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HISTOPATHOLOGICAL CHANGES ON *ETROPLUS MACULATUS (BLOCH, 1795)* DUE TO ZINC EXPOSURE

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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. Heavy metals get bio concentrated in organisms that may cause health problems in humans via the food chain. The heavy metal zinc causes most serious environmental contaminations and effects due to its rampant use. In this study sublethal effects of the heavy metal, zinc on the histopathological parameters in fresh water fish, Etroplus maculatus was investigated. The 96 hour LC_{50} value of zinc was determined by Probit analysis, that was found to be 12.4 mg/L. The liver and kidney samples were collected from fish was exposed to sublethal concentrations of zinc on 14th and 28thdays.The structural deformities observed in the liver tissue showed swelling of hepatocytes, vacuolar degeneration, necrosis, nuclear hypertrophy and cirrhosis with acute haemorrhage where as, kidney showed degenerated renal capsule and renal tubule, edema, necrosis and haemorrhage in the interstetium. The histpathological changes become prominent as the days and concentration of exposure increases. The study thus establishes that zinc is harmful to Etroplus maculatus even in sublethal concentrations and effective management strategies are to be evolved and implemented to protect our water bodies and the organisms from problems of heavy metal pollution.

Keywords: *Etroplus Maculatus; Zinc Toxicity; Histopathological; Acute Hemorrhage; Necrosis.*

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1. Introduction

Recent reports confirm that pollution is the largest environmental cause of disease and death in the world today, responsible for estimated 9million premature deaths. Increased demand for food and the need to sustain the ever increasing world population had led to a massive increase in both agricultural and industrial activities. The quality of aquatic ecosystem has deteriorated markedly

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over last two decades and the water sources available on the earth, has caused significant effects on the aquatic plants and animal life (1). An agricultural, domestic and industrial effluent generally contains a wide variety of organic and inorganic pollutants such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids. Among this heavy metals are considered the most hazardous of all environmental pollutants because of their long half-life period, non-biodegradability, bioaccumulation and biomagnification properties. Among the heavy metals lead, mercury, arsenic and cadmium are highly toxic whereas zinc, copper, chromium, iron and manganese are required by the organisms in trace amounts but above the tolerable level it becomes toxic. The coastal ecosystems of Kerala, along with the unique backwaters are facing severe metallic and organic pollution problems affecting the very survival of biological communities in the region. The heavy metal zinc causes most serious environmental contaminations and effects due to its rampant use (2) ,which include electroplaters, smelting and ore processors, drainage from active and inactive mining operations, domestic and industrial sewage, combustion of fossil fuels and solid wastes, road surface runoff, corrosion of zinc alloys and galvanized surfaces, and erosion of agricultural soils in the production of brass, noncorrosive alloys, and white pigments; in galvanization of iron and steel products; in agriculture as a fungicide and as a protective agent against soil zinc deficiency and therapeutically in human medicine. So understanding the toxic effects of Zn in the environment will be useful for developing suitable water quality standards for critically in suggesting specific management plans to revive and restore the affected coastal aquatic systems.

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 were maintained in 500L tanks disinfected with potassium permanganate solution. Then fishes 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 ($ZnSO_4.7H2O$) 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 fishes were observed regularly and the numbers of death in all tanks were recorded daily for a period of 96h. The 96h LC_{50} value was calculated by Probit analysis with a 95% confidence limit [4]. Two sub lethal concentrations of zinc such as 1/5th and, 1/15thof 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 14th and 28th day of exposure. Histopathological techniques and staining procedures were done by standard methods [5, 6]. Liver and kidney samples were collected on the 14th and 28th 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

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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 LC_{50} values for 24, 48, 72 and 96 hours were determined by Probit analysis and 96 h LC_{50} value of zinc was found to be 12.4 mg/l. No mortality was recorded in the 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 LC_{50}) 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 0.826mg/l). The liver of control fish showed normal architecture with homogenous cytoplasm (Figure 1.1). Hepatocytes were polygonal cells with a prominent spherical central nucleus having densely stained nucleolus. Severe necrotic and inflammatory changes were noticed in the liver of fish in highest concentration and longest exposure.

