ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 13-16

www.fisheriesjournal.com
Received: 03-07-2016
Accepted: 04-08-2016

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Histopathological changes in the gills of fresh water fish, *Catla catla* exposed to electroplating effluent

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Abstract
Histopathology changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, one of the great advantage of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs including gills that are responsible for vital functions. Such as respiration, accumulation, biotransformation of metals in fish and may cause many diseases structural alternations and functional changes in the organs of animals. Histology acts as an integrated parameter, providing a more complete evaluation of the organisms health, effectively monitoring the effects of exposure to environmental pollutants. The electroplating effluent was collected from vellalore station, Coimbatore. The fresh water fish *Catla catla* was selected as the test animals. $1/10^{th}$ of 96 hrs LC_{50} was taken as sublethal concentration of electroplating effluent. After the stipulated period of exposure (24, 48, 72 and 96 hrs) fishes were sacrificed and gill was isolated and used for histopathological studies.

Keywords: Histopathology, gill, electroplating effluent, sublethal, *Catla catla*

1. Introduction
Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [1]. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histopathological biomarkers in environmental monitoring and this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [2]. Histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Gills are suitable organ for histological examination in order to determine the effect of pollution.

Gills are the major organ for osmotic regulation, excretion and respiration in fish. The gills of fish are located on each side of the head beneath a gill-covering operculum and are composed of finger-like filaments attached to a cartilaginous gill bar. Numerous, delicate, leaf-like structures, the lamellae, project from each filament and these consist of minute capillaries covered by a single layer of thin epithelial cells. The epithelium forms a barrier between the fish's blood and the surrounding water. Gills are generally considered a good tissue indicator of the water quality and are appropriate for the assessment of environmental impact [3, 4]. Histopathological studies are performed to evaluate the direct effects of contaminants on fish in laboratory bioassays [5, 6]. However, despite its broad range of distribution on coasts throughout the Americas and its suitability for aquaculture, few studies have reported the effects of pollutants on the species *T. Carolinus* [7, 8].

Therefore, Histopathology is the gold standard when defining toxicological effects, but it is invasive, time consuming and expensive. Furthermore, as histological testing is often impractical in human subjects, using biomarkers with a known histological distribution may fill the need of localizing toxic injury to distinct organs or tissues. Therefore, histopathological evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism. The present study reviews the most important histo-cytopathological alterations in various organs of fish, which have been used as biomarkers in various pollution monitoring programs [9].

2. Materials and Methods

Fresh water fish, *Catla catla* were exposed to 24 hrs, 48 hrs, 72 hrs and 96 hrs to a sublethal concentration of electroplating effluent. At the end of exposure period, fish were randomly selected for histopathological examination. They were collected from the Aliyar fish farm, pollachi stocked during October 2015 and acclimatized for a time period of `10-15 days in the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 26±50°C and the pH was maintained between 6.6 and 8.5.Fish were fed twice daily alternately with rice bran and oil cakes. For the present study, matured adult fishes were exposed to 1/10th concentrations of LC_{50} of electroplating effluent for 24, 48, 72 and 96 hrs continuously. Three replicates of ten fishes for each exposure of the electroplating effluent were used. In these aquaria water was replaced daily with fresh treatment of electroplating effluent. Each experiment was accompanied by its respective control.

Three groups of fishes were exposed to 1/10th of the electroplating effluent for 24, 48, 72 and 96 hrs. Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of exposure period, fish were randomly selected for histopathological examination. Tissues of gills were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's 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 thickness stained with Hematoxylin Eosin, dissolved in 70% alcohol [10] and were mounted in Canada Balsam. The photographs at 200x magnification were taken with computer aided microscope (Intel play Qx3, Intel Corporation, Made in China).

3. Results and Discussion

Gill histology of control fish revealed the intact nature of both primary and secondary gill lamellae. The secondary lamellar surface was covered with simple squamous 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 with the central supporting axis. They were situated laterally on either side of the interbranchial septum. The secondary lamellae on both sides were highly vascularized and covered by a layer of cells with uniform interlamellar spaces.

When the fish was exposed for 24 hours to the short term exposure of electroplating effluent, there was degeneration of epithelial lining. After 48 hours of exposure, degeneration changes in the secondary lamellar of gill was noted. After 72 hours of exposure, there was fusion of secondary lamellae with irregular lamellar spaces. After 96 hours of exposure, structural alterations such as epithelial proliferation, lamellar fusion and necrosis were observed. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae, Moreover, aggregations of inflammatory cells were noticed in gill filaments Also dilation and congestion in blood vessels of gill filament were observed. Atrophy of secondary lamellae was seen.

