RESEARCH ARTICLE

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Toxicological and Histopathological Impacts of Textile Dyeing Industry Effluent on a Selected Teleost Fish *Poecilia Reticulata*

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ABSTRACT
The textile effluent has been proved to impart adverse effects on the aquatic environment and its fauna. The aim of the present study was to determine the lethal concentration of a textile industry effluent to the freshwater fish *Poecilia reticulata* by toxicity bioassay method. The LC_{50} values of the effluent for 6, 12, 24, 48, 72 and 96 hours were found to be 73.081, 67.030, 60.398, 55.521, 49.211 and 39. 726% respectively. The fishes exhibited abnormal behaviors including erratic swimming, hyper-excitation, rapid opercular movement and thick mucus covering which indicated the toxicity of the effluent. The effluent induced histopathological changes also. The primary gill bar was enlarged and secondary gill bar was detached. Liver showed cytoplasmic vacuolation and clustering of nuclei whereas in the intestine disintegration of intestinal villi and infiltration of haemocytes into the lumen was seen.

Keywords: Textile effluent, *Poecilia reticulata*, toxicity bioassay, lethal concentration, histopathological alterations .

INTRODUCTION

Population growth and industrial development are the major causes of contamination of water resources[7]. In developing countries, pollution of water resources has become a serious problem which leads to ecological disorders and causes many physiological as well as biological changes in aquatic animals[21]. The toxic components present in untreated wastewater discharged from textile industries affect the freshwater habitats[2]. This textile wastewater is rich in BOD, COD and suspended solids[33]. In addition, it is rich in starch, bicarbonates, chlorides and metals like copper and chromium which cause mortality as well as sub lethal stress affecting the growth of aquatic organisms. Dyestuff and other chemicals like caustic soda, soda ash, hydrochloric acid and sodium hypochlorite besides exerting severe impact on the soil bio-ecosystem induce mutagenicity and carcinogenicity in human beings also.

Discharge of textile wastewater into aquatic habitats lead to stress and behavioral changes in aquatic organisms, especially in fishes[24]. Fishes live in intimate contact with the aquatic environment and sensitive to a wide variety of toxicants. They serve as valuable models for assessing the effect of various pollutants. A variety of molecular, biochemical and histological responses in fish have been employed as biomarkers of various environmental stresses[12, 16]. There is a trend of using small fish as sentinel vertebrate species for ecotoxicology and biomedical research[15, 34]. To this end, the Zebra fish (*Danio rerio*), fathead minnow (*Pimephales promelas*), mosquito fish (*Gambusia affinis*), guppy (*Poecilia reticulata*) and Japanese medaka (*Oryzias latipes*) have been commonly used as fresh water fish models in ecological studies[9, 20].

Hence an attempt has been made through this study to assess the toxicological and histopthological effects of textile dyeing industry effluent on a selected teleost fish *Poecilia reticulata*.

Materials and Methods

Collection of textile effluent

Untreated textile waste water was collected from a specific point of discharge of a textile industry in Tirupur which uses reactive and dispersive dyes to dye cotton and polyester fabrics. The waste water samples were collected in sterile plastic containers and brought to the laboratory and refrigerated until further use.

Test animal

Adult *Poecilia reticulata* were procured from a private fish farm in Nagercoil. After disinfecting with 0.1% $KMnO_4$ they were kept in aerated tanks for 2 weeks and fed with pelleted fish feed to acclimatize them to laboratory conditions.

Toxicity bioassay

Ten healthy fishes of same size were selected for the toxicological studies. These fishes were introduced into 125 ml cups, which had the effluents at different concentrations ranging from 10% to 100% for 96 hours to determine the mortality percentage. After recording the broad range, the median lethal concentration (LC_{50}) was determined for effluent concentrations of 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80%.

The tests were conducted in triplicate and the effluent media were renewed every 24 hours to avoid depletion of oxygen in the medium. The mortality rate of

P.reticulata was recorded at 6, 12, 24, 48, 72 and 96 hours of exposure to the effluent. The percentage corrected mortality was calculated using Abbott's formula,

Corrected mortality percentage = (Percentage living in control – Percentage living in treatment) / (Percentage living in control)x 100

Probit analysis

The corrected mortality data were analyzed to determine the LC_{50} values (theoretical estimate of the concentration lethal to 50% of the test fishes). The LC_{50} values were obtained by probit regression line, taking test concentrations and corresponding percent mortalities on log value and probit scales respectively. By graphical interpolation LC_{50} values were fixed and their fiducial limits 95% upper (UCL) and lower confidence limits (LCL) were also calculated[10]. Fractions ½ (10.863 ml/dl), ¼ (9.9315 ml/dl) of the mean LC_{50} value (39.726 ml/dl) were used for an exposure period of 96 hours.

