

Impact of Chemical Factory Effluent on the Structural Changes in Gills of Estuarine Fish, *Mugil cephalus*

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Abstract: The present paper study deals with the toxicity of chemical factory effluent on the gills of fish, *Mugil cephalus*. When these fish was exposed to 5, 10, 15, 20 and 25% effluent concentrations, severe gill alteration was observed. But the alteration was less in 5% concentration compared to 10, 15, 20 and 25%. In fish exposed to 5% concentration, hypertrophy, swelling, proliferation of chloride cells were seen. The fish was exposed to 10 and 15%, concentration hyperplasia, fusion of secondary lamellae and disintegration of epithelial cells; but in 20% concentration, lifting up of the epithelium lamellar fusions and necrosis were seen. Whereas in 25% effluent treated fish's gills, disintegration of epithelial cells, desquamated epithelium, haemorrhage and complete damage of epithelial cells of lamellae were noticed. Present study recommends proper dilution of the chemical factory effluent before its discharge.

Key words: Effluent • Histopathology • Gill lamellae • Fish

INTRODUCTION

Rapid industrialization in India has resulted in the substantial increase in the liquid waste (spent wash or effluent) which is traditionally discharged in open land or into nearby natural water, causing a number of environmental problems including threat to plants and animal lives and also creating problems such as surface water logging, ground water contamination and salinizing good quality land due to presence of high quality salt contents [1, 2]. These industrial pollutants alters the natural condition of aquatic medium that causes behavioural changes as well as morphological imbalance of aquatic organisms [3]. Any change in water quality is rapidly reflected in fish gill structure and function, since gills are continuously exposed to ambient water. Gills are the primary sites of gas exchange, acid-base regulation and ion transfer [4]. The gill epithelium consists mainly of three types of cells: pavement or respiratory cells, mucus cells and chloride cells as pointed out by [5]. That gills represent major sites for respiration; they are always in contact with water, which makes them important targets for water pollutants [6, 7]. The authors further stated that

fish gills comprise more than half of the body surface, with an epithelial layer of only a few microns separating the interior of the fish from the external environment. As a result of this, a close association between water and blood occurs so that the gills are strongly affected by environmental contaminants [8]. The gills are the most delicate structures of the teleost body and their vulnerability has thus considerable importance because of their external location and necessarily intimate contact with water, which means that they are liable to damage by any irritant material in the water whether dissolved or suspended [9]. Gills, therefore, are potentially useful to monitor the health of fish [6]. The gills in fish can be a valuable model for assessing the effects of toxicants on cells and tissues as stated by [10] and [11]. They added that gills are highly sensitive to toxicants and are complex in structure and function; therefore, they can reveal how toxicants affect several different cell types that participate in such diverse functions as respiration, ion transport and mucus secretion. Because of their superficial location, gills can be exposed directly to known concentrations of toxicant and effects can be related precisely to toxicant concentration. The discharge of partially or untreated

industrial effluents are responsible for increasing the percentage of water born illness and deformities. The estuarine pollution may lead to marine pollution, which may enter to the deep ocean, thus affecting the marine organisms, which are consumed by human beings [12]. Thus careful management of estuary is the management of ocean and its resources. The authors further added that it is evident that a continued and systematic monitoring of the chemical environment in estuaries is necessary to understand the responses of the organisms to various stresses and to assess the degree of pollution and its variation. Studies on the physical and chemical characteristics of an estuary play a major role in pollution control programme. In view of the above, it was felt that it would be worthwhile to study the changes in gills, the structural target of fishes to environmental toxicants, which would throw a clear light on the extent of damage that is caused. Hence in the present work, we studied the toxic effects of industrial effluents on the gills of important estuarine fish, *Mugil cephalus*.

