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Toxicological effects of the textile industrial effluent to an Indian freshwater fish, *Clarias batrachus*

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Abstract
In this study, the histological effects of textile industrial effluent in the freshwater fish, *Clarias batrachus* was studied after 10, 20, and 30 days period of exposure. The 24, 48, 72, and 96 hours LC_{50} values were found to be 58.476, 53.231, 47.253, and 37.429% respectively. During the experiment, the treated fishes showed abnormal behaviors like erratic swimming, hyper-excitation, rapid opercular movement, and thick mucus covering which indicated the toxicity of the effluent. The observed changes in the gills were epithelial hyperplasia with lamellar fusion, necrosis, vacuolar degeneration, and atrophy of primary and secondary gill lamellae. On the other hand, the liver showed the formation of the number of vacuoles, enlargement of nuclei of some cells, degeneration of cytoplasm in hepatocytes, atrophy, a rupture in blood vessels, and disposition of hepatic cords. The kidney also showed abnormalities like degeneration of proximal and distal convoluted tubule, vacuolation of renal interstitial tissue, necrosis, cellular hypertrophy, and granular cytoplasm.

Keywords: Textile industrial effluent, *Clarias batrachus*, histopathological alterations, LC_{50}; photomicrography

1. Introduction
Many physiological, as well as biological changes in aquatic animals in developing countries, have been occurred due to water pollution which leads to ecological disorders [1]. Specifically, a gradual lowering in biological oxygen demand in the lethal level of declining oxygen from the water bodies [2], the metal, and other pollutants that have been very stable in the environment for longer periods resulting in bio-magnification [3] due to the discharge of toxic wastes into fresh-water reservoirs. The rich BOD, COD, and suspended solids in untreated wastewater discharged from textile industries are affected by the freshwater habitats [4, 5]. As a result of the discharge of textile wastewater into aquatic habitats, aquatic organisms, especially fishes lead to stress and behavioral changes [6]. Bioassay methods can be used to determine the toxicity of any pollutants. Specifically, in the eco-toxicology study, the fish bioassay is considered vital. The fish bioassay study is important to conduct, as they are very sensitive to environmental alterations, and as it is the most organisms of the aquatic food webs and as they are the major source of food items in the world [7, 8]. Bioassays play an important role to give information about the impact of emerging chemicals [9]. Likewise, to detect the primary effects of pollutants in a particular organ, histological biomarkers are known to be sensitive tools [10]. These biomarkers may provide important information from the beginning point of biological effects to its impact on cell physiology [11]. In fish, the structural changes in gills indicate the impact of toxicant, as it is the primary site of toxicant exposure [12]. The liver is the major target area of human chemicals and it is also the second-largest organ in the body [13]. Likewise, the kidney is affected by many harmful substances. Hence, to determine the toxic effect of man-made chemicals, the histopathological study of vital organs like the gill, liver, and kidney is inevitable.
In the present study, to evaluate the impact of sublethal toxicity of industrial effluent in a freshwater fish, *Clarias batrachus*, the histological study was carried out during 10, 20- and 30-days periods of exposure.

2. Materials and methods
2.1 Collection of Fish
Fifty fish specimens of *Clarias batrachus* with an average total length of 26.44± 2.19 cm and

wet body weight 460.77 ± 6.22 g irrespective of the sex were collected from the river Noyyal, Tirupur. About 90% of total cotton knitwear exports from India are contributed by Tirupur, a major textile and knitwear hub of India. The industrial units dispose of wastewater in the river. The river water is also used in various fish farms for culturing fish. The control fish specimens were obtained from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamilnadu, India. The collected alive fish specimens were transported to the Research Laboratory of the Department of Zoology for further analysis.

2.2 Histopathological studies

To avoid dermal infection, fishes were washed with 0.1% $KMnO_4$ solution. The standards of APHA, 2005 were strictly followed for maintaining the fishes [14]. The fishes were exposed to sublethal concentration (3.7429% concentration) for 10, 20, and 30 days. The dead fishes were removed immediately during the experiment to avoid depletion of dissolved oxygen (DO) level which may adversely affect other fishes. The vital tissues like the gill, liver, and kidney of the fishes were taken out for histological examination. A simultaneous control group of fishes was also maintained parallel to the exposed fishes.

