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RESEARCH ARTICLE

TOXICITY OF LEAD EFFECT ON GILLS OF CAT FISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

Heavy metals toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs. Heavy metal pollutants are one of the most common and hazardous water pollutant mainly lead, which has toxic impact on the organs of water bodies viz gills of a common fresh water teleost like sa *Heteropneustus (Saccobranchus) fossilis*. In the order to assess the chronic effects of lead 1/5th of $LC_{50\ for}$ 96 hours as sub lethal concentration for 30 days have been used and the Histopathological alterations examined. Bioassays were performed to determine the LC_{50} values and sublethal concentrations of the lead. The LC_{50} values for *Heteropneustesfossilis* were 15.0 mg/l and 3.0 mg/l. The concentration was selected for chronic studies. The results of this present study indicate that the lead is the main source of pollution in pond water and cause of many diseases in aquatic living organisms. This study will be useful to understand the toxicity and to developing a practical procedure and data estimating to safe concentration to be utilized. This study can help in keeping the environment more suitable, more useful and most hospitable for the life living there.

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INTRODUCTION

Heavy metals pollution has attracted global attention due to the increasing release of industrial effluents and wastes containing heavy metals into fresh water resources and oceans. Reports are available on the occurrence of traces of heavy metals in soil, bottom of streams, lakes, fresh water, rain water and marine water sources. Some of these metals are highly toxic for a wide range of animals including fishes. Others are not acutely toxic but them are resistant to degradation in the environment. Analytical data have shown that heavy metal which are present in aquatic environment in very low concentrations, accumulated by fishes and other aquatic organism up to critical toxic levels. Lead can also affect glucose metabolism as showed by Salmerón-Flores *et al*. (1990), These includes different inorganic and organic compounds of mercury, lead, cadmium, zinc, chromium, copper, arsenic and manganese. Epidemiological and experimental studies have identified mercury, lead and cadmium as primary heavy metal pollutants. Among these heavy metals, lead has a long environmental persistence and never loses its toxic potential, if ingested. Lead is a slow and cumulative poison. It does not usually produce striking symptoms that are easily recognized. Being potential toxicant. Lead is the most ancient poison known to man. Lead acetate was known as "inheritance powder", because of its known use as poison. It was made to ground a sweet-tasting powder, which is almost undetectable in food or drinks when given to make slow death of enemies. Lead poisoning has been described as "aping disease" because of the wide range of symptoms it may produce and the number of other diseases it may imitate.

Lead occurs naturally as a sulfide in galena. It is a soft, bluish-white, silvery gray, malleable metal that melts at 327.5C. It is a highly electropositive metal having a most stable divalent state. It is fairly reactive chemically, dissolves in a number of acids although in metallic form and it is virtually insoluble in pure water but in the presence of air. Lead is attracted by pure water and forms lead hydroxide, which is appreciably soluble. There are many reasons for lead's use other than its abundance and ease in obtaining it. Some of the properties which make it commercially attractive include: easy workability, low melting point, ability to form carbon metal compounds, hold pigments well, very easily recycled, stands up well to the outside weather elements, a high degree of corrosion resistance, and it is inexpensive, etc. Lead compounds are used as a pigment in paints, dyes, and ceramic glazes and in caulk. The major industrial uses such as fuel additives, storage batteries and cable sheathing have contributed greatly to the lead levels in the environment. Drinking water may be appreciably contaminated by the use of lead and poly vinyl chloride (PVC) pipes. Glazed ceramic food wares are another source of lead contamination. Most lead used by industry comes from mined ores ("primary") or from recycled scrap metal or batteries ("secondary"). However, most lead today is "secondary" which obtained from lead-acid batteries. It is reported that 97% of these batteries are recycled. Most of the high levels found throughout the environment come from human activities. Environmental levels of lead have increased more than 1,000-fold over the past three centuries as a result of human activity. The greatest increase occurred between the years 1950 and 2000, and reflected increasing worldwide use of

