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Effects of sub-chronic exposure to zinc nanoparticles on tissue accumulation, serum biochemistry and histopathological changes in tilapia (*Oreochromis niloticus*)

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Abstract

Zinc nanoparticles (ZnNPs) are among the least investigated NPs and thus their toxicological effects are not known. In this study, tilapia (*Oreochromis niloticus*) were exposed to 1 and 10 mg/L suspensions of small size (SS, 40–60 nm) and large size (LS, 80–100 nm) ZnNPs for 14 days under semi-static conditions. Total Zn levels in the intestine, liver, kidney, gill, muscle tissue and brain were measured. Blood serum glucose (GLU), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) were examined to elucidate the physiological disturbances induced by ZnNPs. Organ pathologies were examined for the gills, liver and kidney to identify injuries associated with exposure. Significant accumulation was observed in the order of intestine, liver, kidney and gills. Zn levels exhibited time- and concentration-dependent increase in the organs. Accumulation in kidney was also dependent on particle size; NPs SS-ZnNPs were trapped more effectively than LS-ZnNPs. No significant accumulation occurred in the brain ($p>0.05$) while Zn levels in muscle tissue increased only marginally ($p=0.05$). Significant disturbances were noted in serum GOT and LDH ($p<0.05$). The GPT levels fluctuated and were not statistically different from those of controls ($p>0.05$). Histopathological tubular deformations and mononuclear cell infiltrations were observed in kidney sections. In addition, an increase in melano-macrophage aggregation intensity was identified on the 7th day in treatments exposed to LS-ZnNPs. Mononuclear cell infiltrations were identified in liver sections for all treatments. Both ZnNPs caused basal hyperplasia in gill sections. Fusions appeared in the gills after the 7th day in fish treated with 10 mg/L suspensions of SS-ZnNPs. In addition, separations in the secondary lamella epithelia were observed. The results indicated that

exposure to ZnNPs could lead to disturbances in blood biochemistry and cause histopathological injuries in the tissues of *O. niloticus*.

Keywords

Zinc nanoparticles; Accumulation; Serum biochemistry; Histopathology; *Oreochromis niloticus*

INTRODUCTION

Engineered nanomaterials (ENM) are produced from a variety of materials, such as metals (Zn, Ag, Fe and Cu), metal oxides (TiO₂, ZnO, Fe₂O₃), non-metals (silica and quantum dots), carbon (nanotubes and fullerene), polymers (alginate and chitosan), and lipids (soybean lecithin and stearic acid) (Dreher, 2004; Moore, 2006; Scown et al., 2010). Over the last decade, ENM with unique physical and chemical properties have been used increasingly in the formulations of commercial products (Savolainen et al. 2010; Scown et al., 2010; Sun et al., 2014). Zinc nanoparticles (ZnNPs) are among the one of the most produced nanomaterials. Today, ZnNPs have found use in a vast number of applications or industries, including coatings, first-aid bandages, nanofiber, nanowires, plastics, alloys and textiles (Heinlaan et al., 2008; Savolainen et al., 2010). Products formulated with ZnNPs are also being used as antimicrobial, antibiotic and anti-fungal agents. Potential electrical, dielectric, magnetic, optical, catalytic, biomedical properties of Zn NPs are being investigated to expand their applications (Rasmussen et al., 2010; Savolainen et al., 2010).

Although the field of nanoscience is still in its early stages, nanoscale materials have been detected in aquatic ecosystems that are released from commercial products (Benn and Westerhoff, 2008; Geranio et al., 2009; Ates et al., 2013a). In aqueous environments, NPs undergo numerous transformations, such as aggregation, dissolution, complexation and oxidation-reduction that determine the retention, bioavailability and consequently toxicity of NPs in aquatic organisms (Nel et al. 2006). Beside environmental transformations, size, shape, route of synthesis, chemical composition, and surface coatings also influence the toxicological effects of NPs (Oberdörster et al. 2005; Reijnders, 2008). It is therefore now accepted that the understanding of fate and potential toxic effects of nanomaterials on human and environmental health is a complex task.

Aquatic microorganisms (e.g., phytoplankton and zooplankton) and fish are among the major species that are prone to the exposure to NPs available in the water. Numerous studies have been performed using model species to elucidate the safety and/or toxicological effects of NPs to aquatic microorganisms, and potential transport rates within the trophic food chain (Arujova et al., 2009; Blinova et al., 2010; Ates et al., 2013b; Hao et al., 2013; Imani et al., 2014; Ladhar et al., 2014; Ates et al., 2015). Ingested NPs are mainly stored in guts of planktonic microorganisms. In contrast, ingested NPs are easily transported by blood and accumulate in the organs and tissues of fish (Handy et al., 2008). The variations in blood parameters, tissue metal levels and pathological changes in tissues of the test organisms have been scrutinized to draw inferences about toxicological effects of NPs and the mechanisms of action (Wester et al., 2002; Lai, 2011; Schrand et al. 2012). Often, initial effects are

observed in filtration and detoxification sites, including the gills, liver and kidney. Disturbances in the functions of these organs significantly affect the homeostasis. Potential injuries the gill epithelia lead to suffering even death of the organism. Thus, histopathological changes in the gills are important bioindicators for assessing the health of the fish (Kaya et al., 2013). Similarly, histological damages could be utilized to estimate the functional disorders on liver and kidney that are responsible for detoxification and excretion of toxic substances.

