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MORPHOLOGICAL AND LIVER HISTOLOGICAL EFFECTS OF ZnO NANOPARTICLES ON MOZAMBIQUE TILAPIA

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Abstract

Three different concentrations (30, 50 and 70ppm) of ZnO NPs were exposed to *Oreochromis mossambicus*. Treated fishes showed various skin discolouration and ulceration due to necrosis condition of muscle tissues, damages in Pectoral, Caudal and Dorsal fins than control. HSI values of treated groups showed decreasing HSI results for 30ppm, 50pp, and 70ppm groups as increasing concentrations. Liver Histological results showed congested portal vein (CPV), necrotic hepatocytes and vacuole formation, accumulation of blood cells degradation of liver tissues (DL) in treated fishes. As a novel attempt this study reveals the impact of ZnO NPs on morphology of *O. mossambicus*.

Keywords: ZnO, Morphology, HSI, liver

1. Introduction

Nanotechnology has been defined as using materials and structures with nanoscale dimensions, usually in the range of 1–100 nm (Farre et al., 2009). Nanoparticles are increasingly being used in industrial production as well as scientific, biological and medical area. As the interest in the potential benefits of nanoparticles has increased, there is also increasing concern over their potential toxic effect resulting from use or unintentional release into the environment (Dreher, 2004; Nel, 2006; Nowack and Bucheli, 2007).

Nanoparticles (NPs) can be divided into natural and anthropogenic particles (Nowack and Bucheli, 2007). Nano-sized materials are naturally present from forest fires and volcanoes, viral particles, biogenic magnetite, and even protein molecules such as ferritin. Based on their chemical composition, nanoparticles are separated into carbon-containing and inorganic NPs.The important processes and pathways of NP in the environment are depicted in Figure 1. Release of NP may come from point sources such as production facilities, landfills or wastewater treatment plants or from nonpoint sources such as wear from materials containing NP. Accidental release during production or transport is also possible (Nowack and Bucheli, 2007). Sources to the aquatic environment include waste water discharges, accidental release from factories, and the degradation and wear of products containing oxide nanomaterials. Due to their extremely small size and unique physical properties the behaviour of oxide nanomaterials in the environment, their uptake, distribution and effects within the bodies of living organisms are likely to be different when compared to conventional xenobiotics (Diagomanolin et al., 2004).

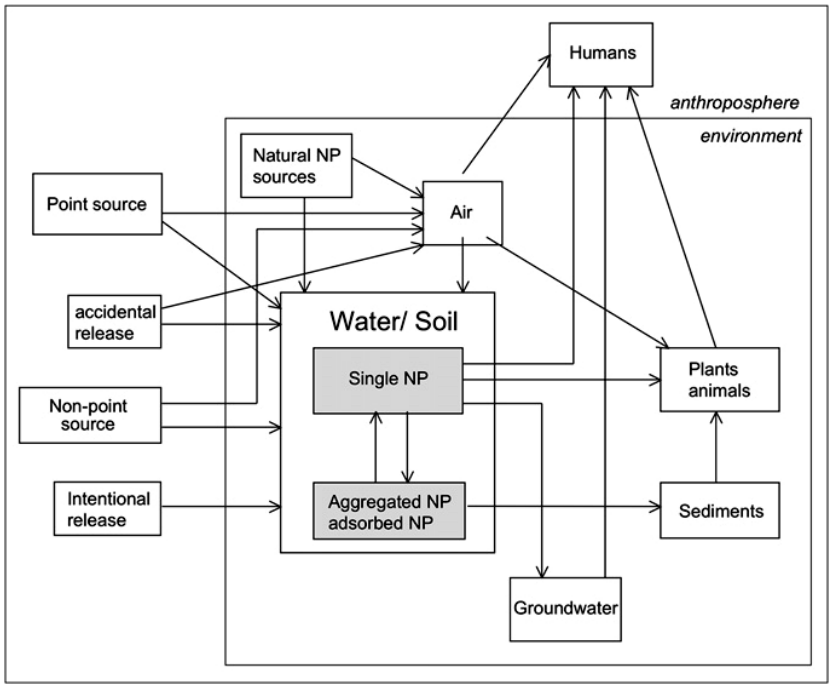

Figure 1. Nanoparticle pathways from the anthroposphere into the environment, reactions in the environment and exposure of humans.