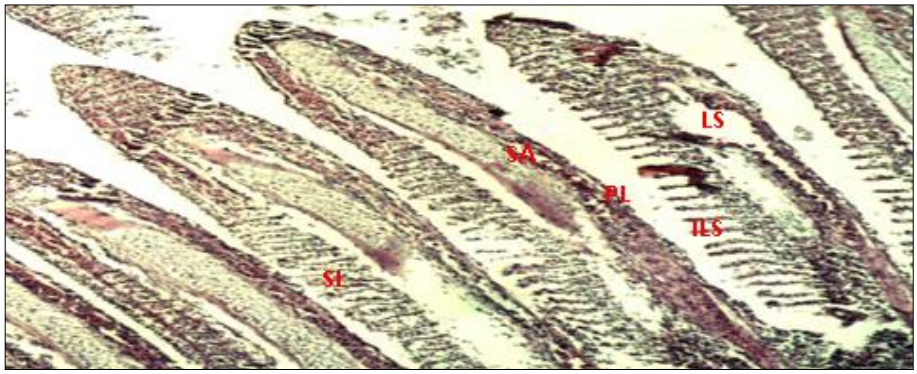
2.3 Microscopic observation

Five fishes were taken from each replicate tank at the end of exposure periods. The gill arches of the fishes were removed from both sides. Fishes were dissected and the liver and kidney were excised quickly and fixed in Bouin's solution as a histological fixative for 24 hours [15]. The specimens were processed as usual in the recognized method of dehydration, cleared in xylene, and finally embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome [16]. The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy (Hamilton compound photomicroscope).

3. Results and discussion

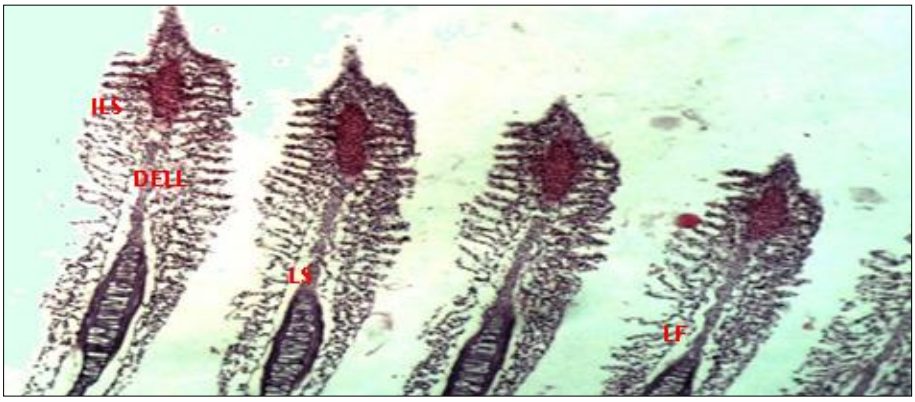
3.1 Histopathological lesions of gills

The gills of fish from the control group showed normal histological structure (Fig.1A). At 3.7429% concentration of textile industrial effluent for 10, 20, and 30 days of exposure, fishes were showed cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae. Other changes that occurred in fishes were epithelial lifting, interstitial edema, and blood congestion in the vascular axis of primary filaments. Also, telangiectasias were observed in gill lamellae (Fig. 1 B, C & D). These pathological responses may be a reaction to toxicants' intake or adaptive responses to prevent the entry of the pollutants through the gill. The observed defense mechanisms are epithelial lifting and proliferation in the epithelial cells which is caused by the increased distance between the external environment and the blood so that it prevents the entrance of contaminants [17]. As a result, oxygen uptake is weakened in fishes. However, to compensate for low oxygen uptake, fishes can increase their ventilation rate [18]. The gas exchange and ionic regulation were adversely affected by the necrosis observed in gill tissue [19, 20]. Due to increased blood flow inside the lamellae, blood congestion, or even an aneurysm may be resulted causing the dilation of the marginal filaments [21].



