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# HISTOCHEMICAL CHANGES IN THE INTESTINE OF *Tilapia mossambica* INDUCED BY ACUTE TOXICITY OF SODIUM FLUORIDE DURING SUMMER

**M. B. BAGALE[1*], K. R. RAO[2] AND N. V. SHAH[2]**

[1]Department of Zoology, Sangameshwar College, Post Box No.52, Solapur-413001, Maharashtra, India.
[2]Department of Zoology, Walchand College of Arts and Science, Solapur-413006, Maharashtra, India.

**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration among all authors. Author MBB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KRR and NVS managed the analyses of the study. Author NVS managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

The objective of this study was to investigate the toxic effect of sodium fluoride (NaF) on freshwater fish, *Tilapia (Oreochromis mossambica)*. Acute toxicity for 24, 48, 72 and 96 hrs. exposure of $LC_0$ value was 30.0 ppm and $LC_{50}$ value was 54.0 ppm for 96 hrs. The histochemical observation revealed that the neutral mucosubstances and glycogen content of intestine *Tilapia mossambica* showed a progressive decrease in staining intensity to Periodic acid Schiff (PAS) and Alcian blue pH-1 - Periodic acid Schiff (AB1- PAS) showed a progressive increase in purple blue staining showed increased acidic mucosubstances (sulfomucins) and decrease neutral mucosubstances and glycogen over time of exposure. The histochemical changes indicate sodium fluoride is toxic to growth and survival of the species.

**Keywords:** Fluoride; *Tilapia mossambica*; acute toxicity; histochemistry; intestine.

## 1. INTRODUCTION

Uncontiminated bodies of fresh water generally have low levels of F, the concentration can increase significantly from fertilize, run-off, mining operations, and industrial emissions. The deposition of fluoride at field sites near areas of industries of and urban sources of fluoride Davison and Blankemare [1]. Fluoride is a general protoplasmic poison but it is not possible yet to describe in detail the mechanism by which it produces the death Hodge and Smith [2].

In normal water fluoride contents range from 0.3 to 5.2 mg/l, but most of the water supplies that were not intentionally fluorinated fluoride contents less than 0.5 mg/l ( National Academy of Science, [3]. Garden [4] reported high fluoride concentration is caused by excess alkalinity and low calcium content. The endemic fluorosis in the world is mainly due to the drinking water [5]. McClurrg [6] studied the acute toxicity of fluoride, cadmium and mercury using conventional 96 hrs $LC_{50}$technique to the estuarine prawn pengeusindicus. He has stated that fluoride generally confined to the skeletal tissue, where as the

**Corresponding author: Email: bagalejyoti6@gmail.com;*

**Table 1. Physicochemical parameter of water used for the experimentation**

| Season | Temperature ºC | pH | mg/l | Total hardness |
|---|---|---|---|---|
| Summer | 28-30 ºC | 7.5 – 8.0 | 5.2 – 5.3 | 110 – 120 |

mercury and cadmium generally deposits in skeletal as well as non skeletal tissue.

In the present investigation histochemical tests were used for localization of chemical products of cellular activities by the intensity of staining reactions. These are glycogen content and acidic mucosubstances (sulformucins) present in the intestine cells of the normal and treated fish, *Tilapia mossambica* at $LC_0$value (30.0 ppm) and $LC_{50}$ value 54.0 ppm for 96 hrs of sodium fluoride for acute exposure for 24, 48, 72 and 96 hrs

## 2. MATERIALS AND METHODS

The fishes, *Tilapia mossambica* were collected from Bheema river at Takli (Solapur district). Takli is located on the south side of the Solapur. It is 28 km away from Solapur Maharashtra State (M.S) India and soon after the fishes were brought to the laboratory. The fishes were maintained in an aerated glass aquaria and acclimatized for four weeks at laboratory conditions. During acclimatization declorinated water was used. Everyday the water was changes twice and excreta and debris were removed. During the period of acclimatization, the fishes were fed with commercial fish food daily. Fishes were exposed to natural photoperiod. Sodium fluoride was used in the present study for experimentation. The predermined $LC_{50}$ value of sodium fluoride at 96 hours was found to be 54ppm. For the determination of $LC_{50}$ 96 hrs, the standard method described by Finney, [7] was used. Acute toxicity tests were conducted and 96 hrs LC50 value was established.

