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A STUDY ON THE EFFECT OF ELECTROPLATING EFFLUENT AND ITS HISTOPATHOLOGICAL AND SEM ANALYSIS IN THE GILLS OF FRESH WATER FISH, *OREOCHROMIS MOSSAMBICUS*

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Abstract: **Histopathological changes used as biomarkers in the evaluation of the health of fish exposed to effluents. One of the advantage of using histopathological biomarkers in environmental monitoring is allows examining specific target organ like gill is responsible for vital functions such as respiration, detoxification, excretion and co-ordination. The freshwater fish, *Oreochromis mossambicus* exposed to electroplating effluent for a period of 7, 14 and 21 days and histopathological changes were analyzed by light and scanning electron microscopy. The main histopathological changes were edema, lifting of lamellar and filamentary epithelia and lamellar fusion, cell proliferation with consequent thickening of the filament epithelium also found in fishes exposed to the electroplating effluent. The severities of the lesions increased with the time of exposure namely the hyperplasia of the epithelial cells with the proliferation of filament are epithelium and fusion of lamellar observed at 21 days. Additionally, several histopathological results obtained by light microscopy were confirmed through scanning microscopy.**

Keywords: Oreochromis **mossambicus, Gills, Histopathology, Electroplating effluent.**

I. INTRODUCTION

Water pollution is the main threat of urbanization, industrialization and modern agricultural practices. Water pollution is any change or modification in the physical, chemical, and biological properties of water that will have a detrimental consequence on living things, is water pollution. Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and ground water), very often by human activities. Water pollution occurs when pollutants (particles, chemicals or substances that make water contaminated.) are discharged directly or indirectly into water bodies without enough treatment to get rid of harmful compounds.

Effluent is an out flow of water or gas from a natural body of water or from manmade structures. Water contaminated by the effluent would cause skin problems and other serious ailments. These industrial effluent containing heavy metals pose a serious threat to the ecosystem. The waste water released from various industrial establishments contributes a major share to the environment pollution.

The generation of large quantities of aqueous effluents many of which contain high level of toxic pollutants. The use of industrial or municipal waste water in agriculture is a common practice of irrigation in many parts of world. Use of these industrial effluent and sewage sludge for agriculture have a common practice in India as a result of which these toxic metals get transferred and accumulated in to plant tissues from soil. However untreated or partially treated waste waters also introduce a huge amount of contaminants particularly heavy metals into agricultural lands. The existence of heavy metals in the environment represents a significant and long term environment hazard since they are not biodegradable and tend to accumulate in living organism.

Histopathological parameters are the one important and specific biomarkers with regard to effects of toxicants on organism. Effluent discharge can also deposit sand and grit into aquatic systems, affecting the physical characteristic of the sediment. The heavy metals present in the effluent leads to the accumulation of toxic substance from lower to higher organisms. So are let out into the aquatic systems as the discharge of effluent waste water has major detrimental effect on the health of aquatic ecosystems [1].

SEM provides detailed high resolution images of the sample by restoring a focused electron beam across the surface and detecting secondary or backscattered electron signal. An Energy Dispersive X-Ray analyser (EDX or EDA) is also used to provide elemental identification and quantitative compositional information [2].

II. MATERIALS AND METHODS

PROCUREMENT AND MAINTANANCE OF FISHES

Healthy specimen of *Oreochromis mossambicus* was produced from Tamilnadu Fisheries Development Corporation (TNDF), Aliyar, Tamilnadu. Fishes acclimated to the laboratory condition were used and the experiment. Fishes of average same size and weight were used for the experiment and both sexes were used for the study.

ACCLIMATIZATION OF THE FISH

Fishes are maintained in a large tank and acclimatized to laboratory conditions for 21 days. Water was changed daily to maintain the oxygen content and to remove the excreta of fishes. Fishes were maintained at room temperature and fed with rice bran and oil cakes daily, at least one hour prior to the replacement of tank water. Feeding was stopped two days prior to the experiment in order to keep the animal more or less in the same state of metabolic requirement.

