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RESEARCH ARTICLE

Effects of Engineered Zinc Oxide Nanoparticles on Freshwater Fish, *Labeo rohita*: Characterization of ZnO Nanoparticles, Acute Toxicity and Oxidative Stress

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A B S T R A C T

Concerns regarding zinc oxide nanoparticles (ZnO-NPs) have gained much attention due to their unique properties and widespread applications in cosmetics, electronics and medicinal industry that may induce an adverse impact not only on specific ecosystem but also on human health. ZnO-NPs were synthesized by co-precipitation method and characterization was done by Scanning electron microscope (SEM), X-ray diffraction (XRD) and Fourier-transform infrared (FT-IR) analysis. SEM showed the hexagonal wurtzite crystal structure of particles. From XRD pattern, average particle size, lattice parameters (a and c), X-ray density and volume of unit cell of zinc oxide nanoparticles were 52.22 nm, (a =3.25 Å and c=5.21 Å), 5.0 g/cm^3 and 54.82 Å^3, respectively. FT-IR confirmed the attached compound of synthesized nanoparticles. The acute toxicity of ZnO-NPs was determined by using fish, *Labeo rohita* as a genetic model during this study. The mean 96-h LC_{50} and lethal concentration were measured as 31.15 and 57.84 mg/L, respectively. Oxidative stress in terms of catalase, lipid peroxidation and superoxide dismutase was also determined in fish gills, muscle, liver and heart after chronic exposure of ZnO-NPs for 80 days and sampling were done on 20, 40, 60 and 80 days. Significantly decreased catalase and superoxide dismutase activity was determined in selected fish organs. However, level of lipid peroxidation was significantly increased in the fish organs as compared to control group. The overall results indicated that induced toxicity mechanism of ZnO-NPs in aquatic ecosystem was oxidative stress.

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INTRODUCTION

Nanotechnology is a rapidly growing industry due to its wide applications in biotechnology, medicine, environmental challenges, energy, and space exploration (Doyle *et al*., 2016). Nanoparticles, products of nanotechnology, can be naturally found and synthesized by different methods and have sizes in the range from 1-100nm (Roco, 2003). Metal oxide nanoparticles have various uses on a large scale in different industries that led their release into the ecosystem affecting several constituents of environmental biota. Exposure of nanoparticles (NPs) to aquatic organisms has revealed possible associated ecological and food chain risks and became main environmental issue (Asghar *et al*., 2015).

Properties of NPs are intimately dependent on the configuration, size ranges, distribution and composition of nanoparticles (Chang *et al*., 2012). Zinc oxide nanoparticles (ZnO-NPs) are widely used in environmental remediation (Saddick *et al*., 2017) and industries (Vinardell *et al*., 2017). The eco-toxicological data on ZnO-NPs is just rising and scanty, after TiO_2 and SiO_2 NPs, they have the third highest worldwide production of 100-1000t/yr (Piccino *et al*., 2012). Zinc atoms discharged from ZnO nanoparticles increases the level of toxicants in exposed tissues and in other internal organs of fish, ultimately causing hazardous effects through oxidative stress system (Ng *et al*., 2017). Mechanisms of NPs toxicity are very complex, cause oxidative stress, resulting in alteration of protein, DNA, lipids and carbohydrates (Afifi *et al*., 2016).

Fish are considered as an important indicator of water quality because of their status in aquatic food webs. Bioaccumulation and trophic transfer of nanoparticles is possible at all levels of marine food chains (Baker *et al*., 2014). Despite of the studies about poisoning of nanomaterials, minute information is present about the metal bioavailability and the impacts of these nanoparticles on organs of fish. In Pakistan, *Labeo rohita* is considered as a most common food source due to its taste. Majority of the freshwater resources are occupied by this fish specie. Now a day, water pollution is becoming a primary concern in riverine ecosystems and underground water. Polluted water adversely affects the freshwater living organisms (Ghaffar *et al*., 2015a, 2015b). *Labeo rohita* is most widely used in research works because it is capable to absorb and fix the toxic metals from the adjacent ecosystem (Hamid *et al*., 2016). It is essential to figure out permissible limits and impact of ZnO-NPs on fish. This present research was carried out to determine the acute toxicity of ZnO-NPs and its effects on activity of lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) in selected organs of *Labeo rohita.* In aquatic environments, organisms are usually exposed for a long duration to sub-lethal concentrations of metals (Javed, 2013). So, the sub-lethal dose ($1/3^{rd}$) for 96-hr LC_{50} of ZnO-NPs was selected to check oxidative stress.

