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# Biomarker Responses of Zinc on Orange Chromidae (Etroplus Maculatus, Bloch, 1795)

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**Abstract:** ***The contamination of the aquatic systems with heavy metals from natural and anthropogenic sources has become a global problem which poses serious threats to ecosystems and natural communities. Acute and chronic toxicities of zinc on teleost fish, Etroplus maculatus and its histopathological impacts on gill and liver tissue were evaluated. The median lethal concentration (96 h; $LC_{50}$) of zinc was calculated as 12.4mg/l by static renewal test method. The fish were exposed to two sub-lethal concentrations of zinc (0.826 mg/l and 2.48mg/l) and gill and liver samples were collected after 14 and 28days of exposure. The structural deformities observed in gills were characterized by epithelial lifting and oedema, lamellar fusion, desquamation and necrosis, whereas, the liver tissue showed swelling of hepatocytes, vacuolar degeneration, focal necrosis, nuclear hypertrophy and cirrhosis with acute hemorrhage. The histopathological change indicates the possibility of bioaccumulation of zinc in the trophic levels of the ecosystem.***

**Keywords:** zinc, histological parameters, Etroplus maculatus, oedema, haemorrhage

## 1. Introduction

Heavy metal pollution from various industries and domestic sources leading to acute and chronic stress to the organisms (1).Similarly bioaccumulation of these metal ions at very low concentrations is a serious problem which is seldom addressed. Zinc is an essential metal however, due to human activities toxicities of such metal is high in trophic levels (2).The present study is planned to evaluate the acute and chronic toxicity of heavy metal zinc and the sublethal effects in exercising histopathological changes in Etroplus maculatus.

## 2. Materials and Methods

The proposed study followed a static renewal bioassay method to determine the 96hr $LC_{50}$. A toxicant free control was also maintained simultaneously. The media were renewed every 24 hours. Etroplus maculatus, a common fish abundantly available in Kerala was selected for the study. Fishes are maintained in 500L tanks disinfected with potassium permanganate solution. Then fish were acclimatized for 14 days to a temperature of 27°C, pH of 7. The oxygen saturation was maintained by aerating the holding tank with aquarium pump. The fishes were fed once daily with a commercial feed and the water was changed one hour after feeding. Zinc stock solution was made from hydrated Zinc sulphate ($ZnSO4.7H_2O$) manufactured by Merck India Limited, Mumbai and added subsequently to the water in experimental tanks to obtain desired test concentrations. Prior to the toxicity experiment, a range finding test was carried out. The acute toxic levels of zinc were determined by static renewal test [3]. The fish irrespective of sex with a weight of 7 - 8g and length 4 – 6cm were selected for the experiment. Ten healthy and active fishes of more or less similar size were randomly selected from the holding tank and were transferred to each experimental tank which contained 20L of dechlorinated tap water. The fish were observed regularly and the numbers of death in all tanks were recorded daily for a period of 96h. The 96h LC50 value was calculated by Probit analysis with a 95% confidence limit [4]. TwoGIOI1q sub lethal concentrations of zinc such as $1/5^{th}$and, $1/15^{th}$of 96 hour $LC_{50}$ were used for the experiment (2.48 mg/L & 0.826mg/L respectively) and each experiment was conducted in triplicate.

Fishes were caught and anaesthetized on$14^{th}$and$28^{th}$ day of exposure. Histopathological techniques and staining procedures were done by standard methods [5, 6]. Liver and gill samples were collected on the $14^{th}$ and $28^{th}$ day of exposure. They were cleaned in saline and fixed in 10% neutral buffered formalin for 24 h. After fixation, the tissues were graded in an ascending alcohol series and cleared in xylene. The tissues were embedded in paraffin wax. After paraffin infiltration, the sections were cut to a 5-micron thickness using a rotary microtome and sections were examined under OLYMPUS CH 20i microscope with Olympus E 420 camera with 40x magnification and photographs were taken. Mayer's hematoxylin staining method was used.

## 3. Results

The acute toxicity response of the selected heavy metal, zinc on the fish, Etroplus maculatus have been evaluated and their LC50 values for 24, 48, 72 and 96 hours were determined by Probit analysis and 96 h $LC_{50}$ value of zinc was found to be12.4 mg/l. No mortality was recorded in control media. The percentage mortality showed an increasing trend with increase in dose of toxicant as well

as increase in duration of exposure. From the acute toxicity (96 hour LC50) values for zinc, the sublethal concentrations were found out as $1/5^{th}$ and $1/15^{th}$ of $LC_{50}$ (2.48 mg/l and 0.825mg/l).