ZnO nanparticles used in a variety of different areas such as electronic, biomedical, pharmaceutical, cosmetic, environmental, catalytic and materials applications such as UV lasers, gas sensors, photoprinting, electrochemical nanodevice, sunscreen lotion cosmetics and medicated creams (Ravichandrika et al., 2012). The production, use and disposal of manufactured nanoparticles (NPs) will inevitably discharges into air, soil and aquatic ecosystem.

The Mozambique Tilapia, *Oreochromis mossambicus* has been described by many researchers as a suitable bioassay organism (Nussey, 1998; Barnhoorn, 2001) and extensively used in biological and behavioural research studies (Skelton, 1993)**.** Accumulated heavy metals may lead to morphological alterations in the tissues of fish (Monteiro et al., 2005). Morphological studies acts as a indicator to identify the health status of an animal by external appearance. It helps to distinguish the affected individual from the normal fishes (Seymore, 1994). The hepato somatic index (HSI) is the weight of the liver expressed as a percentage of

body weight (Slooff et al., 1983). HSI evaluation considered as the role of both endogenous and exogenous factors (Schmitt and Dethloff, 2000).

Histopathological studies are very sensitive and crucial parameter, which reflects the effect of toxicants on organ (Mathur and Gupta, 2008; Abdel-Warith *et al*., 2011). The selection of liver cells as appropriate targets is due to their cytological sensitivity as biomarkers of organic contaminants and environmental pollution. Ultra structural alterations of fish hepatocytes have repeatedly been used as monitor systems to study sub-lethal effects of organic contaminants with great success (Braunbeck and Volkl, 1993). To date, however, there is a lack of data and understanding about their environmental fate, bioavailability and biological effects. As a novel attempt, the morphological and HSI studies on ZnO NPs treated fishes are carried out in this study.

2. MATERIALS AND METHODS

2.1. Experimental Fish

The fresh water fish *Oreochromis mossambicus* (Figure 2) were collected from Cauvery River (latitude 10º 51´ and longitude 70º 30´) in Tiruchirappalli, India. A group of healthy *Oreochromis mossambicus* (29.2±0.41 g) were stocked in a large cement tank containing chlorine free well water (APHA 1998) for 15 days with aeration. Fishes were acclimatized in Environmental Research laboratory (Jamal Mohamed College, Tiruchirappalli) for three weeks before initiation of experiment and separated into groups (n=10). The water was changed on alternate days and the fishes are fed ad libitum on a formulated fish diet prepared from ground nut oil cake and rice bran in the laboratory. The fecal matter and other waste materials are siphoned off daily to reduce ammonia content in water.

Figure 2. Experimental fish *Oreochromis mossambicus*

2.2. ZnO nanoparticles suspension

Based on the literature about toxicity of ZnO nanoparticles three different concentrations (30, 50 and 70ppm) were prepared and sonicated for 30 min in a bathtype sonicator (100W, 40KHz) to disperse the particles **(**Wang 2004). The sonicated nanoparticles exposed to the fish groups in respective concentrations and control group maintained separately.

