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EFFECT OF HEAVY METAL NICKEL ON ULTRASTRCUTURAL CHANGES IN THE GILL OF THE ESTUARINE FISH *LIZA PARSIA*

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ABSTRACT

Effect of 10 % sublethal concentration (12.4 ppm; 96 h LC50) of nickel on the gill ultra structure of Liza parsia after the exposure period of 30 days was studied. The SEM studies revealed many morphological changes in the gill of Liza parsia such as epithelial hypertrophy, fusion of secondary lamellae, necrosis and degenerating microridges with mucous opening due to chronic exposure of the nickel.

INTRODUCTION

These xenobiotic molecules have been found in natural systems and they have a great impact on the environmental quality as they result in a toxicity risk to non-target organisms especially fishes. They can enter the food chain when they become accumulated in aquatic organisms (Pant & Singh, 1983; Heger *et al*., 1995 and Madhab Prasad *et al*., 2002). In fish, gills are critical organs for their respiratory and osmo regulatory functions. Respiratory distress is one of the early symptoms of pesticide poisoning (Mc Donald, 1983). Fish gills comprise a large part of fish body that contacts the external environment and they play an important role in the gas and ion exchange between the organism and environment. They are also an important way of uptake of toxic compounds into the organism (Witeska *et al.,* 2006). Thus, the gills are the very first site where pesticides induced lesions may occur which may result in an impaired gas and ion exchange. Subsequently, heavy metals enter the blood in which they may affect the blood cells.

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Current interest in the field of pesticides and heavy metals detoxification lies on observations under scanning electron microscope, since such observations would lead to a better understanding of the morphological changes, induced in the gills at ultra structural levels, as well as the functions of various cells in the gills. Kimura and Kudo (1979) and Kendall and Dale (1979) have made extensive studies on ultra structure of the normal gills of *Salmo gairdneri*. Very few workers have observed the morphological changes in the gills following exposure to pollutants. Crespo (1982) and Temmink *et al.* (1983) studied in detail the morphological changes in gills of *Salmo canicula* and *Salmo gairdneri* induced by zinc and chromate. Muthukumaravel *et al* (2008) studied ultra structure of gills of *Oreochromis mossambicus* affected by copper sulphate. In order to have an overall pathological picture, the study of the extensive gill surface needs special attention. In the present study mode of action of heavy metal, nickel in surface architecture of the gill of *Liza parsia* has been investigated using scanning electron microscopy.

MATERIALS AND METHODS

The fish, *Liza parsia* fingerlings (Wt: 10 ± 0.5 g; Length 7 ± 1cm) were collected from the Muthupet estuary, Tamil Nadu.

They were acclimatized for 15 days in large cement tanks (Temperature: 24 ± 2 °C, pH: 8.9 ± 0.3 and Dissolved oxygen: 6.9 ± 0.7 mg/l.) previously washed with 1% potassium permanganate. The water was renewed every 24 h. Stock solution of nickel was prepared and the toxicity tests were conducted following method of Finney (1964). Based on the acute toxicity studies (96 h), LC_{50} value for test fish was found to be 12.4 ppm. For histological studies *L. parsia* were reared in sublethal concentrations (10% of 96 hours LC_{50}) for a period of 10, 20 and 30 days. The gill arches were dissected out, washed repeatedly in 0.2M phosphate buffer and then fixed in 3% gluteraldehyde. The dehydration was done in acetone grades and was followed by critical point drying. Ultimately dried gills were mounted on the stub and were sputter coated with gold in a gold coating unit (thickness 100A^{o}) and were examined and photographed using JEOL JSM 6360 scanning electron microscope (SEM) Japan.

RESULTS AND DISCUSSION

SEM study of control gills: In the gills of *Liza parsia* under control the primary gill lamellae exhibited normal architecture and mucous free and uniform branching of secondary lamellae from primary lamellae (Fig.1). The gill filaments bear micro ridges on the surface epithelium (Fig.2).

