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Mercury-Induced Morphological Changes in the Respiratory Surface of an Asian Freshwater Catfish, *Saccobranchus fossilis*

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Mercury (Hg), the so called a black listed element by environmentalists, is released into the environment by several sources, such as mining and fossil fuel combustion, thermal power projects, by the use of fungicides, bactericides and pharmaceuticals. Mercury has been identified as the most hazardous metal in aquatic environment following the human tragedy of Minimata and later Niigata, in Japan (Ui 1972). In recent years, water pollution due to mercurial compounds has become serious in many parts of the world because of their toxicity, immutable and nonbiodegradable properties, tendency to undergoes biotransformation and bioconcentration, during their transfer through food chain in the trophic levels.

Several studies have shown that the fish gill is morphologically and physiologically affected by a variety of mercury compounds. Prasad (1994) described the surface morphological changes such as thin coat of mucus, contraction of epithelium, formation of interlamellar bridge and diminishing number of microridges in the gills after 24 hr exposure of catfish *Saccobranchus fossilis* to $HgCl_2$ at 0.1 and 0.3 mg/L. Acute poisoning by inorganic mercury is characterized by the morphological changes in gill epithelium during the initial period of exposure. These changes include accumulation of cellular debris in the lamellar epithelium, enlarged epithelial cells and lamellar fusion in freshwater fishes *Puntius sophore* (Khangarot and Somani 1980) and in rainbow trout *Salmo gairdneri* (Wobeser, 1975). The toxic effects of mercury in the gills have been investigated during the short- and long-term exposures by means of the scanning electron microscopic techniques. Pereira (1988) described the surface morphological changes in a windowpane flounder (*Scophthalmus aqueous*) gills after long-term exposure of low Hg concentrations. The gross pathological changes of the gill epithelium induced by Hg are often associated with osmoregulatory, acid-base regulation and respiratory malfunctions (Renfro et al. 1974; Evans 1987).

This paper describes the surface morphological effects of sublethal Hg exposure for 7 d at 50 and 100 μg/L on the gills of a freshwater air-breathing catfish, *S. fossilis* (Bloch), as observed by scanning electron microscope (SEM). The test fish is commonly available in Southeast Asia and used as human diet. The gills were chosen because these are likely to be impaired structurally as they offer a

large surface area that comes in direct contact with the dissolved pollutants in aquatic medium.

MATERIALS AND METHODS

Air-breathing catfish, *S. fossilis* were collected from local source and acclimatized to laboratory conditions for at least 15 d prior to Hg exposure. Fish ranged in size from 14 to 16 cm in total length and from 25 to 30 g in wet weight. At the end of the holding period, they were (5 fish per concentration) were introduced in 60 L glass aquaria and remained there for seven days and were not fed during the Hg exposure period. However, fish were fed during the acclimatization period on a goat liver, zooplankton and dried fish food (Shalimar fish Food Co., Mumbai, India). Fish were fed *ad libitum* once a day for five days a week. Stock solution of Hg was prepared by dissolving the reagent grade mercuric chloride ($HgCl_2$) in deionized water. Exposure concentrations of 50 and 100 μg/L of Hg were prepared from stock solution. Control experiments were run under similar conditions without Hg addition. Test water was changed after every 24 hr of exposure. Background levels of Hg in the tubewell water were below detectable limits (1 μg/L). Mercury concentrations were measured everyday using a UV mercury analyzer. Detail test procedures as mentioned in standard methods for static bioassays (APHA et al. 1998) were followed.

Fish were stunned by a blow to the cranium and gill tissues were removed, rinsed briefly with cold physiological saline to remove adhering blood and mucus and fixed in ice-cold, cacodylate-buffered (0.01 M, pH 7.2) 2.5% glutraldehyde, 1% paraformaldehyde fixative for 4 hr at $4^{O}C$ and post fixed in 3% osmium tetra oxide in the cacodylate buffer for 1 hr at $4^{O}C$. Gill pieces were rinsed, dehydrated in an acetone series and then transferred into an amyl acetone series. Gill samples were dried by the critical point dried method and coated with gold-palladium in sputter coater (Polaron), and examined by Philips 515 scanning electron microscope at 15kV.

