ACUTE COMBINED EFFECT OF ALUMINIUM (AL_2O_3) AND ZINC (ZNO) OXIDE NANOPARTICLE ON SUPEROXIDE DISMUTASES (SOD) AND MALONDIALDEHYDE (MDA) ACTIVITIES OF *CLARIAS GARIEPINUS* EMBRYOS

BY

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DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A DISSERTION SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC) DEGREE OF ANIMAL AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

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CERTIFICATION

This is to certify that this project work was carried out by Blessing Ofure ASUELIMEN, in the Department of Animal and Environmental Biology, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated to God almighty for His grace, wisdom and strength to finish well.

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My sincerest gratitude goes to my parents (Mr and Mrs. Anthony Asuelimen) and my older siblings; Mrs Judith, Perpetual, Jennifer, Benjamin, thank you for your immerse support towards me. God bless you

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ABSTRACTS

The issue of water pollution by nanometals in aquatic bodies has become of increasing concern since the rapid development of metal nanoparticles (NPs). Despite the usage of these nanoparticles, they also have negative impacts on fish. This study sought to assess the acute combined effect of Aluminium and Zinc Oxide nanoparticles on Superoxide Dismutase and Malondialdehyde activities of x catfish (*Clarias garienpinus*) embryo/lavae The Organization for Economic Development (OECD) Fish Embryo Acute Toxicity Test (OECD 236) was employed. Superoxide Dismutase and Malondialdehyde activities were assessed using standard procedures. Fertilized embryos were exposed to different concentrations of Aluminium and Zinc Oxide nanoparticles (0, 0.5, 1, 10 µg/L) for 48 h. The results showed a significant ($p < 0.05$) dose-dependent decrease in MDA and SOD activities in fish embryo/larvae, indicating that nanoparticles-induced a significant reduction in MDA and SOD activities. The findings suggest that Aluminium Oxide and Zinc Oxide nanoparticles could potentially impact the MDA and SOD activities of fish which could invariably affect the survival of aquatic life, especially catfish.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Water pollution with heavy metals and nanoparticles affects various physiological processes in fish, including breeding and development. The effects of waterborne metals on fish are related to their uptake and accumulation by the organism, resulting in metal-induced disturbances in the structure and function of various tissues and organs (Jezierska and Witeska 2001). Early developmental stages of fish, including when the embryos are protected by the eggshell, are particularly sensitive to intoxication. Since African catfish are easier to breed artificially at all stages, it is important to carry out research the effects of these nanoparticles on the physiological and morphological features of their embryo (Sopinka, 2016).

Currently, the potential toxicity of nanoparticles in biological systems is becoming a public concern. Nanomaterials may constitute a new source of environmental pollutants, and research is being focused on the potential negative impact they could produce (Moore, 2006; Shah & Mraz, 2019). Owing to its extremely small size, nanoparticles can penetrate through the cell membranes and cause genotoxicity. The intrinsic chemical reactivity of nanomaterials results in higher production of reactive oxygen species and free radicals, and its production is one of the main toxicity mechanisms of nanoparticles. This may produce not only inflammation and oxidative stress but also damage to proteins and DNA. It has also been demonstrated that nanomaterials have the potential to produce DNA mutation and major structural damage to mitochondria, which could even result in cell death (Majumder & Dash, 2017; Meghani *et al*., 2020; Vicari *et al*., 2018).

The investigation of the proper biomarkers for the best possible diagnoses is very important for researchers. Environmental experiments involving the use of biomarkers are recognized as one of the most powerful tools for the investigation of pollutants (Depledg, 1993)

Biochemical biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to toxic substances (Adams, 2002) They are of great toxicological relevance due to their fast response presented to tests of lethal contamination doses at the lowest biological organization levels, i.e, biochemical/cellular responses (Roach, 2021). They are means to assess the extent of damage induced in fish by the contaminants and determine how these affects the fish survival as well as providing information about effects at higher levels of biological organizations.

The genotoxicity observed with Al_2O_3 NPs may be due to pro-inflammatory effects through a reactive oxygen species (ROS)-mediated mechanism. The increase in myocardial SOD and CAT enzymes activities and GSH concentration in nano-alumina fish suggesting enhanced oxidative stress state in myocardium (Prabhakar, 2010). Adverse effects of ZnONPs can be mainly attributed to generation of ROS leading to membrane damage directly or indirectly. Heavy metals accumulated in the fish tissues may catalyze reactions that generate reactive oxidative species (ROS) which result in environmental oxidative stress.

Research on fish enzymes has demonstrated that antioxidant systems could provide relevant indices in explaining the sensitivity of some fish species to pollution (Di Giulio *et al.,* 1993). Antioxidants have a very sensitive role in maintaining cell homeostasis and, when these defenses are impaired or surmounted, oxidative stress products, namely reactive oxygen species (ROS), may induce DNA damage, enzymatic inactivation and peroxidation of cell constituents. Fish often increase the levels of protective antioxidants enzymes, as well as non-enzymatic free radical scavengers for preventing abnormality caused by ROS. Among the biomarkers of oxidative stress are Superoxide dismutases (SODs) and Malondialdehyde (MDA).

Superoxide dismutase (SODs) are essential enzymes that play a crucial role in the antioxidant defence systems of living organisms, including catfish embryos. These enzymes are responsible for neutralizing harmful superoxide radicals, which are highly reactive molecules produced during normal cellular processes and can cause oxidative damage to cells and tissues (kalef,2019). In African catfish embryo, superoxide dismutase serve as defense mechanisms against oxidative stress which can be induced by various environmental factors, including exposure to nanoparticles or other pollutants. The presence and activity of SODs in catfish embryos help maintain redox balance and protect vital cellular components from oxidative damage, thereby promoting healthy embryonic development. Understanding the role of SODs in catfish embryos is essential for comprehending the impact of oxidative stress and identifying potential ways to protect and conserve these important aquatic organisms in the face of environmental challenges.

Malondialdehyde (MDA) is a commonly used biomarker to assess oxidative stress in living organisms, including catfish embryos. MDA is a reactive compound formed during lipid peroxidation, which occurs when cellular lipids are exposed to oxidative damage by free radicals or other reactive oxygen species (Alderman *et al,* 2010)

In the context of catfish embryos, measuring MDA activities provides valuable information about the level of lipid peroxidation and oxidative stress experienced by these early life stages. Elevated MDA levels in catfish embryos indicate increased oxidative damage to lipid molecules, which can be a consequence of various environmental stressors, such as exposure to nanoparticles or pollutants. (Boxall *et al,*2004)

The study was therefore carried out to assess the acute combined effects of Aluminum Oxide and Zinc Oxide nanoparticles on superoxide dismutases (SOD) and Malondialdehyde (MDA) activities of catfish embryo as biomarkers for oxidative stress for nanometals exposure.