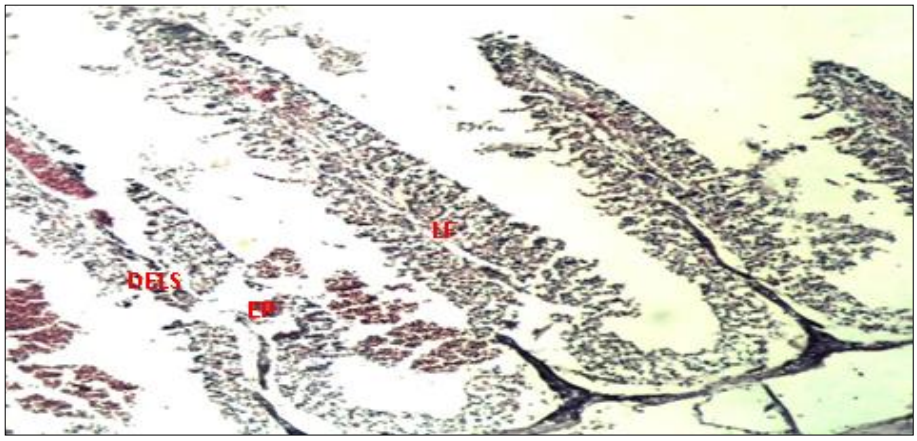
PL - Primary Lamellae, SL- Secondary Lamellae, LS - Lamellar Space, ILS - Inter Lamellar Space, SA- Supporting Axis

Fig 1A: Control gill section of *Clarias batrachus*



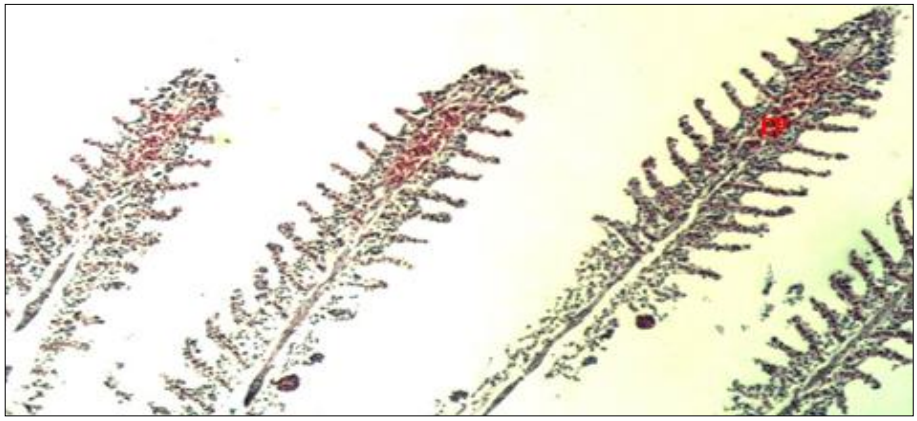
LF- Lamellar Filament, LS- Lamellar Space, DEL- Degeneration of Epithelial Lining, ILS - Inter Lamellar Space

Fig 1B: Gill section of fish exposed to 10 days of textile industrial effluent



LF- Lamellar Filament, DEL- Degeneration of Epithelial Lining, EP- Epithelial Proliferation

