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# Antioxidant and malondialdehyde levels in the tissues of *Heterobranchus longifilis* following lethal and sublethal exposure to zinc oxide nanoparticles

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ABSTRACT: Increased industrial and domestic usage of zinc oxide nanoparticles (ZnO-NPs) informed their great demand, though scanty information exists on their environmental fate. Therefore, the impacts of ZnO-NPs on the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), and malondialdehyde (MDA) levels in the blood, gill and liver of *Heterobranchus longifilis* (Mean length ± SD, 10.28 ± 1.34) exposed to lethal concentrations (0.00, 60.00, 80.00, 100.00 and 120.00 mg/l) and sublethal concentrations (0.00, 6.00, 8.00, 10.00 and 12.00 mg/l) of ZnO-NPs were examined for 96-h and 45 days, respectively. The results showed that during lethal exposure, SOD and GPx activities in the tissues of ZnO-NPs-exposed fish changed insignificantly ($p > 0.05$) compared to the control, except in liver where GPx changed significantly ($p < 0.05$). MDA levels in the blood and liver significantly increased, while no such change was observed in the gill. During sublethal exposure, SOD activities significantly increased at 10 and 12 mg/l after 15 days exposure, while after 30 days there was no significant change. However, after 45 days of exposure, significantly higher activities were recorded at the groups treated with 10 and 12 mg/l. GPx activity in the blood did not show a significant increase after 15 days exposure, but after 30 and 45 days the activity increased significantly than the control. Compared with the control, GPx activity in the gill neither show significant concentration- nor time-dependent difference between the first period of 15 days and the subsequent 15 days (i.e., 30 days) of exposure, until after 45 days at 12 mg/l of ZnO-NPs. In the liver, GPx activity increased significantly as ZnO-NPs concentration and exposure period increased compared with the control. In all the tissues, significantly higher MDA levels than the control was not observed until after 45 days exposure at 12 mg/l of ZnO-NPs. These results suggest that ZnO-NPs concentrations greater than those used in this study may produce deleterious effects on the antioxidant system during short- or long-term exposure and may thus, weakens the adaptive threshold of the fish.

**Keywords**: Zinc oxide nano-particles, *Heterobranchus longifilis*, Toxicity, Lethal, Sub-lethal, antioxidant enzymes.

## Introduction

Nano-particles (NPs) are used in medicine, agriculture and industrial sectors of the economy, and have helped in proffering solutions to myriad of problems ranging from diagnosis and treatment of diseases to manufacturing of consumer products. The utilisation of nanoparticles has led to their continuous production without regulation, thereby increasing their disposal to the environment. In aquatic environment, organisms may become more vulnerable owing to persistent exposure to the risk of toxic effects of nanoparticles either through their discharge from industries, runoff or direct use by human. Zinc oxide nanoparticles (ZnO-NPs)

are popular and frequently utilised nanoparticles in the industries for the production of commercial and medicinal goods due to their distinguished physicochemical properties such as rigidity, photostability, and biodegradability. They serve as photocatalyst, energy generator, solar panel devices, sunscreen, and are also useful in the production of ceramics, textiles, rubber and cosmetics (Wang *et al*., 2005). Zimmermann *et al*. (2011) reported that they are also used in the manufacture of paints and in the treatment of wastewater. ZnO-NPs has also been found useful in the processing of meat, vegetable production and packaging (Asghar *et al*., 2015). Their smaller size confer on them the advantage of distribution in the environment and this has been a cause for concern globally. The increased production and use of ZnO-NPs may facilitate their likelihood to wreak havoc in aquatic environment.

Aitken *et al*. (2006) reported that excessive utilisation of nanoparticles in wastewater treatment triggered debilitating effects on aquatic organisms and human that depend on them. In Nigeria, the environmental regulation agencies (NIS, 2007; NESREA, 2011) encumbered with the responsibilities of setting the threshold values for some chemicals used in the daily lives of Nigerians have not included information on the threshold limits for nanoparticles in aquatic systems. Unfortunately, this paucity of information makes the existing regulations difficult to fully address the safety concerns associated with nanoparticles. Thus, hindering the holistic understanding of the protection and conservation of aquatic ecosystems as well as their resident fish species.

The endogenous antioxidant system is important in animal metabolism as they are involved in defensive mechanisms against the impacts of xenobiotics, which may have significantly induced their alterations due to free radicals generated by reactive oxygen species (ROS). Banaee *et al*. (2015) reported that the antioxidant capacity of tissues is critical in combating free radicals and ensuring normal metabolic functions in fish. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been reported to be of great importance in combating free radicals triggered by ROS (Tripathi *et al*., 2006). SOD is one of the first major defensive steps against free radicals' damage. It catalyzes the conversion of super oxide radical ($O_2^{\cdot-}$) to hydrogen peroxide ($H_2O_2$) (Ruas *et al*., 2008). Glutathione peroxidase (GPx) is involved in the detoxification of hydrogen peroxide. These antioxidants (SOD and GPx) are used as biomarkers of toxicity of xenobiotics in fish since they defend the cells against cellular impairment (Kaya *et al*., 2015). Generally, the hazardous nature of nanoparticles and the risk of ZnO-NPs could particularly portend on aquatic organisms is connected with the induction of oxidative stress (Connolly *et al*., 2016). Oxidative stress induction could be determined by measuring the level of malondialdehyde, and by evaluating the alterations in the antioxidant system due to free radicals' disruption (Kaya *et al.*, 2015).

