



## Research Article

**Sugar Mill Effluent Induced Histological Changes in Gill of *Channa punctatus***

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**Abstract:** Pollution is an undesirable change in surrounding environment which affects human life in many ways. We tried to control these factors to improve our living quality. However, many times, the pollution is due to such reasons which cannot be avoidable like daily need product preparation plants. Sugar is a part of our life now. Sugar mills produce large quantities of undesirable byproducts which pollute our surroundings. Finally, these pollutants go to water bodies and pose effect on aquatic organisms. Keeping these points in view, the effect of sugar mill effluent is observed on gill histology of freshwater fish *Channa punctatus*.

**Keywords:** Histological analysis, *Channa punctatus*, LC<sub>50</sub>, Sugar mill effluents.

## 1. Introduction

Histological biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in that ecosystem. The alterations in cells and tissues in fish have recurrently used biomarkers in many studies as such changes occur in all the invertebrates and vertebrates inhabiting aquatic basins. Histological biomarkers embody tissue lesions arising as a result of a previous or current exposure of the organism to one or more toxins. Sugar mills are associated with effluent characterized by biological oxygen demand and suspended solids, the effluent is high in ammonium content. India is the largest producer of sugar in the world and per capita consumption of sugar in the country is 13.4/kilograms per annum, there are about 500 operating sugar mills, located mainly in the state of Uttar Pradesh, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. The Chhata Sugar Mills (Mathura) have been taken as a case study for this study purpose to justify the pollutional standards. *Channa punctatus* (Bloch.) is an easy handling fish, it is easily available and can be maintained in laboratory aquaria. It is highly sensitive to less amount of any toxicant or pollutant, thus it is selected as a model for the present study. Hence it is necessary to explore the toxic effects of Chhata sugar mill effluents on gill histology of fish *Channa punctatus* (Bloch.).

## 2. Material and Methods

### 1.1 Experimental Fish


The air-breathing teleost *Channa punctatus* (Bloch.) have been selected for the present investigation. Fishes were collected from Government Fish Farm, Laramada village, Agra and other local freshwater resources. The experiments were done at Research Laboratory of Zoology Department, Agra College, Agra.

### 1.2 Maintenance and Feeding of Experimental Fish

The experimental fishes *Channa punctatus* (Bloch.) were kept in clean large glass aquaria measuring 75cms X 37.5cms X 37.5cms. The water, used for keeping fishes, was stored before one week to remove unfavourable gases. Dechlorinated water was used throughout the experiment. Fishes were kept in aquaria at the temperature ranging from 30°C to 35°C. The experimental fishes were acclimatized to the laboratory conditions for one week prior to experiment. The water of aquaria was changed every alternate day. The fishes were fed on readymade fish food. The food was given daily two times and feeding was disrupted 24 hours prior to the experiment.

### 1.3 Experimental Chemical

Sugar mill effluents collected from Chhata sugar Mill, Mathura which contains various organic and inorganic effluents was used for the histochemical experimentation.

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**1.4 LC<sub>50</sub> determination**

In order to estimate the LC<sub>50</sub>, the fishes of different experimental sets have been treated with different concentrations of Sugar mill effluent. Five concentrations 100ml/25L, 200ml/25L, 300ml/25L, 400ml/25L and 500ml/25L have been selected and for each concentration, the mortality number of fishes at different time intervals viz. 24 hrs, 48 hrs, 72 hrs and 96 hrs (Table 1).

**1.5 Tissue Collection**

The control and experimental fish; *Channa punctatus* (Bloch.) were killed under light chloroform anesthesia. They were dissected carefully and the brain was taken out for histological examination accordingly.

**1.6 Histological Study**

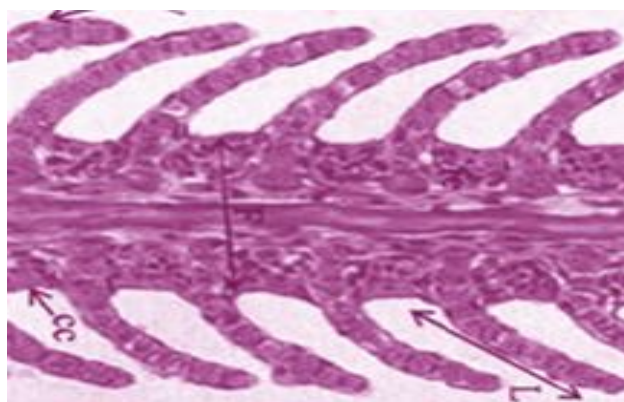
All the tissues were fixed in the Bouin’s solution. After washing and dehydration, the tissues were embedded in paraffin wax. The sections were cut at 5 microns and stained with haematoxylin and eosin (Humason, 1979). Sections were examined under trinocular research microscope and photomicrographs were taken.

