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Elecrophoretic Pattern and Histopathological Study of Muscle Tissue Tilapia, *Oreochromis mossambicus* Exposed to Textile Dyes

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Abstract: ***The research was conducted on fresh water fish Oreochromis mossambicus (Peters) to check out the electrophoretic pattern and histological changes in muscle tissue. In the present study, electrophoretic protein pattern was also demonstrated which proved harmful impacts of the textile dyes on the tissues of the present studied fish. Histological analysis of muscle tissue revealed significant morphological alterations such as degeneration of secondary lamellae, lamellar fusion due to hyperemia of epithelial cells.***

Keywords: Textile dyes, *Oreochromis mossambicus*, Protein pattern, Histopathology

1. Introduction

In urban aquatic environment fish might be exposed to various substances, in the meantime delivered by various types of anthropogenic activities. Natural changes in fish that are identified with the introduction or to the impacts of contaminants are called biomarkers and their utilization has prompted great results in ecological risk assessment [1]. Histopathological changes have been generally utilized as biomarkers as a part of the assessment of the safety of fish presented to contaminants, both in the research center [2,3] and in field studies [4,5].

Histopathological biomarkers in ecological monitoring is that this classification of biomarkers allows examining particular target organs, including gills, kidney, muscle and liver that are responsible for vital functions in the fish [6]. Further, the changes found in these organs are normally easier to identify the functional ones [7] and cause severe damage to animal health [8].

Previous research mentioned that the exposure of fish to pollutants (agricultural, business and sewage) resulted in numerous pathological alterations in different tissues of fish. Histopathological modifications have been located in the muscle of fish exposed to various toxicants [9,10].

Numerous histopathological changes had been located (i) in the liver of *Gymnocephalus cernua* amassed from Elbe Estuary contaminated via home, business and agricultural pollutants [11] (ii) *Oreochromis niloticus* gathered from the southern area of Lake Manzalah contaminated with industrial and agricultural pollution [12] (iii) *Coregonus clupeaformis* uncovered to nickel [13] (iv) Corydoras paleatus exposed to Methyl parathion [14] (v) *Clarias gariepinus* uncovered to lead [15] and (vi) *Oncorhynchus mykiss* exposed to Copper sulphate [16].

Histopathological modifications have been mentioned inside the gills of different fish exposed to one-of-a-kind toxicants [17,18]. Histopathological alterations have been suggested within the gut of fish uncovered to one of a kind toxicants [19,20]

Fish are widely used to assess the health of aquatic ecosystems and physiological modifications function biomarkers of environmental pollution [21]. The modifications inside the fish gill are some of the most generally recognized responses to environmental pollutants [22,23]. Liver plays a essential position inside the metabolism and excretion of xenobiotic compounds. The morphological alterations occurred in the liver of freshwater fish because of extraordinary toxic conditions [24].

Metals can either increase or decrease hepatic enzyme sports and can cause hepatic modifications, depending on the metal type and concentration, fish species, duration of exposure and different factors [25]. Heavy metals from industries disturb the aquatic surroundings and results in environmental health hazards [26,27]

Non-essential metals are generally powerful pollutants and their bioaccumulation in tissue result in intoxication, decreased fertility, tissue harm and disorder of a variety of organs [28]. Histological changes related to heavy metals in fish had been studied with the aid of many authors [29].

Heavy metals can't be destroyed through biological degradation. When exposed to higher concentration, organs of aquatic animals may accumulate heavy metals [30,31].These pollutants purpose unfavorable effects and mortality in aquatic organism [32].

Several histopathological changes were observed in the liver of common carp, *Cyprinus carpio* and *Ctenopharyngodon idellus* exposed to zinc and copper [33].

Histopathological studies have reported changes are either adaptive or degenerative. Adaptive changes include lysosomal proliferation and reticulum development. Degenerative modifications consist of losses in integrity of

mitochondria, plasma or nuclear membrane, disintegration of endoplasmic reticulum and development of autophagic vacuoles in liver of zebra fish exposed to sublethal copper sulphate concentration [34].

The aim of the present investigation is to study the electrophoretic pattern and histopathological effect of muscle tissue of *Oreochromis mossambicus* exposed to textile dyes

2. Materials And Methods

Experimental animal

External Characters of *Oreochromis mossambicus*
Freshwater fish tilapia, *Oreochromis mossambicus* is laterally compressed. It has a deep body with lengthy dorsal fins. The front part of the dorsal fin is the most difficult to handle because it has spines on it. It has weak banding and the colour is yellow. It is broadly used as food and has been added for aquaculture. They are hardy, clean to develop and are the 1/3 maximum vital fish in aquaculture after carps and salmonids. They do no longer migrate because of their high sustainability and therefore they are economically important as a food supply. They are raised as a food supply due to their speedy increase and tolerance for excessive stocking densities and poor water quality.

