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Histopathological Changes in the Gill of Freshwater Fish, *Rasbora daniconius* Exposed to Paper Mill Effluent

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Abstract: The fishes, *Rasbora daniconius* were exposed to lethal concentration at 9.5% (LC_{50} of 96 hrs) for 96 hrs and sublethal concentrations [1.9% (1/5) and 0.95% (1/10) LC_{50} of 96hrs] of paper mill effluent for 30 days to study the impacts on the histology of gill. Many pathological changes were observed in the gill lamellae such as bulging at the tip of primary lamellae, epithelial hypertrophy, Fusion, curling, reduction in secondary gill lamellae, disorganization and rupture in secondary lamellae, swelling in pillar, mucous, and chloride cells. Hemorrhage at the primary and secondary lamellae.

Key words: Paper mill effluent · Gill · Histopathology · *Rasbora daniconius*

INTRODUCTION

The ever increasing industrialization, urbanization and development consequent pollution of water has been brought a measurable water crisis. Today most of the rivers in world receive millions of liters of sewage, domestic waste, industrial effluent and agricultural waste containing substances varying in their characteristic from nutrients to highly toxic substances.

The industrial effluent contributes a lot to water pollution as well as aquatic animals. Polluted state of the water resources has led to steady decline in aquatic flora and fauna particularly fishes and has affected the irrigated land.

In Maharashtra first paper mill was started as "Deccan Paper Mill" at Pune in 1951. Now there is tremendous expansion in these industries during last twenty five years. Controversially, the paper industry as it stands now as one of the largest major industries and contributes a lot towards the water pollution.

Due to high chemical diversity of the organic pollutants in paper and pulp mill effluent, a high variety of toxic effects on aquatic communities in recipient water courses have been observed [1, 2]. A significant number of these substances have been classified as carcinogenic, mutagenic and clastogenic [3]; and endocrinic [4]. The pollutants concerned also kill fish or affect their reproductive physiology [5], or may induce male-biased sex ratios among fish embryos [6].

Very few studies have been carried out in connection with the histopathological effects of pulp and paper mill effluent in fish. Fujiya [7] described extensive histological damage in fish, *Sparus macrocephalus* exposed to water contaminated by a Japanese kraft mill. Lesions were found in the liver, kidney, spleen and intestine. Monteith *et al.*, [8] demonstrated deterioration of the fine ridged structure on the secondary lamellae of the gills in fish exposed to dehydrobietic acid, an important toxic component of paper mill effluent. Khan *et al.*, [9] reported the occurrence of fin necrosis, kidney tumors, anemia change in parastiofauna, low condition factors and organ somatic indices of winter flounder living in the vicinity of a pulp and paper mill. Discharge of untreated pulp and paper effluent in to receiving waters is known to be toxic to some aquatic organisms. Manifestations of toxicity in fish includes fin necrosis, increase of parasites, changes in physiology, detoxifying enzyme activities, hematology, osmoregulation a nd reproduction [10 to 13]. Lesions have also observed the gill and liver of effluent exposed fish [14].

In the fish it is observed that the external organs are affected due to the toxic chemicals causing loss of equilibrium, increase in opercular movement, irregular movement and finally lead to death. This may be attributed to the significant damage to the internal organs. Industrial effluent pollute aquatic ecosystem and find their way in the body of aquatic animals by means of gills, digestive tract and general body surface. Effluent

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accumulates in the different tissues of body. Therefore, it is necessary to study the detail histopathological alternations or changes in structure produced by industrial effluent in different organs of fishes thoroughly, investigate them in order to assess the extent of damage.

Histopathological studies have been conducted to establish fundamental relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments [15].

Therefore, the main objective of study is to assess the histopathological alterations in gill of fish, *Rasbora daniconius* exposed to lethal and sublethal concentrations of the paper mill effluent.

MATERIALS AND METHODS

Experimental Fish: The *Rasbora daniconius were* obtained from Godavari river at Kaigaon Toka (latitude 19° 37.463 and longitude 75° 01.409) 45 km away from Aurangabad (MS). The fishes were kept in glass aquaria, acclimatized for the period of four weeks. During period of acclimatization the fishes were fed after every 24 hours on pieces of live earthworms. Healthy fishes showed active movement were only considered for the experimentation.