Feeding was stopped 24 hrs. before the acute toxicity testing. The well acclimatized healthy fishes weighing 10-16 gm in weight and 5-9 cm in length were divided into 3 groups each containing 10 fishes. The fish Tilapia (*Oreochromis mossambica*) were exposed to two different concentrations grouped in two sets of sodium fluoride and one set as control. In the first group the fishes were exposed to $LC_0$ concentration for 96 hrs for 30 ppm. In the second group the fishes were exposed to $LC_{50}$concentration for 96 hrs 54 ppm.

The organ intestine was removed from control $LC_0$ and $LC_{50}$ groups were carried out after internal at 24, 48, 72 and 96 hrs used for acute toxicity and fixed into carnoys fixative from all experimental groups along with control. The tissue were fixed into carnoys fixative for 2-3 hrs. The fixation of tissue was followed by washing under running tap water for overnight. The tissue were dehydrated using different alcohol grades and clear in xylene. Then the tissue were transferred for cold embedding followed by hot embedding at $58^0$C for 30 minute and ½ hr. The trimmed blocks were used for sectioning. The respective sections were cut at 4 to 5 micron and various histochemical techniques were employed to scheme given by spicer et al. [8].

PAS staining technique was used as described by Mc Manus, [9] Hotchkiss, [10] for detection of neutral mucosubstances and glycogen and AB pH 1 -0- PAS sequencial staining technique, Spicer et al. technique was used sulfomucins and PAS reactive neutral mucosubstances, glycogen in this tissue.

Physicochemical parameter of water used for the experimentation and for control was analyzed during the toxicity test according to APHA [11] and mentioned in Table 1 for summer season are as follows.

## 3. RESULTS

### A) Histochemical observation of control intestine and treated for summer PAS technique

The data of some important histochemical staining technique employed to study the intestine of control and treated fish *Tilapia mossambica* have been recorded in Table 2 according to visually estimated staining intensities and shade with four plus ( ++++ ) representing the strongest activity.

a) Histochemical observation of control intestine PAS technique

In the PAS staining technique the intestinal mucous secreting cells or goblet cells showed indicating presence of neutral mucosubstances and glycogen. The columnar cells or enterocytes showed weak reactivity to PAS indicating very less amount of glycogen and mucosubstances. The connective tissue of neutral mucossubsstances and glycogen. The serosa showed moderate staining indicating presence of glycogen and neutral mucosubstances (Fig. 1, Photo 1).

b) Histochemical alterations due to sodium fluoride at $LC_0$and $LC_{50}$ concentrations in intestine of *Tilapia mossambica*.

In the present study, the histochemical alterations in the intestine after 24, 48, 72 and 96 hrs of exposure to the sodium fluoride at $LC_0$ and $LC_{50}$ toxicity were recorded. The following are the detailed histochemical observations during different time intervals for PAS staining.

