PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (4) 603-610 April 2022

Toxicological Implication of Zinc Oxide Nano-Particles on Nutritional Composition and Depuration Potential of *Heterobranchus longifilis*

***ABDULKAREEM, SI; OWOLABI, OD**

*Fisheries and Aquatic-ecotoxicology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria. *Corresponding Author Email: *Corresponding Author Email: Abdulkareem.si@unilorin.edu.ng*

ABSTRACT: Zinc Oxide nano-particles (ZnO-NPs) are more useful in the production of commercial goods than other nano-particles because of their unique properties. The effluents of ZnO-NPs get into the aquatic ecosystems and accumulate in fish tissues causing serious health consequences. This study was therefore designed to investigate the effect of ZnO-NPs exposure on the nutritional composition and depuration potential of large African catfish (*Heterobranchus longifilis*). The nutritional composition evaluated include, proximate composition, mineral content, fatty acids and amino acids profiles after exposing to varying (0.0, 6.00, 8.00, 10.00, 12.00 mg/l) concentrations of ZnO-NPs to juveniles' catfish (*H. longifilis*) for 60 days and depurating for 30 days to evaluate recovery using standard methods. The results revealed that proximate composition and amino acid profiles decreased significantly ($P < 0.05$) after 60 days of exposure to ZnO-NPs, but gradually improved after 30 days of depuration. This implies that ZnO-NPs has a great influence on the nutrient values of *H. longifilis*, but the fish were able to regain the lost nutrients, however, the ability of *H. longifilis* to recover from adverse condition is time dependent.

DOI: https://dx.doi.org/10.4314/jasem.v26i4.7

Impact factor: http://sjifactor.com/passport.php?id=21082

Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

Dates: Received: 27 February 2022; Revised: 13 March 2022; Accepted: 07 April 2022

Keywords: Depuration, *Heterobranchus longifilis*, Nutrient composition, and Zinc oxide nano-particles

Fish has been known to be the most nutritious and a good source of animal protein, carbohydrate, fats and oil, minerals, vitamins A, D, iodine and water which are essentials for bodybuilding, reproduction and good health (Shalini *et al*., 2012). Fish contains high values of polyunsaturated fatty acids and the essential amino acids, methionine (Shalini *et al*., 2012). However, fish production cannot meet the nations demand due to decline in production which is caused by various human activities through agricultural practices, indiscriminate discharge of domestic wastes and untreated wastes by industries as a result of increased population (FAO, 1996). As the population increases, the wastewater that will be discharged into the water bodies will also increase hence increase in the number of people that will be subjected to the consequences of the pollution (WHO/UNICEF, 2010). In most developing countries more than 70% of industrial wastes dumped into water bodies are untreated and this could be dangerous to the existence of aquatic organisms. Amongst these pollutants, emergent nano-particles recently used in the production of most commercial goods by many industries could also find their ways into the water bodies. Even though the production level and use of nano-particles in Nigeria industries is low, materials that are manufactured with these nanoparticles are still imported. The use of this nano or disposal of the waste or empty cans discarded in the environment may find their ways through run-off into the aquatic environment which may have adverse effects on human health and even the environment at large. Among the environmental pollutants, nano-particles of metal oxides are of particular concern due to their potential toxic effects on living organisms because of their ability to persist, bioaccumulate and biomagnify in animal tissues in aquatic ecosystems. This creates an immense threat to the existence of aquatic organisms particularly fish and even the ecological integrity of the water bodies which in turn increase the exposure of higher animals including man that consume aquatic products to serious health risks. Occurrence of these nano-metal oxides in the water bodies cause depletion in the oxygen level by preventing oxygen diffusion into the

