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Toxic impact of lethal concentration of lead nitrate on the gills of air-breathing catfish *Heteropneustes fossilis* (Bloch)

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ABSTRACT

Toxicopathological impact of lethal concentration (925 mg/l) of lead nitrate on the gills of air-breathing catfish *Heteropneustes fossilis* was analysed. Following exposure the gills exhibited rapid alterations that include detachment and lifting of the epithelial linings from the surfaces of the gill filament (primary, PL) and respiratory (secondary, SL) lamellae. This led to extensive haemorrhage from the gills. Thus the quantity of blood flowing across the gills decreased substantially. Simultaneously, uncontrolled regeneration of the PL and SL occured, leading to extensive hyperplasia of the epithelial cells lining the PL, and SL. Consequently, the gill filaments appeared as a cylindrical solid mass of cells with very little or almost no free surface left on the SL for gaseous exchange. The goblet mucous cells also exhibited periodic fluctuations in their density and staining behaviour. The chloride cells showed periodic fluctuation in their number at different stages of exposure. The density of the chloride cells is inversely proportional to the thickness of the epithelial lining of the PL and SL. Due to prolonged exposure, the neighbouring SL fused together and the entire gills appeared as solid mass of undifferentiated cells. Subsequently, the ladder-like arrangement of the pillar cells-blood capillaries of the gills also collapsed, causing asphyxiation and the death of the fish.

Key words: gills, *Heteropneustes fossilis*, histopathology, lead nitrate toxicity, India

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Introduction

The gills are not only the prime organs for gaseous exchange; they also perform several other physiological functions including osmoregulation and excretion. Changes in environmental parameters often damage this vital organ because of its delicate structure. Recent review articles (DUTTA et al., 1996; WENDALAAR BONGA, 1997) on ambient toxicants in fish have clearly demonstrated that increased concentrations of several heavy metals seriously damage the gills of teleostean fish. HEMALATHA and BANERJEE (1997a, 1997b) have studied the toxic impact of the trace element zinc ($ZnCl_2$) on the gills and accessory respiratory organs of *Heteropneustes fossilis*. However, almost no data are available on the toxic impact of lead salts on the respiratory organs of air breathing fish (including *Heteropneustes fossilis*). The data concerning lead toxicity are mainly related to studies with mammalian subjects and to air-borne pollutants. Therefore in this study efforts have been made to examine the toxicity of lead nitrate on the branchial respiratory organs of *H. fossilis*. PARASHAR and BANERJEE (1999a) have recently attempted to investigate the toxic impact of this lead salt on the accessory respiratory system of this air-breathing teleost.

Materials and methods

Healthy specimens of *H. fossilis* (38-42 g body weight, 18-20 cm in length) were collected from a single population from a local fish market at Varanasi and were acclimated for one month in tap water in large plastic aquaria in the laboratory. They were fed with minced goat liver on alternate days and the water was renewed after every 24 h, leaving no faecal matter, unconsumed food or dead fish, if any. Feeding ceased 24 h prior to the commencement of the experiment with the starvation regime continuing throughout the period of the experiment.

Prior to the commencement of the experiment, 96h median lethal concentration (96h LC_{50}) of lead nitrate (Glaxo India Ltd., Mumbai; 99% pure) was estimated following the Trimmed Spearman-Karber Method (HAMILTON et al., 1977) and was determined at 925 ppm.

Eight groups of 14 fish each were exposed to 925 mg/l (96h LC_{50} value) of lead nitrate for estimation of lethal toxicity. Each group was exposed separately to 50 l of lead salt solution, prepared in tap water (having dissolved O_2, 6.2 mg/l, pH 7.5, water hardness 23.4 mg/l and water temperature 24 ± 2^{o}C). Eight identical groups of 10 fish each were kept in separate aquaria containing 50 l of plain tap water (without lead salt) as controls.

After each of the exposure periods of 0 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h, and 96 h, five fish each from the respective experimental, as well as control, aquaria were sacrificed. The entire second gill from both the sides of the fish were fixed in 10% neutral formalin, aqueous Bouin's fluid and Helly's fluid. Six μm paraffin sections were stained with Ehrlich's or Harris haematoxylin and eosin (H&E) for routine histopathological analysis. Certain carbohydrate moieties were visualised by periodic acid-Schiff (PAS), alcian blue pH 2.5 (AB 2.5), AB 2.5/PAS, alcian blue pH 1.0 (AB 1.0), Bismarck brown (BB) and salivary amylase/PAS techniques (Table 1) (PEARSE, 1985).

