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ORIGINAL ARTICLE

HISTOPATHOLOGICAL CHANGES IN GILL TISSUE OF *ARIUS MACULATUS* EXPOSED TO INDUSTRIAL EFFLUENT FROM CUDDALORE DISTRICT, TAMILNADU

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ABSTRACT

In the present study, *Arius maculatus* were exposed with sub lethal concentration industrial effluents from Cuddalore District. The histopathological changes were observed in the gill tissues of *Arius maculatus* after exposure with industrial effluents. The present study shows that necrosis of secondary lamella, damaged secondary lamellae and the secondary lamellae in the effluents trreated fish

Keywords: Industrial effluents, Histopathology, Gill, *Arius maculatus*

1.INTRODUCTION

The toxicity of chemicals in the aquatic environment is determined by interplay of inter-organismic, intra-organismic and environmental factors. On the organismic level, species, age, sex, health status, trophic level, ecological niche, toxicant level, inductive status, and physiology are determinant factors in the assessment of risk. Similarly on the environmental front, water quality, distribution, temperature, light, absorption, and solubility are the major attributes. The play of environmental factors upon the physico-chemical nature of toxicants, availability of toxicants and physiology of aquatic organisms provides a sliding scale with countless variations adding to the complexities of real hazard assessment.

Effluent and run off from fields comprising chemicals of versatile nature, exert their toxic effects on fish population by depleting the dissolved oxygen, altering the pH, salinity and changing the carbondioxide content (Soundarapandian *et al.*, 2009; Sankaran *et al.*, 2011) thereby directly or indirectly affecting the life cycle as well as the metabolic pathways of the fish at the biochemical level (Puvaneswari *et al.*, 2009). Man- made pollution is perhaps the biggest threat to the estuaries, in many instances; estuaries are being used as "sewers and sinks" for untreated waste water. Pollution of estuaries is difficult to assess because of the special qualities of this ecosystem; estuarine pollution is different from river pollution as the pollutants remain trapped in the ecosystem for a long period due to tidal isolation; and pollution damage of estuaries is the product of man's as well as nature's activities.

Fish is an important as well as cheap source of animal protein and hence farming of fish has become imperative to meet the growing demand for protein worldwide. In recent years in India too, intensive aquaculture practices are on the increase, and aquaculture programmes at present largely depend on riverine and estuarine seed resources (Meehan, 2002). Wide variable effects of different toxicants on a given species (Dalela, 1977; Savinov *et al.*, 2003) and the variable effects of the given toxicants on different species (Ruangsomboon and Wongret, 2006) warrants, no generalization be made on the toxic effects of disposed industrial wastes surrounding aquatic environment.

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasi *et al.*, 1992; Kumaresan and Karuppasamy, 2011b). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al.*, 2004; Lopes *et al.*, 2001).

2.MATERIALS AND METHODS

Arius maculatus was collected from three different stationsat Uppanar estuary and they were immediately transferred to ice box.

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From the experimental station of Uppanar estuary, fish were caught either using cast net or hand lines. Cast net are made of nylon. The mesh varies from 10 to 15 mm. Hand lines with mono filament and hooks were used with prawn or mussel as bait for the capture of *Arius maculatus*. The fish were transported to the laboratory and maintained in the same way as the control fish collected from Perumal Lake. The water was renewed once in two days.

Procurement of fish

Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar brackish water by operating cast net. The fish were acclimatised in the aquaria of 120 litres capacity containing well aerated sea water (salinity 28 ‰; pH 7.69; oxygen content 4.32 mg/l) and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed withchopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal lake (Plate 1A) were used as control and Uppanar brackishwater area was selected as experimental site (Plate 1B).

Acute Toxicity Test

The raw and partially treated effluent was collected from the discharging point of industries surrounding the Uppanar eaturry for acute toxicity test. In the acute toxicity bioassay, mortality could be observed within a short period. LC_{50} was calculated by the following method of Finney (1978) to observe mortality and behavioural response of the test fish, *Arius maculatus* on exposure to effluents of different concentrations.

Static acute toxicity was employed to evaluate the adverse effects of industrial effluents surrounding the Uppanar estuary on the fish, *Arius maculatus* under standardised laboratory conditions.