**Corresponding Author Email: Abdulkareem.si@unilorin.edu.ng*

water bodies thereby affecting respiration of the fish, hence, hypoxia. Ability of the metal oxide to bioaccumulate in fish tissues could affect the quality of the affected fish, thereby making it unfit for human consumption. The effect of nano metal oxides on the aquatic environments evidently showed future deleterious effects on the aquaculture environments which eventually caused the decline in open water capture fishery production. The nano size and large surface area of metal oxide nano-particles allow their easy penetration and bio accumulation into cells which make them toxic to animals (Li *et al*., 2013 and 2014). Among the commonly used nano-particles are zinc oxide nano-particles (ZnO-NPs) mainly because of their special physicochemical properties such as electrochemical stability, photo stability and rigidity make them useful as electronics, solar panel devices and use in ceramic and in paints (Zimmermann *et al*., 2012). ZnO-NPs are also used in the process of producing and packaging of meat and vegetable products (Asghar *et al*., 2015). Release of these ZnO-NPs into the aquatic environment may therefore cause ecological disorders and decline in the quality of aquatic organisms. Fish are commonly used for the ecotoxicological assessment of the quality of the aquatic environment. They serve as bioindicators of environmental pollution because of their link in the food chain (Lopes *et al.*, 2001). Fish also serve as a major test organism and are particularly useful for the assessment of waterborne and sediment deposited toxins where they may provide advanced warning of the potential danger of new chemicals and the possibility of environmental pollution (Powers, 1989). Accumulation of ZnO-NPs in the tissue of fish may reduce the nutritional value, thus contamination of water bodies where fish reside deserve greater attention. Fish health is essential to the success of the aquaculture industry, an industry of growing importance in protein production for humans (Hart, 1996). There is therefore the need to carry out experiments on the consequence of ZnO-NPs on *H. longifilis* in relation to their nutrition level. *Heterobranchus longifilis* has a high value of proteins, carbohydrates, fats and oil, minerals, fatty acids, amino acids and vitamins. Accumulation of ZnO-NPs in the tissue of this fish may reduce the nutritional value (Shalini *et al*., 2012). The objective of this study was therefore designed to investigate the effect of ZnO-NPs exposure on the nutritional composition and depuration potential of large catfish (*Heterobranchus longifilis*).

MATERIALS AND METHODS

Toxicity Experiment: Juveniles of *Heterobranchus longifilis* (average weight 19.40 ± 3.65 g and average length 12.45 ± 0.13 cm) were obtained from a commercial fish farm in Lagos, and transported in plastic aquaria from the farm to the laboratory in the Department of Zoology, Faculty of Life Sciences, University of Ilorin. The fish were not fed throughout the day until the next day to avoid indigestion and mortality. The fish were fed twice daily at 9.00 am and 5.00 pm with commercial feeds (Copens 2 mm) at 4% of initial body weight (Abdulkareem *et al*., 2015). A powdery commercial ZnO-NPs with average particle size of less than 100 nm purchased from Sigma Aldrich (USA) was used for the experiment. The nano-particle was characterized by Akanbi-Gada (2019) as (CAS number: 1314-13-2; Product number:544906; Colour: whitish; Surface area: 15-25 m2/g; Percentage ZINC: 79.1-81.5%; Shape; rod-shaped). The powder was dispersed into distilled water and the suspending solution of ZnO-NPs was vigorously stirred with a magnetic stirrer for about two hours to break suspension or precipitates forming the stock solution. The fish were acclimatized to laboratory conditions under 24℃ and kept in a tank of 1000 L capacity containing chlorine-free bore-hole water for fourteen days before the commencement of the experiment. The water was fully aerated with aerating pumps throughout the experimental periods. The concentration of the ZnO-NPs was renewed every 24 h to maintain the toxicity level and to reduce disease outbreak and mortality. Feeding was stopped 24 hours before the commencement of the experiment (USEPA, 1996). The water quality parameters such as temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and conductivity were monitored throughout the experimental periods according to the procedure of APHA (1995). Based on the result of the acute tests (Abdulkareem and Owolabi, 2019), the test fishes were exposed to varying concentrations (0.0, 6.0, 8.0, 10.0 and 12.0 mg/l) measured from the stock solution of ZnO-NPs for sixty days. Five different groups of 30 fish each were used for the experiment, four groups were exposed to varying concentrations (6.0, 8.0, 10.0 and 12.0 mg/l) of ZnO-NPs and the fifth group without exposure served as the control group.

Depuration Experiment: The ZnO-NPs-exposed-fish were transferred into fresh bore-hole chlorine-free water to evaluate the rate of depuration of the accumulated Zn by the fish. The fish were fed and renewed with fresh water every 24 h. After 30 days of the depuration experiment, five fish from each aquarium were randomly picked for nutritional composition assay.

Analysis of nutrient composition: The nutrient composition was evaluated by taking five fish each from different concentrations (0.00, 6.00, 8.00, 10.00

and 12.00 mg/l) of the two experiments on the 15th, 30th, 45th and 60th day of the experimental period, and 15th and 30th of the depuration period. The fish were oven-dried in an electric oven at 60ºC for 24 hours. The dried fish were milled independently into powder form using an electric grinder. Milled samples were placed in cellophane bags and kept in a desiccator prior analysis. One gramme (1 g) each of the powder was analyzed for proximate composition such as moisture, crude protein, total lipids, total ash, carbohydrate, and crude fibre in the control and the ZnO-NPs exposed-fish according to AOAC (2000).

One gramme (1 g) of the powder was analyzed for the composition of amino acids such as cystine, threonine, methionine, valine, tyrosine, isoleucine, histidine, arginine, and aspartic acid in both the control and the ZnO-NPs-exposed fish. Amino acids were analyzed using the method described by Simpson *et al.* (1976) (Auto analyzer, JLC-500V).