EVALUATION OF MEDIAN LETHAL CONCENTRATION (LC_{50})

Batches of 10 healthy fishes were exposed to different concentrations of electroplating effluent to calculate the LC_{50} value [3]. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 10-50 ml of the electroplating effluent were chosen and the number if dead or affected fish in each set up was counted at regular intervals up to 24 hours.

Appropriate narrow range of concentration 1-5 ml was used to find the median lethal concentration, using a minimum of 10 fishes for each concentration and mortality was recorded for every 24 hours up to 96 hours. It was found as 7.9 mg/l for 96 hours.

Four groups of fishes were exposed to 0.79 mg/l (sublethal concentration of 96 hours LC_{50} value) concentration of electroplating effluent for 7, 14, and 21 days respectively. Another group was maintained as control at the end of each exposure period, the gills are separated for Histopathological and SEM analysis.

HISTOPATHOLOGICAL ANALYSIS

Histological techniques were followed based on the method of Bancroft and Stevens (1997). Gills were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean tissues. They were fixed in aqueous Bouins solution for 48 hrs, processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ in thickness stained with Haemotoxylin, Eosin, dissolved in 70% alcohol and were mounted in Canada Balsam [4].

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SEM ANALYSIS

Representative sample of gills were fixed at 40^0C in 4% glutaraldehyde buffered with 0.1 m cacodylate, pH 7.4 during 2 hrs for SEM. After proper rinses and dehydration in ethanol, the tissues were then critical point dried with CO_2 and mounted in to aluminium stub and coated with gold palladium the preparations were then examined under Fey Quanta 400 EFEM.

III. RESULTS AND DISCUSSION

Effects of electroplating effluent on Histopathological alterations and SEM analysis in the gill of fresh water fish, *Oreochromis mossambicus* under long term exposure.

The exposure of the fresh water fish *Oreochromis mossambicus* 7.9 ml of electroplating effluent. Effluent on long term exposure leads to the formation of histopathological lesions of varying intensities on the gill tissue. In the present investigation the histopathological changes in the gill of *Oreochromis mossambicus* in normal conditions and exposed conditions to long term period of effluent at selected periods have been observed.

HISTOPATHOLOGICAL ANALYSIS ON GILL:

CONTROL

Gill histology of control fish revealed the intact nature of both primary and secondary gill lamellae. The secondary lamellar surface was covered with simple squalors epithelial cells and capillaries separated by mucous cells. Each primary gill lamellae was flat leaf like in structure. It consisted of double rows of secondary lamellae on both sides were highly vascularised and covered by a layer of cells with uniform inner lamellar spaces.

LONG TERM EXPOSURE

When fish exposed for 7 days to the long term exposure of electroplating effluent there was degeneration of epithelial lining. After 14 days of exposure degeneration changes in secondary lamellae of gill was noted and there was fusion of secondary lamellae with irregular lamellae spaces. After 21 days structural alternations such as epithelial proliferation, lamellar fusion and necrosis were observed. Edematous changes characterized by epithelial detachment, were observed in the gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments and also dilation and congestion in blood vessels of gill filaments were observed. Atrophy of secondary lamellae was seen.

SEM ANALYSIS ON GILL:

CONTROL

Numerous regularly arranged slender fingers like secondary gill lamellae were observed along the length of the primary gill filament under Scanning Electron Microscope. The lamellae of the control fish were not fussed together and had a relatively large surface area of exchange with water. SEM showed the presence of denticle of gill rackers parenchyma cells of the primary gill filaments from control fish had inner surfaces with distinct microridges, where as pavement cells of the secondary lamellae had smooth surfaces.

LONG TERM EXPOSURE

Damages like fusion and clumping of secondary gill lamellae were observed after 7 days of exposure to electroplating effluent under Scanning Electron Microscope, on exposure to electroplating effluent under SEM, on exposure to electroplating effluent for 14 days corrosion of epithelial cells, more mucous secretion and acute destruction of denticular structures were observed. Under SEM in the fish up to 21 days, the changes were observed in the gill of *Oreochromis mossambicus* were swelling, fusion of lamellae hemorrhages on the gill filament. Severe corrosion of epithelial layer and high mucous secretion. The gill racker denticular structures were totally damaged and were uprooted from their bases.