leaded gasoline. Most of the lead released into the environment which came from vehicle exhaust. In 1979, cars released 94.6 million kilograms of lead into the air in the United States only. Lead can enter into the environment through releases from mining lead and other metals, and from factories that make or use lead, lead alloys, or lead compounds. Lead is released into the air during burning coal, oil, or waste. Once lead gets into the atmosphere, it may travel long distances, if the lead particles are very small. Lead is removed from the air and particles falling to land by rain and ultimately reached to surface water. Fairly good amounts of lead from lead pipe or solder may be released into water when the water is acidic or "soft". Lead may remain stuck to soil particles or sediment in water for many years. Movement of lead from soil will also depend on the type of lead compound and on the physical and chemical characteristics of the soil. Sources of lead in surface water or sediment include deposits of lead-containing dust from the atmosphere, waste water from industries that handle lead (primarily iron and steel industries and lead producers), urban runoff, and mining piles. The levels of contaminated with lead, if animals eat contaminated plants or animals, most of the lead that they eat accumulates in their bodies. It is believed that mankind has used lead for over 6000 years. Lead mining probably predated the Bronze or Iron Ages, with the earliest recorded lead mine in Turkey about 6500 BC. The oldest artifact of smelted lead is a necklace found in the ancient city site in Anatolia. The estimated age of this necklace is 6,000 to 8,000 years ago. A lead statue displayed in the British Museum, discovered in Turkey, dates from 6500 BC (Hunter, 1978). Lead's toxicity was recognized and recorded as early as 2000 BC and the widespread use of lead has been a cause of endemic chronic plumbism in several societies throughout history. Gross morphological and histopathological changes induced by lead exposure in embryos of the African catfish Clarias gariepinus were examined by Osman *et al*. (2007). Pandey *et al*., (2008) studied effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a fresh water fish, Channa punctatus Bloch. Lead induced gill malformation in African catfish Clarias gariepinus was reported by Osman *et al*., (2007). Devi and Banerjee (2007) studied toxicopathological impact of sublethal concentration of lead nitrate on the aerial respiratory organs of murrel, *Channa striata*. Lead induced DNA breakage in embryonic stages of the African catfish Clarias gariepinus were studied by Osman et at., (2008).

The Greek philosopher Nikander of Colophon in 250 BC reported on the colic and anemia resulting from lead poisoning. Hippocrates related gout to the food and wine, though the association between gout and lead poisoning was not recognized during this period (450-380 BC). Later during the Roman period, gout was prevalent among the upper classes of Roman society and is believed to be a result of the enormous lead intake. The Romans conducted lead mining on a massive scale and had several huge lead mine and smelter sites. In ancient Rome, lead poisoning was a disease of the wealthy who used lead extensively: leaden cooking utensils and pots, leaden wine urns, lead plumbing, vessels used to concentrate grape juice, containers used to store wine, and lead-based makeup. In those days there were no substances to act as preservatives for the wines. Lead is naturally sweet in taste and was found to enhance both the color and bouquet of wine. The Romans shipped wines all over their empire, as far away as northern Germany. A preservative was needed to prevent bacteria from turning the wines into vinegar. The Greeks added pine tree resin to their wines but the Romans preferred sweet Sapa, a boiled down concentrate of grape juice. The problem with Sapa was that the kettle used in boiling unfermented grape juice into a concentrate was made of lead, which leaches into the liquid because of the high acidic content of the grape juice. The final product, Sapa, is a Sweet aromatic syrup containing about one gram of lead per liter. Because of its sweet taste, many Romans used it as a sweetening agent in many dishes. When taken together, all the pathways of lead in Roman society, and the intake of lead in Roman times is estimated to have varied from about 35 mg/day to about 250 mg/day, compared to today's daily intake of 0.3mg in the United States in the 1980's (National Academy of Sciences 1980). There are many distinguished historians who now believe that this high exposure to lead was a contributing force in the decline of the Roman Empire. With the more recent scientific research proving that lead is a highly neurotoxicant and analyzing the strange behavior of most Roman leaders and the upper classes, a good case can be made for lead's role in a declining Roman society. What is ironic is the fact the during Roman times lead poisoning was primarily a disease of the affluent while, today it is an affliction of primarily the poor communities. In the German city of Ulm, during the late 1690's, there was a severe outbreak of colic, an illness characterized by a variety of symptoms, including excruciating abdominal pain. Ulm's official physician noted that at a local monastery the monks who did not drink wine were healthy while those that did developed colic. Since the monks lived together, ate the same food, and drank the same wine, they provided the astute doctor with an ideal setting for investigating the cause of the disease. Every time he visited the monks, he was offered a glass of wine until he too developed colic. Upon a detailed investigation he found the culprit to be the agent used to sweeten the wine, litharge, a white oxide of lead. In 1763, a physician at the court of King George III, discovering that lead fittings used to press cider caused an outbreak of colic.