1.2 Aim and Objective

The aim of this study is to evaluate the combined effect of Aluminum Oxides and Zinc Oxide Nanoparticles on superoxide dismutase (SOD) and Malondialdehyde (MDA) activities of catfish (*Clarias gariepinus*) embryo

The objectives are to

1. Evaluate the combined effect of Aluminum Oxides and Zinc Oxide Nanoparticles on superoxide dismutase (SOD) activities of the catfish (*Clarias gariepinus*) embryo
2. Evaluate the combined effect of Aluminum Oxides and Zinc Oxide Nanoparticles on Malondialdehyde (MDA) activities of the catfish (*Clarias gariepinus*) embryo

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aquaculture and Nanoparticles

Aquaculture is becoming a much larger and more intensive industry with fewer farms, and this growth is expected to accelerate in the future (Naylor *et al*., 2000; 2003; Naylor and Burke, 2005). The share of the world supply of several species of fish, crustaceans and molluscs has increased from 3.9% of total production weight in 1970 to 33% in 2005. Equivalent to a serving of 16.7 kg (equivalent to live weight) per person. Of this, 47% comes from aquaculture.

Nanoparticles are particles with dimensions in the nanometer range (1 to 100 nanometers). Due to their small size, they often exhibit unique properties compared to their bulk counterparts. Nanoparticles find application in a wide range of industries including electronics, medicine, energy, material science, cosmetic and more due to their advantageous properties. In medicine nanoparticles can be engineered to deliver drugs directly to specific cells, reducing side effects and improving treatment efficacy. In electronics, nanoparticles can enhance the performance of displays, improve the efficiency of batteries and enable the development of smaller and more powerful devices. Nanoparticles can be used in the energy sector for improved catalysis in chemical processes and increased efficiency in solar cells. The cosmetic industries utilize nanoparticles for sunscreen that offer better protection while maintaining a more transparent appearance on the skin. Due to the surge in nanotechnology, there have been significant increases in the number of various NPs released into the aquatic environment. Aquatic ecosystems are susceptible to environmental contamination since they are at the receiving end of contaminants, particularly from runoff sources. Identified sources of NPs in the aquatic environment include production facilities, production processes, wastewater treatment plants, and accidents during transport. In addition, aquatic ecosystems are known to sequester and transport contaminants, including NMs. Baun *et al*. showed that NPs may adhere to algae which may then be consumed by filter feeders and transfer to higher trophic levels. In the aquatic environment, NPs may aggregate thus reducing the NPs available for direct uptake in the aqueous phase by aquatic organisms. However, aggregated NPs may settle into sediment thereby posing a threat to benthic organisms. In the aquatic environment, NMs are generally associated with sediments. Sediments and soil represent porous environmental matrices which typically have large specific surface areas.

2.2 Environmental implications of nanoparticles in the environment

Manufactured nanomaterials, which are released in the environment, are most likely to get accidentally in contact with a human organism by aspiration (in the cases of aerosols and fine solid nanoparticles), by direct contact with the skin and eyes or via the GI tract (by consuming contaminated water or food). Inhaled nanoparticles are usually captured by alveolar macrophages, but also may cross the alveolar–blood barrier and can be found to be distributed to the liver, heart, spleen, and brain (Shinde *et al*. 2012). Some nanoparticles may cross the GI, blood barrier and travel to distant organs (liver, kidneys, brain, lung, and spleen) via blood circulation (Choi *et al*. 2010; Shinde *et al*. 2012).

Nanoparticles also may affect microorganisms, plants, or animals, if released in the environment in significant quantities. Thin coatings, immobilized nanoparticles on surfaces, or nanomaterials embedded in solid matrices (nanocomposites) and nanoporous membranes may not pose a great health risk, but free nanostructures (of various shapes, spheres, polygonal, tubes, rods, prisms, cubes, etc.) could be toxic (Bystrzejewska Piotrowska *et al.* 2009). Among them, carbon nanostructures, oxide, and metal nanoparticles make the majority of nanoparticles in use. The potential toxic effects of these nanomaterials, as well as nanomaterials containing toxic heavy metals (such as Cd-based semiconductor nanoparticles), are briefly considered in this chapter. It should be taken into account that chemically inert nanoparticles may be capable of entering living cells by endocytosis and accumulate there, thus disturbing the intracellular functions and causing toxicity. Nanoparticles that adhere to the cell membrane may disturb its structure and function, while nanotubes and other nanostructures with long aspect ratio may pierce cell membranes.

Apart from the effects of nanoparticles in the biological and physiological make-up of aquatic organisms, Nanoparticles have been known to :

reduce fish production by causing oxidative stress in the fish thereby leading to a reduction in the fish production lead to high mortality rates in the fish, due to high oxidative stress of the fish as a result of the presence of nanoparticles, most organisms could not survive the impact thereby lead to high mortality rate and which can decrease fish yield

Economic loss: with the reduction of fish yield can negatively results in the loss of the fish farmer. There is a high potential risk of an increase of nanoparticles in aquatic habitats even in the wild, where they are affected by abiotic factors, such as temperature, pH, and ionic strength. Those agents alter the chemistry of NPs and influence their fate in the environment. Furthermore, Nanoparticles tend to quickly aggregate in the water column through sedimentation, which promotes exposure of benthic organisms to this type of compounds (Załęska-Radziwiłł *et al*). Single and combined exposure of aluminum and zinc oxide nanoparticles has been shown to cause sublethal effects in the freshwater fish, Carassius auratus, which is evident by the alteration of antioxidant defense system in gill and liver tissues. In another study, Al_2O_3NPs has been demonstrated to induce cytotoxicity and oxidative stress in dose-dependent manner. The uptake and accumulation of nanometals in fish involve complex processes influenced by various factors. When Nanometals are present in water fish can absorb them through their gills and skin, ingestion of food containing nanoparticles.

