



HISTOLOGICAL CHANGES OBSERVED IN THE TISSUES OF *CIRRHINUS MRIGALA* EXPOSED TO OIL EFFLUENT

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ABSTRACT

The present investigation was to estimate the acute toxicity of oil effluent on *Cirrhinus mrigala* and to assess the lethal levels. The 120 hrs median lethal concentration of oil effluent were found to be 20ppt for *Cirrhinus mrigala*. Further experiments were conducted with sub lethal concentration of (1/10th conc. of LC₅₀) oil effluent which were evaluated from the LC₅₀ value. After treatment the fishes were reared in ideal condition, then sacrificed dissected at different predetermined interval during the accumulation period, (i.e.) 1st day to 20th day for *Cirrhinus mrigala* in oil effluent treatment for histological studies. The Histological changes were carried out under sub lethal concentration of (1/10th conc. of LC₅₀) effluents in Gill, Liver and Muscle. The following changes occur; the primary and secondary gill lamellae were affected. The photomicrograph of muscle depicted pronounced intramuscular odema with minor dystrophic changes. The liver showed varied degree of hepatic cirrhosis as evidenced by vacuolization, space formation and resulting haemorrhage.

KEY WORDS

Oil Effluent, *Cirrhinus mrigala*, Gill, Liver, Muscles

INTRODUCTION

The effluent when discharged into receiving water body without adequate treatment can cause irreversible changes like high temperature and increased pH of the received stream. The soluble chemicals and dyes present in waste water will interfere with the green aquatic plants. Colloidal organic matter and soluble inorganic salts will increase turbidity of this water (Hussein *et. al.*, 2004). Toxic effects of the chemicals and textile wet processing effluent can be seen on the receiving water bodies e.g. sodium sulphide, free residual chlorine, azo dyes and heavy metal like chromium are all toxic to fish and aquatic animals as well as resistant to bio-degradation. (Hussain *et. al.*, 2004). Water pollution induces pathological changes in fish. As a pointer of exposure to contaminants, histology represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects.

Direct discharge of industrial effluents in to rivers and run off from fields in to the ponds, lakes and rivers are causing serious concern about water pollution particularly with respect to inland fisheries. These effluents and their toxic effects on aquatic animals by depleting the dissolved oxygen, altering the pH, salinity, CO₂ content, and thereby directly and indirectly affecting the life cycles as well as the metabolic activities of the aquatic animals at the biological levels (David and Ray 1960; Stephen *et. al.*, 1987).

Toxicological and pathological studies the variations in the histology are exploited for the evaluation of physiological state of the animal. Histological alterations in marine organisms have been identified as useful biomarkers for environmental contamination (Hinton and Lauren 1990 (a, b); Hinton *et. al.*, 1992, Munday and Nowak, 1997). Histological changes appear as a medium-term response to sub-lethal stressors, and

histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs (Johnson *et. al.*, 1993). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Sindermann 1979; Bucke *et. al.*, 1996). Gills (Mallatt 1985; Poleksic and Mitrovic-Tutundzic 1994), kidney (Oronsaye, 1989; Bucher and Hofer 1993), liver (Hinton and Lauren 1990; Myers *et. al.*, 1993; ICES 1997) and skin (Vethaak 1994) are suitable organs for histological examination in order to determine the effect of pollution. Jiraungkoorskul *et. al.*, (2002) reported histopathological changes in the liver and gills of Nile tilapia, *Oreochromis niloticus*, exposed to glyphosate herbicide.

Gills are the first organs which come in contact with environmental pollutants. Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver. Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. (Athikesavan *et. al.*, 2006; Fernandes *et. al.*, 2007). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes, thus any harm in the gills leads to impairment of vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes (Ortiz, *et. al.*, 2003).

Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale, *et. al.*, 2011, Maryam, *et. al.*, 2013). Histological changes not only give an early indication of water pollution hazard, but also provide useful data on nature and degree of damage to cells and tissues (Shaikh, 2010). The gills of aquatic organism represent primary target of disturbance by pollutants as they are in direct contact with the external medium to perform gaseous exchanges and ionic regulations. This organ has linking sites to promote its regular functions, and these sites link to toxic substances with differentiated charges, triggering, mechanical responses and toxic effects to the organism (Jadhav, *et. al.*, 2007). Allen (1995) observed the histological changes in gills of the *O. mossambicus* due to cadmium toxicity and observed ruptured gill lamellae. Vijayalaxmi and Tilak (1996) while

working on *Labeo rohita* found pathological lesions in gill exposed to pesticides.

Saksena and Pandey (1993) also have observed toxic effect of copper on the histological effect on gills of a freshwater fish, *Labeo rohita*. (Victor *et. al.*, 1990) observed the changes in the gill tissue of freshwater prawns, *Macrobrachium idea* exposed to mercury. Hence, the present study was undertaken to study the effects of oil effluent on histological changes in the gill, liver and muscle of *Cirrhinus mrigala*.

Fish is the one of the abundant populations of an aquatic environment. They are easily susceptible to any alteration in the physico-chemical characteristics of the habitat (Sadiq Bukhari *et. al.*, 2012). Fish gills, food and skin are generally recognized as three possible routes for any chemical substance from aquatic environment to enter the fish (Tao *et. al.*, 2000). NPs absorbed into the blood via gills and then reach the brain through the blood-brain barrier (BBB) via systematic distribution (Hu and Gao, 2010). Histopathological studies are very sensitive and crucial parameter, which reflects the effect of toxicants on organ (Abdel-Warithet *et. al.*, 2011). Histological biomarkers are closely related to others of stress since many pollutants either toxic or non-toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism (Velkova-Jordanoska, 2002). As a tool for assessing endocrine disrupting effects in fish, histopathology has also been applied in other studies such as endocrine disruption in the ovaries and testes of zebra fish (Van der Ven *et. al.*, 2003).

MATERIALS AND METHODS

Collection of Experimental Animal

The fish, *Cirrhinus mrigala* (Length 9.2±0.003cm, Weight 10.3±0.002 g) fresh water food fish were segregated and procured from Karanthai (Golden Fish Farm) farm, Thanjavur, Tamil Nadu, India and were transported in aerated polythene bags to the laboratory. The fishes were acclimatized to lab condition for 3 days before treatment with oil effluent.

Determination of LC₅₀

The mortality rate of *Cirrhinus mrigala* was recorded at 24, 48, 72, 96 and 120 hrs exposure to the oil effluent. The percentage for corrected mortality was calculated using the Abbott's formula (1952).

$$\text{Corrected mortality (\%)} = \frac{\text{Percentage living in control} - \text{percentage living in treatment}}{\text{Percentage living in control}} \times 100$$

The corrected mortality data was analyzed to determine the LC₅₀ values for 24, 48, 72, 96 and 120 hrs and were calculated by probit analysis method (Finney, 1971).

Arithmetic graph was plotted with oil effluent concentration on the X-axis and percentage of mortality on the Y-axis, from this graph LC₅₀ 120 hours value was calculated.

Acute toxicity test

The purpose of this study was to test the acute toxicity of oil effluent on *Cirrhinus mrigala* and to evaluate the lethal levels of oil effluent. At first tentative experiment were conducted to fix the minimum concentration of oil effluent to obtain maximum mortality for *Cirrhinus mrigala* over 120 hour's duration. After confirming the minimum concentration, identified size of *Cirrhinus mrigala* were placed in different tubs (each group consists of 6 animals in 10 litre capacity plastic tubs) and exposed to different concentration of oil effluent which ranges from 10 ppt – 30 ppt at an interval of 5 ppt for *Cirrhinus mrigala* for a period of 120 hour. In addition to that a control was also maintained simultaneously. The Histological studies carried out under sub lethal (1/10th conc. of LC₅₀) concentration in gill, liver and muscle.

