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# HISTOLOGICAL ALTERATIONS IN SELECTED TISSUES OF *TILAPIA MOSSAMBICA* (CICHLIDAE) AS A BIOMARKER OF FERTILIZER POLLUTION

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**Abstract:** Acute toxicity of factamfos and its histopathological impacts on gill, liver and kidney of a cichlid fish, *Tilapia mossambica* were evaluated in the present study. Specimens were collected from a fish farm and exposed to different concentrations of factamfos to determine the median lethal concentration (LC50). The LC 50 for 96 hr exposure was found to be 0.73 g/l. Histology of gill, liver and kidney of the fish was assessed after exposing the clams in sub lethal concentration of factamfos, viz., 0.36 g/l, for about 14 days along with a control. After the exposure time period the tissues were excised, processed and stained in haematoxylin and eosin. Histological alterations in gill, liver and kidney at different levels could be identified. Rupture of gill lamellae and irregular gill lamellae in the gill, vacuolation and hepatocyte hypertrophy in the liver and glomerular expansion and dilation of Bowman's capsule in the kidney were the observed deleterious changes in the fish exposed to factamfos. The results showed that the fertilizer (Factamfos) pollution adversely affects the fish and causes destruction of various organs.

**Key words:** Cichlid teleost, Agriculture, Factamfos, Median Lethal Concentration, Histopathology.

## INTRODUCTION

Aquatic ecosystems are at risk of contamination due to anthropogenic activities such as treatment of agricultural lands with fertilizers and pesticides, unmanaged industrial effluents and domestic wastes. Different environmental pollutants are likely to affect biological systems in different ways according to their chemical properties.

The use of fertilizer in aquaculture and agriculture results in high fish production and crop production. But excessive use of fertilizers in various forms and quantities has got the potentialities of changing the aquatic medium affecting the tolerance limit of aquatic flora as well as fauna, thereby posing a danger to the ecosystem (Palanichamy *et al*., 1985). The continuous exposure to these chemicals for extended time periods can cause chronic stress, starting from the cellular and sub-cellular levels of organization to ultimate death of the species. All species are tolerant to a certain amount of the environmental variation and beyond the tolerable limits, characteristic biochemical and physiological responses related to the ultimate survival or death of the individual are elicited (Elder and Collins, 1991).

The biological monitoring is the systematic use of biological responses to evaluate the changes in the environment with the intent to use this information in a quality control programme (Phillips, 1980). In recent years, increasing emphasis has been given for the evaluation of the relationships between contaminant exposure and observable biological effects on aquatic organisms. Adverse biochemical and physiological changes in an organism result in histopathological alterations. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular changes in the affected organism. Thus it provide a powerful tool to detect and characterize the biological end points of toxicant (Hinton and Lauren, 1990; Hinton *et al*., 1992; Moore and Simpson, 1992).

Histopathological lesions in various tissues have been well studied in different fish exposed to various toxicants (De Flora *et al*., 1991; Alazemi *et al*., 1996; Al- Salim and Al- Niacem, 2002; Stentiford *et al*., 2003; Thophon *et al*., 2003; Iqbal *et al*., 2004; Camargo and Martinez, 2007; Nayan, 2012; Ahemed, 2013). But the studies on the toxicity of fertilizers on aquatic fauna are limited. The present study is aimed to determine the toxic and histopathological effect of a fertilizer, factamfos on a cichlid teleost fish, *Tilapia mossambica*.

## MATERIALS AND METHODS

### Collection and acclimatization of fish

Specimens, *Tilapia mossambica* were collected from a fish farm and brought to the laboratory. In the laboratory the fish were immediately released in to an aquarium tank containing aerated tap water. They were maintained there for about 6-7 days in a static condition. Fish were fed on artificial feed daily. Faecal matter and other unwanted particles were removed from the tank daily. The water medium was changed at 24 hrs interval to remove the metabolic pollutants. During the final acclimatization period of 24 hrs fish were not fed. After acclimatization only healthy fish were used for the experiment.