Once inside the fish, Nano metals can accumulate in different tissues and organs. This accumulation is influenced by factors such as metals physiochemical properties, the fish's species, size, age and metabolic rate. Certain metals might be more likely to accumulate in specific organs. Silver nanoparticles in zebrafish can accumulate these particles in their tissues, including the gills, liver and brain. The accumulation of silver nanoparticles has been observed to affect their behavior, metabolism and oxidative stress responses. Copper nanoparticles in rainbow trout can cause increase in copper accumulation in their gills and liver. This accumulation can lead to impaired gill function and oxidative stress, affecting the fish's ability to respire and potentially impacting their overall health. Gold nanoparticles in tilapia showed an accumulation of these particles in their gills, liver and intestine. The accumulation of gold nanoparticles was associated with changes in gene expression related to inflammation and cellular stress. Zinc oxide nanoparticles in carp: there is the displayed accumulation of these particles in their gills, liver and muscles tissues. The accumulation of zinc nanoparticles led to an altered enzyme activities in the liver and oxidative stress responses. Common carp exposed to iron oxide nanoparticles exhibited accumulation of these particles in their gills, liver and intestines. The accumulation of Iron oxide nanoparticles was associated with histopathological changes in the gills and liver. In a study on Labeo rohita, a rise in total protein was found in chromium oxide(Cr_3O_4) and cobalt oxide(Co_3O_4) treated groups.35 When young salmons were exposed to silver nanoparticles (Ag-NPs), it was observed that there was an increase in stress molecules HSP 70 levels with increase in silver nanoparticle concentration. Na+/K+ ATPase inhibition on exposure to silver nanoparticles was observed in Salmon that increased with increase in silver nanoparticles (AgNP) concentration resulting in osmoregulatory failure. At an increased concentration of silver nanoparticles (Ag-NPs) (100 μg/L), necrosis of gill lamellae was observed and 73% of the organisms died.46 In juvenile zebrafish, the LC-50 was reached at a concentration of 1.78 mg/L for dissolved Cu and 0.71 mg/L for Cu-NPs showing that the metallic-NPs are more lethal than the soluble forms. It is very evident that nanoparticles (NPs) cause harmful effects such as Na+/K+ ATPase inhibition, oxidative stress and disruption of tissue elements.23 Mechanical effects of nanoparticles (NPs) on fishes began to be studied in the late 2000s. In fishes, it was observed that nanoparticles seldom excreted via kidney, but they were able to be eliminate with bile (Ahamed ,2011). Gold nanoparticles (Au NPs) because of their special properties, have wide applications in drug delivery, imaging and labelling and for diseases diagnostics like diabetes, cancer and Alzheimer. Experiments also revealed that gold nanoparticles (Au NPs) showed no toxicity to embryos of zebrafish when compared to the other nanoparticles (NPs) like silver, copper and platinum. Gold nanorods coated with cetyltrimethylammonium bromide-coated induced death and allowed down the embryonic developmental process, which included decreased body length, deformities in the tail, delayed eye, pericardial edema, as well as head and tail elongation development. However on the other hand, some scientists put forth their argument that gold nanoparticles (Au NPs) were harmful to fishes and their exposure to Au NPs caused lethality to embryo, developmental toxicity, neurotoxicity and immunotoxicity. Gold nanoparticles (Au NPs) (10 nm) showed movement throughout the body of zebrafish embryos. These NPs showed accumulation as clusters, the sizes of which were dependent on the concentration, however, their effects on the embryo development were not dependent on the concentrations. The resulting malformations due to gold nanoparticles (Au NPs) occurred as a result of random distribution of these particles during the development within the cells. Gold nanoparticles (Au NPs) were found to be lesser in toxicity when compared to silver NPs. Toxicity of gold nanoparticles (Au NPs) with positive charges (coated using N,N,N trimethylammoniumethanethiol) in development of fish embryos was studied. It was seen that exposure to positively charged gold nanoparticles (AuNPs) caused malformation in eye development as well as pigmentation resulting in behavioural changes and damages the nervous system. Overall, the observations showed that the surface charge and coating of the gold nanoparticles (Au NPs) are closely associated with their toxic effects in the zebrafish. Silver Nanoparticles (Ag NPs) are widely used in many consumer products like in medical imaging, clothing, antimicrobial agents and bactericidal applications. Silver nanoparticles (Ag NPs) induced that neurotoxic effects were observed during in vitro studies on zebrafish different from Ag+ ones. It was observed that silver ions hindered the development of the swim bladder, also resulting in several deformities whereas the effects of silver nanoparticles (Ag NPs) were much lower. The fish behaviour showed modification when exposed to light stimulus, where the PVP-coated NPs which were the smallest, showed hyperactivity whereas the PVP-coated NPs that were the largest, showed hypoactivity.Silver ions that were released by Ag NPs caused increased death and deformities. Silver nanoparticles (Ag NPs) also play a role in inhibition of cell differentiation with their interaction with acetylcholine.

The monovalent silver ion impairs neurodevelopment in zebrafish. Ag NPs concentration was mainly observed around the liver, in the interstitial tissue (between the intestine and the liver), in blood vessels on secondary ion mass spectrometry (SIMS) analysis. Transcription profiles revealed that both Ag NPs and AgNO3 affected the common pathways hence indicating similar targets like the phototransduction system. It was seen that pigmentation of the embryos was not affected, however deformation of the spine and the heart and yolk sac oedemas were observed in the embryos. Various effects on development were also observed. Silver nanoparticles (Ag NPs) pass through the chorion (an envelope that surrounds the developing embryo). Malformations during the embryo development when subjected to silver nanoparticles (Ag NPs) were also observed. Silver nanoparticles (Ag NPs) caused mortality and slowed down the hatching process as well as depleted the levels of glutathione. Titanium dioxide nanoparticles (TiO_2 NPs) cause early hatching, hence reduction in the normal duration is required for normal process of hatching. Studies on adsorption of titanium dioxide nanoparticles (TiO_2 NPs) onto zebrafish embryos was done and it was observed that it obturated chorion pore canals completely and the hatching rate is reduced. In silicon investigations have revealed the effect of titanium dioxide nanoparticles (TiO_2 NPs) synthesized in industries on the variation of accumulation of lipid and reactive oxygen species (ROS) in the developing embryos, providing an in depth in vivo cytotoxicity analysis at the molecular level. One of the studies appreciated the role of titanium dioxide nanoparticles (TiO_2 NPs) (size less than 25 nm) in the development of *Danio rerio* embryos and larvae when ingested. First nanoparticles were ingested by adding them to commercially prepared food and in another batch, the fishes were allowed to feed algae that were subjected to titanium dioxide nanoparticles (TiO2 NPs). At decreased concentrations, hatching was premature and their effects on young ones were not effective.

Metal oxide nanoparticles have the potential to affect the developmental as well as acute toxicity. Copper oxide nanoparticles (CuO NPs) exposure was found to cause unusual phenotypes like retarded epiboly and smaller eyes and head. Copper oxide nanoparticles (CuO NPs) and zinc oxide nanoparticles (ZnO NPs) were found to be more harmful to the embryos and the young fishes when compared to their respective salt. The studies on the potential toxicity on exposure of copper oxide nanoparticles (CuO NPs) on the fish embryos revealed that the produced reactive oxygen species (ROS) was generated by copper nanoparticles (Cu NPs) in a concentration-dependent manner. The effect of copper oxide nanoparticles (CuO NPs) on neuronal differentiation in zebrafish and their liver development indicated that even a exposure for a short-term to high doses of copper oxide nanoparticles (CuO NPs) resulted in neurotoxicity as well as hepatotoxicity in the larvae and embryos shown as slowed retinal neuronal differentiation and hepatic hypoplasia. Due to their special properties, the zinc oxide nanoparticles (ZnO NPs) are highly known for their antimicrobial property, transparency, efficiency of photocatalytic activity, increased isoelectric point biocompatibility. They find their applications in sunscreens, cosmetics, photonics, electrical appliances, ceramics etc. Exposure of zinc oxide nanoparticles (ZnO NPs) showed toxic effect on fish embryos as well as larvae resulting in slowed hatching, damage to the tissue, tail deformation, decreased larval body size at less concentrations and mortality of embryo observed at increased concentrations. Delay in hatching, skin ulcer and increased death of zebrafish were observed on treatment with zinc oxide nanoparticles (ZnO NPs).The harmful effects of zinc oxide nanoparticles (ZnO NPs), polyethylene glycol coated NPs (ZnO-PEG) as well as chitosan coated ZnO NPs (ZnO-CTS) on embryos of zebrafish were studied. Chitosan coated ZnO NPs (ZnO-CTS) and polyethylene glycol coated NPs (ZnO-PEG) were seen to show fewer deposition on egg surface and chitosan coated ZnO NPs (ZnO-CTS) resulted in an increased rate of survival in the embryos. Zn^{2+} ions are thought to be the main reason for the harmful effects of these zinc oxide nanoparticles (ZnO NPs), although their role is still debatable. Few scientists proclaimed that the role of Zn2+ ions is not fully responsible for the toxicities of zinc oxide nanoparticles (ZnO NPs), but the main toxicities are caused by zinc oxide nanoparticles (ZnO NPs) themselves. Conversely, the others concluded that Zn2+ released was the primary source of zinc oxide nanoparticles (ZnO NPs) toxicity17,60. However, more studies are still required to come to a generalized interpretation. Reactive oxygen species (ROS) that is produced by the zinc oxide nanoparticles (ZnO NPs), is also given as another potential explanation for its toxicity. A study on zebrafish embryos revealed that on exposure to zinc oxide nanoparticles (ZnO NPs), there was increase in ROS generation, subsequently there is upregulation, reduction in bcl-2/bax ratio, decrease in the membrane potential of mitochondria, release of cytochrome c into the cytosol as well as activation of caspases and caspase occurs, showing that zinc oxide nanoparticles (ZnO NPs) caused an increased ROS production, subsequently the apoptosis pathway is controlled by the caspases and mitochondria. Embryos of zebrafish mortality and induced malformations were observed as a result of exposure to nickel nanoparticles (Ni NPs). The intestine was seen to reduce in thickness in association with the nickel nanoparticles (Ni NPs). Though the nickel solution showed no effect, nickel nanoparticles (Ni NPs) (30, 60 and 100 nm) and soluble nickel were shown to affect the skeletal muscles. Hence toxic effect of Ni NPs (30, 60, or 100 nm) did not differ much from the soluble nickel. However, huge particles aggregates of Ni NPs (60 nm) showing a dendritic shape were especially fatal on skeletal muscles as well as intestine.

