

TOXICOGENOMICS OF NANOPARTICLE IN FRESHWATER FISH

Submitted in partial fulfillment of the requirement for the award of
Bachelor of Technology degree in Biotechnology

By

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **POTTI BHOO MIKA LEKHYA** (Reg. No.: 39230006), who carried out the project entitled "**TOXICOGENOMICS OF NANOPARTICLES IN FRESH WATER FISH**" under our supervision from **August 2022 to April 2023**.

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ABSTRACT

Due to its unique properties, including anti-corrosion, anti-bacterial, having low electron conductivity, and having great heat resistance, zinc oxide plays a significant role in modern industry. Therefore, the goal of this study is to synthesize zinc oxide nanostructures utilizing the ionotropic gelation process in the most useful methods possible and to characterize the nanostructures. The simplest method is ionotropic gelation, which may control particle size and morphology by methodically observing reaction parameters. Zinc acetate dehydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), sodium hydroxide (NaOH), and distilled water were employed as the medium for the ionotropic gelation process that produced ZnO nanoparticles. XRD, EDAX, FESEM and TEM were used to characterize ZnO nanoparticles. The elemental composition of the prepared ZnO nanoparticles was analyzed using Energy-dispersive X-ray spectroscopy (EDAX). The EDAX spectrum confirms the presence of Zn and O in the prepared sample. The results of the EDAX evaluation demonstrate that the ZnO nanoparticles have good purity with (zinc content 55.38%, oxygen content 44.62%). The XRD result spectrum, as shown, shows mainly oxygen and zinc peaks, indicating natural crystallinity. FESEM images show that the synthesized ZnO has a rod-like structure. The resulting ZnO nanoparticles are uniform and consistent in size, consistent with the XRD results showing good crystallinity. ZnO nanoparticles were successfully synthesized by ion-channel gelation in the Nano size range from 81.28 nm to 84.98 nm.

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LIST OF ABBREVIATIONS

ACRONYM	- EXPANSION
ZnO	- Zinc oxide nanoparticle
LC50	- Lethal concentration
XRD	- X-ray diffractometer
FE-SEM	- Emission scanning electron microscope
TEM	- Transmission Electron Microscope
FTIR	- Spectrum of ZnO nanoparticle
$\text{Zn}(\text{CH}_3\text{CO})_2 \cdot 2\text{H}_2\text{O}$	- Zinc acetate dihydrate
NaOH	- Sodium hydroxide
ICP-MS	- Coupled plasma atomic emission spectroscopy

CHAPTER 1

1. INTRODUCTION:

The word nano has been gotten from a Greek expression "nanos" significance overshadow. Nanotechnology has been momentarily characterized as planning, portrayal, creation and use of designs/gadgets whose shape and size are confined to nanometer (nm) level. Nanoparticles (NPs) have different applications in a few business items viz. gadgets, beauty care products, drug conveyance, medication, agribusiness, industry and ecological remediation. As indicated by a gauge there exist around 1015 purchaser items produced using nanomaterials. Designed nanoparticles (ENPs) are another class of natural pollutants that can possibly hurt earthly environments, the hydrosphere, and the air. ENPs can influence amphibian life, change the science of the dirt, and move to surface or ground water. Their stress on the biological system is additionally expanded by squander water effluents, spillover from downpour, and inadvertent spills. Their conduct in amphibian biology is affected by actual cycles such accumulation, disintegration, retention, and surface change. Warmth, pH, saltiness, and delicate quality are normal ecological elements that influence a life form's entry into biogeochemical cycles. Besides, the presence of regular natural material (NOM) can adjust the bioavailability, electrostatic cooperations, and steric shock of ENPs when they collaborate with oceanic biota.

1.1 TOXICOGENOMICS

Toxicogenomic is a term, how a genome reacts to contaminants and stresses (water et al. 2003). It integrates traditional toxicology with genomics, transcriptomics, proteomics, metabolomics, and bioinformatics Three main objectives of toxicogenomic are as follows (Waters et al., 2000). Recognize the link between environmental stress and the vulnerability of people to illness. Identify useful biomarkers for illness and toxic chemical exposure. Describe the toxicity's molecular origins.

1.2 THE FUTURE OF TOXICOGENOMICS

Genomic innovations offer an opportunity to assess the dangerous connection between an individual's openness to the climate and their gamble of fostering an infection. Find biomarkers of looming destructive impacts that are more exact and delicate than those that are presently available. Lay out a coherent starting point for risk evaluation. Consider the more straightforward ID of specific powerlessness polymorphisms and their association with ecological illnesses.

1.3 NANOPARTICLES

An overall meaning of a nanoparticle (NP) is a molecule having no less than one aspect between 1 nm and 100 nm with extraordinary qualities that are unmistakable from those of the substance where it is created. They have a few expected involves in mechanical designing, hardware, transportation and correspondences, imaging, tidying up toxins, and compound and organic sensors, as well as medication and quality conveyance frameworks (Buzea et al. 2007) NPs are used in a wide range of consumer products. For instance, nanoscale silver particles (Ag NPs) are progressively utilized in socks, home devices, water therapy, clinical gear, and sterilization in view of their antibacterial characteristics. There are many nanotechnology-put together merchandise with respect to the market at this moment, and the quantity of new items in the light of nanotechnology is constantly developing rapidly. Its commercialization of nanoparticles is growing rapidly, which can expand its openness to the climate and coming about conceivable natural strain. The improper removal of nanoscale things could bring about the presentation of another wellspring of contamination to the oceanic climate, which could imperil amphibian animals given the quickly developing nanotechnology organizations. Through the sewage framework, numerous nano-sized merchandise will track down their direction into freshwater and marine conditions. For example, washing garments may rapidly deliver Ag NPs, and weighty downpours can rapidly deliver Ag NPs off building veneers. The surrounding fixations assessed by the openness models are projected to be during the tens to many ng L⁻¹ territory in

water (Tejamaya et al. 2012).

One of the huger components of sea-going biological systems is fish, which likewise offers the principal pathway for human openness to and bioaccumulation of NPs. A portion of the impacts that NPs are utilizing on fish might be an impression of their expected threats to individuals and different vertebrates (Dorea. 2008). Various exploration has been led considering NPs' harmfulness to fish, and a lot of information has been accumulated on the natural effects of NPs from various points. The chance of their natural movement being communicated in vivo is brought about by the retention and bioaccumulation of NPs in many fish organs, such as the gill, liver, brain, and digestive tracts. Different components, including processes, that are related with the vulnerabilities welcomed on by activity, characterization of the openness measurements, portion reaction connections, and the physicochemical changes that the NPs displayed over the entire openness process.

Theoretically, NPs can be delivered from any substance might be utilized to make NPs, albeit the greater part are produced from progress metals, including silicon, carbon (counting carbon nanotubes and fullerenes), and metal oxides. Through the course of vectorization, they can cling to different impurities in a waterway. They simpler to tie to both inorganic and natural mixtures because of their incredibly high surface to volume proportion. ENPs can likewise be biomagnified through the established pecking order and bio transformed by ocean microorganisms. Thus, fathoming the unsafe signs of ENPs in sea-going animals than in other exploratory animals is significantly really testing.

Because of their special ecological way of behaving, like collection, disintegration, and different qualities of the NPs, there are as yet a few discussions on the progressions in openness dose, morphology, and other characteristics changes to the surface science and structure, and so on. Various points have been utilized to inspect different discoveries. This section will give an outline of late exploration in the field, which might support enlightening both general and specific revelations about the impacts of nanotoxicology on Fish.

1.4 UPTAKE AND ACCUMULATION OF ENGINEERED NANOPARTICLES IN FISH

The amphibian environment is one of the final nanoparticle sinks, therefore potential hazards from NPs may be welcomed on by their retention and bioaccumulation in living creatures. Unmodified NPs typically do not disperse well in water. The oceanic compartment often has NPs in the form of a colloidal suspension. They tend to accelerate out of arrangement as totals that can arrive at a few micrometers in size. Normal natural macromolecules can possibly stop or dial back this pattern (Griffith et al. 2008). Unmodified metal oxides have a lower bioavailability because of NP conduct, for example, conglomeration and collaboration with natural material, which limits their retention and bioaccumulation in oceanic biota. Particle carriers are not expected to be engaged with the organic retention of NPs; in any case, endocytosis is a possible system. The most likely NP openness components for fish in an oceanic environment included feeding and direct openness through the fish's skin, respiratory system, or chorion of developing organisms. as per concentrates on the retention and bioaccumulation of produced nanoparticles. The essential openness pathway for NPs in the gastrointestinal frame Work is comprised of watery openness, direct dinner consumption, and trophic exchange through pecking order (Li et al. 2009) delineates the portion related development of dark iron particles in the stomach when grown-up medaka were presented to nano- iron in water for 14 back to back days, exhibiting the use immediate taking care of impact on NPs assimilation from the water. In the wake of being uncovered Fullerene(C60) and single-walled carbon nanotubes (SWCNT) were both seen as being ingested by amphibian species in the water segment (Oberdorster et al. 2005). Trophic transmission of NPs is currently receiving a great deal of research attention as a wise area for NPs ecological fate. To reveal insight into this, numerous species in the sea-going framework, like fish, phytoplankton, and zooplankton, should be examined. The likely dangers to people may at last outcome from the biomagnification of NPs in the oceanic pecking order Zhu et al. discovered that freshwater pecking orders with both low and high trophic level animals may be used in recreation studiosto see whether

nanoscale TiO₂ particles could be transferred from daphnia (*D. magna*) to zebrafish (*D. retio*). There were no biomagnifications of this type of NPs in this pecking order, as evidenced by the biomagnification components' quality being below

However, TiO₂ NP body weights were larger in food-openness clusters than in watery openness clusters, indicating that dietary use may address a crucial avenue for potential nanomaterial openness for seagoing organisms at a higher trophic level (Zhu et al. 2010). Another study conducted by Cederval et al. (2012) revealed the emergence of synthetic polystyrene nanoparticles. through the natural pecking order, from green growth to zooplankton to fish. When contrasted with the control, the top customer in the nanoparticle openness bunch showed modified lipid digestion and eating conduct, exhibiting the unfavorable effects of NPs on higher trophic level species along the pecking order (Cederval et al. 2012). The fish's respiratory framework gives the biggest contact surface to the sea- going NPs. Sea-going NPs may doubtlessly stick and collect in gill tissues because of direct openness. As indicated by Li et al. (2009)'s review, abundant dark iron particles were consumed by the medaka gills when they were presented to oceanic nano-iron, coming about in histopathological changes in the gill tissues. As an energizer in oceanic frameworks, NPs might make fish bodily fluid be discharged in the gills, which will prompt the conglomeration of particles in the tissues' target. According to Smith et al. (2007), newly dispersed SWCNT may quickly grow in the rainbow trout's gills, producing nanoparticle totals linked to the mucoprotein. An engaging technique for concentrating on the in vitro assimilation of NPs by the fish respiratory framework is the gill cell model. We observed that administered Ag NPs were taken up by rainbow trout gill cells. Various types of nanoparticle coatings were related to silver absorption and transport rates over refined epithelial layers (Farkas et al. 2011).