Paper Mill Effluent: The Paper mill effluent was collected directly from the Kaigaon paper mill at releasing site 45 Km away from Aurangabad. The percentage concentration of test solution is obtained by using formula [16], which is as follow.

$$Volume\ percent = \frac{volume\ of\ effuent}{V_E + V_{DW}} \times 100$$

V_E = Vol. of Effluent, V_{DW} = Vol. of Dilution water.

Determination of lethal (LC_{50}) and sublethal concentrations: The LC_{50} value for 96 hrs was determined by renewal bioassay following probit analysis [17], due to its advantage over other bioassay techniques. This method has advantage of replacing the toxicant solution a fresh every 24 h so that metabolic waste (ammonia) which itself highly toxic can be removed. The LC_{50} values for the 96 hr period of paper mill effluent was found to be 9.5% concentration and 1.9% and 0.95% concentration (1/5 and 1/10 of the LC_{50} values for 96 hrs) was selected as the sublethal concentrations for chronic studies.

Histological Biomarkers: *Rasbora daniconius* (length 8 to 8.5 cm and weight 4 to 4.5 gm) were exposed to lethal (96 hrs LC_{50}) concentration of paper mill effluent at 9.5% concentration for 96s hr and sublethal concentrations of paper mill effluent (1.9% and 0.95%) for 30 days. At the end of exposure period the fishes survived were sacrificed dissect carefully to isolate gill and fixed in bounis fluid. After 24 hrs they were processed following the standards technique. Tissues were embedded in paraffin wax and serial section of 4-6 μm thickness were cut, deparaffinised and stained in haematoxylin and counterstained with eosin. The sections were examined under light microscopy, using Takashima and Hibiya [18] as a reference, and photographed using a digital camera.

RESULTS

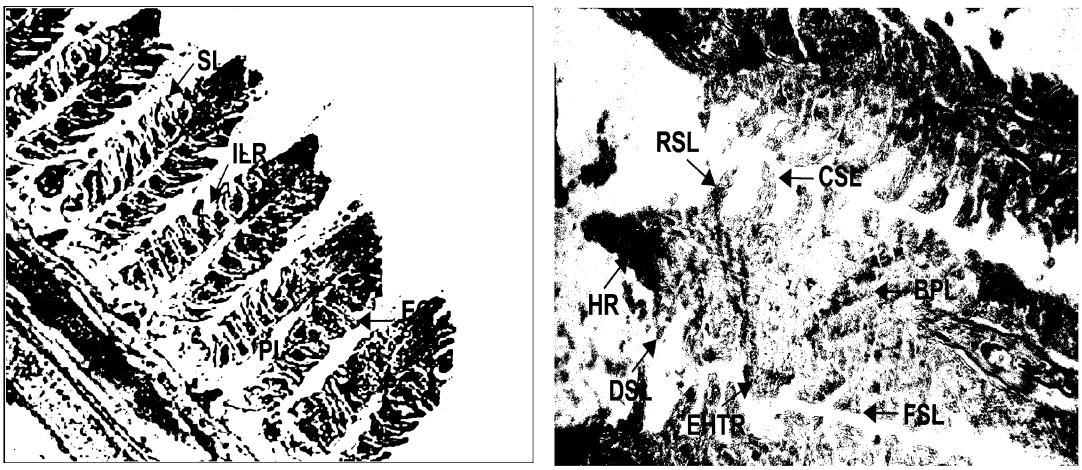
The results of acute toxicity test for *Rasbora daniconius* exposed to different concentrations of the paper mill effluent showed that the LC_{50} for 96 hr was 9.5% concentration which means that the this effluent was highly toxic to the fish.

The toxicity of this effluent is synergistically to physical factors of medium i.e. high COD and BOD besides low pH and dissolved oxygen (DO).

The histological structure of the normal gill characterized by the presence of primary lamellae along with secondary lamellae, shaft and rackers confirming the general architecture of the tissue (Fig. A).