MATERIALS AND METHODS

Synthesis and characterization of ZnO-NPs: Zinc sulfate ($ZnS0_4.7H_2O$) and sodium hydroxide (NaOH), obtained from Merck via local distributors for synthesis of ZNO-NPs, were added drop by drop in distilled water having the ratio (1:2) with continuous mixing by a magnetic stirrer for 12 hours. After filtration, precipitates were firstly rinsed, and then de-hydrated at 100°C in an oven Model (Shel-Lab). The powder collected from this procedure was heated up to 2 hours at 500°C in furnace (SNOL-LHM01). Morphology of ZnO-NPs was checked by using Scanning Electron Microscope (SEM) (JEOL-JSM 5910). XRD technique was used to determine structural properties like average particle size, lattice parameters (a, b), density of X-ray and volume of unit cell were determined by following formulas:

$$t = 0.9\,\lambda / B\cos\theta \quad (1)$$

$$1/d^2 = [(4/3)\{h^2 + hk + k^2\}/a^2] + l^2/c^2 \quad (2)$$

$$D = 2M/N_a\,a^2.c \quad (3)$$

$$V = a^2.c\sin\theta \quad (4)$$

t= particle size; λ= wavelength of X-rays; B= full width at half maximum; θ = diffraction angle; a and c= lattice parameters. M= NPs molar weight, Na= Avogadro's number and V= volume of unit cell. Fourier-Transform Infrared was used to observe atomic configuration.

Collection and maintenance of test organism: This research work was done in the wet laboratory of Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. Healthy group of *Labeo rohita* (90-day old) having the same weight and length were selected after acclimation of 15days. Fish were fed with commercial feed (30% Digestible protein and 3Kcal/g Digestible energy) twice a day.

Acute toxicity: Test concentrations of ZnO-NPs were 0, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 mg/L used for fish, *Labeo rohita* to check acute toxicity. To obtain the test suspensions, ZnO-NPs were dispersed in deionized water through sonicator (100W, 40 kHz) for 60 minutes immediately prior to use. Ten fish were exposed to all mentioned concentrations for 96 h in a 3.5L container with 2.5L of test solution. To maintain fixed level, all the test mixtures were altered after 24 hours. Control group was free from test solution. Fish were not fed during testing durations to reduce the sorption of the nanoscale ZnO in solid feed and feces. During the trial, temperature 29-30°C, pH, 7.5, DO, 5-7 mgL^{-1}, total hardness 290ppm and 12:12 day/night photoperiod were maintained.

Oxidative stress biomarkers: To check concentation specific oxidative stress, *Labeo rohita* was exposed to sub-lethal dose ($1/3^{rd}$ of 96-h LC_{50}) for 80 days. Sampling was done after 20, 40, 60 and 80 days interval, to check oxidative stress in terms of catalase (CAT), superoxide dismutase (SOD) lipid peroxidation level (LPO) in the gills, muscle, heart and liver of fish. All organs of *labeo rohita* were homogenized separately, in chilled phosphate buffer saline (1/4 ratio weight/volume) through a Homogenizer. After the homogenization, mixture was centrifuged (10,000 revolution per minute, at 4°C for 15 min.). Then transparent supernatants were selected for further studies. Activity of CAT and SOD were observed by using the method of Weydert and Cullen (2010) with slight alterations. LPO level was checked by calculating Thiobarbituric acid reactive substances (TBARS) contents in selected organs using the method of Gatta *et al*. (2000).

Statistical analyses: All experiments were performed with three replicates. Probit analysis method was executed with 95% confidence interval for determination of 96-h LC_{50} and lethal concentration by following method of Hamilton *et al*. (1977). To determine statistical differences and similarities among variables, Analysis of Variance (ANOVA) and comparison of means were done by Tukey's/Student Newnan-Keul tests. Results were shown as mean±S.E. Data were analyzed by using SPSS (Statistical Package for Social Sciences) version 16.0.