Results

Control. H. fossilis possesses four pairs of typical teleostean gill arches, each bearing two rows of gill filaments (primary gill lamellae, PL). Each PL bears a series of alternately arranged secondary lamellae (SL) (Fig. 1)

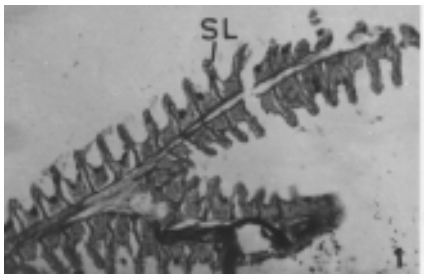

Fig. 1. Structural organization of a part of the second gill of control fish. Note the branching of the gill filament. AB&PAS; ×125.

Fig. 2. Normal distribution of carbohydrate moieties in its different cellular constituents especially in its mucous cells (MCs) of the gill. Note the presence of MCs in the secondary lamellae (SL) also (arrows). AB&PAS; ×520.

on both its sides. The SL at the distal end of the gill filament (PL) mostly remain fused together. The respiratory lining of the SL on each side consists of a thin layer of epithelium which rests on a basement membrane covering the pillar cell - blood channel (PC-BC) system and which constitutes the main vascular component of the gills (Figs. 2, 3). The mucous cells (MCs) are mostly observed in the inter-lamellar epithelium between two SL (Fig. 2) and at the distal tip of the PL. The histochemical properties of the MCs of the gill epithelia are summarized in Table 1.

Table 1. Summary of histochemical alterations shown by the goblet mucous cells of the gills of *H. fossilis* exposed to different periods of exposure to lethal concentration of lead nitrate solution

Staining techniques	Exposure periods							
	Control	3h	6h	12h	24h	48h	72h	96h
PAS	4+C; 4+P	2-3+C; 2-4+P	1-2+C; 2-3+P	3+C; 3-4+P	3+C; 3-4+P	3+C; 3-4P	3-4+C; 3-4+P	3+C; 3-4+P
AB 2.5	1-2+C; 1-2+P	4+C; 4+P	3-4+C; 3-4+P	3-4+C; 3-4+P	3+C; 4+P	3+C; 3+P	3-4+C; 3-4+P	3-4+C; 3-4+P
AB 2.5/PAS**	3+C; 4+P	3-4+C; 4+P	3-4+C; 4+P	3-4+C; 4+P	3+C; 4+P	4+C; 4+P	4+C; 4+P	3-4+C; 4+P
BB	4+C; 4+P	4+C; 4+P	4+C; 4+P	3-4+C; 3-4+P	3-4+C; 3-4+P	2-3+C; 3+P	2-3+C; 3+P	2+C; 2-3+P

Symbols and abbreviations: ** = variously stained reactions from various shades of magenta, violet to greenish blue, in the same or different mucocytes; C = cell content; P = cell periphery; AB 2.5 = alcian blue at pH 2.5 for acidic mucopolysaccharides; BB = Bismarck brown reaction for water-stable mucoproteins; h = hour; PAS = per iodic acid/Schiff reaction for neutral glycoproteins; AB 2.5/ PAS = alcian blue pH 2.5/periodic acid Schiff for acidic and neutral glycoproteins; - = to; 0 = negative reaction; 1+ = weak reaction; 2+ = moderate reaction; 3+ = strong reaction; 4+ = very strong reaction.

Experimental gill. Following exposure the gills showed extensive damage in their lamellar configuration, even though the gills continue to regenerate repeatedly after every wear and tear, especially during the initial stages. The ionocytes (chloride cells) showed fluctuation in their density at different stages of exposure. Generally, the density of these ionocytes decreases, invariably in the thickened epithelia.

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results

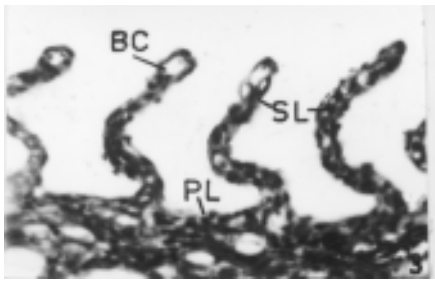

Fig. 3. Magnified view of the gill of control fish showing its structural organization. H&E; ×480.

