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Research Article

EFFECT OF TEXTILE EFFLUENTS ON DIGESTIVE ENZYME ACTIVITY AND HISTOLOGY OF INTESTINE OF THE FRESH WATER FISH *CYPRINUS CARPIO*

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ABSTRACT

The toxic effect of textile effluent on the digestive enzymes and histology of intestine of *Cyprinus carpio* was studied. The fish were exposed for 10, 20 and 30 days in 10% and 30% sublethal concentrations of 96 h LC_{50} of textile effluent. The amylase, protease and lipase activity was significantly decreased after chronic exposure to textile effluent. These changes occurred predominantly in the 30 days exposure. Several pathological changes were noticed in the intestinal tissues of textile effluent treated fish. In *Cyprinus carpio* treated with textile effluent for a period of 10 days, the changes observed in the intestine of fish were ruptured and shapeless villi, vacuolization, histolysis of columnar cells and loss of brush border. In 20 days treated, fish the lamina propria showed inflammation, necrosis and appeared to be fused with the epithelial layer. At the end of 30 days treatment, several necrotic lesions in the epithelial layer, disintegrated columnar epithelium and loss of brush border were obvious.

Keywords: Textile effluent, Fish, Digestive enzyme activity, Intestine histology.

INTRODUCTION

Industrial effluents such as textile mill, paper, distillery, oil mill and tannery effluents have been a major concern in water pollution' abatement programme in India primarily because of the discharge of large quantities of effluents in the nearby water bodies causing fish mortalilty (Sprague and Mclease, 1968). Effluents from industries are normally considered as the main industrial pollutants containing Organic and inorganic compounds, acids, alkalies, suspended Solids and other materials.

The textile mill effluent contain toxic Chemicals, possible hazards to aquatic organisms associated with this, requires a careful evaluation. To understand the mechanism of action of these toxic substances, the qualitative and quantitative changes in metabolism must be studied at the level of organisms (Metelev *et al*., 1983). In the present work, an attempt was made to evaluate the long term exposure effect of textile effluent on the histology of gill and liver of the freshwater fish *Cyprinus carpio*.

MATERIALS AND METHODS

Animal maintenance

The fish, *Cyprinus carpio* (Weight, 35g; Length 10 cm) were collected from the Chellikuruchi Lake, and it is located in Pudukkottai Ullur near Adirampattinam, Tamil Nadu. They were acclimatized for 15 days in large cement tanks (Temperature – 28 ± 2°C**;** total hardness – 518 ± 23 mg/l; DO - 5.6 ± 0.2 mg/l; salinity - 1.2 ± 0.13 ppt and pH - 7.8 ± 0.04) previously washed with 1% potassium permanganate. The water as renewed every 24 h. The LC_{50} of urea for 96 h was found out by using Probit method (Finney, 1971).

Preparation of stock solution and determination of 96 h LC_{50} value textile effluent

Stock solution of textile effluent was prepared by dissolving 1 ml of textile effluent in an appropriate amount of water (1 ppt). For the determination of median tolerance

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limits or LC_{50} different concentrations of were prepared from the stock solution. Three replicates were maintained for each concentration and 10 fishes of equal size and weight were introduced. The test water was renewed at the end 24 h and freshly prepared textile effluent was added to maintain the concentration of textile effluent at a constant level. The mortality was recorded after 24, 48, 72 and 96 h, and median lethal concentration (LC_{50}) values were calculated by the Finney method (1978). $1/10^{th}$ value of the LC_{50} value for 96 h was taken as the sublethal concentration (Sprague, 1971).

Sublethal studies

For sublethal toxicity tests 80 fishes were selected and divided into four groups (one control and three experimental) with 20 fish in each aquarium filled with water. The desired concentration (1/10 and 1/30 of 96h LC_{50}) of the toxicant was added directly in order to maintain constant concentration of the toxicant. The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. At the end of the stipulated periods (10^{th}, 20^{th} and 30^{th} day) of exposures fish were randomly selected and sacrificed for histological studies.

Estimation of digestive enzymes

Amylase and protease activities were assayed according to the Ishaaya (1986) methods and Lipase enzyme activity was assayed by Myrtle and Zell (1975) method.