The great gout epidemics of the eighteenth century in England were traced to the popular port wines from Portugal which were heavily leaded. Poorly glazed pottery used to store beers and wines resulted in chronic colic outbreaks in Germany when the lead leached out into the brew. Even today, some wine seals are made of lead and some leaded crystal decanters can leach lead into the liquor. Presence of lead and its salts in soil and different water bodies have been reported by a number of workers (Archer and Barratt, 1976; Bryan, 1976; Benon *et al*, 1978; Alloway, 1990; Corp and Morgan, 1991 and Hendriks *et al*., 1995). Uptake, absorption, accumulation, tissue distribution and excretion of lead in different animal species have been reported by Brudevold and Steadman (1956), Berlin *et al*. (1966), Kelliher *et al*. (1973), Garber and Wei (1974), Malik and Fremlin (1974), Araki and Honma (1976), Rastogi and Clauser (1976), Ragan (1977), Barton *et al*. (1978), Coelman *et al*. (1978), Finley and Dieter (1978), Roels (1978), Khera *et al*. (1980), Watson *et al*. (1980), Aungest *et al*. (1981), Barton and Conract (1981), Steenhout and Pourtois (1981), Custer *et al*. (1984), Mesmar (1987), Patterson *et al*. (1987), Dodd-Smith *et al*. (1992), Kottferova and Korenekova (1998), Braeckman *et al*. (1999), Hernandez *et al*. (1999), Myklebust and Pedersen, (1999), Rauleau *et al*. (1999), Himeno *et al*. (2000). Kazimirova and Ortel (2000), Sakai *et al*. (2000), Wartvinge (2000), Kundiev *et al*. (2001) and Wade *et al*. (2002b). Kehoe *et al*. (1983) studied absorption and excretion of lead in infants and children. Blus *et al*. (1995) reported the accumulation and effects of lead and cadmium in water fowl and passerines of Northern Indaho.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS: For the present study, living specimens of fresh water teleost fishes, *Heteropneustesfossilis* (Bloch) were collected from the unpolluted fresh water resources through the fishermen. All the fishes were allowed to acclimatize to the laboratory conditions for four to five days. Prior to experimentation, they were treated in 1% potassium permanganate solution for 15 minutes to disinfect the fishes. Healthy fishes measuring 14 to 16 cm. in length and 80 to 100 gm. in weight were selected and maintained in laboratory glass aquaria in dechlorinated tap water, having pH 7.4, hardness 160 ppm ($CaCO_3$), alkalinity 87 ppm. The fishes were fed twice daily with commercial fish pellets and small aquatic animals during the tenure of the experiments. The temperature of water was maintained between 18 to 24 ^{0}C and water was renewed on alternate days.

TOXICANTS USED: Lead acetate of analytical grade was selected as the toxicants for this study, and obtained from BDH, England. Stock solutions were prepared by dissolving 2 - gm. lead acetate in 1 - liter double distilled water drunk with wine made from grapes grown near a busy highway, excessed simultaneously.