One gramme of the powder was analyzed for fatty acid composition such as arachidonic acid, linoleic acid, palmitic acid and stearic acid in both the control and the ZnO-NPs exposed-fish. Saponification was done by preparing methyl esters with 7 % boron trifluoride in a methanol solution (BF_3-methanol) using 50% ethanol. The fatty acid profile was then determined by using a gas liquid chromatography.

One gramme of the powder was analyzed for the mineral constituents such as calcium, iron, phosphorus, sodium, zinc, magnesium and copper in both the control and the ZnO-NPs exposed-fish. Atomic absorption spectrophotometer (AAS) and flame photometry were used to determine the micro mineral composition after digestion of the fish by Perkins Elmer Atomic Absorption Spectrophotometer model 2900 (US), (AOAC, 2000).

Statistical analysis: The values obtained from the experiment were expressed as mean SE. One way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1955), and statistical significance was calculated using ANOVA of SPSS version -16.0 and the charts was drawn using Microsoft Excel of Microsoft Office Corporation (2003) (Duncan, 1955).

RESULTS AND DISCUSSION

The results obtained from the study revealed significant increase ($P < 0.05$) in the values of total dissolved solid (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), conductivity and turbidity as the concentration of ZnO-NPs increased. However, the values of DO decrease with increase in the concentration of ZnO-NPs, except in the values of temperature and pH with no significant difference ($P > 0.05$) compared to control (Table 1). A gradual increase in the values of DO with decrease in those of BOD and COD were recorded as the depuration period increased to 30 days (Table 1).

Table 1: - Mean water quality parameters during exposure of *Heterobranchus longifilis* for 60 days and depuration period for 30 days.

CONC. (mg/l)	TDS (mg/l)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	Conductivity (µ S/cm)	Turbidity	TEMP (℃)	PH
Exposure (60 d)								
0	1.20 ± 0.58 [a]	8.08 ± 1.13 [d]	4.68 ± 0.58 [a]	30.28 ± 1.13 [a]	58.21 ± 1.72 [a]	1.44 ± 1.03 [a]	25.0 ± 0.58 [a]	7.0 ± 1.15 [a]
6	5.10 ± 0.53 [a]	4.4 ± 0.52 [c]	7.12 ± 1.12 [b]	36.9 ± 1.15 [b]	62.19 ± 2.34 [b]	5.14 ± 0.05 [b]	26.9 ± 0.45 [a]	7.0 ± 0.58 [a]
8	5.50 ± 1.20 [a]	3.64 ± 0.42 [b]	9.44 ± 1.23 [c]	40.2 ± 0.13 [c]	68.18 ± 0.52 [c]	5.47 ± 0.04 [b]	26.9 ± 0.13 [a]	6.5 ± 2.10 [a]
10	6.10 ± 1.12 [a]	2.87 ± 1.15 [a]	12.74 ± 1.12 [d]	48.6 ± 0.16 [d]	86.82 ± 1.16 [d]	7.31 ± 0.58 [c]	26.9 ± 1.11 [a]	6.5 ± 0.32 [a]
12	6.70 ± 0.57 [a]	2.25 ± 1.12 [a]	12.88 ± 1.45 [d]	53.28 ± 0.18 [e]	118.10 ± 1.10 [e]	9.28 ± 1.12 [d]	26.9 ± 0.23 [a]	6.2 ± 1.32 [a]
Depuration (30 d)								
0	1.10 ± 2.13 [a]	8.15 ± 2.13 [c]	5.90 ± 0.82 [a]	30.24 ± 2.13 [a]	58.14 ± 2.14 [a]	1.43 ± 2.18 [a]	25.0 ± 0.58 [a]	7.0 ± 0.05 [a]
6	4.80 ± 0.36 [b]	5.12 ± 2.15 [b]	6.98 ± 0.32 [b]	32.39 ± 2.65 [a]	60.15 ± 4.52 [b]	5.04 ± 1.18 [b]	25.0 ± 0.58 [a]	7.0 ± 0.08 [a]
8	4.96 ± 0.58 [b]	4.33 ± 1.04 [a]	9.13 ± 0.72 [c]	36.21 ± 0.82 [b]	65.09 ± 1.23 [c]	5.16 ± 0.53 [b]	25.0 ± 0.58 [a]	7.0 ± 1.04 [a]
10	5.80 ± 0.09 [b]	3.45 ± 2.15 [a]	11.45 ± 0.34 [d]	38.67 ± 0.58 [b]	78.31 ± 1.10 [d]	6.58 ± 2.36 [c]	24.0 ± 0.58 [a]	7.0 ± 1.15 [a]
12	6.20 ± 1.10 [c]	3.14 ± 2.03 [a]	12.02 ± 0.67 [d]	45.72 ± 1.15 [c]	112.17 ± 2.12 [e]	7.84 ± 2.06 [d]	24.0 ±0.58 [a]	7.0 ± 0.14 [a]