The liver of control fish showed normal architecture with homogenous cytoplasm possessing centrally placed nucleus (Figure 1.1). Severe necrotic and inflammatory changes were noticed in the liver of fish in highest concentration and longest exposure. The significant histological changes observed in 0.826mg Zn/l towards the 14days of exposures include the degeneration of hepatocytes and vacuolar degeneration.(Figure 1.2).After 28 days of exposure the changes are more prominent such as, necrosis, haemorrhage and degeneration of hepatocytes (Figure 1.3)

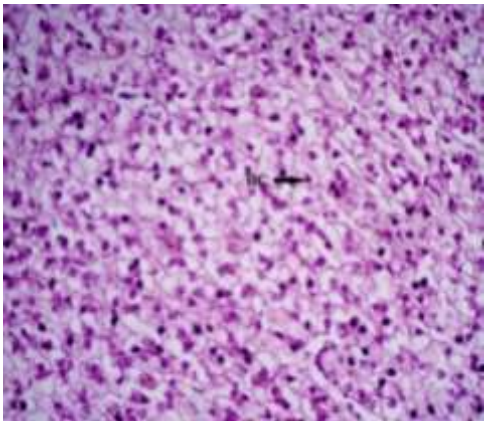

**Figure (1.1) Normal histology of Liver of E.maculatus. Showing normal hepatocyte cell (HC) (H&E 40x)**

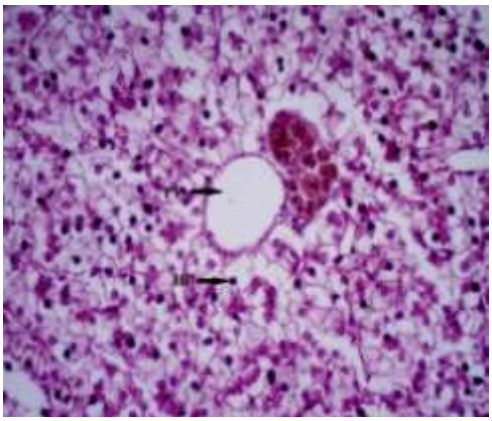

**Figure (1.2) Histopathological alterations of Liver of E.maculatus exposed to 0.826 mg/l zin for 14 days; HC (hepatic cell), PV( permanent vacuolar degeneration ). (H&E 40x)**

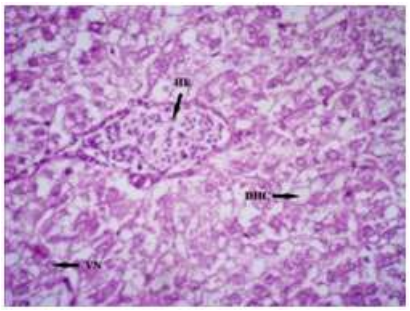

**Figure (1.3) Histopathological alterations of Liver of E.maculatus exposed to zinc 0.826mg/l for 28 days; Necrosis(VN), haemorrhage(HE), Degenerated hepatocyte (DHC).**

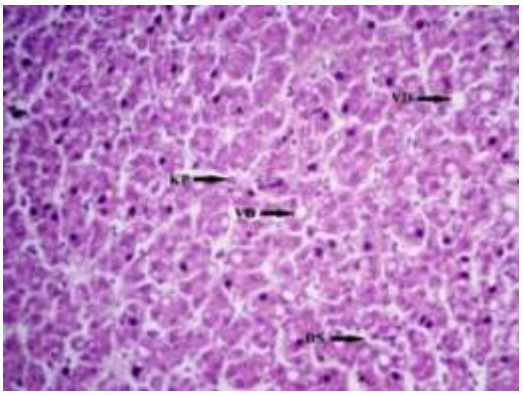

**Figure (1.4) Histopathological alterations of the Liver of E.maculatus exposed to 2.48 mg/l of zinc for 14days. Vacuolar degeneration (VD), Kupffer cell (KP), Degenerated sinuses (DS) (H&E 40x)**

At higher concentration, 2.48mg Zn/l, after 14 days (Fig1.4) and 28 days (Fig1.5) the variations are vacuolar degeneration, degeneration of sinuses, appearence of kupffer cell and necrosis.

The gill of control fish (Fig, 1.6) shows normal structure with primary and secondary gill lamellae. On exposure to 0.826mg Zn/l after 14 days (Figure 1.7) shows congested secondary lamellae and oedema in several places. After 28 days (Figure 1.8) these changes becomes more severe and inflammation is produced.