**3. Results and Discussion**

The calculated value of LC<sub>50</sub> was 257.59ml/25L for *Channa punctatus* (Bloch.). The sublethal concentration for treatment was 1/10th of LC<sub>50</sub> i.e. 25.75ml/25L.

**Table 1. Mortality of *Channa punctatus* (Bloch.) at different time intervals after treatment with different concentrations of sugar mill effluents.**

S. No.	Concentration (ml/25L)	No. of fishes	24 hrs	48 hrs	72 hrs	96 hrs
1	100	10	0	0	0	0
2	200	10	0	1	1	3
3	300	10	0	2	3	6
4	400	10	1	3	5	8
5	500	10	1	4	7	10



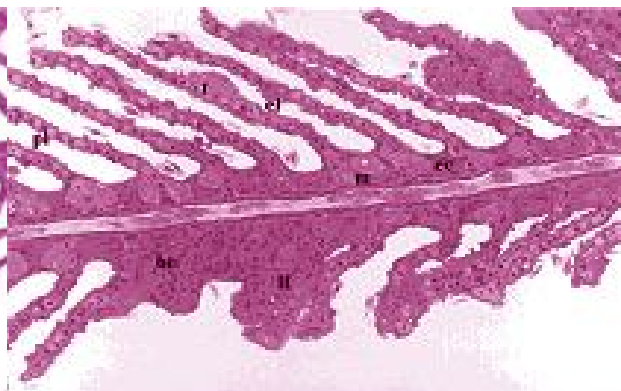
**Plate 1a. Control**



**Plate 1b. 24 hrs**



**Plate 1c. 48 hrs**



**Plate 1d. 72 hrs**



Plate 1e. 96 hrs



Plate 1f. 1 week

(T-Tumour; EP-Epithelium; LF-Lamellar fusion; SL-Secondary Lamellae; PL-Primary lamellae; AN-Aneurysm; ES-Epithelial Separation; M-Mucous Cell.)

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads. A histological investigation may, therefore, prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem. Histological study of the gills shows a typical structural organization of the lamella in the untreated fish. However, fish exposed to sugar mill effluents show several histological alterations, such as; lamellar epithelium lifting, epithelial proliferation, lamellar axis vasodilation, edema in the gill filaments, fusion of lamellae and lamellar aneurysm.

Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals present in this type of effluents. The results of this study confirm the occurrence of edema due to independent exposure of fish to sugar mill effluents, also in other fish species (Bury *et al.*, 1998). The lifting of lamellar epithelium is other histological change observed, probably induced by the incidence of severe edema in gills, also reported by Arellano *et al.*, (1999); Pane *et al.*, (2004) and Schwaiger *et al.*, (2004). Our results have also been supported by Forlin *et al.*, (1995) who worked on kraft mill effluent contaminated fish. These studies are in accordance with the present observation in *Channa punctatus* (Bloch.). Gill edema with lifting of lamellar epithelium could serve as a mechanism of defense because separation of the epithelia of the gill lamellae increases the distance across which waterborne pollutants can diffuse to reach the bloodstream. Cell proliferation with thickening of gill filament epithelium is one histological change found in fish exposed to intoxicant by Arellano (1999) which may lead to the lamellar fusion as observed in this study. Such results were also found in fish exposed to other pollutants Randi *et al.*, (1996); Van den Heuvel *et al.*, (2000) and Rosety-Rodríguez *et al.*, (2002), they reported that

edema in epithelial lifting, as well as lamellar fusion, are defence mechanisms in fish that reduce the branchial superficial area in contact with the external environment.

The changes in gill tissues found in the present experiments were mild to moderate congestion of the primary lamellae and hyperplasia of branchial plates. The changes were indicative of diminished oxygen supply to the test fish, resulting in hypoxic respiratory responses. Although not lethal, gill damage caused by environmental pollutants is important from the aspect of morbidity as it retards growth and affects reproduction.

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