Hence, the configuration of Nickel (NPs, ion, or aggregates) is more important than its size. Few studies also examined the cause of toxicity of nickel nanoparticles (Ni NPs) in the larvae of zebrafish to determine whether nickel nanoparticles (Ni NPs) toxicity was due to itself or whether it was due to Ni_2+ ions that was released from the nickel nanoparticles (Ni NPs). The results of these studies show that the Ni^{2+} was primarily responsible for the toxicity of nickel nanoparticles (Ni NPs). Platinum nanoparticles (Pt NPs) exposed to zebrafish embryos resulted in a dose dependent decrease in heart rate and curvatures of the axis and delayed hatching. The platinum nanoparticles (Pt NPs) accumulation in embryos was seen at be at decreased concentration on comparison with the silver (Ag) and silver (Au) nanoparticles, because their minute size caused them to be retained in very less amount in the embryos. Iron oxide nanoparticles (Fe2O3 NPs) find their applications in biomedical field like targeting drug delivery, cell labelling, gene delivery and magnetic resonance imaging.The harmful effects of these NPs in the early stages of development of zebrafish were observed. It was observed that a concentration of 10 mg/L and greater was shown to initiate the developmental toxicity in these embryos, hatching delay, causing deformation and death.Aluminium nanoparticles (Al NPs) and aluminium oxide nanoparticles (Al_2O_3 NPs) have found their applications in optoelectronics and electronic industry, in biomedical field as well as in drug delivery. Aluminium nanoparticles (Al NPs) and aluminium oxide (Al_2O_3) bulk showed little or no toxicity at all to the zebrafish embryos and their larvae.

Oxidative stress results from the exposure of cells to aluminum oxide nanoparticles that lead to inflammation, oxidation of lipids followed by cell death (Ju-Nam and Lead, 2008). The large surface area of nanoparticles is one of the reasons behind the production of reactive oxygen species (ROS), hence the smaller the size of the nanoparticles the greater the oxidative stress induced (Gliga *et al.,* 2014; Mittal and Pandey, 2014). The induction of enzymatic and non-enzymatic antioxidants can occur as an adaptive onset of the redox defense system (Anjum *et al.,* 2014; Srikanth *et al*., 2014).

2.3 Effects of Oxidative Stress on Different Biological Processes in Fish

In the development of fish, upon exposure to contaminants, it even showed increase in gene expressions as well as capase 3 activity and the ability to produce apoptosis through the developmental processes of Zebrafish (Zhu *et al* , 2020). They affect the fish metabolism causing impairment of its metabolism which may result to both offspring quantity and quality reduction (Jezierska *et al*, 2016). Heavy metals often induce a delay in the hatching process, premature hatching, deformation and death of newly hatched larvae.

2.4 Effects of Oxidative Stress on the Physiology and Metabolic Processes of Fish

Stress, through the action of corticosteroids, may reduce immunocompetence by influencing lymphocyte numbers and antibody production capacity, and affects reproduction by altering levels and patterns of reproductive hormones that influence maturation (Jezierska *et al*, 2008). Stress may also alter metabolic scope in fish and affects growth which results in catabolic or gluconeogenic effect of corticosteroids. In juvenile Chinook Salmon (*Oncorhynchus tshawytscha*), the plasma concentration of cortisol and glucose increased due to handling stress which was applied to them repeatedly at 3 hour interval over 6 hour . It was also reported that acid stress along with application of handling stress can increase the plasma cortisol level and decrease the plasma sodium levels in juvenile *O. mykiss* (Barton *et al*, 2011). The plasma glucose, cortisol and chloride levels were markedly altered when *O. mykiss* were reared in both wild and hatchery conditions. Fish transport from one place to another also elevates plasma cortisol and, plasma glucose level, and also alters the balance of sodium and chloride ions in them. Plasma cortisol and blood lactate also elevate significantly when fish are exposed to progressive hypoxia (Hansen, 2016). There are various physiological effects of nanoparticles dissolved in ambient water of fish. These nanoparticles can cause respiratory toxicity, disturbances of trace elements present in tissues and inhibits sodium and potassium ion ATPas (Nikinmaa, 2007). Chronic exposure to sublethal level of “Fullerene aggregates” can result in oxidative stress resulting in inhibited growth in freshwater fish *C. auratus* (Heise, 2007). There are studies showing an increase in haemoglobin and red blood cell (RBC) count in blood of fish exposed to hypoxia. Hypoxia also effects on adenosine triphosphate (ATP) production leading to a reduced production of ATP. Exposure of freshwater O. mykiss to copper reduces the plasma concentration of sodium, potassium and calcium ions in fish and also results in increased secretion of mucus in gill, liver and kidney tissues (Lushchak, 2007). Fish gill morphology is also markedly affected by chemical and physical irritants in the surrounding water (e.g. various toxicants, extremes of temperature or pH). Histopathological studies have revealed gill lesions under such exposure which include changes in gill epithelium (lifting, necrosis, hyperplasia, hypertrophy, rupture), bulbing or fusing of gill lamellae, hypersecretion and proliferation of mucocytes, changes in chloride cells and gill vasculature (Zeng, 2016). Salinity changes in the ambient of fish can cause increased innate immune response and a depressed adaptive immune response in fish. Some fish anesthetics like benzocaine, 2-phenoxyethanol, MS-222 (Sandoz), metomidate, and carbon dioxide gas were also investigated in O. mykiss. A severe hypoxia developed with the cessation of breathing in deep anaesthesia which leads to a rise in the partial pressure of blood carbon dioxide, adrenaline concentration, and a fall in blood pH . The hematological parameters were altered when fish were orally administered with gum Arabic showing significant changes in thrombocyte count (Zhang, 2010). The hematological parameters along with micronuclei induction and the pathological marker enzyme activities were greatly altered when C. punctatus were exposed to thermal power plant effluents containing heavy metals like Fe, Cu, Zn, Mn, Ni, Co and Cr. The plasma cortisol during stress elevates and plays an important role in up regulating pathways involved in energy-substrate mobilization including gluconeogenesis and simultaneously down regulates energy demanding pathways including growth and immune function. Socially isolated D. rerio faces acute stress which can be evidenced physiologically by looking into the cortisol levels which is elevated in isolated fish compared to the grouped ones. Socially isolated fish also showed a decreased immune response compared to the grouped ones. A recent study has reported that cortisol level elevates when an adult naive D. rerio is in contact with a stimulus fish (predator or non-predator) as compared to fish housed in visual contact with the stimulus fish. One of the studies on D. rerio revealed that Na ion balance and hydrogen ion secretion is regulated during acid exposed to the fish (Richards, 2011).