RESULTS AND DISCUSSION

No fish from the control and treated groups died during the 7 d exposure to mercury. The gills of *S. fossilis* are fairly well developed and consists of folds, viz. gill filament (primary gill lamellae), gill lamellae (secondary gill lamellae), (Figure 1). The epithelium covering of the primary lamellae shows a more complex microridges pattern ((Figure 2). The epithelial cells of the efferent artery side of the primary lamellae have clearly well-defined cell boundaries and from a pentagonal or hexagonal patterns ((Figure 3). Pavement cells showing characteristic, concentric pattern of microridges cover the primary lamellae. The plasma membrane is folded into a mazelike pattern. Epithelial pores, especially in the region between the secondary lamellae, interrupt the sheet of the epithelial cells at some places. These pores are considered as the openings to the mucous cells. On afferent artery side, there is high frequency of pores due to presence of chloride and mucous cells. The epithelial surfaces which cover the secondary

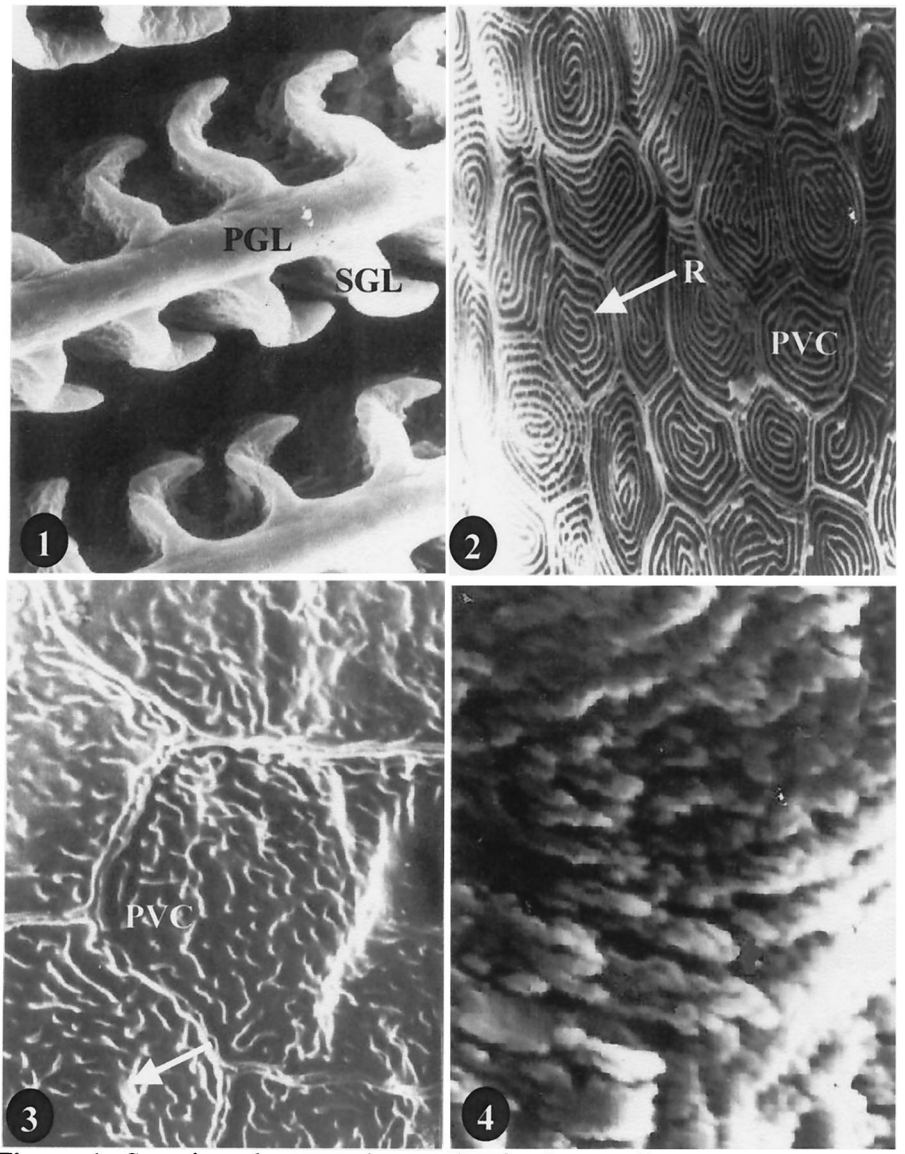

Figure 1. Scanning electron micrograph of primary gill lamella (PGL) and numerous secondary gill lamellae (SGL). The secondary gill lamellae covered by epithelial cells. x 440. **Figure 2.** SEM photograph of surface architecture of primary gill lamellae. The surface ultrastructure consists of long microridges (R) separated by intervening grooves arranged in a maze-like pattern. Also note the uniform pattern of hexagonal squamous pavement cells (PVC) ornamented by complex network of microridges. x 3540. **Figure 3.** SEM of surface of secondary gill lamellae consists intermittent ridges (Arrow). Note the cluster of long-microvilli. PVC, pavement cells. x 2540. **Figure 4.** Higher magnified view of secondary lamellar epithelial surface reveals microvilli separated by grooves of variable dimension and pores (P). x 6200.