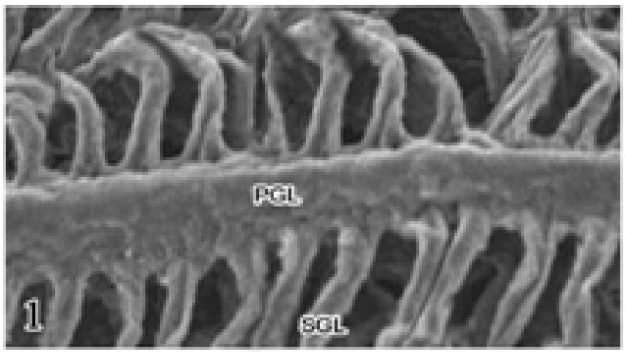

Fig.1. Scanning electron micrographs of the gill of Liza parsia (control). Normal architecture of gill. Primary gill lamella (PGL), secondary gill lamellae (SGL) (X800- scale bar 50 µm)

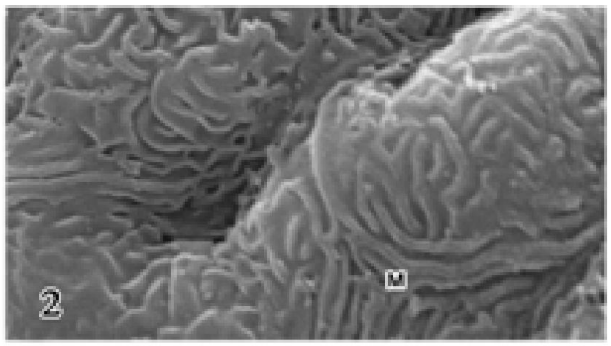

Fig.2. Microridges (M) on the normal gill epithelium. (X350 scale bar 10 µm)

Histological alterations of gill in nickel under SEM observation: The damages, fusion of secondary lamellae and edema of primary lamellae, were observed after 10 days of exposure (Fig.3). On exposure to nickel for 20 days, epithelial hypertrophy, fusion of secondary lamellae and necrosis were observed (Fig.4). In fish, treated up to 30 days, the changes observed in the gill of *Liza parsia* were deformation and edema of primary and secondary lamellae, fusion adjacent lamellae (Fig.5) and degenerating microridges with mucous opening (Fig.6). The gills, participate many important functions in the fish, such as respiration, osmoregulation and

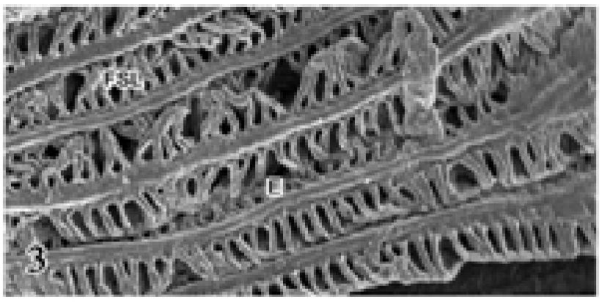

Fig. 3. Fusion of secondary lamellae (FSL), edema(E) of the gill of 10 days nickel treated fish (X75-scale bar 50 µm)

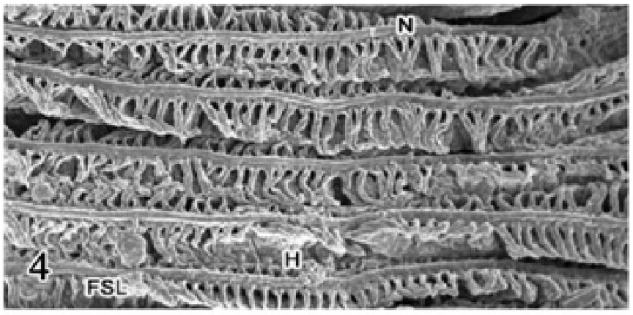

Fig. 4. Hypertrophy (H), fusion of secondary lamellae (FSL) and necrosis(N) of the gill of 20 days nickel treated fish (X75 - scale bar50 µm)