Mean (± S.E, n=3) with the same superscript in the same column are not significantly different (P > 0.05); TDS = Total dissolved solids; DO = Dissolved; Temp. = Temperature; BOD = Biochemical oxygen demand; COD = Chemical oxygen demand; Standard for water quality by FEPA (1991), WHO (2004) and NESTREA Limits are DO = 4-6 mg/l; BOD = 4-6 mg/l; COD = 30 mg/l; pH = 6.5-9.0; Temp. = 20-33 ^{0}C; TURB. = 4 mg/l

The proximate composition of the whole body of *H. longifilis* showed a significant decreasing ($P < 0.05$) trend in the concentrations of protein, lipid, ash and moisture as the exposure period increased from 15 to 60 days except in the concentration of fibre with no significant decrease ($P > 0.05$) compared to the control (Figure 1). Higher contents of protein, lipid, fibre, ash and moisture were observed in the fish exposed to the lowest concentrations of ZnO-NPs while the lowest contents of the nutrients were revealed in the highest concentration of ZnO-NPs. However, the nutrient contents gradually increased as the ZnO-NPs-exposed fish were removed from exposure media and introduced to fresh water for a period of 30 days (Figure 1).

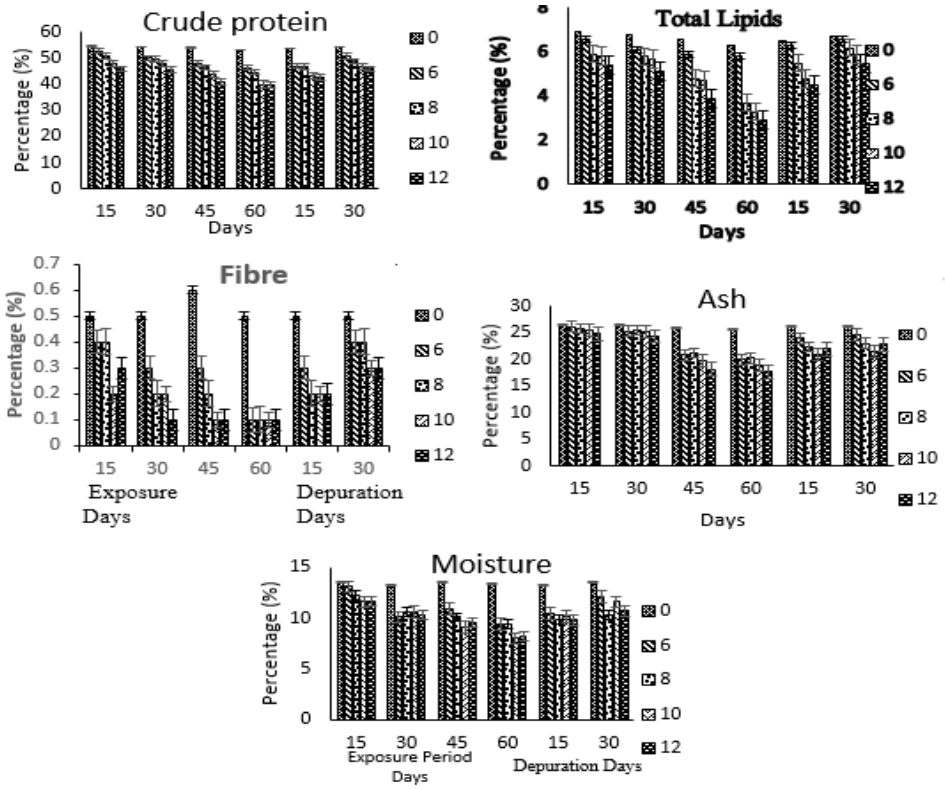


Fig 1: Proximate composition of whole *Heterobranchus longifilis* exposed to ZnO-NPs for 60 days and depuration for 30 days