The histopathological effect of Cyphenothrin on the gill of *Lebistes reticulates*. The result showed necrosis, degeneration of secondary lamellae due to odema, shortening of secondary lamellae [5]. Cell proliferation with thickening of gill filament epithelium may lead to the lamellar fusion alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cell, besides partial fusion of secondary lamellae are examples of defense mechanisms since in general, these results increases the distance between the external environment and the blood and thus serve as a barrier to the entrance of

contaminants. As a result of the increased distance between the water and blood the oxygen uptake is impaired. Hypertrophy, hyperplasia, fusion of adjacent lamella and telengeasteases of the gill were noticed when fish exposed to electroplating effluent for chronic test. hyperplasia, lamellar fusion, curling and bulging of tips of primary gill lamellae, exudation of erythrocytes, when the fish, *Cyprinus carpio* was exposed to sublethal concentration of Aldrin [6].

The proliferation of pavement cells, mucous cells and chloride cells seem to be protective which limit the accesses of with the branchial surface, on the other hand they may also block respiratory gas exchange and lead to animal smoothening, uplifting of epithelium, necrosis and increased density cells on the secondary lamella hyperplasia and hypertrophy between the external environment and the blood and thus serve as barrier to the entry of the contaminants. These alterations more commonly associated with chronic exposures than acutely lethal exposures, greatly increase the blood to water diffusion distance decrease inter lamellar distance and lead to a total reduction in the diffusive conductance of the gill to respiratory gases [7].

Hyperplasia, degeneration of pillar cells necrosis of gill epithelium and fusion of gill filaments was clearly assessed [8]. The effects of pollutants are generally manifested in the survival, reproduction or growth due to physiological alterations in the aquatic animals. In the present study when fishes were exposed to sublethal concentrations of the effluent, they showed abnormal movements and behavior including erratic swimming, hyper excitation, rapid movements and thick mucus covering over the body surface. Toxic substance can disturb the osmoregulatory function of aquatic organism. The repture and necrosis of gill epithelium may be due to the deleterious effect of the low pH the effluent. However hyperplasia, hypertrophy, lamellar fusion, and sloughing of gills may be the defence response of the fish to the effluent toxicity [9].

SEM analysis in the Gill of Fish *Oreochromis mossambicus*

Damages like fusion and clumping of secondary gill lamellae were observed after 7 days of exposure to electroplating effluent under Scanning Electron Microscope, on exposure to electroplating effluent under SEM, on exposure to electroplating effluent for 14 days corrosion of epithelial cells, more mucus secretion and acute destruction of denticular structures were observed. Under SEM in the fish up to 21 days, the changes were observed in the gill of *Oreochromis mossambicus* were swelling, fusion of lamellae hemorrhages on the gill filament. Severe corrosion of epithelial layer and high mucus secretion. The gill racker denticular structures were totally damaged and were uprooted from their bases [10].

IV. CONCLUSION

The above findings and the results of the present study indicate that the gill is a sensitive tissue gets affected easily by the pollutants. The histology of gill tissue of *Oreochromis mossambicus* showed various degrees of deterioration when compares to control. The severity of the lesions increased with the time of exposure.