Classification:
Phylum : Chordata
Class : Actinopterygii
Order : Perciformes
Family : Cichlidae
Genus : *Oreochromis*
Species : *mossambicus*
Binomial name : *Oreochromis mossambicus* (Peters.)

Maintenance of experimental fish
Freshwater suitable for eating fish tilapia *Oreochromis mossambicus* changed into used as the experimental model to evaluate the toxicity of textile dyes. The fishes had been procured from the neighborhood lake at Kolathur, Chennai, Tamilnadu. They have been added to the laboratory and acclimatized underneath laboratory situations for duration of 3 weeks and fed ad libitum.

Experimental Design
The fishes had been maintained in circular plastic tubs packed with 56 litres of dechlorinated tap water. The tubs have been disinfected with potassium permanganate solution and wash thoroughly prior to introduction of fishes to prevent any fungal contamination. Feeding became stopped 24 hours earlier than the graduation of the toxicity test to keep the animals greater or less inside the identical metabolic kingdom.

The adult healthy tilapia *Oreochromis mossambicus* weighing 50 – 75 g and about 8 – 10 cm in length had been selected and divided into 4 groups of 30 fishes each. The fishes were tested carefully for any pathological signs. The fishes were grown and maintained in 56L of tap water.

Group I: control fishes maintained in dechlorinated toxicant tap water
Group II: Fishes maintained at 0.5 ppm concentration of textile dyes
Group III: Fishes maintained at 1.0 ppm concentration of textile dyes
Group IV: Fishes maintained at 1.5 ppm attention of textile dyes

The control and experimental animals have been fed with industrial meals pellets with ingredients consisting of fish meal, wheat flour, soyabean meal, corn meal, yeast, nutrients and minerals. The proximate evaluation of business food pellet consisted of crude protein 28%, crude fat 2.0%, crude fiber 5%, crude ash 9% and moisture 10%. Water was modified day by day at 18.00 hours which facilitated the removal of nitrogenous waste excreted by means of the fishes and for the elimination of unconsumed food.

After renewal of water the specified amount of textile dye solution from the inventory with attention of a thousand ppm changed into brought to every of experimental tank to preserve the toxic awareness of the water medium.

On the end of each 7, 14 and 21 days six fishes had been sacrificed by cervical decapitation. After decapitation the desired tissues – liver and muscle were dissected out and washed thoroughly with 0.9N saline solution. Tissues have been weighed and homogenized in Tris – HCl buffer using mortar and pestle. The homogenate of the tissues become centrifuged at 2500 rpm for 15 mins in a refrigerated excessive speed centrifuge and clear supernatant became used for assay of enzyme interest and evaluation of electrolytes and vitamins.

For histological research the muscle tissue had been collected in 10% formalin. For electrophoretic protein sample, complete tissues was used to isolate extracellular protein and then subjected to SDS – page evaluation

Electrophoretic protein pattern of muscle
The extracellular protein fraction was isolated from the liver and muscle homogenate which was then subjected to SDS PAGE to identify the change in the protein pattern of the control and textile dye treated tissues.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)
SDS-Poly acrylamide gel electrophoresis was carried out by the method of (Laemmli, 1970). The tissue protein became mixed with pattern buffer (1.75 ml of Tris – HCl, 0.5 ml of Glycerol, a hundred µl of 2% SDS, 250 µl of β-mercaptoethanol, pinch of bromophenol blue and 0.4 ml of distilled water had been combined and sterilized) in 2:1ratio, boiled in a water bathtub for three min, cooled and brought to the wells then the energy supply turned into related with cathode inside the higher tank and anode inside the lower tank. Electrophoresis was carried out at room temperature with steady voltage and 20 mA current supply changed into maintained till the tracer dye reached 0.5 cm above the lower end. The gel was stained with silver staining method of Blum et al. (1987). After staining, the gels had been stored in 7 % (v/v) acetic acid.

Histopathological assessment

For histological studies the muscle tissue muscle were collected in 10% formalin. They were processed routinely for paraffin embedding and sectioned to 5m thickness for staining by Haematoxylin and eosin (H & E) method (Luna 1968) for histopathological examination.

3. Results

Protein Pattern in Muscle tissue

Protein pattern of muscle tissue of control group of fish, *Oreochromis mossambicus* is illustrated in Figure 1. Protein pattern of muscle tissue of group of fish exposed to 0.5, 1.0 and 1.5 ppm of textile dyes for 7 days is illustrated in Figure 2. Figure 3 and Figure 4 depict the protein pattern of muscle tissue of group of fish exposed to 0.5, 1.0 and 1.5 ppm of textile dyes for 7, 14 and 21 days respectively.