Fig 1C: Gill section of fish exposed to 20 days of textile industrial effluent

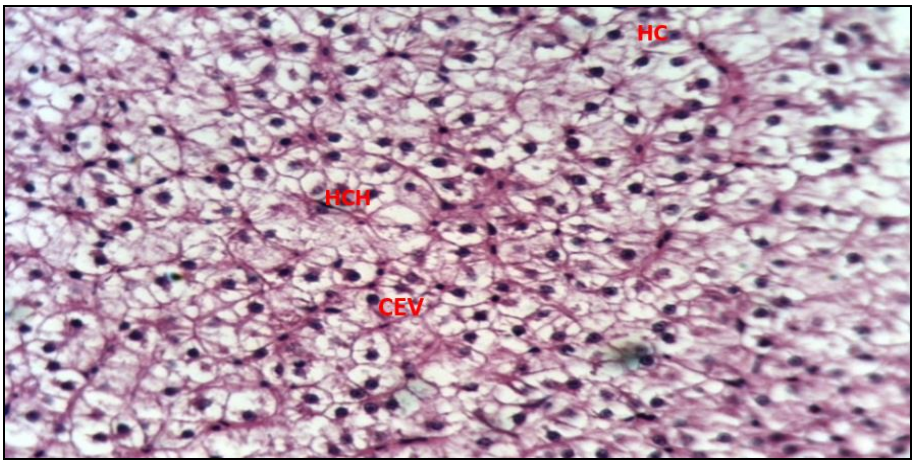


EP- Epithelial Proliferation, DEL - Degeneration of Epithelial Lining

Fig 1D: Gill section of fish exposed to 30 days of textile industrial effluent

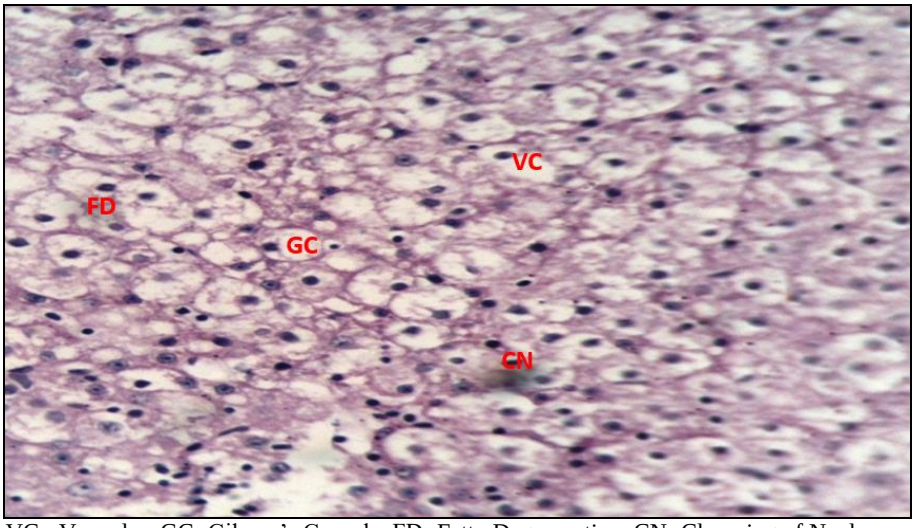
3.2 Histopathological lesions of the liver

The liver serves several basic functions like metabolism, storage, and secretion of bile which is the main metabolic factory of the body [22]. Figure 2A shows the normal histology of the liver. But, the *Clarias batrachus* exposed to textile effluent showed remarkable histological alterations as compared to control. During 10 days of exposure, necrosis occurred as a result of the localized death of liver cells (Fig 2 B). On the other hand, during 20 days period of exposure, the fish exhibited vacuolation in the cytoplasm, degeneration of the cell membrane, mild infiltration of leukocytes, darkly stained specks of necrotic nuclei, extensive pyknosis, and swollen hepatocytes (Fig 2 C). Figure 2 D revealed that there were large vacuoles in the cytoplasm, moderate infiltration of leukocytes, and the nuclei continued to be pyknotic in 30 days period. The vascular dilation, intravascular hemolysis, and thrombus formation in the blood vessels with subsequent stasis of blood resulted in cellular degeneration and necrosis in the liver [20]. Moreover, the increased vacuolization of the hepatocytes can be described as a signal of a degenerative process [23]. Similar observations were made in Gourami fish (*Trichogaster trichopterus*) upon exposure to 0.30 mg/l concentrations of paraquat [24] and in freshwater fish, Rohu (*Labeo rohita*) exposed to thermal power station effluent [25].



