singhi is one of the important fresh water air breathing cat fish in the sub tropic of India belongs to the order Cypriniformes, family Heteropneustidae under the group of siluroid fishe. It is widely available in marshy and derilict water having low level of dissolved oxygen. The presence of accessory respiratory orgsn(ARO) also enable them to temporarily stay out of water for hours together through aerial mode of respiration. This ability is being exploited in marketing of the fish in live condition wall of the supra branchial chamber is formed by the fused gill lamellae of the gill arches. The gill lamellae born by their respective hemibranch s fuse to form the fans. The curious disposition of the 3rd and 4th fan in the suprabranchial cavity causes it to be divided into an anterior smaller and posterior larger chamber. The air sacs which extend on either side of the body right upto the middle of the tail region and provide the main air-breathing organ of the fish, is the product of gill mass and as such can be looked upon as the modified 5th gill. The gill mass remains present at the posterior extremity of the air sacs even in the adult condition and adds to its length. The pillar cells of the ARO differing from those of the gills and retaining certain embryonic features is due to the facts that they are not derived from the gill lamellae once formed at the gills but from the gill mass which persist in the embryonic form at the submit of the air sacs even in the adult condition.

By acquiring the effect of toxic pollutant in aquatic medium, its discharge into nearby water resources may be regulated to protect aquatic life. Several reports are available on the toxicity of various insecticides to fishes (Eisler and Edmonds, 1971, Joshi2002,Singh etal., 1990; Banerjee, 2004). Further, it is also well known from the works of Jones (1964), Verma etal(1993). The susceptibility of fish to pesticides is also frequently influenced by pH and /or temperature of water. Several reports have also appeared in recent years suggesting that various concentrations of different heavy metals may cause significant changes in metabolic process of the fish. Metabolic activity of an organism is increased by its oxygen utilization. Various environmental factors and stresses alter metabolic rate of animals which would be indicated by oxygen utilization rate. Fry (1957) has considered the rate of oxygen consumption as an index to denote the intensity of metabolism. A change in respiratory parameter has been taken up as an index for the harmful effect of pollutant (Sastry etal, 1982). Several biologists have measured the oxygen uptake rate in water breathing fishes taking into account the different variables such as temperature, body size, different stages of life cycle, respiratory surface area, nutrition and other factors causing changes in energy requirement and consequently the oxygen consumption by the fish (Munshi and Singh 1992). In air breathing fishes, however the gas exchange is bimodal with water via buccopharynx, gill, and skin and with atmospheric air through accessory respiratory organs, but the relative role of gill and air breathing organ in respiration

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varies from species to species. Hence any change in normal respiratory epithelium would ultimately affect the rate of oxygen uptake. Therefore direct contact with respiratory surface area of fish with the lead nitrate polluted water, an alteration in the normal respiration as well as diffusion capacity of gills and accessory respiratory organs is evidenced (Brown etal1986; Munshi and Singh1992Murphy etal1987).Recently changes in respiratory behaviour and metabolic rate of heavy metals induced fishes have drawn the attention of several biologists (wilson etal1993), Haider and Indraj (1986) have stated that the changes produced by various pollutants look alike superficially their harmful effect on different genera of fish is not of the same magnitude. Further it is also well known from the work of Munshi and Singh (1968) that mucous secretion is known to have an important function in the maintenance of respiratory activity of the gills in fishes. The normal function of the mucous cell, is influenced by pathogenic and environmental agents.

Similarly biological studies on various fish tissues are also of specific interest as they constitute a rich source of high nutrient and caloric value. Mazon etal., (2002) have stated that in fish, stressful situation elicites neuroendocrine responses which interm induce alteration in carbohydrates metabolism. Since both catecholamines and adrenocorticosteroids are secreted in increased amount due to stressful stimuli, they ultimately elicite marked changes in carbohydrate energy reserves of the fish, (Bakthavathsalam, 1987).