Histopathology of the Gill of *Catla Catla*

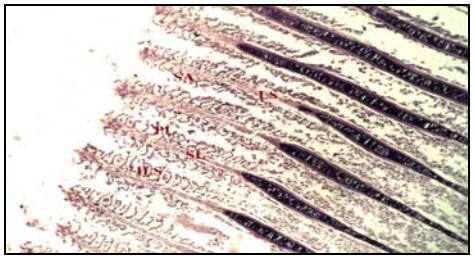

Fig 1: Control Gill section of *Catla catla*

PL - Primary lamellae
SL - Secondary lamellae
LS - Lamellar space
ILS - Inter lamellar space
SA - Supporting axis

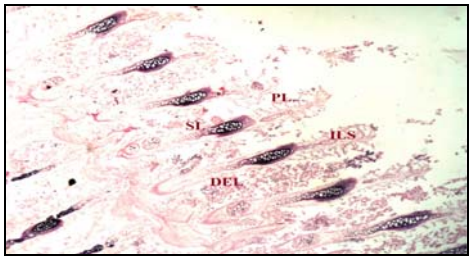

Fig 2: Gill section of fish exposed to 24 hours of electroplating effluent

SL - Secondary lamellae
DEL - Degeneration of epithelial lining
ILS - Inter lamellar space
PL - Primary lamellae

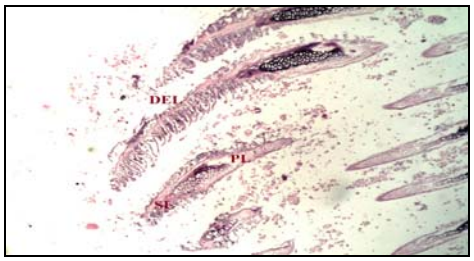

Fig 3: Gill section of fish exposed to 48 hours of electroplating effluent

SL - Secondary lamellae
DEL - Degeneration of epithelial lining
PL - Primary lamellae

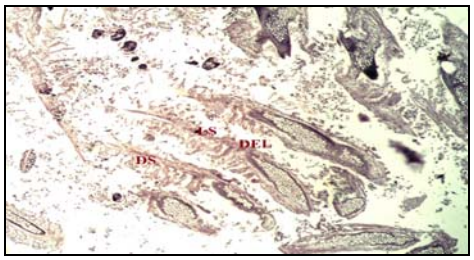

Fig 4: Gill section of fish exposed to 72 hours of electroplating effluent

LS - Lamellar space
DEL - Degeneration of epithelial lining
DS - Degenerated secondary lamellae

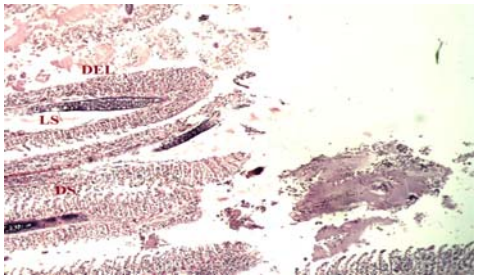

Fig 5: Gill section of fish exposed to 96 hours of electroplating effluent

LS - Lamellar space
DEL - Degeneration of epithelial lining
DS - Degenerated secondary lamellae

In gills degeneration of epithelial lining was observed in 24 hour observation, In 48 hour degeneration of secondary lamella will occur. In 72 hour fusion of secondary lamellae with irregular lamellar spaces will occur. In 96 hours structural alteration, epithelial proliferation, lamellar fusion and necrosis can be observed. Dilation and congestion in blood vessels of gill filament and atrophy of secondary lamellae also observed.
The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion [11]. It is possible that the damage of the gills could be a direct result of the salts, heavy metals, pesticides, sewage and fertilizers, which are conveyed to the water [12]. They are directly exposed to poisons occurring in the external environment, which often cause pathology in fish [13]. The gills are among the most vulnerable structures of the teleost fish because of their external location and intimate contact with the water. So, they are liable to damage by any irritant materials whether dissolved or suspended in the water [14]. In the gills, only a few micrometers separate the blood from the water [15] which not only facilitates the exchange of gases, but also allows the gill tissue to be exposed to variations in the environment. Consequently, the presence of toxic substances in the environment causes alterations in the vital functions carried out by the gills and alterations in the morphologic structure of these organs [16].
Gill histopathology may be indicative of general stress from exposure to various xenobiotics, metals, organic pollutants, toxic algae, and suspended solids. The histopathological changes in this tissue, in general, are a response to exposure to non-specific contaminants [17]. Hypertrophy observed in the gills and lamellar fusion have also been reported by [18] in their studies with Liza saliens (Risso) and Piaractus brachypomus (Cuvier), respectively the author stated that changes in the lamellar epithelium constitute a defense mechanism that increases the diffusion distance between blood and contaminants in water and that these changes affect epithelial permeability, which in turn affects osmoregulation. The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants [19]. The histopathology of gill in control fish, the structure of the gill bearing four pairs of gill lamellae and both the sides were supported by bony structure and primary lamellae. The secondary lamellae showed numerous channels of blood capillaries, each separated by single layered pillar cells when observed in vertical section. The laminar epithelium was thick followed by basement membrane below which the pillar cells enclosed blood spaces, large number of mucous cells were present on the epithelial gill rackers, where asprimary lamellae had comparatively small and less number of mucous cells.

4. Conclusion
The mode of action of electroplating effluents leading to varied effects on different tissues of the fish, *Catla catla*. The severity of damage of organs depends on the toxic potentiality of a compound present in the electroplating effluent. Thus, when fish is happened to exposed to effluent, they cause irreparable architectural changes in the vital organs like gill making the fish less fit for better survival. These histopathological changes can alter various physiological activities of fish such as release of various enzymes and consequently metabolism is affected.

6. Acknowledgements
The authors are grateful to Department of Zoology, Kongunadu Arts and Science College for guiding and providing necessary help for conducting this research studies.

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