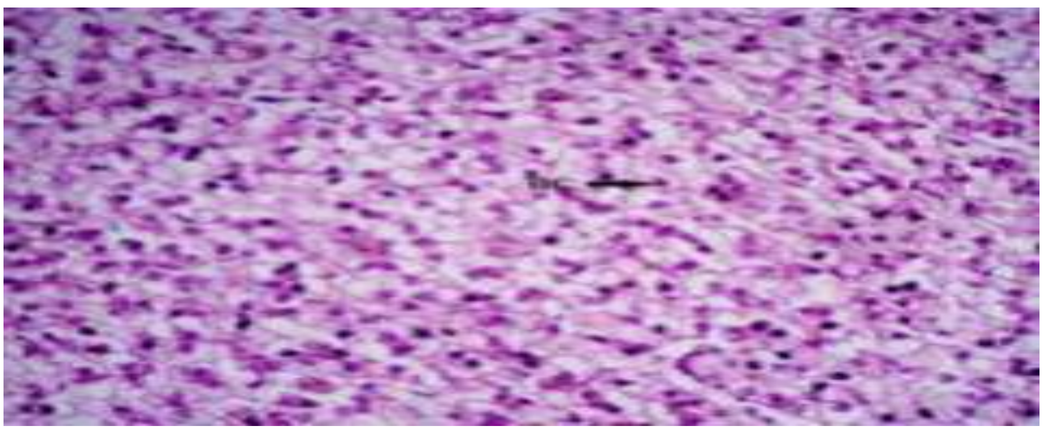

Figure 1.1: Normal histology of Liver of E.maculatus.Showing normal hepatocyte cell (HC) (H&E 40x)

On the 14th day in lowest sublethal concentration (0.826mg/l) showed compactly arranged hepatocytes with intense vacuolar degeneration (Figure 1.2). On long term exposure for 28 days, (Figure 1.3) in lowest dose of zinc, intense vacuolar degeneration and hepatocellular necrosis were more prominent. Hypertrophied bile duct and haemorrhage were more prominent at this stage. At the end of 14th day of exposure at highest concentration (2.48mg/l of Zn), hepatocytes were swollen and contained vacuoles of varying sizes (Fig. 1.4). Hepatocytes have vescicular nuclei, vacuolar degeneration was probably necrotic. The kupffer cells were present more in number. The changes induced by sublethal concentration of zinc were severe after 28 days (Fig. 1.5). The most pronounced changes were that the cytoplasm of hepatocytes got disintegrated and due to its absence they became hollow. Hypertrophied hepatocytes with pyknotic nuclei in which the nuclei underwent shrinkage with condensation of chromatin were also detected in the liver tissue of fishes subjected to the highest sublethal concentrations. The entire liver tissue became a necrotic spongy mass and thus the liver on the whole showed kupffer cells and vacuolar degeneration.

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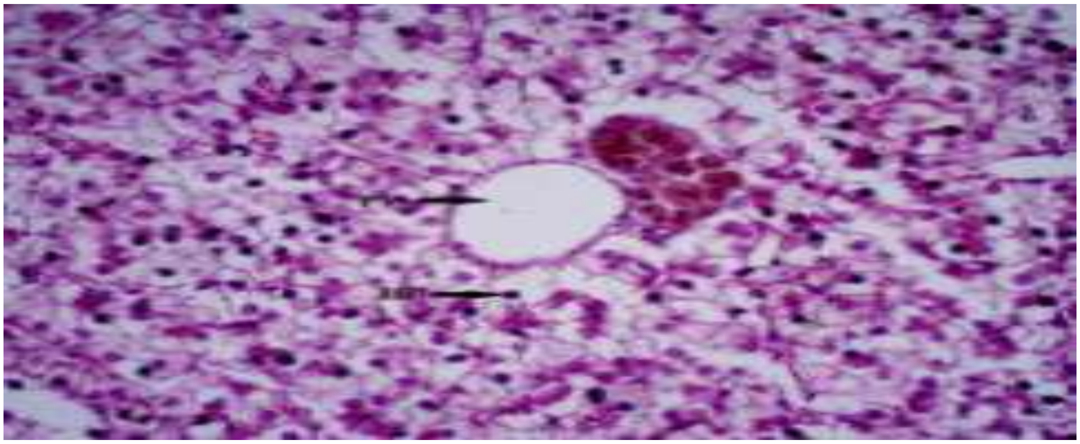