On exposure to2.48mg Zn/l after 14 days (Fig1.9), the changes are congested secondary gill lamellae, Oedema and haemarrage is seen. The epithelial lifting and detachment and desquamation are more visible on 28 days (Fig1.10), of exposure. Lamellar aneurism, oedema and inflammation are more prominent as the concentration and days of exposure increases.

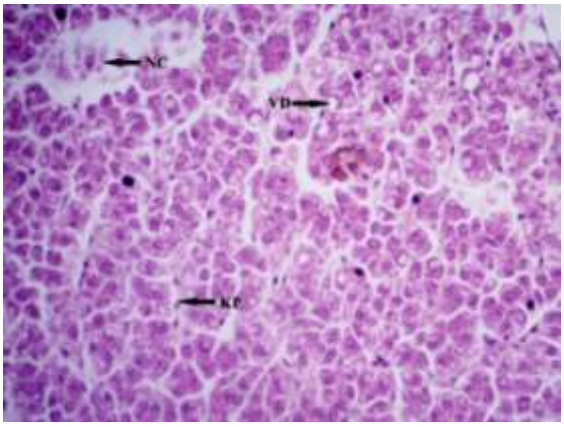


**Figure (1.5) Histopathological alterations of the Liver of E.maculatus exposed to 2.48 mg/l of zinc for 28 days. Vacuolar degeneration (VD), necrosis (NC), Kupffer cell (KP) (H&E 40x)**


**Figure (1.6) Normal histology of gill showing primary gill lamellae (PGL) and secondary gill lamellae (SGL) (H&E 40x)**

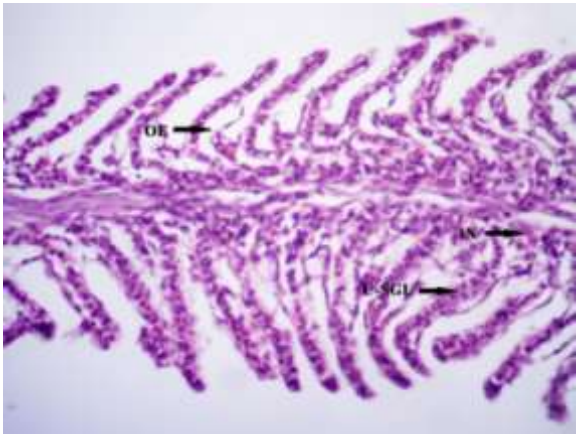


**Figure (1.7) Histopathological alterations of Gill of E.maculatus exposed to 0.826 mg/L zinc of for 14 days)showing Clubbed secondary gill lamella (CSGL), Oedema(OE) Inflammation, (IN) (H&E40x)**

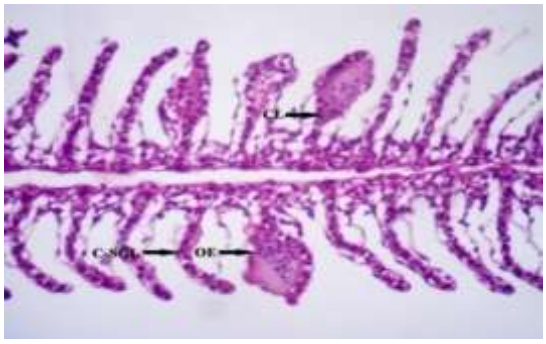


**Figure (1.8) Histopathological alterations of Gill of E.maculatus exposed to 0.826mg/L of zinc for 28days. Congested secondary gill lamellae (CSGL, CL), Oedema (OE). (H&E 40x)**

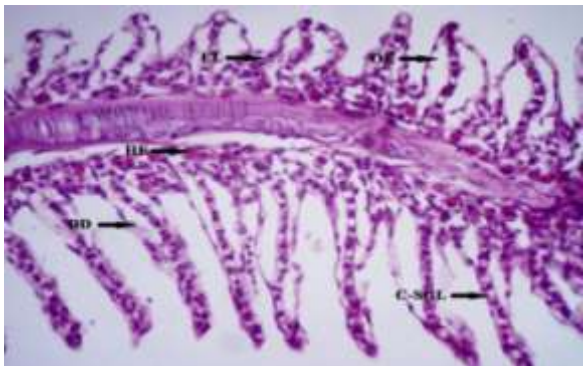


**Figure (1.9) Histopathological alterations of gill of E.maculatus exposed to 2.48 mg/L zinc for 14 days. Haemorrhage (HE) Epithelial lifting(EL) Congested secondary gill lamellae(CSGL) Oedema (OE) detachment and desquamation(DD). (H&E 40x)**