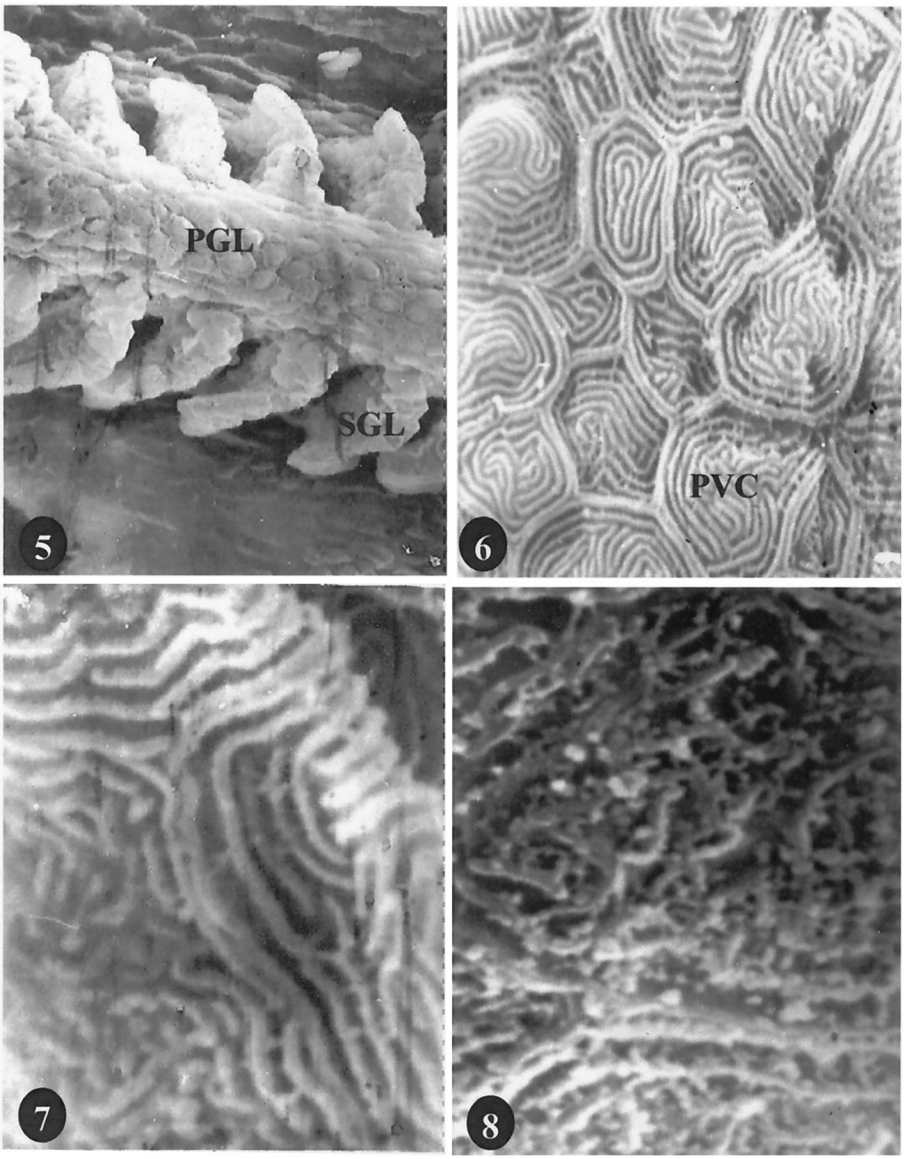

Figure 5. A low magnification of SEM of primary gill lamellae (PGL) and few branching secondary gill lamellae (SGL) of fish exposed to 50 μg/L of Hg for 7 d. Secondary lamellae show the hyperplasia, fusion and focal swelling in PGL at many places. x 440. **Figure 6.** Ultrastructural surface view of primary gill lamellae showing less regular arrangement of microridges. The hexagonal pattern of epithelial pavement cell (PVC) microridges also altered due to 50 μg/L of Hg exposure for 7 d. x 3540. **Figure 7.** The magnified view of surface of secondary gill lamellae surface of fish exposed to 50 μg/L of Hg. x 3540. **Figure 8.** SEM of secondary gill lamellae surface view showing fragmented microvilli following exposure to 50 μg/L of Hg. x 3540.