In the essential take-up pathways for amphibian NPs might be displayed in examinations on the NP dispersion in grown-up fish, which uncovered that NPs are oftentimes observed to be significantly packed in the gills and stomach. The presence of NPs in different tissues, including the blood, liver, and

cerebrum, gives proof that NPs are shipped all through the organs. An examination investigation of sodium selenite and selenium nanoparticles uncovered hyperaccumulation of the nanosized structure in the liver, gills, muscles, and whole body of the medaka fish, demonstrating the precise effects of nanoparticle retention in fish (Li et al. 2008). Numerous NP bioaccumulation routes have also been discovered. Zebrafish could accumulate CuO NPs, as shown by Griffitt et al. (2007), but under a scope of openness settings, fish tissues ingested next to no TiO₂ or other metal oxides. In any case, rainbow trout kidneys consumed huge measurements of intravenously conveyed TiO₂ NPs (Scown et al. 2009). The bioavailability of unaltered metal oxides is diminished because of NP conduct, for example, total and collaboration with organic material, which thus limits the admission of these substances by fish. Little degrees of unaltered metal oxide NP retention in fish do occur, regardless of whether bioavailability is compelled. They can get gathered NPs that have settled to the riverbed or sea floor for benthic or pelagic fish. Rather than pelagic, nonfilter-taking care of species that live higher in the water section, channel taking care of animals effectively catch particles from the water segment (Johnston et al. 2010).

An especially delicate model, the fish undeveloped organism, might be utilized to evaluate the formative poisonousness of NPs. For a wide range of nanomaterials, the retention and scattering of NPs in fish undeveloped organisms is a general peculiarity (Lee et al. 2007). It is accepted that the break of the chorion obstruction or the launch of chorionic pores is the essential technique for NP ingestion by the undeveloped organism. As per Lee et al. (2012), a solitary Ag NP (30-72 nm) diameter could accidentally enter the developing organisms through chorionic pores and remain there throughout the course of the development of the organism (120 h post preparation).

According to research on NPs, they can be found in the chorion, oil droplets, yolk, and nerve bladder of the immature organism (Kashiwada 2006). According to a model developed by researchers (Kashiwada 2006; Lee et al. 2007), nanoscale particles were discovered to be picked up by the medaka chorion before moving into the yolk sac and body. Moreover, different

examinations shown that nanoparticles could enter creating zebrafish through chorion pore waterways After being transported through the chorion layers and internal mass, the NPs were constrained in the zebrafish egg's blood, mind, heart, and yolk. Both supported the ingestion and take-up are the focus of transmission electron microscopy (TEM) and trial neutron enactment (Bar-Ilan et al. 2009; Laban et al. 2010); (Asharani et al. 2008; Lee et al. 2007). In the real world, NPs may coexist alongside various contaminants. The impact of C60 totals on sea-going harmfulness and bioaccumulation of other naturally critical contaminations was uncovered by Baun et al. (2008). Because of their different bioavailability's, the sub- atomic structure of the solvent contamination might fluctuate the poisonousness welcomed on by nanoparticles. Therefore, describing NPs in fluid suspension and collaborations with different substances are urgent for assessing nano- chances. To survey NPs' poison levels, one should know about fish retention as well as their biodistribution all through tissues, cells, and subcellular compartments.

1.5 ENGINEERED NANOPARTICLES' SYSTEMIC TOXICITY TO FISH

Rapid growth in the use of nanoscale materials is accompanied by growing concern over nanotoxicity brought on by accidental and occupational exposure. Some people are upbeat about how NPs will affect biological processes. For example, Zhu et al. (2006) demonstrated that fathead minnow exposed to 0.5 g mL⁻¹ C60 while stirring for 48 hours did not exhibit any obvious physiological effects. Meanwhile, opposition voices are becoming increasingly louder in view of potential environmental dangers brought on by NPs. Numerous articles have offered copious evidence to demonstrate the many toxicities that NPs may cause. The most evident and obvious effect that so many NPs may have is lethality. Numerous reports of additional consequences in animals include reversible testis destruction, immunotoxicity, neurotoxicity, renal toxicity, and pulmonary injury. Developmental, Geno, and immunotoxicity as well as other toxicities are largely explored in relation to aquatic nanotoxicity utilizing fish as a model.

CHAPTER 2

2. REVIEW OF LITERATURE

2.1 DEVELOPMENTAL TOXICITY

In research on ecological toxicology, the fish undeveloped organism and hatchlings are much of the time used as the touchy model for evaluating formative harmfulness. Various examinations have been finished to decide the formative harmfulness of specific produced metal NPs. In zebrafish undeveloped organisms presented Critical alterations, including heart malformations, yolk sac edema, and eye/head irregularities, were observed in response to Ag NPs at concentrations between 0.02 nM and 0.2 nM. Among the effects of Ag NP openness were yolk sac edema with enlarged and augmented yolk sac head edema, extreme eye mutations with relating increment (little eyes) and asymmetric eyes, strange tail, and spinal line flexure with almost 180degree twist, and fanfold distortions with scattered and unusually organized fin folds. When different Ag NP kinds were applied to fish models, this formative poisonousness was evident. Additionally, it is suggested that the early-stage formative courses were closely related by the way that various different types of misshapen lngs, such as fin fold, tail, and yolk sac edema, routinely coincided with one serious deformity (such as the heart, eye, or head) in the distorted fishes (Lee et al. 2012). Similar outcomes were achieved in various further research projects. Olivia and colleagues (2012) found that Ag NPs caused morphological deformities in creating zebrafish undeveloped organisms, like bent tails, modest heads, and a more modest yolk sac. In the yolk syncytial layer, metallothionein 2 (Mt2), a weighty metal pressure reaction quality, showed a significant increment (Chen et al. 2004). Molecule size and dose are two factors that might be essential for the poison levels. Additionally, it is suggested that the early stage formative courses were closely related to one another. Additionally, it is suggested that the early stage formative courses were closely related to one another by the way that various different types of miss happenings, such as fin fold, tail, and yolk sac edema, routinely coincided with one serious deformity (such as the heart, eye, or

head) in the distorted fishes (Lee et al. 2012). Similar outcomes were achieved in various further research projects. Olivia and colleagues (2012) 75-91% of incipient organisms might develop into solid fish. The portion range where distortions could happen was between 0.02-0.2 nM.

Notwithstanding zebrafish, a few extra fish models have been utilized to concentrate on the formative harmfulness of Ag NPs broadly. When undeveloped medaka fish (*Oryzias latipes*) and fathead fish (*Pimephales promelas*) were subjected to Ag NPs at the minnow stage, significant morphological abnormalities, delayed improvement, and edema were seen. (Laban et al. 2010; Wu et al. 2010) uncovered in medaka undeveloped organisms and hatchlings the ordinary misshaping impacts welcomed on by openness to Ag NP. Slow blood stream, a hazy yolk sac, draining in numerous region hemostasis in the Cuvier conduits and of the larval body among these irregularities. Pericardial edema and cylindrical heart were side effects of serious flow peculiarities. All Ag NP-uncovered bunches showed a high pervasiveness of eye irregularities, including microphthalmia, anophthalmia, cyclopia, exophthalmia, and anophthalmia. In the wake of being presented to the best centralization of Ag NPs, torn egg cortex was the most serious injury that was seen. The osmotic sicknesses (yolk sac, head, heart, and gallbladder edema) were the most frequently seen hurt in fish undeveloped organisms presented to NP, and they were constantly present close by additional serious anomalies. Edema is a profoundly normal event in a few fish undeveloped organisms. Presented to Ag nanoparticles (Laban et al. 2010, Laban et al. 2009, Asharani et al. 2008, Bar-Ilan et al. 2009, Lee et al. 2007). It is thought to be brought on by a problem with osmoregulation (Kiener et al. 2008). At the point when these metabolic and physiological cycles are hampered at a vital point in the early improvement of the medaka, typical embryogenesis can be eased back, which might prompt various irregularities. As per Peters et al. (2007), a large number of the irregularities found in fish undeveloped organisms might be brought about by an upset circulatory framework and osmoregulation. These NPs might go about as nucleation locales, gather with approaching NPs to make greater totals, block chorion pore channels, and

disrupt film transport in the event that they are stuck (Lee et al. 2007). As both an outcome, the chorion loses its capacity to keep a mechanical hindrance and the osmotic equilibrium is upset, causing edema and formative irregularities in the medaka undeveloped organisms treated with Ag NP. As indicated by the biochemical review, openness to Ag NP at the beginning phases of early-stage advancement prompted oxidative pressure, which might have additionally added to the improvement of the disfigurement. There are reports that other made NPs can likewise actuate formative poisonousness notwithstanding Ag NPs. For example, broke up C60 at 1.5 mg L⁻¹ focus adjusted early-stage advancement, eased back the speed of incubating, and caused edema in hatchlings (Zhu et al. 2007; Cheng et al., 2009). It was acknowledged that osmoregulation and circulatory system disturbance were the main causes of many of the fish anomalies. Further research should be conducted to determine the effects of formative interruption on fish while considering the recently developed NPs' capacity to pollute oceanic frameworks.