2.3. Morphological studies and HSI calculation

No aeration and feed condition were maintained in control and treated group. During experimental period, the fishes were closely observed and their changes were noted. Morphological studies done by visual observation of treated and control fish at the end of 96hrs in normal sunlight. Treated and control group fishes were weighed and liver tissues was dissected out and used for further histological analysis.

$$HSI = \frac{Liver\ Weight\ (gm)}{Total\ Body\ Weight\ (gm)}\ x\ 100$$

2.4. Liver Histological analysis

Liver tissues are dissected and immediately fixed in Bouin's fixative for 48 h. The preserved tissues were processed by a routine histological method (Gurr, 1962), dehydrated in an alcohol series, cleared in xylene, infiltrated with liquid paraffin at 58º C, and finally embedded in

paraffin blocks. The blocks were trimmed and sectioned at 5-8 mm thick cut on a rotary microtome (Weswox MT Chennai, India), were stained with the Harris' Hematoxylin and counter-stained with Eosin (H&E stain). Then the slides were mounted with DPX and observed under a light microscope (LEICA).

2.5. Statistical analysis

The obtained values were expressed as the Mean ± SD using the SPSS software package for windows (version 17.0) and reasonable interpretations were made following.

3. Results

3.1. Morphological observation

Treated fishes showed various skin discolouration and ulceration due to necrosis condition of muscle tissues, damages in Pectoral, Caudal and Dorsal fins (Figure 3) resulted in distinguishing of web tissues between the rays which resulted in disturbed swimming activity. Damaged fin rays leads to disturbed escaping activity from predators and food hunting acitivty, Dilated eyes resulted in blurred vision and transparency, Gill haemorrhages leads to disturbed oxygen absorptions were observed in all 30, 50 and 70ppm treated fishes than control fish.

4.2. Hepato Somatic Index (HSI)

Whole fish body weight was weighed before dissection at the end of the experiment (96hrs). Dissected liver tissues were weighed and used for the calculation of hepato somatic index. HSI values of control fishes showed 1.80 where the treated groups showed decreasing HSI results as 1.23, 0.95 and 0.49 (Table 1) for 30ppm, 50pp, and 70ppm groups respectively. Figure 4 showed decreasing values of HSI among the control and ZnO exposure groups.