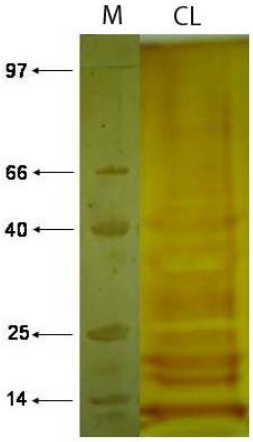

Figure 1: Protein Pattern of Muscle of Control group of fish

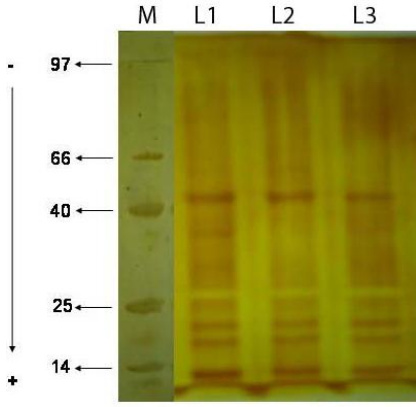

Figure 2: Protein Pattern of Muscle of fish treated with textile dyes for 7 days

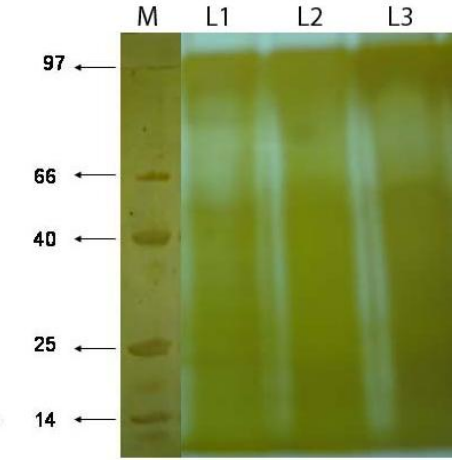

Figure 3: Protein Pattern of Muscle of fish treated with textile dyes for 14 days

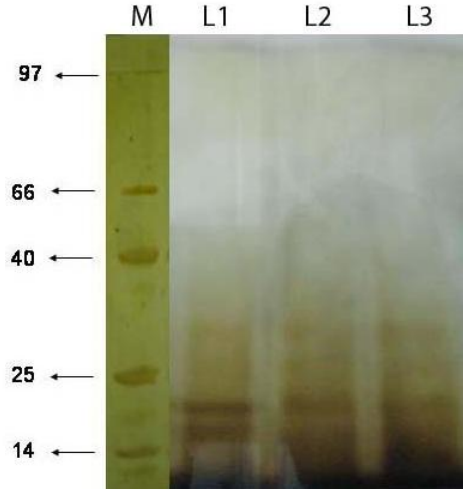

Figure 4: Protien Pattern of Muscle of fish treated with textile dyes for 21 days

M – Marker, CL – Control Lane L1 – Lane 1 (0.5ppm), L2 – Lane 2 (1.0ppm), L3 – Lane 3 (1.5ppm)

Lane 1, Lane 2 and Lane 3 of Figure 2 exhibited bands which were similar to that of control lane in Figure 1.

The bands at approx. MW of 40 was prominent that band in the control lane. The band in Lane 1, Lane 2 and Lane 3 of Figure 3 were not distinguishable and no distinct bands were observed in Figure 4 which is suggestive of loss of protein in nutritionally valuable muscle tissue of fish, *Oreochromis mossambicus* due to the influence of textile dyes.

4. Histopathology of Muscle

The exposure of 0.5 ppm of textile dyes for 7 days to the group of fish resulted in degeneration of muscle bundles and splitting of muscle fibres which is shown in Figure 6. Degeneration, necrosis and atrophy of muscle bundles and splitting of muscle fibres were the histological changes observed in the group of fish exposed to 1.0 ppm of textile dyes for 7 days which is shown in Figure 7.