Thus, the available investigation related with the effect of heavy metals on the respiratory biology of fishes with special reference to histopathological changes in the accessory respiratory organ. Lead nitrate induced histopathological changes are insufficient to establish the exact toxicological effects of heavy metals on suprabranchial chamber and air sac in *H. fossilis*. Industrial uses of lead nitrate included heat stabilisation in nylon and polyesters and in coating photothermographic paper. It is also used in gold cyanidation and rodenticides.

Larsson etal., (1981) investigated the histopathological changes due to heavy metals in association with disturbance in osmotic and ionic balance. They also elucidated the chronic exposure to sublethal concentrations of lead nitrate resulting the damage of organs of respiration and excretion like kidney through impared metabolism of ions like sodium, potassium, calcium, magnesium, chloride and inorganic phosphate in the flounder, *Platichthys flesus*. Pool (1981) proposed that other heavy metals like Cd also possesses strong affinity for sulphur, dithiol and other S-groups in the biological materials. Under laboratory conditions, hyperglycaemia is a typical symptom of cadmium poisoning, as reported in an Indian fresh water cat fish *H. fossilis* (Sastry and Subhadra - 1982).Histological alteration in liver and intestine of *Sartherodon mossambicus* in response to Hg toxicity was depicted by Naidu etal., (1983). They recorded engorged blood sinusoid, vocuolation, rupture, granular degeneration of hepatocytes, edemafocal necrosis and proliferation in fibroblast in liver. Jhingaran (1985) narrated air breathing fish, *Clarias batrachus* to be more refractive to cadmium toxicity than the non-airbreathing ones, viz., *Labeo rohita.* Dubale and shah (1979) exposed the Channa punctatus to different concentration of cadmium nitrate (0.01 to 0.05 ppm) and observed hepatopathic effects such as necrosis of hepatocytes. The reason behind the observation of Dubale and Shah (1979) were envisaged that these might happen due to the effect of cadmium on sulphur. Besides the work as mentioned above as toxic effect of notable heavy metals like Cadmium,Hg,on respiratory organs, the effect of lead nitrate in respiratory epithelium of accessory respiratory organ in an air breathing cat fish *H.fossilis* is significantly noticed.

II. MATERIALS AND METHODS

Equal size (length-20 cm and weight about 30 gm) of test fish, *H.fossilis* bought from the fisherman of the local market and kept for two weeks in laboratory condition for acclimatization in aquarium. The fish were fed with tubifex and chopped goat liver during laboratory acclimatization. No mortality were noticed in this periods. The experimental fish were pre treated with 1%Methylene blue for 15 minutes to avoid disinfection. Dechlorinated tap water is used as test water. Three test glass battery jar used as test container in which first container contained no heavy metal and acted as control. Reagent grade Lead nitrate was obtained from scientific supplier, its calculated amount dissolved in 10 litres of water. Before diluting them, few drops of methanol used in it. Two series of lead nitrate with 30 ppm and 60 ppm concentration were prepared for experiment.

A. Exposure Procedure

Five fishes of almost equal body size were exposed to 30 ppm and 60 ppm of lead nitrate for 72 and 96 hours of duration. During the period of exposure, both the control and experimental fish were fed with 3% chopped goat liver of the total body weight of the fish. Replacement of test water performed every alternate day to prevent any chance of adverse effect of the faecal matters of the fish. After experiment , the desired tissues from the suprabranchial chamber and air sacs were collected from both the control and experimental specimen and processed separately for histological studies. The tissues were fixed in Bouins fixative for different histopathological studies. Parffin section of 6 micron were stained using delafields haematoxylin-eosin stain. Some selected slides showed different types of histological effect were photomicrographed as test result.

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B. Observation And Discussion

Behavioural Manifestation:- Behavioural studies on the experimental fish *H.fossilis* exposed in to the various concentration of Lead nitrate, the fish showed restlessness,respiratory distress and rapid swimming than control. Increased opercular movement and gulping activity was evident. During the early stage of exposure,excessive secretion of mucous into medium was observed. In the 60 ppm of lead nitrate exposure,the fish exhibited lethargy and erratic swimming,suggesting loss of equilibrium.

