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EFFECT OF UNTREATED EFFLUENT ON HISTOPATHOLOGICAL CONSTITUENTS OF FRESH WATER FISH, TILAPIA OREOCHROMIS MOSSAMBICUS

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ABSTRACT

The physicochemical parameters (pH,BOD, COD, TDS, TSS, total chromium and Hexavalent chromium) of untreated tannery effluent and its effects on the histoptathological constituents present in gills, liver and muscle of *tilapia* were studied. The results of analysis of physico chemical parameters of untreated tannery effluent revealed that the parameters were found to be higher than the permissible limits of CPCB(1995) thereby indicating the high pollution potential of the tannery effluent. LC50 analysis was conducted for different lengths of time of at various concentrations. Histopathological observations were made on gills, muscle and liver tissues of tilapia fish at two intervals of 15 and 30 days. The gills of fish exposed to untreated tannery effluent for 30 days resulted in histopathological changes such as fusion and shortening of secondary lamellae and epithelial necrosis. In liver, it showed severe disorganization of hepatic cells, damaged cell membrane, necrosis, sinusoids were observed. Likewise, In muscle, there was vacuolar degeneration of muscle bundle with aggregation of inflammatory cells, atrophy of muscle bundles and splitting of muscle fibres.

KEYWORDS: ***Tannery effluent, Tilapia, physicochemical parameters, LC50, histopathology.***

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INTRODUCTION

Industrialization is an important tool for the development of any Nation. With the rapid growth of industries in the country, pollution of natural water body with industrial waste has been increased tremendously[1]. The pollution of water courses is due to the discharge of waste water from industries such as tanneries, pulp, paper, textile, petroleum, and chemical industries[2]. Leather production is a major industry in India which makes a significant contribution to the earning countries foreign exchange. During the process of leather making, several chemicals like $Cr(SO_4)_3$, NaCl, H_2SO_4 etc., are extensively used. Therefore the resultant effluent is enriched with chromium and salts. Chromium as an inorganic pollutant is a transition metal and exists in several oxidation states, with trivalent Cr^{3+} and hexavalent Cr^{6+} species being the most common forms. Furthermore when the two species of chromium (trivalent and hexavalent) are compared, differences in their chemical properties are observed. Indeed chlorinated phenols (e.g. 3, 5-dichlorophenol) as an organic pollutant associated with the tanning industry have been found to be highly toxic and affect the cellular components of the organism exposed to such waste. Heavy metals present in effluent discharged by the leather industries are the major group of toxic and non degradable substances. On reaching the aquatic ecosystem, they pose a serious threat to the aquatic species especially fish with altering physicochemical characteristics of water and production food products of fish. Tannery waste water is generally treated by various physico-chemical and biological methods and by a combination of both. Physical and chemical processes are frequently employed to treat contaminated sites, but often do not destroy contaminants.Tilapia has typically laterally compressed, deep bodies. Like other species, their lower pharyngeal bones are fused into a single tooth-bearing structure. A complex set of muscles allows the upper and lower pharyngeal bones to be used as a second set of jaws for processing food, allowing a division of labor between the "true jaws" (mandibles) and the "pharyngeal jaws". This means they are efficient feeders that can capture and process a wide variety of food items[3]. Their mouths are protruisible, usually bordered with wide and often swollen lips. The jaws have conical teeth. Typically tilapia have a long dorsal fin, and a lateral line which often breaks towards the end of the dorsal fin, and starts again two or three rows of scales below. Other than their temperature sensitivity, tilapia exist in or can adapt to a very wide range of conditions. Fishes have a great significance in the life of mankind, being a most important source of protein and providing other useful products. The need for fish arises from the need for protein in diet. Most of the research work has been carried out so for using different effluents on aquatic species except tannery effluents on fish. Keeping this in view, a preliminary work has planned and executed to analyse physicochemical parameters of untreated tannery effluent and also to study the histopathological changes that occurs in different organs (gills, muscle and liver) of fish, *Tilapia (Oreochromis mossambicus*) by exposing to untreated tannery effluent.