Histology

On 10, 20 and 30^{th} day fish were taken out, sacrificed and the intestine was excised out. The tissues was fixed in Bouin's fluid and then they were processed (Gurr, 1959) and embedded in paraffin wax (58-60°C). Serial sections of 8 µm thickness were cut and deparafinshed sections were stained in haematoxylin and counterstained with aqueous eosin. The stained slides were examined for histopathological changes and were photomicrographed.

RESULTS AND DISCUSSION

The effect of textile effluent on the digestive enzymes of fresh water fish, *Cyprinus carpio* was studied. The amylase activity was significantly decreased after chronic exposure to textile effluent (Table 1). The amylase activity in the control fishes was 6.52 mg of maltose/g per hr at 37°C temperature. The chronic exposure to textile effluent showed amylase activity of 1.67 mg of maltose/g per hour at the end of 30 days. Masarrate and Lomte (1977) found decrease in amylase activity in *Lamellidens marginalis* and explained that mercuric chloride and copper sulphate are potent inhibitors of amylase activity. Kumar and Bhattacharya (1977) reported marked inhibition of amylase activity of liver of fish, *Anabas testudineus.* Sastry and Malik (1979) observed elevated activity of amylase of stomach, intestine and liver while decrease in enzyme activity in phyloric caeca of the fish *channa punctatus* exposed to 3.1 ppm of diazin for 96 hr. Lomte and Patil (1989) observed a decrease in amylase activity in the army worm, *Mythimna separate* after pesticide treatment.

In the present investigation the decrease in the activity of protease was observed when *Cyprinus carpio* was exposed to textile effluent (Table 2). The protease activity was found to be 6.97 mg of tyrosine/gm of protein/hr at 37°C. Textile effluent showed a decrease in protease activity. Leo and Sabapathy (1990) noted a decrease in protease activity in *perna viridis*.

In the present study, lipase activity was significantly decreased after chronic exposure to textile effluent (Table 3). Patil (1993) observed decrease in amylase, lipase and protease activity in *Lamellidens marginalis* after heavy metal stress. Zambare and Mahajan (2001) reported decrease in lipase activity during heavy metal stress in *Corbicula striatella*. Nalina Sundari *et al.* (1986) reported depletion in the amylase, and protease activity in *spodoptera litura* when exposed to *Vinca rosea* extract. The result of the present investigation are in harmony with the results of some workers like Sontakke (1992) in *Thiara tuberculata*. Bhamre (1993) in *Parreysis favidens*, Jadhav (1993) in *Corbicula striatella*, Patil (1993) in *L. marginalis*.

In the present investigation, depletion in the amylase, protease and lipase activity was observed in *Cyprinus carpio.* The depletion in the enzymatic activity may be due to the damage caused by the textile effluent to the cells of alimentary canal (Sultana and Lomte (1997). Decrease in enzyme activity may be related to hormonal level in the fish body. There might be decrease in the hormonal level which might have suppressed the enzyme activity (Tomake, 1998). The vital activities like energy synthesis and the metabolism are carried out by the biocatalysts namely the enzymes. In the present study a decline in the rate of amylase activity was noticed in *Cyprinus carpio* when exposed chronically to sublethal concentration of the heavy metal textile effluemt. Similar observation was made by Cheng Thomas and Rodrick (1975) in *Crassostrea virginica* and *Mercenaria mercenaria.* The variations in the amylase activity of crystalline style in the short necked clam *Ruditape sphillippinarum* were noted by Hara Kenji *et al.* (1979). Rosoiu Natalia and Corneliu Covache (1981) studied the partially purified alpha amylase from the hepatopancreas and the whole body of *Mya arenaria.* Lomte and Patil (1989) observed a decrease in amylase activity in the army worm, *Mythima separate* after pesticide treatment. Selvarani studied the digestive enzymes of marine bivalves *Donax cuneatus* and *Perna viridis.* Zambare and Mahajan (2001) recorded a decreased rate of amylase activity in the freshwater bivalve *Corrbicula striatella.*

Babu and Murugan (1999) reported depletion in the amylase, protease and lipase activity in *Atractomorpha crenulata* when exposed to the azadirachtin. The finding of the present investigation was in concurrence with the observations made by Sontakke (1992) in *Thiara tuberculata*, Bhamre (1993) in *Parreysia favidens*, Jadhav

(1993) in *Corbicula striatella* and Patil (1993) in *L. marginalis*.