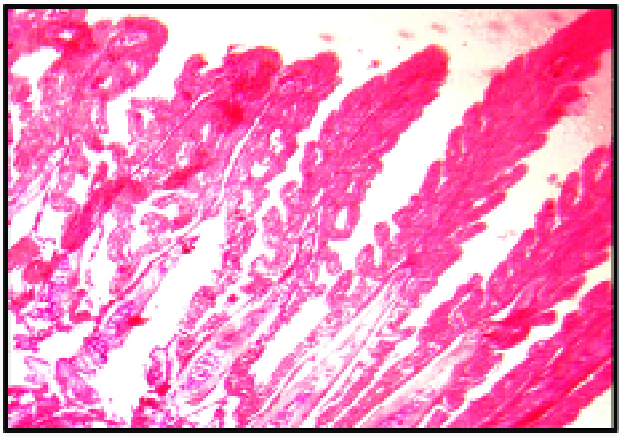

Fig. 1. Transverse section of the gill of Hetoeropneustes fossilis form control group showing normal histology of primary gill lamellae. H/E Stain, X-150

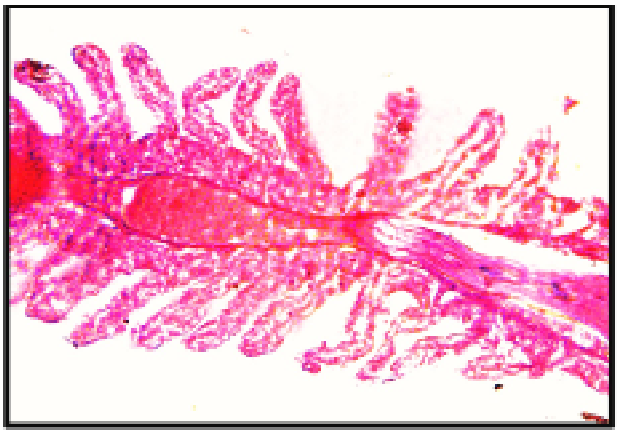

Fig 2. Enlarged view of a primary gill lamella of control fish. Note the normal histoarchitecture of secondary lamellage, gill filament and afferent and efferent edges. H?E Stain, X-400

METHODS OF HISTOPATHOLOGY: Light Microscopic Studies-For light microscopic studies, fishes from the experimental and control groups were dissected and the tissues gill were taken out carefully, washed in 1% saline solution to eliminate mucus and blood deposits. All these tissues after cutting in pieces of required thickness fixed immediately in 10% buffered neutral formalin and alcoholic Bouin's fluid for 12 - hr. The tissues now washed with continuously running water for 12 hr. to remove traces of formalin and the yellowish colour of picric acid. After dehydration in graded alcoholic series tissue were cleared in xylene and for infiltration placed in xylene plus wax and then in pure wax. The blocks of tissues were prepared by embedding in paraffin wax (M.P. 58^0-60^0C). Serial sections of 5-6 µ thickness of all the tissues were cut. For routine staning, Delfieldhaematoxylene and alcoholic eosin were used. The experiments were repeated thrice to confirm the validity of results.

Electron Microscopic Studies: The ultrastructural studies were carried out in Regional Electron Microscope Facility at All India Institute of Medical Sciences, New Delhi. For ultrastructural studies, the fishes of control and experimental groups were dissected ventrally and heart was exposed to perfuse intracardially. The perfusion was initiated with normal saline which is followed by 3% glutaraldehyde (GA) solution in 0.1M phosphate buffer for about 10 minutes to give proper preservation of organs for ultrastructural studies. The experimental tissues gill removed and placed in GA solution for 2 hr. at room temperature. The tissues were washed in 0.1M phosphate buffer, and placed into buffer over night at 4^0C. Final trimming of the tissues to appropriate size was done in the buffer. The trimmed tissues of about 1 mm. thickness were post fixed in 1% OsO_4 (A stock solution of 2% OsO_4 was prepared by dissolving 1 gm in 50 ml. of double distilled water and stored at 4°C in tightly stoppered brown coloured bottle. To a part of 2% OsO_4 solution added an equal part of 0.2 M phosphate buffer to achieve 1% OsO4, prepared fresh before use) for 2 hr. at 4°C.