2.2 GENOTOXICITY

Genotoxicity is the term used to depict the capacity of synthetic specialists to change the hereditary material of a cell, prompting transformations. Considering that NPs might go through cell layers and enter the phone core, expanding harm to hereditary material is a huge concern NP poisonousness in organic medium is related. In this regard, the study of potential NP genotoxicity, including the impacts of various NP sizes and union procedures, is becoming more and more well-known (Lima et al. 2012). Ag NPs have received extensive research as common designed NPs using a few natural models. Using the comet test, DNA damage brought on by Ag NPs was discovered in a polychaeta living in the dregs. When compared to microAg and ionic-Ag treatment, nano-Ag treatment showed the most genotoxic influence, whilst the latter was the least genotoxic (Cong et al. 2011). Chromosomal abnormalities and aneuploidy were brought on by silver nanospheres with a breadth of 30 nm. in the medaka cell line. Medicines with this nano silver caused harms in 8, 10, 8, 16, and 15.8% of metaphases as

well as 10.8, 15.6, 24, and 24 all out deviations in 100 metaphases, separately (Savvy et al. 2010). Various levels of Ag NP openness prompted chromosomal anomalies like chromatid injury, isochromatic sore, chromatid trades, and centromere spreading. This outcome was predictable with different examinations that found titanium nanoparticles caused Rainbow trout cells exhibit genotoxicity (Vevers and Jha 2008). The discrepancy may result from the varied genotoxic potential of titanium versus silver. nanoparticles or the differing awareness of testing methods like the micronucleus and chromosomal abnormality measures. The guard against oxidative pressure welcomed on by openness to Ag NP includes CrGST qualities. The majority of them changed their guidance levels according to the varying openness dosages (0.2-1 mg L1) and timings (12-72 h). The best mRNA articulation was seen in the Delta3, Sigma4, and Epsilon1 GST classes, confirming their efficacy as NP openness biomarkers (Prakash et al. 2011). It was discovered that just a small number of other anticipated NPs might influence how the objective quality was released. When larval zebrafish were exposed to C60 suspension (with water as the dissolvable), eight qualities were down-controlled, and three of them were connected to electrical explanation to well-known capabilities. The intensity shock quality is modified, which recommends a boundless pressure reaction. Down- guideline of the RNA restricting element quality and the Kruppel-like variable (KLF) quality shows that record is adjusted all around the world in light of C60 openness. The Sp1 arrangements that the KLF group of record factors ties to have been connected to apoptosis, angiogenesis, and tumor genesis as well as development related signal transduction pathways. The humble effects of the openness situation utilized in this study's examination might be deduced from the minuscule modifications in worldwide quality articulation seen in zebrafish hatchlings presented to C60. Different results might be seen with longer C60 dosages or other openness settings (various species, life history stages). Henry et al. 2007, p. Fish models are frequently used to evaluate how harmful natural contaminants are, which increases the risk to human health. Genotoxicity welcomed on by NPs in a fish model might conceivably affect the two people and other oceanic creatures. Understanding the component of

genotoxicity as to molecule assimilation and disintegration is the focal point of additional review. The impact of changing the molecule's size and functionalization on genotoxicity is likewise thought about.

2.3 IMMUNOTOXICITY

The resistant framework can recognize contaminations and other unfamiliar items that enter the body. The insusceptible framework is equipped for recognizing NPs too. Whenever NPs have entered the fish by the gill, skin, or blood, they will cooperate with the safe framework. NPs are taken up by resistant cells through endocytic and phagocytic pathways and are then put away in the cells' endosomes, lysosomes, and phagosomes. Neutrophils were displayed to phagocytose fullerene through the connection and actuation of FC receptors (Moore 2006; Dobrovolskaia and McNeil 2007). (Moore 2006; Dobrovolskaia and McNeil 2007). Neutrophil phagocytosis is unaffected by NP transporter size because neutrophils may unquestionably phagocytize NP totals up to 3 μ m. (Kumazawa et al. 2002). It has been seen that nanoparticles influence safe cell movement and disrupt the inborn insusceptible framework's transformative conducted research on ecological toxicology, the fish undeveloped organism and hatchlings are every now and again used as the touchy model for evaluating formative poisonousness. Various examinations have been finished to decide the formative poisonousness of specific made metal NPs. In zebrafish undeveloped organisms presented to huge deformities, including as heart anomalies, yolk sac edema, and eye/head irregularities, were observed in Ag NPs at concentrations between 0.02 nM and 0.2 nM. The impacts of Ag NP openness included yolk sac edema with enlarged and expanded yolk sac head edema, extreme eye contortions with relating increment (little eyes) and dissymmetric eyes, strange tail and spinal rope flexure with almost 180-degree twist, and finfold deformations with muddled and unusually organized finfolds. This kind of formative harmfulness showed itself in fish models exposed to several Ag NP kinds. Additionally, it is suggested that the early stage formative courses were closely related to one another by the way that various different types of misshapenings, such as finfold, tail, and yolk sac edema, occasionally

coincided with one serious disfigurement (such as the heart, eye, or head) in the distorted fishes (Lee et al. 2012). Comparative outcomes were attained in many additional examinations. Ag NPs, according to Olivia et al. (2012), led to morphological changes. imperfections in creating zebrafish undeveloped organisms, like turned tails, modest heads, and a more modest yolk sac. In the yolk syncytial layer, metallothionein 2 (Mt2), a weighty metal pressure reaction quality, showed an extensive increment (Chen et al. 2004). Molecule size and dose are two factors that might be critical for the poison levels. As indicated by Kerry et al. (2012), the standard unsafe effect of Ag NPs on the development of zebrafish incipient organisms is portion subordinate. When presented to Ag NPs at focuses lower than 0.02 nM, 75-91% of incipient organisms might develop into sound fish. The portion range where disfigurements could happen was between 0.02-0.2 nM. The lethal effect prevailed when the openness measurements surpassed 0.2 nM. (Lee et al. 2012).

Notwithstanding zebrafish, a few extra fish models have been utilized to concentrate on the formative harmfulness of Ag NPs broadly. At the point when undeveloped fathead minnows medaka fish and (*Pimephales promelas*) (*Oryzias latipes*) were presented to Ag NPs, huge morphological anomalies, postponed advancement, and edema were seen (Laban et al. 2010; Wu et al. 2010) uncovered in medaka undeveloped organisms and hatchlings the typical twisting impacts welcomed on by openness to Ag NP. Slow blood stream, an obscure yolk sac, draining in numerous regions of the larval body, and hemostasis in the channels of Cuvier is among these irregularities. Pericardial edema and rounded heart were side effects of extreme flow oddities. All Ag NP-uncovered bunches showed a high commonness of eye irregularities, including microphthalmia, anophthalmia, cyclopia, exophthalmia, and anophthalmia. In the wake of being presented to the best convergence of Ag NPs, torn egg cortex was the most serious injury that was seen. The osmotic diseases (yolk sac, head, heart, and gallbladder edema) were the most frequently seen hurt in fish undeveloped organisms presented to NP, and they were constantly present close by additional extreme anomalies. Edema is an exceptionally normal event in a few fish undeveloped organisms.

Presented to Ag nanoparticles (Laban et al. 2010, Laban et al. 2009, Asharani et al. 2008, Bar-Ilan et al. 2009, Lee et al. 2007). According to Kiener et al. (2008) it is thought that an osmoregulation problem is what brings it on. Whenever these physiological and metabolic cycles are interfered with during a crucial stage of the medaka's early development, conventional embryogenesis can be eased back, which might prompt various irregularities. As indicated by Peters et al. (2007), large numbers of the irregularities found in fish undeveloped organisms might be brought about by an upset circulatory framework and osmoregulation. These NPs might go about as nucleation locales, collect with approaching NPs to make greater totals, block chorion pore trenches, and impede layer transport in the event that they are stuck (Lee et al. 2007). As both an outcome, the chorion loses its capacity to keep a mechanical hindrance and the osmotic equilibrium is upset, causing edema and formative irregularities in the medaka undeveloped organisms treated with Ag NP. As indicated by the biochemical review, openness to Ag NP at the beginning phases of undeveloped advancement prompted oxidative pressure, which might have likewise added to the improvement of the deformation. There are reports that other produced NPs can likewise actuate formative harmfulness notwithstanding Ag NPs. For example, disintegrated C60 at 1.5 mg L⁻¹ fixation modified early stage improvement, eased back the speed of incubating, and caused edema in hatchlings (Zhu et al. 2007); Cheng et al. (2007). It was accepted that a large number of the fish irregularities were brought about by osmoregulation and circulatory framework disturbance. While pondering the recently made NPs' capability to contaminate oceanic frameworks, further examination should be finished to test for the impacts of formative disturbance on fish

Recession of the arrival of neutrophil extracellular snares (NETs) and essential granule degranulation (up to a 40-half drop in movement contrasted with control). Interleukin11 and myeloperoxidase quality record was upgraded by hydroxylated fullerenes and TiO₂ treatment in fish (12-and 3-overlay increments, separately; 5-crease declines) (2011) and (2012), Jovanovic et al; These findings are consistent with observations made in resistant cells

exposed to Au NPs (Bartneck et al. 2009) . Recently, it was shown that single- and multi-walled carbon nanotubes can stimulate. Rainbow trout safe cells exhibit the IL-1 trait (Klaper 2010). Young largemouth bass (*Micropterus salmoides*) and fathead minnows (*P. promelas*) communicated CYP2 family isozymes in their livers subsequent to being upset in water, which additionally supported LPO in the cerebrum and impressively expanded LPO in the gill (Oberdoster 2004) Metallothionein A (MT-A), Na/K ATPase, and Heat Shock Protein 70 (HSP 70) were chosen for quantitative quality articulation analysis by qPCR as sensitive markers of general pressure, metal permeability, and osmoregulatory detrimental effect. HSP 70's recruitment suggests an unavoidable pressure reaction. According to reports, when fish are exposed to TiO₂ NPs, oxidative pressure occurs, causing irritation, cell damage, and hereditary harm. Increased plasma glucose, decreased plasma Na and Cl, and altered gill MT-A, Na/K ATPase, and HSP70 quality expression were all clearly impacted (Lee 2007; Scown et al. 2009). Collected Ag welcomed on fixation subordinate reaction expansions in common pressure markers, such as plasma glucose and HSP 70 gill quality articulation (Farmen 2012).

2.4 LIPID METABOLISM DISRUPTION

The lipid peroxisome is a biomarker for damage in living things after sublethal openings. According to Imanaka et al. (2000), the peroxisomal films contain PMP70, a carrier protein for long-chain acyl Co-A_n unsaturated lipid. Modifications in this protein may be a sign of changes in cell flagging linked to lipid digestion. PMP70 levels in fathead minnows were shown to be significantly decreased in an investigation after 96 hours and 0.5 g mL⁻¹ of nC60 exposure. While the CdSe/ZnS QDs-uncovered fish did not significantly differ in hepatic all-out glutathione, lipid peroxidation, or the expression of qualities involved in metal digestion (Blickle et al. 2014), lower levels of PMP70 may indicate less lipid transport into peroxisomes and further restraint of the maintenance cycle.

A quality of adipocyte improvement is intracellular lipid development. As per reports, morphological modifications and an adjustment of quality

articulation designs happen during cell separation (Rosen 2000). An earlier examination checked that HTT2800 essentially influences the gathering of intracellular lipids according to Tsukahara and Haniu (2011) in C2C12 cells.

2.5 ENGINEERED NANOPARTICLES ARE SPECIFICALLY TOXIC TO TARGET ORGANS IN FISH

These exceptional mixtures might be retained, shipped, and bioaccumulated in various tissues when the fish is presented to a sea-going climate that has been tainted with NP, prompting various natural outcomes and poison levels. Fish presented to NPs have given indications of an assortment of sublethal impacts, for example, respiratory harmfulness, changes to follow components oxidant pressure and Na^+/K^+ ATPase restraint in tissues. NP openness has the potential to promote infections in a number of organs, including the gill, liver, hematologic system, vision and visual system, and mind.

