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Histopathological studies on *Etroplus maculatus* (Cichlidae) exposed to a fertilizer, Factamfos

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Abstract
Toxicological studies were conducted to assess the toxicity of the fertilizer, factamfos and its effect on gill, liver and kidney of the fish, *Etroplus maculatus*. Fish were exposed to asdifferent concentrations of factamfos to find out the median lethal concentration (LC_{50}). LC_{50} was found to be 265 ppm. In chronic toxicity studies, fish having almost uniform size were exposed to sub lethal (26.5 ppm) concentration of factamfos along with a control for a period of 20 days. After the exposure time, the tissues, gill, liver and kidney, were excised, processed and stained in haematoxylin and eosin. Histological alterations of these tissues in different levels, such as, gill filament rupture, severe degeneration and more prominent fusion of lamellae; severe necrosis and hypertrophy in the liver tissues; fusion of renal corpuscles and renal tubule and glomerular expansion could be observed. It revealed that like pesticides, the fertilizer also causes destruction of various organs and thus adversely affects the fish population in the ecosystem.

Keywords: Kuttanad, agrochemicals, factamfos, Cichlid fish, histology

Introduction
Water pollution affects plants and organisms living in the water bodies. In almost all cases the effect is damaging not only to individual species and populations, but also to the natural biological communities. Aquatic organisms accumulate pollutants directly from contaminated water and indirectly via food chain. All species are tolerant to a certain amount of environmental pollution and beyond the tolerable limits, characteristic biochemical and physiological responses starting from the cellular and sub-cellular levels of organization to the ultimate survival or death of the individual are elicited [1].
Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of the pollutants [2, 3]. The effects of these xenobiotics on fish depend on a number of factors such as type of fish species exposed, exposure time, concentration and the type of toxicant used, life cycle stage of fish exposed to the toxicant and environmental factors such as dissolved oxygen concentration, salinity, hardness of the water, temperature, etc. [4-7]. Any change in the environment determines its survival by causing deleterious influence on biochemical and physiological activities of the fish. Histopathological studies of fish exposed to pollutants revealed that fish organs are efficient indicators of water quality [8-12].
Kuttanad is a Lowlying area in the southern end of Vembanad Lake covering Kottayam, Alappuzha and Pathanamthitta districts. The major occupation in Kuttanadu is farming. Rice is the important agricultural product, giving Kuttanadu the moniker, "The Rice Bowl of Kerala". Farm productivity is directly proportional to use of agrochemicals, various agricultural chemicals used in agriculture. It includes a broad range of pesticides, synthetic fertilizers and other chemical growth agents. The use of these chemicals in paddy cultivation in Kuttanad benefits the crop production. But the heavy use of these chemicals in paddy farming causes many health risks on ecosystem. Toxic effects of pesticides are widely studied. But the studies on fertilizers are meagre. So the present study is aimed to understand the toxic effect of the fertilizer, Factamfos on a Cichlid fish, *Etroplus maculatus*, predominant in Kuttanad area.

Materials and Methods
Collection and acclimatization of fish
The fish, *E. maculatus* were collected from the fish farm and transported to the laboratory. They were maintained in an aquarium tank containing aerated tap water and acclimatized for one week. The water medium was changed at 24 h interval to remove the food wastes, faecal matter and other metabolic wastes. Feeding was stopped 24 h prior to the commencement of the experiment.

Determination of LC_{50}

Preliminary experiments were conducted by exposing ten fish each in different concentrations of factamfos *viz*., 50, 100, 200, 300, 400, and 500 ppm. Later, in the final trial, fish were exposed to narrow range of concentrations, 210, 220, 230, 240, 250, 260 and 270 ppm. Mortality of the fish during the experiment was recorded as percentage and the dead ones were removed from the tank. LC_{50} was determined by probit analysis which was run by using the SPSS version 20.0 statistical system [13] and was found to be 265 ppm.

Histopathology

Sub lethal concentration, 1/10th (26.5 ppm) of the median lethal concentration, was selected for the histopathological investigation. Fish having almost uniform size were exposed to 26.5 ppm concentration of factamfos along with a control set up devoid of toxicant for a period of 20 days. In chronic toxicity experiments, the test animals were fed during the entire period. The experimental medium in the test chambers was replaced daily to maintain the concentration of the toxicant constant.

At the end of the experiment, the tissues, *viz*., gill, liver and kidney were excised from the experimental fish and fixed in Bouin's fixative for 24h. The tissues fixed were then dehydrated in alcohol series, cleared in xylene and embedded in paraffin. Paraffin sections having 5µ thickness 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

Rice cultivation in Kuttanad is of an intensive nature compared to many other parts of the state. Nearly 90% of the farmers sow high-yielding varieties necessitating the use of high levels of chemical fertilizers, which are expected to give immediate results in agricultural production. In agriculture field, there are different types of fertilizers used to boost up the health of plant. It include synthetic fertilizers, organic fertilizers, potassium based fertilizers, nitrogen based fertilizers, time relase fertilizers and fertilizer with pesticides. The inorganic fertilizers, *viz*., factamfos, urea and potash were extensively used in the field in different quantities.