Several studies have assessed changes in the antioxidant enzyme profile of fish exposed to ZnO-NPs and there seems to be conflict of report. Xiong *et al*. (2011) demonstrated the oxidative effect of both the ZnO-NPs and their bulk on zebrafish, *Danio rerio*, accompanied by a decrease in SOD activity at 5 mg/l concentration. Similar observation was made by Hao and Chen (2012) except there was increased SOD activity in the organs of *Cyprinus carpio* on the seventh day of exposure to ZnO-NPs and reduced after prolonged exposure at the same concentration of ZnO-NPs. Hao *et al*. (2013) also reported the inhibition of SOD activity and increased MDA content of *C. carpio* exposed to sub-acute concentrations of ZnO-NPs.

The report of a parallel study by Kaya *et al*. (2015) indicated evidence of bioaccumulation, oxidative stress and fluctuations of antioxidant defense enzymes in the various tissues of *Oreochromis niloticus*. Benavides *et al*. (2016) and Abdelhazim *et al*. (2017) reported the generation of increased level of lipid MDA, indicating oxidation, and antioxidant depletion in the tissue of *Carassius auratus* and *O. niloticus*, respectively. Shahzad *et al*. (2018) also reported increased lipid peroxidation with attendant antioxidant induction in the tissues of *O. niloticus* exposed to increasing concentrations of ZnO-NPs. Changes in the antioxidant parameters of fish upon exposure to chemical agents is dependent on the species, concentration of the chemical, duration and route of exposure (Sanchez *et al*., 2005). Up till now, a dearth of information exists in literature on the detrimental impacts of ZnO-NPs exposure on the antioxidant and malondialdehyde levels in *Heterobranchus longifilis*.

The African giant freshwater catfish, *Heterobranchus longifilis* is a high-level protein fish food and commercially important in Nigeria, where it is intensively cultured (Suleiman *et al*., 2015). This has

informed its extensive study not only to improve its culture potentials but also to assess the impact of xenobiotics on its physiology. The understanding of the fish's response to any chemical agent would, in no small measure, help in predicting and managing the consequences of exposure, particularly in a rapidly changing environment and thus assist in the conservation of the species. The present study, therefore, investigated the oxidative and antioxidant response of the blood, gills and liver of *H. longifilis* after acute and chronic exposure to ZnO-NPs.

## Materials and Methods

**Chemicals, Fish collection and maintenance**

The powdery form of Sigma Aldrich (USA)-manufactured ZnO-NPs (<100 nm) (CAS number: 1314-13-2; Product number: 544906; Colour: whitish; Surface area: 15-25 $m^2/g$; Percentage zinc: 79.1-81.5 %; Shape: rod-shaped) as characterised by Akanbi-Gada *et al*. (2019) was used for this study. Two hundred and fifty juvenile specimens of *H. longifilis*, with average length of 10.28 ± 1.34 cm were procured from the hatchery of a fish farmer in Ilorin ($10^0$53'0" N, $4^0$ 1' 0" E), metropolis Kwara State, Nigeria and transferred to the Fisheries and Hydrobiology Laboratory, Department of Zoology, University of Ilorin.

Fish were then allowed to acclimatise to a 2-week ambient conditions (at 12h light: 12h dark photoperiod) in an aerated 120 L tank before the experiment. *H. longifilis* were fed two times daily with commercial fish pellets at 3% body weight. The mean physicochemical properties of the water throughout the experiment were as: temperature 27.00 ± 2.08 $^0$C, dissolved oxygen 6.45 ± 1.20 mg/l, pH 7.04 ± 0.06 and total hardness as $CaCO_3$ 26.67 mg/l. Water was replaced every 48 hours to prevent deterioration.

**Experimental design**

Sequel to the presumptive test results, five concentrations (0, 60, 80, 100 and 120 mg/l of ZnO-NPs were prepared through serial dilution in a static renewal acute bioassay using the Organization of Economic Cooperation and Development (OECD, 1992) procedures. Three replicates of twenty fish each were then randomly allocated into the control (devoid of the toxicant) and the prepared lethal concentrations of ZnO-NPs and subjected to a 4-day (96 h) exposure regime. For the chronic assay, three replicates of the same number of acclimatised, randomly selected fish were introduced into the control and four measured sublethal concentrations (6.0, 8.0, 10.0 and 12.0 mg/l) of ZnO-NPs; equivalent to 1/10 each of the concentrations used for the acute assay. Fish were exposed for 45 days and fed twice daily throughout the experiment at 3% body weight. The aquaria containing fish were cleaned and toxicant renewed every day to keep its concentration constant (FAO, 1986).

**Tissue homogenate preparation, malondialdehyde and antioxidant assays**

At the end of 96 h, 15-, 30- and 45-days exposure, random samples of fish were collected from each treatment for bloodletting. Blood was collected using labeled heparinised capillary tubes covered with plasticin to prevent entry of air that can aid clotting. Approximately 0.5ml aliquots of blood were centrifuged for five minutes at 3500g to separate serum for the biochemical analysis. Fish were subsequently sacrificed and the gills and liver carefully excised using dissecting kit. With buffer, the gills and liver samples were washed and then placed in 10% homogenate in 0.1 M phosphate buffer (pH 7.4) and homogenized using Teflon tissue homogenizer. The homogenates were centrifuged at 10,000 rpm, $4^0$C for ten minutes; after which the supernatants were kept in a freezer prior use. Superoxide dismutase (SOD) was determined by the protocols of Misra and Fridovish (1972). Glutathione peroxidase was assayed using the procedure of Koracevic *et al*, (2001). MDA was estimated using the freshly prepared homogenate of each tissue sample following the method of Buege and Aust (1978).