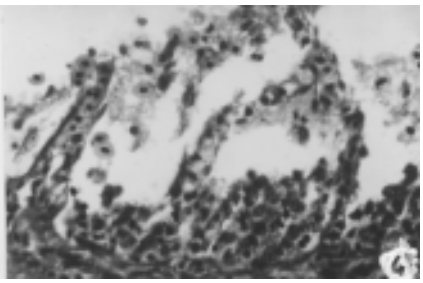

Fig. 4. Part of the second gill showing large scale wear and tear in the SL, causing haemorrhage after 3h of exposure. H&E; ×480.

The blood vessels (BVs) running through the PL show congestion material and appeared bright red due to engorgement of the red blood corpuscles (RBCs).

Within a short span of three hours of exposure, large-scale wear and tear in the SL took place (Fig. 4). This induced severe haemorrhaging. To compensate for the loss, blood rushes into the vascular elements of the SL and PL. The intact BVs thus appear completely engorged with RBCs. The MCs that are usually present in the PL of the gills of the control fish, exhibited periodic fluctuation in their density and staining behaviour (Table

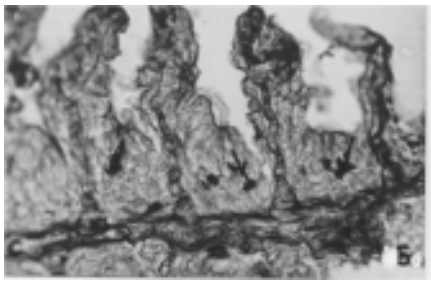

Fig. 5. Regeneration of MCs (arrows) in the deeper layers of the epithelial lining of primary lamellae (PL) that also show hyperplasia after 3^{h}of exposure. AB&PAS; ×520.

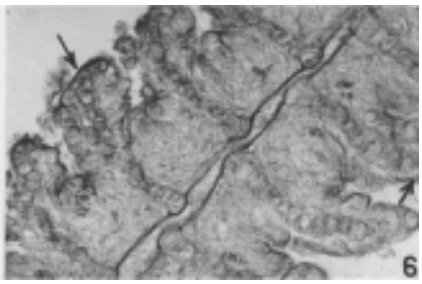

Fig. 6. Thin layer of slime (arrow) covering the surface of the SL after 6h of exposure. AB&PAS; ×520.

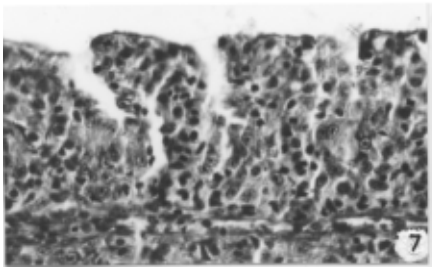

Fig. 7. Extensive hyperplasia due to extensive multiplication of the epithelial cells (ECs) lining the SL and PL after 6h of exposure. Note the greatly disturbed pillar cells (PC)-blood capillary (BC) system. H&E; ×450.

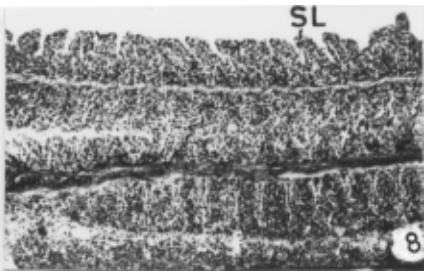

Fig. 8. Further intensification of hyperplasia of the ECs of the PL and SL after 12h of exposure. H&E; ×125.

1) at different stages of exposure (Figs. 5, 6). However, a thin layer of AB 2.5 positive slimy coating continued to cover the surface of the SL at several stages of exposure (Fig. 6). Even though their number decreased marginally after 3 h, MCs were very often found in the inner, as well as in the outer layers of the hyperplastic epithelial lining of the PL, indicating regeneration of new MCs. Simultaneously, regeneration and healing of the damaged gills also takes place at this stage. Uncontrolled regeneration of epithelial cells (ECs) causes hyperplasia of PL, resulting in their increased thickness

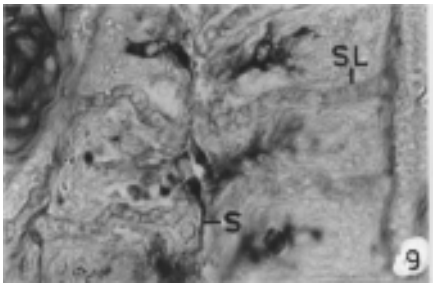

Fig. 9. Magnified view of the stage illustrated in Fig. 8 showing fusion of the epithelial linings of the PL. Note the MCs of the PL pouring their contents into the space between the neighbouring gill filaments. AB&PAS; ×520.