Various fish species (e.g., rainbow trout, carp, and zebrafish) have been used as model animals to investigate toxicological effects of a number of metal and metal oxide NPs, such as Ag (Imani et al., 2014), Cu (Shaw et al., 2012; Al-Bairuty et al., 2014; Sovová et al., 2014), ZnO (Hao et al., 2012, 2013; Ates et al. 2015; Kaya et al., 2016), CuO (Zhao et al., 2011; Ates et al., 2014, 2015), TiO₂ (Hao et al., 2009; Boyle et al. 2013), Fe₂O₃ (Remya et al., 2015; Saravanan et al. 2015; Zhang et al. 2015). To date, studies attempting to elucidate the adverse effects of ZnNPs have been conducted only recently by a handful of groups (Ates et al., 2103; Adel Abdel-Khalek, 2015), therefore, there is a need for comprehensive studies detailing the ecotoxicological effects of ZnNPs. In this study, tilapia were exposed to 1 and 10 mg/L aqueous suspensions of two different sizes (40–60 nm and 80–100 nm) of ZnNPs in a semi-static regime for 14 days. The objectives of the study were to determine the physiological accumulation and distribution of ZnNPs in the organs of tilapia and toxicological impacts on the fish. Serum biochemical variables were examined to elucidate health status of fish. Histology was conducted on the sections of gills, kidney and liver to determine the pathological effects of waterborne exposure to ZnNPs on the tissues.

MATERIALS AND METHODS

Reagents and chemicals

Zinc nanoparticles were purchased as uncoated nanomaterials from Skyspring Nanomaterials, Inc. (Houston, TX) and stored at room temperature. The sizes of the ZnNPs were 40–60 nm and 80–100 nm (99.5% pure), and they are denoted as small size (SS-ZnNPs) and large size (LS-ZnNPs), respectively (Table 1). Deionized water used in the experimental studies was produced by a Barnstead E-pure system with 18.0 MΩ-cm resistivity. Trace metal grade nitric acid (HNO₃, Fisher Scientific) was used for the dissolution of the tissue samples and organs extracted from exposed tilapia. Carbon-coated Cu TEM grids (300 mesh) were purchased from Electron Microscopy Sciences (Hatfield, PA).

Exposure design

Exposure was conducted according to Organization for Economic Cooperation and Development (OECD section 203) test directives (OECD, 2004). A total of 150 tilapia (*O. niloticus*) (average weight of 30.2 ± 4 g) were used in the study. The fish were procured from Çukurova University Faculty of Fisheries, Aquaculture Department Laboratory and acclimated to ambient conditions for one month in 15 stock aquaria (45 × 28 × 80 cm) that contained 60 L rested tap water. During the adaptation period, the fish were fed with commercial fish pellets (35% protein, 10% fat). For exposure, 10 fish were randomly placed

into 15 aquaria with control and four treatments comprising 1 and 10 mg/L concentrations of SS- and LS-ZnNPs. Exposure was carried out with three-recurrences for 14 days. Control fish were maintained in rested tap water throughout. Feeding was terminated 24 h prior to initiating the experiment. No food was provided during the experiment. The exposure concentrations were determined based on the information provided in the literature (Hao et al., 2012). Semi-static approach was followed during the experiment in that 75% of the water was replaced in the morning and 25% was replaced in the evening. In each water replacement, appropriate amounts of ZnNPs were added from freshly made aqueous suspensions to the tanks to maintain the exposure concentrations (Kaya et al., 2015). Temperature and dissolved oxygen levels of the water were measured by a YSI MPS 556 probe and were 25.5 ± 0.5 °C and 5.4 ± 0.1 mg/L, respectively. The pH of water was measured by a HANNA C 200 (HI 83200) photometer daily, and maintained at pH: 6.96 ± 0.1 . Total hardness was around 142 ± 6 mg CaCO_3 /L which was measured by Optizen POP UV-VIS spectrophotometer. Electrolyte levels in tanks were measured on alternating days by a Varian Liberty Sequential ICP-OES instrument. Sampling was made at three intervals; on day 0 (before any chemical application), 7th day and 14th day. On day 0, 10 fish from stock fish aquaria were taken. On the 7th and 14th days, 10 fish from control aquaria and 5 fish from each treatment aquarium (5 fish/aquarium * 15 aquaria = 45 fish) were randomly taken out. All experiments were conducted by following ethical rules.

Preparation and characterization of NP suspensions

Stock suspensions of ZnNPs (10% m/v) were prepared by dispersing appropriate amounts of ZnNPs in deionized water in polypropylene tubes. For dispersion, the suspensions were vortexed and then exposed to ultrasounds for 10 min. Appropriate volumes of the stock suspension were immediately transferred into the glass exposure tanks to yield 1 or 10 mg/L ZnNPs in 30 L tap water. Samples of suspensions were taken from stock and exposure medium to record the images of ZnNPs by transmission electron microscopy (TEM) (Fig. 1). A volume of 10 μL colloidal ZnNPs solution was dropped onto 50 Å thick carbon-coated copper grids and allowed to dry overnight at room temperature. TEM images were recorded by a JEOL–1011 TEM instrument providing a resolution of 0.2 nm lattice with magnification of 50 to 10^6 under the accelerating voltage of 40 to 100 kV (JEOL, Peabody, MA, USA). Hydrodynamic sizes and zeta potentials in the stock solution and exposure medium were characterized by dynamic light scattering (DLS) using a Malvern Nano ZS zetasizer. DLS and zeta potential measurements were made in triplicate for individual suspensions using automated, optimal measurement time and laser attenuation settings. The recorded correlation functions and measured particle mobilities were converted into size distributions and zeta potentials, respectively, using the Malvern Dispersion Software (V5.10; www.zetasizer.com).

Preparation of tissues for zinc analysis

On days 0, 7 and 14 of the experiment, four of the fish were taken from each group, anesthetized with MS-222 and dissected to collect the gills, liver, kidney, intestine, brain and muscle tissue. The organs were washed with deionized water and digested with HNO_3 to determine total Zn accumulation as described elsewhere (Ates et al., 2014, 2015). After measuring the wet weights, samples were dried in an incubator for 2 days at 70 °C. The

dried tissues were digested in 4 mL of 50% HNO₃ in the microwave oven, and diluted to 10 mL after cooling the digests. A volume of 1 mL of the digests was further diluted to 10 mL and analyzed by ICP-MS. Accuracy of the Zn measurements were monitored by analyzing Dogfish (*Squalus acanthias*) muscle (DORM-2) and American lobster (*Homarus americanus*) hepatopancreas (TORT-2) certified reference materials (CRM) obtained from National Research Council of Canada (NRC, Ottawa, Ontario, Canada). The CRMs solutions were prepared similarly by digesting 0.1 g homogenized dry powders in 4 mL of 50% HNO₃. Measured Zn values for the CRMs were found between 92–108% of the certified values. All Zn concentrations were expressed as micrograms per gram (µg/g) in dry weight. Determinations were performed by Spectro-MS ICP-MS instrument (Spectro Analytical Instruments, Kleve, Germany).