**Statistical Analysis**

A parametric (one-way ANOVA) or non-parametric (Kruskall-Wallis) analysis of variance depending on the distribution and homogeneity of data was used to analysed the data. Tukey's test was used to identify the differences, if any, and statistically significant values set at $p < 0.05$.

# Results

The activities of SOD, GPx and the levels of MDA in the blood, gill and liver of *Heterobranchus longifilis* upon 96 h exposure to varying ZnO-NPs concentrations are illustrated in Fig. 1. The activities of SOD showed no significant ($p > 0.05$) increase in all the tissues examined compared to the control. Similar trend was observed with the activities of GPx in the tissues of the exposed fish except in the liver where a significant ($p < 0.05$) concentration-dependent increase was noticed. MDA levels in the blood and liver of ZnO-NPs-treated fish significantly ($p < 0.05$) increased compared to the control, but the MDA level did not significantly ($p > 0.05$) change in the gill.

The activities of antioxidant enzyme, SOD in the blood, gill and liver of *H. longifilis* exposed to ZnO-NPs for 45 days are presented in Fig. 2, while those of GPx are shown in Fig. 3. After 15 days exposure, SOD activities slightly increased with increase in concentration of ZnO-NPs in all the tissues examined but with significant ($p < 0.05$) increase at 10 and 12 mg/l. After 30 days exposure, a similar increasing trend of SOD activities in all the tissues as the concentration of ZnO-NPs increased was observed; but there was no significant ($p > 0.05$) change compared with the control. The values of SOD in the tissues of exposed fish were higher than the control group after 45 days of exposure, but significantly ($p < 0.05$) higher value than the control was recorded only at each of the groups treated with 10 and 12 mg/l ZnO-NPs.

GPx activity in the blood of the exposed fish did not show a significant ($p > 0.05$) concentration-dependent increase than the control after 15 days exposure. After 30 and 45 days, however, there was a significant ($p < 0.05$) concentration- and time-dependent increase in GPx activities in the blood than the control. Compared with the control, GPx activity in the gill of exposed fish neither show significant concentration- nor time-dependent ($p > 0.05$) difference between the first period of fifteen days and the subsequent fifteen days (i.e., 30 days) of exposure, until after elongated days of exposure (45 days) when a significantly higher GPx was recorded; particularly at the highest concentration (12 mg/l) of ZnO-NPs (Fig. 3). In the liver, compared with the control, GPx activity in the exposed fish increased significantly ($p < 0.05$) as the concentration of ZnO-NPs and exposure period increased.

MDA levels in the tissues of ZnO-NPs-exposed *H. longifilis* are described in Fig. 4. In the three tissues (blood, gill and liver) examined, significantly ($p < 0.05$) higher levels of MDA than the control was not observed until after 45 days exposure particularly at higher concentration of ZnO-NPs.