Histological studies

At the end of the treatment period, control and treated fishes were dissected. The gills, liver, intestine and gills were separated and fixed in 10% formalin, washed in distilled water, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax at 56-58^{0}C. The serial sections of the tissues were cut at 5-7 micron thickness and sections were transferred to glass slides, coated with glycerin and stained with hematoxylin and eosin. The stained sections were analyzed and studies using photomicrography [Magnus (WH 10 x /20) 528372].

Results

Probit analyses of the toxicity response of *P. reticulata* to the effluent were used to find out the LC_{50} values under their upper and lower confidence limits (Table l).

Concentration	Mortality Percentage					
(in %)	6	12	24	48	72	96
20						
25						10
30						20
35					10	30
40					20	40
45				10	30	60
50			20	30	50	80
55		10	30	40	60	90
60	10	20	40	70	80	100
65	20	30	60	80	100	
70	40	60	80	100		
75	60	80	100			
80	70	100				

Table 1:- Mortality response of P. reticulate exposed to varying concentration of textile effluent

After 6 hours of exposure to the effluent, 10% mortality of *P. reticulata* was recorded at an effluent concentration of 60 ml/dl. The LC_{50} value for 6 hours of exposure was 73.081, the LCL was 0.8941 and UCL was 0.2765.

In *P. reticulata* exposed to the effluent for 12 hours, 10% mortality was recorded in the concentration of 55ml/gl, 20% in 60ml/dl, 80% in 75ml/dl and 100% in 80ml/dl. The LC_{50} value was 76.030. In *P. reticulata* exposed to the effluent for 24 hours, 20% mortality was recorded in the concentration of 50ml/dl, 40% in 60ml/dl, 80% in 70ml/dl and 100% mortality in 75ml/dl. The LC_{50} value was 60.398.

After 48 hours of exposure to effluent, 30% mortality was seen in 50ml/dl, 70% in 60ml/dl and 100% in 70ml/dl. The LC_{50} value was 55.521. In *P. reticulata* exposed to the effluent for 72 hours, 10% mortality was recorded in the concentration of 35ml/dl, 30% in 45ml/dl, 60% in 55ml/dl and 100% in 65 ml/dl concentration. The LC_{50} value was 49.211. In *P. reticulata* exposed to effluent for 96 hours, 20% mortality was seen in 30ml/dl, 40% in 40ml/dl, 60% in 45ml.dl, 90% in 55ml/dl and 100% in 60ml/dl concentration. The LC_{50} value was 39.726 (Table 2).

Hours of exposure	LCL	LC_{50}	UCL
6	0.8941	73.081	0.2765
12	0.2565	67.030	0.2664
24	0.2437	60.398	0.2574
48	0.2349	55.521	0.2482
72	0.2195	49.211	0.2370
96	0.1923	39.726	0.2149

Table 2:- nhr LC50 and confidence intervals for the fish P. reticulata exposed to textile effluent

The fishes exposed to ½th and ¼th of 96 hour LC_{50} values showed significant histopathological changes. Liver of control fish generally exhibited a normal architecture, with hepatocytes presenting a homogenous cytoplasm, a large spherical nucleolus and variable amount of dispersed and peripheral heterochromatin (Plate 1).

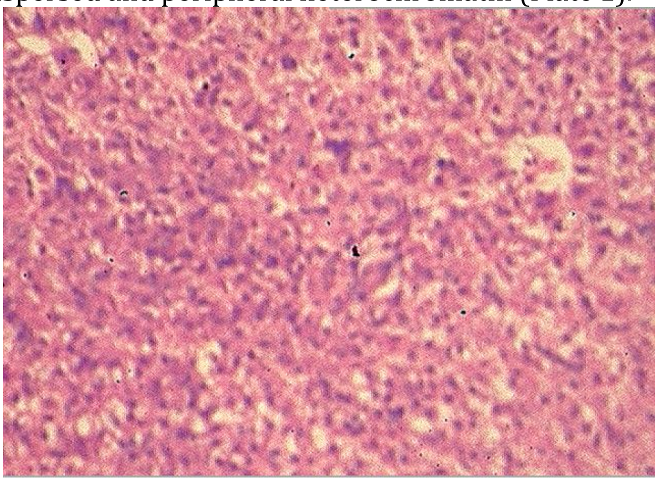

Plate 1 Liver of *P. reticulata* showing normal hepatocyte (×100)

Hepatocytes were located among blood capillaries called sinusoids forming cord-like structure kupffer cells are found on the luminal surface of the sinusoid endothelium.

The liver of fishes exposed to ¼th of 96 hour LC_{50} value of the effluent concentration showed hepatocytes with nuclear pycnosis and haemocyte infiltration. In addition, the liver of treated fish also showed vacuolar degenerative changes with congestion and dilation of sinusoids. The gills showed visible alterations in the histology after exposure to sub lethal concentrations of the effluents. Intestine of *P. reticulate* exposed to ½th and ¼th of LC_{50} value of the effluent for 96 hours showed degeneration and disintegration of villi and infiltration of haemocyte into the lumen (Plates 7 and 8).