2.6 RESPIRATORY TOXICITY

The fish's gill serves as the first line of defense, acting as a channel to sift out potentially harmful combinations and particles. Numerous studies have revealed that the gill is the primary objective organ of NPs given to fish (Scown et al. 2010; Federici et al. 2007; Griffitt et al. 2007). NPs can obstruct the gill epithelium's outer layer and lead to osmoregulatory poisoning. Gill damage happened after being exposed to Cu NPs at 0.1 mg L⁻¹ or higher (Griffitt et al. 2007). Rainbow trout that had been presented to SWCNT had gill pathologies that were portion subordinate, including edema, modified mucocytes, bodily fluid creation, both hyperplasia and (Smith et al. 2007). After being exposed to TiO₂ NPs, the lamellae thickened and produced edoema (Federici et al. 2007). Global quality articulation research in the

Gills of zebrafish revealed that exposure to Ag (1 mg L⁻¹), Cu (0.1 mg L⁻¹), and TiO₂ (1mg L⁻¹) NPs resulted in innovative quality articulation designs and showed that they elicited various natural reactions via various routes (Griffitt et al. 2009). Interestingly, a focus subordinate concealment of Na^+/K^+

ATPase articulation uncovered that when the fish were treated with 20 g L⁻¹ Ag NPs, unfortunate osmoregulation happened. The excitement of the metallothionein A quality in adolescent Atlantic salmon recommended that Ag was caught up in the gills. TiO₂ NPs likewise decreased Na⁺/K⁺ ATPase action, caused edema and thickening of the lamellae, and raised glutathione levels in the gills of rainbow trout (Federici et al. 2007). Cu NPs brought about significant Comparable results were seen in zebrafish presented to Ag NP (Griffitt et al. 2009). Intense gill lamellae corruption was set off by the business Ag NP arrangement at high focuses (100 g L⁻¹), which might have added Fish mortality has reached a critical level (73%) (Farmen 2012). In the RT-W1 fish gill cell line, Ag NPs were likewise found to have oxidative pressure impacts that were receptive to N-acetyl cysteine (George et al. 2012). Moreover, fish presented to different measures of silver particles showed a few similar side effects, proposing that ionic silver might have added to the poisonousness of Ag NPs (Lubick 2008).

2.7 HEPATOTOXICITY

The liver is the main objective tissue with regards with relation to the NP biodistribution in fish. According to research by Li et al. (2008), Se NPs have been found to be many times more hyperaccumulated in the liver than selenite. NPs on fish have been linked to liver damage, according to a few articles. According to Choi et al. (2010), Ag NPs generated histological anomalies in the fish liver tissues, such as hepatic cell rope breakdown and apoptotic alterations. Cu NPs produced numerous diseases in the liver of young rainbow trout, such as pyknotic cores or cytoplasmic vacuoles, minute foci of hepatitis-like cell damage, and an increase in melanomacrophage storage (Genan et al. 2012). In liver cells exposed to TiO₂ NPs, dense atomic patterns (apoptotic bodies), loss of sinusoid space, and foci of lipidosis with mild greasy alteration were seen (Federici et al. 2007). Fish liver tissue can encounter oxidative pressure and lipid peroxidation harm because of NPs. In the liver, P450 isozymes process endogenous substances and opposite lipid peroxidation harm. Fish presented to C60 NPs showed an up-guideline of P450 isozymes in the liver, demonstrating that According to Sayes et al.

(2004), C60 NPs may affect the fish liver by lipid peroxidation. While the PMP70 levels in fathead minnows were significantly reduced in a different review (Oberdorster et al. 2006), the P450 isozymes were not up-regulated. In peroxisomal films, PMP70, a protein that transports long-chain acyl Co-A unsaturated lipid, participates in lipid digestion. Changes in this protein reflect adjustments in cell flagging linked to lipid digestion. Lower levels of PMP70 may indicate impaired maintenance and reduced lipid transport into peroxisomes (Imanaka et al. 2000). Ag NPs decreased levels of the oxyradical-scavenging molecules catalase and glutathione peroxidase 1a in the liver of adult zebrafish while increasing levels of total glutathione and malondialdehyde as a result of cellular lipid peroxidation. Following Ag NP treatment, the proapoptotic properties of Bax, Noxa, and p21 were also up-regulated. According to these findings, Ag NPs stimulate oxidative pressure and hepatic passage in zebrafish (Choi et al. 2010).

2.8 OCULAR AND VISUAL SYSTEM TOXICITY

Au NPs were discovered to interfere with zebrafish eye growth and coloration. Zebrafish embryos subjected to 1.3 nm AuNPs with N, N,N-trimethyl ammonium ethanethiol ligand acquired smaller, malpigmented eyes (TMAT- AuNPs). In Cell death rose noticeably in the eye. According to Kim et al. (2013), this eye injury was caused by the inhibition of the genes *sox10* and *pax6a*, which are involved in pigmentation and eye development, respectively. According to our research, fish larvae treated with NP may have malformed eyes and eyeballs. Japanese medaka exposed to Ag NPs had their eyes' microscopic structure and shape significantly altered. The retinal pigment epithelium remained intact and thickened while the inner nuclear cell layer shrank and the ganglion cells became insufficient. The pale grey eyes (known as pge mutants) occurred with diminished black and silver pigments, as seen in Figure 3.3, and the inner segments of the retina were lacking (Wu et al., 2010). Other investigations have similarly found the toxicity of Ag NPs to fish eyes. For instance, Asharani et al. (2011) discovered that during the development of zebrafish embryos. The eyes were missing or distorted as a result of the capping agent polyvinyl alcohol (PVA) utilized in Ag NPs.

2.9 NEUROTOXICITY

It is considered that NPs may choose to ship beyond the fish's blood-mind barrier. The thickness of the mesencephalon layers, the vein on the ventral surface of the cerebellum, and a few minor changes in the nerve cell bodies were seen following NP exposure. Rainbow trout minds that were given to Cu NPs exhibited irregular necrotic cells between the sub-atomic and granular layer of the cerebellum cortex and enlarged vein width on the ventral side of the cerebellum in the metencephalon (cerebellum) areas (Genan et al. 2012). With the exception of a few necrotic cells in the frontal cortex and some obvious vein expansion, exposure to TiO₂ NPs did not significantly alter that state of mind (Boylea et al. 2013). In any case, the treatment diminished the measures of the metals Cu and Zn in the cerebrum, and there was a propensity toward decreased compound movement. The fixation subordinate increments (twice or more) in thiobarbituric corrosive receptive mixtures (Ski lifts) were noticed (Federici et al. 2007). The consequences of the SWCNT treatment and the TiO₂ NPs treatment were indistinguishable (Smith et al. 2007). The largemouth bass' mind was impacted by lipid peroxidation brought about by C 60 NPs (Oberdoster 2004). Cu NPs fundamentally adjusted plasma and tissue Na⁺ focuses, diminished Na⁺/K⁺

ATPase movement, and impacted the rainbow trout cerebrum's capacity to battle oxidative pressure (Genan et al. 2012)

CHAPTER 3

3. AIM AND OBJECTIVE

The present study aims to prepare ZnO nanoparticles using standard technique and determined their acute toxicity level in Guppy fish (*Poecilia reticulata*).

CHAPTER 4

4. MATERIALS AND METHODS

4.1 CHEMICALS USED

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), sodium hydroxide (NaOH), beaker, magnetic stirrer, hot air oven, muffle furnace, FTIR machine, EDX, UV spectrophotometer.

4.2 ZINC OXIDE NANOPARTICLE SYNTHESIS (ZnO)

0.1M of zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) in 1000ml beaker add 200ml of distil water in beaker add a solvent 3.66 g in the beaker. 0.2 M of sodium hydroxide (NaOH) in 250 ml beaker add 200ml of distill water in beaker add a solvent 1.6g in the beaker. The beaker was placed in a magnetic stirrer for 15 minutes, one at a time, and after the zinc acetate solution had been stirring for 15 minutes, sodium hydroxide was added drop by drop. After 3 hours of stirring, a milky white solution was developed. The supernatant and precipitate should separate after being left in room temperature for the entire night. The precipitate should be placed in a hot air oven until it dries into a solid (powder form). After that, move the power from the beaker to the crucible bowl and hold it there for 4 hours while it is being calcined at a temperature of 600 degrees in muffle furnace. The zinc oxide nanoparticles (ZnO) are prepared. characterization of ZnO nanoparticles.

1. UV – visible spectroscopy
2. X-ray diffraction analysis. (XRD)
3. Scanning electron microscope. (SEM)
4. Transmission Electron Microscope
5. FTIR (Fourier transform infrared rays)

4.3 SYNTHESIS OF ZNO NANOPARTICLES

One gram of zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$] was dissolved in 50 mL of deionized water (A), followed by 4 mg of NaOH pellets in 50 mL of ethanol (B). Furthermore, solutions A and B were thoroughly mixed for 3 hours at 70 °C using magnetic stirring. During the heating process, the color changed to milky white, and a precipitate formed. To remove any impurities, the precipitate was centrifuged at 5000 rpm for 30 minutes and washed three times with absolute ethanol followed by double deionized water. The final sediment was dried on a hot plate at 60 °C for 20 minutes before being ground into powder with a mortar and pestle. Finally, the powder was annealed for 4 hours at 800 °C to produce crystalline ZnO NPs.

4.4 Characterization of Zinc Oxide nanoparticles

Chemically synthesized nanoparticles were characterized to confirm the various properties by UV – Visible spectroscopy, FTIR, XRD, TEM, SEM with EDAX and FTIR.

4.5 UV visible spectroscopy

The optical absorption properties of ZnO were studied using UV-vis spectroscopy. The UV-vis absorption spectra of the samples were measured using a Shimadzu UV 3600 UV-vis-NIR spectrometer (Shimadzu Corporation, Kyoto, Japan) in diffuse reflectance mode with BaSO_4 as a reference. Spectra were collected at room temperature and transformed using the Kubelka-Munk function (Cimitan et al. 2009)

4.6 Fourier-transform infrared spectroscopy (FTIR)

The functional groups present in the plant extract and ZnO NPs were identified using a Fourier transform infrared (FTIR) spectrophotometer (Perkin-Elmer 1725x) and the KBr pellet method.