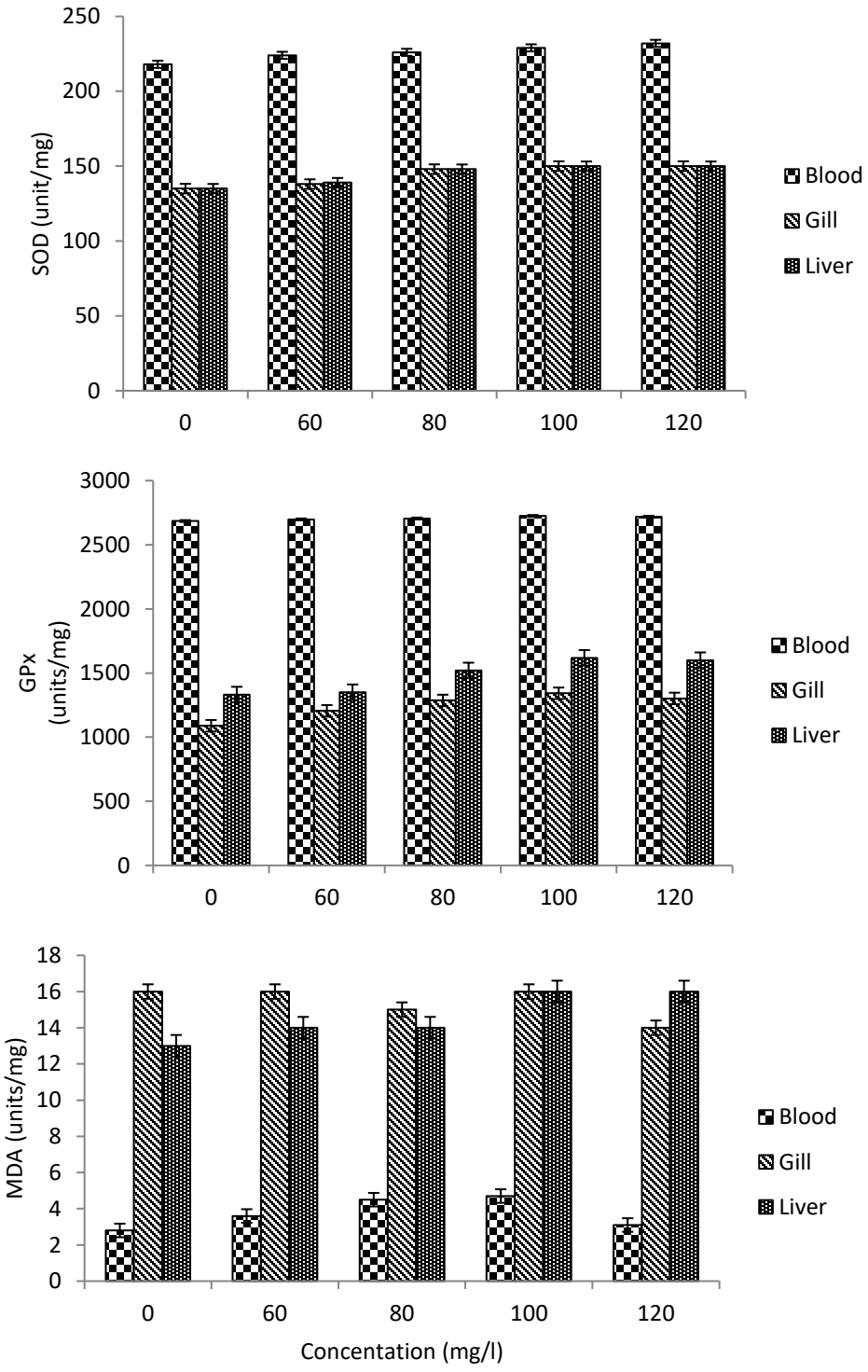


Fig. 1: Activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) levels in the blood serum, gill and liver of *Heterobranchus longifilis* exposed to varying concentrations of zinc oxide nano-particles for 96 h. Values are means ±SE (n=3).

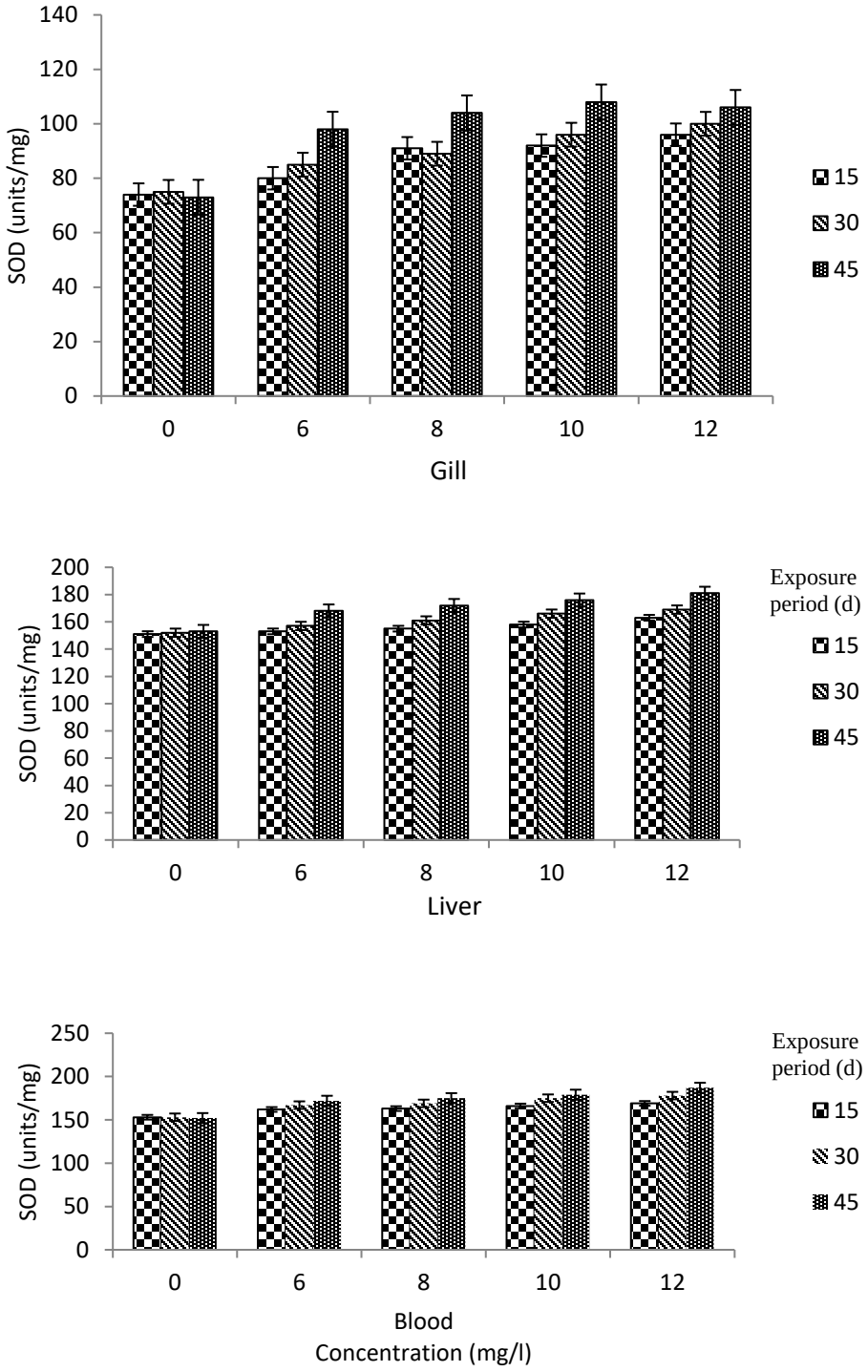


Fig. 2: Activity of superoxide dismutase (SOD) in gill, liver and blood of *Heterobranchus longifilis* exposed to varying concentrations of zinc oxide nano-particles for 45 d. Values are means ±SE (n=3).