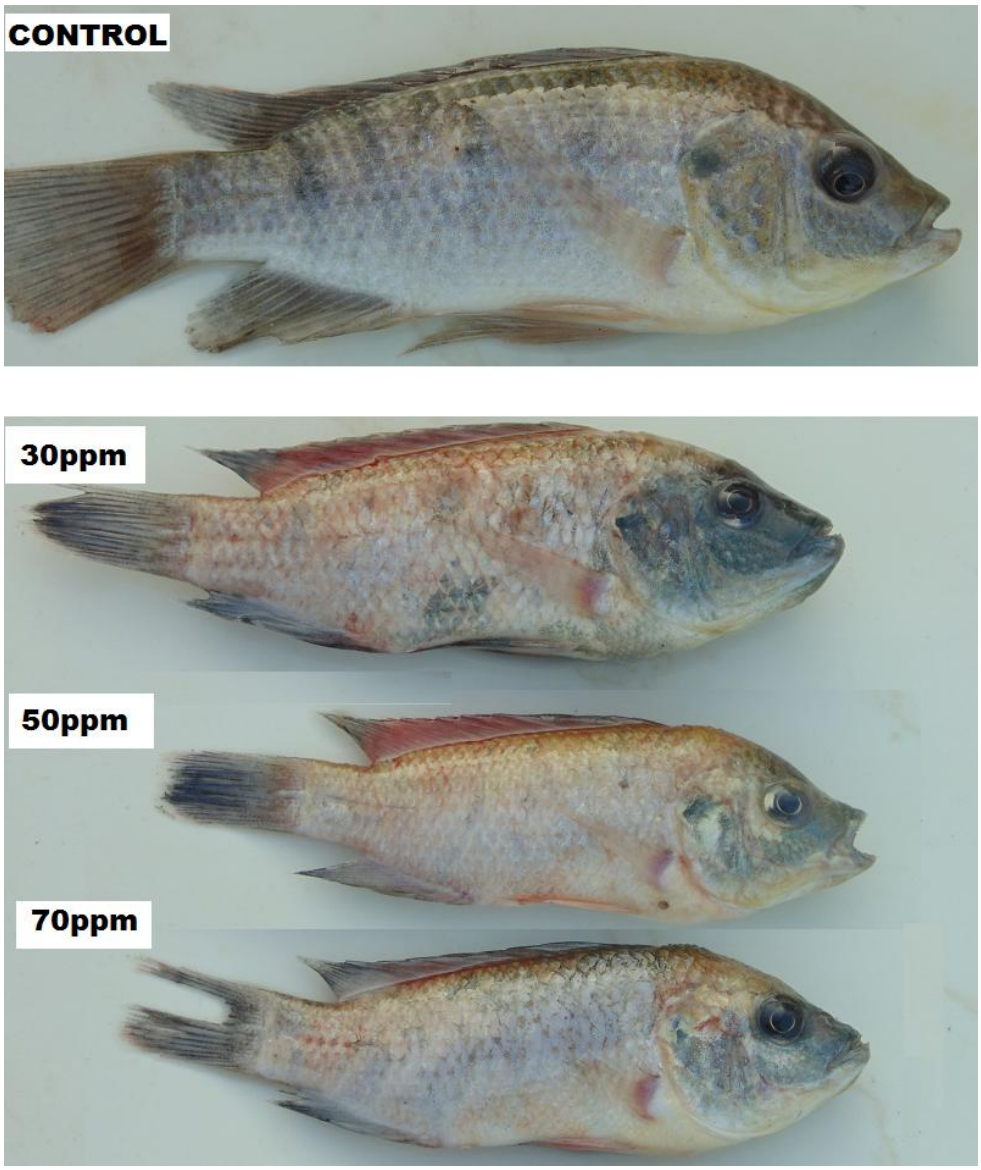

Figure 3. Observation of Skin discolouration and damaged caudal fin in 30, 50 and 70ppm of ZnO nanoparticle treated group and control group.

Group	Body weight (gm)	Liver weight (gm)	HIS
Control	43.28±0.02	0.78±0.09	1.80
30 ppm	34.12±0.03	0.42±0.05	1.23
50ppm	22.02±0.04	0.21±0.06	0.95
70ppm	18.11±0.06	0.09±0.11	0.49

Table 1. Hepato somatic index (HSI) of control and ZnO treated groups of *Oreochromis mossambicus* (n=10)

