Aquatic Science and Fish Resources

http://asfr.journals.ekb.eg

Print ISSN: 2682-4086 Online ISSN: 2682-4108

Evaluating the Effectiveness of Vitamins E and C in Mitigating the Toxic Effects of Zinc Oxide Bulk and Nanoparticles on Fish: A Review.

Amal S. Mohamed [1,2], Hala E. Ghannam[1], Hanan A. Soliman[2] and Adel A. Ellahamy[3*].

[1] National Institute of Oceanography and Fisheries (NIOF), Fresh Water Division, Egypt.

[2] Beni Suef University, Faculty of Science, Biochemistry Division, Egypt.

[3] Department of Fish Processing and Technology, Faculty of Fish Resources, Suez University, Egypt.

ARTICLE INFO

Article history:
Received Sep. 05, 2023
Received in revised form Oct. 27, 2023
Accepted Nov. 05, 2023
Available online Nov. 06, 2023

Keywords
Vitamins E And C
Antioxidants
Oxidative Stress
Chemical Composition
Aquatic Ecosystem.

ABSTRACT

Nanotechnology has noticeably developed with diverse applications in every science, especially using nanomaterial. The development of nanotechnology also hurts the environment as many nanoparticles are discharged into the aquatic environment and cause a serious effect on living organisms. Aquatic animals, particularly fish could serve as biological indicators for ecosystem health. The present study investigated the effect of vitamin (E + C) addition on fish exposed to nanoparticles. The previous studies on the impact of zinc oxide (bulk and nanoparticles) of fish in aquatic ecosystems showed that there were clear differences between the accumulation of zinc oxide bulk particles (ZnOBPs) compared to nanoparticles (ZnONPs). Fish exposed to ZnONPs showed a higher accumulation potency in tissues than in ZnOBPs. Also, the activities of antioxidant defense enzymes, biochemical parameters and proximate chemical composition were affected by exposure to nanoparticles. On the other hand, dietary supplementation with vitamins (E and C) resulted in protective effects against these toxic effects in fish.

1. INTRODUCTION

The contamination of the aquatic environment by toxicants has become one of the most important problems in the world**.** With the advancement of technology and increased industrial activity, the features of pollutants released into ecosystems, particularly the aquatic environment, become increasingly complicated, and their toxicity becomes more harmful **(Çavas and Ergene-Gözükara, 2005**).

Due to increased nanotechnology applications, many nano-metals such as (ZnONPs) are dumped into water environments, which alter their biota (**Isani *et al*., 2013).** Most of the investigations on the behaviour of ZnO-NPs in water systems have been conducted in the laboratory, focusing on freshwater. According to the available data, dissolution is concentration dependant, with the lowest dissolution percentage occurring at the highest ZnO concentrations (**Adam *et al*., 2015**). Biochemical parameters of fish blood have been found to change when exposed to environmental pollution and these parameters are extremely sensitive to these pollutants (**Luskova *et al*., 2002**). Oxidative

* * Corresponding author: **Adel A. Ellahamy**
E-mail addresses: adel.ammar@frc.suezuni.edu.eg

doi: 10.21608/asfr.2023.234468.1053

stress is one of the most often reported nanoparticles toxicity mechanisms **(Mocan *et al*., 2010).** As indicators of contaminants in various freshwater and marine organisms, many antioxidant endpoints have been suggested as their induction indicates a response to pollutants **(Borkovic´ *et al*., 2005**). Acute nanoparticle exposure has also been found to cause toxicity by increasing intracellular ROS (Reactive Oxygen Species) levels and lowering antioxidant responses (**Kaewamatawong *et al*., 2006; Singh *et al*., 2009)**. Vitamins and antioxidants are among the most significant nutrients that influence an organism's immune system, and their availability can help to minimise fish mortality and increase performance. Vitamin E is an effective antioxidant and anti-inflammatory agent that prevents the propagation of oxidative stress ,especially in biological membranes in animals and humans **(Al-Rasheed *et al.,* 2012).** Exposure of fish to nanoparticles induced a deleterious biochemical effects and oxidative stress while dietary supplementation with vitamins (E and C) resulted in protective effects against these toxic effects (**Mohamed *et al*., 2021**). Vitamin E and C as powerful antioxidants protect the fish against Ag-NPs except high dose level **(Iqbal *et al*., 2023).** Addition of vitamin (E and C) considered as an effective antioxidant that protects *O.niloticus* fish from the harmful effects of ZnOBPs and ZnONPs (**Ghannam *et al*., 2022**). Although, the role of vitamins against toxic effects in fish is relatively well documented, there is a lake of information available on the effect of vitamins (E + C) in Mitigating the toxic Effects of Zinc Oxide Bulk and Nanoparticles on fish. So, this work aims to study the role of vitamins (E + C) addition on fish exposed to ZnONPs and ZnOBPs.

2. Pollution of aquatic environment:

Excessive pollution of aquatic habitats has sparked severe environmental and health issues around the world (**McNeil and Fredberg, 2011)**. According to World Health Organization **(WHO, 2011)**, pollution of water happens when undesirable elements enter the water and alter the quality of the water. (**WHO**, **2011**). Waterborne infections account for 80% of all diseases. Water that is unclean and of poor quality causes about 3.1 % of deaths **(Pawari and Gawande, 2015).** The pollutants may raise metal levels in natural water, posing a major threat to both freshwater and marine environments **(El Nemr *et al*., 2012).** Aquatic animals are exposed to nanoparticles via a variety of sources, including food, water, and sediment **(Vali *et al*., 2020)**. Because of the effects of chemicals on the aquatic environment, it is critical to study the impact and effects of these chemicals on aquatic life forms, as well as to monitor pollution and its influence on marine species. This knowledge will assist us in identifying sources of contamination, provide governing bodies with information useful in aquatic ecosystem management, information on which to base regulations concerning the use and chemical compound handling, and, ultimately assisting in protecting aquatic ecosystems from the negative effects of anthropogenic activities (**El-Gazar, 2012)**. Fish is a cheap source of protein, and in many places of the globe essential cash crops are used to assist them in their life tasks such as feeding, breeding, excretion, swimming, and digestion **(Bronmark and Hanson, 2005).** Fish can become agitated and susceptible to disease as water quality deteriorates **(ICAR, 2006).** The effects of NPs on aquatic species, and parameters such as NP characteristics and the presence of other environmental contaminants or stressors can influence NP behavior as described in figure 1 **(Trevisan *et al*., 2022)** .

3. Nanotechnology and Nanoparticles:

Nanotechnology is certainly one of recent century's most important technologies. Due to their nano-scale size, nanotechnology is concerned with materials and systems whose structures and components display

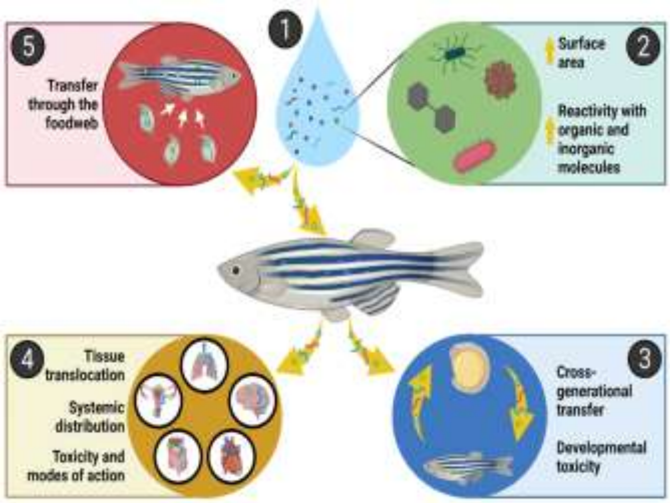

Figure 1. Fate and impacts of nanoplastics (NPs) in aquatic species. (**1**) Mixtures of NPs in the aquatic environment; (**2**) interactions of NPs with environmental molecules; (**3**) impacts of NPs on animal development; (**4**) internal distribution and toxicity of NPs; (**5**) transfer and potential biomagnification of NPs through the food web **(Trevisan *et al*., 2022)**.

innovative and greatly improved physical, biological, and chemical properties, phenomena, and processes **(Chen *et al*., 2012)**. Metal-based nanotechnologies are being more widely employed for environmental remediation and a variety of industrial activities; however, the toxicological effects of metal nanoparticles on aquatic ecosystems are still unintelligible **(Chen *et al*., 2012**). Nanotechnology and nanoparticle production are rapidly expanding, but research into the toxicological effects of nanoparticles on human health and the environment is still in its early stages **(Elsaesser and Howard, 2012)**.