Figure 1.2: Histopathological alterations of Liver of E.maculatus exposed to 0.826 mg/l zinc for 14 days; HC (hepatic cell), PV (permanent vacuolar degeneration).(H&E 40x)

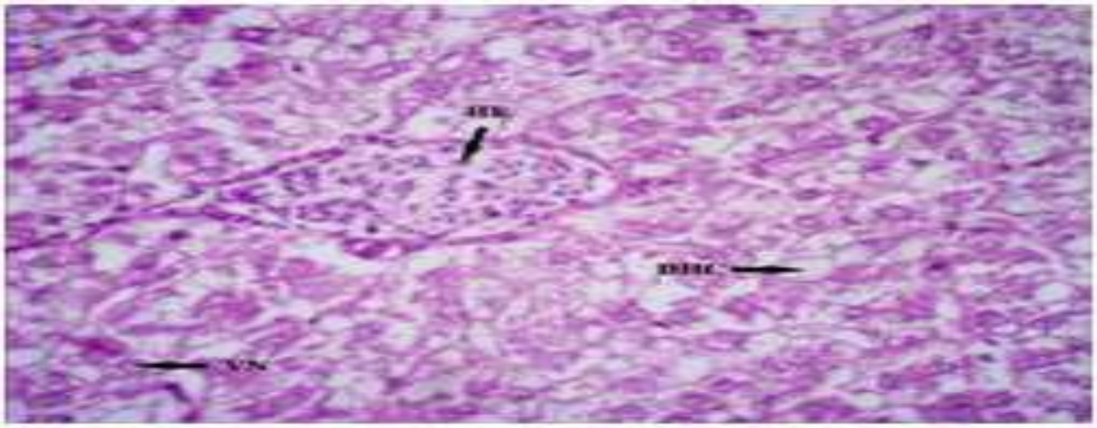

Figure 1.3: Histopathological alterations of Liver of E.maculatus exposed to zinc 0.826mg/l for 28 days; Vascular necrosis (VN), haemorrhage (HE), Degenerated hepatocyte (DHC) (H&E 40x).

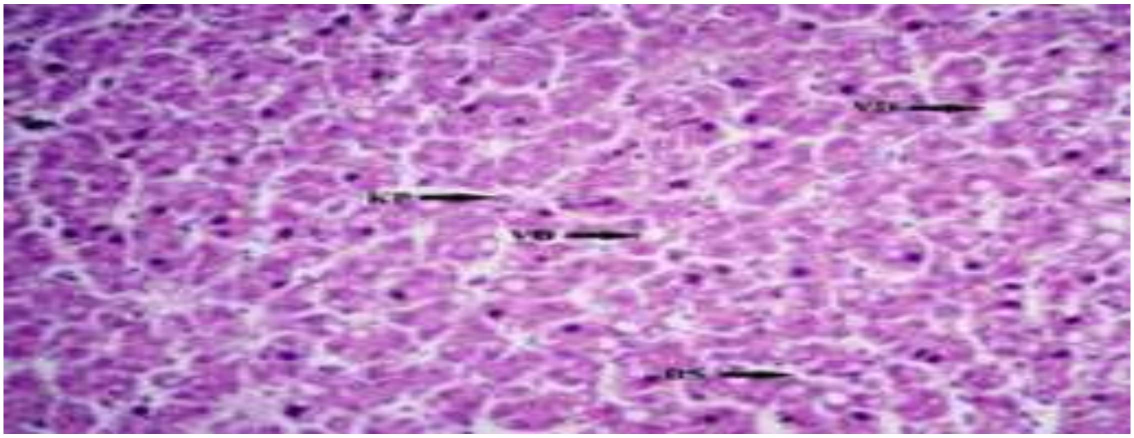

Figure 1.4: Histopathological alterations of the Liver of E.maculatus exposed to 2.48 mg/l of zinc for 14days. Vacuolar degeneration (VD), Kupffer cell (KP), Degenerated sinuses (DS) (H&E 40x)