C. Histopathological Observation

Respiratory epithelium of suprabranchial chamber and air sac showed remarkable alteration like mucous cell hyperplasia after 72 and 96 hour exposure at 30 and 60 ppm of test toxicant. At some places respiratory epithelium of suprabranchial chamber was retracted. Blood capillaries dilation (tilangiectasis) was observed in the form of swollen epithelium. Breakage of micro blood vessels erosion and hyperplasia of epithelium cells and fusion in the vascular papilla were also observed after 96 hours exposure at 60 ppm. The lining of the suprabranchial chamber showed vacuolation, the epithelial lining of air sac were corrugated. It result in the massive sloughing of epithelial cells, lesions thereby affected the microanatomy and histopathological changes in suprabranchial chamber and air sacs. According to Eisler(1971),the breathing distress in the fishes is due to clogging of the gills by mucous precipitation besides direct damage of the gill and other respiratory surfaces by heavy metal ion leading to anoxia, carbon dioxide retention and collapse of blood vessels. Lloyd(1960)pointed out that it is the cellular damage of the gills that cause respiratory distress in the fish and not the mucous coagulation. In this regard, it is reasonable to assume that the damage in the respiratory epithelium is main cause for the death of the fishes because of the breakdown of the vital functions of the body due to decreased efficiency of gaseus exchange through gills and accessory respiratory organ. Wedemeyer(1976) also pointed out that the reduced oxygen uptake occurs due to damage of gills and respiratory epithelium in the fish exposed to heavy metal salts.

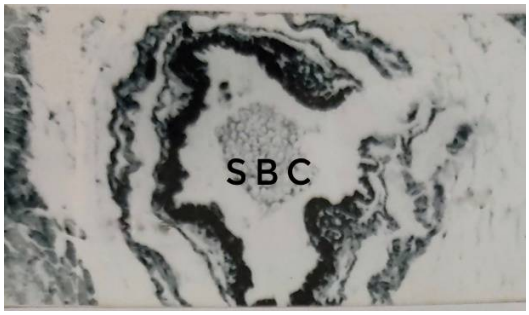


FIG 1

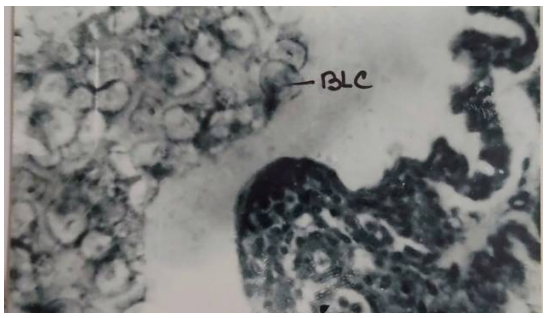


FIG 2



FIG 3

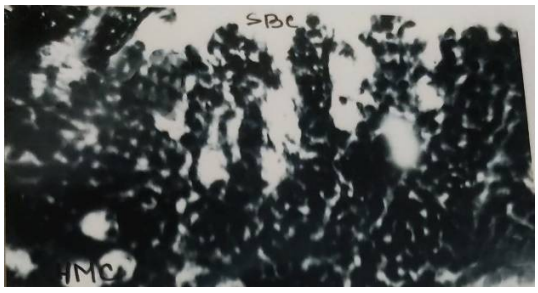


FIG 4

FIG 1 – T.S.of the suprabranchial chamber and posterior part of air sac of H.fossilis shows numerous folds in the epithelial lining of lumen(H/E × 350)

FIG 2 – T.S. of suprabranchial chamber treated with 30 ppm of lead nitrate for 72 hours shows blood capillary dilation (H/E × 350) BLC – BLOOD CAPILLARIES.

FIG 3 – T.S. shows retracted respiratory epithelium of suprabranchial chamber (H/E × 850) R.E.-retracted epithelium. Treated with 60 ppm lead nitrate for 96 hours.