Manufactured nanomaterials are described as particles with a diameter of 1 nm to 100 nm **(Rotello, 2003).** They possess enhanced or even distinctive physicochemical properties, such as nanoscale size effects, as well as unique electric, thermal, mechanical, and imaging properties not seen in bulk counterparts (**Andersson *et al.,* 2012**). In addition, due to a greater surface area per weight, nanoparticles (NPs) tend to be more reactive with other biomolecules and, consequently, more toxic **(Benn and Westerhoff, 2008)**. Increasing NP technology need and application in metal, biological and healthcare industries have been noted (**Jovanović and Palić, 2012**). This is why they are released into the environment, through the production, usage or disposal processes **(Paterson *et al.,* 2011)**, Aquatic ecosystems are their eventual destination, where they can interact with the biota **(Nohynek *et al*., 2007).**

Nanoparticles are the most important commodities in the domestic and industrial fields. Nanoparticles can enter into the bodies of fish and accumulate in tissues and other body organs. Fish is the main bioindicator to the toxic materials present in their environment. The nanoparticles by means of skin and gills enter into the circulatory system and are distributed throughout the fish bodies like brain, liver, kidneys and muscles **(Handy *et al*., 2008**).

4. Zinc oxide bulk and nanoparticles in aquatic environment:

Zinc oxide is commonly utilized in different areas of life due to its diverse properties, in chemical and physical aspects, it is crucial in a large number of applications, including tires, ceramics ,pharmaceuticals, agriculture, paints and some other chemicals **(Asghar *et al*., 2015)**. Zinc (Zn) is well-known for its importance in practically every aspect of biological systems, either directly or indirectly **(Shukla *et al*., 2007).** Zn is required by fish in a particular quantity for optimal growth, but excessive accumulation can be harmful to exposed organisms **(Gupta and Srivastava, 2006; Senthil Murugan *et al*., 2008).**

The fundamental distinctions between nanoparticles and their bulk equivalents are the high surface-to-volume ratio and the physicochemical, optical, reactive, and electrical properties that result. Furthermore, the behaviour, reactivity, and possible toxicity of nanoparticles are determined by the environment in which they exist (**Bian *et al*., 2011**). ZnONPs dissolve in water partially and are likely to contain species that are both soluble and particulate in aquatic systems, implying that these three systems of hazardous action were viable for ZnO. Solubilized Zn+ from ZnONPs has been demonstrated to play a major effect in the cytotoxicity of these NPs (**Heinlaan *et al.,* 2008**).

Because of their potential toxicity and widespread presence in consumer products and industrial pollution, metal oxide nanoparticles have been investigated in depth **(Melegaria *et al*., 2013).** After TiO2 and SiO2 NPs, ZnONPs have the third biggest global production among nanoscale metal oxides (**Piccinno *et al*., 2012**). Zinc oxide nanoparticles (ZnONPs) are a new type of highly functional fine inorganic materials having one dimension that measures 100 nanometers or less (**Elsammad *et al.,* 2014).** They have been widely used in cosmetics, sun screens, foot-care ointments, antimicrobials, food additives, cancer therapy, over-the-counter topical products (mouthwashes and anti-dandruff shampoos ,(fungicide, in paints, in photo electricity and rubber industry (**Guan *et al*., 2012).** Oral exposure can occur directly from food, water or orally administered drugs. (**Osmond and McCall, 2010)**. After gut absorption, they are transported to the blood causing adverse biological reactions in several organs. The major sites of interaction with ZnONPs are lung, liver, kidneys and heart **(Johnston *et al*., 2010).** The principal cause of toxicity was the released free zinc ions from ZnO NPs that partially dissolved in water but did so quite quickly or induced additional impacts **(Poynton *et al*., 2011**). The solubility of nano zinc oxide may have a greater impact on its toxicity. At least three different pathways could be involved in the harmful impacts of metal and metal oxide NPs (**Brunner *et al*., 2006**). First, particles, such as free Zn+ ions from zinc particles, may release hazardous chemicals into exposure media. Second, surface interactions with media can result in the formation of hazardous chemicals, such as chemical radicals or reactive oxygen species. Third, particles or their surfaces may directly interact with and damage biological targets, such as carbon nanotube interactions with membranes or DNA intercalation (**Ma *et al*., 2013**). Nanoparticles may travel from the water column to the aquatic food chain and be accumulated by organisms via internal or external exposure routes, hence it is critical to understand the biological behaviour of nano zinc oxide in the aquatic system **(Lee *et al.,* 2010).** Recent studies demonstrated the immunotoxicity induced by ZnO NPs **(Rashidian *et al.,* 2021)**, while, a safety side for the dietary inclusion of ZnO NPs was addressed in *O. niloticus* **(Mahboub *et al*., 2020; Ghazi *et al*., 2021)**. Although some disagreements found, ZnONPs toxicity mechanisms largely recognized include particle and dissolved free ion impacts **(Ma *et al*., 2013)**. ZnONPs were characterized by X-ray diffraction characterization (XRD, Panalytical X,pert pro PW 3040/60), as shown in Fig. 2 **(Mohamed *et al*., 2021)**.

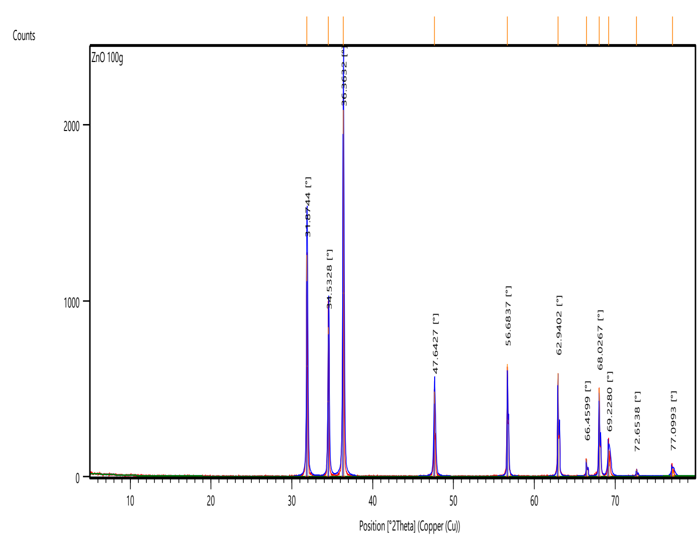

Figure 2. X-ray diffraction (XRD) pattern of ZnONPs as described in **Mohamed *et al*. (2021).**

5. The role of vitamins (E and C) in fish

Vitamins are natural organic substances that are required for appropriate growth and health; often are not synthesized by fish, and must be supplied in the diet **(El-Shebly, 2009).** An important element of animal health research is antioxidant defense against metal-free radical species and their cytotoxic and genotoxic effects (**Mekkawy *et al*., 2012)**.

One of the most significant antioxidants for protecting membrane lipids is vitamin E (α-tocopherol). It is a lipid-soluble antioxidant that protects cellular membranes, lipoproteins, and lipid reserves from oxidation (**Mekkawy *et al*., 2013; Hassaan *et al*., 2014**). The main role of vitamin E as an antioxidant is in scavenging lipid peroxyl radicals which are the chain-carrying species and propagate lipid peroxidation (**El-Demerdash *et al.,* 2004**); and essential to maintain immunity, normal resistance of red blood cells to hemolysis (**Halver, 2002**).

Tocopherols' antioxidant action is linked to the transfer of phenolic hydrogen to lipid free radicals, which slows down autocatalytic lipid peroxidation (**Hamre, 2011; Izquierdo *et al.,* 2016).** Lipid-free radicals react highly with unsaturated lipids, creating a cascade of chemicals that can harm membrane structure and function, as well as change protein molecules (**Kanazawa, 1993**). Although tocopherols can also operate as singlet oxygen quenchers, vitamin E plays a key role in the control of polyunsaturated fatty acid (PUFA) peroxidation as a structural component of cell membranes. After that, a stable tocopherol radical is generated, which is then removed from the cycle when it combines with another peroxyl radical to form non-radical inactive products. This tocopherol radical can then be reduced by vitamin C, resulting in the regeneration of α-tocopherol (**Izquierdo *et al.,* 2016)**. Vitamin E exists in eight naturally occurring forms, four tocopherols and four tocotrienols; the most active form is α-tocopherol (**El-Shebly, 2009**). When fish liver is exposed to contaminants and administered a vitamin E supplemented diet, vitamin E has been proven to considerably enhance GSH levels **(Singh *et al*., 2011; Kadry *et al*., 2012).** This situation may reflect the importance of vitamin E as a strong agent to improve the internal antioxidant system in the body to prevent the attacks of ROS, and making ROS unable to react with the vital macromolecules such as lipid, protein, carbohydrate and nucleic acid in cells preventing the cytotoxicity and genotoxicity of pollutants. Tocopherol is a useful indicator of exposure to metals and organic contaminants that generate oxidative stress (**Palace *et al.,* 2005**). Vitamin E is a potent antioxidant that defends numerous fish tissues from oxidative damage (**Adham *et al.,* 2000**).