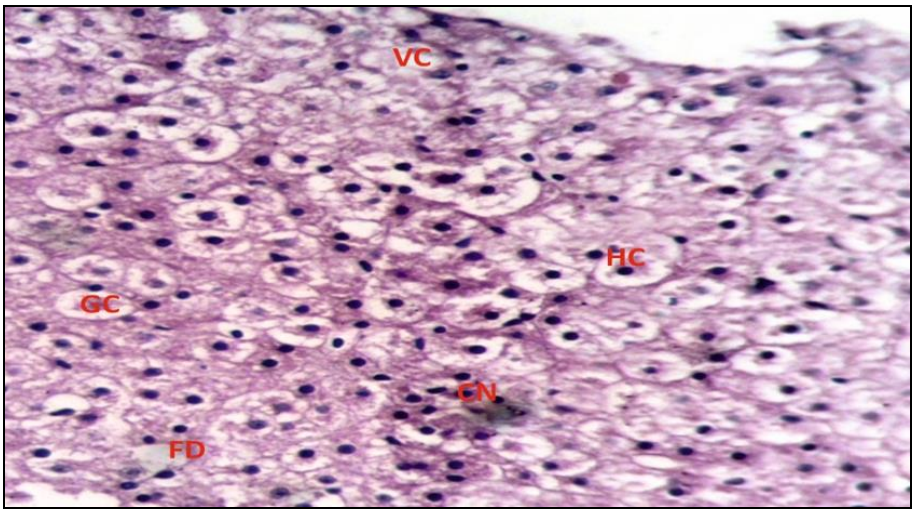
HC- Hepatocyte Cells, HCH- Hepatic Cords, CEV- Central Efferent Vein

Fig 2A: Control liver section of *Clarias batrachus*



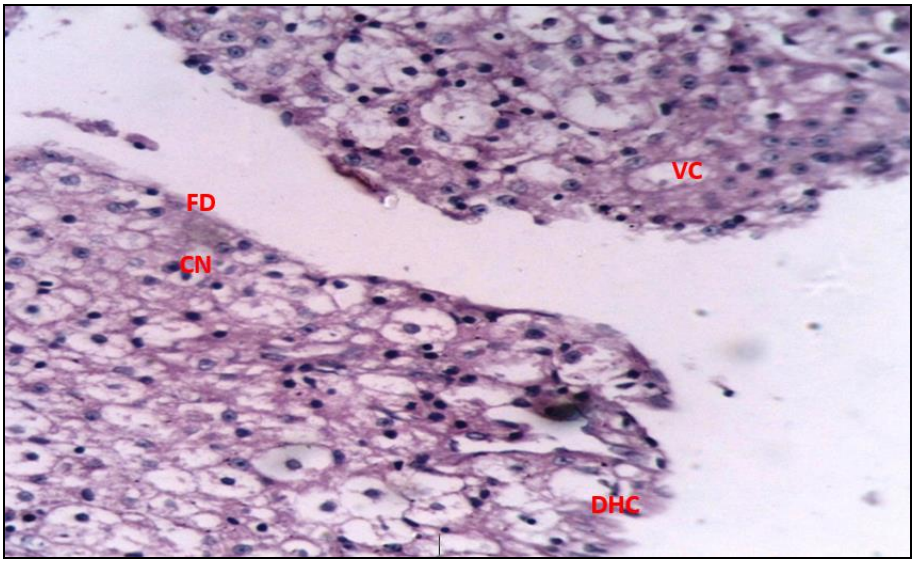
VC - Vacuoles, GC- Gilssen's Capsule, FD- Fatty Degeneration, CN- Clumping of Nucleus

Fig 2B: Liver section of fish exposed to 10 days of textile industrial effluent



HC- Hepatocyte cells, VC- Vacuoles, GC- Gilssen's Capsule, FD- Fatty Degeneration, CN- Clumping of Nucleus