A decrease in the rate of protease activity was observed when *Cyprinus carpio* was exposed to textile effluent. Similar results were observed by Kasi Reddy (1984) in L. *marginalis* when exposed to methyl parathion. Leo and Sabapathy (1990) noted a decrease in protease activity in *Perna viridis*. Sultana and Lomte (1997) recorded the decreased rate of protease activity in L. *marginalis* when exposed to some heavy metals.

The lipase activity was inhibited due to the treatment of textile effluent on *Cyprinus carpio*. The decline of lipase activity was time and concentration dependent. Similar results were observed in the bivalve, *L. marginalis* by Swami *et al.,* (1983) when treated with flodit and metacid. Lipase activity in Scallop hepatopancreas was reported by Itabashi *et al.,* (1994).

The intestine of control *Cyprinus carpio* has a normal lining of simple mucoidal columnar epithelium with villi, which are very thin and slender. The columnar epithelial cells, arranged informally in the intestinal muscosal layer, have a distinct double layered lamina propria inside the villi (Plate 1a).

Several pathological changes were noticed in the intestinal tissues of textile effluent treated fish. In *Cyprinus carpio* treated with textile effluent for a period of 10 days, the changes observed in the intestine of fish were ruptured and shapeless villi, vacuolization, histolysis of columnar cells and loss of brush border (Plate 1b & 2a).

In 20 days treated, fish the lamina propria showed inflammation, necrosis and appeared to be fused with the epithelial layer (Plate 1c & 2b). At the end of 30 days treatment, several necrotic lesions in the epithelial layer, disintegrated columnar epithelium and loss of brush border were obvious (Plate 1d and 2c).

Arora and Kulshrestha (1984) have observed vacuolation and necrosis in the stomach of *C. striatus* due to carbaryl and endosulfan. According to Saxena and Mathur (1986) chronic exposure to rogor produced complete degeneration in the serosa, muscularis, submucosa and broken villi in the intestine of *C. punctatus*.

The damages were more in the fish exposed to 30% sublethal concentrations of mercury than 10% SLC. Similar pathological changes are notated in the intestine of *H. fossilis* by Konar (1977) exposed to phosphomidon. The present findings are in good agreement with the observation of Anithakumari and Shree Ramkumar (1995), Mishra and Jain (1988) Shammi (1992), Wagh and Khalid Shareef (1985) and Thorat (2001).

The intestinal villi in textile effluent treated *Cyprinus carpio* were found to be severely damaged (Fig. 4, 8, 12 and 16). The lamina propria was shrunken and the epithelial layer orientation was found completely collapsed. The tips of the villi were also found to be ruptured. Sudha Singh and Asha Mehrotra (1999) have noted similar pathological lesions of carbaryl treated *Nandus nandus*. Virk *et al*. (1987) have also discussed endrin and carbaryl induced damages in the intestine of *Mystus tengara*.

Histopathological changes due to pesticides are observed by a number of workers (Mandal and Kulshrestha 1980; Saxena and Mathur, 1986; Santhos Kumar, 1994). Arora and Kulshrestha (1984) have observed vacuolation and necrosis in the stomach of *C. striatus* due to carbaryl and endosulfan. According to Saxena and Mathur (1986) chronic exposure to rogor produced complete degeneration in the serosa, muscularis, submucosa and broken villi in the intestine of *C. punctatus*.

The damages were more in the fish exposed to 10% sublethal concentrations of textile effluent in 30 days. Similar pathological changes are notated in the intestine of *H. fossilis* by Konar (1977) exposed to phosphomidon. The present findings are in good agreement with the observation of Anithakumari and Shree Ramkumar (1995), Mishra and Jain (1988) Shammi (1992), Wagh and Khalid Shareef (1985) and Throat (2001).