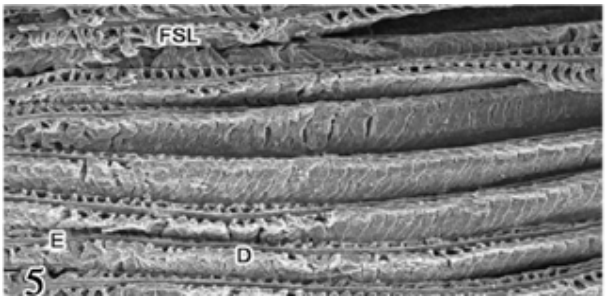

Fig. 5. Deformation (D), edema (E) and fusion of secondary lamellae (FSL) of the gill of 30 days nickel treated fish (X75-scale bar 50 µm)

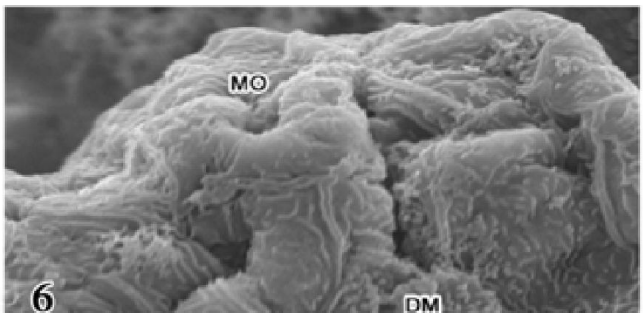

Fig. 6. Degeneration microridges (DM) and mucous opening (MO) of the gill of 30 days nickel treated fish. (X350-scale bar 10 µm)

excretion, remain in close contact with the external environment and particularly sensitive to quality of the water and are considered as the primary target of contaminants (Fernandes and Mazon, 2003). The gills of fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae and congestion in blood vessels of gill filaments due to exposure of nickel. The pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the pollutants through the gill surface. The observed alterations like proliferation of the epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defense mechanisms, since in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Malt, 1985 and Fernandes & Mazon, 2003). The cellular damage observed in the gills in terms of epithelial proliferation, separation of the epithelial layer from supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation (Dutta *et al*., 1996).

The SEM is a technique that allows the study of the damage of surface ultrastructure of the gill epithelium that can not be revealed by light or TEM (Devos *et al.,* 1998; Dutta *et al.,* 1998). The scanning electron micrographs of the gill epithelium also revealed that fish of untreated group showed normal architecture. In contrast, the present study showed that the gills of *Liza parsia* exposed to nickel during thirty days presented a higher occurrence of histopathological lesions such as hypertrophy, fusion of secondary lamellae, edema and mucus openings. These pathological changes may be a reaction to toxicants intake or an adaptive response to present the entry of the pollutants through the gill surface (Mohamed 2009). The damages observed in the gills in terms of hypertrophy, fusion of secondary lamellae and necrosis could cause a decrease in free gas exchange, thus affecting the general health of fish (Skidmore & Tovell, 1972). Similar of these changes in gill epithelia of *Oreochromis niloticus* were ultrastructurally observed by Nath and Kumar (1989). Crespo (1982) in the dog fish, *Scyliorhinus canicula* subjected to zinc sulphate; Temmink *et al.* (1983) in rainbow trout, *Salmo gairdneri* exposed to chromate; Gupta and Dua (2002) in the *Channa punctatus* intoxicated with mercury. Pane *et al* (2004) in *Oncorhynchus mykiss* treated with nickel. Acharya *et al.* (2005) in *Labeo rohita* treated with sublethal acidic (HCl) and alkaline (NaoH) pH. In the study of Muthukumaravel *et al*(2008), copper exposure resulted in marked ultrastructural damage to the respiratory epithelium of gill in *Oreochromis mossambicus* including swelling and fusion of secondary lamellae. Palaniappan *et al.* (2008) observed hypertrophy, hyperplasia, alteration of lamellar surface and fused lamellae in pb exposed *Catla catla*. In the present study, it can be stated that nickel exposure during sublethal treatment produces severe toxic effects on the respiratory organ of the freshwater fish *Liza parsia*. The finding of the present study indicate that ultrastructural changes observer serve as "biomarkers" for assessing herbicide toxicity in aquatic environment.

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