RESULTS

Characterization of ZnO-NPs: Scanning electron microscope (SEM) is important for scanning of the samples and also provides knowledge about the size and shape of particles. Fig. 1 represents that Zinc oxide manufactured NPs contain clear hexagonal wurtzite crystalline arrangement, regular distribution and all particles show well association among them. To measure the size, volume and solidity of manufactured nanoparticles (NPs) were observed under X-ray diffraction technique (XRD). For this method, powdered form of particles was used which have small crystals with indiscriminate alignment. The X-ray diffraction design of ZnO NPs is shown in Fig. 2. The average measured size was 52.22nm for NPs crystallites. At 2θ, diffraction peaks 31.82°, 34.46°, 36.29°, 47.59°, 56.64°, 62.89°, 66.40°, 67.97° referred to as Planes of XRD as (100), (002),

(101), (102), (110), (103), (112) and (201), respectively. Lattice parameters (a and c), X-ray density and volume of unit cell of ZnO nanoparticles were a=3.25Å and b=5.21Å, 5.0 g/cm^3 and 54.82 Å^3, respectively.

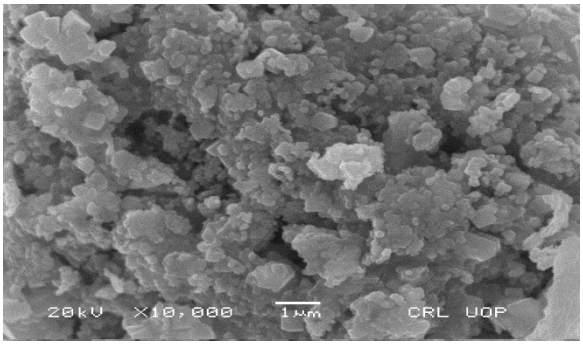

Fig. 1: Scanning electron microscopy (SEM) image of ZnO nanoparticles showed fine hexagonal wurtzite crystal structure.

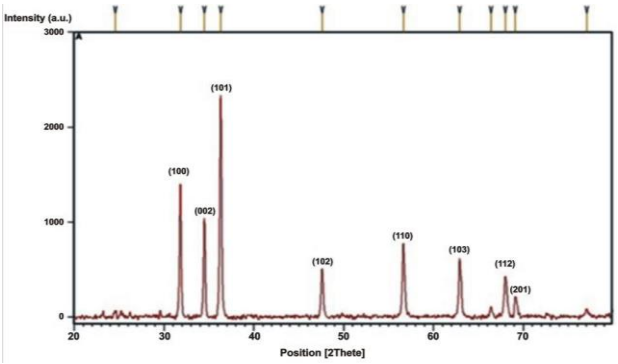

Fig. 2: X-ray diffraction pattern of nanoscale ZnO. Different peaks showed the particle size.

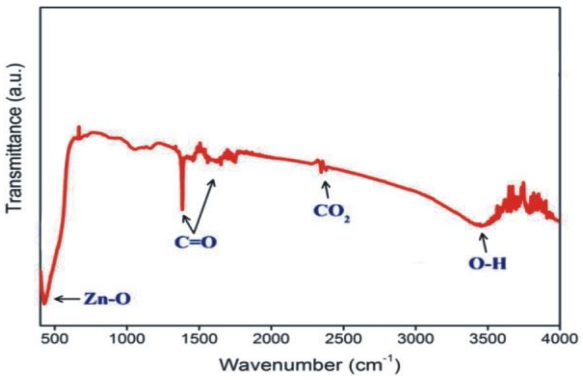

Fig. 3: FTIR spectra of associated with attached molecules with newly synthesized ZnO-NPs.

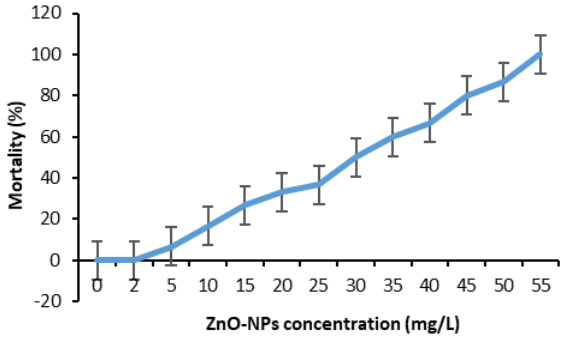

Fig. 4: The % mortality of *Labeo rohita* at different ZnO-NPs concentrations during 96-h acute toxicity tests.