Vitamin C (L-ascorbic acid) is required for normal growth and physiological functioning in animals, including fish, and helps to reduce the genotoxicity of metals (**Jiraungkoorskul and Sahaphong, 2007**). Several in vivo and invitro studies proved that vitamin C is an effective agent for reducing lung injury caused by increased oxidative stress, as well as avoiding protein damage, cytotoxicity, and apoptosis **(Nemenqani, 2015).** Indeed, vitamin C is a powerful water- soluble antioxidant which protects cell membranes and cytosol agents, and is, among other roles, a cofactor in numerous hydroxylating reactions. Due to a lack of L-gulonolactone oxidase, fish cannot synthesis vitamin C (**Sato *et al.,* 1978**).

6. Biochemical parameters in fish:

Biochemical parameters of fish blood have been found to change when exposed to environmental pollution and these parameters are extremely sensitive to these pollutants. Modifications in the biochemical blood profile reflect changes in the organism's metabolism and biochemical processes as a result of the impact of various pollutants, and they allow researchers to investigate the mechanisms of the effect of pollutants. (**Luskova *et al*., 2002**). The Zn toxicity may induce changes in fish blood parameters which impact their growth. Blood chemical analyses are typically essential information to help diagnose and manage crops for health evaluation **(Cnaani *et al*., 2004; Abdel-Tawwab *et al*., 2012).**

The measurement of biochemical parameters in aquatic species is commonly used to track contaminants and their effects on human health. Biochemical characteristics can also be used as indicators of toxicant exposure and consequences in fish (**Vutukuru, 2003**). Blood is a physiological reflector of the entire body, and blood parameters are critical in determining the structural and functional state of animals exposed to toxins **(Ekrem *et al.,* 2012)**. Recent studies have assessed the toxicity of ZnO nanoparticles and Zn ions on different aquatic organisms and reported oxidative stress, bioaccumulation, immunological, histopathological and ultrastructural changes (**Mansouri *et al*., 2018; Sayadi *et al.,* 2020**). Biochemical parameters of fish blood, including :

6.1. Blood glucose

Stress is an energy-intensive process, and the animal mobilises energy substrates to cope metabolically with it **(Vijayan *et al*., 1997**). Glucose is one of the organism's most significant stress indicators: its high blood levels show that the fish are under stress, utilising reserves of energy intensely. Cortisol, a stress hormone, has been found to boost glucose synthesis in fish through both gluconeogenesis and glycogenolysis, and is likely to play a part in the stress-related rise in plasma glucose concentration **(Iwama *et al.*, 1999).** Plasma glucose and cortisol levels, which are regarded as stress markers, both rose during the waterborne copper exposure period and are highly associated with each other **(Monteiro and Roychoudhury, 2005).**

The rise in blood glucose levels is regarded as a valid indicator of environmental stress since it is a general secondary response to the stress of fish to acute harmful effects (**Sepici –Dinc *et al.,* 2009).**

6.2. Transaminases

Transaminases are essential enzymes that serve a significant function in mobilising L-amino acids for gluconeogenesis and serve as bridges between carbohydrate and protein metabolism at altered physiological and pathological conditions (**Manjunatha *et al*., 2015**). Enzyme activity is regarded as a delicate biochemical signal prior to dangerous effects in fish; therefore they have important role during monitoring of water pollution **(Gu¨l *et al*., 2004).** Changes in the activity of enzymes in fish have been employed as indications of intoxication and water pollution (**Kim *et al*., 2008**). As indicators for liver function, AST and ALT are used. These enzymes are nonfunctional plasma enzymes that are normally found in the cells of the liver, heart, kidneys, gills, muscle, and other organs **(Hadi *et al*., 2009).** The actions of AST and ALT are significant in cellular nitrogen metabolism, amino acid oxidation, and hepatic gluconeogenesis (**Murray *et al*., 2003**).

ALT catalyses the amino group transfer from alanine to a-ketoglutarate, resulting in glutamate and pyruvate, whereas AST catalyses the amino group transfer from aspartate to a-ketoglutarate, resulting in glutamate and oxaloacetate (**Moss *et al*., 1986**). Alanine is a non-essential amino acid that is important in the glucose-alanine cycle that occurs between the liver and muscle tissue. Aspartate is an acidic, non-essential amino acid that is included in protein synthesis and a variety of other cellular metabolic reactions. Aspartate aminotransferase is an intracellular enzyme involved in amino acid and glucose metabolism. It can be present in the heart, skeletal muscle, liver, and kidney, with the heart being the most common location. The levels of AST fluctuate throughout time. The first indicator of liver injury is alanine aminotransferase (ALT), which is seen in the enzymes produced in the bloodstream by liver cells **(Ulukaya, 2007).** ALT and AST are commonly employed in diagnosing damage to liver tissues induced by contaminants (**Coppo *et al*., 2003; Chen *et al*., 2004**).

6.3. Kidney function enzymes:

Creatinine is a byproduct of Creatine Kinase in the muscle. The creatinine test has been usually used to identify impaired kidney function and to identify renal damage in animals like fish **(Banaee *et al*., 2011).** In addition, urea is present in all fish, liver is the primary organ of production and the gills appearing to be the main organ of excretion. Fresh water fish release ammonia along with a small quantity of urea as they use urea as an osmotic filter. Urea in fish is synthesized by the liver and excreted primarily by the gills rather more the kidney. Increased blood urea levels have been found to occur in the presence of compromised kidney function, liver illness, and cardiac arrest (**Abdel-moneim *et al*., 2008**).

Urea blood urea nitrogen (BUN) test detects the quantity of blood nitrogen that originates from the waste product urea, which is produced when protein in the body is broken down. The BUN amount elevates when the kidneys are unable to eliminate urea from the blood in a regular manner., Another kidney function enzyme is creatinine which is a waste product of muscle metabolism that rises as kidney function declines. Creatinine concentration is influenced by few factors outside the kidney, making it a better renal function indicator than BUN. Both tests are connected and linked to the complete metabolic profile, or CMP. A blood sample or a urine sample can be used for either test. A kidney or liver disease or condition is indicated by abnormal levels **(Ajeniyi and Solomon, 2014).**

6.4. Cholesterol and Albumin concentration:

The blood cholesterol (CHOL) value are effective by toxic substances ,reproductive, industrial wastes, and pollution, diseases, nutritional status, sex ,month and seasons, year of fish species. Also, the triglycerides (TG) are fatty acid esters of glycerol and are usually found in the presence of more than one type of fatty acid compound. The storage of energy as triglyceride in the fat deposits is very active because triglycerides have high calorific value and low water content. Plasma triglycerides vary with age, sex, and especially with different diets (**Çelik and Bilgin, 2007**).

Albumin is an important serum protein for transportation of steroid hormones (**Shahsavani *et al*., 2010**). Also, the importance of albumin has been described in respect to fish pathology, Albumin: Globulins ratio (A/G) is widely used as an index of physiological state (**Nakagawa, 1978**).

6.5. Oxidative stress biomarkers:

Oxidative stress (OXS) is a practical toxicity measurement metric, since a range of protective responses is easily quantified as altered enzyme or genetic expression in the cells to response to OXS **(Kovochich *et al*., 2007**).

Biomarkers are measures in body fluids, tissues or cells, that indicate biochemical or cellular changes caused by the toxicant or host response existence and extent (**NRC, 1987**). **Peakall and Walker (1994)** described biomarkers as alterations in biological response (ranging from molecular through physiological and cellular responses to behavioral modifications) which can be associated with toxic impacts of environmental chemicals. Fish biomarkers are promising for ERA (Environmental Risk Assessment) as supplements to existing chemical measures (**Vander Oost *et al*., 2003**). Furthermore, the systematic use of several biomarkers has been found to be the most beneficial in assessing the effects of contaminants (**Tsangaris *et al*., 2010**).

Inhaled or injected NPs could penetrate the bloodstream and move to many organs and tissues, generating fears that they could harm biological systems via the "oxidative burst" pathway (**Segal and Abo, 1993**). Although the signaling pathways for the ensuing oxidative burst are unknown, research into the plasma membrane appears to be required for the activation of a multicomponent enzyme system known as NADPHox and the onset of the oxidative burst. This activation produces superoxide anions immediately, which convert to numerous ROS, including hydrogen peroxide, hydroxyl radicals, and peroxynitrites, which can destroy the offending stimuli via oxidative pathways. The excess superoxide anions and its dismutation product, hydrogen peroxide, is maintained in cytoplasmic granules and provides an immediate supply of intracellular oxidants or can diffuse from the microglial plasma membrane where they cause potentially damage neighboring cells' proteins, lipids, and DNA, particularly neurons (**De Giorgi *et al*., 2002; Fernandes *et al*., 2003**).