Food was withheld one day before the toxicity test with a view to avoid the possible change in the toxicity of the pollutants after addition of the effluent into the test tank with 100 litres of sea water having 10 fishes. Mortality was recorded after 24, 48, 72 and 96 hr and five replicates were maintained simultaneously for the purpose. Fishes showing respiratory and lack of response to tactile stimuli were considered, nearing dead and removed immediately. Percentage mortality was calculated and the values were subjected to Probit analysis (Reddy *et al.*, 1992). Confidential limits (upper and lower) of the Regression coefficient with Chi - square test were calculated.

Design of sublethal toxic study

Sublethal studies are helpful to assess the response of the test organism to stress caused by the effluents. Based on acute toxicity test two sublethal concentrations (2% and 6%) on *Arius maculatus* were derived and used as the experimental concentrations. Sublethal of safe level concentration were derived from 96 hr LC_{50} value.

In the present study 2% (1/50) and 6% (1/30) dilution (in *Arius maculatus*) of the 96 hr LC_{50} were selected as sublethal concentrations. The experimental fish were exposed in each concentration for a period of 7, 15 and 30 days. A control batch corresponding to each test group was maintained simultaneously.

Histological study in gill tisues

At an interval of 7, 15 and 30 days, a fish from each concentration (2% and 6%) of the effluent was sacrificed and the tissues of gill was excised out and subjected to standard histological technique of Culling (1957). Each tissue was fixed separately in aqueous Bouin's fluid for histological examinations. After fixation, the tissue were dehydrated in ascending series of alcohol, cleared in methyl salicylate and embedded in paraffin wax. Serial sections were taken at 5 to 8 µ thickness and stained with Ehlrich's haematoxylin with aqueous eosin as the counterstain.