MATERIALS AND METHODS

Sample collection

The untreated tannery effluent was collected from CETP(Common Effleunt Treatment Plant) Pallavaram, Chennai in 5L polythene containers and brought to the laboratory with due care and stored in refrigerator for further analysis. The physicochemical parameters such as colour, odour, pH, Total suspended solids (TSS), Total dissolved solids(TDS), Biochemical oxygen demand (BOD), Chemical oxygen demand (COD) and chromium of untreated tannery effluents were determined by following the standard methods[3]. Healthy fish, Tilapia*, Oreochromis mossambicus* were collected from the local lake at Kolathur, Chennai, Tamil Nadu. They were brought to the laboratory and acclimatized under lab condition for a period of three weeks and fed *ad libitum*. The fishes were maintained in plastic tubs and disinfected with potassium permanganate solution and washed thoroughly prior to induction of fishes to prevent fungal infection. Feeding was stopped 24 hrs before commencement of the toxicity test to keep the animals more or less in the same metabolic state. Healthy adult tilapia around 50-55 grams in weight and about 10-12cm in length were chosen. The fishes were examined carefully for any pathological symptoms. Ten fishes were introduced into plastic tubs containing 100% untreated tannery effluent and this experimental set up was left undisturbed for 24hrs, after which different organs such as gills, liver and muscle were dissected out after exposure and fixed in bouins fluid. They were then passed through graded series of alcohol and xylene and serial sections of the organs were prepared and stained with Haematoxylin and counter stained with Eosin. The histological changes in the tissues were observed comparing with control tilapia fish tissues.

Experimental design

Tilapia fish were incubated at concentration of 32 ppm of untreated tannery effluent as shown in Table-1 separately for 15 days and 30 days respectively and histopathological analysis was carried out on gills, muscle and liver tissue to reveal the significant morphological alterations.

GROUP 1*:* Control fishes maintained in dechlorinated and toxicant free water for 15 and 30 days.

GROUP 2: Fishes maintained at 32 ppm of untreated effluent for 15days.

GROUP 3: Fishes maintained at 32 ppm of untreated effluent for 30days.

Determination of lethal concentration (lc50) of tannery effuent

In the present investigation LC50 was determined and the values are influenced by the age, sex, health of the animal and even laboratory conditions. Each group containing 10 fishes was incubated in various concentrations of untreated tannery effluents and the results were observed at different time periods as shown in the Table-1.

Table 1
LC50 Analysis Of Tannery Effluents

Conc. in ppm	% Mortality at 24Hrs	% Mortality at 48Hrs	% Mortality at 72Hrs	% Mortality at 96Hrs
	R	R	R	R
10	-	-	-	-
20	-	-	10	20
30	-	-	10	20
40	-	30	30	60
50	10	40	50	80
60	30	50	70	100

R - Raw effluent

The results of the analysis of physicochemical parameters of untreated tannery effluent is depicted in Table-2.

Table 2
Physicochemical Parameters of Untreated Tannery Effluents

Sr. No	Parameter	Untreated Tannery Effluent	Permissible limits as per CPCB 1995
1	BOD mg/L (3 days at 27^0c)	830	100
2	pH	6.33	6.0-9.0
3	COD mg/L	2576	250
4	Total Dissolved Solid mg/L	5244	2100
5	Total Suspended Solid mg/L	96	100
6	Total Chromium as Cr mg/L	22.3	2
7	Hexavalent Chromium as Cr^{6+} mg/L	5.92	0.1