Plate 7 Intestine of *P. reticulata* showing normal villus

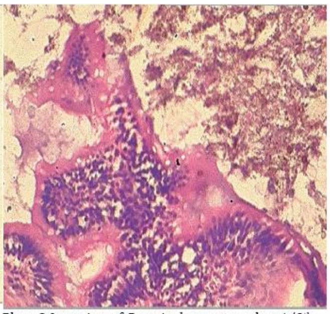

Plate 8 Intestine of *P. reticulata* exposed to 1/2th 96 hour LC_{50} concentration of effluent showing degeneration and disintegration of villi and infiltration of haemocyte into the lumen (×400)

Discussion

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 lethal concentrations of the effluent, they showed abnormal movements and behaviour including erratic swimming, hyper excitation, rapid opercular movements and thick mucus covering over the body surface. Similar results were observed when *Labeo rohita* and *Channa punctatus* were exposed to paper mill effluent[32]. Industrial effluents affect the normal vision, proper body motion and behavior of fishes[5]. Hyper activation in *Oreochromis niloticus* was noticed when exposed to textile industry effluent[2]. Various stressful behaviors like erratic swimming, increased activity, inconsistent jumping were observed in *Cyprinus carpio* exposed to both influent above 25% concentration and effluent above 50% concentration[29].

At 90% effluent concentration when exposed to 96 hours, the fishes started to show escaping refluxes within 24 hours of exposure and they became shaggy and started coming to the surface. At this concentration, 100 percent of the fishes died within 48 hours of exposure. When exposed to the concentrated effluent, 100 percent mortality of the fishes was recorded within 6 hours of exposure. It was noticed that at this concentration, a sudden stress was laid on the animal which resulted in erratic swimming, convulsion, jerky movement and rapid opercular movements. The fishes struggled hard for breathing and tried to leap out of the toxic medium. They tried to engulf atmospheric air and thick mucus covered the body surface. Hyper activity characterized by erratic and short darting movements was generally observed across all exposure concentrations.

These behavioral responses of fish is in response to toxicants present in the sample at different duration of exposure and the prevailing environmental conditions[6]. This also signifies respiratory impairment, an outcome of the impact of wastewater on the gills of fish[3]. There was a gradual loss of equilibrium and eventually 100% mortality occurred at 96 hours for higher effluent concentration of 60%. This could be a consequence of depletion of energy in the body of the exposed animals and an indication of impairment of carbohydrate metabolism wherein organisms that could not tolerate the contaminants enter into a state of coma and subsequent death. Similar observations were reported in juveniles of African cat fish *Clarias geriepinus* when exposed to 70 % detergent effluent[24].

The percentage mortality increased with increasing concentration and increased period of exposure. The results of mortality analysis of influent against *Cyprinus carpio* yielded the derived toxicity indices values (LC_{50}) ranging from 11.11% (v/v) at 96 hr to 25.90% (v/v) at 24 hr, while for the final treated effluent, the values ranged between 36.04% (v/v) and 63.18% (v/v) at 96 and 24 hr respectively[29]. The lethal concentrations (LC_{50}) were inversely proportional to duration of exposure. The mortality rate of *C. carpio* remained directly proportional to duration of exposure, concentration and toxicity factor in cat fish hybrid[11, 27]. The mortality induced in the aquatic fauna by the effluent might not be only due to chloro – lignin and other compounds such as chlorinated resin acids and terpenes but also due to non-specific acids present in the effluents[30].

In this investigation, *P. reticulate* exposed to ½th and ¼th of LC_{50} value for 96 hours showed marked variations in their histological parameters. Histological changes in animal tissues are powerful indicators of prior exposure to environment stressors and are the net result of adverse biochemical and physiological changes in an organism. Histopathology is often the easiest method for assessing both short and long term effects[15]. It provides a rapid method to detect effects of irritants on various organs[17].

The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Liver of fishes exposed to ½ th 96 hour LC_{50} value showed cytoplasmic vacuolation, clustering together of nuclei and hepatocyte degeneration (Plates 2 and 3).