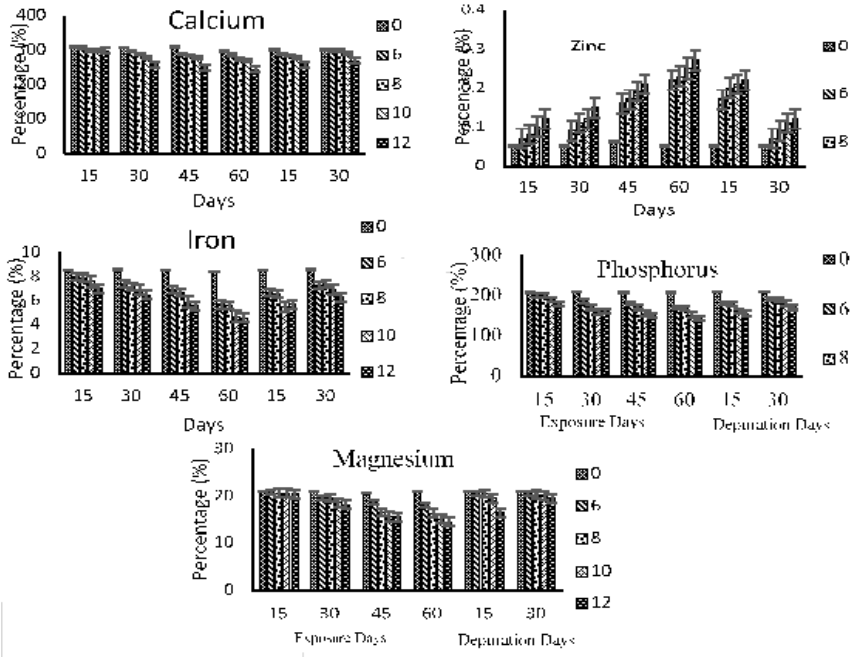
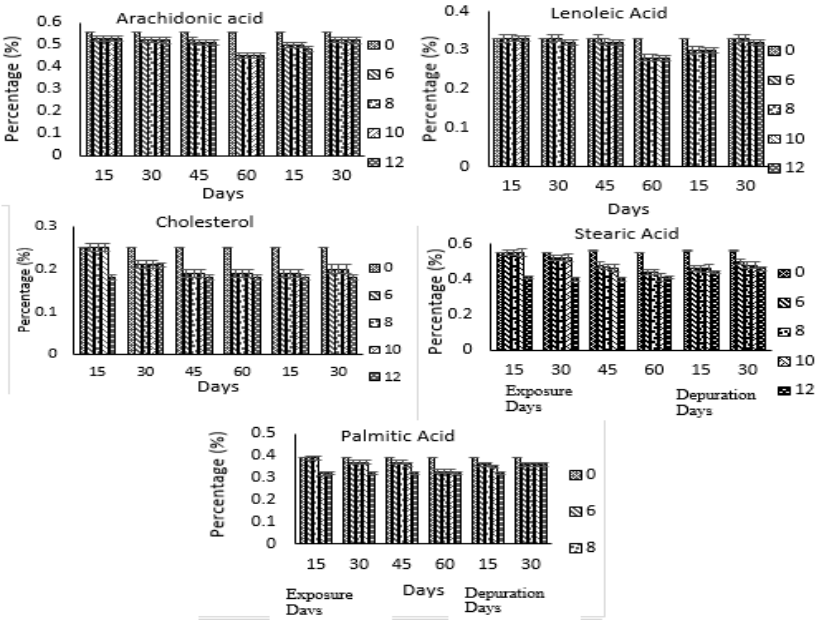


Fig 2: Mineral composition of *Heterobranchus longifilis* exposed to ZnO-NPs for 60 days and depurated for 30 days (ppm)



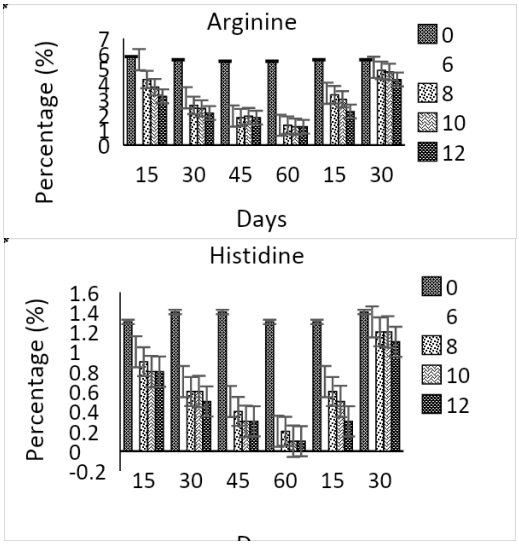
Fige 3: - Fatty acid composition of whole *Heterobranchus longifilis* exposed to ZnO-NPs for 60 days and depurated for 30 days (g/100 g of total