Nanoparticles are transferred to tissues and organisms through the blood in aquatic habitats via fish **(Handy *et al.,* 2008**). It has been demonstrated that ENPs, even at low concentrations, have the capacity to cause oxidative stress by causing the production of free oxygen radicals (ROS) **(Sharma *et al*., 2012; Song *et al*., 2010).** The use of pollution indicators in fish is an efficient technique for monitoring the aquatic environment and diagnosing detrimental effects **(Lasheen *et al.,* 2012).** Several manmade nanomaterials have been suggested to have negative effects on fish health, including oxidative stress, DNA damage, and respiratory problems **(Zhu *et al.,* 2006; Federici *et al.,* 2007; King-Heiden *et al.,* 2009).** ROS-mediated oxidative damage to lipids, proteins, and DNA, in particular, has been linked to the pathogenesis of important diseases such as cancer, inflammatory processes, and immunological disorders (**Young and Woodside, 2001)**.

One of the most dangerous hazardous effects of nanoparticles is oxidative stress and ROS-mediated damage. The production of reactive oxygen species (ROS) by NPs in the body exceeds the body's ability to neutralise and eliminate them, resulting in oxidative stress (**Manke *et al.,* 2013**). A range of extremely reactive chemicals formed from the metabolism of molecular oxygen are described by ROS, for example superoxide radicals and hydroxyl radicals. They interact with macromolecules (proteins, DNA, and lipids) within the cell, with one of the most common impacts being lipid peroxidation, which is the oxidation of the polyunsaturated fatty acids that largely comprise the cell membranes. As (**Kerksick and Willoughby, 2005)** reported that, lipid peroxidation is frequently assessed by measuring the buildup of the byproducts of this process, such as malondialdehyde (MDA)

The toxicity of ZnONPs is due to the disturbance of cellular Zinc homeostasis caused by ZnONP dissolution with elevated amounts of these ions, as well as the generation of reactive oxygen and nitrogen species, which cause oxidative stress, inflammation, DNA damage, and cell death (**George *et al*., 2010; Kao *et al*., 2012)**. Reactive ROS cause lipid peroxidation and changes in antioxidant defence mechanisms by disrupting sensitive biological macromolecules and cell signaling (**Livingstone, 2001).** The antioxidant defence system, which includes catalase (CAT), superoxide dismutase (SOD), and glutathione, is designed to neutralise ROS and protect cells and tissues from oxidative damage **(Asagba *et al.,* 2008; Romeo *et al.,* 2000).**

Catalase (CAT) and superoxide dismutase (SOD) have been found in a wide range of mammalian cells. These enzymes are crucial in shielding the cell against the potentially harmful effects of environmental contaminants (**Kuthan *et al*., 1986**). SOD and CAT are powerful markers for early detection of ambient oxidative pollution; in ZnONP-exposed groups, their activity was considerably reduced. The decrease in SOD and CAT activity, as well as their mRNA expression, has been utilised as an indication of oxidant elimination **(Moreno *et al.,* 2005).**

SODs are metalloenzymes that catalyse the conversion of reactive superoxide anions (O_2+) to hydrogen peroxide (H_2O_2), which is a significant ROS in and of itself. After that, two types of enzymes detoxify H2O2: catalases and glutathione-dependent peroxidases (GPOXs). SOD catalytically scavenges the superoxide radical, which looks to be a key agent of oxygen toxicity, providing protection against one aspect of oxygen toxicity (**Kadar *et al*., 2006**). A unit of SOD activity is defined as the quantity that inhibits the scavenger's decrease by 50% under specific conditions (**Stegeman *et al.,* 1992**).

CATs are hematin-containing enzymes that help remove (H_2O_2), which is converted to molecular oxygen (O2) and water **(Vander Oast *et al*., 2003)**. Unlike certain peroxidases, which may decrease a variety of lipid peroxides in addition to H_2O_2, CATs can only reduce H_2O_2 (**Filho, 1996**). It was demonstrated that peroxisome proliferating compounds (a class of nongenotoxic carcinogens) induce both the activities of H_2O_2-generating fatty acid oxidases and CAT in rodents (**Halliwell and Gutteridge, 1999**).

Biotransformation reaction of toxic chemicals in organisms occurs in three phases (transformation, conjugation and excretion). Glutathion S transferase enzyme (GST) is a phase 2 enzyme system that promotes the conjugation of electrophilic substances or groups to tripeptide glutathione in order to make xenobiotic compounds more hydrophilic for transportation or excretion (**Egaas *et al.,* 1993**). CAT and GST enzymes have been used as parameters to assess environmental pollutant contamination in fish tissues (**Moraes *et al*., 2007**). In addition, SOD and CAT are powerful markers for early detection of oxidative pollution in the environment **(Moreno *et al*., 2005).**

6.6. Bioaccumulation of ZnO (Bulk and nanoparticles) in fish:

Bioaccumulation is the accumulation of a contaminant in an organism, and it takes into account all routes of exposure. The amount of metals ingested by organisms, the way the metals were distributed among different tissues, and the extent to which the metals were maintained in each tissue type are all factors that go into bioaccumulation. As a result, bioaccumulation measures relate to research into methods for detecting the uptake and retention of contaminants such as metals in the organs and tissues of creatures such as fish **(Murugan *et al*., 2008**). The accumulating nanoparticles not only degrade the water quality, but also cause discomfort to aquatic life by generating a variety of biological changes as well as mutagenic and carcinogenic effects **(Lee *et al*., 2009).** Heavy metal residues in fish organs can cause structural damage and, more typically, functional issues **(Jezierska and Witeska, 2006)**.

Zinc is a prevalent pollutant in aquatic systems, and it is linked to urban runoff, industrial discharges, soil erosion, pharmaceuticals, pesticides, and a number of other activities and sources (**Bowen *et al*., 2006**).

Because it cannot be removed biologically and can only be changed from one oxidation state or organic complex to another, the threat of Zn in the environment is exacerbated (**Everall *et al*., 1989**).

Fish are an important part of aquatic environments. They are essential bio indicators of trace element contamination, in addition to being a source of protein for humans (**Rashed, 2001**). Also, it provides a primary exposure route for NPs' uptake and bioaccumulation in humans. Some effects induced by NPs in fish may reflect their possible hazards in other vertebrates including human beings (**Dorea, 2008**). As a result, the use of fish for measuring environmental conditions in aquatic habitats has grown in popularity in recent years (**Adeniyi *et al*., 2008; Palaniappan *et al*., 2010**). Nanoparticles have the ability to clump together in cells like macrophages and hepatocytes, and are thus ingested by aquatic creatures like as fish, crustaceans, mollusks, and artemia **(Ward and Kach, 2009)**. Exposure to these nanoparticles in fish occurs primarily through the gills to blood, skin mucosa, and from gastrointestinal absorption by blood, which leads to other parts of the body such as the kidney, liver, muscles and brain (**Handy *et al.,* 2008**).

The accumulation of Zinc (Zn) from zinc oxide nanoparticles (ZnONPs) in the liver, muscles and gill of fish started to accumulate in the tissues with the increasing dose concentration and maximum concentration was shown in the liver as compared to muscles and gills at high dose level. (**Shahzad *et al.,* 2017)**.

Zinc nanoparticles have high accumulation potency in the fish tissues compared to Zinc bulk particles especially in renal and hepatic tissue which reflected that zinc nanoparticles could easily

penetrate cell membrane with different entering mechanisms (**Abdel-Khalek *et al*., 2016**). Also, ZnONPs have more potential to accumulate Zn in fish tissues than bulk-ZnO and induce oxidative cellular responses (**Hao *et al*., 2013).** Fish groups exposed to ZnONPs accumulated Zn higher than that of ZnOBPs at all exposure periods. However, fish group exposed to the same concentration of ZnO BPs or ZnO NPs and supplemented with vitamins (E and C) showed a significant decreasing ($P \leq 0.05$) in accumulated Zn level compared to the group without supplementation after all exposure periods except 1/8 LC_{50} of ZnO NPs plus vitamins (E + C) at 7 day as described in table 1 **(Mohamed *et al*., 2022)**

Table 1. Zn accumulation (mg/kg dry weight) in muscle tissue of *O. niloticus* exposed to ZnO BPs or ZnO NPs sub-lethal concentrations and vitamins (E + C) supplementation **(Mohamed *et al.,* 2022)**.