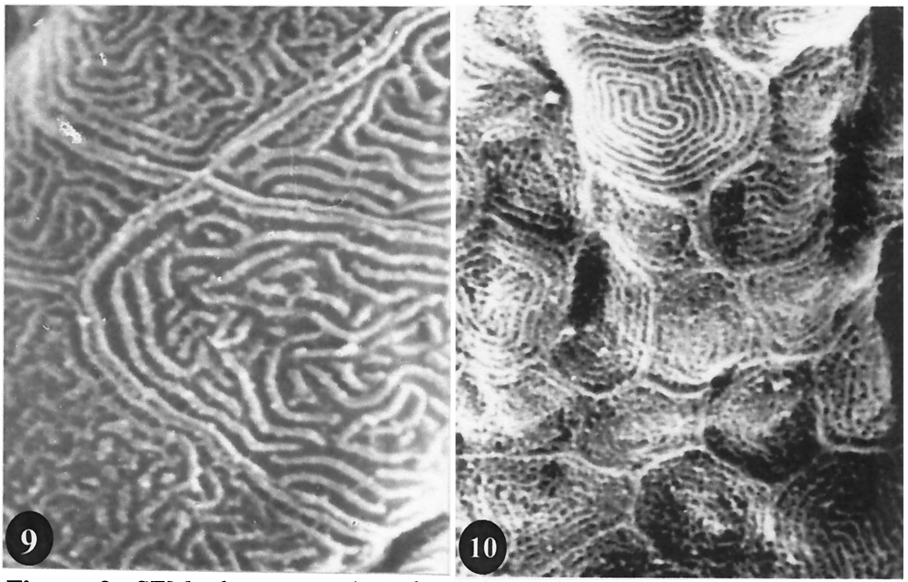

Figure 9. SEM ultrastructural surface view of primary gill lamellae (PGL) showing more degeneration and fragmentation of microridges at 100 μg/L of Hg exposed for 7 d. x 3540. **Figure 10.** SEM of surface of secondary gill lamellae showing complete degenerated and fragmented microvilli following exposure to 100 μg/L of Hg for 7 d. x 3540.

lamellae is made of flat thin cells, with a surface consisting of microridges and cells are pentagonal in shape. The microridges are shorter in height and length than the primary lamellar epithelial cells. Several microridges of the secondary epithelial cells are projected into microvilli-like structure ((Figure 4). The microridges are connected to each other by thinner microridges. A high magnification of the secondary lamellae reveals the presence of ridges separated by intervening grooves and has variable size of pores ((Figure 5). This pseudocentric arrangement of microridges of the pavement cells provides a more extensive lumnel respiratory surface area for gaseous exchanges. The detail surface gill morphology of *S. fossilis* under normal condition has been described by other investigators (Hughes and Munshi 1978).

Gill from fish exposed to 50 μg/L for 7 d showed severe surface morphological changes in the primary and secondary lamellae as compared to controls. The primary lamellar pavement cells microridges became non-uniform in height and in arrangement as compared to the control gills (Figure 6). At many places the microridges in the pavement cells of the primary lamellae lost their shape and size, and cellular debris and mucus hypersecretion were noticed (Figure 7). The pathomorphological alterations in the surface of the secondary lamellae were reflected by loss in the general pattern of pavement cells and microvilli arrangement. Degenerative changes in microridges pattern were common (Figure 8).

Gills from *S. fossilis* exposed to 100 μg/L of Hg for 7 d morphologically different from the low-level Hg exposure and the controls. The structural changes of the primary lamellae from the Hg exposed fish were especially interesting under the scanning electron microscope. The cell boundaries were distinguishable but arrangement of microridges were irregular in the pavement cells (Figure 9).The most striking change was that of the primary lamellar microridges which became more non-uniform and also showed pronounced changes in the length and width. The microridges are shorter or fragmented giving the appearance of microvilli. Epithelial cells of the secondary lamellae appeared swollen and long, continuous microridges boundary between the epithelial cells absent in the 100 μg/L of Hg exposure ((Figure 10). The secondary lamellar microvilli were not clear and lost their identity due to presence of cellular debris.