4.7 GUPPY FISH EXPERIMENTAL

4.7.1 FISH MAINTENANCE

The juvenile guppy fish (*Poecilia reticulata*) were obtained from the Kolathur local fish market in Chennai, Tamil Nadu (latitude: 13,1282 °N and longitude: 80,20769° E;). For one week, these fish were allowed to adapt in tap water under a natural photoperiod of 14:10 h light-dark cycle. Following acclimation, the healthy fish were chosen for further research, which was confirmed by their physical appearance (skin lustre, eyes, colour, and behaviour). All fish were kept at the temperatures, pH levels, and dissolved oxygen (DO₂) levels. Fishes were fed with a commercial food diet twice a day (9:00 a.m. and 3:30 p.m.) (algae powder, shrimp meal, wheat gluten, soya bean, crude protein, and fresh fishmeal). The experiment was carried out in 10:14 dark and light cycles. Throughout the study, 20% of the water was changed daily to avoid either a fungal or bacterial infection, while the remaining 80% was changed once a week.

4.7.2 EXPERIMENTAL SETUP

The present study was carried out in accordance with the existing standards for long-term exposure to Zinc Oxide Nanoparticles (Li et al., 2017). (ZnO). There were four experimental groups: one control group and three ZnO treatment groups (of varying concentrations). The fish in the tanks were exposed to ZnO nanoparticles at concentrations of 2 mg/L (LC50), 4 mg/L (LC50), 6 mg/L (LC50), and 8 mg/L. (LC50). Each group consisted of 20 fish. Before beginning the experiment, the fish were not fed for 24 hours. Every 48 hours, the fish from each study group were sampled. The fish were anesthetized and dissected for organ collection (gills and liver). The collected organs were kept at 80 °C for future research.

4.7.3 LETHAL CONCENTRATION (LC50)

The mortality and acute toxicity were carried out according to the standard protocols Li et al., 2017. The mortality rates were recorded every 24 h for 20 days.

4.7.4 GILLS ZNO CONTENTS ANALYSIS

The collected gills were washed and dried properly. Dried organs were digested in the muffle furnace at 450 °C for 2 hours. To 1 g of ash, 10 mL HCl were added. After the ash was completely taken up by the HCl, the Ale and Rossi 2018 protocol called for the solution to be filtered.

4.7.5 ANALYTICAL DETECTION OF ZnO EXPOSURE IN GILLS

The filtered solution was analyzed for ZnO exposure in the gills of *Poecilia reticulata* using inductively coupled plasma atomic emission spectroscopy (ICP-MS) (Agilent Technologies, 7700).

4.7.6 BIOMARKER ASSAY

Tissue extracts for antioxidant enzyme measurements were obtained by homogenizing the tissues in 0.1 M sodium phosphate buffer, pH 6.5, containing 20% glycerol, 1 mM EDTA, and 1.4 mM dithioerythritol. The glutathione S-transferase (GST) activity was measured using the substrate 1-chloro-2,4-dinitrobenzene. Glutathione reductase (GR) activity was measured by homogenizing the tissues in 0.1 M K-phosphate and 5 mM ascorbate. A 3 mL CAT mixture of 0.05 mL tissue extract, 1.6 mL phosphate buffer (100 mM buffer, pH 7.0), 0.5 mL H₂O₂, and 0.95 mL distilled water was used.

CHAPTER 5

5. RESULT AND DISCUSSION

5.1 SYNTHESIS OF ZNO NANOPARTICLES

A sequence of chemical reactions occurs as a result of the experimental work that has been done. A ZnO colloid should be produced when zinc acetate is completely hydrolyzed with the help of NaOH in an ethanolic solution. The equilibrium between the condensation reaction and hydrolysis led to the final product. Zinc acetate in the solution is hydrolyzed by the heating, resulting in zinc ions and acetate ions. The hydroxyl groups (-OH) of alcohol molecules form a connection with the zinc ions due to the abundance of electrons in oxygen atoms (Yung et al. 2012).

Zinc hydroxide acetate is a byproduct of the hydrolysis reaction that forms in the presence of water and OH ions. At higher temperatures and over an extended period, it can be easily converted into ZnO. Because the compound sodium acetate is water soluble, it might be removed from the finished product. Thus, using the sol-gel technique⁶, it was possible to produce high-purity ZnO nano powder successfully (Bari et al. 2009).

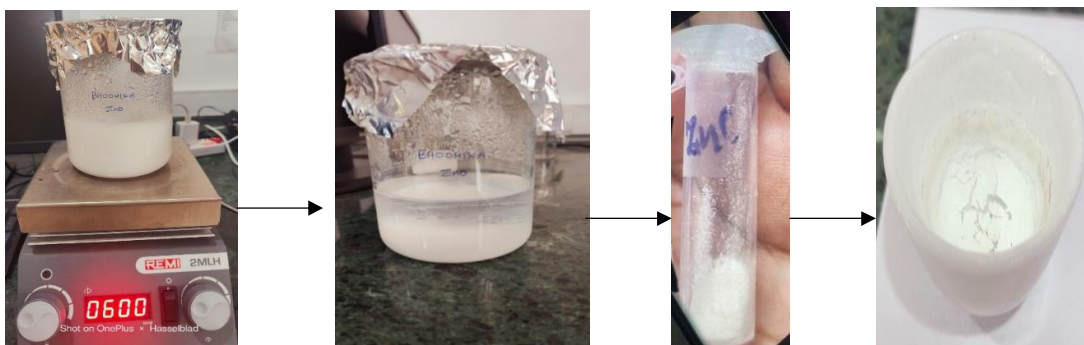


Fig. 5. 1: Synthesis of ZnO nanoparticles

5.2 CHARACTERIZATION OF ZNO NANOPARTICLES:

5.2.1. UV – VIS ABSORPTION SPECTRA

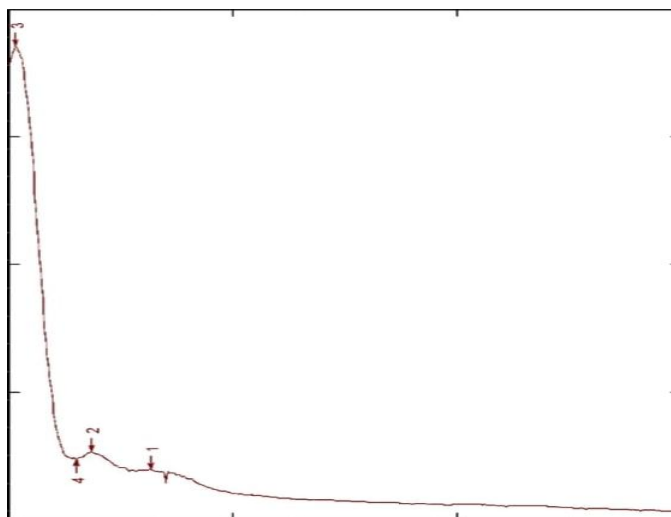


Fig. 5.2: UV-Visible Spectrum of ZnO Nanoparticle

The zinc oxide nanoparticles' UV-Vis absorption spectrum is depicted in Figure 2. The sample's absorption spectra were recorded between 280 and 420 nm. According to the spectra, the absorbance peak for zinc oxide nanoparticles was seen at 340 nm.

5.2.2 X-RAY DIFFRACTOMETER (XRD)

Nanopowder contained the phase, according to an XRD study. As shown in Fig. 6, the XRD results indicate the diffraction of ZnO nano-powder using zinc acetate as a precursor and illustrate the pattern of ZnO nano-powder in various profiles of peak and diffraction angle 2θ . The fraction angle range for the powder's XRD pattern was 20° to 80° . The graphic displays distinct ZnO peaks that represent the crystallinity of nature. According to the presented findings, the synthesised powder, ZnO nano-powder, displayed a single phase with distinct diffraction peaks. (JCPDS card no 36-1451). The highest peak is located at

angles 33.8616° and 36.2695° in the (101) plane, respectively, with intensities of 4847.45 and 4069.75. Zinc Oxide is a component of the ZnO nano-powder that is manufactured.

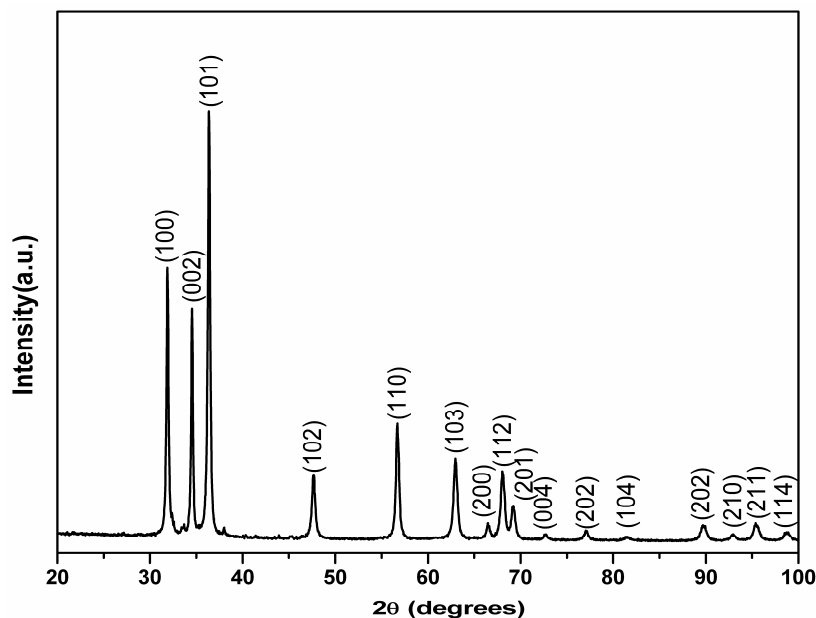


Fig. 5.3: XRD Pattern of ZnO Nanoparticles

5.2.3 FIELD EMISSION SCANNING ELECTRON MICROSCOPE (FE-SEM) AND EDAX

The image of the materials' microstructure is captured using the cutting-edge technology known as field emission scanning electron microscopy (FE-SEM). Because gas molecules tend to affect the electron beam and the produced secondary and backscattered electrons utilized for imaging, FE-SEM is normally carried out in a high vacuum.

Figure 5.4. shows SEM images of ZnO nanoparticles taken at various magnifications. These images demonstrate how ZnO nanoparticles develop. These images support the nanoparticles' roughly spherical shape, and most of

the particles show some faceting. Additionally, it is evident from the images that the nanoparticles are smaller than 10 nm in size, which is in good agreement with the estimated particle sizes (8.32 nm) derived from the Debye-Scherrer formula. Figure 3's SAED pattern (Figure 3) displays clear, bright rings that support nanocrystals' preferred orientation over irregular crystals [29, 32]. Figures 4(a), 4(b), and 4(c) from transmission electron microscopy images also demonstrate the creation of ZnO nanoparticles at various magnifications by demonstrating the hexagonal plane of the produced nanoparticles.