Time (days)	Control	Sub-lethal concentration			Sub-lethal concentration + vitamins (E + C)		
		1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}	1/8 LC_{50}	1/4 LC_{50}	1/2 LC_{50}
ZnOBPs							
7	20.3±0.17^{d}	21.6±0.34^{bc}	22±0.57^{ab}	23.2±0.05^{a}	20.4±0.23^{d}	20.7±0.40^{cd}	20.8±0.46^{bcd}
14	20.9±0.51^{d}	22.2±0.11^{c}	23.6±0.34^{b}	25.3±0.17^{a}	21±0.54^{cd}	21.6±0.34^{cd}	21.7±0.28^{cd}
21	21..2±0.69^{c}	25.4±0.40^{b}	28.9±0.51^{a}	31±1.15^{a}	23.4±0.80^{bc}	23.7±0.98^{bc}	24.9±0.51^{b}
28	21.6±0.23^{e}	28.1±0.57^{cd}	30.7±0.40^{b}	39.7±0.98^{a}	26.9±0.51^{d}	28.1±063^{cd}	29.4±0.23^{bc}
ZnONPs							
7	20.3±0.17^{b}	21.9±0.14^{b}	23.8±1.03^{a}	24.1±0.57^{a}	21±0.28^{b}	21.1±0.05^{b}	21.6±0.17^{b}
14	20.9±0.51^{d}	25.3±0.17^{b}	25.7±0.69^{b}	27.5±0.86^{a}	21.6±0.34^{cd}	22.7±0.11^{c}	22.8±0.23^{c}
21	21..2±0.69^{e}	28.4±0.08^{bc}	29.1±0.63^{b}	31.9±0.51^{a}	23.6±0.92^{d}	23.9±0.25^{d}	26.8±0.28^{c}
28	21.6±0.23^{e}	32.7±0.31^{c}	35.4±0.23^{b}	42.7±1.15^{a}	28.9±0.51^{d}	29.7±0.34^{d}	29.8±0.46^{d}

6.7. Proximate Chemical composition of fish:

The research of fish chemical composition is significant because it determines the fish's keeping quality and technological characteristics. Proximate profile measurements, such as protein, lipids, and moisture content, are frequently required to ensure that they meet food rules and commercial demands. They also have an impact on post-harvest processing and the fish's shelf life (**Jim *et al.,* 2017**). The chemical composition and quality criteria of fish are among the major factors that affect the overall quality of fish. Several factors included; age, species, sexual cycle, physical state, feed, stage of maturity, and environmental conditions have been reported to affect the chemical composition of fish (**Noël *et al.,* 2011).** Information on the chemical and nutritional composition of derived fish products is important and very necessary for nutritionists, biologists and researchers who work together with the food industry to help formulate diets, classify food and support the food industry, research into the conservation and processing of fish. Factors affecting the chemical composition of fish are numerous, and some are intrinsic such as genetic morphological and physiological factors as well as other related environmental quality of the area where they live **(Porto *et al.,* 2016).** Exposure of fish to nanoparticles has more affected proximate chemical composition while supplementation with vitamins (E + C) ameliorate chemical composition **(Mohamed *et al.,* 2022).**

CONCLUSION

Dietary supplementation with vitamins (E and C) against toxic effects in fish have gained interest in recent years. Exposure to ZnOBPs and ZnONPs resulted in harmful effects in fish tissues. Therefore, addition of vitamins E and C considered as an effective antioxidant that protects fish from the harmful effects of ZnOBPs and ZnONPs.

REFERENCES

Abdel-Khalek, A. A.; Hamed, A. and Marie M.A. (2016). The accumulation potency of bulk and nano zinc metal and their impacts on the hematological and histological perturbations of *Oreochromis niloticus*. Water Air Soil Pollut. 227, 6: 206.

Abdel-Moneim, A. M.; Abou Shabana, N. M.; Khadre, S. E. M. and Abdel-Kader, H. H. (2008). Physiological and histopathological effects in catfish (*Clarias lazera*) exposed to dyestuff and chemical wastewater. Academic Journals Inc.

Abdel-Tawwab, M.; El-Sayed, G. O. and Shady, S. H. (2012). Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus (L.)*. Toxicol. Environ. Chem. 94(7): 1368-1382.

Adam, N.; Schmitt, C.; De Bruyn, L.; Knapen, D. and Blust, R. (2015). Aquatic acute species sensitivity distributions of ZnO and CuO nanoparticles. Science of the Total Environment, 526:233–242.

Adeniyi, A. A.; Yusuf, K. A. and Okedeyi, O. O. (2008). Assessment of the exposure of two fish species to metals pollution in the Ogun river catchments, Ketu, Lagos, Nigeria. Environ Monit Assess. 137:451–458.

Adham, K. G.; Hashem, H. O.; Abu-Shabana, M. B. and Kamel, A. H. (2000). Vitamin C deficiency in the catfish *Clarias gariepinus*. Aquaculture Nutrition, 6 (2), 129-139.

Ajeniyi, S. A. and Solomon, R. J. (2014). Urea and creatinine of *Clarias gariepinus* in three different commercial ponds. Nat. Sci. 12, 124-138.

Al-Rasheed, N.; Abdel Baky, N. A.; Al-Rasheed, N.; Shebly, W.; Ahmed, A. M. and Faddah, L. M. (2012). Effect of vitamin E and alpha lipoic acid on nano zinc oxide induced renal cytotoxicity in rats. African Journal of pharmacy and pharmacololgy, 629 (29): 2211-2223.

Andersson-Willman, B.; Gehrmann, U.; Cansu, Z.; Buerki-Thurnherr, T.; Krug, H.F.; Gabrielsson, S. and Scheynius, A. (2012). Effects of subtoxic concentrations of TiO2and ZnO nanoparticles on human lymphocytes, dendritic cells and exosome production. Toxicol. Appl. Pharmacol. 264, 94–103.

Asagba, S. O.; Eriyamremu, G. E. and Igberaese, M. E. (2008). Bioaccumulation of cadmium and its biochemical effect on selected tissues of the catfish (*Clarias gariepinus*). Fish physiology and biochemistry, 34(1), 61-69.

Asghar, M. S.; Qureshi, N. A.; Jabeen, F.; Khan, M. S.; Shakeel, M. and Noureen, A. (2015). Toxicity of zinc nanoparticles in fish: a critical review. J. Biol. Environ. Sci. 7(1), 431-439.

Banaee, M.; Mirvaghefei, A. R.; Majazi Amiri, B.; Rafei. G. R. and Nematdost, B. (2011). Hematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinus carpio*). Journal of Fisheries (Iranian Journal of Natural Resources) 64(1), 1:14.

Benn, T. M. and Westerhoff, P. (2008). Nanoparticle silver released into water from commercially available sock fabrics. Environ. Sci. Technol. 42, 4133–4139.

Bian, S.; Mudunkotuwa, I. A.; Rupasinghe, T. and Grassain, V. H. (2011). Aggregation and dissolution of 4 nm ZnO NPs in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir 27:6059–6068.

Borkovic, S. S.; Saponjic, J. S.; Pavlovic, S. Z.; Blagojevic, D. P. and Milosevic, S. M. (2005). The activity of antioxidant defence enzymes in the mussel Mytilus galloprovincialisfrom the Adriatic Sea. Comp. Biochem. Physiol. C 141, 366–374.

Bowen, L.; Werner, I. and Johnson, M. L. (2006). Physiological and behavioral effects of zinc and temperature on coho salmon (*Oncorhynchus kisutch*). Hydrobiol. 559:161–168

Bronmark, C. and Hansson, L. A. (2005). The biology of lakes and ponds, Oxford University Press, Oxford, pp 245.

Brunner, T. J.; Wick, P.; Manser, P.; Spohn, P.; Grass, R. N.; Limbach, L. K.; Bruinik, A. and Stark, W. J. (2006). In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environmental Science and Technology, 40(14), 4374-4381.

Çavas, T. and Ergene-Gözükara, S. (2005). Induction of micronuclei and nuclear abnormalities in *oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents. Aquat. Toxicol., 74, 264–271.

Çelik, E. Ş. and Bilgin, S. (2007). Bazi balik türleri için kan protein ve lipidlerinin standardizasyonu, Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 23(1 -2):215-229**.**

Chen, C. Y.; Wooster, G. A. and Bowser, P. R. (2004). Comparative blood chemistry and histopathology of tilapia infected with Vibrio vulnificus or Streptococcus iniae or exposed to carbon tetrachloride, gentamicin, or copper sulfate. Aquaculture, 239:421–443.

Chen, P. J.; Tan, S. W. and Wu, W. L. (2012). Stabilization or oxidation of nanoscale zero valent iron at environmentally relevant exposure changes bioavailability and toxicity in medaka fish. Environ. Sci. Technol., 46, 8431–8439.

Cnaani, A.; Tinman, S.; Avidar, Y.; Ron, M. and Hulata, G. (2004). Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *O. niloicus*. Aquacult. Res., 35:1434–1440.

Coppo, J. A.; Mussart. N. B. and Fioranelli, S. A. (2003). Physiological variation of enzymatic activities in blood of bullfrog, *Rana catesbeiana* (Shaw, 1802). Rev. Veter., 12:22–27.

De Giorgi, F.; Lartigue, L.; Bauer, M. K.; Schubert, A.; Grimm, S.; Hanson, G. T.; Remington, S. J., Youle, R. J. and Ichas, F. (2002). The permeability transition pore signals apoptosis by directing Bax translocation and multimerization. FAS EB J., 16, 607–609.