It was showed that the transcript levels of a few antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and some stress proteins like (metallothionein, MT) varied in *Gadus. morhua* when exposed to various ambient oxygen concentrations (Olsvik *et al*, 2013). The mRNA levels of two key antioxidant enzymes (GPx and glutathione S-transferase, GST) in *Paralichthys olivaceus* was increased when exposed to a change in salinity in their ambience. Significant changes of antioxidant gene mRNA levels on heavy metal exposure to fish also disclosed the stress responsiveness of the fish. It was also showed that MT is a stress protein consisting of a sulfhydryl group which is mostly involved in heavy metal homeostasis and detoxification. In *C.auratus* and *salmo trutta*. It was reported that the presence of Cd and Zn in the ambient water significantly increases the mRNA levels of MT, GPx, SOD, catalase and glutathione reductase (GR) in a dose and time dependent manner (Hansen *et al*. and Choi *et al*, 2017). It was also observed that when D. rerio were exposed to a very common pesticide called atrazine at various concentrations, there was a significant increase in the mRNA levels of SOD, catalase (Jin *et al*, 2010). When a fish was exposed to Cd for 24 hour, it promoted enhanced expression of mitochondrial succinate dehydrogenase activity and PGC-1α suggesting Cd induced stress involvement of PGC-1α in fish and it may be associated with mitochondrial function . At low ambient temperature, expression of senescence associated β-galactosidase, lipofuscin and adenosine diphosphate (ADP) ⁄ATP ratio were reduced compared to those reared at high and moderate temperatures, whereas catalase activity, Mn-superoxide dismutase activities, mitochondrial membrane potential and the levels of ATP, ADP, sirtuin 1 (SIRT1) and Forkhead box O expression were elevated. It can be said that cellular metabolism, energy utilization and gene expression are altered at lower ambient temperature, which is associated with the extension of lifespan of the annual fish (Zhang,2010). In a study of salinity stress in *Scophthalmus maximus*, AMPK α1 and α2 genes could be detected in all tested tissues indicating that they are constitutively expressed and significantly altered the gene expression levels of AMPK α1 and α2 mRNA in gill tissues, thereby suggesting that AMPK α1 and α2 played important roles in mediating the salinity stress in S. Maximus. It is observed that ability to reduce metabolic rate in a fish exposed to hypoxic condition is an important component to enhance survivability resulting in reduced energy expenditure. It is studied that hypoxic state leads to significant lowering of ATP production in the cells of the fish to enhance their survivability (Zeng,2011).

2.5 Aluminium Oxide and Zinc Oxide nanoparticles on Catfish Embryo

Most paths of metal toxicity to fish were described for juveniles and adults, but they probably apply also to the embryos as many of their metabolic processes are similar to those in older fish (Cavas 2008)). The main mechanisms of the toxic action of heavy metal nanoparticles like Aluminum and Zinc Oxides are related to the osmotic disturbances and alterations of enzyme synthesis and activity (Vosyliene 2006)). Pro-oxidative properties of these nanoparticles may result in oxidative stress in fish and oxidative damage to the cell membranes. For example; Cadmium, copper and lead also exert a genotoxic effect on fish (Bagdonas and Vosyliene 2006). Detailed investigations revealed very high individual variation in susceptibility of embryos to intoxication: some of them died at early developmental stages, some showed morphological anomalies (part of them failed to hatch) and some developed correctly and hatched as normal larvae (Cavas *et al*. 2005). (Jezierska *et al.* 2005) Studies of the effects of exposures to various concentrations of metals (Cd and Cu) during the entire embryonic period or at certain developmental stages showed that metals induced body malformations and mortality, and the effects were concentration-related. Thus, we can presume that heavy metals in the polluted waters, even at lower concentrations, exert similar effects, but the portion of affected individuals is lower.

(Jezierska *et al*, 2005) after fertilization, the Aluminum and zinc oxide nanoparticles were introduced at different concentration to test for reactions of the fertilized eggs to toxicity. The effects of metals on developing eggs include the increase in frequency of body malformations of embryos. Studies has shown that some results could include; blastomeres unevenness and irregularly distributed, and the entire blastula maybe deformed. Nanoparticles affected the embryos also at the stage of organogenesis. Most commonly observed malformations included craniofacial anomalies, yolk sac malformation, vertebral shortening and curvatures, and cardiac malformations . Metals also caused damage to the blood vessels and hemorrhages

(Jezierska and Słomin 1997; Jezierska and Gorzynska 1998; Słomin 1998; Ługowska 2005).

Various authors have observed similar teratogenic and mutagenic effects of metals on fish embryos. Speranza *et al*. (1977) reported that the blastodisc in Zn-exposed catfish eggs showed abnormal protoplasmic protrusions and in several cases appeared as a crenated disc on top of the yolk. Sarnowski and Jezierska (1999) reported malformations of *Ctenopharyngodon idella* embryos exposed to lead: the blastomeres differed in size and were irregularly distributed, single cells were detached, and the entire blastula was deformed. According to Meinelt and Staaks (1992), damage may already be detected in early embryonic development, including irregular cell divisions, spine distortions and early elevation of the head and tail from the yolk.

Oxidative stress results from the imbalance between the production of Reactive Oxidative Species (ROS) principally from the mitochondria and the ROS buffering activity of some enzymes and some antioxidant molecules (Biller, 2014). Especially the mitochondria is the most sensitive to oxidative stress, thus causing an increase in SOD activities as a defense mechanisms in different aquatic organisms including fish (Tremblay *et al,* 2011). Aluminum oxide nanoparticles are discovered to trigger oxidative stress in aquatic organisms.