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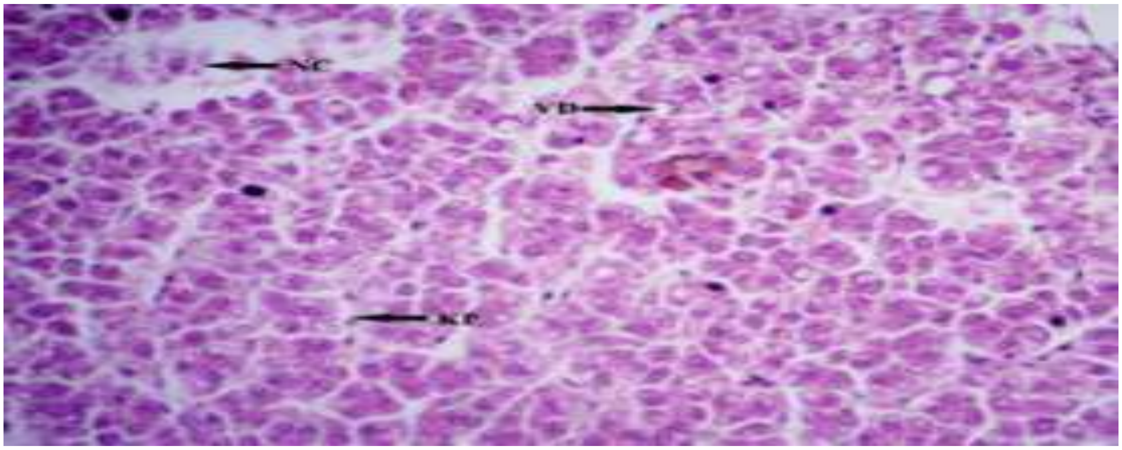

Figure 1.5: Histopathological alterations of the Liver of E.maculatus exposed to 2.48 mg/l of zinc for 28 days. Vacuolar degeneration (VD), necrosis (NC), Kupffercell (KP) (H&E 40x)

The kidney functions not only as an organ of excretion, but also as an organ of conservation and regulation of the body constituents, especially in aquatic vertebrates. The anterior half of the kidney was filled with renal elements, lymphoid and haemopoietic cells. The renal corpuscle consisted of a central glomerulus surrounded by the double layered Bowman's capsule (Fig.1.6). Moderate edema and hemorrhage in the interstitium were the prominent histological changes detected in the sublethal concentration in short-term (14 days) (Fig.1.7). In addition to these changes degeneration and atrophy of renal tubules with oedematous changes and inflammation with severe necrosis were the prominent histological changes detected in the sublethal concentration in long-term (28 days) exposures (Fig. 1.8). Degenerated renal capsule and renal tubule, moderate edema and necrosis in the interstitium are the major changes (Fig.1.9) on 14th day of exposure at 2.48mg/l. After 28th day of exposure (Fig.4.10) the lesions increased. Degenerated renal capsule and renal tubule, moderate oedema and haemorrhage in the interstetium were the major changes.

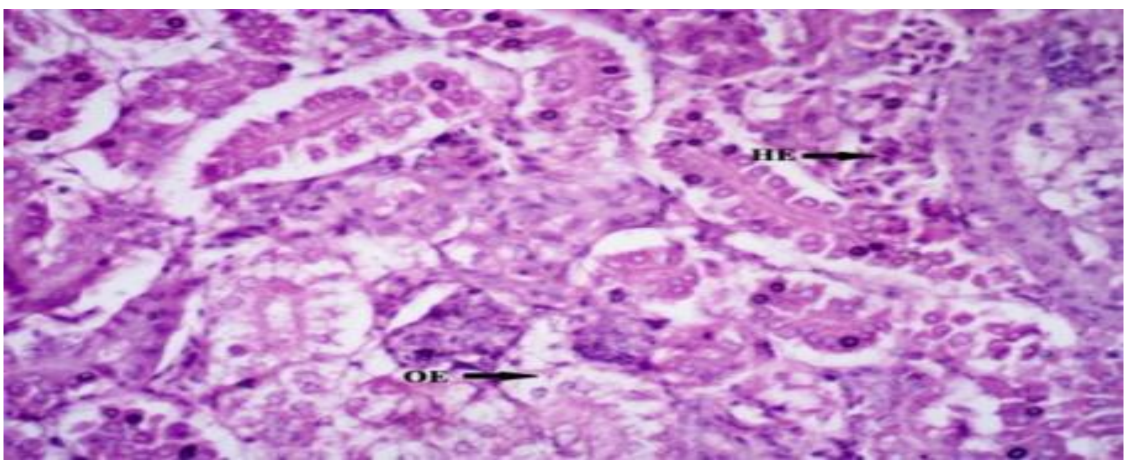

Fig. 1.6: Normal histology of kidney of E.maculatus. Renal tubule (RT), Boman's capsule (BC) (H&E 40x)