Histological Method

The effects of oil effluent on the fish can be studied by histological methods. The gill, liver and muscle were removed from the control and experimental animals for histological observation. Then these organs are fixed in freshly prepared Bouin's fixative for 24 hours. Then they are transferred to alcohol series and processed as per the methods suggested by Epple (1967). Tetrahydrofuran used as a clearing reagent. Ethyl alcohol was used as decolourising agent then it is embedded in Paraffin wax sections of 6µm thickness were taken and stained for light photo microscopic observations.

Results

LC₅₀

The lethal concentration of LC₅₀ value refers the concentration of toxicant at which 50 % of the selected organism or dead in the given exposure of time. The LC₅₀ values are applied for aquatic animals such as fishes. LC₅₀ are represented in the form of ppt or mg / litre.

Acute toxicity LC₅₀

The 120 hrs median lethal concentration of oil effluent on *Cirrhinus mrigala* was 20 ppt. During the experimental period the fishes were restless aggressive and have the tendency to leap out of the tubs (struggle for existence). This may be due to the suffocation out of

oxygen deficiency. Secretion of mucus in the gill chamber should the lesions in gills of fishes. The 120 hrs median lethal concentration of oil effluent were found to be 20 ppt for *Cirrhinus mrigala*.

Control

The morphology of control *Cirrhinus mrigala* are presents numerous gill arches. Arches are supported by mixed bone (a cellular and spongy) and cartilage with associated striated abductor and adductor muscle, facilitating movement of gills. Gill arches showed clear boundaries of hypertrophic zone, growth zone and apical zone which is supported by mucosal epithelium (**Plate 1**). Normal distribution of macrophages, endothelial cells, mucous cells, and chloride cells was observed. Control muscle showed muscle fibers, fibril layers and connective tissue covers the muscle fibres, myofibrils and myofilaments. The common histopathological observations in the gills of *Cirrhinus mrigala* includes ploriferation of the epithelium of the gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, degenerative necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucus cells proliliorahom. Edematous changes, characterized by epithelial detachment in gill filaments and secondary lamellae, associated with aggregations of inflammation cells in gill filaments. Evaluation of toxicology effects of oil effluent on gill of *Cirrhinus mrigala* are presented in (**Plate 1**).

1/10th Conc.

The results of the light microscopic studies showed that the morphological changes caused due to exposure to oil effluent were evident in the gill of *Cirrhinus mrigala* were observed in the control fish. In the fish exposed to 1/10th Conc. of oil effluent, resulted in the primary gill lamella was diluted leading to damage at various places (**Plate 1**). Basal cell layer was reduced. Destruction of mucus cells on the top of gill lamella was observed.

Gills

Light Microscope (LM) examination of the Photomicrograph of the vertical section of the gills (**Plate 1**) showed the arrangement of primary and secondary lamellae processes of which the former was thicker than the latter. The primary gill lamellae are flat leaf like structures with a control rod like supporting axis and a row of secondary gill lamellae on each side of it. The secondary lamellae (SL) were equally spaced along the columnar structures with falt cellular layer attached at their bases with the primary lamellae and free at their distal ends. The normal secondary lamellae epithelium

was simple, consisting of a thin single or double sheet of epithelial cells, blood vessels and a row of pillar cells. The region between the two adjacent secondary gill lamellae is known as inter lamellar region.

In fish exposed to sub lethal ($1/10^{\text{th}}$ Conc. of LC_{50}) concentration of oil effluent at the 5-day interval of the gill photograph (**Plate 1**) showed cyto architectural distortion of the lamellae with overlap of the primary and secondary lamella. Considerable mucous and granulated eosinophilic cells were witnessed in their cytoplasm. Extensive vacuolization were observed with prominent disruption of epithelium. The main response of gill epithelium was reduction in permeability.