MATERIALS AND METHODS

Specimens of *Mugil cephalus* were collected from the Uppanar Estuary, Cuddalore (Lat. 11°42' N; Long. 79°46' E), Tamil Nadu, lying along the Southeast coast and acclimatized to laboratory conditions for fifteen days. Water was changed daily and fish were fed *ad libitum* with flour pellets and ground dried shrimp twice a day. For experimental studies, fish ranging from 12-15 cm in length and weighing 20-30 g were selected. The physico-chemical parameters of the fertilizer factory effluent was estimated according to [13] and are as follows: Colour - Brown; Odour - Pungent; Dissolved Oxygen- $3.2 \pm 0.02 \text{ mg l}^{-1}$. BOD- $13.7 \pm 2.0 \text{ mg l}^{-1}$; pH- 5.7 ± 0.2 . Temperature - $30.0 \pm 2.0^\circ\text{C}$. Salinity- $1.3 \pm 0.30 \text{ ppm}$. Total Hardness- $4.0 \pm 2.0 \text{ mg/l}$. and Total Alkalinity- $40 \pm 2.0 \text{ mg l}^{-1}$ Three groups containing ten healthy fish in each were selected and introduced into the plastic tubs containing 100, 75, 50, 25, 10 and 5% fertilizer factory effluent. The manifestation time and survival time of fish were observed following the method of [14]. Based on the above studies, 10 and 25% effluent concentration was selected for experimental purpose. Fish were exposed to the above mentioned concentrations along with common control. At the end of one hour, alive fish from the control and experimental tubs were taken and gills were separated and fixed in Bouin's fixative for histological studies, following the methods of [9, 15, 16].