The fish exposed to lethal concentration for 96 hrs at 9.5% (LC_{50}) of paper mill effluent showed marked degenerative changes in architecture of gill, fusion of secondary gill lamellae, curling of secondary lamellae, disorganization, ruptures in the secondary lamellae, reduced secondary lamellae and hypertrophy of epithelial cells were observed. The cells of gill lamellae i.e. pillar, mucous and chloride cells showed cloudy swelling. Hemorrhage at primary lamellae and bulging at the tip of primary filament were noticed (Fig. B).

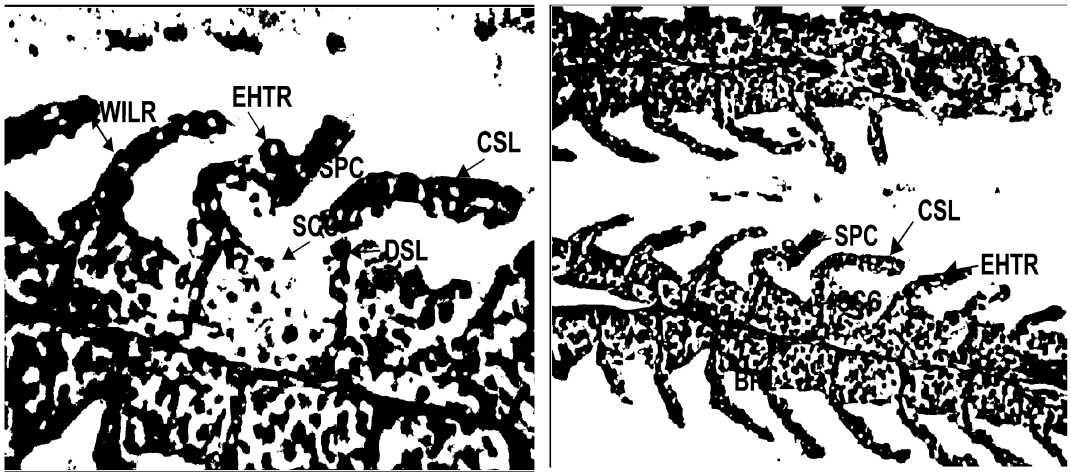
The fish exposed to sublethal concentration (1.9%) of paper mill effluent for 30 days showed marked pathological changes. In the gills, the most common symptom of toxic exposure were curling of secondary lamellae, disorganization, rupture in the secondary lamellae, hypertrophy of epithelial cells and widening of inter lamellar distance. The cells of gill lamellae i.e. chloride, pillar and mucous cells showed cloudy swelling and their nuclei appeared swollen and pyknotic (Fig. C).



(A) (B)

Figure A (100x): Longitudinal section of the gill of control fish, *Rasbora daniconius* showing primary gill lamellae (PL), secondary gill lamellae (SL), pillar cell (PC), epithelial cell (EC), inter lamellar distance, adipose tissue (AT).

Figure B (400x): Figure B. (400x).After exposure lethal concentration at 9.5% (LC_{50} of 96 hrs) of paper mill effluent gill showing fusion of secondary gill lamellae (FSL), curling of secondary lamellae (CSL), epithelial hypertrophy (EHTR), bulging tip of primary gill lamellae, hemorrhage (HR), degeneration of secondary lamellae (DSL) and reduced secondary gill lamellae (RSL).



(C) (D)

Figure C. (400x): After exposure to sublethal concentration at 1.9% (1/5) of paper mill effluent for 30 days, gill showing curling of secondary lamellae (CSL), epithelial hypertrophy (EHTR), swelling of pillar cell (SPC) and swelling of chloride cell (SCC), widening of inter lamellar distance (WILR) and degeneration of secondary lamellae.

Figure D. (400x): After exposure to sublethal concentration at 0.95% (1/10) of paper mill effluent for 30 days, gill showing hemorrhage (HR), curling of secondary lamellae (SCL), epithelial hypertrophy (EHTR), bulging tip of primary lamellae (BPL), swelling of pillar cell (SPC) and swelling of chloride cell (SCC).

Similarly on 0.95% concentration of paper mill effluent treatment for 30 days, exhibited marked pathological changes. The most common symptom of toxic e xposure were fusion of the adjacent secondary lamellae, hemorrhage at the primary lamellae, curling of the secondary lamellae and hypertrophy of epithelial cells. The cells of gill lamellae i.e. pillar, chloride cells showed cloudy swelling and bulging at the tip of primary filament were noticed (Fig. D).