2.6 Challenges Faced in Accessing Nanometal Exposure

Many toxicology studies outline NPs as a potential environmental risk, and the importance of understanding the fate and exposure routes of NPs has been stressed (Sweet and Strohm 2006;

Klaine *et al*. 2008; Colvin 2003; Maynard *et al*. 2006). The lack of knowledge is often highlighted in these toxicological studies; production volumes, emissions to the environment, environmental fate and exposure, and toxic effects are stated as highly uncertain. Issues related to fate and exposure modeling of NPs in water are addressed in this toxicological study. Previous exposure and risk assessments of NPs have not modeled fate processes at the nano level, but at much higher system levels (Mueller and Nowack 2008; Boxall *et al*. 2007). Consequently, NPs are treated as bulk material and material flow analysis is applied, without acknowledging the particulate nature of the material.

2.7 Mitigation Measures On Nanoparticles Pollution

Some effective methods includes;

Filtration and separation Technologies: utilize advanced filtration systems such as membrane filtration, electrostatic precipitators and activated carbon filters to capture nanoparticles from waste water and water bodies. Nanofiltration membranes with specific pore sizes can effectively remove nanoparticles while allowing other substances to pass through. A few types of water filters are effective for removing nanoparticles are carbon filters, reverse osmosis and UV filters and the most effective of them all is carbon filter (deLoach, 2022). It was proven that the polyvinylidene fluoride microporous membrane could be used as a low-cost and environmentally stable adsorbent to capture NPs from water (Jiang *et al*). Using Au-NPs and Ag-NPs as models, they studied four industrial microporous membranes, namely, polyvinylidene fluoride, nylon, mixed cellulose ester, and polyethersulfone, using intermittent adsorption experiments. The polyvinylidene fluoride microporous membrane showed the best removal efficiency . Moreover, this membrane can be used as a filter for the continuous treatment of water samples contaminated with Au-NPs, Ag-NPs, ZrO2-NPs, ZnO-NPs, Fe2O3-NPs, and NiO-NPs (Jiang *et al*). Also, Encourage the development of environmentally synthesis methods for nanoparticles, reducing the generation of harmful byproducts. Employ bio-based or plant-mediated synthesis approaches to minimize toxic waste during nanoparticle production. Implementation of stringent regulations on the production, usage and disposal of nanoparticles to prevent uncontrolled release into the environment (EER, 2021). Establish standard foe nanoparticles concentrations in water bodies, air and soil to ensure safe levels. Promote the recycling of nanomaterials to reduce then Demand for new nanoparticles production and minimize the release of pollutants. Develop efficient methods to recover nanoparticles from industrial wastes and electronic devices for reuse (EER, 2020).

Risk assessment and monitoring: conduct through risk assessments to understand the potential impacts of nanoparticles on environment and health. Regularly monitor the nanoparticles concentrations in different environmental compartments to detect pollution and assess the effectiveness of mitigation strategies (Zhang, *et al*, 2017).

Sustainable Nanotechnology: Encourage research and development of sustainable nanotechnologies that prioritize minimal environmental impact and responsible nanoparticle management.

Public Awareness and Education: raise awareness among industries, researchers, and the general public about the potential risks associated with nanoparticles and the importance of responsible usage and disposal.

Nanoparticle Stabilization: develop strategies to stabilize nanoparticles in various products and applications, reducing the likelihood of their discharge into the environment.

Natural Remediation Processes: investigate the potential of natural processes such as microbial degradation and plant uptake to remove nanoparticles from the soil and water.

Collaboration and Research: foster collaboration between researches, industries and government bodies to develop effective and innovative methods for nanoparticle pollution mitigation and remediation

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Apparatus

Four plastic fish tanks (chemically inert) with suitable capacity. Inverted microscope and/ or binocular with a capacity of at least 80-fold magnification. Test chambers; eg standard twenty-four well plates. Self-adhesive foil to cover the well plates. Incubator or air conditioned room with controlled temperature, allowing to maintain standard temperature in the wells, PH meter Oxygen meter, Equipments for determining the hardness of water and conductivity

Spawn trap: instrument trays of glass, stainless steel or other inert materials to protect the eggs once it is laid; spawning substrate.

Pipettes with widened openings to collect eggs. Glass vessels used to prepare different test concentrations and dilution water (beakers, graduated flasks, graduated cylinders and pipettes) to collect catfish eggs.

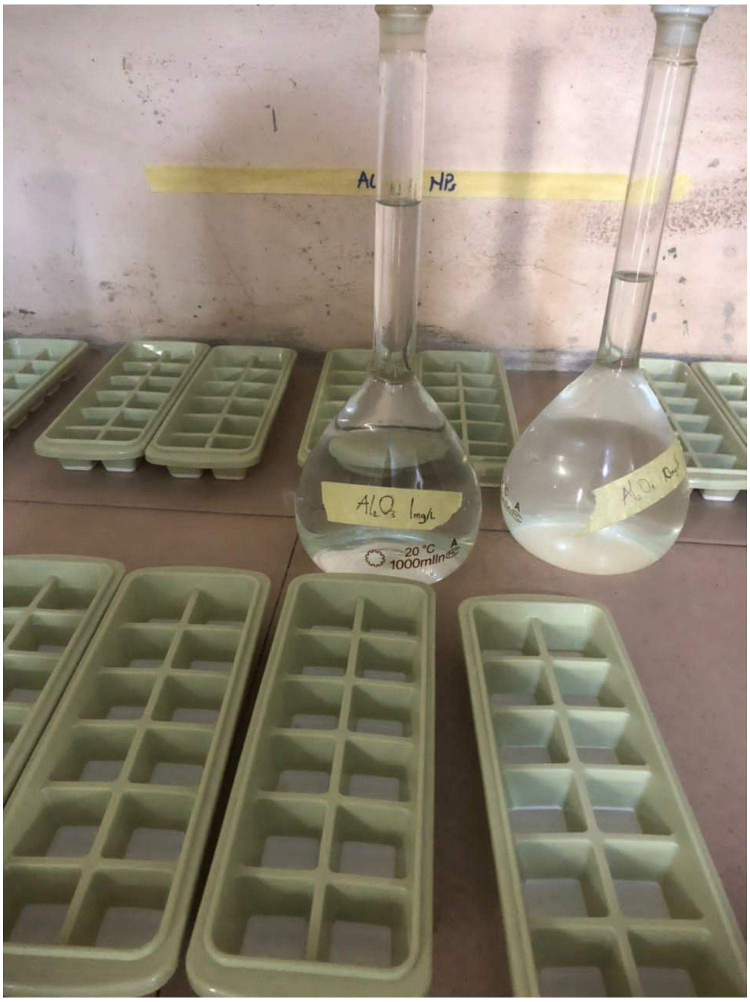

Plate 3. 1: Well plates for the acute toxicity test

3.2 Methods

3.2.1 Fish Selection and Hormonal Induction

A total of 4 healthy 12-month-old adult catfish (Clarias gariepinus) of both sexes (body weights ranged from 2.1 to 4.3 kg) were obtained from a commercial aquaculture facility (Agricultural-Farm Project, University of Benin) and kept in an aquarium system with water temperature (27 ± 2 ◦C). The animals were subjected to a light/dark cycle of 12/12 h respectively and were fed with Durante commercial feed, once times a day for two weeks.