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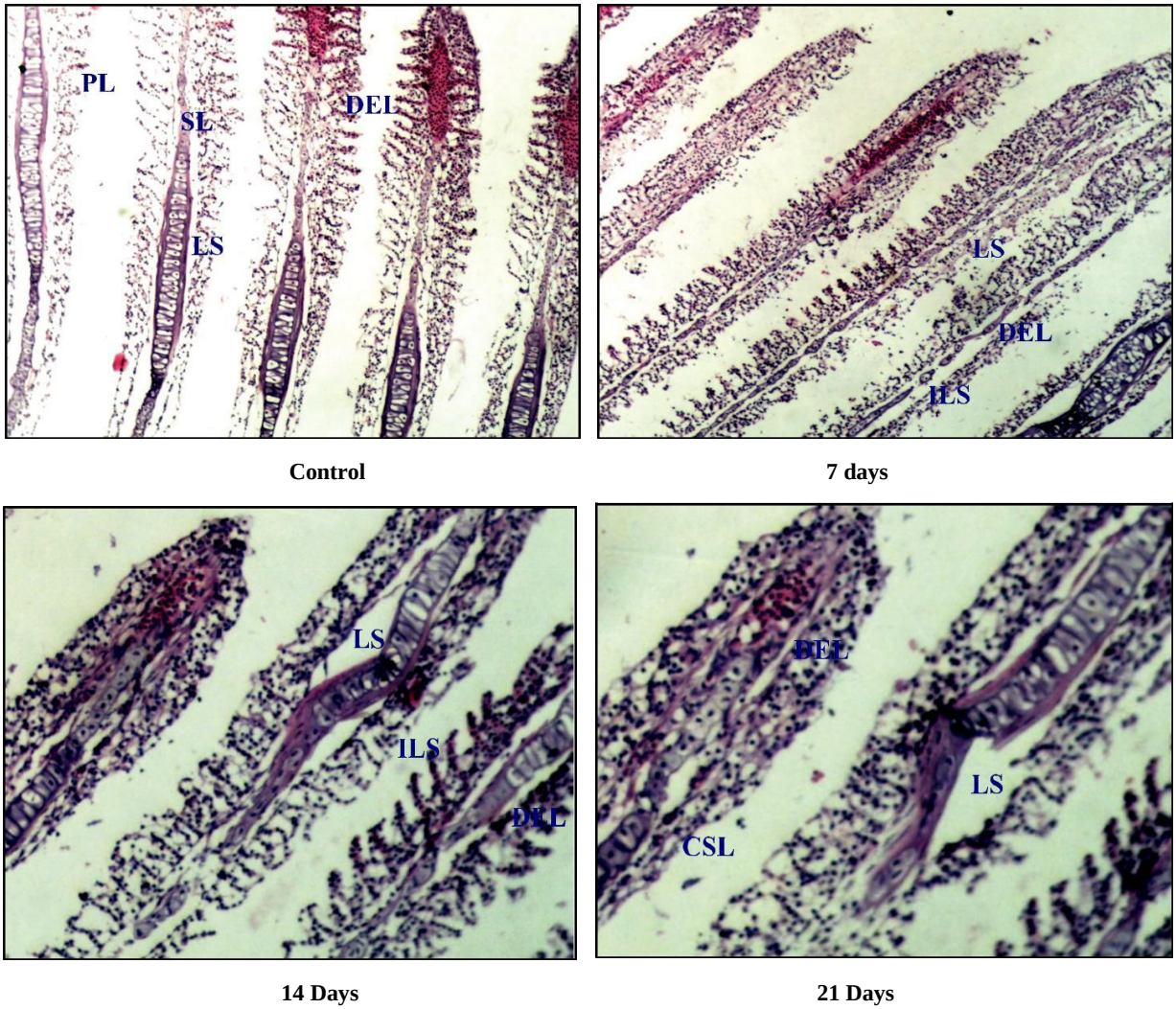

Control **7 days**

14 Days **21 Days**

Fig 1: Effects of Electroplating effluent on Histopathological alterations in the gill of fresh water fish, *Oreochromis mossambicus* under long term exposure.