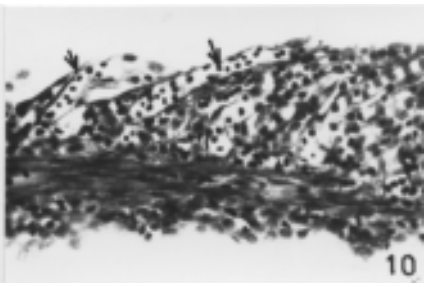

Fig. 10. Coalescence of neighbouring BCs due to collapse of PCs after 24h of exposure. The merged BCs appear as small lakes (arrows) filled with blood material. H&E; ×480.

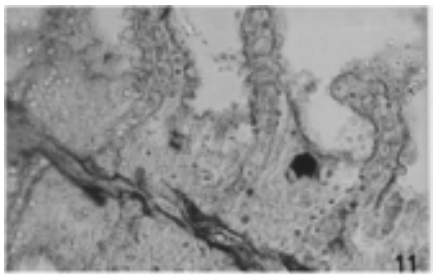

Fig. 11. Distribution of carbohydrate in the epithelial linings of the PL and SL after 24h of exposure. The minute PAS positive granules represent glycogen. AB&PAS; ×520.

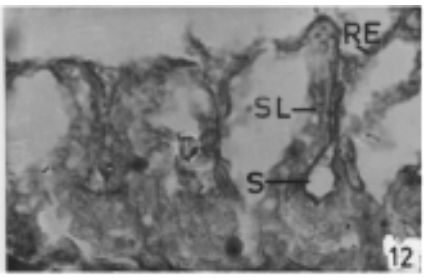

Fig. 12. A layer of slime (S) laid over the surface of the RE after 48h of exposure. H&E; ×480.

within 6 h (Fig. 7). From certain damaged SL, however, haemorrhaging continues. The escaped blood materials frequently become trapped between neighbouring SL of the same and/or adjacent gill filaments. All this resulted in marked histopathological alterations in the gills that often lost the typical ladder-like arrangement of their vascular components (PC-BC) (Figs. 7, 8). These PCs and BCs become haphazardly arranged. At this stage the BCs could mostly be recognised by the presence of the RBCs present within them. At many other sites the BCs appeared as empty spaces. The entire epithelial lining appeared as greatly vacuolated. After 12 h the hyperplasia became more extensive and the space between the neighbouring SL became filled with ECs, which gave the gill filaments a compact (mass of cell-like) appearance (Fig. 8). Neighbouring PLs also often fuse together, leaving almost no space between them (Figs. 8, 9). The voided secretions of the MCs very often become mixed with this blood material.

Subsequently, the ladder-like arrangement of the PC-BC further collapses and after 24 h the number of PCs greatly decreased due to their degeneration. As a result of coalescence the BCs acquired a lake-like appearance (Fig. 10). At many other places, the PCs shrank. Simultaneously, regeneration with uncontrolled hyperplasia of the cells of the respiratory epithelium makes the SLs thickly multi-layered, causing increased barrier distance for exchange of gases. Consequently, the SLs appear as solid