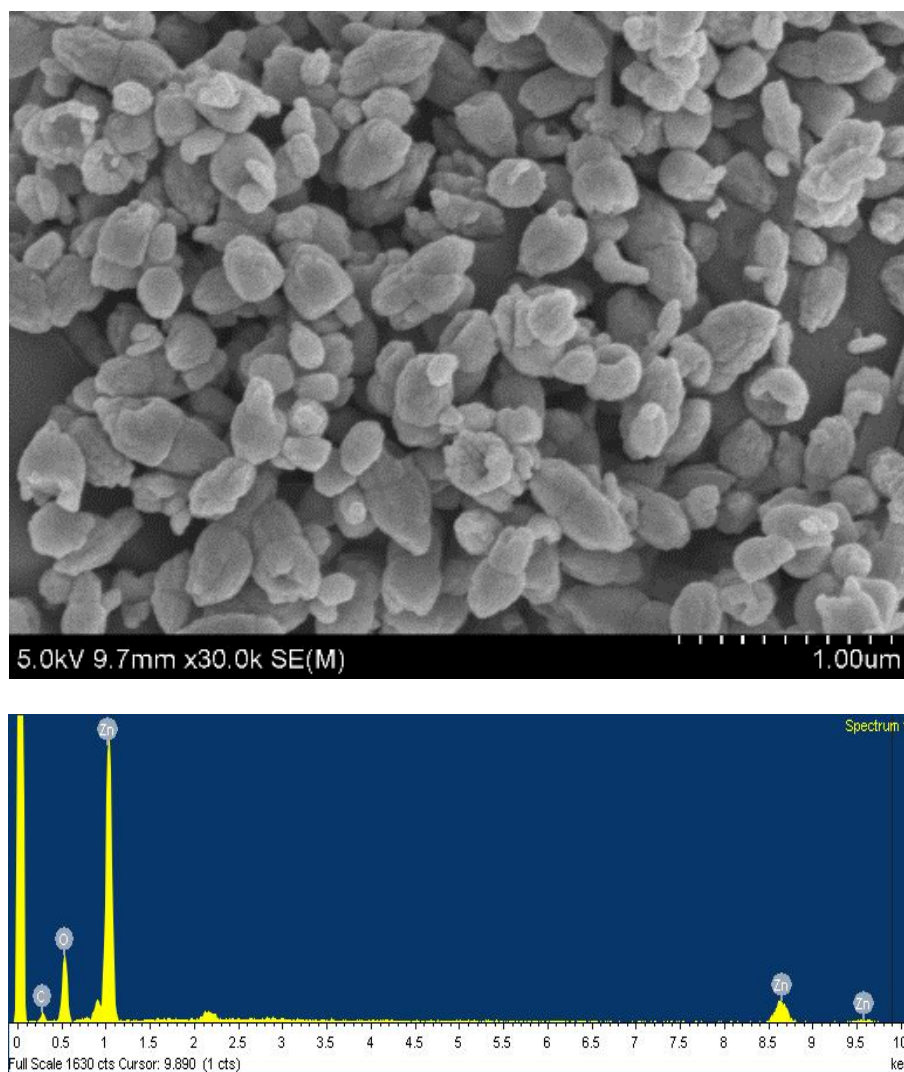


Fig.5.4: (a) FESEM and (b) EDAX Spectrum of ZnO Nanoparticle

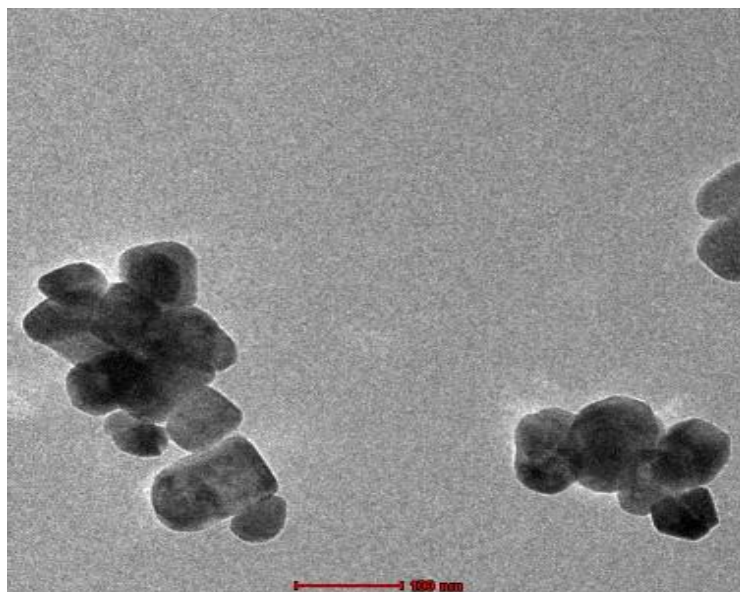


Fig. 5.4(b): Transmission Electron Microscope of ZnO Nanoparticle

Fig. 5.3. shows the XRD pattern ZnO nanoparticles. All diffraction peaks were attributed to ZnO with hexagonal wurtzite structure. The data obtained are in good agreement with the joint committee on powder diffraction standards (JCPDS) card for ZnO (JCPDS 36-1451). The strong and narrow diffraction peaks indicate that the prepared sample has well crystalline in nature. Diffraction peaks correspond to impurities was not detected in the XRD pattern which suggests high quality of the synthesized ZnO nanoparticles. From Fig.1, the peaks at $2\theta = 31.98^\circ, 34.64^\circ, 36.35^\circ, 47.58^\circ, 56.68^\circ, 63.04^\circ, 66.41^\circ, 68.09^\circ, 69.23^\circ, 72.81^\circ, 77.11^\circ, 81.48^\circ, 89.73^\circ, 92.93^\circ, 95.42^\circ$ and 98.69° , corresponds to the following lattice (*hkl*) planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104), (203), (210), (211) and (114) respectively.

The crystallite size (*D*) of stabilized ZnO nanoparticles were calculated from the full width at half-maximum (FWHM) of the most intense peak (101) using the Scherrer's formula and was around 33 nm.

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

where λ is the wavelength of the X-ray radiation (for CuK_α radiation, $\lambda = 1.5418 \text{ \AA}$), β is the FWHM in radians of the XRD peak and θ is the angle of diffraction.

Fig. 5.4 (a) depicts the FE-SEM images of ZnO nanoparticles. The elemental composition of the prepared samples was analyzed using EDAX and spectrum is shown in Fig. 5.4 (b). The EDAX spectrum confirms the presence of Zn and O in the prepared sample.

The size and shape of the ZnO nanoparticles were also confirmed by TEM measurements. TEM images of ZnO nanoparticles were dispersed rod, spherical and hexagonal shape particles with size ranging 15–49 nm (Fig. 5.5).

5.2.4 FTIR SPECTRUM OF ZnO NANOPARTICLES

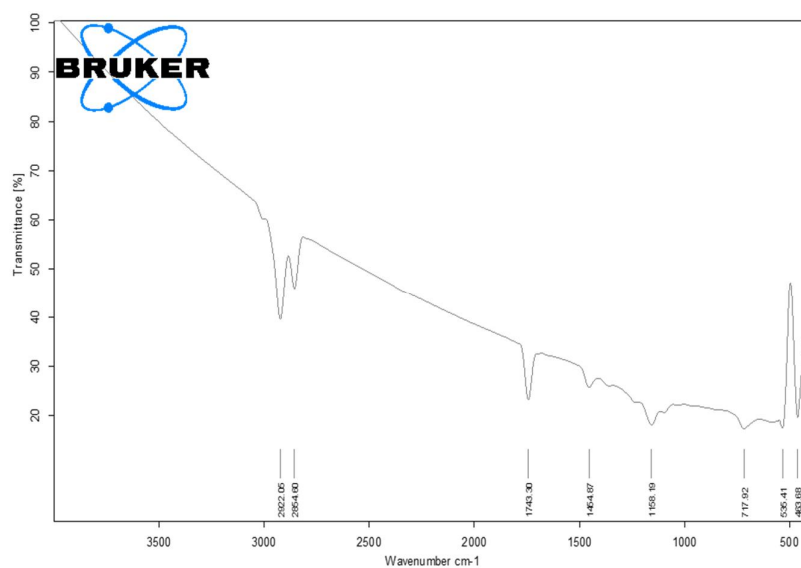
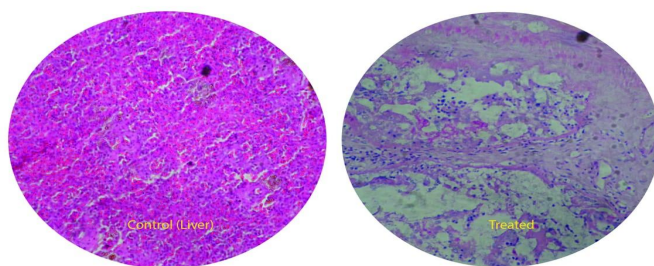
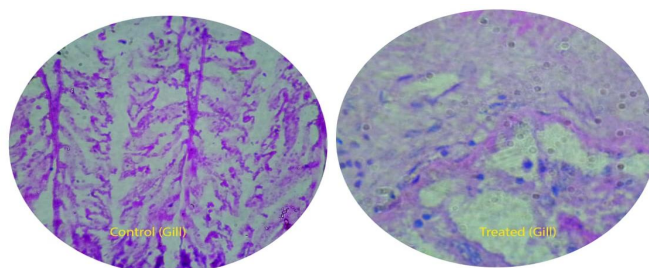


Fig.5.5: FTIR Spectrum of ZnO Nanoparticles

5.2.6 HISTOPATHOLOGY



Histopathological alteration of the gills of *Poecilia reticulata* fish after the exposure to 4mg/L concentrations of ZnO. The atrophy of the liver, The tissues were stained using eosin and magnified with $\times 400$



Histopathological alteration of the gills of guppy fish after the exposure to 4mg/L concentrations of ZnO. Vacuolation of gill lamella, (epithelial cell of gill shedding, and necrosis. The tissues were stained using eosin and magnified with $\times 400$

Fig. 5.6: Histopathological Studies

CHAPTER 6

6 CONCLUSION

The toxic effects of ZnO nanoparticles on aquatic species has been less studied. so, this work could contribute to finding solution to remove nanoparticle from water bodies.