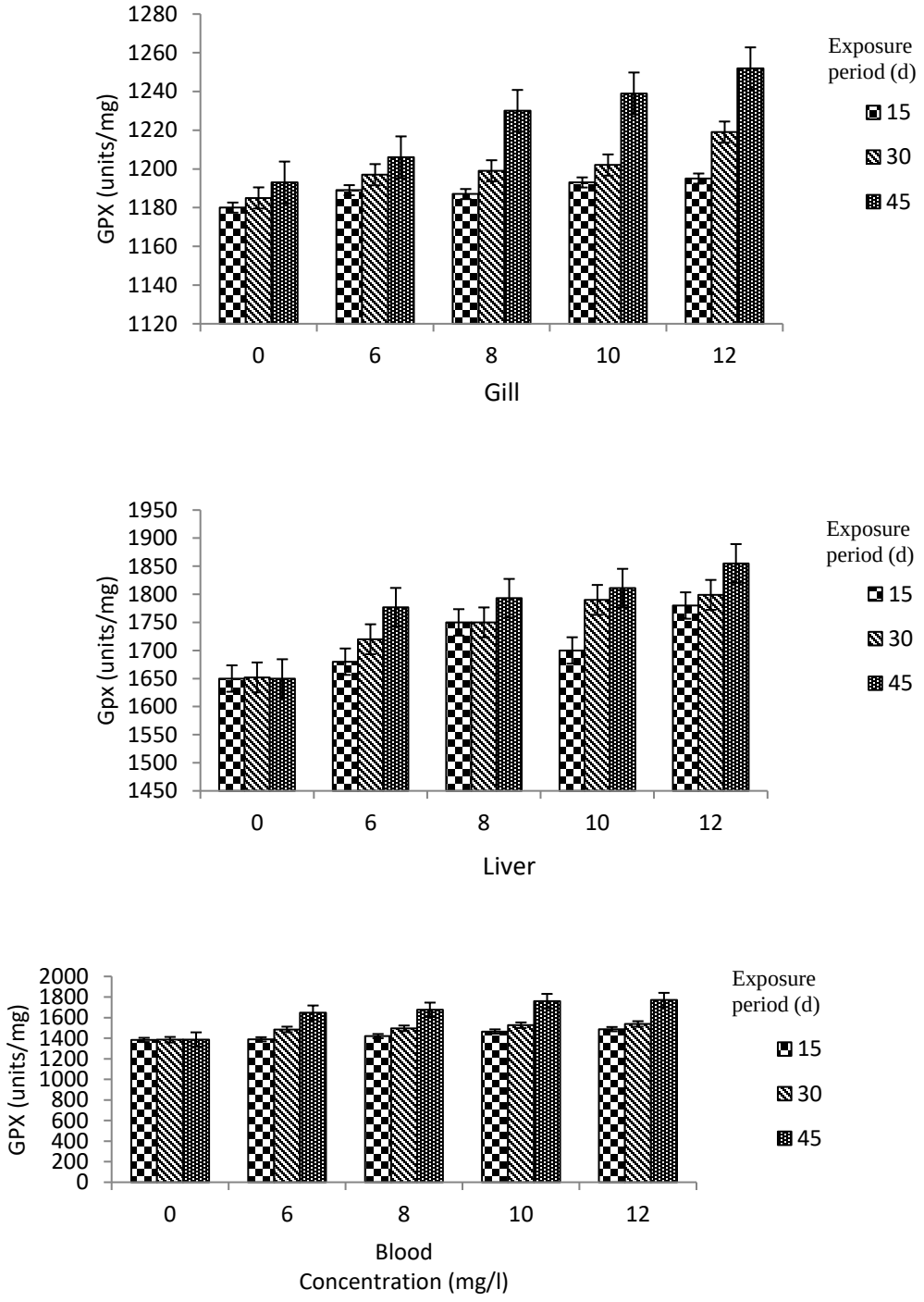


Fig. 3: Activity of glutathione peroxidase (GPx) in blood, gill, and liver of *Heterobranchus longifilis* exposed to varying concentrations of zinc oxide nano-particles for 45 d.