mass. At this stage however, the thickness of the epithelial lining of the PLs decreased. A large number of fine salivary amylase labile – PAS-positive granules increased in the epithelial cells of the PL (Fig. 11). Fusion of the neighbouring SLs was also not so frequently observed (Fig. 11). From this stage onwards a substantial decrease in the number of MCs takes place and after 48h MCs were rarely seen in the inter-secondary lamellar (PL) epithelium. The respiratory epithelium of the SL frequently became lifted from the PC-BC system after 48h. (Fig. 12). The ladder-like PC-BC system of the SL once again started to become dismantled. Large-scale wear and tear again occurs, causing haemorrhage from the secondary lamellae. However, vacuolisation continued to persist between the polygonal cells, and wear and tear often resulted in a considerable decrease in the thickness of the PLs. These polygonal epithelial cells, however, appeared to be metabolically active with well-dilated prominent nuclei. Hyperplasia became more prominent after 72 h with a great increase in the density of the polygonal cells. The PC-BC system was partially re-established at this stage, even though haemorrhage continued from many other sites. A large quantity of coarse glycogen granules were prominently observed in the polygonal cells of the thickened epithelial lining of the PLs, especially in the cells constituting the PC-BC system. No marked change was observed in the density of the MCs at this stage of exposure.

Damage becomes very extensive after 96 h and the PC-BC systems of the SL became greatly collapsed. The highly disturbed neighbouring lamellae fuse between themselves, leaving almost no surface area free for gaseous exchanges. The density of MCs increased at the distal ends of the PLs at this stage of exposure. The outer peripheral lining of the SL and Pl gave a moderate AB 2.5 positive reaction for acidic glycoproteins. However, the glycogen granules disappeared completely.

The MCs exhibited periodic alterations in their density and staining behaviour at different stages of exposure.

Discussion

Gills have an extensive surface area and minimal diffusion distance between dissolved O_2 and blood capillary for efficient gaseous exchange.

This organ system remains protected by a thin layer of mucous coating (HUGHES et. al., 1979; POWELL et al., 1992). Electron microscopic investigations have shown that the surface of the gill epithelia is provided with numerous micro-ridges which anchor the mucus film (HUGHES and WRIGHT, 1970). The number and pattern of the microridges are disturbed following treatment of the fish with various stress conditions, including exposure to heavy metals, and may diminish the capacity of gas exchange by reducing both the lamellar area and micro-turbulence (KARLSSON-NORRGREN et al., 1986a; 1986b).

The immediate morpho-pathological response of the gills of fish exposed to ambient xenobiotics (including metal salts) is often manifested by a significant increase in the density of its MCs (BAKER, 1969; CARDEILHAC et al., 1979; MATEY, 1984; WISE et al., 1987; DUTTA, 1997; HEMALATHA and BANERJEE, 1997a; 1997b). The large quantity of mucous secretion acts as a defence mechanism against several toxic substances (SELLERS et al., 1975; McDONALD, 1983; HANDY and EDDY, 1991; MAZON et al., 1999). The regular sloughing of mucus from the surface of gills into the media helps to remove the bound pathogens, toxicants and foreign matters (POWELL et. al., 1992) (including metal-compounds, LOCK and VAN OVERBEEKE, 1981) which adhere to the gills. Lead is also known to bind with COOH, PO_4, SH and amidazole groups and causes the precipitation of proteins due to the insolubility of lead-protein complex (LUCKEY and VENUGOPAL, 1977). Thus, the periodic sloughing of mucus might be one of the important means for elimination of toxic lead salt from the surface of the gills. WITTERS et al. (1990) noticed that complexion with organic material (e.g. humic acid, PEURANEN et al., 1994) lowers (but not completely prevents) the toxicity of the metals on the gill surface. Thus, the quantity of the xenobiotics available immediately around the gill surface for absorption becomes continually reduced, at least in the initial stages of exposure. PÄRT and LOCK (1983) inferred that the mucous layer creates a microenvironment that may act as ion trap for concentrating trace elements in the water. PLAYLE and WOODS (1989) suggested that because the pH at the gill microenvironment differs considerably from that of the surrounding media, precipitation of the metals on the gills possibly takes place. While substantiating the protective function of fish mucus against hexavalent chromium contamination, ARILLO and MELODIA (1990) suggested that some components of mucus, probably the protein-bound sulphydryl