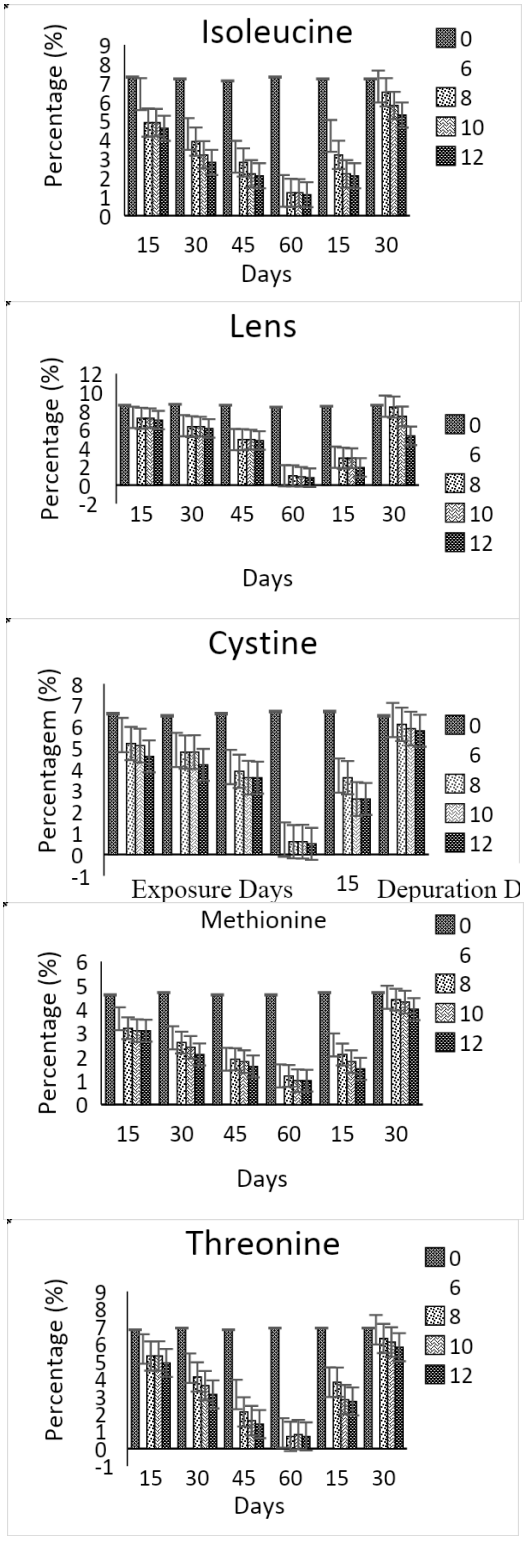
The mineral element composition of the whole body of *H. longifilis* also recorded a significant reduction ($P < 0.05$) in the concentrations of calcium, iron, phosphorus, sodium and magnesium as the ZnO-NPs concentration and exposure period increased (Figure 2). While a significant increase ($P < 0.05$) in the content of zinc occurred as the concentration and exposure period increased. However, a gradual increase in the concentrations of the lost minerals such as calcium, iron, phosphorus, sodium and magnesium were observed in the ZnO-NPs-exposed fish as the depuration period increased from day 15 to 30 days compared to the control. While the content of zinc in the fish body significantly reduced ($P < 0.05$) as the depuration period increased (Figure 2).

The content of fatty acids in the fish exposed to ZnO-NPs showed no significant difference in their contents ($P > 0.05$) with increase in ZnO-NPs concentration, but recorded significant decrease as the exposure period increased. The highest content of fatty acids was observed in the fish after 15 d of exposure to ZnO-NPs, while the lowest content of fatty acids was observed in the group of fish at the end of 60 d of exposure to ZnO-NPs. There was no significant increase ($P > 0.05$) in the content of fatty acids as the fish were introduced into fresh water for recovery for a period of 30 days (Figure 3).

Amino acid contents in the exposed fish were significantly lower ($P < 0.05$) compared to those in the control group. The level of amino acids decreased as the ZnO-NPs concentration and exposure period increased. The concentrations of protein, carbohydrates, moisture, fatty acids, mineral elements and amino acids are therefore concentration and time dependent. However, the content of amino acids increased as the recovery period extended from 15 to 30 days (Figure 4).

Decrease in protein, lipid, and amino acids contents is similar to the report of Alkaladi *et al*., (2014), who exposed *Oreochromis niloticus* to ZnO-NPs. The decrease could be as a result of accumulation of ZnO-NPs in the tissues of *Heterobranchus longifilis* which generated reactive oxygen species (ROS) that modify proteins, lipids and nucleic acids.





Reduction in the profile of protein is also in accord with the report of Hao *et al.*, (2009) who exposed common carp to titanium dioxide nano-particles. This could be attributed to the formation of hydroxyl radicals that damage lipid proteins by a process called lipid peroxidation which greatly affects lipid molecules.