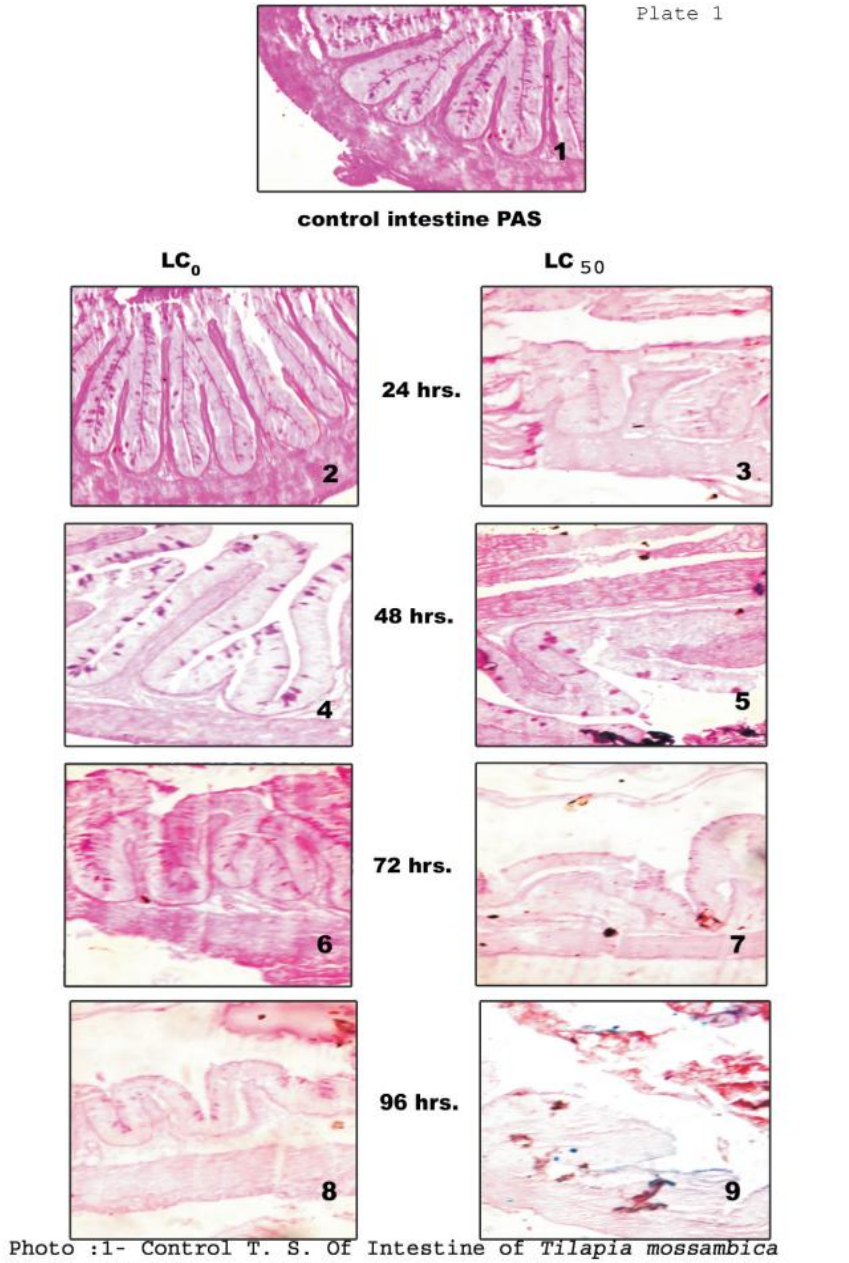


**Fig. 1. Photoplate: 1: T. S. of intestine of *Tilapia mossambica with PAS (400X) after treating with Naf during summer***