The Infrared Spectroscopy (IR) is a technique which is used to observe the phase configuration of metallic ions and also an oxygen binding pattern in the prepared particles. Fig. 3 represents the infrared transmission pattern of all observed samples ranged from 4000-400 cm^{-1} (wave number). The 3000-3500 cm^{-1} wide patches are allotted to O---H elongating and curved vibrations of H_2O, the patches within the range of 2300-2450 cm^{-1} are assigned to the extending vibrations of CO_2 in air. The high pitched top ranging from 1499 to 1580 cm^{-1} described as extending vibrations of C=O. Ultimately, the firm patch observed at 400-500 cm^{-1} appointed to vibrations of Zn-O.

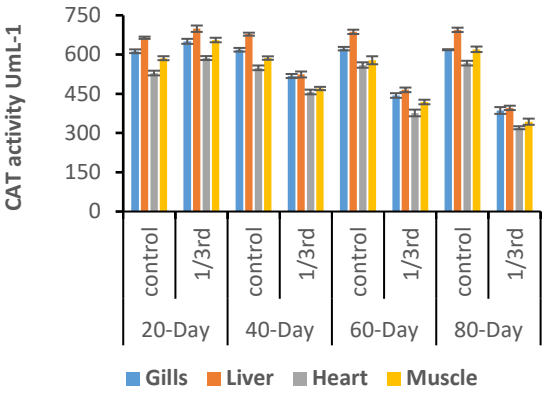

Fig. 5: Effect of Zno-NPs on catalase activity (CAT) (UmL^{-1}) in different organs of *Labeo rohita*. Values are means of three replications and are given with standard error.

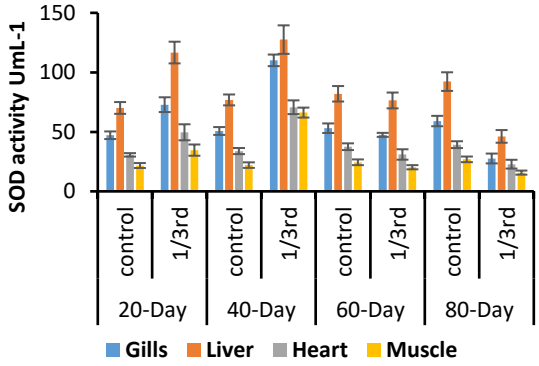

Fig. 6: Effect of ZnO-NPs on superoxide dismutase activty (SOD) (UmL^{-1}) in different organs of *Labeo rohita*. Values are means of three replications and are given with standard error.

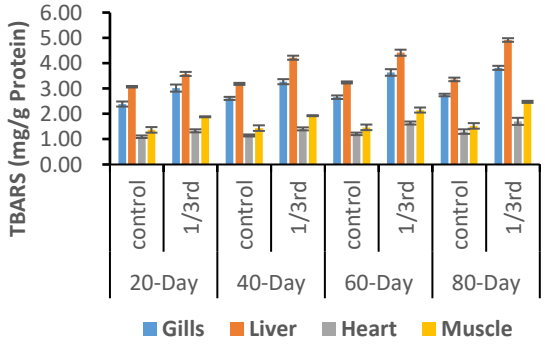

Fig. 7: Changes in TBARS level (mg/g protein) in different organs of *Labeo rohita*. Values are means of three replications and are given with standard error.

Table 1: 96h acute toxicity of ZnO-NPs (mg/L) for *Labeo rohita*

Fish species	MeO-NPs	LC_{50}	95%CI	Lethal conc.	95%CI	Pearson goodness of fit tests		
			(LCL-UCL)		(LCL-UPL)	Chi-Square	DF	P-value
Labeo rohita	ZnO-NPs	31.15	26.29-35.31	57.84	51.39-68.73	3.31045	11	0.986

CI, confidence interval (mg/L); LCL, lower confidence limit (mg/L); UCL, upper confidence interval (mg/L); Lethal Conc., lethal concentrations (mg/L); DF, degree of freedom.