Blood sampling and biochemical analyses

Five (5) of the fish collected in each test period were randomly assigned for blood analyses. Fish were anesthetized with clove oil, a natural product commonly used for anesthesia. The area behind the anus fin was cleaned thoroughly with 70% ethanol to avoid mixing of mucous membrane into the blood. Blood was immediately drawn without hurting the animal through caudal vein using a 5-mL plastic injector syringe and placed into serum tubes with gel. For biochemical analyses, the blood samples were centrifuged for 5 min at 5000 rpm. The serum (e.g. plasma) fraction was separated and stored at –80 °C. Serum biochemical analyses of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), and glucose (GLU) were conducted with Thermo multi-scan Go micro-plate reader using test kits (Bioanalytic Diagnostic Industry, Co.).

Histopathology

Of the 15 fish, remaining 6 were used for histological assessment. The gill, liver and kidney tissues of the fish sampled on days 0, 7 and 14 were examined histopathologically. After fixing in Bouin's solution for 8 h, the tissues were stored in 70% ethanol until the preparation of wax blocks. During the preparation stage, the tissues were passed through 80%, 90% and 96% absolute ethanol, respectively. Tissues were then passed through xylene and embedded in paraffin. Five µm cross-sections were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E) for routine wax histology. Slides were examined under an Olympus CX31 optical microscope. Photographs were captured by a camera attached to an Olympus BX51 optical microscope using Olympus Analysis LS software.

Statistical assessment

Data were analyzed by using SPSS 21 statistical analysis package. One-way analysis of variance (ANOVA) with Tukey or Kruskal Wallis subtests was used to identify the effects of exposure on treatments. Level of significance on intra-group differences was set at $p < 0.05$.

RESULTS AND DISCUSSION

Zinc is an essential trace metal present in all organs, tissues and body fluids serving for various enzymatic reactions and neurological processes in biological systems (Qin et al., 2008; Deshpande et al., 2013). It is also commonly present in foods or added as a nutritional

supplement. As a result, ZnNPs are often considered to be of low toxicity, and so far have received little attention in ecotoxicological studies of nanomaterials (Ates et al., 2013; Abdel-Khalek et al., 2015). Nevertheless, ZnNPs in aquatic systems are likely to dissolve into free Zn ions (Zn^{2+}) (Ates et al., 2013) that are toxic to most cells and neurons (Qin et al., 2008; Plum et al., 2010). In this study, no mortality occurred in the during 60-day exposure. However, inactivity, hiding and escaping behaviors were observed in all fish treated with ZnNPs, which was indicative of the physiological and toxicological disturbances of ZnNPs on tilapia.

Characterization of colloidal properties ZnNPs

TEM images of ZnNPs have shown that both SS-ZnNPs and LS-ZnNPs contained mixtures of spherical and rod-shapes particles (Fig. 1). Aggregation of the NPs was also visible on TEM images, for which estimated sizes from dried colloids are provided in Table 1. As for many other metal-based nanostructures, agglomeration of ZnNPs in water was significant. Results of DSL measurements (Table 1) indicate that the colloidal ZnNPs possessed much larger sizes than dry-particles (TEM). Hydrodynamic sizes were approximately 477 nm and 662 nm for SS-ZnNPs and LS-ZnNPs, respectively. These results are consistent with the zeta potentials that indicate that surfaces of both NPs were negatively charged; yet the values very small to prevent the aggregation of the NPs in water.

Tissue zinc accumulation

The concentration of Zn measured in the tissues of *O. niloticus* exposed to ZnNPs is presented in Fig. 2. Intestines possessed the highest Zn levels followed by the liver, kidney, gills, muscle, and the brain. These results is consistent with other studies that intestines in fish are the major end-points for water-borne and food-borne particles. Exposures of sheepshead minnow, goldfish, rainbow trout, carp, tilapia and zebrafish to various metal oxide NPs, such as CuO, Al_2O_3 , TiO_2 , Fe_2O_3 and ZnO NPs, have also resulted in excessively high accumulation in the intestines (Ramsden et al. 2009; Hao et al., 2013; Boyle et al., 2013; Ates et al., 2014, 2015; Kaya et al., 2015; Zhang et al., 2015). The intestinal Zn levels increased with time and NP concentration. On the 7th day, intestines possessed about 625 $\mu\text{g/g}$ Zn that increased to 1055 $\mu\text{g/g}$ on the 14th day in groups exposed 1 mg/L suspensions to SS-ZnNPs (40–60 nm). The accumulation pattern were similar for the LS-ZnNPs (80–100 nm) that Zn levels increased from 656 $\mu\text{g/g}$ (7th day) to 1120 $\mu\text{g/g}$ (14th day). For 10 mg/L suspensions, total intestinal Zn was about 778 and 978 $\mu\text{g/g}$ on the 7th day for SS- and LS-ZnNPs, respectively, and increased to 1528 $\mu\text{g/g}$ (SS) and 1630 $\mu\text{g/g}$ (LS) on the 14th day. The variation in particle size did not affect the intestinal accumulation ($p>0.05$), though the levels for LS-ZnNPs were relatively high. On one hand, this effect could be attributed to relatively small differences in the sizes of the ZnNPs (e.g., 40–60 nm and 80–100 nm), but it was more likely due to the direct assimilation of NPs through digestive tract. Because no food was provided during the experiment, fish could have fed on the suspending ZnNPs that were accumulated in the guts unselectively of the hydrodynamic size differences. Similarly, Zhang et al. (2015) observed aggregates of NPs in the intestines of zebrafish from chronic exposure (28 days) to Fe_2O_3 NPs. Equally important, there was not any significant accumulation after the 14th day since the guts were completely full with ingested particles.