groups, have a detoxifying function against ambient toxins. These SH groups of mucus seem to bind with the toxicants, playing a fundamental role in their reduction mechanism, especially for occasional and short-term exposure. The mucous coat covering the gill epithelia is composed mainly of glycoproteins that have electro-negative charges (as shown by AB 2.5 and BB positive reactions) at neutral pH. It is perhaps also due to the well established ability of these glycoproteins to trap heavy metal ions (McKONE et al., 1971; COOMBS et al., 1972; VARANASI and MARKEY, 1977; LOCK and VAN OVERBEEKE, 1981). The present investigation has shown that the same or different MCs in the PL of the gills at the same or different stages of lethal (present investigation) as well as sub-lethal concentrations (BANERJEE and PARASHAR, unpublished) of lead salt exposure show varying intensities of PAS and/or AB 2.5 positive reactions indicating synthesis, storage and secretion of mucus containing mostly acidic, or a mixture of neutral and acidic, glycoproteins (Table 1). A shift in the nature of the mucus secreted by the MCs towards acidity and/or weak sulphation as revealed by increased AB 2.5 reaction has been observed following lead salt exposure (Table 1). According to DAOUST et al. (1984), exposure to heavy metals very often alters the chemical composition or thickness of mucous layer that may disturb the normal ability to recognise different cell types. They concluded that this is due to contact stress and may also be due to transformation of electrically charged properties of the epithelial cells which favour adhesion between the cells of two neighbouring SL, which has been a very common manifestation of the toxic impact of a large number of xenobiotics, including lead salt. This leads to extensive fusion of SL, causing a drastic reduction in the respiratory surface area. Several other xenobiotics are also known to induce fusion of the SL of gills (LEINO et al., 1987; DUTTA et al., 1996; WENDELAAR BONGA, 1997). During the present study also, the slimy coatings over the gills showed compositional alterations (Table 1) and sloughed off several times, which might have led to the fusion of the SL (Figs. 7, 8).

According to MALLATT (1985) induced alterations in gill histology are mostly non-specific in nature, which partially represent the damage, and partially the compensatory response of the fish. Examples of the first (representing damage) are necrosis of the epithelial cells of the SL, epithelial lifting, dilatation of the blood sinuses of the SL, and lamellar aneurysm. The

main compensatory responses are hypertrophy and hyperplasia of the respiratory epithelial and chloride cells, hyperplasia of the MCs (including decrease due to exhaustion, followed by an increase in their density) and infiltration of the dilated intercellular spaces by leukocytes. DUTTA et al. (1996), categorised the structural alteration in the gill morphology into two groups: (1) direct deleterious effect of the xenobiotics causing necrosis and rupture of the branchial epithelium. Such type of effect is mostly dose dependent and very often reported under lethal conditions (MALLATT, 1985). They also suggested that death of branchial cells and their rupture usually develops either by autolysis or by rapid lyses caused by the direct action of toxicants on the cells' constituents (ABEL, 1976), and (2) branchial defence response achieved by mucus hyper secretion, chloride cell proliferation, epithelial lifting, swelling, hyperplasia and lamellar fusion. Other changes emphasised by them include vascular stasis, leukocyte infiltration, and lymphatic space dilation. However, very little is known about the toxic impact of lead salts on the functional morphology of the gills. The present investigation indicates that due to continuation of the toxic impact of lead salt, the protective role of the thin layer of slime collapses and fails to prevent the penetration of lead salt, subjecting the cellular constituents lining the extensive surface area of the gills to the toxicity of the heavy metal. This leads to various degrees of wear and tear, which causes damage to the delicate protective device of the gill epithelia of *H. fossilis* (Figs. 4, 5). The epithelial layer covering the PC-BC systems of the SL is finally ruptured, causing direct exposure of the blood in the BCs to the surrounding media, resulting in loss of blood and other body fluids. Subsequently, the typical ladder-like structures of the PC-BC infrastructure of the SL of the gills are also lost. Very often, after 24h the damaged PCs degenerate and become lost, causing merging of the neighbouring BCs, which appear like lakes. According to PEURANEN et al. (1994) any discontinuity of epithelial lining of the gill due to massive wear and tear may lead to a negative ion balance and to changes in the haematocrit and mean cellular haemoglobin values of the blood. The lead salt in *H. fossilis* also causes an overall decreased quantity of blood material (including those in the gills and air-breathing organs), leading to anaemia. Other important damage to the gill epithelia following exposure to various xenobiotics includes detachment of the

respiratory epithelium from the basement membrane, which causes the formation of non-tissue spaces and increased width of the SL (KARLSSON-NORRGREN et al., 1985; HEMALATHA and BANERJEE, 1997a; 1997b). These non- tissue spaces in the gills were filled with a fluid consisting of myelin bodies and cellular debris. The non-tissue spaces, along with hypertrophied epithelial lining, results in inadequate gas exchange and consequently a reduced diffusion capacity, although they also create an additional barrier for the prevention of penetration of the water-borne xenobiotics. Identical lifting of the respiratory epithelia of the SL of the gills and/or ARO has also been observed in *H. fossilis* subjected to desiccation stress (PARASHAR and BANERJEE, 1999b; 1999c) and lead nitrate exposure (PARASHAR and BANERJEE, 1999a).