REFERENCES

- [1] Antunes A.M., T. L. Rocha, F. S. Pires, M. A. Freitas, V. R. M. C. Leite, S. Arana, and S. M. T. Sabo'ia-Morais (2017). J. Appl. Toxicol. 37, (9), 1098.
- [2] Asharani, P. V, Lianwu, Y., Gong, Z., and Valiyaveetil, S. (2011). Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. *Nanotoxicology*, 5, 1, 43–54.
- [3] Asharani, P. V., Wu, Y. L., Gong, Z. Y., and Valiyaveetil, S. (2008). Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology*, 19, 1–8.
- [4] Aliko V., G. Hajdaraj, A. Caci, and C. Faggio (2015). Braz. Arch. Biol. Technol. 58,(5), 750.
- [5] Battez A.H., R. González, J. L. Viesca, J. E. Fernández, J. M. DíazFernández, A. Machado, R. Chou, and J. Riba (2008). Wear 265, (3–4), 422.
- [6] Bar-Ilan, O., Albrecht, R. M., Fako, V. E., and Furgeson, D. Y (2009). Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. *Small*, 5, 1897–1910.
- [7] Bartneck, M., Keul, H. A., Zwadlo-Klarwasser, G., and Groll, J. (2009). Phagocytosis independent extracellular nanoparticle clearance by human immunecells. *Nano Lett.*, 10, 59–63.
- [8] Baun, A., Sørensen, S. N., Rasmussen, R. F., Hartmann, N. B., and Koch, C. B. (2008). Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C-60. *Aquat. Toxicol.*, 86, 3, 379–387.

- [9] Blickley, T. M., Matson, C. W., Vreeland, W. N., Rittschof, D., Di Giulio, R. T., and McClellan-Green, P. D. (2014). Dietary CdSe/ZnS quantum dot exposure in estuarine fish: bioavailability, oxidative stress responses, reproduction, and maternal transfer. *Aquat. Toxicol.*, 148, 27–39.
- [10] Boylea D, Al-Bairutya G. A., Ramsdena, C. S., Slomanb, K. A., Theodore, B. H., and Handya, R. D. (2013). Subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles are associated with gill rather than brain injury. *Aquat. Toxicol.*, 126, 116–127.
- [11] Bari, A. R., Shinde, M. D., Vinita. D. & Patil, L. A (2009). Effect of Solvents on the Particle Morphology of nanostructured ZnO. *Indian J. Pure Appl. Phys*, 47, 24-27.
- [12] Buzea, C., Pacheco, I. I., and Robbie, K. (2007). Nanomaterials and Nanoparticles: sources and toxicity. *Biointerphases*, 2, 17–71.
- [13] Burgos-Aceves M. A, A. Cohen, G. Paoletta, M. Lepretti, Y. Smith, C. Faggio, and L. Lionetti (2018). *Sci. Total Environ.* 645, 79.
- [14] Burgos-Aceves M. A., A. Cohen, Y. Smith, and C. Faggio (2018). *Ecotoxicol. Environ. Saf.* 148, 995–1000.
- [15] Bao S., Q. Lu, T. Fang, H. Dai, and C. Zhang (2015). *Appl. Environ. Microbiol.* 81,(23), 8098.
- [16] Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*, 22(1), 25-34.
- [17] Bucher, F., & Hofer, R. (1993). The effects of treated domestic sewage on three organs (gills, kidney, liver) of brown trout (*Salmo trutta*). *Water Research*, 27(2), 255-261.

- [18] Buzea, C., Pacheco, I. I., and Robbie, K. (2007). Nanomaterials and Nanoparticles:sources and toxicity. *Biointerphases*, 2, 17–71.
- [19] Cimitan, S, Albonetti, S, Forni, L, Peri, F, Lazzari, D: Solvothermal synthesis and properties control of doped ZnO nanoparticles. *J. Colloid. Interface Sci.* 329, 73–80 (2009).
- [20] Cedervall, T, Hansson, L, Lard, M, Frohm, B, and Linse S. (2012).Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS One*,7, 2, e32254.
- [21] Chen, W., John, J., Lin, C., Lin, H., Wu, S., and Chang, C. (2004). Expression of metallothionein gene during embryonic and early larval development in zebrafish.*Aquat. Toxicol.*, 69, 215–227.
- [22] Cheng, J., Flahaut, E., and Cheng, S. H., (2007). Effect of carbon nanotubes on development zebrafish (Danio rerio) embryos. *Environ. Toxicol. Chem.*, 26, 708– 716.
- [23] Cong, Y., Banta, G. T., Selck, H., Berhanu, D., Valsami-Jones, E., and Forbes, V.
- [24] E. (2011). Toxic effects and bioaccumulation of nano-, micron- and ionic-Ag in thepolychaete, Nereis diversicolor. *Aquat. Toxicol.*, 105, 403–411.
- [25] Colvin VL. “The potential environmental impact of engineered nanomaterials”. *Nature Biotechnology* 21 (2003): 1166-1170.
- [26] Chang Y. N, X. Zhang, J. Zhang, and G. Xing (2012). *Materials* 5, 2850.
- [27] Dobrovolskaia, M. A., and McNeil, S. E. (2007). Immunological properties ofengineered nanomaterials. *Nat. Nano*, 2, 469–478.
- [28] Dorea, J. G. (2008). Persistent, bioaccumulative and toxic substancesin fish: human health considerations. *Sci. Total Environ.*,400, 93–114
- [29] Dorrington.T, J. Zanette, F. L. Zacchi, J. J. Stegeman, and A. C. Bainy

- (2012). *Aquat. Toxicol.* 15, 106.
- [30] Fabrega J., *et al.* "Silver nanoparticles: behaviour and effects in the aquatic environment". *Environment International* 37 (2011): 517-531.
- [31] Farkas, J., Christian, P., Gallego-Urrea, J. A., Roos, N., Hassellöv, M., Tollefsen, K. E., and Thomas, K. V. (2011). Uptake and effects of manufactured silver nanoparticles in rainbow trout. *Aquat. Toxicol.*, 101, 117–125
- [32] Farmen, E., Mikkelsen, H. N., Evensen, O., Einset, J., Heier, L.S., Rosseland, B. O., Salbu, B., Tollefsen, K. E., and Oughton, D. H. (2012). Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low $\mu\text{g L}^{-1}$ concentrations of Ag nanoparticles. *Aquat. Toxicol.*, 108, 78–84.
- [33] 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.
- [34] Genan, A. Al-B., Benjamin, J. S., Richard, D. H., and Theodore, B. H. (2012). Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.*, 126, 104–115.
- [35] George, S., Lin, S., Ji, Z., Courtney, R. T., Li, L. J., Mathew, M., Huan, M., Wang, X., Zhang, H., Xia, T., Nathan Hohman, J., Lin, S., Jeffrey, I. Z., Paul, S.W., and Andre, E. Nel. (2012). Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. *ACS Nano*, 6, 3745–3759.
- [36] Griffitt, R. J., Hyndman, K., Denslow, N. D., and Barber, D. S. (2009). Comparison of molecular and histological changes in zebrafish gills

exposed to metallic nanoparticles. *Toxicol. Sci*, 107, 2, 404–415.

- [37] Griffitt, R. J., Luo, J., Gao, J., Bonzongo, J.-C., and Barber, D. S.(2008). Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environ. Toxicol.Chem.* 27, 9, 1972–1978.
- [38] Griffitt, R. J., Weil, R., Hyndman, K. A., Denslow, N. D., Powers, K., Taylor, D., Barber, D. S., and David, S. (2007). Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ. Sci. Technol.*, 41, 8178–8186.
- [39] Griffitt, R. J., Weil, R., Hyndman, K. A., Denslow, N. D., Powers, K., Taylor, D., Barber, D. S., and David, S. (2007). Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ. Sci. Technol.*, 41, 8178–8186.
- [40] Ghane M., B. Sadeghi, A. R. Jafari, and A. R. Paknejhad (2010). *Int. J. Nano Dimens.* 1, (1), 33.
- [41] Griffitt R. J., R. Weil, K. A. Hyndman, N. D. Denslow, K. Powers, D. Taylor, and D.S. Barber (2007). *Environ. Sci. Technol.* 41, 8178.
- [42] Henry, T. B., Menn, F. M., Fleming, J. T., Wilgus, J., Compton, R. N., and Sayler, G.S. (2007). Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. *Environ. Health Perspect.*, 115, 7, 1059–1065.
- [43] Isani. C, M. L. Falcioni, G. Barucca, D. Sekar, G. Andreani, E. Carpenè, and G. Falcioni (2013). *Ecotoxicol. Environ. Saf.* 97, 40.
- [44] Jovanović, B., Anastasova, L., Rowe, E.W., and Palić, D. (2011a). Hydroxylated fullerenes inhibit neutrophil function in fathead minnow (*Pimephales promelas* Rafinesque, 1820). *Aquat. Toxicol.*, 101, 474–

- [45] Jovanović, B., and Palić, D. (2012). Immunotoxicology of nonfunctionalized engineered nanoparticles in aquatic organisms with special emphasis on fish— review of current knowledge, gap identification, and call for further research. *Aquat. Toxicol.*, 118– 119, 141–151.
- [46] Kashiwada, S. (2006). Distribution of nanoparticles in the see through medaka (*Oryzias latipes*). *Environ. Health Perspect.*, 114, 1697-1702.
- [47] Kerry, J. L., Lauren, M. B., Prakash, D. N., Tanvi, D., Pavan, K. C., and Xu, X. N. (2012). *In vivo* quantitative study of sizedependent transport and toxicity of single silver nanoparticles using zebrafish embryos. *Chem. Res. Toxicol.*, 25, 5, 1029– 1046
- [48] .Kiener, T. K., Selptsova-Friedrich, I., and Hunziker, W., (2008). Tjp3/zo-3 is critical for epidermal barrier function in zebrafish embryos. *Dev. Bio.*, 316, 36–49
- [49] Kim, K. T., Zaikova, T., Hutchison, J. E., and Tanguay R. L. (2013). Gold nanoparticles disrupt zebrafish eye development and pigmentation. *Toxicol. Sci.* 133, 275–288.
- [50] Klaper, R., Arndt, D., Setyowati, K., Chen, J., and Goetz, F. (2010) . Functionalization impacts the effects of carbon nanotubes on the immune systemof rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.*, 100, 211–217.
- [51] Kumazawa, R., Watari, F., Takashi, N., Tanimura, Y., Uo, M., and Totsuka, Y., (2002). Effects of Ti ions and particles on neutrophil function and morphology. *Biomaterials*,
- [52] Keller A, S. McFerran, A. Lazareva, and S. Suh (2013). *J. Nanopart. Res.* 15, 1692.
- [53] Kodric-Brown. A and P. F. Nicoletto (2001). *Am. Nat.* 157, (3), 316.