DISCUSSION

The impact of untreated effluent discharged by pulp and paper mills hinge not only on the toxic components but also on other variables such as oxygen demand, pH, colour and suspended solids, which singly or collectively might produced a variety of biological effects [19].

Gill was the primary target organ of the effluent. Death of the fish correlated with extensive fusion of the gill lamellae and was probably due to impaired gas exchanges. Fusion of gill lamellae is common symptom of gill damage, generally related to rapid poison [20]. Several toxicants particularly resin acids, untreated fatty acid and chlorinated phenolic have been identified in kraft pulp effluent and may be responsible for the lesion the gill lesions [21].

Couillard *et al.* [22] observed hyperplasia in the gill of rainbow trout following acute and sublethal exposure to untreated bleached kraft mill effluent. Fusion of the lamellae is a common symptom of the gill damage, generally related to rapid poisoning [23, 24]. Several chemicals, particularly resin acids, untreated fatty acids and chlorinated phenolic have been identified in kraft pulp mill effluent may responsible for gill lesions [25].

Haniffa and Sundaravadhanam [26] observed partial destruction of gill epithelium, pillar cells, acidophil mast cells, blood cells, blood capillaries, cartilage cells, separation of epithelial layer of secondary lamellae from basement membrane, mucous cells destroyed and gill filaments were completely covered by thick mucous layer in distillery effluent treated *Barbus stigma.* Adeymo [27] observed severe necrosis, hypertrophy and vacuolation of hepatocytes in cassava mill effluent treated fish, *Clarias batrachus.* Wade *et al.* [28] reported that following 96 hr- toxicity assay of Cassava (Manihot esculenta Crantz) effluent on the Nile tilapia, histopathological examination of kidney, gill and liver of the treated fish indicated damages, ranging from odema and telangiectasis of the gill lamellae and gill hyperplasia to vacuolation of the liver cells and necrosis.

Dhanapalkiam *et al.* [29] studied the gill lesions in the major carp, *Labeo rohita* exposed to lethal and sublethal concentrations of tannery effluent and reported severe damages to gill architecture such as fusion and clumping in the middle and distal parts of the primary lamellae, swelling and deterioration of the cells. Swelling of primary and secondary epithelial cells was evident in sublethal concentration. Susithra *et al.* [30] noticed bulging of the hyperemic secondary lamellae in the lumen of the accessory respiratory organ, necrosis and swelling of the respiratory epithelium leading to hemorrhages and fusion of secondary lamellae of cadmium chloride exposed fish, *Heteropneustes fossilis.* Peebua *et al.* [31] studied the histopathological alteration in the gill of *Oreochromis niloticus* exposed to alachlor. Gill alteration includes edema of the epithelial cell system, aneurism with some ruptures, hypertrophy and hyperplasia of epithelial cells.

The objective of the histological assessment of the gill is to verify the possible damage caused to the organism by paper mill effluent, evidencing alterations resulted from the acute and chronic toxicity. The gills have a large superficial area through which gaseous exchanges between the blood and the external medium take place [32]. Beside the respiratory function, this organ performs other vital functions such as osmoregulation and excretion [33].

The present study reveals extensive damage to the gill architecture of treated fish compared with gill of control fish. The changes like bulging tip of primary lamellae, epithelial hypertrophy. Fusion, curling and reduction of secondary gill lamellae, disorganization and rupture in secondary lamellae, swelling in pillar, mucous, and chloride cells and their nuclei appear swollen and pyknotic. Hemorrhage at primary and secondary lamellae in the paper mill effluent treated fish. The pathological changes in the gills might have resulted due to shifting from aerobic to anaerobic pathway in tissue respiration of fish under stress. Histological evidences in the present study are correlated to some extent with the work [34, 38].

The results obtained during this study, it can be concluded that the gill histology of *Rasbora daniconius* appears to be sensitive monitoring tool to aquatic health. Fish histopathology could therefore make valuable contribution in the monitoring of aquatic ecosystems and should form an important part of environmental impact assessment in the environmental management process.

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