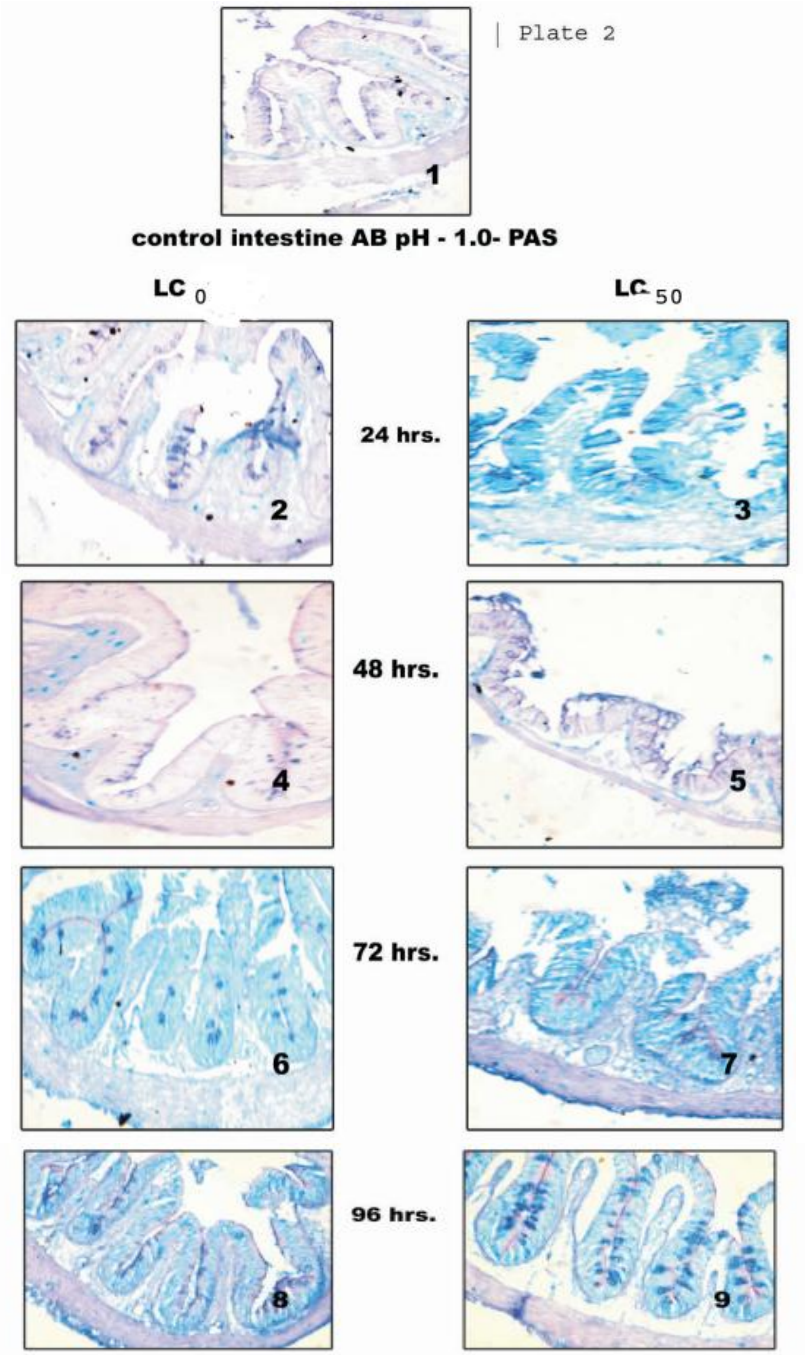


**Fig. 2. Photoplate 2: T. S. of intestine *Tilapia mossambica* with AB pH-1.0-PAS (400X) after treating with Naf during summer**

After 24 hrs.of exposure to $LC_0$ concentrations of sodium fluoride the histochemical changes in the intestine, of the goblet cells were showed slightly decreased glycogen. The connective tissue and muscle mucosubstances and glycogen. The serosa showed less amount of neutral mucosubstances and glycogen (Fig. 1 Photo 2).

After 24 hrs.of exposure to $LC_{50}$ of concentration of sodium fluoride the goblet cells were showed decreased neutral mucosubstances and glycogen. The columnar cells showed negligible amount of glycogen. The connective tissue, muscle fibres and serosa showed very very weak amount of neutral mucosubstances and glycogen (Fig. 1, Photo 3).

After 48 hrs.of exposure to $LC_0$ concentration of sodium fluoride, the intestinal goblet cells showed further decrease in PAS staining so that there observed moderate amount of neutral mucosubstances and glycogen. The columnar cells showed negative reaction with PAS indicating absence of glycogen. The connective tissue showed moderate to weak amount of neutral mucosubstances and glycogen. The muscle fibres and serosa showed moderate amount neutral mucosubstances and glycogen (Fig. 1, Photo 4).