Acute toxicity of ZnO-NPs: The temperature of water was controlled at 29-30°C during the study period. Dissolved oxygen and pH of water were examined (5-7mg/L and 7.5, respectively). After every 24 hours, dead fish were examined and instantly removed from the test solutions to prevent pollution in environmental condition. The toxicity of nano-scale zinc oxide in *Labeo rohita* was increased as the particle concentration increased, showing dose dependent response (Fig. 4). The mean 96-h LC_{50} and lethal concentration of Zinc oxide nanopowder for *Labeo rohita* were calculated as 31.15 and 57.84mg/L, respectively at 95% confidence interval (Table 1).

Oxidative stress biomarkers

Catalase (CAT) activity: CAT activity in specified organs of *Labeo rohita* was measured after the exposure to sub-lethal dose ($1/3^{rd}$ of 96-hr LC_{50}) for 20-day, 40-day, 60-day and 80-day intervals (Fig. 5). Fish showed significant increase over 20-day followed by a sharp decrease at 40, 60 and 80 days in CAT activity than that of control group. Due to the high concentration of ZnO-NPs, CAT became inactivate, which enhance the creation of ROS (reactive oxygen species) in fish. Our results demonstrated that effects of ZnO-NP were directly proportional to the duration of exposure.

Superoxide dismutase (SOD) activity: Sub-lethal exposure of Zinc oxide nanoparticles caused significant variable activity of super oxide dismutase in specified organs of *Labeo rohita (*Fig. 6). Fish organs showed significant increase after 20, and 40 days followed by a sharp decrease at 60 and 80 days as compared to control group in SOD activity which enhance the creation of ROS (reactive oxygen species). In our present study, level of superoxide dismutase was in the following trend: liver > gills > heart > muscles.

TBARS assay: The levels of TBARS as an effective symbol to detect lipid peroxidation have been used during this research. Chronic exposure of ZnO-NPs showed significant variability in induction of TBARS level in body organs of *Labeo rohita*. Level of TBARS increased significantly with increase of exposure duration than control (Fig. 7).

DISCUSSION

In all environmental problems, pollution is a most severe danger to human-beings and world ecosystems. Fish show fundamental role in the transfer of energy from lower trophic levels to higher and have been utilized as bio-indicator of aquatic environmental status (Monteiro *et al*., 2010). Nanoparticles show peculiar behavior in comparison with bulk forms of similar chemical configuration due to its smaller size, higher reactiveness and greater surface sphere per unit mass. Chemical pattern and high surface reactivity of nanoparticles resulted in toxicity of aquatic systems (Ramskov *et al*., 2015).

Finding of LC_{50} concentration is most beneficial to evaluate the safety limits and tolerable limits of several toxicants (Prentera *et al*., 2004). Previous studies also showed acute lethal value always measured in mg/L not in ug/L. It is indicated that metal oxide nanoparticles are less soluble and cause less pollution. Present work shows acute toxicity of nanoscale ZnO for *Labeo rohita* as 31.15 mg/L. In contrast to our research, 96 h LC50 of ZnONPs in zebra fish were determined as 4.92 mg/L (Asghar *et al*., 2015). The difference in toxicity may be due to different physico-chemical properties of nanosacle ZnO that will affect toxicity mechanism of nanoparticles. Many researchers indicated that pollution of ZnO nanoparticles has close relation with its soluble free ions while some research articles reported that the ZnO nanoparticles was more toxic as compared to its ionic form (Wong *et al*., 2010). Miao *et al*. (2010) investigated level of ZnO-NPs toxicity at various pH (7-9) and reported that ZnO-NPs showed elevated toxicity at low pH due to excess dissolution of Zn ions in exposure medium.

Antioxidants are an essential part to examine the water pollution and prior to the occurrence of harmful impacts in fish, enzymatic activities work as susceptible biochemical indicators. One of the most frequently studied topics of NPs toxication is oxidative stress. Present study showed fluctuations in CAT and SOD activity as duration increases. *Labeo rohita* showed significant increase after 20 days followed by a sharp decrease at 40, 60 and 80 days in CAT activity than that of control group. This result is consistent with Abdelazim *et al*. (2018) who reported significantly decreased CAT, GPx, and GST activities in Nile Tilapia after the exposure of ZnONPs. ZnO nanoparticles act as an inhibitor in Catalase activities which suggested that hydrogen peroxide (H_2O_2) produced through SOD was not directly eliminated via Catalase. Abdel-Khalek (2015) suggested that CAT activity in different organs of fish was disturbed due to frequent contact with metals and instability of superoxide free radicals. Our results are also matched with Xia *et al*. (2013) who reported significantly decreased CAT activity at concentrations equal to or higher than 160 mg/L.