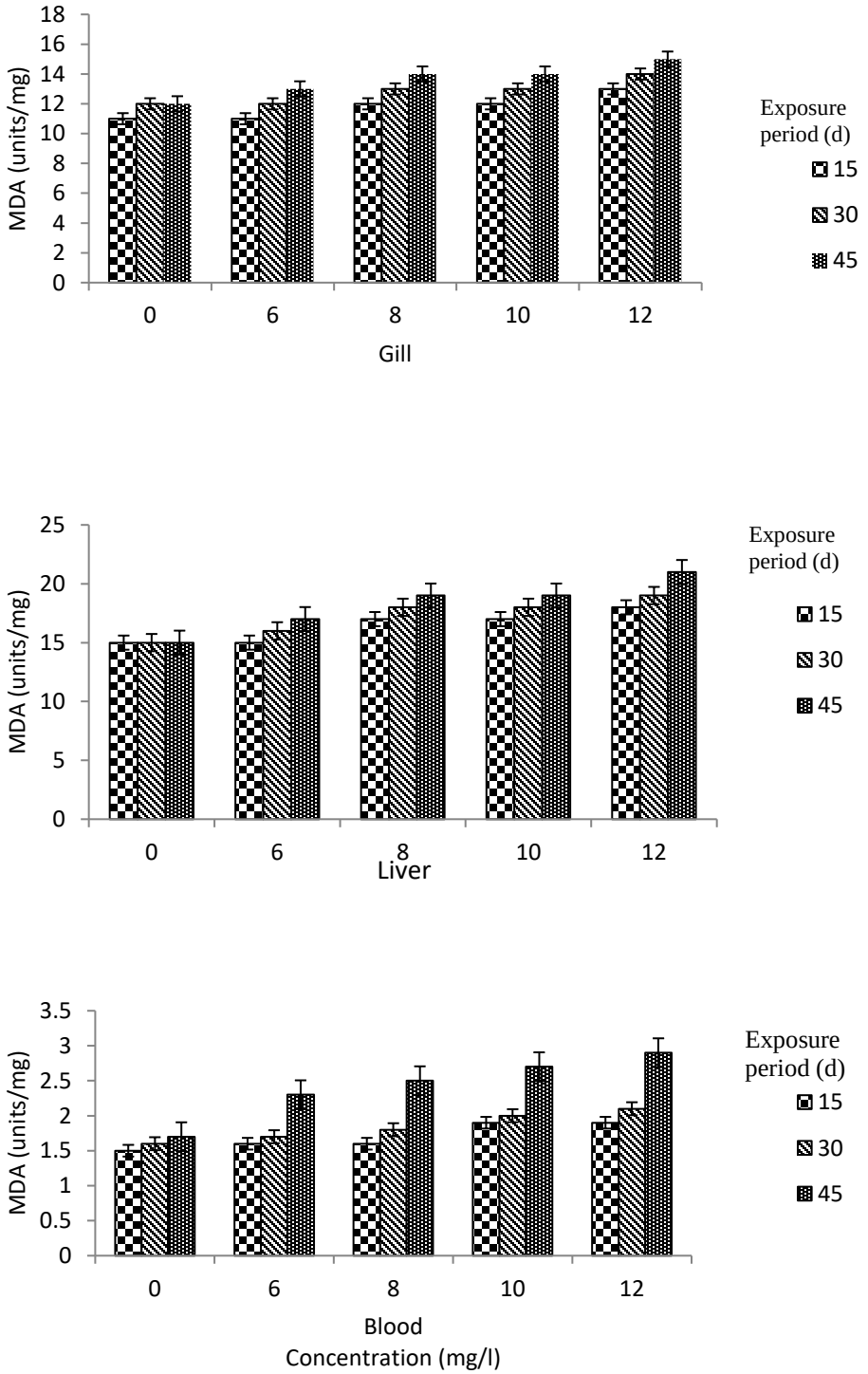


Figure 4: Activity of malondialdehyde (MDA) in blood, gill and liver of *Heterobranchus longifilis* exposed to varying concentrations of zinc oxide nano-particles for 45 d. Values are means ± SE (n=3).

## Discussion

Aquatic organisms are being decimated due to the accumulation of chemical agents which may have been washed into their habitats and thus, suffer from tissue damage as a result of physiological and biochemical alterations (Sula *et al*., 2020; Faria *et al*., 2021). To counteract the effects of these toxic chemicals, fish have evolved complex defensive mechanisms in form of antioxidants. The induction or inhibition of these antioxidants are widely accepted as biological indicator of xenobiotic-induced peroxidative injury in fish tissues, and have been used in diagnosing the negative impacts of xenobiotics in aquatic environment (Lasheen *et al*., 2012).

SOD and GPx are used as biomarkers of lipid peroxidation in organisms exposed to chemical perturbations. These enzymes provide the first line of defence against formation of oxyradicals by converting superoxide radicals into hydrogen peroxide and subsequently into water and molecular oxygen. The induction of these enzymes in the tissues of ZnO-NPs-exposed groups at 10 and 12 mg/l is an indication of oxidative stress through the production of reactive oxygen species (ROS). Thus, the enhanced SOD and GPx activities in this study is suggestive of ZnO-NPs-induced adaptive response to eliminate the formation of lipid peroxidation for polyunsaturated fatty acids which might have damaged the cell membrane. Several reports have earlier indicated that exposure to ZnO-NPs and other nanoparticles in aquatic organisms can exacerbate the liberation of ROS which could cause oxidative damage to biological systems (Ferreira *et al*., 2005; Bobori *et al*., 2020). The formation of ROS could be ascribed to the accumulations of the ZnO-NPs in the tissues of *H. longifilis*. Previous results had earlier confirmed that fish accumulates nanoparticles in different tissues from the surrounding milieu (Mansouri *et al*., 2016; Canli and Canli, 2019; 2020). Elevated levels of SOD and GPx in the tissues after 45 days exposure, particularly at the highest concentrations of the toxicant, may be associated with the tolerance and/or detoxification ability of *H. longifilis* especially under a long-term ZnO-NPs exposure regime. However, the apparent similarities in values of the two antioxidants in the ZnO-NPs-exposed fish to the control at each of the exposure period and concentration is suggestive of the scavenging potency of the antioxidants against ZnO-NPs-induced oxygen radicals, thus reducing its hazardous effects by sustaining the antioxidants' optimal levels. Increased activities of SOD and GPx have been reported previously in the tissues of fish exposed to nanoparticles (Saddick *et al*., 2015).