### Determination of LC50

Factamfos manufactured by FACT, Cochin was collected from a fertilizer store at Kottayam. For preliminary experiment ten fish of almost same size were exposed to 6 different concentrations of factamfos (0.05 g/l, 0.2 g/l, 0.4 g/l, 0.6 g/l, 0.8 g/l, 1 g/l). Fish mortality induced by these concentrations was noted. On the basis of observed mortality in the preliminary trial, a series of narrow range concentrations (0.68 g/l, 0.7 g/l, 0. 72 g/l, 0.74 g/l, 0.76 g/l, 0.78 g/l) were selected for final experiment to determine LC50. Ten healthy acclimatized fish were selected and exposed to these concentrations for 96 hrs. The control experiment without the toxicant was also maintained simultaneously. The mortality of animals in each concentration of the toxicant was recorded as percentage and the dead animals were removed from the tank. LC50 was determined by using probit analysis (Finney, 1971).

### Histopathology

For histopathology experiment a sub lethal concentration of factamfos, viz., 0.36 g/l were selected based on the 96 hr LC50 value. Fish were exposed to this concentration for 14 days along with a control experiment. The test animals were fed during the entire period of experiment. The experimental medium in the test chambers was replaced with appropriate concentrations of the fertilizer daily to maintain the concentration of the toxicant. At the end of 14$^{th}$ days gill, liver and kidney were excised from the exposed fish and fixed in bouin's fixative for 24 hrs. The tissues fixed were then dehydrated in alcohol series, cleared in xylene and embedded in paraffin. 5µ thick paraffin sections were cut and stained with haematoxylin and eosin. The stained sections were examined under a light microscope and the histopathological changes observed in the tissues were photographed.

## RESULTS AND DISCUSSION

The 96 hr. LC50 value of *T. mossambica* exposed to factamfos was found to be 0.7 g/l (Fig. 1). Sub lethal concentration used for the experiment was 0.36 g/l. The three tissues selected viz., gill, liver and kidney of *T. mossambica* for the study exhibited alterations.

The histology of gill in control fish *T. mossambica* is given in Fig. 2. Gill consists of two rows of gill filaments and is attached to the opposite sides of the interbranchial septum. Fila-

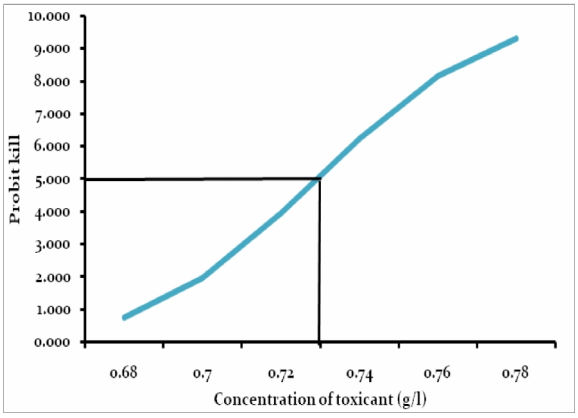


**Fig. 1.** Median lethal concentration of *T. mossambica* exposed to factamfos

ments have a central cartilaginous support, afferent and efferent arterioles and a thin epithelial covering. The primary lamella is a fold of epithelium and consists of a central vascular core. Secondary lamellae originate on both the surfaces of primary lamellae and are oriented perpendicular to the filaments. The secondary lamellae possess numerous channels of blood capillaries. The thin epithelial covering of the secondary lamellae lies on a basement membrane supported by pillar cells.

Fish gills are critical organs which perform respiratory, osmoregulatory and excretory functions. The gills of freshwater fish are the largest fraction of the total body surface area, which are the first target of waterborne pollutants due to the constant contact with the external environment (Hughes, 1984). On exposure to factamfos gills of *T. mossambica* showed alterations like rupture of gill lamellae and irregular gill lamellae (Fig. 3). Besides, gill showed marked oedema with vacuolated wall. The secondary lamellae are also showed rupture and desquamation of epithelial cells. Pandey and George (1977) observed hyperplasia, desquamation of the epithelial cells, fusion of secondary gill lamellae, and congestion of blood sinuses in the gill of an estuarine mullet, *Liza parsia* exposed to lead. According to Hinton and Lauren (1990) the lifting of the epithelium from the basement membrane of the gill arch, filaments and lamellae is the most important sign of ecological degradation. The pathological changes may be a reaction to toxicants intake.