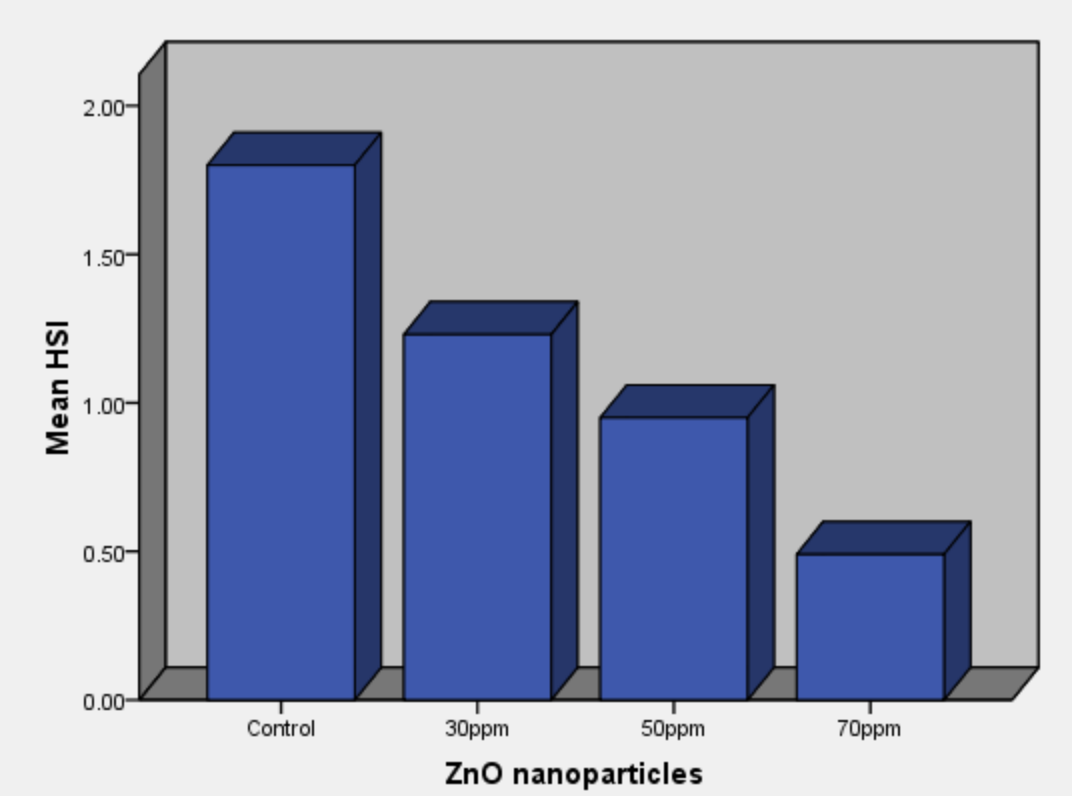

Figure 4. Bar diagram of Hepato Somatic Index (HSI) values of control and ZnO nanoparticles treated groups

4.3. Liver histology

Control liver tissues showed normal hepatocyte arrangements with normal portal vein arrangement. In 30ppm treated fishes showed congested portal vein (CPV), necrotic hepatocytes and vacuole formation. In 50ppm treated groups showed increased blood congestion in portal vein (CPV), necrotic condition and vacoulation, accumulation of blood cells (circle). In 70ppm group showed severe necrosis (N), degradation of liver tissues (DL) and increased vacoulation (Figure 5).