Lipid peroxidation has been adduced as the main reason for the loss of functional integrity in organisms during the formation of oxyradicals (Storey, 1996) and has been used in the assessment of oxidative damage (Kaloyianni *et al*., 2019; 2020). In this study, the induction of lipid peroxidation in the tissues of ZnO-NPs-exposed fish as reflected by the high malondialdehyde (MDA) levels is an indication that the fish is under oxidative stress. However, this seemed not to have manifested during the early days of exposure due to the similarities observed in MDA value between the exposed fish and the control until after 45 days at higher toxicant concentrations. Increased levels of MDA are indicative of lipid components' vulnerabilities to the reaction of free radicals, thereby increasing lipid peroxidation. The increase in MDA levels may be explained by the disproportionate generation of ROS, and could be linked to the diminution of the antioxidants owing to their leakage into the blood circulation (Ural, 2013; Saddick *et al.*, 2015). Similar to this result, increased MDA have also been observed in the tissues of fish (Benavides, 2016; Abdelhazim *et al.*, 2017; Shahzad *et al.*, 2018).

In conclusion, this study showed that ZnO-NPs at varying concentrations induced detrimental effects on the antioxidant system of *H. longifilis*. However, the elevated activities of SOD and GPx indicate resistance to the toxic effects even at higher concentrations and longer period of exposure. ZnO-NPs concentrations used in this study may, therefore, be considered as ecologically relevant; suggesting that concentrations greater than these under short-term or long-term exposure may produce deleterious effects on the antioxidant system, and thus, weakens the adaptive threshold of the fish.

# References


Abdelhazim AM., Saadeldin, IM., Swelum, AA. Oxidative stress in the muscles of the fish Nile tilapia caused by zinc oxide nanoparticles and its modulation by vitamins C and E. Oxid. Med. Cell. Longev., 2018; https://doi.org/10.1155/2018/6926712

Aitken RJ., Chaudry MQ., Boxall ABA., Hull M. Manufacture and use of nanomaterials: current status in the UK and global trends. Occ. Med. 2006; 56: 300-306.

Akanbi-Gada MA., Ogunkunle CO., Vishwakarma, V., Viswanathan K., Fatoba PO. Phytotoxicity of nano-zinc oxide to tomato plant (*Solanum lycopersicum* L): Zn uptake, stress enzymes response and influence on non-enzymatic antioxidants in fruits. Environ. Technol. Inno. 2019; *14, 100325*. https://doi.org/10.1016/j.eti.2019.100325

Asghar MS., Quereshi NA., Jabeen F., Khan MS., Shakeel and Noureen, A. Toxicity of zinc nanoparticles in fish: a critical review. J. Biodiv. Environ. Sci. 2015; 7 (1): 431-439.

Banaee M., Sureda A., Shahaf S., Fazilat N. Protective effects of silymarin extract on malathion-induced zebra cichlid (*Cichlasoma nigrofasciatum*) hepatoxicity. Iranian J. Toxicol. 2015; 9 (28), 1239-1246.

Benavides M., Fernandez-Londeiro J., Coelho P., Lodeiro C., Diniz MS. Single and combined effects of aluminum ($Al_2O_3$) and zinc oxide (ZnO) nanoparticles in a freshwater fish, *Carassius auratus*. Environ. Sci. Pollut. Res. 2016; 23 (24): 24578-24591.

Bobori D., Dimitriadi A., Karasiali S., Feidantsis K., Tsoumaki-Tsouroufli P., Mastora M et al. Common mechanisms activated in the tissues of aquatic and terrestrial animal models after $TiO_2$ nanoparticles exposure. Environ. Int. 2020; 138: 105611.

Buege JA., Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-310.

Canli EG., Canli M. Nanoparticles ($Al_2O_3$, CuO, TiO2) exposures in freshwater fish (*Oreochromis niloticus*). Environ. Toxicol, Pharmacol. 2019; 62: 181-187.

Canli EG., Canli M. Investigations of the nervous system biomarkers in the brain and muscle of freshwater fish (*Oreochromis niloticus*) following accumulation of nanoparticles in the tissues. Turk. J. Fish & Aquat. Sci. 2020; 44:90-103.

Connolly M., Fernandez, M., Conde, E., Torrent F., Navas, JM., Fernandez-Cruz ML. Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout. Sci. Total Environ. 2016; 551/552: 334-343.

Faria M., Prats, E., Ramirez Jrr, Bellot M., Pagano M., Valls A., Gomez-Canela C., Porta et al. Androgenic activation, impairment of the monoaminergic system and altered behaviour in zebrafish larvae exposed to environmental concentrations of fenitrothion. Sci. Total Environ. 2021; 775: 145671.