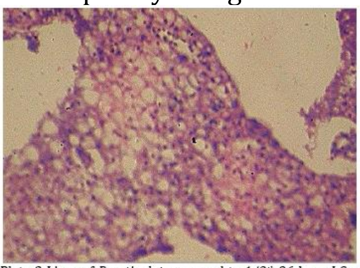

Plate 2 Liver of *P. reticulata* exposed to 1/2th 96 hour LC_{50} concentration of effluent showing cytoplasmic vacuolation, clustering of nuclei and hepatocyte degeneration (×100)

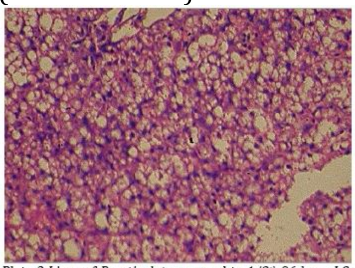

Plate 3 Liver of *P. reticulata* exposed to 1/2th 96 hour LC_{50} concentration of effluent showing cytoplasmic vacuolation, clustering of nuclei and hepatocyte degeneration (×400)

Small vacuoles were noticed in the hepatic cells of brown trout fry and adult guppies exposed to 0.0032-3.2 pm DDT[19]. Extensive degeneration of cytoplasm with pyknosis were observed in the liver tissue of *Heteropneustus fossilis* when subjected to acute thiodon toxicity[18, 23]. It appears to be a general feature of the liver of intoxicated fish that the degree of structural heterogeneity is enhanced with increasing concentration of the toxicant[14]. Increase in liver size, degeneration of hepatocytes with cytoplasmic vacuolation and hypertrophy were noticed in P. reiculata exposed to 96 hrs at higher concentrations of textile effluent. Similar observations were recorded in gourami fish (*Trichogaster trichopterus*) upon exposure to 0.30 mg/l concentrations of paraquat[4].

Gill is a suitable organ for histological examination for determining the effect of a pollutant[12]. Gill covers more than 60% surface area of the fish and its external location renders it the most vulnerable target organ for pollutants[26]. Toxic substances can disturb the osmoregulatory function of aquatic organisms[28,1]. The rupture and necrosis of gill epithelium may be due to the deleterious effect of the low pH of the effluent. However hyperplasia, hypertrophy, lamellar fusion, mucus secretion and sloughing of gills may be the defence responses of the fish to the effluent toxicity[2, 22] (Plates 4, 5 and 6).

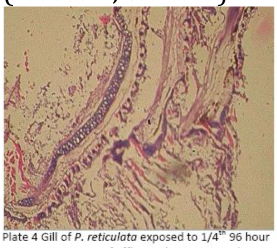

Plate 4 Gill of *P. reticulata* exposed to 1/4th 96 hour LC_{50} concentration of effluent showing vacuolation and enlargement of primary gill bar, detachment of secondary gill lamellae from gill bar (×100)

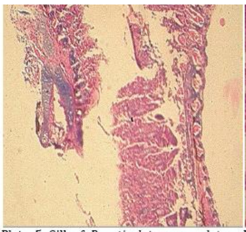

Plate 5 Gill of *P. reticulata* exposed to 1/2th 96 hour LC_{50} concentration of effluent showing lamellar degeneration and blood accumulation (×100)

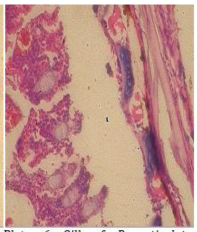

Plate 6 Gill of *P. reticulata* exposed to 1/2th 96 hour LC_{50} concentration of effluent showing lamellar degeneration and blood accumulation (×400)

Fish exposed to paraquat showed hyperplasia of gill lamellae and increased thickness of the gill lamellae resulting in fusion and necrosis[4].

In the control fishes there is a typical structural organization of the parechymatous cell appearance of the hepatocytes. The histological abnormalities observed in fishes exposed to effluent were oedema, cellular infiltration and necrosis which showed a progressive architectural distortion. The vacuolization of heptocytes might indicate an imbalance between the rate of synthesis of substances in the Parenchymal cells and the rate of their release into the circulation system[13, 31]. In the present study the muscle of the fish also showed necrotic and degenerative changes when exposed to sublethal concentration of the effluent due to acute toxicity exerted by the effluent on the muscle cells.

As with gills, skin and muscle tissue come in close contact with pollutants dissolved in water. Hence, reactions in the muscle cells were spontaneous. The fishes showed an increased level of mucous over the body which was reflected by the hyperplastic glandular cells in the dermis of exposed fish. The appearance of inflammatory cells indicated secondary defence mechanism in the body of fish. Separation of muscle bundles and intracellular oedema were noticed in *Labeo rohita* exposed to hexachlorocyclohexane[8].

The present study has been able to establish the fact that exposure of *P. reticulata* to even sublethal concentrations of the textile effluent can induce various toxicological effects and histological degradations which depend on the period of exposure and concentration of the toxicant. In view of the toxicity effect of this effluent, it can be inferred that indiscriminate discharge of textile effluents into natural water bodies can induce damages to the tissues and organs which might make all the living entities in polluted environment vulnerable to diseases, eventually leading to death. Therefore there is need for the adoption of proper treatment of the textile effluents prior to their discharge into the environment.

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