Kidney is the major filtration site in fish responsible for trapping the particles from blood. Liver primarily serves for detoxification of toxins but is also a filtration site as kidney. Zn concentrations in the liver and kidneys of the controls were virtually constant around 40 and 71 µg/g, respectively (Fig. 2). Treatments showed time- and concentration-dependent accumulation. Zn levels in the liver of treatments exposed to 1 mg/L suspensions were about 106 µg/g for SS-ZnNPs and 123 µg/g LS-ZnNPs on the 7th day and increased to 221 and 236 µg/g on the 14th day, respectively. For 10 mg/L suspensions, Zn levels for SS-ZnNPs increased from 200 µg/g (7th day) to 370 µg/g (14th day), and from 270 µg/g (7th day) to 380 µg/g (14th day) for LS-ZnNPs. Kidney Zn levels for 1 mg/L suspensions of SS-ZnNPs increased from 160 µg/g (7th day) to 212 µg/g (14th day) and from 254 µg/g (7th day) to 403 µg/g (14th day) for 10 mg/L suspensions. For LS-ZnNPs, Zn levels in the kidney did increase from 155 to 175 µg/g from 7th to 14th day of exposure for 1 mg/L suspensions, and from 245 to 285 µg/g for groups exposed to 10 mg/L suspensions. However, it was evident that total Zn concentration was significantly lower than for SS-ZnNPs indicating that LS-ZnNPs were not trapped in the kidney as effectively as the SS-ZnNPs ($p < 0.05$). This effect was not observed in the liver under the same time and concentration regime ($p > 0.05$, see Fig. 2). These results could indicate that accumulation of toxic substances including particles appears to be dependent on the filtering mechanisms of the organs. In addition, liver and kidney receive high blood flow, yet the liver possesses lobular structure and is at the central location within the circulatory system where xenobiotic exchange between the blood and hepatocytes reaches the maximum. This aspect differentiates the liver from intestines and branchial pathways, rendering it an early target organ for toxins (Di Giulio and Hinton, 2008). Indeed, the results demonstrated that liver accumulated marginally more ZnNPs compared with kidney ($p = 0.05$). These findings are consistent with those reported for ZnO NPs exposures on carp (Hao et al., 2013) and tilapia (Kaya et al., 2015).

Gill epithelia are the first interface between the contaminant and the fish. Because of the large surface area, gills have been reported to accumulate NPs extensively from water (Ramsden et al., 2009; Zhao et al., 2011; Hao et al., 2013; Boyle et al., 2013; Ates et al., 2014, 2015; Kaya et al., 2015). In this study, accumulation of ZnNPs on the gills increased with time and NP concentration ($p < 0.05$), but was not affected from differences in NP size ($p > 0.05$). For instance, Zn concentration was 156 µg/g on the 7th day and increased to 185 µg/g on the 14th day for 1 mg/L suspensions SS-ZnNPs. Similarly, exposure to 1 mg/L suspensions of LS-ZnNPs resulted in accumulation equivalent to 124 µg/g Zn (7th day) that increased to 181 µg/g on the 14th day. In case of 10 mg/L sessions, gills contained significantly higher Zn on the 7th day for both ZnNPs compared with 1 mg/L suspensions. On the 14th day, LS-ZnNPs exhibited increasing accumulation ($p < 0.05$) while that for SS-ZnPs was marginal ($p = 0.05$) relative to the values on the 7th day. This accumulation could be explained with the retention of ZnNPs on the gill mucosa (Shaw and Handy, 2011).

The Zn concentrations in the muscle tissue of exposed tilapia were much lower compared to organs, ranging between 50 µg/g (7th day) to 85 µg/g (14th day) for SS-ZnNPs, and 62 µg/g (7th day) to 93 µg/g (14th day) for LS-ZnNPs. In general, white muscles of fish are the least affected organs from the contaminants due to their low blood perfusion rates (Di Giulio and Hinton, 2008). Yet, the accumulation in the muscle tissue of the treatments were statistically higher than in controls ($p < 0.05$). These results are consistent with accumulation of other

metal oxide NPs, such as CuO, ZnO and TiO₂ NPs in the muscle tissue of carp, tilapia and rainbow trout, respectively (Ramsden et al., 2009; Zhao et al., 2011; Hao et al., 2013; Kaya et al., 2015). Among the examined organs, brain did not show any accumulation (see Fig. 2). Zn levels were not significantly different from those of controls till the end of the study ($p>0.05$). This could suggest that NPs in the blood stream were too large to pass the blood-brain barrier (Shaw et al., 2012; Ates et al., 2014, 2015). Nonetheless, the metal levels have been reported to increase in the brain of fish exposed to NPs (Zhao et al., 2011; Ladhar et al., 2014; Kaya et al., 2015). Copper on the brain of carp for instance were noted to increase after the 30th day of exposure to CuO NPs (Zhao et al., 2011), whereas Cd levels showed increases after 60 days in zebrafish brain in an exposure to CdSe NPs (Landhar et al., 2014). These findings could suggest that accumulation of NPs in the brain tissue is very slow in comparison to other target organs that notable internalization occurs under chronic exposure regimes.