Ferreira M., Moradas-Ferreira P., Reis-Henriques MA. Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. Aquat. Toxicol. 2005; 71: 39-48.

Hao L., Chen L. Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. Ecotoxicol. Environ. Saf. 2012; 80: 103-110.

Hao L., Chen L., Zhong N. Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (Cyprinus carpio): a comparative study with its bulk counterparts. Ecotoxicol. Environ. Saf. 2013; 91: 32-60.

Kaloyianni, M., Feidantsis, K., Ntelie, I., Stergiou, P., Tsoulia, T., Dimitriadi, A., Antonopoulou, E., Bobori, D. Biochemical and molecular responses of cyprinids in two Mediterranean lacustrine ecosystems: Opportunities for ecological assessment and biomonitoring. Aquat. Toxicol. 2019; 211: 105-115.

Kaloyianni M., Dimitriadi, A., Ovezik, M., Stamkopoulou, D., Feidantsis, K., Kastrinaki, G., Tsiaoussis, I., Koumoundouros, G., Bobori, D. Magnetite nanoparticles effects on adverse responses of aquatic and terrestrial animal models. J. Hazard. Mater. 2020; 383. https://doi.org/10.1016/j.hazmat.22019.121204

Kaya H., Aydin F., Gurkan M., Yilmaz Z., Ates M., Demir, V., Arslan Z. Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreochromis niloticus*). Environ Toxicol. Pharmacol. 2015; 40: 936-947.

Koracevic D., Koracevic G., Djordjevic V., Andrejevic S., Cosic V. Method for the measurement of antioxidant activity in human fluids. J. Clin Pathol. 2001; 54: 356-361.

Lasheen MR., AbdelGawad FKh., Alaneny AA., AbdElbary HMH. Fish as bioindicators in aquatic environmental pollution assessment: a case study in Abu-Rawash Area, Egypt. World Appl. Sci. J. 2012; 19: 265-275.

Mansouri B., Maleki A., Johari SA., Shahmoradi B., Mohammadi E., Shahsavari S., Davari B. Copper bioaccumulation and depuration in common carp (Cyprinus carpio) following co-exposure to $TiO_2$ and CuO nanoparticles. Arch. Environ. Contam. Toxicol. 2016; 71 (4): 541-552.

Misra H.P., Fridovish, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 1972; 243: 3170-3175.

NESREA. National Environmental (Surface and Groundwater quality) Regulations. National Environmental Standards and Regulation Enforcement Agency. 2011; 37 pp.

NIS (Nigerian Industrial Standard). *Nigerian standards for drinking water quality*. (NIS 554, 2007, 748 ICS 13.060.20). 2007; Standard Organisation of Nigeria (SON) Governing Council.

OECD. Organisation for Economic Cooperation and Development. *Guidelines for testing of chemicals*. *Guideline* no. 1992; 203: 10. fish, acute toxicity test.

Ruas CBG., Dos Santos Carvalho C., De Araujo HSS., Espindola EIG., Fernandes MN. Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. Ecotoxicol. Environ. Saf. 2008; 71(1): 86-93.

Saddick S., Affifi M., Zinada OAA. Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*. Saudi J. Biol. Sci. 2015; 24 (7): 1872-1678.

Sanchez W., Palluel O., Meunier L., Coquery M., Porcher JM., Ait-Aissa S. Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. Environ. Toxicol. Pharmacol. 2005; 19: 177-183.

Shahzad K., Khan MN., Jabeen F., Kosour, N., Chaudhry AS, Sohail M., Ahmad N. Toxicity of zinc oxide nanoparticles (ZnO-NPs) in tilapia (*Oreochromis mossambicus*): tissue accumulation, oxidative stress, histopathology and genotoxicity. Int. J. Environ. Sci. Technol. 2018; https://doi.org/10.1007/s13762-018-1807-7

Storey KB. Oxidative stress: animal adaptations in nature. Braz. J. Med. Biol. Res. 1996; 29: 1715-1733.

Sula E., Aliko V., Barcelo D., Faggio C. Combined effects of moderate hypoxia, pesticide and PCBs upon Crucian Carp fish, *Carassius carassius* from a freshwater lake-*In situ* Eco-physiological Approach. Aquat. Toxicol. 2020; 228: 105644; https://doi.org/10.1016/j.aquatox.2020.105644

Suleiman, B., Maruff L., Oniye SJ. Radiographic studies on morphological anomalies in artificially spawned *Heterobranchus longifilis* Valenciennes, 1840 F1 generation. Sokoto J. Vet. Sci. 2015; 13 (1): 9-16.

Tripathi BN., Mehta SK., Amar A., Gaur JP. Oxidative stress in *Scenedesmus* sp. during short-and long-term exposure to $Cu^{2+}$ and $Zn^{2}$. Chemosphere 2006; 62: 538-534.

Wang, J., Cao J., Fang B., Lu P., Deng S., Wang H. Synthesis and characterization of multipod, flower-like and shuttle-like ZnO framework in ionic liquids. Mater. Lett. 2005; 59: 1405-1408.

Xiong D., Fang T., Yu L., Sima X., Zhu W. Effects of nano-scale $TiO_2$, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci. Total Environ. 2011; 409: 1444-1452.

Zimmermann YS., Schaffer A., Hugi, C., Fent K., Corvini PFK., Lenz M. Organic photovoltaics: potential fate and effects in the environment. Environ. Int. 2011; 49: 128-140.