After 48 hrs.of exposure to $LC_{50}$ concentration of sodium fluoride, the goblet cells showed decreased amount of neutral mucosubstances and glycogen. The columnar cells, muscle fibres and serosa showed weak reaction to PAS indicating weak amount of neutral mucosubstances and glycogen (Fig. 1, Photo 5).

After 72 hrs.of exposure to $LC_0$ concentration f sodium fluoride, the goblet cells were showed decreased neutral mucosubstances and glycogen. The columnar cells showed slightly increased neutral mucosubstances and glycogen. The connective tissue, muscle fibres and serosa all showed decline in staining intensity to PAS indicating decreased neutral mucosubstances and glycogen (Fig. 1, Photo 6).

After 72 hrs. of exposure to $LC_{50}$ concentration sodium fluoride, the goblet cells were showed reduced neutral mucosubstances and glycogen. The columnar cells and the connective tissue showed negative reaction to PAS. The muscle fibres and serosa showed very weak reaction to PAS (Fig. 1, Photo 7).

After 96 hrs. of exposure to $LC_0$ concentration sodium fluoride, the goblet cells showed further reduction in neutralmucosubstances and glycogen. The columnar cells and the connective tissue showed negative reaction to PAS. The muscle fibres and serosa showed very less neutral mucosubstances and glycogen (Fig. 1, Photo 8).

After 96 hrs.of exposure to $LC_{50}$ concentration of sodium fluoride, the goblet cells showed negligible amount mucosubstances and glycogen. The connective tissue, muscle fibres and serosa showed negative reaction with PAS. ( Fig. 1, Photo 9).

**B) Histochemical observation of control intestine and treated intestine for summer AB1 – PAS technique.**

a) Histochemical observation of control intestine AB1-PAS staining technique

In the AB1-PAS staining technique the intestinal goblet cells showed moderate purple blue staining indicating moderate amount acidic mucosubstances sulformucins and moderate amount of neutral mucosubstances and glycogen. The columnar cells showed negligible amount of acidic mucosubstances, sulfomucins and neutral mucosubstances and glycogen. The connective tissue showed very weak reaction to PAS and AB1 indicating very little amount of acidic mucosubstances and neutral mucosubstances and glycogen. The muscle to PAS and AB1 indicating less amount of acidic mucosubstances and neutral mucosubstances and glycogen (Fig. 2. Photo 1).

b) Histochemical alterations due to sodium fluoride at $LC_0$ and $LC_{50}$ concentrations in intestine of *Tilapia mssambica.*

In the present study, the histochemical alterations in the intestine after 24hrs., 48hrs., 72 hrs. and 96 hrs. of exposure to sodium fluoride $LC_0$and $LC_{50}$ toxicity were recorded. The following are detailed hisochemical observation during different time intervals for AB1-PAS technique.

After 24 hrs.of exposure to $LC_0$ concentration of sodium fluoride, the intestinal goblet cells showed slightly in amount of acidic mucosubstances, sulfomucins and slightly decrease in neutral mucosubstances and glycogen. The columnar cells, connective tissue, muscle fibres and serosa showed negligible amount of glycogen and poor quantity of acidic mucosubstances, like that observed in control (Fig. 2. Photo 2).

After 24 hrs.of exposure to $LC_{50}$ concentration of sodium fluoride the goblet cells showed increased intensity increased amount of acidic mucosubstances and decreased amount of neutral mucosubstances and glycogen. The columnar cells showed slightly

increased acidic mucosubstances sulfomucins and decreased glycogen. The connective tissue showed slightly increased acidic mucosubstances and very less amount of neutral mucosubstances and glycogen. The muscle fibres and serosa showed small amount of acidic mucosubstances and glycogen (Fig. 2. Photo 3),

After 48 hrs. of exposure to $LC_0$ concentration of sodium fluoride, the intestinal goblet cells and columnar cells showed similar changes observed after exposure to 24 hr of $LC_0$ as shown in figure. The connective tissue showed slightly decreased neutral mucosubstances and glycogen. The muscle fibres and serosa showed weak reaction to AB and PAS indicating very less amount of neutral mucosubstancess and glycogen and acidic mucosubstances, sulfomucins. (Fig.2 Photo 4).