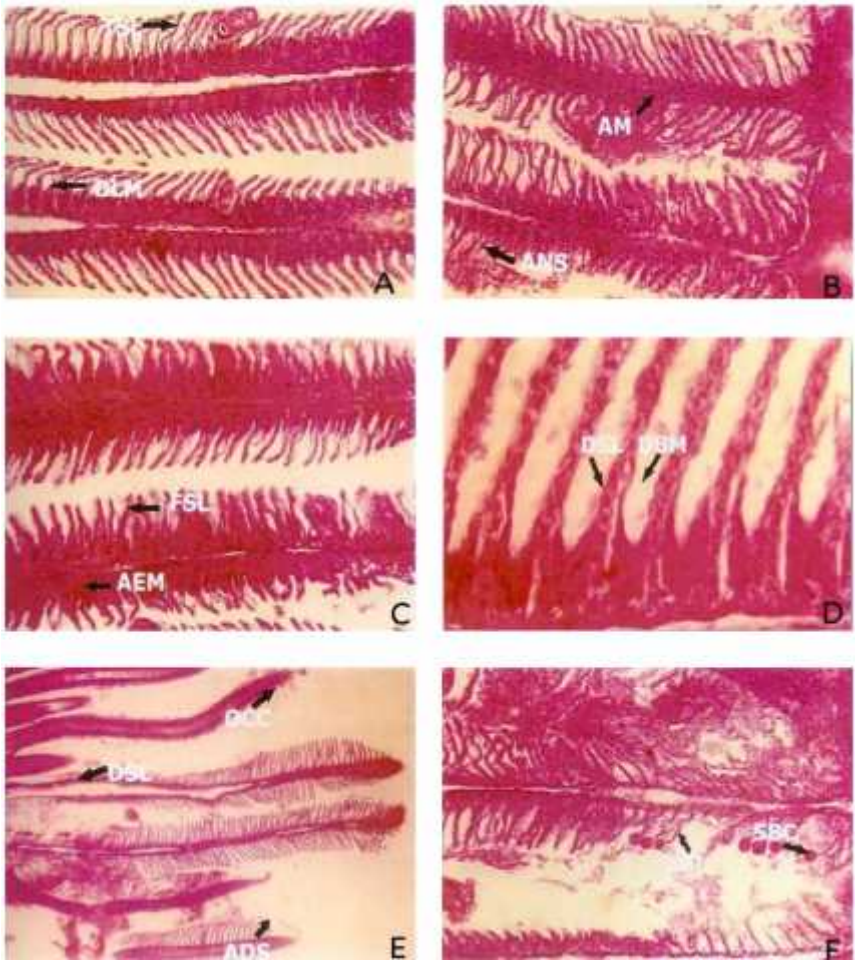
3.RESULTS

The gill of control specimen of *Arius maculatus* (Plate 26-A) comprised of laterally compressed leaf - like gill filaments called primary lamellae arranged alternately on either side of a branchial cartilage and septum. Each primary lamella enclosed a row of secondary gill lamellae on both sides perpendicular to its long axis. Furthermore, the primary lamella comprised of a central core of cartilagenous rod with lining epithelial cells and blood cells. The secondary lamellae consisted of a layer of flattened epithelial cells attached to the basement membrane and are found associated with contracted pillars called pilaster and blood spaces or sinusoids. Besides, each secondary lamellae was characterised by a central axis with lateral barb like laminae extending throughout the length and the length of the laminae decreased towards the free end. The interbranchial space between the secondary lamellae and interlaminar base were uniform in the control samples.

Gills of fish exposed to 2% and 6% effluent showed respective variations in the respiratory surfaces of gill arches or lamellae. For instance the gills exposed to 2% concentration for 30 days showed marked necrosis than 7 days and 15 days ,consequently the fanner showed necrosis of secondary lamellae in which only the central cartilage was retained. Between 7 days and 15 days of exposure, the later seemed to influence more secretion of mucous thereby all the secondary lamellae were fused amidst with excess secretion of mucous 7 days of exposure caused the dissolution of cell membrane to the extent that the cellular outline of secondary lamellae became increasingly difficult to distinguish. Contrastingly, 6% concentration of effluent brought about histolysis in all the 3 intervals of exposure. Nevertheless, the damage was more severe during 30 days of exposure resulting in absolute dissolution of secondary lamellae, sinusoids and branchial epithelium. In fact 7 days of exposure stimulated over secretion of mucous and its accumulation between the interlamellar spaces and consequently the secondary lamellae became slender with occasional necrosis in the proximal half. In the case of 15 days of exposure, the secondary lamellae showed the dissolution of lamellar membrane. Exposure of gills to raw effluent in the field condition necessitated excess secretion of mucous, excessive necrosis of branchial epithelium and infiltration of epithelial and blood cells, and dissolution of blood vessels, sinusoids and basal lamina (Plate 1).

Fig. 1 Shows Histology of control fish, Arius maculatus

Light micrographs on the histopathology of the gill of *Arius maculatus* exposed to 2% (A, C & E) and 6% (B, D & F) sublethal concentrations of the effluent for an exposure period of 7 days (A&B); 15 days (C&D) and 30 days (E&F) (80X).



4.DISCUSSION

Histopathological studies with light microscopy and electron microscopy are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants. Toxic effects are often due to physical changes in the tissue at the cellular or ultra structural levels and can only be speculated upon, unless they are visualized. Histological studies on fish revealed that toxicants produced pathological changes such as neurobiotic changes in the liver, tubular damage of kidney and gill lamellae abnormalities (Karuppasamy, 2000c). The heavy metal such copper, zinc, mercury and nickel are persistent pollutant and are discharged into freshwater from industrial municipal and agricultural effluents (Chen and Lin, 2001).

Gill is the primary site of osmoregulation and respiration in aquatic vertebrates. It is the main target organ which gets affected easily when the organism is exposed to dissolved heavy metals. Gill covers more than 60% surface area of the fish and its external location renders it the most vulnerable target organ for the pollutants (Roberts, 1989).

Branchial responses were also noticed in a number of teleosts under prolonged exposure to the sublethal concentration of heavy metals (Karuppasamy, 2000c; Krishnani *et al.,* 2003). The oedematous separation of gill epithelium for the basement membrane of pillar cells in treated mullets may probably be due to the increased or lowered efficiency of the epithelial cells in maintaining normal water balance (Roberts, 1989). The proliferative thickening (hyperplasia) of the gill epithelium of *Liza parsia* exposed to effluent for longer duration appears to be a general safety measure against irritation by the environmental toxicants (Fernandes *et al.,* 2007; Pandey *et al.,* 2008). Multiple lamellar telangiectases (aneurysms) and the filling up of the interlamellar spaces by hyperplastic epithelium under prolonged treatment tend to explain the high mortality of the fish after day 10, probably due to asphyxia (Mazon *et al.,* 2002).