Fig 2C: Liver section of fish exposed to 20 days of textile industrial effluent

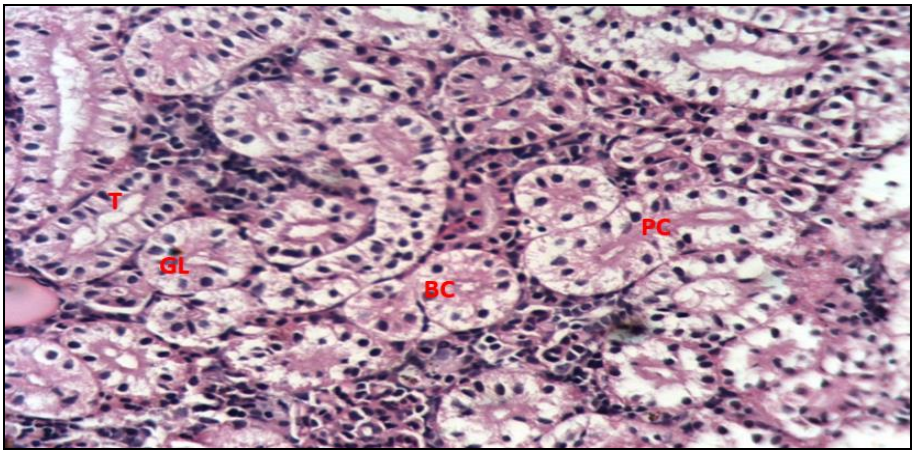


VC- Vacuoles, DHC- Degenerated Hepatocyte Cells, FD- Fatty Degeneration CN- Clumping of Nucleus

Fig 2D: Liver section of fish exposed to 30 days of textile industrial effluent

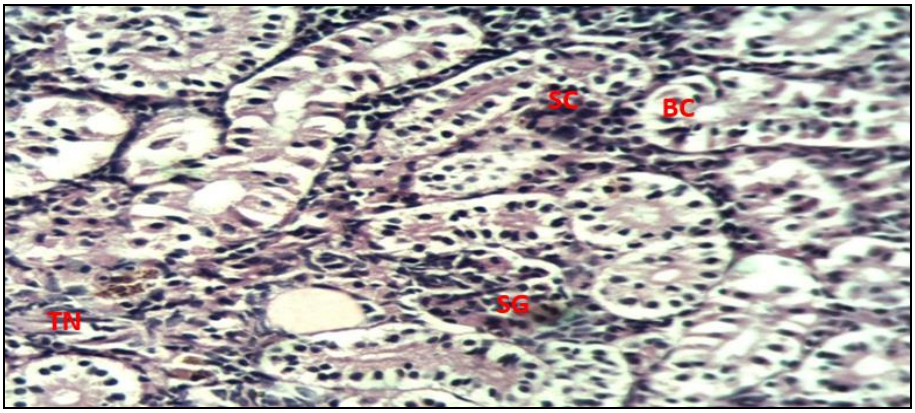
3.3 Histopathological lesions of the kidney

Figure 3A shows the kidney of the control group *Clarias batrachus*, well-expanded glomeruli, well-organized blood vessels, and hematopoietic tissues. But, variations were seen in the kidney of *Clarias batrachus* on sub-lethal exposure of textile industrial effluent. All treated fishes showed glomerular shrinkage. Specifically, the exposure produced a gap between the glomerulus and Bowman's capsule and increased tubular lumen in the kidney of *Clarias batrachus.* Pyknotic nuclei, hyaline degeneration of tubular epithelium, disorganized tubules, hydrophobic swelling, intact tubules vacuolation, and eccentric nuclei were also seen in the kidney of *Clarias batrachus*. (Figure 3B,3C & 3D). The damage was severe in fishes exposed to 30 days with the effluent. The damages observed in the present investigation are similar to the study using cypermethrin in different fish species [26]. The enlargement of the proximal tubules and a reduction of the Bowman's space which impaired the functioning of the kidney in the present study agreed with the results of the studies with fathead minnows collected from an effluent-dominated stream [27] and in freshwater fish, *Cirrhinus mrigala* exposed to the detergent Tide [28].