Dorea, J. G. (2008). Persistent, bioaccumulative and toxic substances in fish: human health considerations. Sci. Total Environ.,400, 93–114.

Egaas, E.; Skaare, J. U.; Svendesen, N. O.; Sandvik, M.; Falls, J. G.; Dauterman, W. C.; Collier, T. K. and Netland, J. (1993). A comparative study of effect of atrazine on xenobiotic metabolizing enzymes in fish and insect, and on the in vitro phase 11 atrazine metabolisms in some fish, insect, mammals and one plant species. Comp, Biochem .Physio., 106:141-149.

Ekrem, S.; Celik, H.; Kaya, S. Y.; Mehmet, A. and Arinc, T. (2012). Effects of zinc exposure on the accumulation, haematology and immunology of Mozambique tilapia, *Oreochromis mossambicus*. African J. Biotech., 12(7):744-753.

El Nemr, A.; Khaled, A.; Moneer, A. A. and El Sikaily, A. (2012). Risk probability due to heavy metals in bivalve from Egyptian Mediterranean coast. Egypt. J. Aquat. Res., 28, 67–75.

El-Demerdash, F. M.; Yousef, M. I.; Kedwany, F. S. and Baghdadi, H. H., (2004). Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and b-carotene. Food Chem. Toxicol., 42, 1563–1571.

El-Gazar, M. G. (2012). Impact of Some Environmental Pollutants on biodhemical functions of *Tilapia sp.* At different hot spot. Area of Alexandria, Egypt, master. thesis, Faculty of Science, Ain shams University.

Elsaesser, A. and Howard, C. V. (2012). Toxicology of nanoparticles.Adv. Drug Deliv. Rev., 64 (2), 129–137.

Elsammad, N. M.; Abd Elhaleem, A. H.; Hassan, S. h.; Elshaer, M. and Badawi, A.M. (2014). Evaluation of The Protective Effect of Zinc Oxide /Ascorbyl Palmitate NanoComposite on Cadmium- Induced Hepatotoxicity and Nephrotoxicity in Rats. Int. J. Pharm. Sci. Rev . Res., 29: 232-239.

El-Shebly, A. A. (2009). The role of antioxidant (Vitamin E) in the control of lead (Pb) pollution and ehancement of growth within Nile Tilapia (*Oreochromis niloticus*). Int. J. Appl. Res. Vet. Med., 7, 97–101.

Everall, N. C.; MacFarlane, N. A. A. and Sedgwick, R. W. (1989). The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, Salmo trutta L. J. Fish Biol., 35:27–36.

Federici, G.; Shaw, B. J. and Handy, R. D. (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout, (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquat. Toxicol., 84, 415–430.

Fernandes, M. A.; Santos, M. S.; Vicente, J. A.; Moreno, A. J.; Velena, A.; Duburs, G. and Oliveira, C. R. (2003). Effects of 1, 4-dihydropyridine derivatives (cerebrocrast, gammapyrone, glutapyrone, and diethone) on mitochondrial bioenergetics and oxidative stress: a comparative study. Mitochondrion, 3(1), 47-59.

Filho, D. W. (1996). Fish antioxidant defences - a comparative approach. Braz. J. Med. Biol. Res., 29, 1735_/1742.

George, S.; Pokhrel, S.; Xia, T.; Gilbert, B.; Ji, Z.; Schowalter, M.; Rosenauer, A.; Damoiseaux, R.; Bradley, K. A.; Mädler, L. and Nel, A. E. (**2010)** Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. ACS Nano., 29-4:15.

Ghannam, H.; Mohamed, A.; Soliman, H.; ElGaar, D. and Ellahamy, A. (2022). Protective role of vitamins (E+ C) on Cholesterol and Albumin in Oreochrmis niloticus subjected to Zinc oxide Bulk and Nanoparticles. *Aswan University Journal of Environmental Studies*, *3*(3), 293-302.

Ghazi, S.; Diab, A. M.; Khalafalla, M. M. and Mohamed, R. A. (2021). Synergistic Effects of Selenium and Zinc Oxide Nanoparticles on Growth Performance, Hemato-biochemical Profile, Immune and Oxidative Stress Responses, and Intestinal Morphometry of Nile Tilapia (Oreochromis niloticus). Biol. Trace Elem. Res., 1-11.

Gu¨l, S.; Belge-Kurutas, E.; Yldz, E.; Sahan, A. and Doran, F. (2004). Pollution correlated modifications of liver antioxidant systems and histopathology of fish (*Cyprinidae*) living in Seyhan Dam Lake, Turkey. Environ. Int., 30, 605–609.

Guan, R.; Kang, T.; Lu, F.; Zhang, Z.; Shen, H. and Liu, M. (2012). Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles. Nanoscale Res. Lett., 7: 602.

Gupta, P. and Srivastava, N. (2006). Effects of sub-lethal concentrations of on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus (Bloch*). J. Environ. Biol. 27:211–215.

Hadi, A.; Shokr, E. and Alwan, F. (2009). Effects of aluminum on the biochemical parameters of fresh water fish *Tilapia zillii*. J. Sci. Appl., 3: 33–41.

Halliwell, B. and Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine, third ed. Oxford University Press, Oxford, UK.

Halver, J. E. (2002). The vitamins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, CA, pp. 61–141.

Hamre, K. (2011). Metabolism, interactions, requirements and functions of vitamin E in fish. Aquac. Nutr., 17, 98–115.

Handy, R. D.; Owen, R. and Valsami-Jones, E. (2008). The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and futureneeds. Ecotoxicology, 17, 315-325

Hao, L.; Chen, L.; Hao, J. and Zhong, N. (2013). Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): a comparative study with its bulk counterparts. Ecotoxicol. Environ. Saf., 91: 52-60.

Hassaan, M. S.; Goda, A. M. A-S.; Mahmoud, S. A. and Tayel, S. L. (2014). Protective effect of dietary vitamin E against fungicide copperoxychloride stress on Nile tilapia, *Oreochromis niloticus (L.),* fingerlings. Int. Aquat. Res., 6, 1: - 6-1.

Heinlaan, M.; Ivask, A.; Blinova, I.; Dubourguier, H. C. and Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacterial *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere, 71(7), 1308-1316.

ICAR (2006). Indian council of Agricultural Research . Handbook of fisheries and aquaculture.

Iqbal, K. J.; Majeed, H.; Iqbal, K. J.; Asghar, M.; Azmat, H.; Fatima, M.; ... and Davies, S. J. (2023). Administration of vitamin E and C enhances immunological and biochemical responses against toxicity of silver nanoparticles in grass carp (Ctenopharyngodon idella). *Plos one*, *18*(4), e0284285.

Isani, G.; Letizia, M.; Barucca, G.; Sekar, D.; Andreani, G.; Carpene, E. and Falcioni, G. (2013). Comparative toxicity of CuO nanoparticles and CuSO4in rainbow trout. Ecotoxicol. Environ. Saf., 97, 40–46.

Iwama, G. K.; Vijayan, M. M.; Forsyth, R. B. and Ackerman P. A. (1999). Heat shock proteins and physiological in fish. Am. Zool. 39:901 – 909.

Izquierdo, M. S.; Ghrab, W.; Roo, J.; Hamre, K.; Hernandez-Cruz, C. M.; Bernardini, G.; Terova, G. and Saleh, R. (2016). Organic, inorganic and nanoparticles of Se, Zn and Mn in early weaning diets for gilthead seabream (*Sparus aurata; Linnaeus, 1758*). Aquac. Res.

Jezierska, B. and Witeska, M. (2006). The metal uptake and accumulation in fish living in polluted waters. In Soil and water pollution monitoring, protection and remediation (pp. 107-114). Springer, Dordrecht.

Jim, F.; Garamumhango, P. and Musara, C. (2017). Comparative analysis of nutritional value of *Oreochromis niloticus* (*Linnaeus*), Nile tilapia, meat from three different ecosystems. Journal of Food Quality, 2017.

Jiraungkoorskul, W. and Sahaphong, S. (2007). Efficacy of ascorbic acid reducing waterborne copper toxicity in butterfish (*Poronotus triacanthus*). J. Biol. Sci., 7, 620–62.

Johnston, H. J.; Hutchison, G.; Christensen, F. M.; Peters, S. and Stone, H. S. (2010). A review of the in vivo and in vitro toxicity of silver and gold particulates. Particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev. Toxicol., 40, 328-346.

Jovanović, B. and Palić, D. (2012). Immunotoxicology of non-functionalized engineered nanoparticles in aquatic organisms with special emphasis onfish–review of current knowledge, gap identification, and call for further research. Aquat. Toxicol., 118–119, 141–151.

Kadar, E.; Costa, V. and Santos, R. S. (2006). Distribution of microessential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. Sci. Total Environ., 358, (1-3), 143-150.