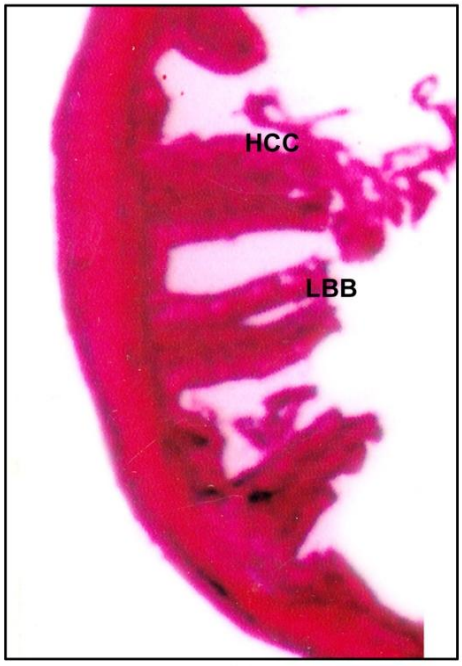
The intestinal villi in urea treated *Cyprinus carpio* were found to be severely damaged (Plate 1, b, c, d). The lamina propria was shrunken and the epithelial layer orientation was found completely collapsed. The tips of the villi were also found to be ruptured. Sudha Singh and Asha Mehrotra (1999) have noted similar pathological lesions of carbaryl treated *Nandus nandus*. Virk *et al*. (1987) have also discussed endrin and and carbaryl induced damages in the intestine of *Mystus tengara*. Inbarani and Seenivasan (1998) have also observed similar pathological lesions in the intestine of phosphomidon treated *S. mossambius*.

Table 1. Levels of amylase activity in the alimentary canal of *cyprinus carpio* on exposure to sublethal concentrations of textile effluent (µg glucose/mg/hr).

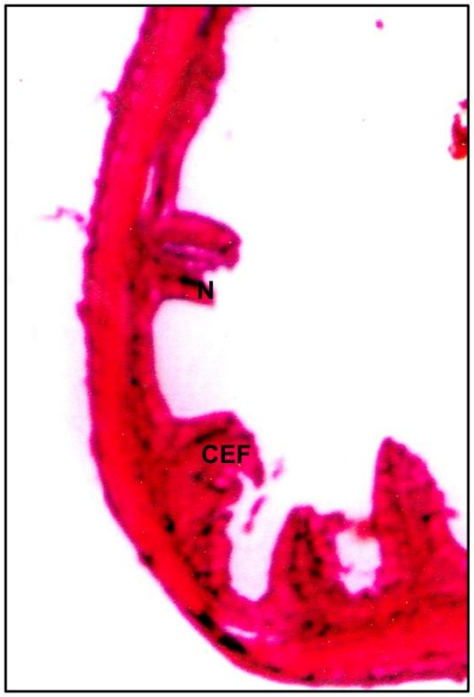
Experimental Group	10 Days	20 Days	30 Days
Control	6.44±0.071	6.52±0.061	6.47±0.039
10% SLC	5.36±0.077	4.37±0.051	3.12±0.028
% Variation	-16.27	-32.97	-51.77
30% SLC	4.12±0.037	2.92±0.029	1.67±0.028
% Variation	-36.02	-64.87	-74.18

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control.

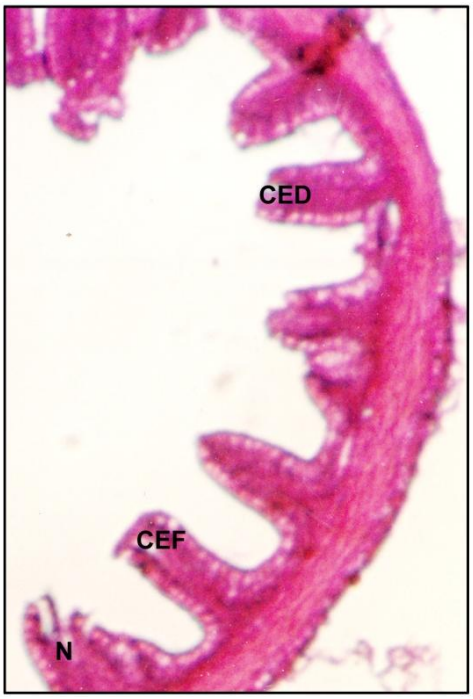
(a) Control
MC - Mucus cells
CE - Columnar epithelium
LP - Lamina Propria
BB - Brush border



(b) 10 days treated
LBB - Loss of brush border
HCC - Histolysis of columnar cells