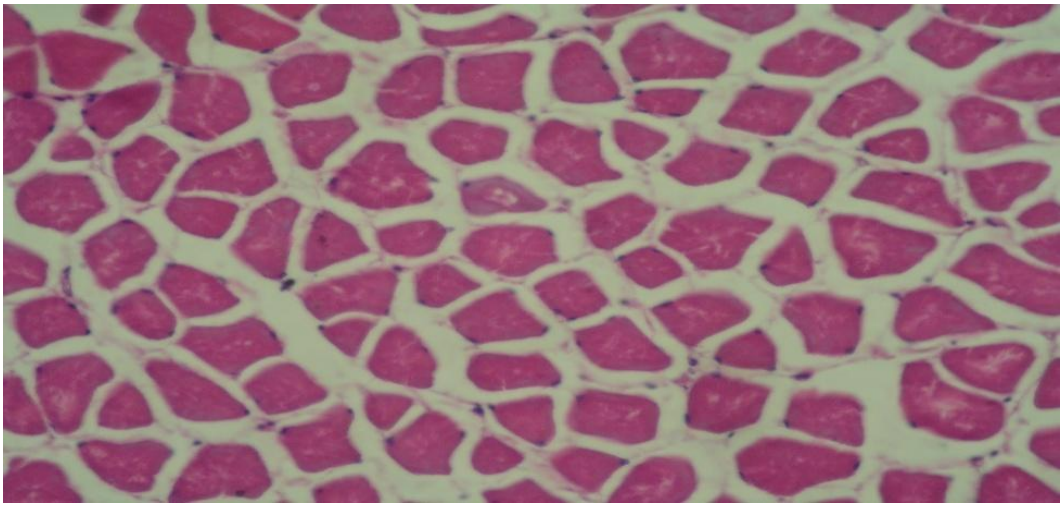

Figure 5: Photomicrograph of Muscle of Control group of fish (X40)

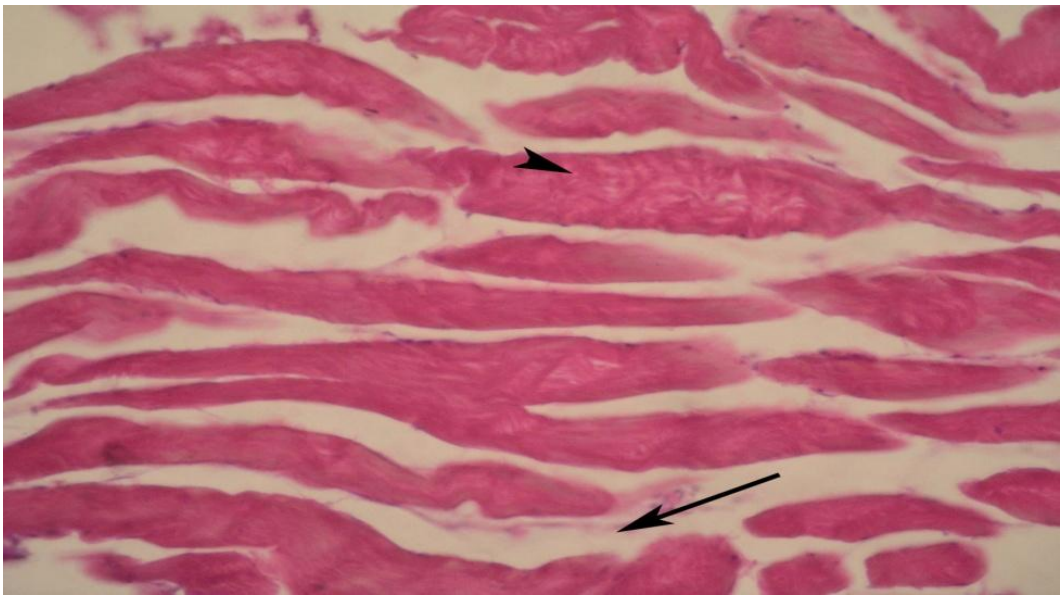

Figure 6: Photomicrograph of Muscle of fish exposed to 0.5 ppm of textile dyes for 7 days (X40)

Stained with H & E Arrow - Splitting of muscle fibres, Arrow head – Muscle bundles degeneration