- [54] Korai, A. L., Lashari, K. H., Sahato, G. A., & Kazi, T. G. (2010). Histological lesions in gills of feral cyprinids, related to the uptake of waterborne toxicants from Keenjhar lake. *Reviews in Fisheries Science*, 18(2), 157-176.
- [55] Laban, G., Nies, L., Turco, R., Bickham, J., and Sepúlveda, M. S. (2010). The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. *Ecotoxicology*, 19, 185–195.
- [56] Lee, K. J., Nallathamby, P. D., Browning, L. M., Osgood, C. J., and Xu, X. H. N. (2007). *In vivo* imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. *ACS Nano*, 1, 133–143.
- [57] Lee, K. L., Browning, L. M., Nallathamby, P. D., Desai, T., P. K., and Xu, X. H. N. (2012). In vivo quantitative study of sized-dependent transport and toxicity of single silver nanoparticles using zebrafish embryos. *Chem. Res. Toxicol.*, 25, 1029–1046
- [58] Lima, D. R., Seabra, A. B., and Duran, N. (2012) Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. *J. App. Toxicol.*, 32, 11, 867–879.
- [59] Lubick, N. (2008). Nanosilver toxicity: ions, nanoparticles – or both? *Environ. Sci. Technol.*, 42, 8617.
- [60] Li, H., Zhou, Q, Wu, Y., Fu, J., Wang, T., and Jiang, G.(2009). Effects of waterborne nano-iron on medaka (*Oryzias latipes*): antioxidant enzymatic activity, lipid peroxidation and histopathology. *Ecotox. Environ. Saf.*, 72, 684–692.
- [61] Lima, D. R., Seabra, A. B., and Duran, N. (2012) Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. *J. App. Toxicol.*, 32, 11, 867–879.

- [62] Lubick, N. (2008). Nanosilver toxicity: ions, nanoparticles – or both? *Environ. Sci. Technol.*, 42, 8617.
- [63] Mallat, J. (1985). Fish gill structural changes induced by toxicants and others irritants: a statistical review. *Canadian Journal of Fish and Aquatic Science*. *Canadian Journal of Fisheries and Aquatic Science*. 42, 630-648.
- [64] M. F. Vajargah, A. M. Yalsuyi, A. Hedayati, and C. Faggio (2018). *Microsc. Res. Tech.* 81, (7), 724–729.
- [65] Moosavi M. J. Shamushaki and V. A. J. (2015). *J. Aquacult. Res. Dev.* 6, (2), 305.
- [66] M. J. Moosavi and V. A. J. Shamushaki (2015). *J. Aquacult. Res. Dev.* 6, (2), 305.
- [67] M. M. Chorehi, H. Ghaffari, S. A. Hossaini, E. H. N. Niazie, M. F. Vajargah, and A. Hedayati (2013). *Int. J. Aquat. Biol.* 1, (6), 254.
- [68] M. M. H. C. M. Valko, H. Morris, and M. T. D. Cronin (2005). *Curr. Med. Chem.* 12, (10), 1161.
- [69] Moore, M. N. (2006). Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ. Int.*, 32, 967–976.
- [70] Oberdorster, E., Ortiz-Acevedo, A., Xie, H., Pantano, P., Baughman, R. H., Dieckmann, G. R. (2005). Exposure of fathead minnow to fullerene and singlewalled carbon nanotubes. *Toxicol. Official J. Soc. Toxicol.*, 84, S1, 325.
- [71] Oberdorster, E., Zhu, S., Blickley, T. M., McClellan-Green, P., and Haasch, M. L. (2006). Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C60) on aquatic organisms. *Carbon*, 44, 1112–1120.

- [72] Oberdoster, E. (2004). Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Perspect.*, 112, 1058–1062.
- [73] Olivia, J. O., Blair, D. J., Julian, M., Mohammed, B., Jamie, R. L., Tetsuhiro, K., and Charles, R. T. (2012). Effects of particle size and coating on nanoscale Ag and TiO₂ exposure in zebrafish (*Danio rerio*) embryos. *Nanotoxicology.*, 2, 1–10.
- [74] Prakash, M., Gopalakrishnan, N., and Jinhee, C. (2011). Identification, characterization and expression profiles of *Chironomus riparius* glutathione S- transferase (GST) genes in response to cadmium and silver nanoparticles exposure. *Aquat. Toxicol.*, 101, 550–560.
- [75] Peng C., C. Shen, S. Zheng, W. Yang, H. Hu, J. Liu, and J. Shi (2017). *Nanomaterials* 7, (10), 326.
- [76] Pan. X, J. E. Redding, P. A. Wiley, L. Wen, J. S. McConnell, and B. Zhang (2010). *Chemosphere* 79, 113.
- [77] Peters, L. E., MacKinnon, M., Van Meer, T., van den Heuvel, M. R., and Dixon, D. G., (2007). Effects of oil sands process-affected waters and naphthenic acids on yellow perch (*Perca flavescens*) and Japanese medaka (*Orizias latipes*) embryonic development. *Chemospher.*, 67, 2177–2183.
- [78] Rosen, E. D, Walkey, C. J., Puigserver, P., and Spiegelman, B. M. (2000). Transcriptional regulation of adipogenesis. *Genes Dev.*, 14, 1293–1307.
- [79] Saber, T. H. (2011). Histological adaptation to thermal changes in gills of common carp fishes *Cyprinus carpio* L. *Rafidain Journal of Science*, 22, 46-55.
- [80] Santos A.P. R, T. L. Rocha, C. L. Borges, A. M. Bailaõ, C. M. Almeida Soares, and S. M. T. Sabo´ia-Morais (2017). *Chemosphere* 168, 933.

- [81] Sayes C, Fortner J, Guo W, Lyon D, Boyd A, Ausman K, Tao, Y. J., Sitharaman, B., Wilson, L. J., Hughes, J. B., West, J. L., and Colvin, V. L. (2004). The differential cytotoxicity of watersoluble fullerenes. *Nano Lett.*, 4, 10, 1881–1887.
- [82] Scown, T. M., Santos, E. M., Johnston, B. D., Gaiser, B., Baalousha, M., Mitov, S., Lead, J. R., Stone, V., Fernandes, T. F., Jepson, M., Aerle, R. van, and Tyler, C. R. (2010). Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. *Toxicol. Sci.*, 115, 521–534.
- [83] Scown, T., Van Aerle, R., Johnston, B., Cumberland, S., Lead, J., Owen, R., and Tyler, C. (2009). High doses of intravenously administered titanium dioxide nanoparticles accumulate in the kidneys of rainbow trout but with no observable impairment of renal function. *Toxicol. Sci.*, 109, 372.
- [84] Shaw, B. J., and Handy, R. D. (2011). Physiological effects of nanoparticles on fish: a comparison of nanometals versus metal ions. *Environ. Intern.*, 37, 1083–1097.
- [85] Song, G. W., Hou, Y., Gao, L., Lin, Z., Zhang, Q., Niu, R., Ma, L., Mu, L., and Wang (2016). *Bot. Stud.* 57, (3), 2.
- [86] Smith, C. J., Shaw, B. J., and Handy, R. D. (2007). Toxicity of single walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. *Aquat. Toxicol.*, 82, 94–109.
- [87] Tejamaya, M., Römer, I., Merrifield, R. C., and Lead, J. R. (2012). Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environ. Sci. Technol.*, 46, 7011–7017.
- [88] Tsukahara, T., and Haniu, H. (2011). Nanoparticle-mediated intracellular lipid accumulation during C2C12 cell differentiation. *Biochem. Biophys. Res. Commun.*, 406,

- [89] Vajargah M. F. and A. Hedayati (2017). *Transylv. Rev. Syst Ecol. Res.* 19, (3), 85.
- [90] Vajargah M. F, A. M. Yalsuyi, A. Hedayati, and C. Faggio (2018). *Microsc. Res. Tech.* 81, (7), 724–729.
- [91] Vevers, W. F., and Jha, A. N. (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO₂) nanoparticles on fish cells in vitro. *Ecotoxicology*, 17, 411–421.
- [92] Waters, MD., Olden, K., Tennant, RW. Toxicogenomic approach for assessing toxicant-related disease. *Mutation Research* (2000);544:415.
- [93] Waters, MD., Selrik, JK., Olden, k. The impact of new technologies on human population studies . *Mutation Research* (2003);544:349-360.
- [94] Whatmore RW. "Nanotechnology-what is it? Should we be worried?" *Occupational Medicine, Oxford* 56 (2006): 295-299.
- [95] Wise, J. P. Sr, Goodale, B. C., Wise, S. S., Craig, G. A., Pongan, A. F., Walter, R. B., Thompson, W. D., Ng, A. K., Aboueissa, A. M., Mitani, H, Spalding, M. J., and Mason, M. D. (2010). Silver nanospheres are cytotoxic and genotoxic to fish cells. *Aquat. Toxicol.*, 97, 34–41.
- [96] Wu, Y., Zhou, Q., Li, H., Liu, W., Wang, T., and Jiang, G., (2010). Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. *Aquat. Toxicol.*, 100, 160–167.
- [97] Yalsuyi A.M., A. Hedayati, M. F. Vajargah, and H. Mousavisabet (2017). *J. Environ. Treat. Tech.* 5, (2), 83.
- [98] Yung K., Ming, H., Yen, C. & Chao, H (2012). Synthesis of 1D, 2D and 3D ZnO Polycrystalline Nanostructures Using Sol-Gel Method. *J. Nanotechnol.*, 1-8.

- [99] Zhang . Li, H, J.,Wang, T., Luo,W., Zhou, Q., and Jiang, G. (2008). Elemental selenium particles at nano- Li size (nano-se) are more toxic to medaka (*Oryzia latipes*) as a consequence of hyperaccumulation of selenium: a com, H., Zhang, J.,Wang, T., Luo,W., Zhou, Q., and Jiang, parison with sodium selenit. *Aquat. Toxicol.*, 89, 251–256
- [100] Zhou . Li, H, Q, Wu, Y., Fu, J., Wang, T., and Jiang, G.(2009). Effects of waterborne nano-iron on medaka (*Oryzias latipes*): antioxidant enzymatic activity, lipid peroxidation and histopathology. *Ecotox. Environ. Saf.*, 72, 684–692.
- [101] Zhu, S., Oberdorster, E., and Haasch, M. L. (2006). Toxicity of an engineered nanoparticle (fullerene, C60) in two aquatic species, *Daphnia* and fathead minnow. *Mar. Environ. Res.*, 62, S5–S9.
- [102] Zhu, X., Wang, J., Zhang, X., Chang, Y., and Chen, Y. (2010). Trophic transfer of TiO₂ nanoparticles from *daphnia* to zebrafish in a simplified freshwater food chain. *Chemosphere*, 79, 928–933.
- [103] Zhu, X., Zhu, L., Li, Y., Duan, Z., Chen, W., and Alvarez, P. J. J. (2007). Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC60) and fullerol. *Environ. Toxicol. Chem.*, 26, 976–979.