Serum biochemistry

Variations in serum glucose (GLU) levels are important parameters used as biomarkers of stress in fish (Kaya et al., 2014). The increase of the GLU levels is an indicator of depletion of glycogen reserves and glycolysis capacity as a result of decrease in certain enzymes activities, such as phosphofructokinase, lactate dehydrogenase and citrate kinase under stressful conditions. Decreasing glycolysis capacity leads to reduced glucose breakdown and glycogen formation reduction (i.e., increased glucose levels in the blood) (Almeida et al., 2001; Adel Abdel-Khalek et al., 2015). The results from biochemical analysis of serum samples are summarized in Table 2. Within the 7 day period, glucose (GLU) levels fluctuated in treatments and the changes were not statistically different from those of controls ($p>0.05$). On the 14th day, in contrast, glucose values increased significantly in treatments exposed to 10 mg/L suspensions of SS-ZnNPs but decreased in treatments exposed to 1 mg/L suspensions of LS-ZnNPs ($p<0.05$). On the other hand, serum glucose levels increased progressively till the conclusion of exposure (28th days) in Nile tilapia (*Oreochromis niloticus*) exposed to 90 mg/L suspensions of ZnNPs (< 50 nm) (Adel Abdel-Khalek et al., 2015). These results could be explained with dose-dependent suppression of glycolysis by ZnNPs. Similarly, exposure to 2.4 mg/L suspensions of ZnO NPs caused increases in serum glucose levels in common carp (*Cyprinus carpio* L.) (Lee et al., 2013). In another study concerning the exposure of silver carp (*Hypophthalmichthys molitrix*) to AgNPs, serum glucose concentrations increased significantly on the 3rd and 7th days of exposure, but returned to similar levels on the 14th day (Shaluei et al., 2013).

The activities of serum enzymes, such as glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) aid in identifying health status of fish and thus are advocated to provide early warning of adverse effects in stress conditions (Firat et al., 2011; Kaya et al., 2014). Transaminases, such as GOT and GPT play a significant role in protein and amino acid metabolisms and are released into the plasma in cases of tissue damage or dysfunction caused by metal toxicity. Hence, changes of the activities of these enzymes in the plasma or in extracellular fluid are considered sensitive indicators of even low level cellular damage (Vaglio and Landriscina, 1999; Levesque et al., 2002; Kaya et al., 2014). In this study, GPT activity was found to be suppressed on the 14th

day only in fish exposed to 1 mg/L suspensions of LS-ZnNPs ($p < 0.05$), while no stimulation or suppression was noted in other groups in comparison to controls ($p > 0.05$). GOT activity possessed similarities among the treatments and the controls on the 7th day ($p > 0.05$), but decreased in fish exposed to LS-ZnNPs ($p < 0.05$). In contrast, 90 mg/L suspensions of ZnNPs induced significant increases in both GOT and GPT levels in *O. niloticus* on the 7th day indicating that ZnNPs could be more toxic at elevated levels (Adel Abdel-Khalek et al., 2015). Lee et al. (2013) reported that GPT rates decreased at the end of the study in a twelve-week long exposure of carp to 0.3 to 2.4 mg/L suspensions of ZnO NPs, while GOT levels increased only in fish treated with 2.4 mg/L colloids of the ZnO NPs.

LDH catalyzes the interconversion of lactic and pyruvic acids, and thus plays an important role in regulation of energy metabolism, especially in exposure environmental pollutants (Atli et al., 2015). In this study, LDH rates increased on the 7th day for treatments exposed to 10 mg/L suspensions of LS-ZnNPs ($p < 0.05$). No significant disturbances were observed on the 14th day regardless of the size and concentration of the ZnNPs ($p > 0.05$). Similarly, ZnO NPs were not overtly toxic to carp (Lee et al., 2013). In another study, 0.2 to 0.4 mg/L suspensions of AgNPs increased serum LDH rates in *Oncorhynchus mykiss* at the end of the study (8th day) (Imani et al., 2014). These results suggest that GOT, GPT and LDH activities could vary largely on experimental settings mediated by the relative toxicity of the NPs, duration of exposure and the resilience of the fish species to toxic effects.

Histopathological observations

Histopathological anomalies and lesions in tissues arising from exposure are important bio-indicators in determining the toxicity of substances (Handy et al., 2011). In this study, histopathological changes in kidney, liver and gills were investigated to elucidate the pathological injuries caused by ZnNPs. The results are summarized in Table 3. The gill, liver and kidney cross-sections of controls showed normal pathology. In contrast, tubular deformations and mononuclear cell infiltrations were identified in kidney sections of the exposed fish for both ZnNPs. Furthermore, significant melanomacrophage aggregation was observed at the end of the study in groups exposed to 1 and 10 mg/L suspensions of SS-ZnNPs (e.g., 40–60 nm). Glomerular expansion was observed in kidney sections on the 7th day, especially for suspensions of SS-ZnNPs, while glomerular deformations were identified in treatments exposed to the suspensions of LS-ZnNPs (Fig. 3). On the 7th day, mononuclear cell infiltrations and fatty changes were observed in liver sections of treatments for both ZnNPs. On the 14th day, hepatocellular deformation and focal fatty changes were also observed in the liver sections of all treatments. Similar liver pathologies were observed on the 7th day of the study for treatments exposed to 10 mg/L suspensions of SS-ZnNPs (Fig. 4). In gill sections, generally basal hyperplasia and clubbing of the tips of secondary lamellae were traced in all treatments. Lamellar fusions were evident on the 7th day for fish exposed to 10 mg/L suspensions of SS-ZnNPs. Gill sections incised on the 14th day possessed epithelial lifting in all concentrations of LS-ZnNPs (Fig. 5).

Gills of fish are important organs in toxicological studies owing the fact that they are the sites for direct contact with the environment. In a study, McGeer et al. (2000) reported that suspensions of CuNPs caused hyperplasia, fusion and epithelial separations in the secondary

lamella of the gills of rainbow trout. Similarly, exposure ZnO NPs caused inflections at the tips of secondary lamella, basal hyperplasia, fusion and epithelial separations in *O. niloticus* (Kaya et al., 2016). The changes identified in gill histopathology in this study are consistent with the existing literature. Gill pathologies for all treatments were similar that became more significant with increasing ZnNP concentrations. First lamellar fusion was identified on the 7th day in treatments exposed to 10 mg/L suspensions of SS-ZnNPs, which could indicate that SS-ZnNPs could be more toxic.