Kadry, S. M.; Marzouk, M. S.; Amer, A. M.; Hanna, M. I.; Azmy, A. H. and Hamed, H. S. (2012). Vitamin E as antioxidant in female african catfish (*Clarias gariepinus*) exposed to chronic toxicity of atrazine. Egypt. J. Aquat. Biol. Fish, 16 (2), 83–98.

Kaewamatawong, T.; Shimada, A.; Okajima, M.; Inoue, H.; Morita, T. and Inoue, K. (2006). Acute and subacute pulmonary toxicity of low dose of ultrafine colloidal silica particles in mice after intratracheal instillation. Toxicol. Pathol., 34, 958–965.

Kanazawa, K. (1993). Tissue injury induced by dietary products of lipid peroxidation. In: Corongiu, F. (Ed.), Free Radicals and Antioxidants in Nutrition. Richelieu Press, London, pp. 383–399.

Kao, Y. Y.; Chiung, Y. M.; Chen, Y. C.; Cheng, T. J. and Liu, P. S. (2012). Zinc oxide nanoparticles interfere with zinc ion homeostasis to cause cytotoxicity. Toxicol. Sci., 125 :462–472.

Kerksick, C. and Willoughby, D. (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exerciseinduced oxidative stress. Journal of the International Society of Sports Nutrition, 2(2): 38-44.

Kim, S. G.; Park, S. W.; Jang, G. S.; Lee, S. S. and Chung, M. H. (2008). Effect of dietary Benzopyrene on growth and nematological parameters in guvenile Rock fish, *subustes schlegeli* Bull, Environ. Cont. Toxical., 81: 470 – 474.

King-Heiden, T. C.; Wiecinski, P. N.; Mangham, A. N.; Metz, K. M.; Nesbit, D. and Pederse, J. A. (2009). Quantum dot nanotoxicity assessment using the Zebra fish Embryo .Environ. Sci. Technol., 43, 1605–1611.

Kovochich, M.; Xia, T.; Xu, J.; Yeh, J. I. and Nel, A. E. (2007). Principles and procedures to assess nanomaterial toxicity. In: Wiesner, M.R,. Bottero, J.Y. (Eds.), Environmental Nanotechnology: Applications and Impacts of Nanomaterials. Mc Graw Hill, New York, pp. 205–229.

Kuthan, H.; Haussmann, H. J. and Werringlover, J. (1986). A spectrophotometric assay for superoxide dismutase activities in crude tissue fractions. Biochem. J., 237: 175- 180.

Lasheen, M. R.; AbdelGawad, F. K.; Alaneny, A. A. and AbdElbary, H. M. H. (2012). Fish as Bio indicators in aquatic environmental pollution assessment: a case study in Abu-Rawash Area, Egypt.World Appl. Sci. J., 19, 265–275.

Lee, S. W.; Kim, S. M. and Choi, J. (2009). Genotoxicity and ecotoxicity assays using the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius to screen the ecological risks of nanoparticle exposure .Environmental Toxicology and Pharmacology, 28, 86–91.

Lee, W.; Kim, S. W.; Kwak, J.; Nam, S.; Shin, Y. and An, Y. (2010). Research Trends of Ecotoxicity of Nanoparticles in Soil Environment. Toxicology Research, 26(4), 253–259.

Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollut. Bull., 42, 656-666.

Luskova, V.; Svoboda, M. and Kolarova, J. (2002). The effects of diazinon on blood plasma biochemistry in carp (Cyprinus *carpio L.*). Acta. Vet. Brno., 71:117 -123.

Ma, H.; Williams, P. L. and Diamond, S. A. (2013). Ecotoxicity of manufactured ZnO nanoparticles–a review. Environ. Pollut., 172: 76-85.

Mahboub, H. H.; Shahin, K.; Zaglool, A. W.; Roushdy, E. M. and Ahmed, S. S. A. (2020). Efficacy of nano zinc oxide dietary supplements on growth performance, immunomodulation and disease resistance of African Catfish, *Clarias gariepinus*. Dis. Aquat. Org., 142: 147-160.

Manjunatha, B.; Tirado, J. and Selvanayagam, M. (2015). Sub-lethal toxicity of potassium cyanide on Nile Tilapia (*Oreochromis niloticus*): Biochemical response. International Journal of Pharmacy and Pharmaceutical Sciences, 7, (3): 379-382.

Manke, A.; Wang, L. and Rojanasakul, Y. (2013). Mechanisms of Nanoparticle- Induced oxidative stress and toxicity. Bio Med Research International, 2103(15): 650-665.

Mansouri, B.; Johari, S. A.; Azadi, N. A. and Sarkheil, M. (2018). Effects of waterborne ZnO nanoparticles and Zn2+ ions on the gills of rainbow trout (*Oncorhynchus mykiss*): bioaccumulation, histopathological and ultrastructural changes. Turk. J. Fish. Aquat. Sci., 18, 5: 739-746.

McNeil, D. G. and Fredberg, J. (2011). Environmental water requirements of native fishes in the Middle River catchment, Kangaroo Island, South Australia. A report to the SA Department for water. South Australian Research and Development Institute (aquatic sciences), Adelaide. SARDI Publication No. f2011/000060-1. SARDI research report series, (528), 50.

Mekkawy, I. A. A.; Mahmoud, U. M. and Mohammed, R. H. (2013). Protective effects of tomato paste and vitamin e on atrazine-induced hematological and biochemical characteristics of *clarias gariepinus* (burchell, 1822). Glo. Adv. Res. J. Environ. Sci. Toxicol., 2, 11–21.

Mekkawy, I. A. A.; Mahmoud, U. M.; Wassif, E. T. and Naguib, M. (2012). Protective roles of tomato paste and Vitamin E on Cadmium-induced histological and histochemical changes of liver of *Oreochromis niloticus* (Linnaeus, 1758). J. Fisher. Aqua. Sci., 7, 240–265.

Melegaria, S. P.; Perreaulut, F.; Costa, R. H.; Popovic, R. and Matiasa, W. G. (2013). Evaluation of toxicity and oxidative stress induced bycopper oxide nanoparticles in the green alga; *Chlamydomonas reinhardtii*. Aquat. Toxicol., 142–143, 431–440

Mocan, T.; Clichici, S.; Agoston-Coldea, L.; Mocan, L.; Simon, S. and Ilie, I. R. (2010). Implications of oxidative stress mechanisms intoxicity of nanoparticles (review). Acta Physiol. Hung., 97, 247- 255.

Mohamed A. S.; Soliman H. A.; Ghannam H. E. (2021). Ameliorative effect of vitamins (E and C) on biochemical alterations induced by sublethal concentrations of zinc oxide bulk and nanoparticles in Oreochromis niloticus. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *242*, 108952.

Mohamed, A. S.; Ghannam, H. E.; El-Lahamy, A. A. and Soliman, H. A. (2022). The protective role of vitamins (E+ C) on Nile tilapia (Oreochromis niloticus) exposed to ZnO NPs and Zn ions: Bioaccumulation and proximate chemical composition. *Annals of Animal Science*, *22*(2), 633-642.

Monteiro, P. M. S. and Roychoudhury, A. N. (2005). Spatial characteristics of sediment trace metals in an eastern boundary upwelling retention area (St .Helena Bay, South Africa). A hydrodynamic-biological pump hypothesis. Estuarine Coastal and Shelf Science, 65, 123 – 134.

Moraes, B. S.; Loro, V. L.; Glusczak, L.; Pretto, A.; Menezes, C.; Marchezan, E. and de Oliveira Machado, S. (2007). Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). Chemosphere, 68(8), 1597-1601.

Moreno, I.; Pichardo, S.; Jos, A.; Gomez-Amores, L.; Mate, A.; Vazquez, C. M. and Camean, A. M. (2005). Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally. Toxicon, 45(4), 395-402.

Morphometry of Nile Tilapia (*Oreochromis niloticus*). Biol. Trace Elem. Res., 1-11.

Moss, D. W.; Henderson, A. R. and Kochmar, J. F. (1986). Enzymes; principles of diagnostic enzymology and the aminotransferases. In: Tietz, N.W. (Ed.), Textbook of Clinical Chemistry. Saunders, Philadelphia, PA, pp. 663_678.

Murray, R. K.; Granner, D. K.; Mayes, P. A. and Rodwell, V. W. (2003). Harper's Illustrated Biochemistry, 26th Edition ,McGraw-Hill, Medical Publishing Division. 818 p.

Murugan, S. S.; Kanippasam, R.; Poongod, K. and Puvaneswari, S. (2008). Bioaccumulation of zinc in Freshwater fish Chana punctatus (Bloch) after chronic exposure. Turk. J. Fish. Aquat. Sci., 8: 55-59**.**

Nakagawa, H. (1978). Classification of Albumin and Globulin in Yellow tail plasma. Bulletin of the Japanese Society of Scientific Fisheries, 44, (3): 251-257.