The 96 h LC_{50} value of *E. maculatus* exposed to factamfos was found to be 265 ppm (Figure 1). The tissues excised *viz*., gill, liver and kidney from the experimental fish exposed to sub lethal concentration (26.5ppm) of the toxicant exhibited alterations

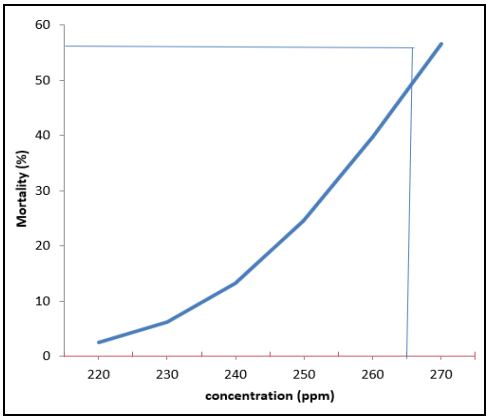

Fig 1: Median lethal concentration (ppm) of *E. maculatus* exposed to factamfos

The histological appearance of the gill of *E. maculatus* exposed to factamfos showed severe alterations. In normal gill, interbranchial septum bears rows of gill filaments on either side. The primary lamella is a fold of epithelium and consists of a central vascular core. Secondary lamellae originate from primary lamellae and are oriented perpendicular to both the surfaces (Figure 2). On 20th day of exposure, gills showed filament rupture, severe degeneration and more prominent fusion of lamellae with the adjacent lamellae (Figure 3).

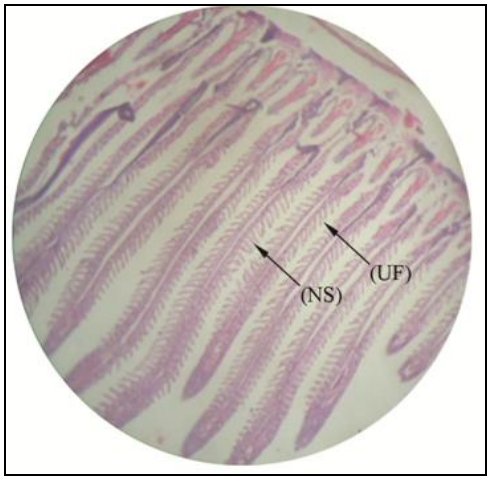

Fig 2: Gill filament of *E. maculatus* showing unfused filaments (UF), normal secondary lamellae (NS).

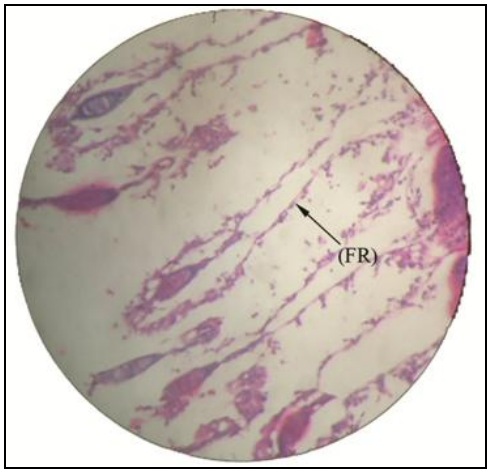

Fig 3: *E. maculatus* exposed to sublethal concentration of factamfos showing filament rupture (FR)

The gills of freshwater fish, which perform respiratory, osmoregulatory and excretory functions, are the largest massive organ directly exposed to waterborne pollutants [14]. The pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants through the gill surface [15]. Several histological alterations such as lifting of the epithelium from the basement membrane of the gill arch, filaments and lamellae [16], fusion or rupture of the gill lamellae [8, 17, 18], etc. were reported in fish exposed to different toxicants.

The liver of fish is a dense organ, consists of lobules of tubular glands, having nucleated polygonal hepatocytes. Biliary canaliculi are found in between hepatic cells. The monitoring of the histological changes in fish liver is a highly sensitive way to assess the effect of anthropogenic agents. In the present investigation the liver tissue showed normal

structure of hepatic cells, connective tissue, and hepatic mass granulation (Figure 4). But *E. maculatus* exposed to sublethal dose of factamfos showed degenerative changes in the liver such as severe necrosis and hypertrophy (Figure 5). Fat deposition around blood vessels, and hemorrhage was also seen. Vacuolation of hepatocytes is associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization [16]. Hepatocytes were found swelled and dissociated in arsenic exposed teleost fish [19, 20]. Several authors have reported the histological alterations in liver of fish exposed to industrial pollutants [21, 8, 22].