PL - Primary lamellae

SL - Secondary Lamellae

LS - Lamellar Space

DEL - Degeneration OF Epithelial Lining

ILS - Inter Lamellar Spa

CSL - Collapsed Secondary Lamellae

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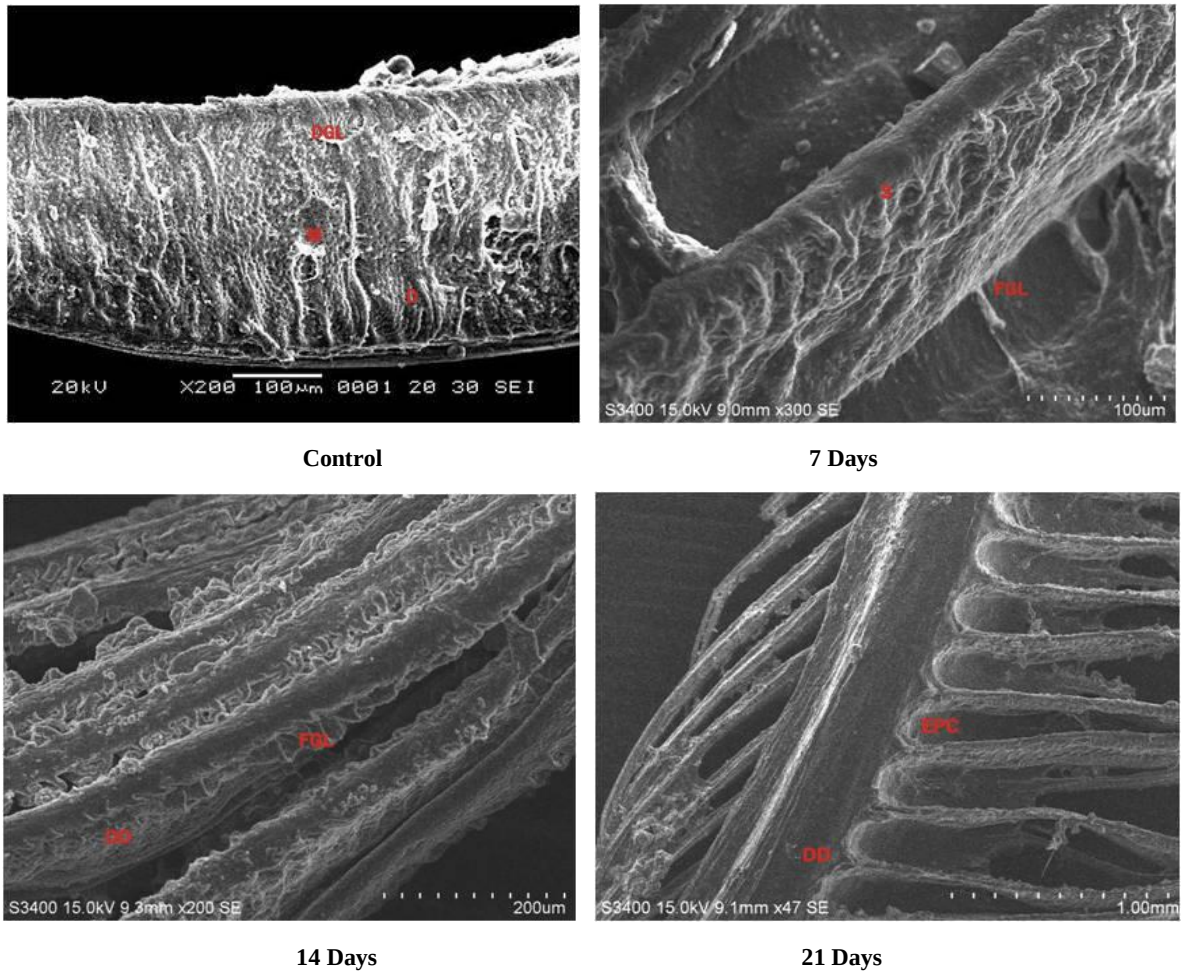

Control **7 Days**

14 Days **21 Days**

Fig 2: Effects of Electroplating effluent on SEM (Scanning Electron Microscopic) analysis in the gill of fresh water fish, *Oreochromis mossambicus* under long term exposure.

D - Denticular structure
M - Mucus secretion
FSL - Fusion of gill lamellae
DGL - Degenerated gill lamellae
DSL - Degeneration of secondary lamellae
EPC - Erosion of epithelial cells
S - Swelling of secondary lamellae
DD - Destruction of denticular structure