The organ most associated with the detoxification and biotransformation process is the liver due to its function, position and blood supply. The liver of fish is a dense organ, consists of lobules of tubular glands. Its size, shape, and volume are adapted to the space available in the body cavity. Hepatocytes are polygonal in shape having a distinct central nucleus with densely staining chromatin margins and a prominent nucleolus. Biliary canaliculi are found in between hepatic cells. Liver histology of the control fish T. mossambica is given in Fig. 4. The liver tissue showed normal structure of hepatic cells, connective tissue, and hepatic mass granulation.

The teleost liver shows alterations in histoarchitecture, biochemistry, and physiology on exposure to various environmental pollutants (Thophon *et al*., 2003; van der Oost *et al*., 2003). In the present investigation *T. mossambica* exposed to factamfos showed many deteriorative changes in liver such as hepatocyte hypertrophy, vacuolated cytoplasm, and dilation of sinusoids (Fig. 5). Small spaces were also appeared in between hepatic cords. The changes in liver histology identified in the study may be the result of various biochemical lesions. According to Hinton and Lauren (1990) vacuolation of hepatocytes is associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization. Swelled and dissociated hepatocytes were also reported in arsenic exposed teleost fish (Pedlar *et al*., 2002; Lam *et al*., 2006). The monitoring of the histological changes in fish liver is a highly sensitive and accurate way to assess the effect of xenobiotic compounds in field and experimental studies. Several histological alterations were reported in liver of fish exposed to industrial pollutants (Mukherjee and Bhattacharya, 1975; Ahmed *et al*., 2013).

The histology of kidney in control fish is shown in Fig. 6. The kidney of fish consists of Bowman's capsule, proximal convoluted tubules and distal convoluted tubules. It is characterized by the vacuolation of the epithelial cells of the proximal convoluted tubules and distal convoluted tubules. The glomerular tissue was closely arranged with renal tubules including distal and collecting tubules and intact interstitial cells.

The teleostean kidney is an important organ to be affected by contaminants in the water (Thophon *et al*., 2003), because of its important role in excretion of harmful materials. Glomerular expansion and dilation of Bowman's capsule and renal tubules were noticed in the kidney of *T. mossambica* exposed to sub lethal concentration of factamfos (Fig. 7). Disturbance at the cellular levels of its biological organization by toxicant exposure can lead to cell injury, resulting in degenerative and neoplastic changes in target organs which can be lead to tissue necrosis (Pacheco and Santos, 2002). Several deleterious changes like shrinkage of glomerulus, and dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degen-

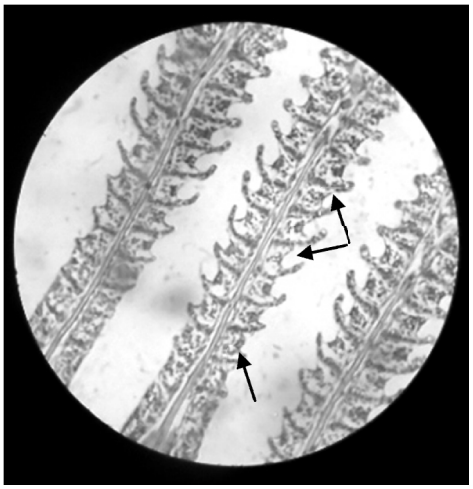

**Fig. 2.** Primary and secondary lamellae of gill filament of *T. mossambica*

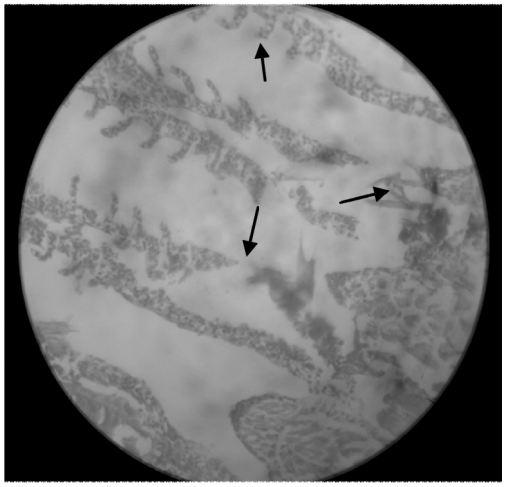

**Fig. 3.** Gill of *T. mossambica* exposed to sublethal concentration of factamfos. Arrows indicate the rupture of primary and secondary gill lamellae.