Liver

The photomicrograph of the liver (**Plate 1**) showed large polyhedral cells within the network of minute canaliculi. There was irregular distribution of bile duct blood capillaries and sinusoids that are filled with erythrocytes. Hepatocytes surrounding the control vessels appeared to be lightly arranged in a rosette pattern with 10 to 12 cells in each group. Hepatic cells

are roundish polygonal containing clear spherical nucleus. They are located among sinusoids forming cord like structure, known as hepatic cell cords. In fish, these structures are generally obscure. The fish exposed to sub lethal ($1/10^{\text{th}}$ Conc. of LC_{50}) concentration of oil effluent (**Plate 1**) showed varied degree of hepatic cirrhosis as evidenced by vacuolization, space formation and resulting haemorrhage. Hypertrophy of hepatocytes and clumping was also clearly evident in the treated tissues of *Cirrhinus mrigala*. At $1/10^{\text{th}}$ Conc. of LC_{50} of oil effluent for the same exposure, acute and extensive necrosis of liver cells were observed with indistinct cell boundaries in many places and pyknotic nuclei.

Muscles

On exposure to sub lethal concentration of oil effluent (**Plate1**) marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed sub lethal ($1/10^{\text{th}}$ Conc. of LC_{50}) concentration of oil effluent led to pronounced intramuscular odema with minor dystrophic changes.

Fig. 1: Determination of LC_{50} (120 hours) for oil effluent in *Cirrhinus mrigala*