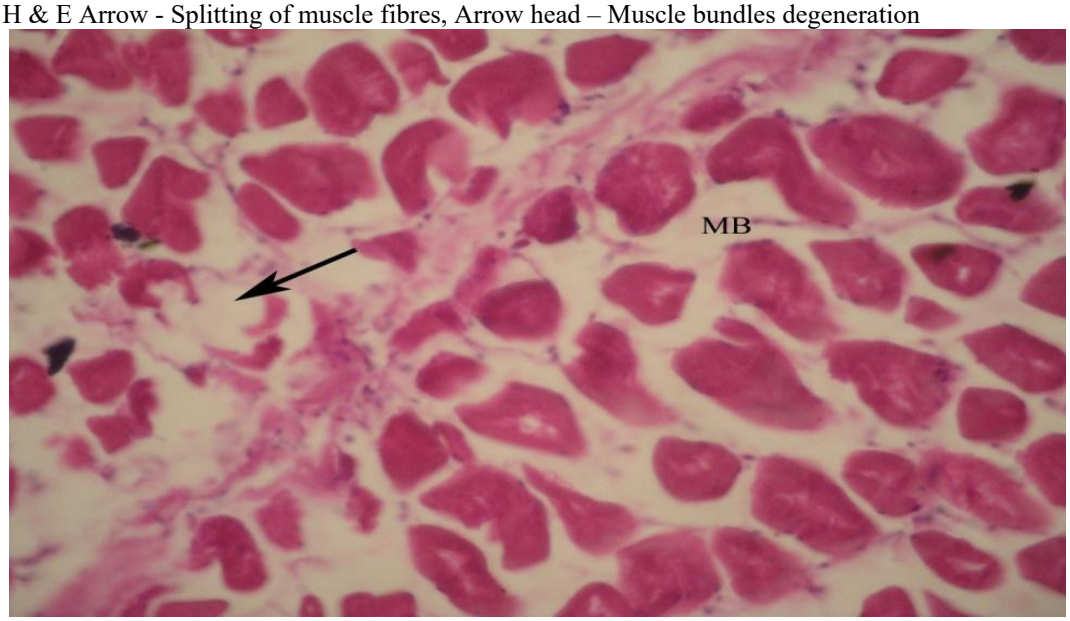

Figure 7: Photomicrograph of Muscle of fish exposed to 1.0 ppm of textile dyes for 7 days (X40)
Stained with H & E Arrow – Degeneration, MB – Muscle Bundles

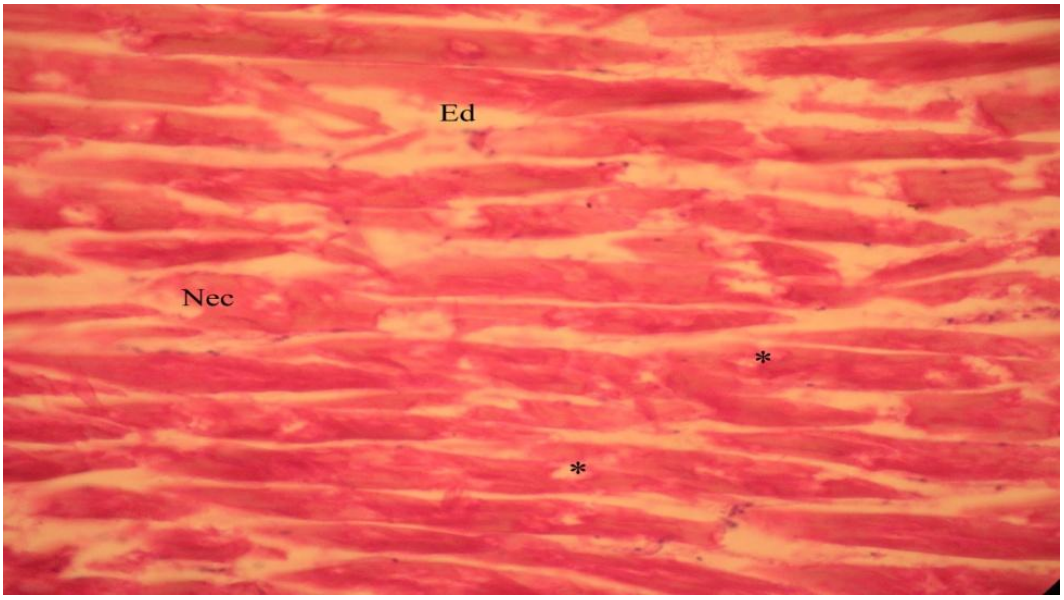

Figure 8: Photomicrograph of Muscle of fish exposed to 1.5 ppm of textile dyes for 7 days (X40)

Stained with H & E Ed – Edema, Nec – Necrosis, * - Vacuolar degeneration in muscle bundles The histological alterations such as edema between muscle bundles, vacuolar degeneration of muscle bundles and necrosis were observed in the muscle of group of fish exposed to 1.5 ppm of textile dyes for 7 days which is shown in Figure 8.

Degeneration of muscle bundles and necrosis were observed in the muscle of present studied group of fish exposed to 0.5 ppm of textile dyes for 14 days as depicted in Figure 9.

Splitting of muscle fibres was observed in the muscle of group of fish exposed to 1.0 ppm of textile dyes for 14 days as depicted in Figure 10.

The muscle of present studied group of fish exhibited vacuolar degeneration in muscle bundles due to exposure of 1.5 ppm of textile dyes for 14 days as depicted in Figure 11.

The exposure of group of fish exposed to 0.5 ppm of textile dyes for 21 days resulted in degeneration and necrosis of muscle bundles and atrophy of muscle bundles as shown in Figure 12.

The histological change in muscle of group of fish exposed to 1.0 ppm of textile dyes for 21 days was degeneration of muscle bundles as shown in Figure 13.

Severe muscle damages such as splitting of muscle fibres and necrosis were observed in the group of fish exposed to 1.5 ppm of textile dyes for 21 days as shown in Figure 14.