RESULTS AND DISCUSSION

The result revealed that untreated tannery effluent is slightly brownish in colour and odour is little offensive. The colour and odour could be due to decomposition of organic or inorganic matter[4]. A large number of pollutants can impact colour and odour to the receiving water and making them unaesthetic and unfit for domestic consumption[5]. The pH of untreated tannery effluent is 6.33 indicating acidic in nature. Discharge of such effluent with acidic pH into ponds, river etc., for irrigation may be detrimental to aquatic biota such as zooplankton and fishes.TSS level of untreated tannery effluent is 96 mg/L indicating that the value of TSS is compared to the permissible limit (100mg/L) as prescribed[6] for effluent discharge. The amount of TSS elevated the density and turbidity of water there by affects osmoregulation and interferes with photosynthesis by preventing sunlight.[7]TDS of untreated tannery effluent is 5244 mg/L and this value is higher than the permissible limits[6]. The high levels of TDS recorded in the present study may be due to high inorganic salt contents present in the untreated tannery effluent and also renders it unsuitable for irrigation hence further treatment or dilution would be required.[5,8]BOD level of untreated tannery effluent is 830mg/L which is higher than the_permissible limit[6]. Increase in BOD which may cause hypoxia conditions and leads to adverse effects on aquatic biota. High BOD level has been reported in tannery effluent[9] and in fertilizer effluent[10].The present study showed high levels of COD 2576 mg/L and this value of untreated tannery effluent is beyond the permissible limit[6]. This indicates that the effluent is unsuitable for the existence of aquatic organisms due to the reduction of DO content[11].Chromium level of untreated effluent is 22.3 mg/L which is higher than the permissible limit[6]. The effects of untreated tannery effluent on histopathological constituents of gills, liver and muscle for 15 days and 30 days are studied.

Gills

The gills are important organs for respiration, osmoregalation, acid-base balance and nitrogenous waste excretion[12]. Gills are generally considered as good indicators of water quality, being used as models for studies of environment impact, such as xenobiotic agents. Gills are the first target of water borne pollutants due to constant contact with the external environment[13]. The gills of control fish is depicted in Figure 1. The gills of fish exposed to 32 ppm of untreated tannery effluent for 15 days in the present study resulted in histopathological changes such as fusion of secondary lamellae (FSL), shortening of secondary lamellae (SSL) epithelial necrosis and narrowed water channels (WC) in the gills as shown in the Figure 2, which is in correlation with the observations of different fresh water fish exposed to copper[14-17]

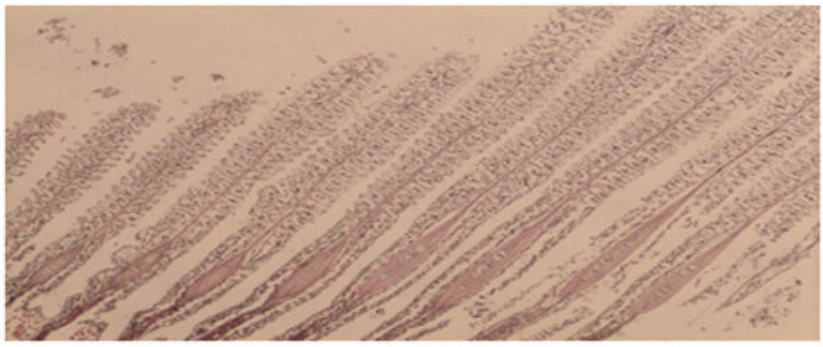

Figure 1
Photomicrograph of Gill of Control group fish(x40), Stain with H&E

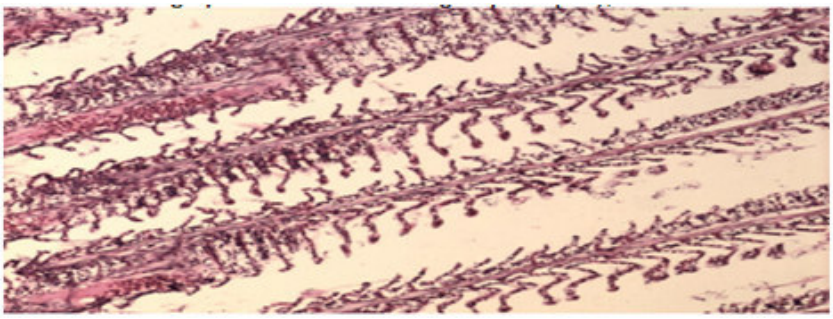

Figure 2
Photomicrograph of Gill of fish exposed to 32ppm untreated tannery effluent for 15days(x40)

The gills of tilapia fish exposed to 32ppm for 30days is shown in Figure 3. Histopathological observations such as hyperplasia (H), degenerative epithelium (DE), necrosis (N), fusion of secondary lamellae (FSL) were observed the gills of tilapia fish. The observed histopathology of gills correlated with many of the previous investigations. Reduction in size and shape of nucleus with degenerative changes in parenchyma cells with necrosis were observed in Cyprinus carpio due to heavy metals[18].