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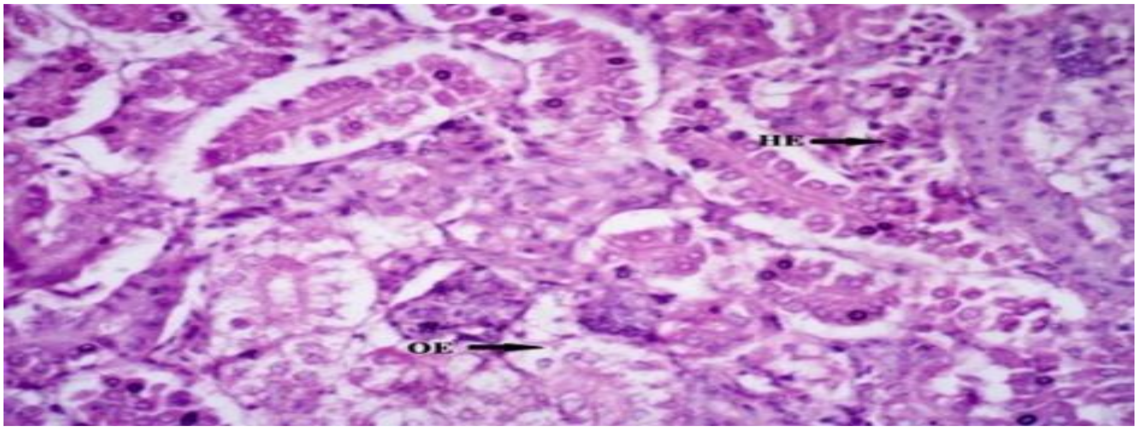

Fig.1.7: Histopathological alterations of Kidney of E. maculatus exposed to 0.826 mg/L of zinc for 14days. Oedema (OE), Haemorrhage (HE). (H&E 40x)

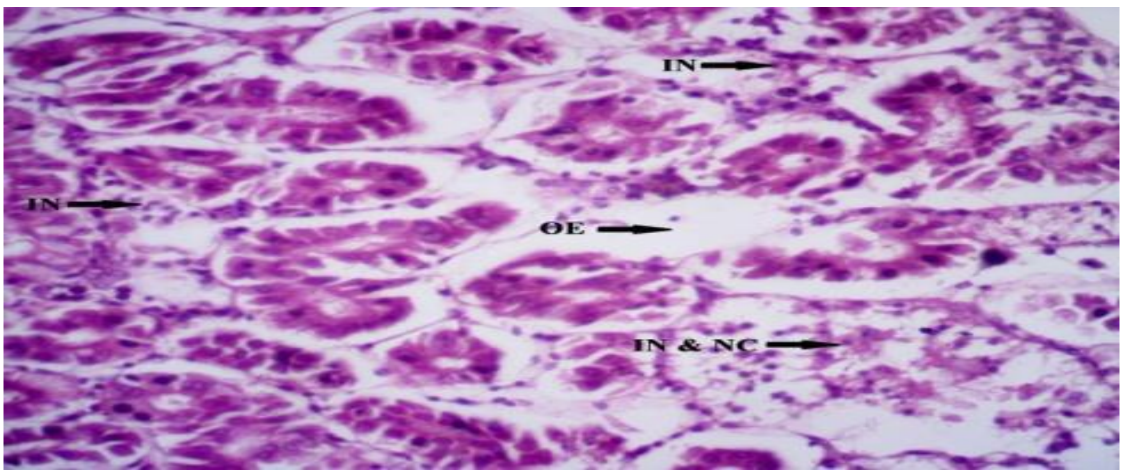

Fig.1.8: Histopathological alterations of Kidney of E.maculatus exposed to 0.826mg/L of zinc for 28days. Oedema (OE), Inflammation (IN), Necrotic tissue (NC) (H&E 40x)