Liver is a target organ of metal toxicity. Common pathological injuries observed in the liver of exposed fish include hepatocellular degeneration, vacuolization, fatty changes, changes in sinusoidal areas and necrosis (Mc Geer et al., 2000; Handy et al., 2002). In this study, initially inflammatory reaction in all treatments was noted with an increase in mononuclear cell infiltration count. Significant degenerations in hepatocytes were observed especially at the end of the 14th day from 10 mg/L suspensions of SS-ZnNPs. Tissue damage recorded as fatty change also became more significant in time in fish treated with LS-ZnNPs. Pathological changes in the kidney were related to tubular deformations in kidney sections of all treatments. Significant differences in pathological effects were observed with increasing exposure period and NP concentration. Frequent melanomacrophage aggregations were observed in kidney sections on the 14th day for treatments exposed to 1 mg/L suspensions of SS-ZnNPs substantiating the fact that ZnNPs could be toxic to kidneys of fish.

CONCLUSIONS

The present study is among the first detailing the potential consequences of water-borne exposure to ZnNPs from the points of view of metabolic assimilation to toxicological impacts on tilapia (*O. niloticus*). Based on the experimental evidence, it is concluded that aqueous suspensions of ZnNPs could accumulate substantially in the critical organs, and induce significant adverse effects on fish blood biochemistry and pathological injuries in gill, liver and kidneys. Total body burden and toxic effects were found to increase with exposure time and NP concentration. Size-dependent effects were also observed, especially for kidney, but further investigations are needed with possibly size-wise more distinct ZnNPs to draw definitive conclusions about the size-induced accumulation and toxic effects of ZnNPs. The results, though informative, indicate the ZnNPs are not totally benign, and thus further investigations should be conducted with commercial and custom-made ZnNPs to understand the limits and mechanisms of toxic effects on fish and other microorganisms of aquatic ecosystems.

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REFERENCES

- Adel Abdel-Khalek A, Kadry M, Hamed A, Marie MA. Ecotoxicological impacts of zinc metal in comparison to its nanoparticles in Nile tilapia; *Oreochromis niloticus*. J Basic Appl Zoology. 2015; 72:113–125.
- Almeida JA, Novelli EL, Dal Pai Silva M, Junior RA. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. Environ Pollut. 2001; 114:169–175. [PubMed: 11504339]
- Aruoja V, Dubourguier HC, Kasemets K, Kahru A. Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae *Pseudokirchneriella subcapitata*. Sci Tot Environ. 2009; 407:1461–1468.
- Ates M, Daniels J, Arslan Z, Farah IO. Effects of aqueous suspensions of titanium dioxide nanoparticles on *Artemia salina*: assessment of nanoparticle aggregation, accumulation, and toxicity. Environ Monit Assess. 2013a; 185:3339–3348. [PubMed: 22810381]
- Ates M, Daniels D, Arslan Z, Farah IO, Rivera HR. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. Environ Sci: Processes and Impacts. 2013b; 15:225–233.
- Ates M, Dugo MA, Demir V, Arslan Z, Tchounwou PB. Effect of copper oxide nanoparticles to sheephead minnow (*Cyprinodon Variegatus*) at different salinities. Digest J Nanomater Biostruct. 2014; 9:369–377.
- Ates M, Daniels J, Arslan Z, Farah IO. Comparative study of accumulation, toxicity and trophic transfer of CuO and ZnO nanoparticles through waterborne and dietary exposure of goldfish (*Carassius auratus*). Environ Toxicol. 2015; 30:119–128. [PubMed: 24860999]
- Atli G, Ariyurek SY, Kanak EG, Canli M. Alterations in the serum biomarkers belonging to different metabolic systems of fish (*Oreochromis niloticus*) after Cd and Pb exposures. Environ Toxicol Pharmacol. 2015; 40:508–515. [PubMed: 26310509]
- Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol. 2008; 42:4133–4139. [PubMed: 18589977]
- Blinova I, Ivaska A, Heinlaan M, Mortimer M, Kahru A. Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environ Pollut. 2010; 158:41–47. [PubMed: 19800155]
- Boyle D, Al-Bairuty GA, Ramsden CS, Sloman KA, Henry TB, Handy RD. Subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles are associated with gill rather than brain injury. Aquat Toxicol. 2013; 126:116–127. [PubMed: 23178178]
- Deshpande JD, Joshi MM, Giri PA. Zinc: The trace element of major importance in human nutrition and health. Int J Med Sci Public Health. 2013; 2:1–6.
- Di Giulio, RT., Hinton, DE., editors. The toxicology of fishes. CRC Press; 2008.
- Doaa MM, Hanan HAE. Histological changes in selected organs of *Oreochromis niloticus* exposed to doses of lead acetate. J Life Sci Biomed. 2013; 3:256–263.
- Dreher KL. Health and environmental impact of nanotechnology: Toxicological assessment of manufactured nanoparticles. Toxicol Sci. 2004; 77:3–5. [PubMed: 14756123]
- Firat O, Cogun HY, Yuzeroglu TA, Gok G, Firat O, Kargin F, Kotemen K. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. Fish Physiol Biochem. 2011; 37:657–666. [PubMed: 21229307]
- Geranio L, Heuberger M, Nowack B. The behavior of silver nanotextiles during washing. Environ Sci Technol. 2009; 43:8113–8118. [PubMed: 19924931]
- Hao L, Chen L. Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. Ecotoxicol Environ Safety. 2012; 80:103–110. [PubMed: 22425733]
- Hao L, Wang Z, Xing B. Effect of sub-acute exposure to TiO₂ nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (*Cyprinus carpio*). J Environ Sci. 2009; 21:1459–1466.
- Hao L, Chen L, Hao J, Zhong N. Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): a comparative study with its bulk counterparts. Ecotoxicol Environ Safety. 2013; 91:52–60. [PubMed: 23375439]