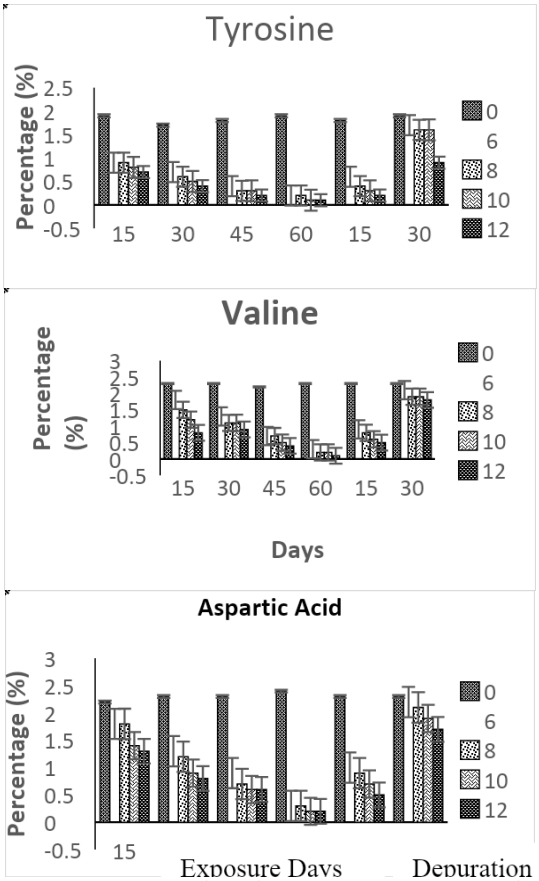


Fig 3: - Amino Acids profiles of whole *Heterobranchus longifilis* exposed to ZnO-NPs for 60 days and depurated for 30 days (g/100 g dry basis)

Generation of ROS could also damage proteins leading to structural alterations and alterations of enzyme activities (Yoshikawa and Naito, 2002). Decrease in the protein, lipid, amino acids and moisture contents in the exposed fish may reduce the quality, texture, colour and taste of the fish as was reported by Shalinl *et al*., (2012). Reduction in the value of lipids could also be due to oxidation of fat (Aranilewa *et al*., 2005). Oxidative stress induced by ZnO-NPs lead to excessive use of energy which altered the levels of protein, amino acids and fatty acids and also decreased amino acids content as was reported by Hao *et al.*, (2013). This could be due to their utilization for energy formation and other metabolic reactions as a result of over reactivity to avoid the toxicant (Shobha Rani and Janaiah, 1991). Recorded decrease in the protein content also agrees with the report of David *et al*., (2004), which could be as a result of liver damage caused by accumulation of ZnO-NPs that inhibit protein synthesis. The formation of hydroxyl radicals which cause damage to lipid protein could greatly have an effect on lipid molecules.

Increase in the zinc content in the fish exposed to ZnO-NPs is in accordance with the report of Kaya *et al*., (2015), who exposed *O. niloticus* to ZnO-NPs, this could be as a result of accumulation of zinc in the muscle and tissues of the fish. The nutritional quality in the exposed fish could decrease as the concentration and exposure period increased due to decrease in the mineral contents. The increased value of zinc in the fish muscle which is above the acceptable level in the fish as reported by Zhang et al., (2007) could also be toxic to the fish which are transferred to the next level in the food chain. The gradual increase in the contents of protein, moisture, fatty acids, mineral elements and amino acids shows that *H. longifilis* has the ability to regain the lost components when rescued from a polluted environment, but the recovery is time dependent. This may therefore give the fish the opportunity of improving its quality, texture, colour and taste.

Conclusion: The values of nutrients composition recorded a significant decreasing ($P < 0.05$) trend as the ZnO-NPs concentration and exposure period increased. A gradual increase in the lost nutrients occurred as the depuration period increased. These findings revealed that *H. longifilis* have the ability to naturally recover from the adverse condition imposed by ZnO-NPs toxicity, but recovery is time dependent.

REFERENCES

Abdulkareem, SI; Oraezue, PI; Olaniyan, AT (2015). Histopathological effects of lethal and sub-lethal concentrations of glyphosate on gills and liver of African catfish, *Clarias gariepinus*. *J. of Aqua. Scs*. 30 (1), 53-61

Abdulkareem, SI; Owolabi, OD (2019). Acute toxicity of zinc oxide nanoparticles on blood cell morphology, haematology and histopathology of *Heterobranchus longifilis*. *Anim. Res. Inter.*16 (1), 3174-3185.

Akanbi-Gada, MA; Ogunkunle, CO; Vishwakarma, V; Viswanathan, K; Fatoba, PO (2019). Phytotoxicity of nano-zinc oxide to tomato plant (Solanum lycopersicum L): Zn uptake, stress enzyme response and influence on non-enzymatic antioxidants in fruits. *Environ. Tech. Inno*. 14: 100-325.

Alkaladi, A; Afifi, M; Youssef, Y; Osama, M; Zinada, A **(**2014). Ultra structure alteration of sub-lethal concentrations of zinc oxide nanoparticals on Nile Tilapia (*Oreochromis niloticus*) and the protective effects of vitamins C and E. *Life Sci. J.* 11(10), 257-262. (ISSN: 1097-8135).