Number of fishes used = 6/each concentration

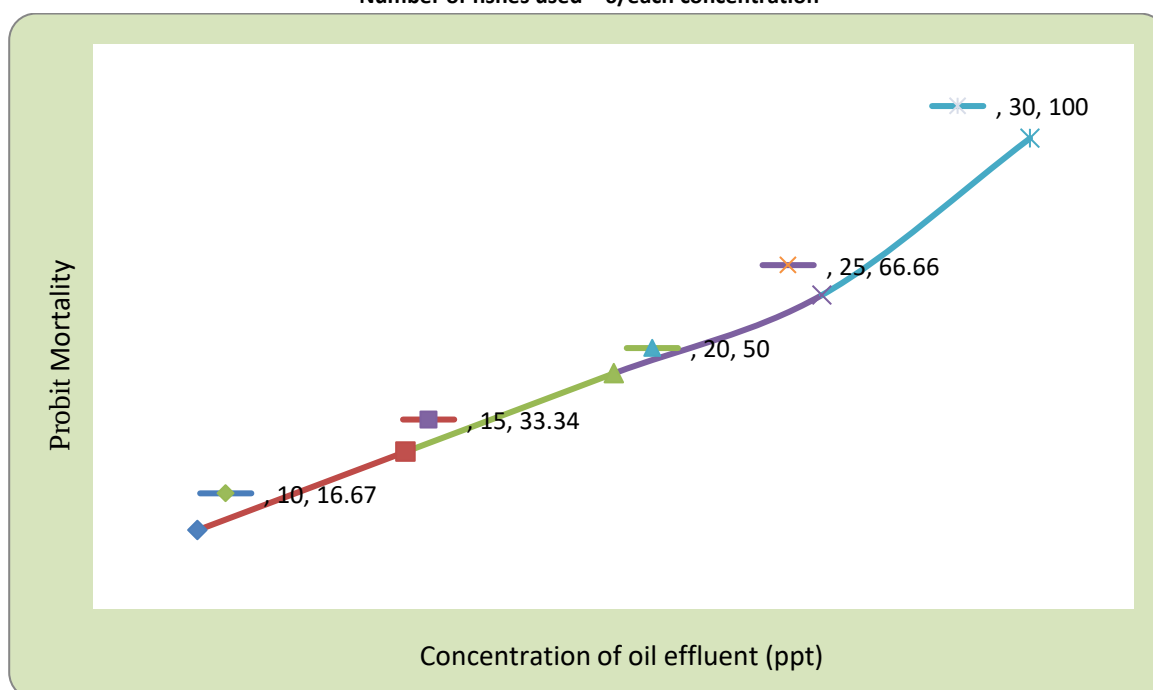
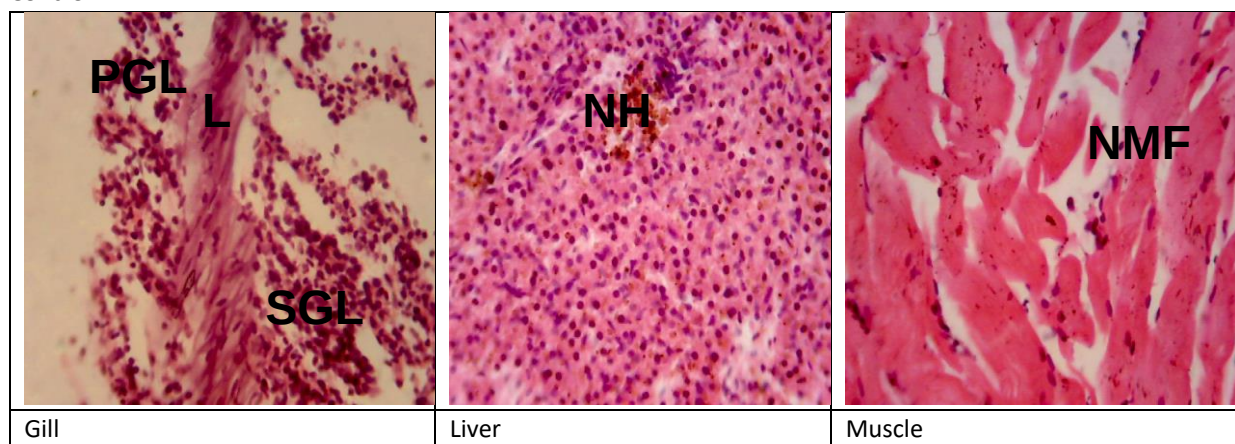
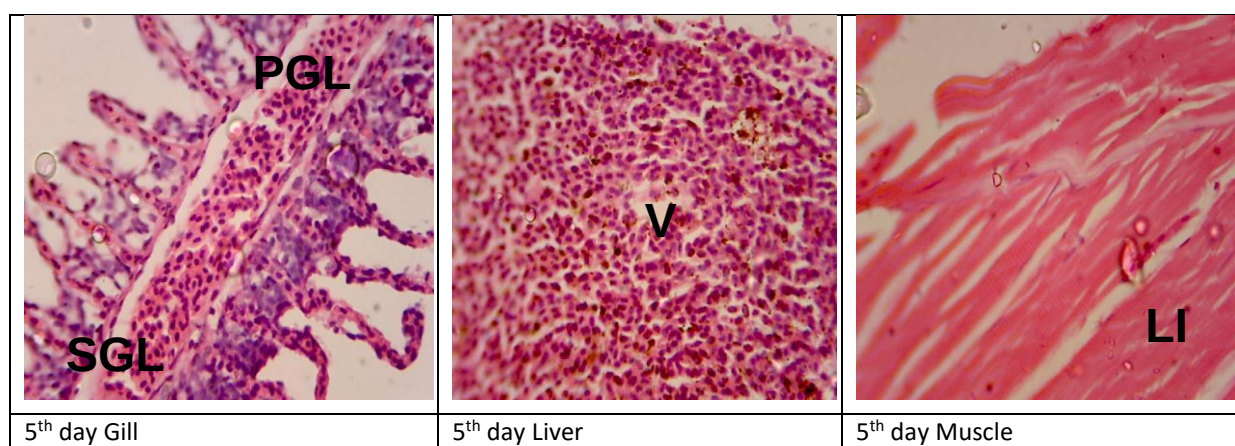
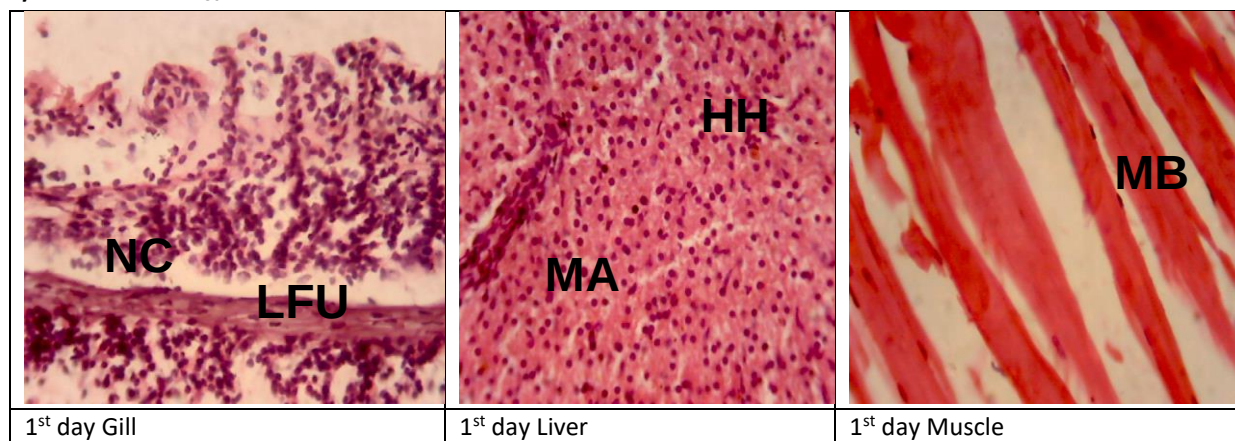