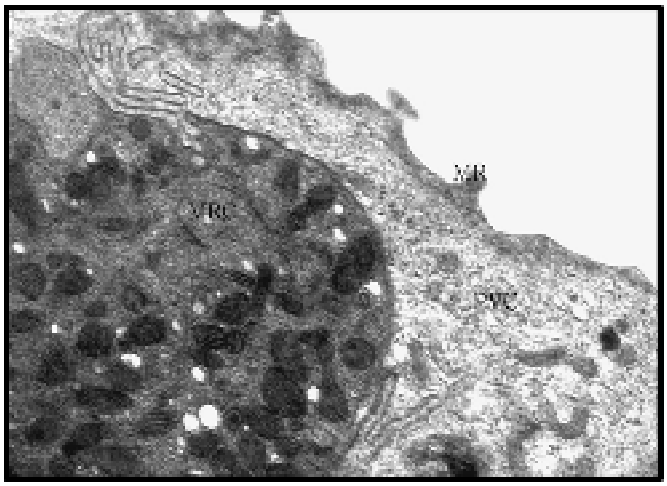

Fig. 3. Electron microphotograph of part of gill filament of fist form control group showing normal structure of PVC with micrordges and mitochondria cell.X-11300

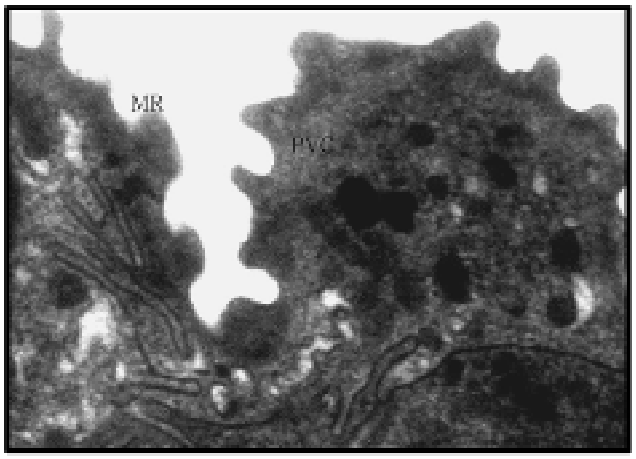

Fig. 4. Portion of a PVC cell of a control fish as seen under electron microscope showing a number of tubular elements, lysosomes and microridges. X-18700

The tissues which contained about 95% water were processed for dehydration in alcoholic series of 30, 50, 70, 80, 90, 95 percent and absolute grades for two changes of 15 minutes in each grade at 4°C. For clearing, the tissues were given two changes of 5 minutes each in xylene at room temperature. Infiltration was carried out at the room temperature with the embedding medium [Araldite (Cy212)-10 ml, DDSA (HY964) - 10 ml, Accelerator (DMP30) -0.4 ml.]. Added the gradients and stir vigorously to thoroughly mixed them) and xylene mixed as follows

- 1 Part embedding medium + 2 Parts of xylene, kept for ovemight.
- 2 Parts embedding medium + 2 Parts ofxylene, kept for 1 hr.
- 3 Parts embedding medium + 1 Part ofxylene, kept for 1 hr. under vacuum.
- Two changes in pure embedding medium each for 1 hr. at 50°C.

Embedding of the tissues was carried out in pure embedding medium in rubber molding trays. The embedded blocks were kept at 50°C in a special oven for 12 to 24 hr. for polymerization. The temperature was then raised to 60°C for 24 to 48 hr. for complete polymerization. The blocks were trimmed and semi thin sections (5 to 2 µm thickness) were cut by the help of Reichert OMU-2 Ultra microtome. The semi thin sections floated in water were lifted with a glass rod and placed on a clean glass slide. The slide was placed on a hot plate at about 80°C and dried.