- Handy RD, Runnalls T, Russell PM. Histopathologic biomarkers in three spined sticklebacks, *Gasterosteus aculeatus*, from several rivers in Southern England that meet the freshwater fisheries directive. *Ecotoxicology*. 2002; 11:467–479. [PubMed: 12521142]
- Handy RD, Henry TB, Scown TM, Johnston BD, Tyler CR. Manufactured nanoparticles: their uptake and effects on fish—a mechanistic analysis. *Ecotoxicology*. 2008; 17:396–409. [PubMed: 18408995]
- Handy RD, Al-Bairuty G, Al-Jubory A, Ramsden CS, Boyle D, Shaw BJ, Henry TB. Effects of manufactured nanomaterials on fishes: a target organ and body systems physiology approach. *J Fish Biol*. 2011; 79:821–853. [PubMed: 21967577]
- Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A. Toxicity of nanosized and bulk ZnO, CuO and TiO₂ to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere*. 2008; 71:1308–1316. [PubMed: 18194809]
- Imani M, Halimi M, Khara H. Effects of silver nanoparticles (AgNPs) on hematological parameters of rainbow trout, *Oncorhynchus mykiss*. *Comp Clin Pathol*. 2014; 24:491–495.
- Kaya H, Çelik E, Gürkan M, Yılmaz S, Akbulut M. Effects of subchronic exposure to phosalone on oxidative stress and histopathological alterations in common carp (*Cyprinus carpio*, L., 1758). *J Toxicol Environ Health Part A*. 2013; 76:853–864. [PubMed: 24053362]
- Kaya H, Çelik E, Yılmaz S, Tulgar A, Akbulut M, Demir N. Hematological, serum biochemical, and immunological responses in common carp (*Cyprinus carpio*) exposed to phosalone. *Comp Clin Pathol*. 2014; 24:497–507.
- Kaya H, Aydın F, Gürkan M, Yılmaz S, Ates M, Demir V, Arslan Z. Effects of zinc oxide nanoparticles on bioaccumulation and oxidative stress in different organs of tilapia (*Oreochromis niloticus*). *Environ Toxicol Pharmacol*. 2015; 40:936–947.
- Kaya H, Aydın F, Gürkan M, Yılmaz S, Ates M, Demir V, Arslan Z. A comparative toxicity study between small and large size zinc oxide nanoparticles in tilapia (*Oreochromis niloticus*): Organ pathologies, osmoregulatory responses and immunological parameters. *Chemosphere*. 2016; 144:571–582. [PubMed: 26398925]
- Ladhar C, Geffroy B, Cambier S, Treguer-Delapierre M, Durand E, Brèthes D, Bourdineaud JP. Impact of dietary cadmium sulphide nanoparticles on *Danio rerio* zebrafish at very low contamination pressure. *Nanotoxicology*. 2014; 8:676–685. [PubMed: 23883150]
- Lai DY. Toward toxicity testing of nanomaterials in the 21st century: a paradigm for moving forward. *WIREs Nanomed Nanobiotechnol*. 2011; 4:1–15.
- Lee JW, Kim JE, Shin YJ, Ryu JS, Eom IC, Lee JS, Kim Y, Kim PJ, Choi KH, Lee BC. Serum and ultrastructure responses of common carp (*Cyprinus carpio* L.) during long-term exposure to zinc oxide nanoparticles. *Ecotoxicol Environ Safety*. 2014; 104:9–17. [PubMed: 24632117]
- Levesque HM, Moon TW, Campbell PGC, Hontela A. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquat Toxicol*. 2002; 60:257–267. [PubMed: 12200090]
- McGeer JC, Szebedinszky C, McDonald DG, Wood CM. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 2: tissue specific metal accumulation. *Aquat Toxicol*. 2000; 50:245–256. [PubMed: 10958958]
- Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ Int*. 2006; 32:967–976. [PubMed: 16859745]
- Nel A, Xia T, Moedler L, Li N. Toxic potential of materials at nanolevel. *Science*. 2006; 311:622–627. [PubMed: 16456071]
- Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Yang H. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol*. 2005; 2:8. [PubMed: 16209704]
- Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. *Int J Environ Res Public Health*. 2010; 7:1342–1365. [PubMed: 20617034]
- Qui Y, Thomas D, Fontaine CP, Colvin RA. Mechanism of Zn²⁺ efflux in cultured cortical neurons. *J Neurochem*. 2008; 107:1304–1313. [PubMed: 18808447]