Superoxide dismutase is helpful in the disintegration of free radical superoxide by changing it into hydrogen peroxide (H_2O_2), after which is decayed by CAT at higher levels (Arimoto *et al*., 2005). Low activity of SOD under strong stress of metals can lead to accumulation of more reactive oxygen species in animals that ultimately results in damage of cell (Phull *et al*., 2018). Present study showed significant increase in SOD activity after 20, and 40 days followed by sharp decrease at 60 and 80 days as compared to control group. This is in accordance with previous study that SOD activity was significantly reduced after 100 mg/L exposure of ZnO-NPs that results in excessive ROS in cell (Zhao *et al*., 2013). This process can also be demonstrated that ZnO-NPs caused ROS synthesis which the antioxidant system could not eliminate. Alteration in SOD activity in target organ was observed in this study. Xiong *et al*. (2011) reported that

ZnO-NPs showed significantly reduced SOD level in liver and increased levels in gut of adult zebrafish, indicating that the nanopowders affected the SOD activity of target tissues specifically.

In present study, lipid peroxidation level was increased in target organ of fish after exposure of ZnO-NPs. In the same line of our data, Abdelazim *et al*. (2018) reported more lipid peroxidation in the muscle of treated Nile Tilapia after ZnO nanopowder exposure. More MDA contents in selected organs of carp after 50 mg/L ZnO NPs for 10 and 14 days, showed the oxidative stress of ZnO nanopowder in cells of fish (Hao and Chen, 2012). Rise in Lipid peroxidation level may be attributed to the change in mechanism of antioxidant system to inhibit the available radicals formation (Kim *et al*., 2010). The measurement of lipid peroxidation supplies a comparative measurement of the all possible toxicants to drive oxidative harm. Abdel-Khalek (2015) investigated significant rise in both gills and liver lipid peroxidation level after Zn bulk and nanoparticles exposure after different durations. It has been mostly recognized that free radicals that produced due to stress are damaging element, which results in high lipid peroxidation and inactivation of different enzymes (Valko *et al*., 2004).

Conclusions: Acute toxicity of ZnO nanopowder to *Labeo rohita* was determined at 96h (LC_{50}) as 31.15 mg/L. Zinc oxide nanoparticles disturbed the functions of many enzymes that provide defense due to excess of ROS (reactive oxygen species). After the exposure of 1/3rd of 96-h LC_{50} of ZnO nanopowder, reactivity of LPO, CAT and SOD in *labeo rohita* significantly fluctuated and showed a tissue specific response. The well-known functions of synthesized metal oxide nanoparticles develop an interest towards the protection of freshwater biota and mankind. It is necessary to promote this work to understand the impacts of different ecological factors on pollution of nanoparticles and a distinct mechanism of toxicity.

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Authors contribution: SA executed the research. SA supervised and planned the research. KA and AZ helped in conducting research in their laboratories.

REFERENCES

Arimoto T, Kadiiska MB and Sato K, 2005. Synergistic production of lung free radicals by diesel exhausts particles and endotoxin. Am J Respir Crit Care Med 171:379-87.

Abdel-Khalek AA, 2015. Antioxidant responses and nuclear deformations in freshwater fish, *Oreochromis niloticus*, facing degraded environmental conditions. Bull Environ Contam Toxicol 94:701-8.

Asghar MS, Qureshi NA, Jabeen F, *et al*., 2015. Toxicity of zinc nanoparticles in fish: a critical review. J Biol Environ Sci 7:431-9.

Abdelazim AM, Saadeldin IM, Swelum AAA, *et al*., 2018. Oxidative stress in the muscles of the fish Nile tilapia caused by zinc oxide nanoparticles and its modulation by vitamins C and E. Oxid Med Cell Longev 2018:1-9.

Afifi M, Saddick S and, Zinada OAbu, 2016. Toxicity of silver nanoparticles on the brain of *Oreochromis niloticus* and *Tilapia zillii* Saudi J Biol Sci 23:754-60.

Baker TJ, Tyler RCR and Galloway TS, 2014. Impacts of metal and metal oxide nanoparticles on marine organisms. Environ Pollut 186:257-71.