In the present study the gill exhibit several pathological changes like erosion of secondary lamellar epithelium, hypertrophy, distortion of secondary lamella, hyperplasia, fusion, vacuolization and necrosis on exposure to the effluents. Lifting of the gill branchial epithelium which was observed in light microscopic study is one of the most common responses to water pollution (Haaparanta *et al.,* 1997). Similar observations were made in fish exposed to various toxicants (Chezhian *et al.,* 2010; Karuppasamy, 2000c).

The gill lesions in fish induced by toxicants are mediated through inflammation (**Wong and Wong, 2000; Pandey *et al.,* 2008**) on central regulatory systems. Mucous formation in the gill is a primary result of interactions of total solids in the mucous producing cells which results in the secondary destruction of the respiratory system that ultimately affects the CO_2 exchange (Haniffa and Sundaravadanam, 1984). Stress caused by variations in the environmental conditions induces the proliferation of mucus cells and increased mucous secretion (Fernandes *et al.,* 2007).

Cyprinus carpio when exposed to sublethal concentration of raw distillery effluent showed destruction and thinning of interlamellar epithelium that may be due to the destruction and disappearance of the interlamellar cells. Santhakumari (1990) has also observed the destruction and disappearance of the interlamellar cells leading to the thinning of the epithelium in the gill *Anabas testudineus* exposed to mercury. In the present study the gill epithelium is completely desquasated, the shapeless secondary lamellae are observed with hyperplasia and are broken at several places. The destruction in the arrangement of the pillar cells and red blood cells observed by Christy (1995) in *Catla catla* exposed to chromium.

Velmurugan *et al.* (2009) reported that the histopathological effects of dichlorvos on the gill and liver tissues in *Cirrhinus mrigala* were determined by light microscopy. They also reported hyperplasia, desquamation and necrosis of epithelial, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae, curling of secondary lamellae and aneurism in the secondary lamellae were observed in gill tissues exposed to dichlorvos. Hepatic lesions in the liver tissues of fishes exposed to dichlorvos were characterized by cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy. Ashok Kumar Gupta and Ashwani Kumar, (2006) reported that the histopathological lesions in the selected tissues such as gills, kidney and eye of the fingerlings of *Cirrhinus mrigala* exposed to a sublethal concentration of mercury. The primary gill lamellae showed degeneration in the middle and distal region. In distal region, spherical structure showing complete degeneration and fusion of pillar, epithelial, mucus producing gland and blood cells in the secondary gill lamellae. Degeneration and necrosis in glomerulus, interstitium tissue and epithelium lining of renal tubules in kidney have also been seen in mercury treated fingerlings.

Sirimongkolvoraku, (2012) observed that the histopathology alterations were observed in the gill, kidney and liver. Alterations like hyperplasia, epithelial lifting and telangiectasis were found in gill. The kidney lesions were shown cloudy swelling, tubular narrowing and hyaline droplet. Anomalies such as nuclear pyknosis, cytoplasmic vacuolation and melano-macrophages aggregation were found in liver due lead. Athikesavan *et al.* (2006) observed that nickel chloride on histopathological changes were studied in the gill, liver and kidney of the nickel treated freshwater fish *H. molitrix*. The nickel showed a tissue specific alteration in the tissues. Mucus proliferation, fusion of the gill lamellae and hypertrophy of gill tissues were observed. Lack of normal palisade arrangement was followed by necrosis in hepatocytes. Degeneration of blood vessels, vacuolation, hypertrophy, pyknotic nuclei and lesion were observed in liver tissues. Degeneration of tubular cells, hyperplasia was observed in kidney tissues.

Bharat Bhusan Patnaik *et al.* (2011) studied histological studies in organs like gill, liver and muscle of *Cyprinus carpio* communis were made to assess tissue damage due to sublethal concentration of heavy metals lead and cadmium after 28 days of exposure. In lead treated gill, disintegration and fusion of primary lamellae, extensive vacuolization with disruption of epithelial lining was observed, whereas on sublethal exposure to cadmium, hyperplasia of branchial arch, vacuolization and congestion of blood vessels were well marked in the lead treated fish. Both lead and cadmium treated fish showed marked thickening and separation of muscle bundles with severe intramuscular oedema more pronounced in sublethal treatment of cadmium.

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