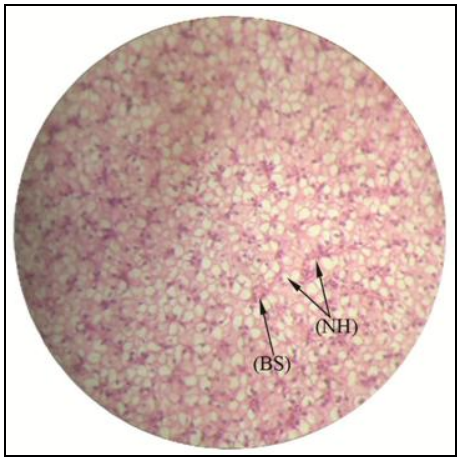

Fig 4: Liver of *E. maculatus* showing normal hepatocytes (NH) and blood sinusoids (BS)

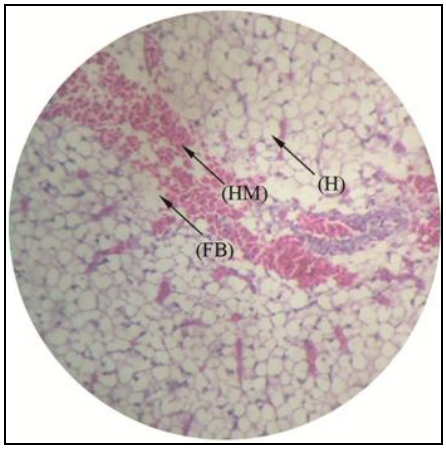

Fig 5: Liver of *E. maculatus* exposed to sublethal concentration of factamfos showing hypertrophy of cells (H), hemorrhage (HM), and fat deposition (FB).

The teleostean kidney is an important organ to be affected by contaminants in the water [23]. The kidney of fish consists of Bowman's capsule, proximal convoluted tubules and distal convoluted tubules. The glomerular tissue was closely arranged with renal tubules and intact interstitial cells (Figure 6). In *E. maculatus* exposed to factamfos, the kidney showed severe necrosis, fusion of renal corpuscles and renal tubule and glomerular expansion (Figure 7). Since a large volume of blood flows through the kidney, lesions found in this organ can be a useful sign of environmental pollution. Several authors have reported deleterious changes like shrinkage of glomerulus, and dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium [24], 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 [12] of different teleost fish species. The effects of pollutants on fish kidneys have been studied in several species and the severity of damage depends on the sensitivity of the species to the substances present in the environment [8, 25, 26].

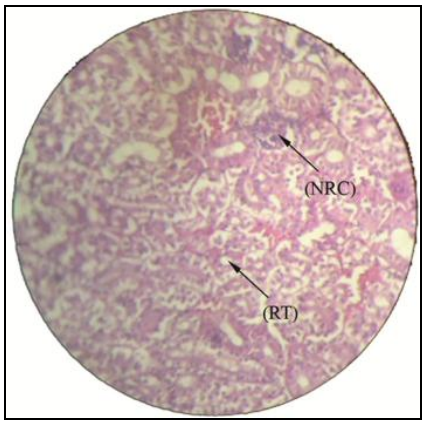

Fig 6: Kidney of *E. maculatus* showing normal renal corpuscles (NRC), renal tubules (RT).

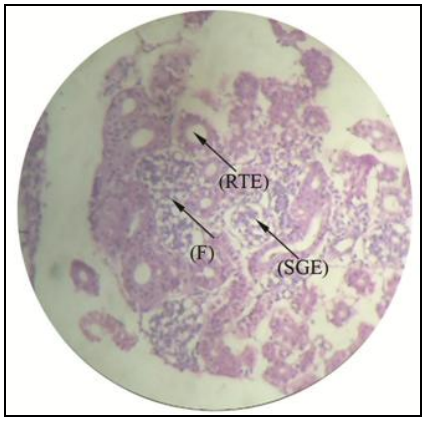

Fig 7: Kidney of *E. maculatus* exposed to sublethal concentration of factamfos showing renal tubule expansion (RTE), slight glomerular expansion (SGE) and fusion (F).

Conclusion

All histopathological observations in the present study indicated that exposure to sub lethal concentrations of the fertilizer; factamfos caused destructive effect on the gill, liver and kidney *of Etroplus maculatus*. The biological monitoring is the systematic use of the responses of the organisms to evaluate the toxic effect of the external chemical agent with the intent to use this information in a quality control programme. So the information on different levels of responses of organisms with respect to pollutant stress is a necessary pre-requisite for the proper management of fertilizer application in agriculture and aquaculture.

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