Chang YN, Zhang M, Xia L, *et al*., 2012. The toxic effects and mechanisms of CuO and ZnO nanoparticles. J Mater 5:2850-71.

Doyle JJ, Ward JE and Mason R, 2016. Exposure of bivalve shellfish to titania nanoparticles under an environmental-spill scenario: Encounter, ingestion and egestion. J Mar Biol Assoc UK 96:137-49.

Gatta PP, Pirini M, Testi S, *et al*., 2000. The influence of different levels of dietary vitamin E on sea bass *Dicentrarchus labrax* flesh quality. Aquacult Nutr 6:47-52.

Ghaffar A, Hussain R, Khan A, *et al*., 2015a. Hemato-biochemical and genetic damage caused by triazophos in fresh water fish, *Labeo rohita.* Int J Agric Biol 17:637-642.

Ghaffar A, Hussain R, Khan A, *et al*., 2015b. Butachlor induced clinicohematological and cellular changes in fresh water fish Labeo rohita (Rohu). Pak Vet J 35:201-206.

Hamid A, Khan MU, Yaqoob J, *et al*., 2016. Assessment of mercury load in river Ravi, urban sewage streams of Lahore Pakistan and its impact on the oxidative stress of exposed fish. J Bio Environ Sci, 8:63-72.

Hamilton MA, Russo RC and Thurston RV, 1977. Trimmed Spearman-Karber method for estimating median length concentration in toxicity bioassays. Environ Sci Technol 11:714-9.

Hao LH and Chen L, 2012. Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. Ecotoxicol Environ Saf 80:103-10.

Javed M, 2013. Chronic effects of nickle and cobalt on fish growth. Int J Agric Biol 15:575-9.

Kim HY, Kim JK, Choi JH, *et al*., 2010. Hepatoprotective effect of pinoresinol on carbon tetrachloride induced hepatic damage in mice. J Pharmacol Sci 112:105-12.

Monteiro D, Rantin F, Kalinin A, 2010. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxa, *Brycon amazonicus* (Spix and Agassiz, 1829). Ecotoxicology 19:105-23.

Miao AJ, Zhang XY, Luo Z, *et al*., 2010. Zinc oxide engineered nanoparticles: dissolution and toxicity to marine phytoplankton. Environ Toxicol Chem 29:2814-22.

Ng CT, Yong LQ, Hande MP, *et al*., 2017. Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human beings fibroblasts and *Dorsophila melanogaster*. Int J Nanomed 12:1621-37.

Phull AR, Nasir B, Haq Iul, *et al*., 2018. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Biol Interact 281:121-36.

Piccino F, Gottschalk F, Seeger S, *et al*., 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanopart Res 14:1-11.

Vinardell PM, Llanas H, Marics L, *et al*., 2017. In vitro comparative skin irritation induced by nano and non-nano zinc oxide. Nanomaterials 7:56-64

Prentera J, Macneila C, Dicka JTA, *et al*, 2004. Lethal and sub-lethal toxicity of ammonia to native, invasive and parasitized freshwater amphipods. Water Res 38:2847-50.

Roco MC, 2003. Nanotechnology: convergence with modern biology and medicine. Curr Opin Biotechnol 14:337-46.

Ramskov T, Thit A, Croteau MN, *et al.,* 2015 Biodynamics of copper oxide nanoparticles and copper ions in an oligochaete–part I: relative importance of water and sediment as exposure routes. Aquat Toxicol 164:81-91.

Saddick S, Afifi M and OAbu Z, 2017. Effect of zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii.* Saudi J Biol Sci 24:1672-8.

Valko M, Izakovic M, Mazur M, *et al*., 2004. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 226:37-56.

Wong SWY, Leung PTY, Djurisic AB, *et al*., 2010. Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. Anal Bioanal Chem 396:609-18.

Weydert CJ and Cullen JJ, 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cells and tissues. Nat Protoc 5:51-66.

Xia J, Zhao HZ and Lu GH, 2013. Effects of selected metal oxide nanoparticles on multiple biomarkers in *Carassius auratus.* Biomed Environ Sci 26:742-9.

Xiong DW, Fang T, Yu LP, *et al*., 2011. Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafifish: acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409:1444-52.

Zhao X, Wanga S, Wub Y, *et al*., 2013. Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquat Toxicol 136-137:49-59.