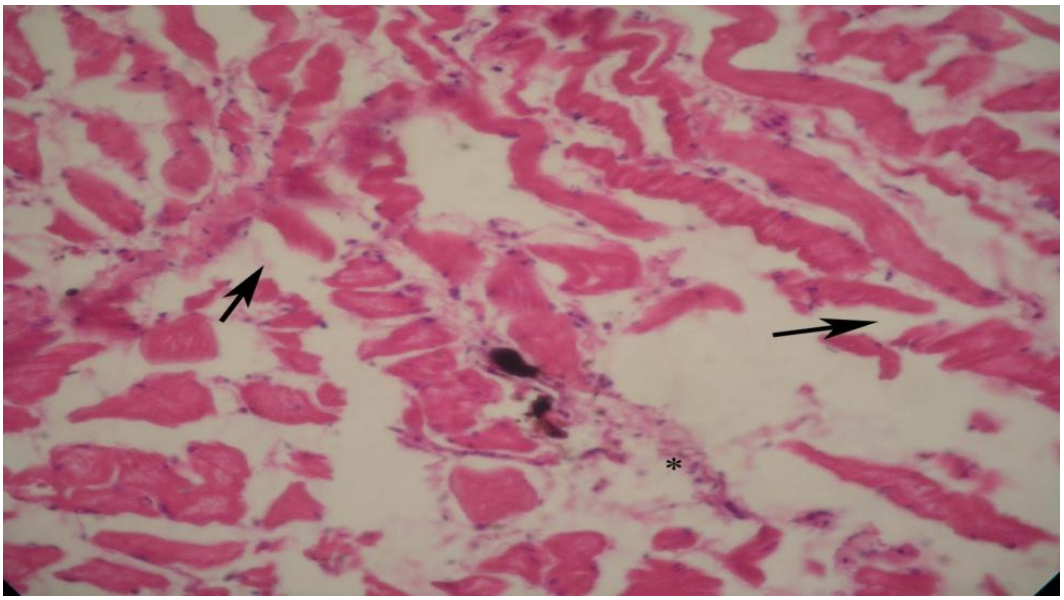

Figure 9: Photomicrograph of Muscle of fish exposed to 0.5 ppm of textile dyes for 14 days (X40)

Stained with H & E Arrow – Muscle bundles degeneration

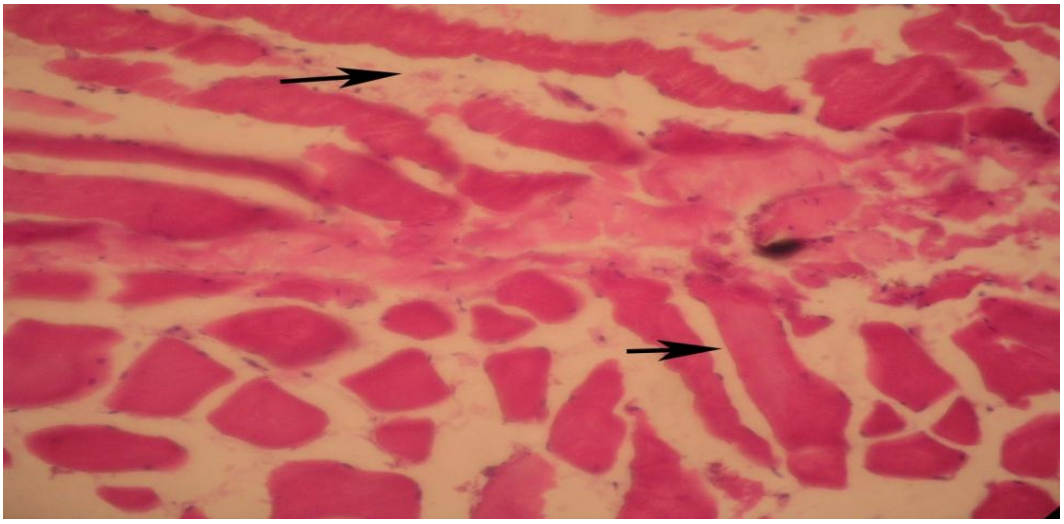

Figure 10: Photomicrograph of Muscle of fish exposed to 1.0 ppm of textile dyes for 14 days (X40)