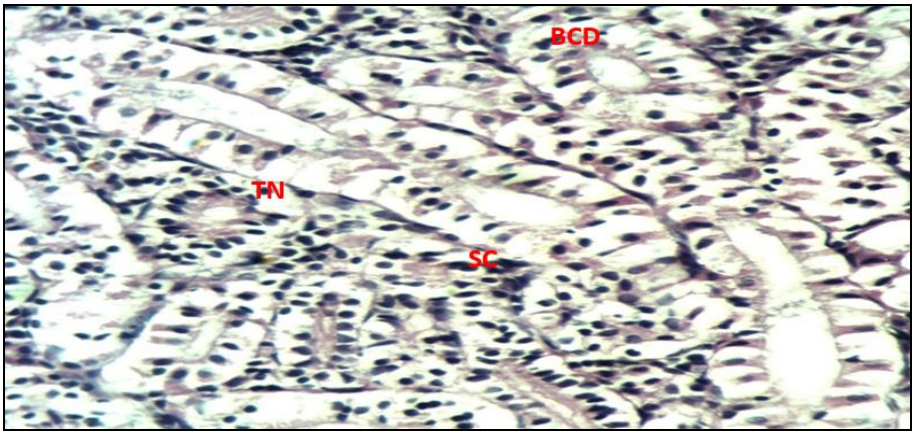
GL- Glomeruli, LC- Lymphoid Cells, PC- Parenchyma Cells, BC- Bowman's Capsule, T- Tubules

Fig 3A: Control kidney section of *Clarias batrachus*



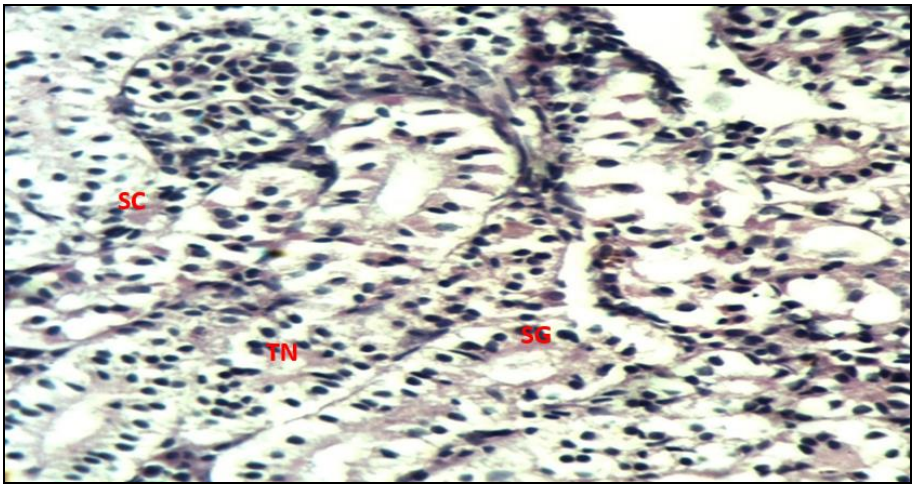
BC- Bowman's Capsule, SC- Shrunken of Cells, SG- Shrunkened Glomerulus, TN- Tubule's Nucleus

Fig 3B: Kidney section of fish exposed to 10 days of textile industrial effluent



BC- Bowman's Capsule, SC- Shrunken of Cells, TN- Tubule's Nucleus

Fig 3C: Kidney section of fish exposed to 20 days of textile industrial effluent



SC- Shrunken of Cells, SG- Shrunkened Glomerulus, TN- Tubule's Nucleus

Fig 3D: Kidney section of fish exposed to 30 days of textile industrial effluent

4. Conclusion

The industrial effluent adversely affected the health status of the fish, *Clarias batrachus* in the present study. The histological alterations found during sublethal treatment indicate the severe impact of the effluent. The histopathological parameter alterations can be used as an ideal tool in aquatic toxicology. Moreover, it is very much essential to precede further toxicity study for a better definition of effluent toxicity in aquatic organisms.

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