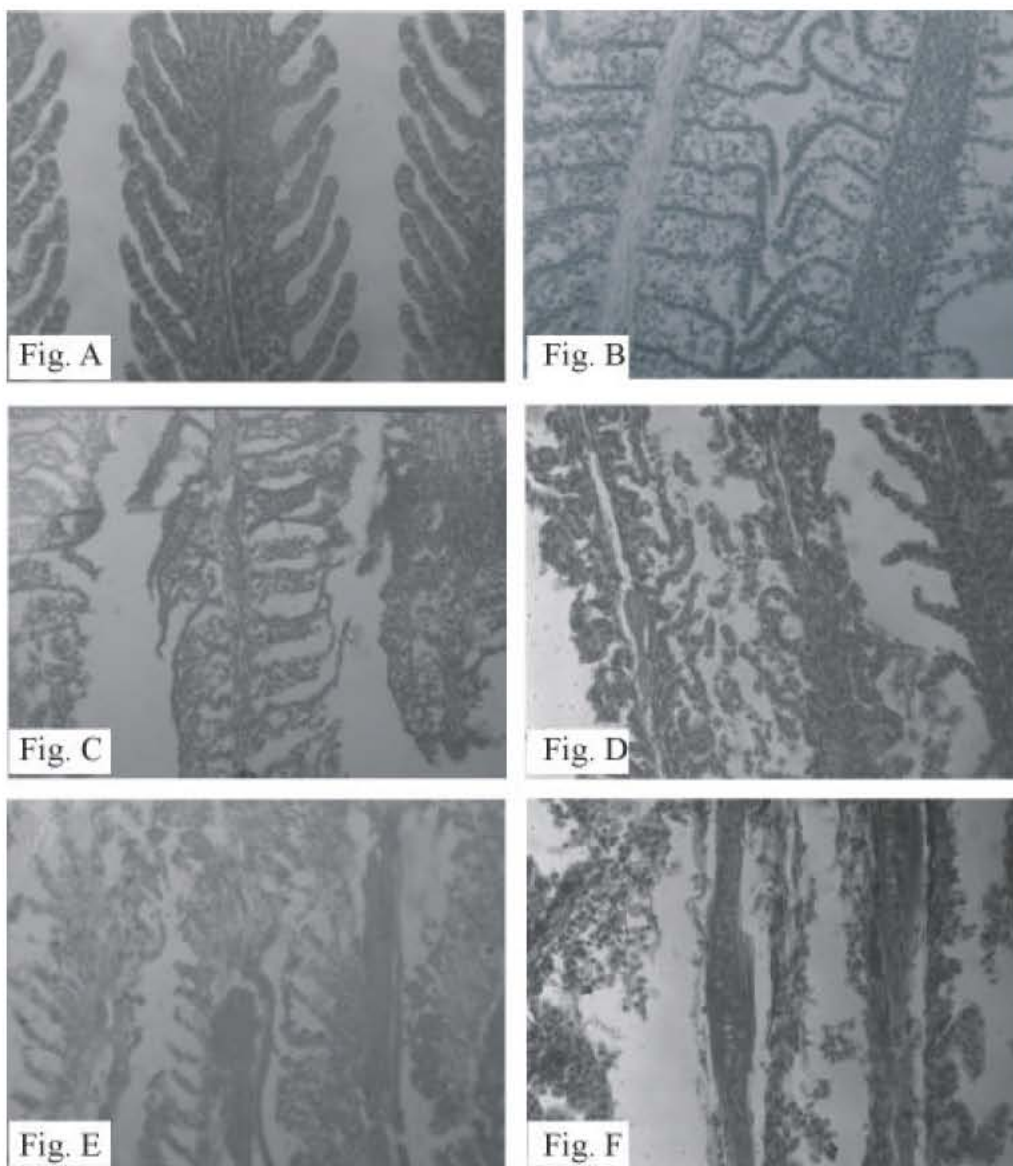
RESULTS AND DISCUSSION

In the present study, when fish were exposed to 40% crude factory effluent concentration, they survived for 20 minutes. In 5 % concentration and, they were survived for 10 hours in 10, 15, 20 % and 30 % concentration and they survived for 6, 5, 3 and 2 hours, respectively, whereas in concentrations of 50, 75 and 100% effluent, the fish survived for 15, 10 and 5 minutes, respectively. Based on this observation 5, 10, 15, 20 and 25 % were selected for experimental purpose. In control fish, primary lamellae appeared normal and mucus free with well-defined secondary lamellae branched from them (Fig. A). In the fish, *Mugil cephalus* exposed to 5% concentration, hypertrophy, swelling, proliferation of chloride cells were observed (Fig.B).

The fish was exposed to 10% and 15%, concentration hyperplasia, fusion of secondary lamellae and disintegration of epithelial cells were seen (Fig.C and D) ; but in 20% concentration, lifting up of the epithelium lamellar fusions and necrosis were observed (Fig.E).

Whereas in 25% effluent treated fish's gills, disintegration of epithelial cells, desquamated epithelium, haemorrhage and complete damage of epithelial cells of lamellae were observed (Fig.F).

Examination of tissues after death from fish and other aquatic organisms may serve to identify the cause of death and possibly the causative agent as opined by [17]. Gill lesions can be divided into two groups *i.e.* the direct deleterious effects of the irritants [18] and the defence responses of the fish [19]. Separation of epithelial cells and necrosis were reported in brook trout, *Salvelinus fontinalis* exposed to acute toxic levels of toxicant [20]. Shortened and thickened secondary lamellae, primary lamellar swelling, droplets of mucus and fused adjacent secondary lamellae in Sunapee trout, *Salvelinus alpinus oquassa* subjected to pH 4.0 [21]. Hyperplasia, shortened and thickened respiratory lamellae, fused adjacent primary lamellae were observed by [22] in peal dace, *Semotilus margarita* and fathead minnows, *Pimephales promelas* exposed to low acid pH of 5.16. Fused secondary lamellae and hyperplasia in fish, brown trout, *Salmo trutta* exposed to pH 3.0 and 4.9 [23]. In the present study, rupture and necrosis of gill epithelium of fish may be due to direct deleterious effect of low pH of the effluent. However, hyperplasia, hypertrophy, lamellar fusion and mucus secretion and sloughing of gills may be defence responses of the fish to the effluent toxicity [7].



The intimate contact of gill with polluted water may lead to alterations in the normal gill epithelium [24]. Many noxious compounds and ions have been shown to damage the respiratory epithelium of gills [25-27]. The mitochondria-rich chloride cells (also called ionocytes) are believed to be the principal site of transepithelial influxes of ions and $\text{Na}^+\text{K}^+\text{ATPase}$ is an important enzyme in the regulation of the ion balance [28,29].

[Matey 30] Pavement cells may also be the site of Na^+ and Ca^{2+} uptake, especially if the number of chloride cells is reduced. The mucus cells are of two different types: the ordinary mucus cells and the rodlet cells. They are essential for fish respiration and osmoregulation and also

play a protective role. They are typical unicellular glands, which form a thin layer on the gill surface and separate the epithelium membranes from the water. Phospholipids are the most prevalent class of membrane lipids and their structure and ratios are very important for membrane function. We believe that changes in the ion concentration of water can affect the phospholipid composition of fish gills. Various environmental factors can affect the gill structure in fish: hypoxia, hyperoxia, pH, ion concentration, heavy metals and other pollutants. Further, an increase in water colour, humic substances and dissolved organic matter (DOM) may raise the complexation level of metal ions, thus acting as protection and preventing their penetration into the gills [31].