Females were induced to spawn using 0.5 ml kg–1 Ovulin hormone.

Plate 3. 2: female fish(C. gariepinus) in a tank

The eggs were obtained by stripping the mature female 9 h after induced ovulation at 26 ◦C.

The eggs were obtained by stripping the mature female 9 h after induced ovulation at 26 ◦C.

Plate 3. 3: The process of stripping in fish

After stripping the male sperm sac was extracted and then was inseminated to the eggs and was kept in saline solution to maintain natural environment for fertilization.

3.2.2 Embryo Acute Toxicity Test

Embryo-larval acute toxicity test was carried out using the protocol according to OECD guideline 236-fish early life stage toxicity test (OECD, 2013). Fertilized eggs were randomly transferred into three 24 well-plates. Each well contained twenty (20) embryos. Catfish embryos were exposed to 0.0, 0.5, 1.0, 5.0 and 10 µg L− 1 of Aluminium Oxide and Zinc Oxide nanoparticles in 5 ml of fish water with three replicates per concentration. The control groups contained only distilled water. The fish embryos were exposed to a static renewal test with the test solutions renewed every 24 h (Nagel, 2002). The water quality parameters of the experimental water were determined according to APHA (2005) and mean values ranged from 25.20 to 27.00° C (temperature), 5.60–6.40 (pH), 10–20 µs cm–1 (Electrical conductivity), 6.00–6.50 mg L–1 (dissolved oxygen), 10 mg L–1 (total hardness), 0–0.06 mg L–1 (salinity), 8–60 mg L–1 (total suspended solid).

3.3 Determination of MDA Concentration

The concentration of MDA was determined according to the method of Guttridge and Wilkins (1982), a modification of the procedure used by Placer, *et al*., (1966). The principle that underlies this assay is that MDA – a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in the presence of an acid, forms a pink or reddish complex that is measured spectrophotometrically at 532 nm.

3.3.1 Calculation of MDA Concentration

The MDA concentration of each sample was calculated as shown

$$\frac{O.D \times V_t \times 1000}{a \times V \times L \times Y}$$

where,

O.D = Absorbance of sample test at 535 nm

V_t = Total volume of the reaction mixture

a = Molar extinction coefficient of the product

L = Light path = 1.0 cm

V = Volume of sample homogenate used

Y = mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

3.4 Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in an aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O^{2-}). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of enzyme activity.

Calculation

$$\%\ Inhibition = \frac{O.D_{test} - O.D_{reference}}{O.D_{test}} \times \frac{100}{1}$$

$$Enzyme\ Activity\ (units/mg\ protein) = \frac{\%\ inhibition}{50 \times Y}$$

Where Y = mg of protein in the volume of the sample.

A unit of SOD activity was taken as the amount of SOD required to cause 50 % inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

3.5 Statistical analysis

Statistical and graphic analyses were carried out using Excel. Data for MDA activities and SOD activities were presented as means ±SE based on replicates results.

CHAPTER FOUR

4.0 RESULTS

4.1 MDA Activities of *Clairas garienpinus* Embryo

The catfish embryo exposed to ZnO and Al_2O_3 nanoparticles demonstrated a decrease in the malondialdehyde activities (Fig. 1). MDA levels in the control group was higher than that for 0.5mg/L, 1mg/L, 5mg/L and 10mg/L Mean MDA concentrations were 0.066, 0.082, 0.076 and 0.059 nmol.mg^{-1} in the control, 0.5mg/L, 1mg/L, 5mg/L and 10mg/L respectively

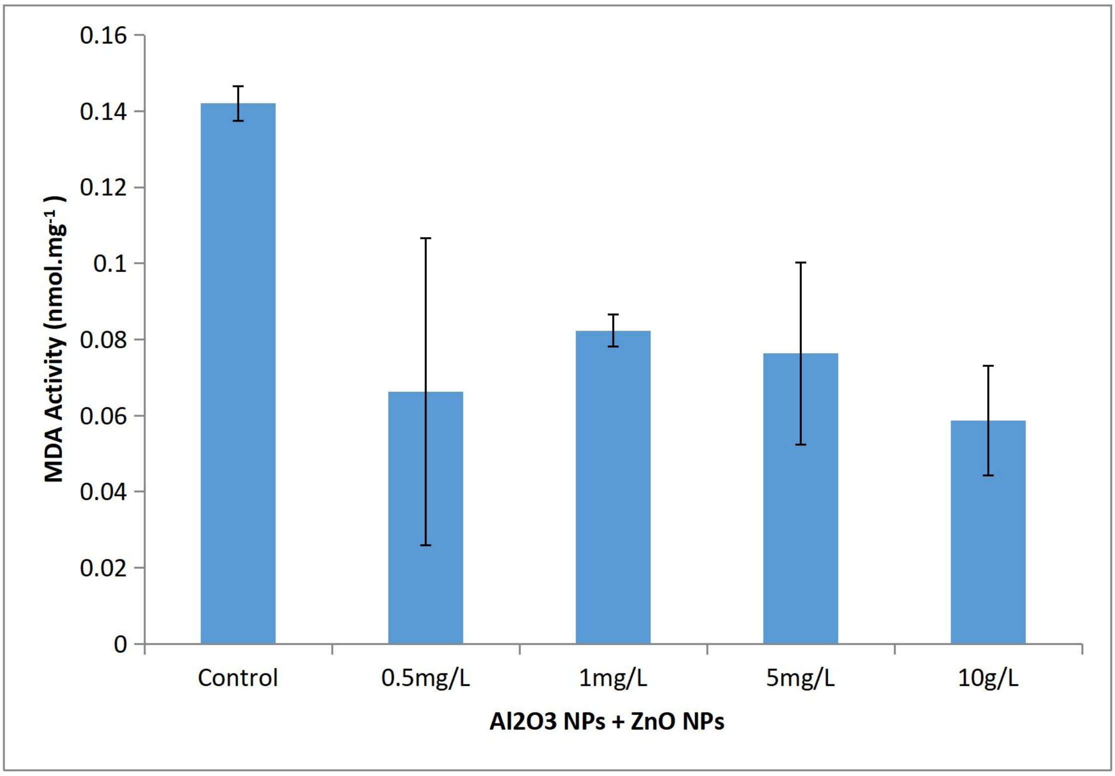

Figure 4. 1: Mean MDA activities of *Clairas gariepinus* embryo exposed to the combined mixture of Aluminium Oxide and Zinc Oxide Nanoparticles

4.2 SOD activities of *Clarias garienpinus* Embryo in Nanoparticles

The catfish larvae/embryo exposed to combined mixture of Aluminium Oxide and Zinc Oxide Nanoparticles