The semi thin sections were stained with toluidine blue and area was selected under light microscope for TEM studies and accordingly the blocks were further trimmed by the help of Reichert TM 60 trimmer. The finally trimmed each block was fitted in the specimen block holder of the Reichert Jung Ultra cut-E Ultra microtome. The ultra-thin sections 90 to 150 nm (Golden) thickness were obtained by using diamond knife and stretched on the water by exposing them to chloroform, and lifted from below on the surface of specially made metal (copper)matted grids (100 to 300 mesh size).

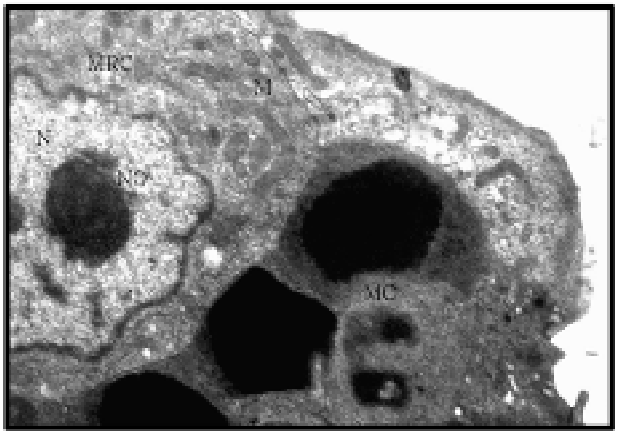

Fig. 5. Electron micrograph of gill filament of control fish showing mucus cell with mucus droplets, MRC and PVC. X-11300

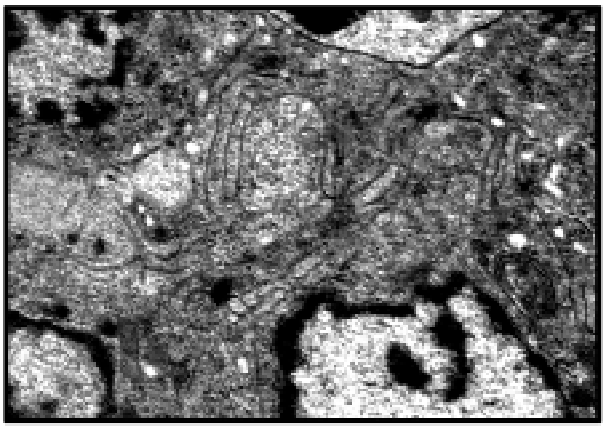

Fig. 6. Reticular elements providing support to adjacent endothelial cells. X-17600

For a good contrast a double staining method, using uranyl acetate and lead citrate was adopted. The sections on grids were observed under the Philips CM-10 Transmission Electron Microscope and photographed accordingly.