The qualitative stereo scan morphological alterations observed in the present study clearly show the direct effect of toxic Hg ions on the gills of *S. fossilis* following 7 d of exposure at 50 and 100 μg/L. The surface morphological changes increased number of focal swelling in epithelial cells and irregular arrangement of microridges and microvilli patterns of primary and secondary lamellae. These SEM observations of this study are in good agreement with the results reported by Pereira (1988). He described surface epithelium morphological changes in the gills of windowpane flounder exposed for 60 days to 5 or 10 μg/L of Hg. These degenerative changes and cell swelling increased as mercury concentrations increases from 50 μg/L to 100 μg/L of Hg. Olson et al. (1973) described morphological changes such as vaccuolation and degeneration of gill epithelial cells after 6-8 weeks exposure of rainbow trout to either $HgCl_2$ or CH_3HgCl_2 at low concentrations. The microridges fragmentation and swelling in gill epithelial cells have also been reported with other pollutants, for example, when fishes were exposed to copper (Khangarot and Tripathi 1991; Cerqueira and Fernandes 2002). The pathomorphological changes in microridges may affect gill function through reduction in respiratory surface area, osmoregulation and adherence of mucus to the gills (Heath 1995). By observing such an interesting change in microridge patterns of the Hg-treated gill lamellae, the conclusion drawn in view of the functional interpretation is that the fragmented microridges of the gill epithelium reduced the mechanical adhesion of the water molecules to the respiratory surfaces and ultimately decrease the active diffusion of the respiratory gases from water to the blood and vice-versa, through epithelium, basement membrane and flanges of pillar cells. This may resulting gradual decline of oxygen-carrying capacity and hence cause anoxia and ultimately respiratory failure.

The histological study on a Indian freshwater fish *Puntius sophore* showed major pathological changes at 0.25 mg/L of Hg after short-term exposure (Khangarot and Somani 1980). These changes are the separation of gill epithelium from the basement membrane and pillar cell system, swelling of epithelium cells, fusion of secondary lamellae, degeneration of epithelium layer of gill lamellae and hyperplasia of epithelial cells. Interestingly, similar observations were reported for rainbow trout following exposure to Hg as mercuric chloride (Wobeser 1975). The separation of intact epithelial layers from the underlying pillar cells is the

most common reaction to the sublethal pollutant exposure and the hyperplasia of epithelial cells is the second (Mallat 1985). Some of these light microscopical findings were confirmed by the present scanning electron microscopical study on *S. fossilis.*

One of the common responses of the *S. fossilis* gills to Hg was the mucus hypersecretion. Thin layer was observed on the fish gills at the time of tissue fixation for SEM study. The mucus coatings over the respiratory epithelium protect *S. fossilis* by checking the further entrance of toxic Hg ions from water medium. The increased mucus secretion is helpful in attenuating the osmotic influence of the environmental stress (Evans 1987). Moreover, the mucus, deposit being an effective complexing agent, but affords only limited protection to the fish under heavy metal poisoning (Varanasi et al. 1975). Toxic metal ions such as Hg are generally entered into the fish body via gills, but in presence of excess mucus; their diffusion is reduced by 50% (Part and Lock 1983). Fish gills are considered the primary target organs of all pollutants due to their extensive surface area in contact with external medium. Furthermore, the gills are the main site for gas exchange and other important functions such as ionic and osmotic regulation and acid-base equilibrium, surface morphological changes in gill structure involve respiratory disturbances and electrolyte imbalance. Fish exposed to metals showed a reduction in the lamellar surface area and oxygen diffusion capacity (Heath 1995; Mazon et al. 1999)

In *S. fossilis*, the extensive cellular disarrangement and irregular pattern of microridges, necrotic changes in the gill lamellae, hyperplasia and the excessive secretion of mucus may cause impairment of respiration and osmoregulatory functions of gills. For example, exposure of killifish (*Fundulus heteroclitus*) to 125 μg/L of $HgCl_2$ for 24 hr completely blocked the sodium uptake from the solution (Renfro et al. 1974). Pereira (1988) also reported the marked fragmentation of pavement cell microridges pattern and focal swelling of respiratory epithelial cells at 10 μg/L of Hg after 60 d of exposure in windowpane flounder.

It appears that acute mercury exposure produced toxic effects on the fish gill, both morphological and physiological. Morphological effects involve fragmentation of microridges and microvilli in epithelial cells of gill filament and gill lamellae hyperplasia, swelling, necrosis, degeneration and fusion of gill filament and gill lamellae. The physiological effects involve a reduction in blood ionic levels and inhibition of Na, K^- and Cl^{-1} ions (Mallat 1985). It is suggested that Hg has direct effect on the fish gills and via this route it enters in the blood vascular system later on have detrimental effects on other sensitive organs such as kidney, central nervous, cardiac, muscular and immune systems.

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