AOAC **(**2000). Official methods of analysis of AOAC International. 17th edition. Gaithersburg, MD, USA, *Assoc. of Anal. Comm.*.

APHA (American Public Health Association) American water works Association and Water pollution control federation (1995). Standard methods for Examination of Water and wastewater, 19th Edn. *Ameri. Pub. Hth. Assoc.* Washington D.C.

Aranilewa, ST; Salawu, SO; Sorungbe, AA; Ola-Salawu, BB (2005). Effect of Frozen Period on the Chemical Microbiology and Sensory Quality of Frozen Tilapia Fish.

Asghar, MS; Qureshi, NA; Jabeen, F; Khan, MS; Shakeel, M; Noureen, A (2015). Toxicity of zinc nanoparticles in fish: a critical review. *J. of Bio. and Environ. Sc*, 7 (1): 431–439.

David, M; Mushigeri, SB; Shivakumar, R; Philip GH (2004). Response of *Cyprinus carpio* (Linn) to sub-lethal concentration of cypermethrin: Alterations in protein metabolism profiles. *Chemos.* 56: 347-52.

FAO (1996). "Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". (*World Food Summit,* 1996)

Hao, L; Wang, Z; Xing, B (2009). Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in juvenile carp (Cyprinus carpio). *J. Environ. Sci.* 21:1459–66.

Hao, L; Chen, L; Hao, J; Zhang, N (2013). Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): A comparative study with its bulk counterparts. *Ecotox. and environ. saf*.

Hart, LJ (1996). Subacute Immunotoxic effects of the environmental contaminants 7, 12 dimethylbenzanthracene (DMBA), hexachlorocy clohexane (Lindane), 2, 3, 7, 8 – tetrachlorodibenzop – dioxin (TCDD) on spleen and pronephros celuarity and morphology and functiona acyivity of macrophages contained in these hematopoietic organs in the cichid tilapia fish (oreochromis nilotians). M.Sc. Thesis in the faculty of the Virginia – Maryland Regional

College of veterinary Medicine, Virginia Polytechnic Institute and State University.

Kaya, H; Fatih, A; Mert, G; Sevdan, Y; Mehmet, A; Veysel, D; Zikri, A (2015). Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreochromis niloticus*) *Environ. Tox. Pharm.* 40(3): 936-947.

Lopes, C; Gassanova, S; Delabar, JM; Rachidi, M (2001). The cask/lin-2 Drosophila homologue, camguk, could play a role in epithelial patterning and in neuronal targeting. *Biochem. Biophys. Res. Commun.* 284 (4): 1004-1010.

Powers, DA (1989). Fish as model systems. *Sc.* 246:352–358.

Li S, Canas-Carrell, JE; Irin, F; Atore, FO; Green, MJ (2013). Determination of multi-walled carbon nanotube bioaccumulation in earthworms measured by a microwave-based detection technique. *Sc. of Total Environ.* 445–446, 9–13.

Li, S; Wallis, LK; Ma, H; Diamond, SA (2014). Phototoxicity of TiO2 nanoparticles to a freshwater benthic amphipod: are benthic systems at risk? *Sc. of Total Environ.* 466–467, 800–808.

Shalini, S; Dorstyn, L; Wilson, C; Puccini, J; HO, L; Kumar, S (2012). Impaired antioxidant defense and accumulation of oxidative stress in caspase-2-deficient mice. *Cell Death Differ* 19: 1370–1380.

Shobha Rani, VR; Venkateshwaralu, P; Janaiah, C (1991). Impact of sublethal concentration of Malathion on certain aspects of metabolism in freshwater fish *Clarias batrachus* (Linn). Comp. *Physiological. Ecol.* 15(1): 13-16.

Simpson, JA; Behan, PO; Dick, HM (1976). Studies on the nature of autoimmunity in myasthenia gravis: Evidence for an immunodeficiency type. *Annals of the New York Acad. Sci.* 274: 382-389.

USEPA (1996). Ecological effects test guidelines OPPTS 850.4200 toxicity test. *United States Environ. Protec. Agency* EPA 712-C-96-154.

WHO; UNICEF (2010). World Health Organization and United Nations Children's Fund. Progress on sanitation and drinking water.

Yoshikawa, T; Naito, Y (2002). What is Oxidative Stress? *J Japan Med. Assoc. 45*(7): 271-276.

Zhang, X; Hongwen, S; Zhang, Z; Niu, Q; Chen, Y; Crittenden, JC (2007). Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chem.* 67: 160–6.

Zimmermann, YS; Schäffer, A; Hugi, C; Fent, K; Corvini, PFX; Lenz, M (2012). Organic photovoltaics: potential fate and effects in the environment. *Environ. Int.* 49: 128–140.