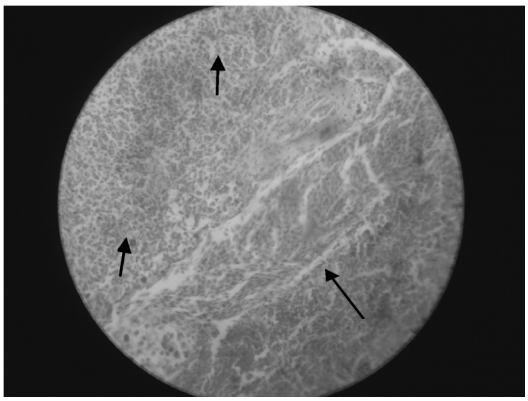

**Fig. 4.** Liver of T. mossambica showing hepatocytes and central vein (long arrow)

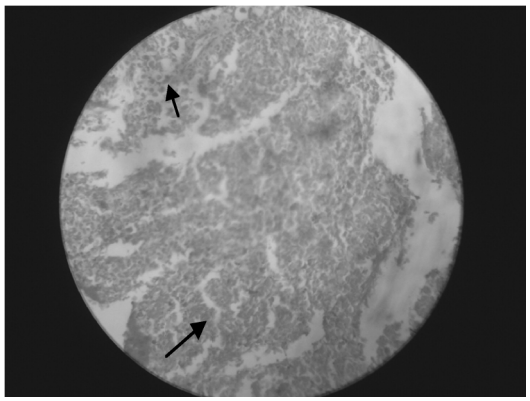

**Fig. 5.** Liver of T. mossambica exposed to sublethal concentration of factamfos. Arrows indicate hepatocytes hypertrophy and intercellular space

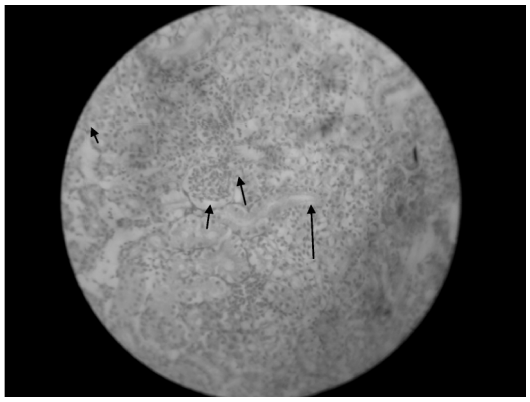

**Fig. 6.** Kidney of *T. mossambica* showing glomerulus, Bowman's capsule, distal convoluted tubule (short arrow) and proximal convoluted tubule (long arrow)

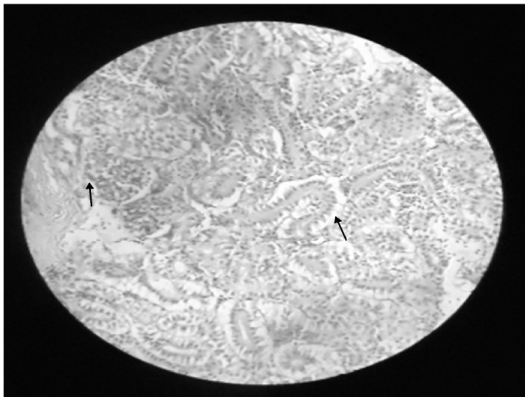

**Fig. 7.** Kidney of *T. mossambica* showing glomerular expansion and dilation of renal tubules

eration of tubular epithelium of the kidney in *Cyprinus carpio* exposed to sub lethal concentration of dimethoate have been reported by Nayan (2012). Velmurugan *et al*. (2007) observed pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubule, contraction of the glomerulus and expansion of space inside the Bowman's capsule in the kidney of *Cirrhinus mrigala* exposed to monocrotophos

All histopathological observations in the present study indicated that exposure to sub lethal concentrations of factamfos caused destructive effect on the gill, liver and kidney tissues of *T. mossambica*. Thus it is understood that monitoring and understanding the histopathology of various tissues of fish exposed to these toxicants, would be helpful in minimizing the loss in fish production and thus providing safe guard to public health. To check the continual introduction of these chemicals into the food chain, more cautious application measures of these agrochemicals should be employed.

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