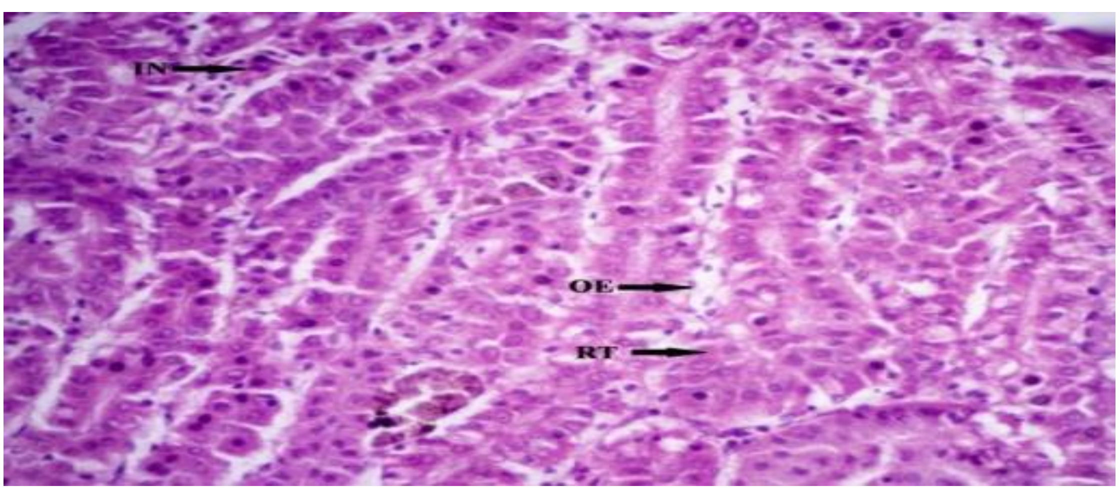

Fig.1.9: Histopathological alterations of kidney of E.maculatus exposed to2.48mg/L zinc for 14 Days. Renal tubule (RT) Inflammation (IN) Oedema (OE). (H&E 40x)

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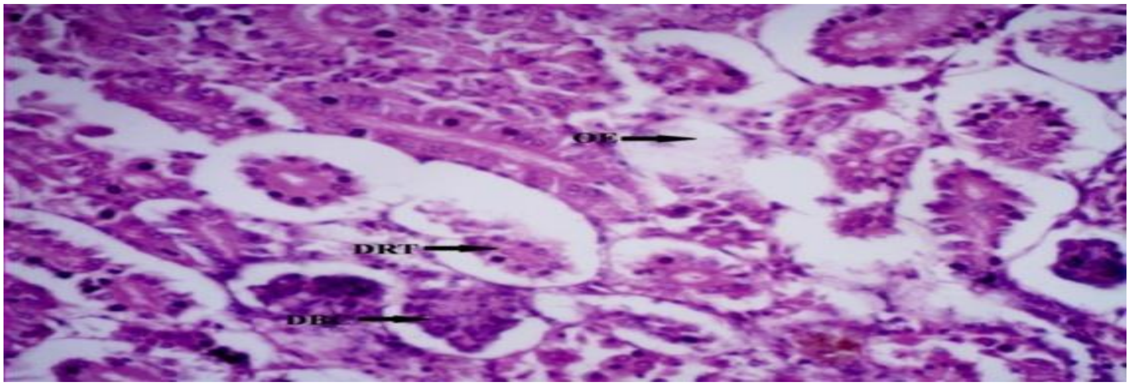

Fig.1.10: Histopathological alterations of kidney of Emaculatus exposed to2.48mg/L zinc for28 Days. Damaged renal tubule (DRT), Oedema (OE), Damaged Bomans capsule (DBC) Inflammation (IN). (H&E 40x).