Different inorganic ions in water may also change gill reactions. For example, aluminum and iron in acidic water cause gill damage by hypertrophy and mucus secretion [32]. Heavy metal ions can readily bind to the gills of freshwater fish and disrupt their ionoregulatory and respiratory functions [31]. At the same time metals, such as mercury, form strong complexes with a number of common ligands found in water, e.g. OH, Br. and $+NH_4$ [37]. The possibility of inorganic Hg passing through the fish gill is very slight. The complexation of inorganic Hg by DOM is probably very important in determining the uptake of Hg by the gills [31]. Lamellar fusion could be protective as it diminishes the amount of vulnerable gill surface area in fish [34]. Fusion of secondary lamellae and swelling of Primary and secondary lamellae increases the diffusion distance [35] and reduced surface area [36]. Fusion of primary lamellae at the distal end and thickened and shortened secondary lamellae observed in the present study may be involved in reducing the impact of acid toxicity in the present case supporting the observation of the above authors. The mechanism by which cells recognize and aggregate with one another at the cell surface is still unknown [37,38]. The authors further stated that the term 'mutual recognition' in a cell population, 'surface coding' and 'preferential affinities' have been used to explain such mechanisms; it could be explained that alterations in the charge of glycoproteins coating the lamellae due to the presence of salicylic acid residues of mucin at low pH, favour attraction between the cells of adjacent lamellae causing fusion. A similar reason may be attributed for the fusion of primary and secondary lamellae in the present study too.

Swelling of primary and secondary lamellae, fusion of adjacent secondary lamellae, increased mucus production, progressive loss of microridge pattern, secondary lamellae appeared thickened and shortened with extremely rough surface and considerable mucus in brook trout, *Salvelinus fontinalis* exposed to pH 4.5 and 5.0 for 456 h [39]. A similar observation was made by [40,41] in brown trout, *Salmo trutta* [20]. Hypertrophy and separation of epithelial cells from the supporting pillar cells in brook trout, *Salvelinus fontinalis* in chronic acid pH (pH 5.5) treatment and this condition greatly increased the diffusion distance (water-blood distance) [27]. Toxicants appear to cause loss of adhesion between the epithelial cell and the underlying pillar cell system accompanied by a collapse of the structural integrity of the secondary lamella [41]. Thickening of the lamellar epithelium

increased diffusive distance of the gill. The thickening of the gill epithelium (*via*. cell hypertrophy) is sometimes considered to be an indicator of cell degeneration and eventually necrosis [35,32]. The lifting and hypertrophy of cells greatly increased the diffusion distance (water blood distance) [42]. Hypertrophied gill tissue exhibited an adaptive response and reduced the ion permeability through gills of fish exposed to low pH [32,43]. Hypertrophy in the gills is a common response when fish are exposed to low pH [35,44]. The gill damages were accompanied by either mucus cell hypertrophy or hyperplasia and were most pronounced at pH 4.3 [42]. In the present (long term) study, hypertrophy noticed may be in response to prevention of diffusion across gill epithelium which finds support from the above authors. That a layer of edema has the primary effect of increasing the diffusion distance between blood and water and thus reducing the affected area to an essentially dysfunctional state [43]. Gill epithelial swelling, complete disquamated lamellae and blood capillary pillar cells would account for the impairment of oxygen, carbon-di-oxide exchange and for the hypoxia as reported by [44]. Swelling and disquamated epithelium of gills were observed in the present study, which might have caused impairment of oxygen or carbon-di-oxide exchange [19] stated that lifting up of epithelium is a protective effect, resulting in increased water blood diffusing distance and hindered toxicant uptake. Ramesh [48] reported that separation and lifting up of the epithelium may be a defence response of the fish in response to toxicants. Similar situation may prevail in fish from long term treatment in the present study thus finding the support from the observations of the above authors. Toxicants at lower levels given for a prolonged time causes severe damage to the branchial system of fish than to short term treatment [48], which finds support from the work of [46]. In the present study also, extensive cellular hyperplasia filling the entire interlamellar spaces, haemorrhage and complete damage of epithelium were more in long term studies when compared to that of the short term ones.

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