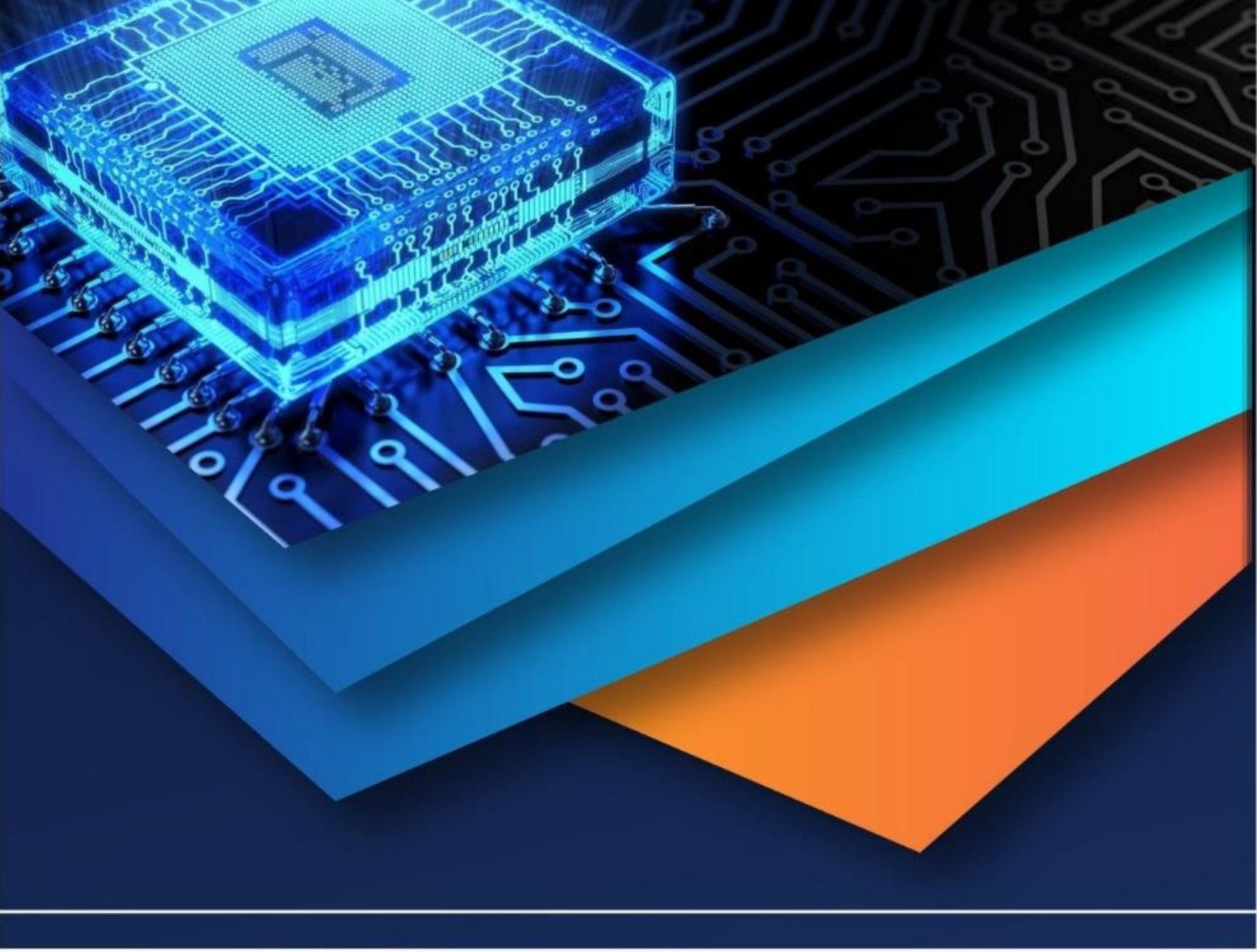
FIG 4 – T.S.of suprabranchial chamber treated with 60 ppm of lead nitrate for 96 hours (H/E × 850) shows hyperplasia of mucous cells. SBC- suprabranchial chamber, HMC- hyperplasia of mucous cells

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