4. Discussion

In present study, acute and chronic toxicity effects of zinc on freshwater fish, *E.maculatus* was elucidated. 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 environmental condition. In the present study, it was observed that, significant deformations in liver such as the degeneration of hepatocytes, vacuolar degeneration, necrosis and haemorrhage on exposure to different sublethal concentrations of zinc. 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]. Vacuolization of hepatocytes is an important change occurring in the fish on exposure to sub lethal concentrations of zinc. The vacuolization of hepatocytes was reported in many metal exposed liver tissues as a result of excessive deposit of fat in cytoplasm [12]. Kidney is an important dynamic organ not only for excretion but also for so many functions such as ionic regulation, osmoregulation etc. In kidney, most of the alterations were seen in the renal tubules and renal corpuscle. The pathological changes observed in kidney, especially shrinkage of glomerulus, tubular necrosis, nuclear pyknosis in the convoluted portion lead to impaired kidney functions. Renal tissue is highly susceptible to histological damage because of delicate renal functional units and high renal blood supply. These findings from the present study are in agreement with that described by Bhatnagar *et al*., (2007) and Ayoola (2008) [13, 14]. Zinc is toxic for *E.maculatus* and the toxicity increases with increasing chemical concentration as well as exposure time. The empirical and theoretical models developed based on the different toxicity tests derived through investigations could be a significant tool for the planners, policy makers

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and conservation specialists to regulate the ever increasing issues of metal contamination and general health of aquatic ecosystems.

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References

[1] Bijoy Nandan, S., C. M. sumitha and B. Girish Kumar 2013. Manganese induced haematological and histopathological changes in Oreochromis mossambicus (Peters, 1852), 32:555-562
[2] Bijoy Nandan, S. and P. J. Nimila 2012. Lindane toxicity: Histopathological, behavioural and biochemical changes in Etroplus maculatus (Bloch, 1795). Marine Environmental Research., 76: 63-70.
[3] Au, D. W. T. 2004. The application of histocytopathological biomarkers in marine pollution monitoring: A review, Mar. Poll. Bull., 48: 817- 834
[4] APHA., Methods for the examination of water and waste water (American Public Health Association, 15th Edn. New York, Washington, 2005)
[5] Finney, D.J., Probit analysis, Second (Edn.), Cambridge University Press, Cambridge, UK, (1971)
[6] Bucke D., Some histological techniques applicable to fish tissues, Symposium of Zoology Society, London, Vol.30, In: Mawdesley-Thomas. LE. (Edn.). 10, Diseases of fish, 10 (Academic Press, New York,1972) 153-189.
[7] Amoozadeh E, Malek M, Rashidinejad R, Nabavi S, Karbassi M, et al.(2014) Marine organisms as heavy metal bioindicators in the PersianGulf and the Gulf of Oman. Environ Sci Pollut Res 21: 2386-2395
[8] Anu, P. R., P. R. Jayachandran, P.K. Sreekumar and S. Bijoy Nandan 2014. A Review on HeavyMetal Pollution in Cochin Backwaters, Southwest Coast of India, International Journal of Marine Science, 10: 92-98
[9] Ciji, P. P. and Bijoy Nandan 2014. Toxicity of copper and zinc to Puntius parrah (Day, 1865). Marine Environmental Research, 93: 38-46
[10] Hinton DE, Laurén DJ. Integrative histopathological effects of environmental stressors on fishes. American Fisheries Society Symposium 1990;8:51–66.
[11] Loganathan, K., B. Velmurugan, J. HonggrayHowrelia, M. Selvanayagam and B. B. Patnaik 2006. Zinc induced histological changes in brain and liver of Labeo rohita (Ham.). J. Environ. Biol., 1:107-110
[12] Abdel-Khalek, A. A 2015, Risk assessment, bioaccumulation of metals and histopathological alterations in Nile tilapia (Oreochromis niloticus) facing degraded aquatic conditions, Bulletin of Environmental Contamination and Toxicology,94, 77–83.
[13] Bhatnagar, C., Bhatnagar, M and Regar, B.C 2007, Flouride - induced histopathological changes in gill, kidney and indestine of fresh water teleost, Labeo rohita. Res.Rep.Fluoride, 40(1): 55-61.
[14] Ayoola SO. 2008. Toxicity of glyphosate herbicideon Nile tilapia (Oreochromisniloticus) juvenile. African Journal of Agricultural Research, 3:825-834.

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