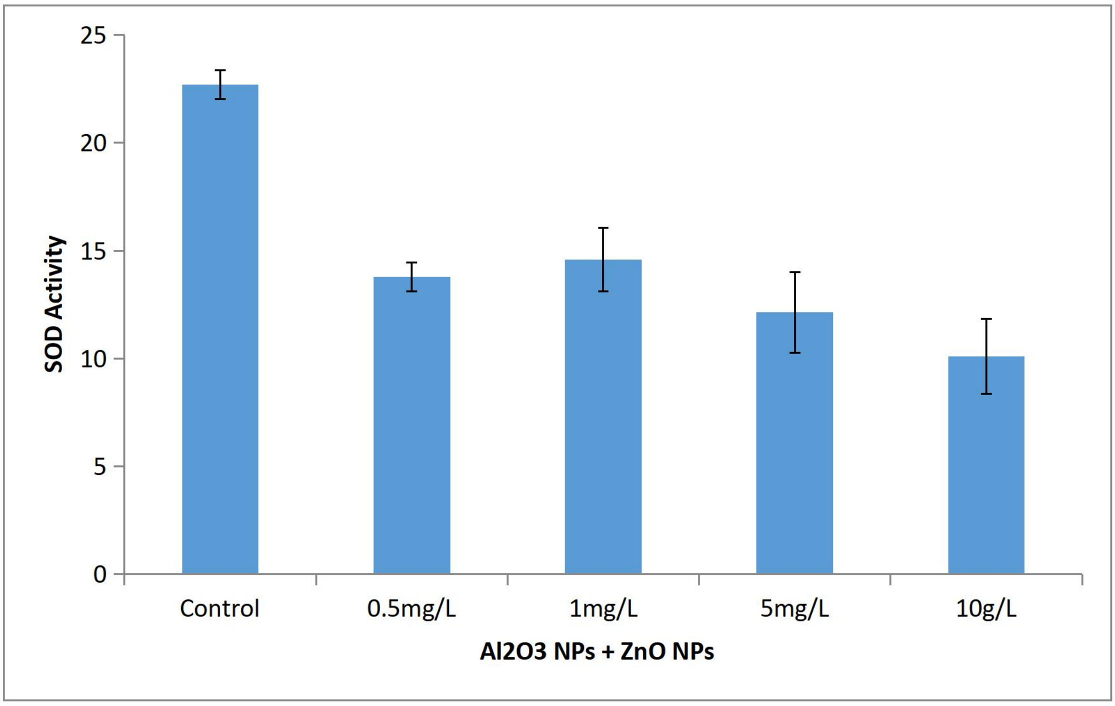

Figure 4. 2: Mean SOD activities of *Clairas gariepinus* embryo exposed to the combined mixture of Aluminium Oxide and Zinc Oxide Nanoparticles.

CHAPTER FIVE

5.1 DISCUSSION

The emergence of nanoparticles in water bodies are increasing by the day and because their impacts are high on aquatic organisms including fish are high, the need for alarm is necessary (EER, 2021). This present study reports the acute toxicity on Aluminium Oxide and Zinc Oxide nanoparticles on the SOD and MDA activities of the African Catfish embryo/larvae (Cliaras gariepinus). Reactive oxygen species (ROS) were produced in cells or tissues as a result of nanoparticle exposure, which has been well-documented (chitra *et al*, 2018). Several variables, including internalisation of nanoparticles, size and surface area, solubility, aggregation, agglomeration, bioconcentration, and biomagnification, influence ROS formation. Exposure to high concentration of nanoparticles for a period of time cause an increase in oxidative stress (Zhu *et al*, 2013). Single and combined exposure of aluminium and zinc oxide nanoparticles has been shown to cause sublethal effects in the freshwater fish. This study reveal that upon exposure to Aluminium and Zinc Oxide nanoparticles resulted in the increase in oxidative stress of the catfish embryo/larvae. Exposure of catfish embryo/larvae to Aluminum Oxide and Zinc Oxide nanoparticles for 48hrs caused decrease in the activities of the antioxidant enzymes such as Superoxide dismutase, catalase and Malondialdehyde. SuperOxide dismutase is the first set of defensive enzymes against oxidative stress(Lammer *et al*, 2009). The decrease in the SOD activities could be caused by the generation of excessive Relative Oxygen Species (ROS) which is as a result of the exposure to aluminium oxide and zinc oxide nanoparticles. The total depletion of SOD upon longer exposure could result into the depletion of other antioxidant enzymes like MDA and catalase (Bachman, 2002). Research investigating fish enzymes has uncovered that the antioxidant system could serve as environmental indicators of pollution (Di Guilio, 1993). Antioxidant play an important role in maintaining balance in cellular level and when these defenses are reduced, it can results in the production of reactive oxygen species (ROS) which can lead to DNA damage, enzyme deactivation and peroxidation of cellular component (van der Oost *et al*. 2003). Thus, this study reflects on the nanotoxicity of Al2O3 +ZnO NPs on African Catfish (Clairas gariepinus) embryo/Larvae.

Enzymatic activities in response to Oxidative stress caused by ambient environment in aquatic ecosystem are suitable biomarkers for contamination in toxicological studies (Biller, 2014). MDA and SOD activities are important and useful as biochemical biomarkers in the assessment of toxicity (Zhu, *et al*, 2018). In this study, at different concentration of Al2O3 + ZnO NPs (0.5mg/L, 1mg/L, 5mg/L and 10g/L), for 48hrs, there was a decrease in the SOD and MDA activities of the embryo whivh signifies increase in oxidative stress and the decrease in thus SOD and MDA activities could result into apoptosis and eventually affect the developmental activities of the larvae. These results are similar with Jezierska *et al*, 2005 report on the oxidative stress for Aluminium Oxide and Zinc Oxide for Zebrafish such as reduced activities in the embryo at different concentration. In our studies it was noticed that the MDA activities reduction wasn't at a significant value at different concentration, we can deduce that the impact of these nanoparticles towards MDA activities isn't massive and it is similar to Jezierska *et al*, 2005 report on Zebrafish. The SOD activities were notably diminished following 48 hours of exposure to these nanoparticles (NPs), which is indicative of heightened oxidative stress and elevated NP concentrations. Moreover, given that SOD acts as the initial defense enzyme against oxyradicals, it can serve as an early indicator of oxidative stress in the context of environmental pollution. Imbalances in the antioxidant system have been linked to Al2O3 + ZnO NP exposure, as evidenced by prior studies (Mendonça *et al*. 2011; Xing *et al*. 2011; Diniz *et al*. 2013). . It can also be hypothesized that the observed decreasing trend in SOD is a consequence of exhaustion of the detoxification mechanisms (Jacobson and Reimschuessel 1998). It is also noteworthy in this study that the decrease in MDA activities could easily be interpreted as the negative impact of nanoparticles on oxidative stress. The results indicate that the NPs of metallic oxides, with the contribution of Zn ions, are a potential risk for the biota and aquatic ecosystem.

RECOMMENDATION

The use of proper remediation measures in cases of nanometal pollution should also be encouraged and education of people on proper waste disposal is of utmost importance.

CONCLUSION

The present toxicological studies reveal that nanoparticles resulted inare oxidative stress in catfish embryos exposed to a combined mixture of Al_2O_3 and ZnO nanoplastics as shown in the, decrease in the SOD and MDA activities. The study demonstrate that the toxicity of Al_2O_3 and ZnO nanoplastics are highly dependent on the concentration of the nanoplastics the fish is exposed to. This studies, therefore, provides an understanding of the possible environmental impacts of nanoplastics on the SOD and MDA activities of *Clairas garienpinus* embryos Further research is however encouraged.

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