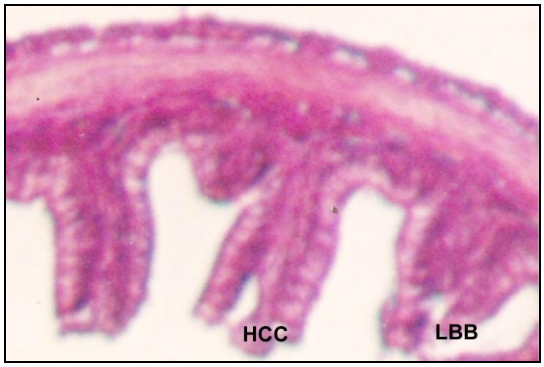


(c) 20 days treated
N - Necrois
CEF - Columnar epithelial

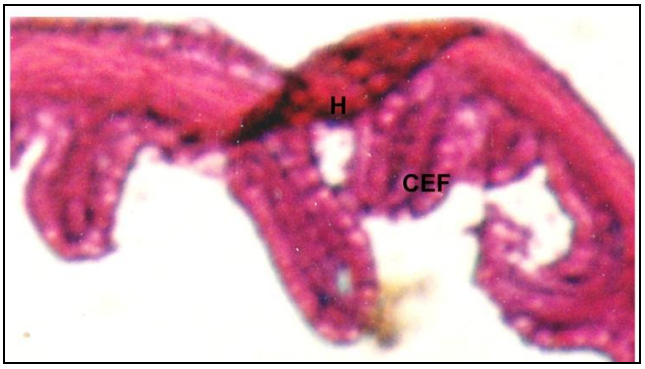


(d) 30 days treated
CED - Columnar epithelium disintegration
CEF - Columnar epithelial fusion
N - Necrois

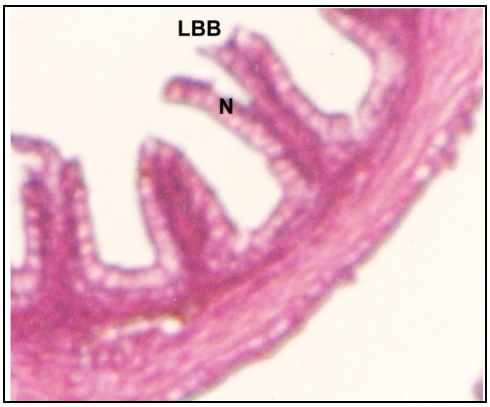
Plate – 1. Histopathological lesions in the intestine of *Cyprinus carpio* exposed to 10% sublethal concentration of textile effluents at different durations.



(a) 10 days treated
HCC - Histolysis of columnar cells
LBB - Loos of bruh border



(b) 20 days treated
20 days treated
H - Histolysis
CEF - Columnar epithelial fusion



(c) 30 days treated
LBB - Loos of bruh border
N - Vacuolization

Plate 2. Histopathological lesions in the intestine of *Cyprinus carpio*exposed to 30% sublethal concentration of textile effluent at different durations.

Table 2. Levels of protease activity in the alimentary canal of *cyprinus carpio* on exposure to sublethal concentrations of textile effluent (µg tyrosine/mg/hr).

Experimental Group	10 Days	20 Days	30 Days
Control	6.95±0.081	6.87±0.067	6.97±0.048
10% SLC	5.15±0.029	4.36±0.032	3.71±0.026
% Variation	-25.89	-36.53	-46.77
30% SLC	4.17±0.042	3.28±0.038	2.16±0.023
% Variation	-40.00	-52.25	-69.01

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control.

Table 3. Levels of lipase activity in the alimentary canal of *cyprinus carpio* on exposure to sublethal concentrations of textile effluent (µg oleic acid/mg/hr).

Experimental Group	10 Days	20 Days	30 Days
Control	3.91±0.019	3.87±0.021	3.94±0.033
10% SLC	2.88±0.014	2.16±0.019	1.68±0.028
% Variation	-26.34	-44.18	-57.36
30% SLC	2.18±0.021	1.64±0.041	1.08±0.027
% Variation	-44.24	-57.62	-72.58

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control.

CONCLUSION

The results in the present study showed that the textile effluent has been proved to be harmful to fishes. Therefore, the information obtained may be useful for management and monitoring of textile effluent contamination in the aquatic environment.

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