Stained with H & E Arrow – Splitting of muscle fibres

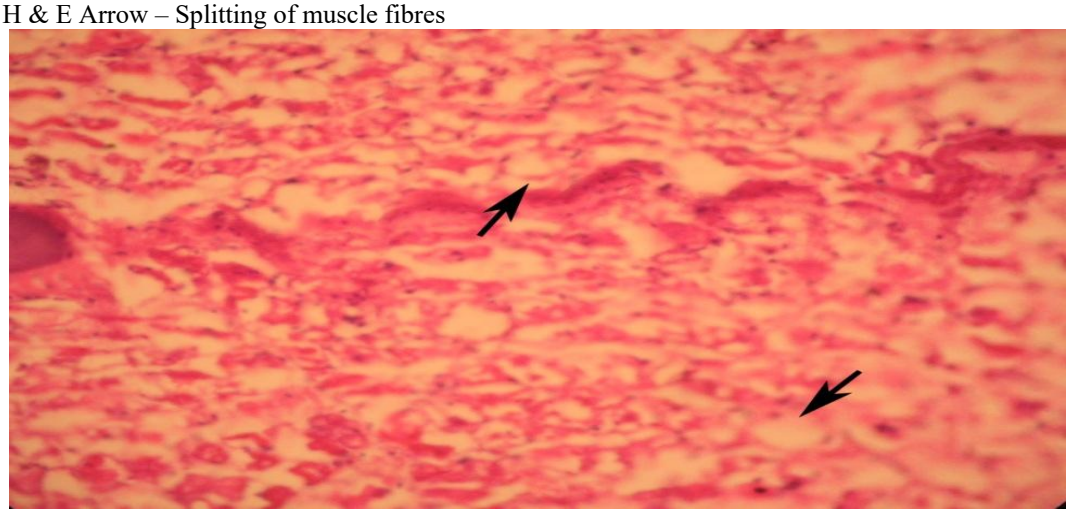

Figure 11: Photomicrograph of Muscle of fish exposed to 1.5 ppm of textile dyes for 14 days (X40)

Stained with H & E Arrow – Vacuolar degeneration

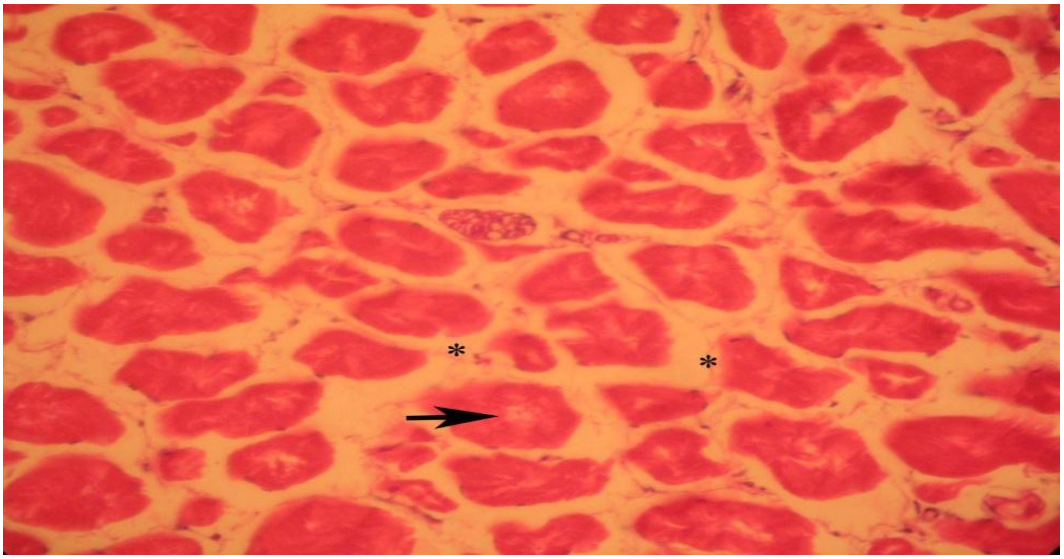

Figure 12: Photomicrograph of Muscle of fish exposed to 0.5 ppm of textile dyes for 21 days (X40)