After 48 hrs of exposure to $LC_{50}$ concentration of sodium fluoride, the goblet cells showed slightly increased acidic mucosubstances sulfomucins and decreased neutral mucosubstances and glycogen. The columnar cells showed negligible amount glycogen and acidic mucosubstances. The connective showed same pattern of staining as observed in Plate 2. Fig. 4, indicating increased acidic mucosubstances and decreased neutral mucosubstances and glycogen. The muscle fibres showed slightly increased acidic mucosubstances and slightly decreased neutral mucosubstances and glycogen (Fig. 2 Photo 5).

After 72 hrs.of exposure to $LC_0$ concentration of sodium fluoride, the goblet cells, columnar cells showed increased acidic mucosubstances and decreased neutral mucosubstances and glycogen. The connective tissue as well as muscle fibres and serosa all showed slightly increased acidic mucosubstances and decreases neutral mucosubstances and glycogen (Fig. 2. Photo 6).

After 72 hrs.of exposure to $LC_{50}$ concentration of sodium fluoride, showed the goblet cells were increased highly (secretion) of acidic musosubstances, sulfomucins and low level neutral mucosubstances and glycogen. The columnar cells also showed increased more amount of acidic mucosubstances, sulfomucins and very small amount (neutralmucosubstances) and glycogen. The connective tissue showed more increased acidic mucosubstances and reduction in neutral mucosubstances and glycogen. The muscle fibres showed more increased acidic mucosubstances and decreased neutral mucosubstances and glycogen. The serosa showed increased acidic mucosubstances and reduction in neutral mucosubstances and glycogen (Fig. 2. Photo 7).

After 96 hrs.of exposure to $LC_0$ concentration of sodium fluoride, the intestine goblet cells showed highly increased acidic mucosubstances, sulfomucins, and decreased neutral mucosubstances and glycogen. The columnar cells showed increased acidic mucosubstances and poor content of glycogen decreased. The connective tissue increased acidic musubstances and decreased neutral mucosubstances and glycogen. The serosa showed increase mucosubstances and decreased neutral mucosubstances and glycogen (Fig. 2. Photo 8).

After 96 hrs.of exposure to $LC_{50}$ concentration of sodium fluoride, the goblet cells showed dark blue staining indicating greatly acidic mucosubstances, sulfomucin and reduction in neutral mucosubstances and glycogen. The columnar cells showed increased acidic mucosubstances, the sulphated mucins were very high in concentration and very low amount of neutral mucosubstances and glycogen. The connective tissue, muscle fibres and serosa also showed increased acidic mucosubstances and decreased neutral mucosubstances and glycogen (Fig.2. Photo 9).

## 4. DISCUSSION

Histochemical studies provide reliable information about different chemical constituents, their natural cytochemical architecture within the cell and explain probable functional role of these constituents in the cell. Number of physiological functions are accelerated by enzymes, coenzymes, proteins and lipid changes in these constituents provide most valuable informations for the toxicological studies.

The studies of Ribelles et al. [12] 1995 have shown that the quality of gut mucosubstances is directly related to environmental condition, which in turn may directly affect the function of the alimentary tract. The presence of mucosubstances, especially those sulphated in the intestine, possibly regulate the transfer of proteins as well as ions and fluids.

In our histochemical study, it was observed that, when the fish *Tilapia mossambic*a were exposed to toxic stress environment of sodium fluoride the intestinal secretion of neutral mucosubstances, glycogen was decreased. Sodium fluoride altered the concentration of neutral mucosubstances, glycogen in the cells and tissue under utilization of neutral mucins, glycogen to overcome the toxic stress on the cellular metabolism.