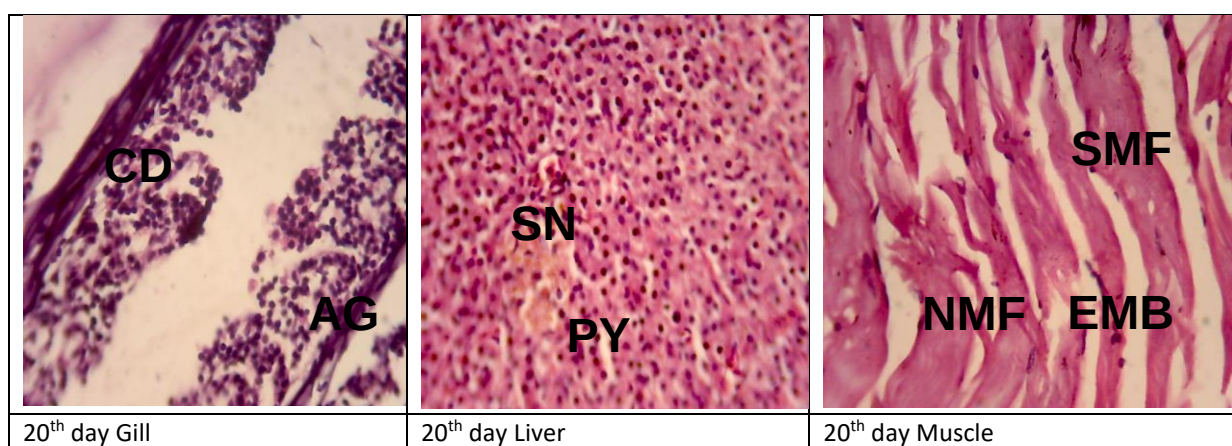
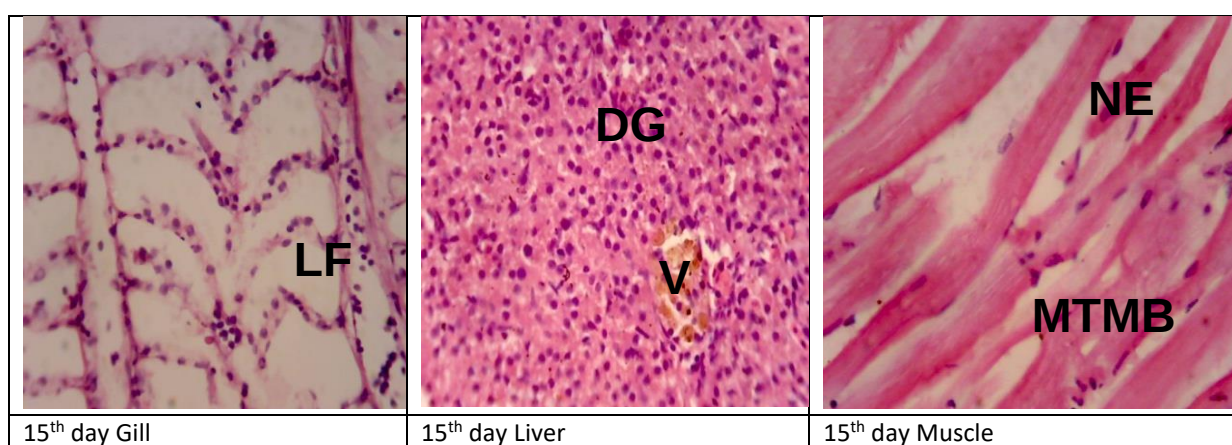
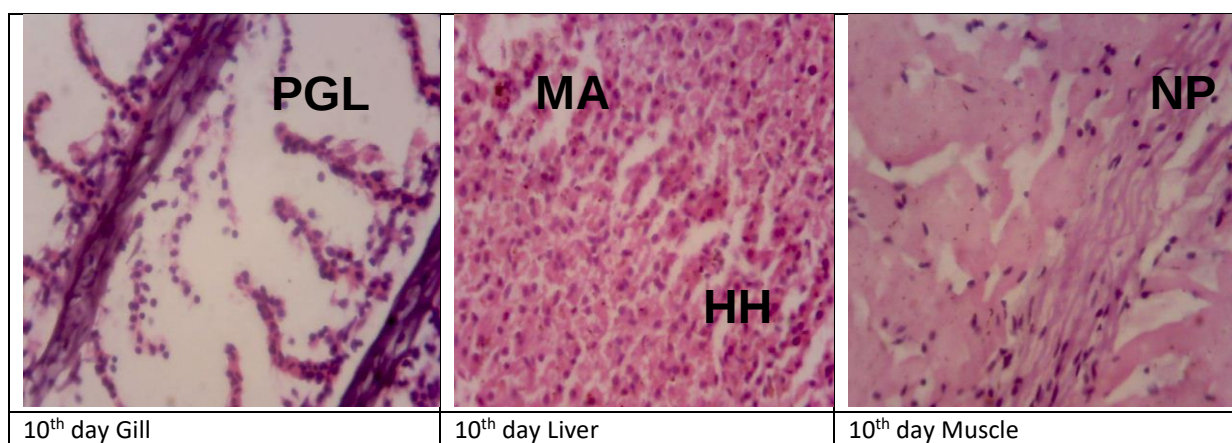
Plate 1: Histological Changes of *Cirrhinus mrigala* in the tissues of gill, liver and muscle exposed to oil effluent