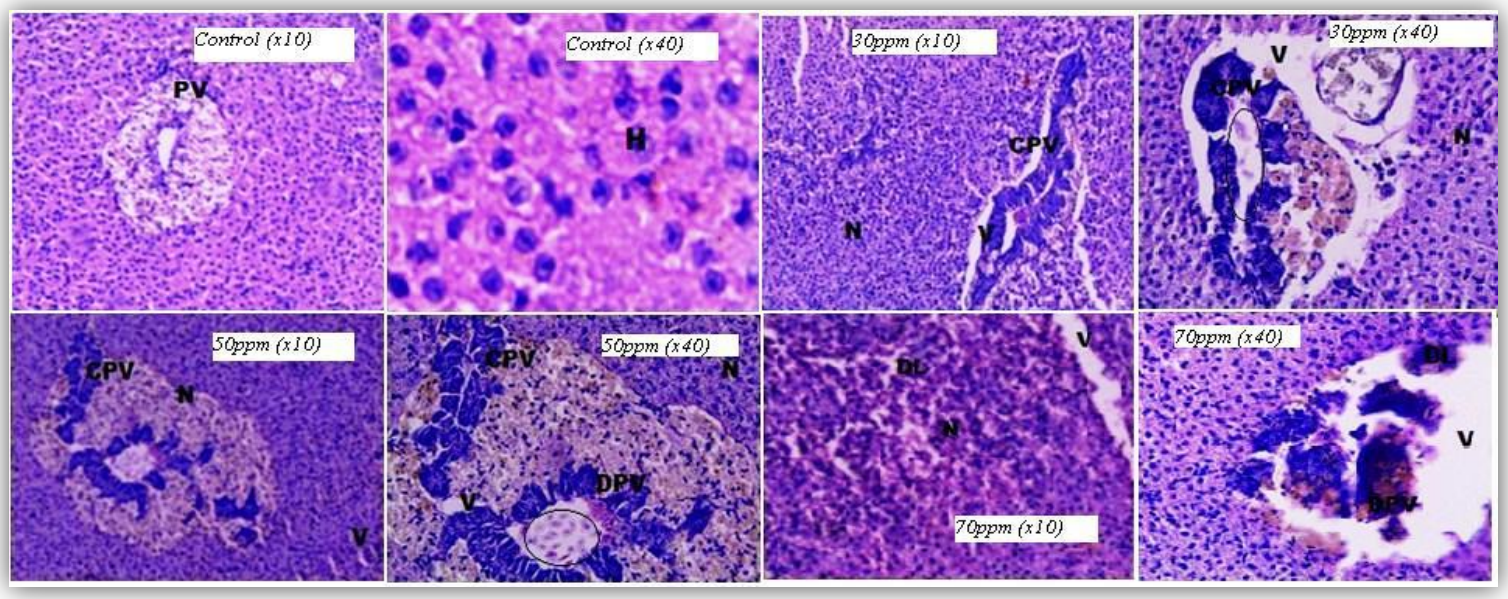

Figure 5. Histological liver observation of control and ZnO nanoparticles treated groups of freshwater fish *Oreochromis mossambicus*

5. Discussion

Morphological changes have been established as sensitive indicator of stress in aquatic organisms. Morphological changes like injury or lesions, degenerative changes in the skin and muscle have been observed in fishes may be due to replacement by loose connective tissue elements (Banerjee, 1997). Gul (2005) reported that following exposure of *O. niloticus* to chlorpyrifos-methyl, exposed fish were pale and discoloured in comparison to control fish. In the pesticide-exposed fish showed darker than control fish. Fish skin was continuously contact with the aquatic environment the epidermis, which was covered with a layer of mucus that forms an additional barrier to potentially harmful substances, protects the animal against external effects (Whitear, 1986).

The ZnO nanoparticles treated fish liver showed some patches of cellular necrosis, changes in sinusoid space, etc., are well-known (Figueiredo-Fernandes et al., 2007; Handy et al., 1999) and often explained by Zn-induced oxidative stress in the liver tissue (Hoyle et al., 2007). Authman and Abbas (2007) reported vacuolar degeneration in the hepatocytes and necrosis. Degeneration and necrosis of hepatocytes may be due to the cumulative effect of the metals and increase in their concentration in liver. The cellular degeneration in the liver may be due to oxygen deficiency as a result gill degeneration and vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed 2001).

The histological lesions due to cadmium and zinc poisoning were reported by Van Dyk et al. (2007) in *O. mossambicus*. Loganathan et al. (2006) and Radhakrishnan and Hemalatha, (2010) have also observed the histological changes in the liver of zinc treated *L. rohita* and cadmium chloride treated *Channa striatus*. Athikesavan et al. (2006) reported the histological lesions in the liver of *Hypophthalamichthys molitrix* exposed to nickel, such as degeneration of

blood vessels hypertrophy, vacuolisation necrosis and pyknotic nuclei of the exposed fish. Many reports revealed a variety of changes in the liver of *O. niloticus*, resulting from exposure to different toxic chemicals (Figueiredo-Fernandes et al., 2007). Mario et al., (2010) revealed that the Bleached Kraft paper mill waste water caused histological changes in the hepatic tissue of both *Carassius auratus* and *Dicentrarchus labrax*.

4. CONCLUSION

ZnO NP exposure (even in ppm conc.) cause morphological changes in the adult *Oreochromis mossambicus.* Higher concentration showed high morphological deformities in fishes. Changes in the treated fishes Liver weight clearly explained the degeneration of hepatocytes. NPs exposure liver tissues showed disturbances in the hepatocyte cell arrangements and necrosis condition proved that ZnO NP exposures cause histological abnormalities in liver tissues of fish *O. mossambicus*. As a novel attempt, this study revealed the impact of ZnO NPs on morphology of *O. mossambicus*.

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