**Table 2. Histochemical staining reaction on intestine in control and sodium fluoride intoxicated fish, *Tilapia mossambica* during summer season**

| Sr. no. | Histochemical techniques | Tisssue/ Cells | Control group | Animal groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Experimental groups | | | | | | | |
| | | | | $LC_0$ | | | | $LC_{50}$ | | | |
| | | | | Fluoride exposure period | | | | Fluoride exposure period | | | |
| | | | | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. |
| 1 | PAS | GC | ++++P | ++P | +++P | +++P | ++P | ++P | +++P | ++P | +P |
| | | CC | +++P | ++P | +P | ++P | - | ++P | ++P | - | - |
| | | CT | ++P | ++P | ++P++P | ++P | - | ++P | ++P | - | - |
| | | M | ++P | ++P | +P | ++P | +P | ++P | ++P | +P | - |
| | | S | ++P | ++P | | ++P | +P | ++P | +P | +P | - |
| 2 | AB pH1-PAS | GC | ++PB | ++PB | +++PB | +++PB | +++PB | +++PB | +++PB | ++++PB | ++++PB |
| | | CC | +PB | +PB | +PB | ++PB | ++PB | ++PB | ++PB | +++PB | +++PB |
| | | CT | +PB | +PB | +PB | ++PB | ++PB | ++PB | ++PB | +++PB | +++PB |
| | | M | +PB | +PB | +PB | ++PB | ++PB | ++PB | ++PB | +++PB | +++PB |
| | | S | +PB | +PB | +PB | ++PB | ++PB | ++PB | ++PB | +++PB | ++++PB |

*++++ = Very intense; +++ = Intense; ++ = Moderate*
*- = No reaction (negative) + = Poor*
*GC = Goblet cells; CC = Columnar cells; CT = Connectivetisse*
*M = Muscle fibres; S = Serosa; P-Pink; PB- Pinkish blue (Purple blue)*

On the other hand, the toxic effect of sodium fluoride caused enhancement in the concentration of acidic mucosubstances, sulfomucins in the cells of intestine of *Tilapia mossambica*. The quantity and quality of acidic mucosubstances was increased in the targeted cells and tissues.

According to Murray et al. [13] different mucosubstances have been correlated with assorted digestive function. Neutral substances combined with alkaline phosphate assist in the digestion and emulsification of food Clarke and Witcomb [14]. Grav et al. [15], suggested that presence of neutral mucins may indicate absorptive functions and Anderson [16] reported that mucosubstances may provide cofactors required for the breakdown of food.

Increase of acidic mucosubstances of intestine of young giltheads, sparus aura, L., induced by acute action of the anionic tensioactive alkyl benzene sulphonate by M. Rosety et al. [17] who reported that the acidic mucosubstances content in the intestine of treated fish exhibited variable changes of different dose level. Ribelles 1995 a.b, [18] reported histopathological and histochemical changes produced by sodium dodecyl sulphate (SDS) in the liver and intestine of giltheads (*Sparus aurata*) in.

## 5. CONCLUSION

Polluted environment bring impact on natural balance in aquatic ecosystem and also cause many physiological, histological, histopathological and histochemical changes in the organisms.

Ample amount of neutral mucosubstances , glycogen present in most of the cells of controlled group of fishes.

Sodium fluoride exposed fish *Tilapia mossambica* showed altered histochemical picture in the cellular constituents of intestine. Histochemical alteration evaluates the sodium fluoride stress and toxicity on organ intestine for acute toxicity of fish *Tilapia mossambica.* Sodium fluoride decreased the concentration of neutral mucosubstances, glycogen in the cells and tissues under study. This depletion may be to the higher utilization of neutral mucosubstances, glycogen to overcome the toxic strees on the cellular metabolism. Sodium fluoride on the other hand enhanced concentration of acid mucosubstances, such as slufomucins in the cells.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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