Stained with H & E Arrow – Necrosis of muscle bundles, * - Atrophy

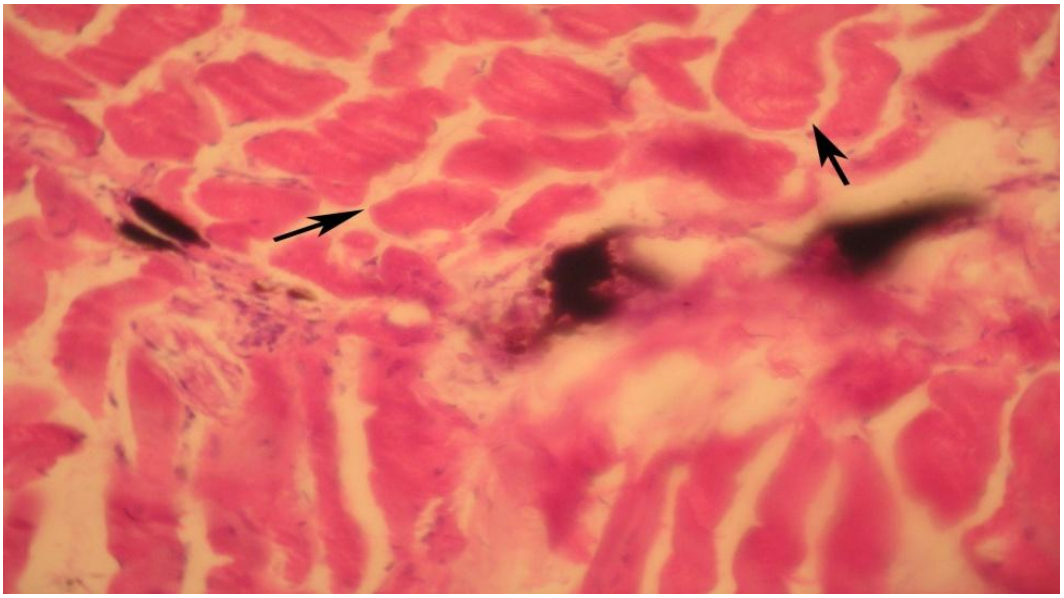

Figure 13: Photomicrograph of Muscle of fish exposed to 1.0 ppm of textile dyes for 21 days (X40)

Stained with H & E Arrow – Degeneration of muscle bundles

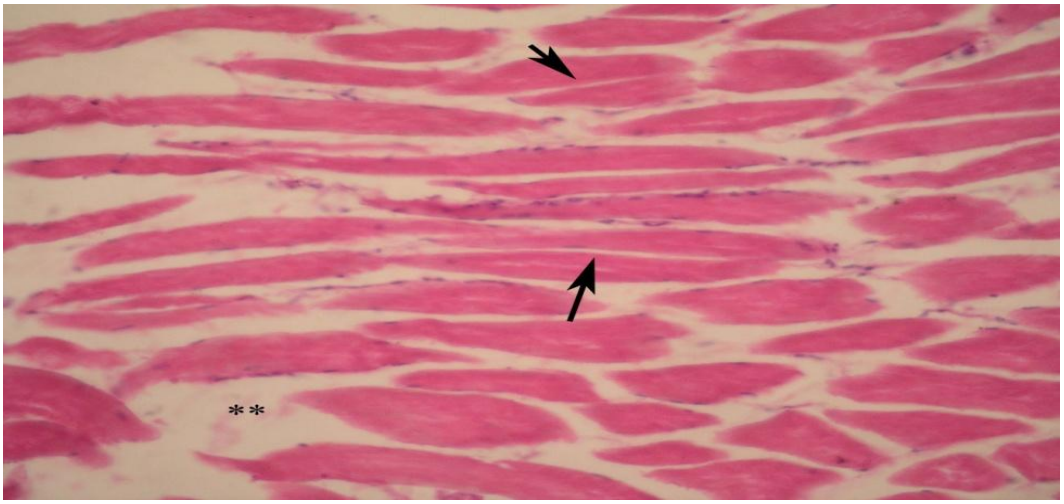

Figure 14: Photomicrograph of Muscle of fish exposed to 1.5 ppm of textile dyes for 21 days (X40)

Stained with H & E Arrow – Splitting of muscle fibres, * - Focal area of necrosis

5. Discussion

The protein pattern of muscle tissue of group of fish exposed to 0.5, 1.0 and 1.5 ppm of textile dyes for 7, 14 and 21 days was observed.

Lachapelle *et al*. (1993) observed a decline in albumin secreted by hepatocytes by approximately 38% as a result of mercury toxicity. Decreased liver and muscle proteins in *Tilapia mossambicus* by electrophoresis after malathion treatment [36].

The pathological alterations observed in the present studied fish were in agreement with observations in fish muscle due to the exposure of different pollutants [37].

Disintegration of muscle bundles with aggregations of inflammatory cells between them and focal area of necrosis, vacuolar disintegration in muscle bundles and atrophy of muscle bundles, edema between muscle bundles and splitting of muscle fibres were observed histopathological alteration in muscle of *Tilapia zilli* and *Solea vulgaris* from lake Qarun which is contaminated with different contaminants [38].

Deterioration of muscle bundles, focal area of necrosis, atrophy and vacuolar degeneration of muscle bundles were the histopathological changes observed in *Oreochromis niloticus* and *Lates niloticus* procured from Lake Nasser, Egypt contaminated with different heavy metals [39].Destruction and vacuolation of muscle cells was observed in *Oreochromis* spp. exposed to chromium was observed [40]

6. Conclusion

The protein pattern exhibited by electrophoresis suggested depletion of protein synthetic pathway in the muscle tissues of group of fish exposed to textile dyes.

Histopathological alterations under the influence of textile dyes can be used as a sensitive model to monitor problems in aquatic pollution. The sub- lethal effects detected in the histological alteration of tissues are significant in relation to the health of tilapia, *Oreochromis mossambicus* exposed to textile dyes.

Histopathological study offers a definitive toxicological effect of toxicants on the aquatic organisms. From the observations made in the present study, extensive use of textile dyes should be restricted and alternative use of natural dyes should be appreciated.

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