Control



1/10th Conc. of LC₅₀





AG= Abnormal gill tip

CD= Cartilage damage

DG= Degranulation and degeneration

EMB= Edema of muscle bundles

HH= Hypertrophy of hepatocytes

LFU= Lamella fusion

LI= Leucocytic infiltration

MA= Metal accumulation

MB= Muscle bundles

NE= Necrosis

NH= Normal hepatocytes

NMF= Necrotic muscle fibres

NP= Nuclear proliferation

PGL = Primary gill lamella

PY= Pyknotic nuclei

SGL= Secondary gill lamella

SMF= Shrinkage of muscle fibres

SN= Severe necrosis

MTMB = Marked thickening of muscle bundles

V= Vacuolation

NC= Necrosis in cytoplasm

DISCUSSION

The experimental fishes exposed to sub lethal concentrations of oil effluent exhibit histological changes in the gill, liver and muscle due to the accumulation of oil effluent within the organism body at sublethal levels lead to histological lesion in the body fish, *Cirrhinus mrigala*. Similar observations were made by Hemalatha and Banerjee (1997) and Gupta and Kumar (2006) gill lesions in zinc treated *Heteropneustes fossilis* and mercury treated *Cirrhinus mrigala* respectively. Kaoud and El-Dahshan (2010) observed severe hyperplasia in secondary gill lamellae which lead to complete embedding in adjacent lamellae in copper, cadmium, lead and mercury treated *Oreochromis niloticus*.