Nemenqani, D. (2015). The Ameliorating Effect of Vitamin C against Cardiopulmonary Toxicity of Zinc Oxide Nanoparticals. Int. Res. J. Appl. Basic Sci., 9 :1102-1109.

Noël, L.; Chafey, C.; Testu, C.; Pinte, J.; Velge, P. and Guerin, T. (2011). Contamination levels of lead, cadmium and mercury in imported and domestic obsters and large crab species consumed in France: differences between white and brown meat. J. Food. Comp. Anal., 24: 368–375.

Nohynek, G. J.; Lademann, J.; Ribaud, C. and Roberts, M. S. (2007). Grey Goo on the skin Nanotechnology, cosmetic and sunscreen safety. Crit. Rev. Toxicol., 37, 251–277

NRC, (1987). Committee on Biological Markers of the National Research Council. Biological markers in environmental health research. Environ Health Persp.74: 3-9.

Osmond, M. J. and McCall, M. J. (2010). Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. Nanotoxicology, 4: 15–41.

Palace, V. P.; Doebel, C.; Baron, C. L.; Evans, R. E.; Wautier, K. G.; Klaverkamp, J. F.; Werner, J. and Kollar, S. (2005). Caging small-bodied fish as an alternative method for environmental effects monitoring (EEM). Water Qual. Res. J. Canada, 40(3):328–333.

Palaniappan, P. R.; Nishanth, T. and Renju, V. B. (2010). Bioconcentration of zinc and its effect on the biochemical constituents of the gill tissues of *Labeo rohita*: An FT-IR study. Infrared Phys. Technol., 53, 2: 103-111.

Paterson, G.; Ataria, J. M. and Hoque, M. E. (2011). The toxicity of titanium dioxide nanopowder to early life stages of the Japanese medaka (*Oryzias latipes*). Chemosphere, 82, 1002–1009.

Pawari, M. J. and Gawande, S. (2015). Assessment of Underground water Quality around Hadapsar region in Pune, Maharashtra.

Peakall, D. W. and Walker, C. H. (1994). The role of biomarkers in environmental assessment (3). Vertebrates Ecotoxicol., 3:173-179.

Piccinno, F.; Gottschalk, F.; Seeger, S. and Nowack, B. (2012). Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. Journal of Nanoparticle Research, 14(9), 1109.

Porto, H. L. R.; de Castro, A. C. L.; Filho, V. E. M. and Rádis-Baptista, G. (2016). Evaluation of the Chemical Composition of Fish Species Captured in the lower Stretch of Itapecuru River, Maranhão, Brazi, ntl. J. Adv. Agri and Envi., Engg., 3(1), 181-186.

Poynton, H. C.; Lazorchak, J. M.; Impellitteri, C .A.; Smith, M. E.; Rogers, K.; Patra, M.; Hammer, K. A.; Allen, H. J. and Vulpe, C. D. (2011). Differential gene expression in Daphnia magna suggests distinct modes of action and bioavailability for ZnO nanoparticles and Zn ions. Environmental Science and Technology, 45, 762-768.

Rashed, M. N. (2001). Monitoring of environmental heavy metals in fish from Nasser Lake. Environ. Int., 27:27–33.

Rashidian, G.; Lazado, C. C.; Mahboub, H. H.; Mohammadi-Aloucheh, R.; Proki´c, M. D.; Nada, H. S. and Faggio, C. (2021). Chemically and Green Synthesized ZnO Nanoparticles Alter Key Immunological Molecules in Common Carp (*Cyprinus carpio*) Skin Mucus. Int. J. Mol. Sci., 22, 6: 3270.

Romeo, M.; Bennani, N.; Gnassia-Barelli, M.; Lafaurie, M. and Girard, J. P. (2000). Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. Aquat. Toxicol., 48, 185-194.

Rotello, V. M. (2003). Nanoparticles: Building Blocks for Nanotechnology, first ed. Springer, New York.

Sato, M.; Yoshinaka, R. and Ikeda, S. (1978). Dietary ascorbic acid requirement of rainbow trout for growth and collagen formation. Bull. Jpn. Soc. Sci. Fish., 44, 1029–1035.

Sayadi, M. H.; Pavlaki, M. D.; Martins, R.; Mansouri, B.; Tyler, C. R.; Kharkan, J. and Skakari, H. (2020). Bioaccumulation and toxicokinetics of zinc oxide nanoparticles (ZnO NPs) co-exposed with graphene nanosheets (GNs) in the blackfish (*Capoeta fusca*). Chemosphere, 269: 128689.

Segal, A. W. and Abo, A. (1993). The biochemical basis of the NADPH oxidase of phagocytes. Trends Biochem. Sci., 18, 43–47.

Senthil Murugan, S.; Karuppasamy, R.; Poongodi, K. and Puvaneswari, S. (2008). Bioaccumulation pattern of zinc in freshwater fish *Channa punctatus* (Bloch.) after chronic exposure. Turk. J. Fish. Aquat. Sci., 8:55–59.

Sepici -Dinc, E. A.; Benli, A. C. K., Selvi, M.; Sarıkaya, R. S.; ahin, D.; Ozkul, I. A. and Erkoc, F. (2009). Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio L.*) fingerlings: biochemical, hematological, histo pathological alterations .Ecotoxicol. Environ. Saf., 72:1433.

Shahsavani, D.; Kaxerani, H.; Kaveh, S. and Kanani, H. (2010). Determination of some normal serum parameters in starry Sturgeon (*Acipenser stellatus*) during spring season. Comp. Clin. Pathol., 19: 57-61.

Shahzad, K.; Khan, M. N.; Jabeen, F.; Kosour, N.; Sohail, M.; Khan, M. K. A. and Ahmad, M. (2017). Bioaccumulation of manufactured titanium dioxide (TiO 2), copper oxide (CuO) and zinc oxide (ZnO) nanoparticles in soft tissues of tilapia (*Oreochromis mossambicus*). Punjab. Univ. J. Zool., 32(2), 237-243.

Sharma, V.; Anderson, D. and Dhawan, A. (2012). Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). Apoptosis, 17, 852-70.

Shukla, V.; Dhankhar, M.; Prakash, J. and Sastry, K. V. (2007). Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. J. Environ. Biol., 28:395–397.

Singh, M.; Sandhir, R. and Kiran, R. (2011). Effects on antioxidant status of liver following atrazine exposure and its attenuation by vitamin E. Exp. Toxicol. Pathol., 63 (3),(269 - 276).

Singh, N.; Manshian, B. and Jenkins, G. J. S., (2009). Nano genotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials, 30, 3891–3914.

Song, W.; Zhang, J.; Guo, J.; Zhang, J.; Ding, F.; Li, L., and Sun, Z. (2010). Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicol. Lett. 199, 389-397.

Stegeman, J. J.; Brouwer, M.; Richard, T. D. G.; Forlin, L.; Fowler, B. A.; Sanders, B. M. and van Veld, P. A. (1992). Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 235_/335.

Trevisan, R.; Ranasinghe, P.; Jayasundara, N. and Di Giulio, R. T. (2022). Nanoplastics in aquatic environments: impacts on aquatic species and interactions with environmental factors and pollutants. *Toxics*, *10*(6), 326.

Tsangaris, C.; Kormas, K. and Strogyloudietal, E. (2010). Multiple biomarkers of pollution effects in caged mussels on the Greek coastline. Compar Biochem Physiol Part C: Toxicol Pharmacol., 151, 3, 369 -378.

Ulukaya, E. (2007). Biyokimya. 3. Baskı, Nobel Yayın Dağıtım. s.536, İstanbul.

Vali, S.; Mohammadi, G.; Tavabe, K. R.; Moghadas, F. and Naserabad, S. S. (2020). The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (Cyprinus carpio): Bioaccumulation, hematology, serum biochemistry and immunology, antioxidant enzymes, and skin mucosal responses. *Ecotoxicology and environmental safety*, *194*, 110353.

Vander Oost, R.; Beyer, J. and Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol., 13: 57-149.

Vijayan, M. M.; Cristina, P.; ereira, E.; Grau, G. and Iwama, G. K. (1997). Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comp Biochem. Physiol., 116C: 89 –95.

Vutukuru, S. S. (2003). Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton). Bulletin of environmental contamination and toxicology, 70(1), 0118-0123.

Ward, J. E. and Kach, D. J. (2009). Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Marine Environmental Research, 68(3), 137–142.

WHO (2011). The risks and benefits of fish consumption. Report of the joint FAO/WHO expert consultation, 25-29 January 2010, Rome, Italy (No. FIPM/R978 (En)). World Health Organization. p. 50.

Young, I. S. and Woodside, J. V. (2001). Antioxidants in health and disease. J. Clin. Pathol. 54, 176–186.

Zhu, S.; Oberdörster, E. and Haasch, M. L. (2006). Toxicity of an engineered nanoparticle (fullerene, C60) in two aquatic species, Daphnia and fathead minnow. J. Mar .Environ. Res., 62, 5–9.