Due to continuation of lethal lead nitrate exposure, uncontrolled regeneration of the gills takes place and the PCs become haphazardly arranged. Consequently, the space between the neighbouring SL becomes almost entirely filled with polygonal epithelium and the gill filaments appear as a solid mass of cells. The tips of the neighbouring PL also fuse, leaving no space between them. Uncontrolled hyperplasia of the polygonal cells of the respiratory epithelium makes the SL multi-layered thick, the free surface of the SL is entirely lost and the SLs appear as solid mass. This decreases the respiratory efficiency of the fish by increasing the blood-oxygen barrier distance. However, this also delays the penetration of the xenobiotics by increasing the diffusion distance of the xenobiotics via the gill epithelium. Between the polygonal cells extra-cellular vacuolisation is regularly seen and these polygonal cells appear to be metabolically active, while lifting of the respiratory epithelium from the gill surface is not generally seen following exposure to a lethal concentration.

During the present investigation, the number of the chloride cells in the epithelial linings of both the PL and SL of *H. fossilis* increased significantly following exposure to lead nitrate solution. RAJBANSHI and GUPTA (1988) also observed an increased number of chloride cells in *H. fossilis* following exposure to water-borne copper. An increased number of chloride cells in the SL following exposure to heavy metal salts has also been observed by SHEPARD and SIMKISS (1978), LAUREN and McDONALD (1987a; 1987b), KARLSSON-NORRGREN et al. (1985). The increased number of chloride cells

in the gills of fishes following exposure to a variety of toxicants have been summarised by DUTTA et al. (1996). Following $ZnCl_2$ exposure, the number of such cells present in the respiratory epithelia of the SL of the gills also increases at several stages of exposure (HEMALATHA and BANERJEE, 1997a). An increase in the number of chloride cells following exposure to several metal salts (SARDET et al., 1979; ORONSAYE and BRAFIELD, 1984; KARLSSON-NORRGREN et al., 1986a; 1986b) and other xenobiotics (LEINO and McCORMICK, 1984; LEINO et al., 1987; FISCHER-SCHERL and HOFFMANN, 1988) and bacterial gill diseases (KUDO and KIMURA, 1983; DUTTA et al., 1996) has commonly been observed. An increase in the concentration of any positive charge-bearing element in the environment (either H+, metallic ions or flocculants, etc.) causes multiplication of chloride cells.

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SAŽETAK

Analiziran je toksopatološki učinak letalne koncentracije (925 mg/l) olovnog nitrata na škrge indijskog smeđeg soma *Heteropneustes fossilis* (Bloch). Ubrzo nakon izloženosti olovnom nitratu na škrgama je utvrđeno odvajanje i podizanje epitelnog sloja od površine škržnog filamenta (primarno, PL) i respiratornih lamela (sekundarno, SL). To je dovelo do opsežnog krvarenja na škrgama pa se bitno smanjila količina krvi što protječe kroz njih. Istodobno je došlo do nekontrolirane regeneracija PL i SL što je dovelo do snažne hiperplazije epitelnih stanica koje okružuju PL i SL. Škržni filamenti su izgledali kao cilindrične nakupine stanica s vrlo malo ili gotovo bez slobodne površine preostale za izmjenu plinova na SL. Promjene su bile utvrđene i na vrčastim stanicama s obzirom na njihovu gustoću i svojstva bojenja. U različitim fazama izloženosti utvrđene su i povremene promjene u broju kloridnih stanice. Gustoća kloridnih stanica bila je obrnuto proporcionalna debljini epitelnog sloja PL i SL. Nakon produžene izloženosti spojile su se susjedne SL pa su škrge izgledale poput čvrste mase nediferenciranih stanica. Kao posljedica toga kolabirale su stupnjevito poredane stanice krvnih kapilara škrga što je dovelo do uginuća ribe.

Ključne riječi: škrge, *Heteropneustes fossilis,* histopatologija, olovni nitrat, toksičnost, Indija