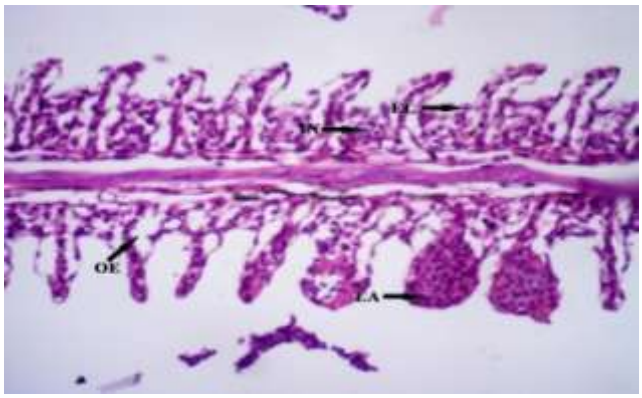


**Figure (1.10)Histopathological alterations of gill of E.maculatus exposed to 2.48 mg/l of Zn for 28 days**

Inflammation (IN), Epithelial lifting (EL), Lamellar aneurism (LA) Oedema (OE). (H&E 40x)

## 4. Discussion

In present study we elucidated acute and chronic toxicity effects of zinc on freshwater fish, E.maculatus. The 96 h $LC_{50}$ value of zinc was found to be 12.4 mg/litre. Fish mortality significantly increased in the elevated dose of zinc, with significant effect of the exposure duration (7).The 96 h $LC_{50}$ value of zinc in E.suratensis is 28.97mg/l (8 ) and that of Puntius parrah is 8.46mg/l (9). This difference in zinc toxicity might be due to the difference in species and environment condition.

In the present study, we observed significant deformations in liver such as the degeneration of hepatocytes, vacuolar degeneration, necrosis and haemorrhage on exposure to different sublethal concentrations of zinc. Liver is the main organ of various key metabolic pathways. The teleost liver is one of the most sensitive organs which show alterations in histoarchitecture, biochemistry, and physiology following exposure to various types of environmental pollutants. The histological alterations noticed in the liver are in accordance to the concentration of zinc and days of exposure. Liver is considered as the most important organ associated with detoxification and bioaccumulation process. The histological changes identified within the hepatocytes in this study may have been the result of various biochemical lesions. Vacuolation of hepatocytes are associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization [10]. Necrosis, haemorrhage, degeneration of hepatocytes and pyknosis in the liver tissue were witnessed in Labeo rohita exposed to zinc [11]. Vacuole formation, degeneration of hepatic cells and necrotic lesion are present in Heteropneustes fossilis exposed to sewage for 180 days. Bile stagnation in the liver of most fish is due to damage in the liver. This lesions, converts the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes [12], indicates that the bile is not being released from the liver. This accumulation of bile leads to damage to the hepatic metabolism [12]. Zinc is toxic for E.maculatus and the toxicity increases with increasing chemical concentration as well as exposure time.

Fish gills have many important functions including exchange of gases, transport of many mono and divalent ions, excretion of waste nitrogen, and uptake and excretion of various xenobiotics [13]. The oedema of the gill epithelium is one of the main structural changes due to heavy metal exposure. The present study also agrees with these findings. Swelling and rupture of lamellae were observed due to heavy metal accumulation of haemocytes. The first sign of gill damage is detachment of chloride cells from underlying epithelium. The subepithelial space enlarges because of detachment of epithelial cells from basal lamina. Water to blood distance can more than double, making gaseous exchange more difficult [14]. Marked proliferation of mucus cells, curling of secondary lamellae, dialation of gill filaments are observed on long term exposure of zinc. These alterations have been reported for other species and sometimes referred as a first sign of pathology [15]. Oedematous changes characterized by epithelial lifting and total degeneration were detected in sublethal concentrations in the present study agrees with similar observations in Oncorhynchus mykiss exposed to nickel reported.[16] Gills are particularly vulnerable to environmental toxicants because of their external location and close contact with water and because of their permeability, which makes them the principal sites of the uptake of toxicant from the medium. Injuries in gill tissues as reported due to zinc exposure may reduce the oxygen consumption and disrupt the osmoregulatory function of the fish. Histological lesions have been used as sensitive and reliable indicators to determine the health status of aquatic species. Overall, the results are in accordance with duration and concentration. The results suggest that these biomarkers are useful for assessing the impact of metal pollution in the aquatic environments and thus effective management strategies are to be evolved and implemented to protect our water bodies and aquatic organisms from the arms of these heavy metal pollution and its toxic effects.

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