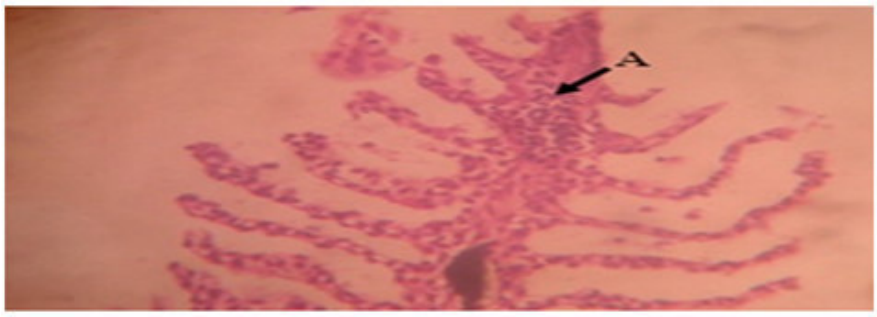

Figure 3
Photomicrograph of Gill of fish exposed to 32ppm untreated tannery effluent for 30days(x40) aneurysm(A) in the primary lamellae

The pathological changes may be in response of the fish gills to toxicants intake or adaptation response to pollutants[19].The histopathological changes of the gills likely resulted hypoxia, respiratory failure problems with ionic acid base balance[20,21]. WHO reported necrosis, degeneration of secondary lamellae, in the gills of Lapistes reticulates exposed to cyphenothrin. The observed histopathology of gills in the present studied tilapia fish exposed to tannery effluent correlated with the gills of silver carp, *Hypophthmichthys molitrix* exposed to nickel chloride[22]. These pathological changes may be a response of the fish gills to toxicants intake or an adaptive response to pollutants and may be due to increased capillary permeability[23].

Liver

The liver is associated with the detoxification and biotransformation process and blood supply and it is also of the organs most affected by contaminants in the water[24]. Liver has an important detoxification role of endogenous waste products as well as externally derived toxic chemicals and heavy metals[25-26].The control group of liver of fish is depicted in Figure 4.

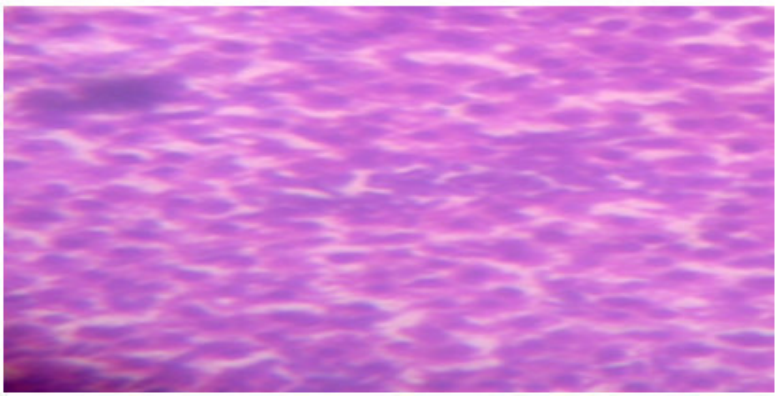

Figure 4
Photomicrograph of Liver of Control group fish (x40), Stain with H&E

Histological changes were observed in the liver tissue of *tilapia* fish exposed to 32ppm of untreated tannery effluent for 15days was the focal area of necrosis and degenerative nuclei as depicted in the Figure 5.