Histopathological studies are also useful in evaluating the pollution potential of heavy metal pesticides, since trace amount of these chemicals which do not bring animal mortality over a given period, were capable of producing considerable organ damage (Kumar and Pant, 1984; Jaykumar, 2002). Despite much information available on the histological changes caused by heavy metal pesticides, the mode of action on the vital organs is still not fully understood. The difference between the control and the experimental tissues were studied critically.

The freshwater forms very important media for the production of protein-rich fishes, prawns and crabs. But the fresh water media are ecologically deteriorating due to discharge of industrial effluents (Thingran1974). The disposal of industrial effluents in the aquatic environment is toxic to fishes. Mortality of fishes has been recorded in rivers receiving various pollutants. Abnormal physico-chemical characteristics of industrial effluents are responsible for mortality of fishes (Mishra *et. al.*, 1988; Pawar 1988).

These histological observations are quite comparable to pathological lesions induced in gills by mercuric chloride in *Acipenser persicus* fry (Khoshnood *et. al.*, 2011), by lead and cadmium treatment in *Cyprinus carpio* (Patnaik *et. al.*, 2011), *Lates calcarifer* (Thophon *et. al.*, 2003), *Brachydanio rerio* and *Salmo gairdneri* (Karlson-Norgren *et. al.*, 1985). Patel and Bahadur (2010) also noted severe gill lesions in copper treated *Catla catla*.

The liver is an important vital organ through which most of the important metabolic functions are occurring and the entry of toxicants primarily affects the liver. Toxic induced changes in the liver of the fishes can be regarded as an index for the identification of pollution present in the environment (Cough1975). During the present study marked histopathological lesions were observed in the liver of *Cirrhinus mrigala* exposed to sub lethal concentrations of oil effluent. At sublethal concentration histopathological lesions were mild and observed as degeneration of hepatocytes, cell wall rupture, necrosis, picnosis and cytoplasmolysis. Parenchymal cells leading to appear smaller in size, cytoplasm becomes granulated and vacuolated, damaged and empty blood vessels, and swelling of liver cord. These severe effects in sub lethal concentration may be due to the possibility of more accumulation of effluent liver.

Depletion of glycogen in the tissues is an indication of typical stress response of the fish challenging with pesticides (Rao, 2006b). Decrease in muscle glycogen content of *Channa punctatus* exposed to lambda cyhalothrin and permethrin were reported by Saxena and Gupta (2005). The reductions in liver and gill glycogen content were also observed in *C. batrachus* exposed to cypermethrin (Begum, 2005).

The high accumulation of lead and cadmium in the liver, which Gbem *et. al.*, (2001) also noted in their findings, is related to the fact that liver plays a role in accumulation and detoxification. 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 (Hawkes, 1980). Although, according to Friberg *et. al.*, (1971), fishes are known to possess sequestering agent (metallothionein), the bioaccumulation of these trace elements in the liver tissue reaches a proportion in which the function of the liver is impeded, thus resulting in gradual degeneration of the liver and syncytial arrangement. The surface area of the liver cell is also decreased, which may be due to increase in intra biliary fibre-connective tissue. The vacuolization observed are zones of total cell degeneration. Similar degenerating changes were observed in liver of both sexes of *C. carpio* after exposure to HgCl₂ at 0.1 ppm for 45 and 60 days (Masud *et. al.*, 2001, 2003).

As with gills, muscle tissue also come in close contact with pollutants dissolved in water. Hence, reactions in the ultra structure of the muscle were spontaneous. Separation of muscle bundles was an interesting observation. Initial stimulus of lead and cadmium can induce hyperactivity and excitability in animals, leading to release of lactic acid and subsequent muscular fatigue (Das and Mukherjee, 2000)

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