OBSERVATION

HISTOLOGY OF GILLSOF *HETEROPNEUSTES FOSSILIS:* The fish gills evolved into the first vertebrate gas exchange organ and is essentially composed of a highly complex vasculature, surrounded by a large surfacearea epithelium that provides a thin barrier between a fish's blood and aquatic environment. The gills of *Heteropneustesfossilis* are located near the head region and arecomposed of five paired gill arches on both lateral sides of the pharynx. Anchored to the gill arches is a complex arrangement of epithelial, circulatory, and neural tissues. Gill filaments are the basic functional unit of gill tissue and long and narrow projections lateral to the gill arch that taper at their distal end. Each filament is supplied with blood from an afferent filamentalartery that extends along the filament. Blood in this vessel also travels across the filament's breadth through numerous folds on the dorsal and ventral surfaces of the filament-termed lamellae, which are perpendicular to the filament's long axis. Blood that crosses the lamellae drains intoan efferent filamental artery that runs along the length of the filament and carries blood in the opposite direction to that in the afferent filamentalartery. The region of the filament that contains the afferent blood supply is commonly referred to as the afferent edge, whereas the region that collects efferent blood is referred to as the efferent edge. These two terms are synonymous with trailing edge and leading edge, respectively, relative to water flow across the filament. Gill filaments contain three distinct vascular systems: (1) the respiratory circulation which receives the entire cardiac output and perfuses the secondary lamellae; (2) a nutrient system that arises from the post lamellar circulation and perfusesfilamental tissues; (3) a network consisting of sub epithelial sinusoids surrounding afferent and efferent margins of the filament and traversing the filament beneath the interlamellar epithelium. Lamellae are evenly distributed along a filament's length, and the spaces between lamellae are channels through which water flows. Each individual lamella reveals that itis essentially composed of two epithelial sheets, held apart by a series of individual cells, termed pillar cells. The spaces around the pillar cells and between the two epithelialla yers are perfused with blood, flowing as a sheet, not through vessels. Lamellae dramatically increase thes urfacearea of the gill filament epithelium and result in a small diffusion distance between the blood that perfuse each lamella and the respiratory water. Moreover, blood flow through the lamellae is countercurrent to water flow between them. The gill epithelium that covers the gill filaments and lamellae providesa distinct boundary between a fish's external environment and extracellular fluids and also play a critical role in the physiological functions of the fish gills. As seen under electron microscope the gill epithelium of *Heteropneustesfossilis* is composed of several distinct cell types.

These are pavement cells (PVCs) and chloride cells (mitochondrion-richcells), which comprise >90% and <10% of the epithelial surface area respectively. Pavement cells(PVCs) cover the vast majority of the gill filament surface area and are assumed to be important for gas exchange because they are thin squamous, or cuboidal cells with an extensive apical(mucosal) surface area. The apical membrane of PVCs is characterized by the presence of microvilli and microplicae (microridges). These apical projections likely in creasethe functional surface area of the epithelium and may also playa role in anchoring mucous to the surface. Typically, PVCs donot contain many mitochondria but are rich in cytoplasmicvesicles and have a distinct Golgi apparatus. The intercellular junctions between PVCs and adjoining cellsare extensive or multistranded which makes the junctions "tight" and presumably relatively impermeable to ions. In contrast to PVCs, chloride cells occupy a much smaller fraction ofthe branchial epithelial surface area, but they are considered to be the primary sites of active physiological processes inthe gills. Whereas PVCs are found in all regions of gill filaments, chloride cells are usually more common on the afferent (trailing) edge of filaments, as well as the regions that run between individual lamellae, termed the interlamellar region. Chloride cellsare usually not found on the epithelium covering the lamellae. Some ultrastructural characteristics of these cells are presence of thee etensivebasolateral membrane infoldings that form a tubular system associated with numerous mitochondria, and the sub apical tubule vesicular system.

CONCLUSION

Lead is a heavy metal and important environmental toxicant and hazardous water pollutants that cause lead poisoning. Lead poisoning is a medical condition caused by increased levels of lead in the body. Lead interferes with a variety of body processes and is toxic to the nervous system, tissues and many organs of the body. In this study, we investigated the toxic impact of lead poisoning on the on the gills of the common freshwater teleost catfish Heteropneustes focusis. Lead 1/5 LC50 at sub-lethal concentrations over 96 hours was examined for histopathological changes over 30 days to assess chronic effects. Bioassays were performed to determine LC50 values and sublethal lead concentrations. The LC50 values for Heteropneustes focusis were 15.0 mg/l and 3 mg/ 1 for chronic studies. Sub lethal concentrations of lead acetate, i.e. 3 mg/l for 30 days, caused significant histological changes in fish gills. Epithelial lifting, lamellar fusion, hyperplasia, lamellar aneurysm, mucosal and

chloride cell hypertrophy, chloride cell injury, apoptosis and necrosis of epithelial cells, lamellar vasodilation, blood reflux into edematous spaces - these are the changes observed in this study. In conclusion, we can say that this study gives detailed information about the toxic effects of lead on the freshwater fish Heteropneustes focusis.

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