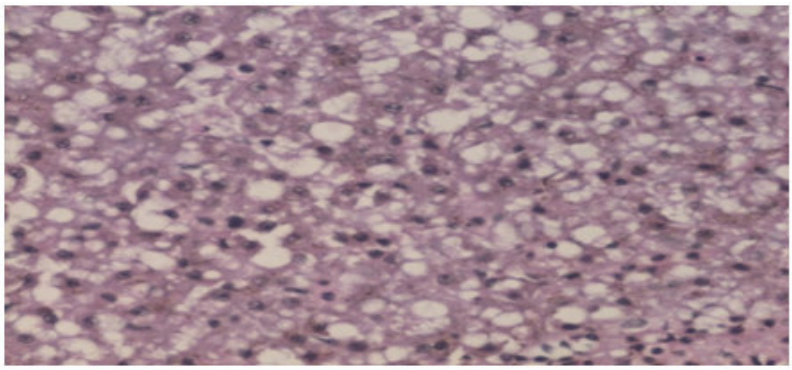

Figure 5
Photomicrograph of Liver of fish exposed to 32ppm of untreated tannery effluent for 15days(x40)

Histopathological alternations were observed in the liver of *tilapia* fishes exposed to 32ppm of untreated tannery effluent for 30days with necrosis and cytoplasmic vacuolation were shown in the Figure 6.

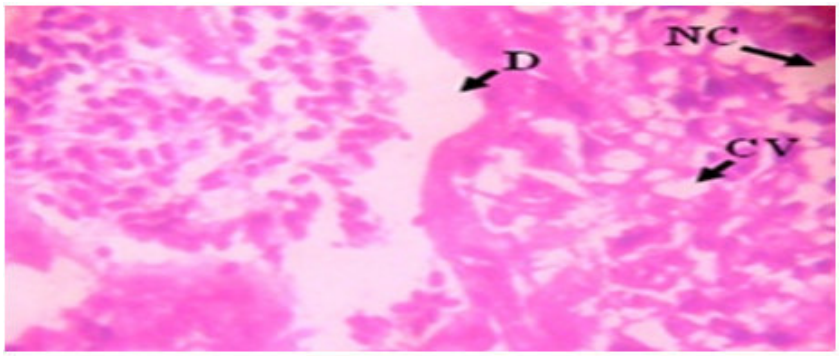

Figure 6
Photomicrograph of Liver of fish exposed to 32ppm of untreated tannery effluent for 30days (x40) necrosis (NC), cytoplasmic vacuolation (CV) and vascular dilation (D)

Vacuolar degeneration in the hepatocytes, focal area of necrosis and aggregation in inflammatory cells between hepacocytes, dilation and congestion in blood sinusoids and intravascular hemolysis in hepatic blood vessel, observed in the tilapia zilli and solca vulgaris from the lake qarun contaminated with different pollutants[27]. This may be attributed to degeneration and necrosis of the hepatocytes due to cumulative effect of heavy metals in the tannery effluent. The alteration in the liver tissue is often associated with degenerative necrotic condition. With exposure to tannery effluent, the present studied liver showed severe disorganization of hepatic cells, damaged cell membrane, necrosis, sinusoids with congestion is in accordance with the observed histopathological changes in liver[28].

Muscle

The control muscle tissue is depicted in Figure 7. The pathological alterations were observed in the present studied tilapia fish at 32ppm concentration for 15days and 30 days exposure.

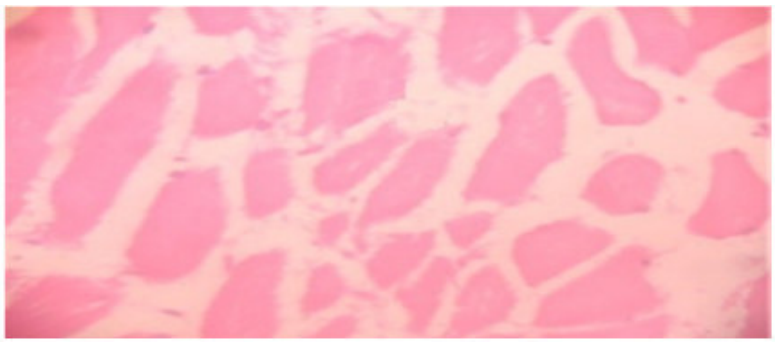

Figure 7
Photomicrograph of muscle of Control group fish (x40) Stain with H&E

The histological alterations such as splitting of muscle bundle and degeneration of muscle bundles is shown in Figure 8 at the concentration exposed to 32pmm of untreated tannery effluent for 15days.

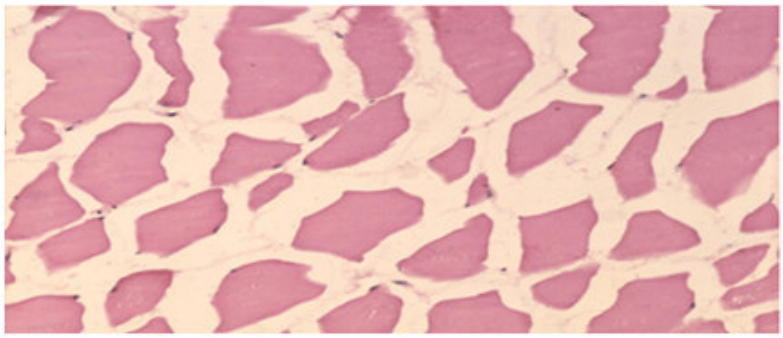

Figure 8
Photomicrograph of muscle of fish exposed to 32ppm of untreated tannery effluent for 15days (x40)

The histological alterations such as vacuolar degeneration necrosis and atrophy of muscle tilapia fish exposed to 32ppm of untreated tannery effluent for 30days as shown in Figure 9.

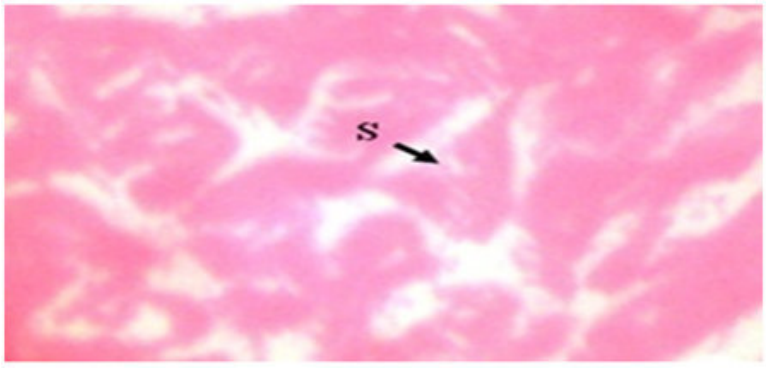

Figure 9
Photomicrograph of muscle of fish exposed to 32ppm of untreated tannery effluent for 30 days (x40) splitting of muscle fibres (S)

The pathological alterations observed in the present studied fish were in agreement with observations in fish muscle due to the exposure of different pollutants[29-30].Destruction and vacuolation of muscle cells was observed in *Oreochromis* species exposed to chromium[25]. Degeneration of muscle bundle with aggregation of inflammatory cells between them and focal area of necrosis, vacuolar degeneration in muscle bundles and atrophy of muscle bundles, splitting of muscle fibers were observed in muscle of tilapia zilli and Solea vulgaris from lake Qarun which is contaminated with different contaminants[27].

CONCLUSION

Thus from the results of the present study it can be inferred that the data obtained for physicochemical parameters such as PH, BOD, COD,TDS, TSS are higher, suggesting that untreated tannery effluent should be treated or it should be diluted before disposal, so that it does not hamper the aquatic organisms which may affect the food chain. Hence from the result of the present study, it can be inferred that the histopathological alterations were observed in the gills followed by liver and muscle tissues compared to that of control tissues. This could be of the fact that gills are the first organ which comes to contact with the untreated tannery effluent and more over untreated sample could have affected the gills first [31, 32].Histopathological study offers a definitive and promising technique on the aquatic organisms. From the observations made in the present study, extensive use of untreated tannery effluent should be diluted before disposal to the environment.

CONFLICT OF INTEREST

Conflict of interest declared none.

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