- Ramsden CS, Smith TJ, Shaw BJ, Handy RD. Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical disturbances in the brain. *Ecotoxicol.* 2009; 18:939–951.
- Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opin Drug Deliv.* 2010; 7:1063–1077. [PubMed: 20716019]
- Remya AS, Ramesh M, Saravanan M, Poopal RM, Bharathi S, Nataraj D. Iron oxide nanoparticles to an Indian major carp, *Labeo rohita*: Impacts on hematology, ionoregulation and gill Na^+/K^+ ATPase activity. *J King Saud University Sci.* 2015; 27:151–160.
- Rosseland, BO., Staurnes, M. Physiological mechanisms for toxic effects and resistance to acidic water: an ecophysiological approach. In: Steinberg, CEW., Wright, RF., editors. *Acidification of freshwater ecosystems: implications for the future*. Vol. Chapter 16. Chichester: John Wiley and Sons Ltd.; 1994.
- Saravanan M, Suganya R, Ramesh M, Poopal RK, Gopalan N, Ponpandian N. Iron oxide nanoparticles induced alterations in haematological, biochemical and ionoregulatory responses of an Indian major carp *Labeo rohita*. *J Nanopart Res.* 2015; 17:274.
- Savolainen K, Alenius H, Norppa H, Pylkkänen L, Tuomi T, Kasper G. Risk assessment of engineered nanomaterials and nanotechnologies-a review. *Toxicology.* 2010; 269:92–104. [PubMed: 20105448]
- Scown TM, Van Aerle R, Tyler CR. Review: Do engineered nanoparticles pose a significant threat to the aquatic environment? *Crit Rev Toxicol.* 2010; 40:653–670. [PubMed: 20662713]
- Schrand AM, Dai L, Schlager JJ, Hussain SM. Toxicity testing of nanomaterials. *Adv Exp Med Biol.* 2012; 745:58–75. [PubMed: 22437813]
- Shalvei F, Hedayati A, Jahanbakhshi A, Kolangi H, Fotovat M. Effect of subacute exposure to silver nanoparticle on some hematological and plasma biochemical indices in silver carp (*Hypophthalmichthys molitrix*). *Hum Exp Toxicol.* 2013; 32:1270–1277. [PubMed: 23632006]
- Shaw BJ, Handy RD. Physiological effects of nanoparticles on fish: a comparison of nanometals versus metal ions. *Environ Int.* 2011; 37:1083–1097. [PubMed: 21474182]
- Shaw BJ, Al-Bairuty G, Handy RD. Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): physiology and accumulation. *Aquat Toxicol.* 2012; 116–117. 90–101.
- Sovová T, Boyle D, Sloman KA, Péreza CV, Handy RD. Impaired behavioural response to alarm substance in rainbow trout exposed to copper nanoparticles. *Aquat Toxicol.* 2014; 152:195–204. [PubMed: 24792150]
- Sun TY, Gottschalk F, Hungerbühler K, Nowack B. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environ Pollut.* 2014; 185:69–76. [PubMed: 24220022]
- Vaglio A, Landriscina C. Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. *Ecotoxicol Environ Safety.* 1999; 43:111–116. [PubMed: 10330329]
- Wester PW, Van der Ven LTM, Vethaak AD, Grinwis GCM, Vos JG. Aquatic toxicology: opportunities for enhancement through histopathology. *Environ Toxicol Pharmacol.* 2002; 11:289–295. [PubMed: 21782612]
- Zhao J, Wang ZY, Liu XY, Xie XY, Zhang K, Xing BS. Distribution of CuO nanoparticles in juvenile carps (*Cyprinus carpio*) and their potential toxicity. *J Hazard Mater.* 2011; 197:304–310. [PubMed: 22014442]
- Zhang Y, Zhu L, Zhou Y, Chen J. Accumulation and elimination of iron oxide nanomaterials in zebrafish (*Danio rerio*) upon chronic aqueous exposure. *J Environ Sci.* 2015; 30:223–230.

Fig. 1. TEM images of (a) SS-ZnNPs (40–60 nm) and (b) LS-ZnNPs (80–100 nm) recorded from stock suspensions of the ZnNPs in water.

Fig. 2.

Zinc accumulation (dry-weight) in *O. niloticus* tissues on 0, 7, and 14th day exposure to aqueous suspensions of SS-ZnNPs (40–60 nm) and LS-ZnNPs (80–100 nm). For given times, concentrations with different lowercase letters are significantly different ($p < 0.05$); one-way ANOVA followed by Tukey or Kruskal-Wallis post-hoc test).

Fig. 3.

O. niloticus kidney. a. control, b. 1 mg/L SS-ZnNPs (7 days), c. 10 mg/L SS-ZnNPs (7 days), d. 1 mg/L SS-ZnNPs (14 days), e. 10 mg/L SS-ZnNPs (14 days), f. 1 mg/L LS-ZnNPs (7 days), g. 10 mg/L LS-ZnNPs (7 days), h. 1 mg/L LS-ZnNPs (14 days), i. 10 mg/L LS-ZnNPs (14 days) (MA: Melanomacrophages aggregation, T: Tubule, GE: Glomerular expansion, MCI: Mononuclear cell infiltration, TD: Tubular deformation, GD: Glomerular deformation, H: Hemorrhage, N: Necrosis), scale bar 20 mm, H&E.

Fig. 4.
O. niloticus liver. a. control, b. 1 mg/L SS-ZnNPs (7 days), c. 10 mg/L SS-ZnNPs (7 days), d. 1 mg/L SS-ZnNPs (14 days), e. 10 mg/L SS-ZnNPs (14 days), f. 1 mg/L LS-ZnNPs (7 days), g. 10 mg/L LS-ZnNPs (7 days), h. 1 mg/L LS-ZnNPs (14 days), i. 10 mg/L LS-ZnNPs (14 days) (MCI: Mononuclear cell infiltration, FC: Fatty change, SV: Sytoplasmic vacuolation, SD: Sinuzoidal dilation, HD: Hepatocellular deformation), scale bar 20 μ m, H&E.

Fig. 5.
O. niloticus gill. a. control, b. 1 mg/L SS-ZnNPs (7 days), c. 10 mg/L SS-ZnNPs (7 days), d. 1 mg/L SS-ZnNPs (14 days), e. 10 mg/L SS-ZnNPs (14 days), f. 1 mg/L LS-ZnNPs (7 days), g. 10 mg/L LS-ZnNPs (7 days), h. 1 mg/L LS-ZnNPs (14 days), i. 10 mg/L LS-ZnNPs (14 days) (PL: Primary lamellae, SL: Secondary lamellae, BH: Basal hiperplasia, CT: Clubbing of tip of secondary lamellae, CD: Capillary dilation, LF: Lamellar fusion, EL: Epithelial lifting, stars: Water channel), scale bar 20 μ m, H&E.

TABLE I

Size distribution, polydispersity index (PI) and surface charge of Zn NPs estimated from aqueous suspensions. Data expressed as mean of three measurements (n = 3) where relative standard deviation was < 10%

Nanoparticle	Size (nm)		PI	Zeta value (mV)	
	Reported	TEM		DLS	
SS-ZnNPs	40–60	35–110	0.53	477	–0.17
LS-ZnNPs	80–100	90–190	0.43	662	–0.59

Table II

Effect of subchronic exposure to SS-ZnNPs (40–60 nm) and LS-ZnNPs (80–100 nm) on glucose (GLU), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH) levels in *O. niloticus* on days 0, 7 and 14. For given times, concentrations with different lowercase letters are significantly different ($p < 0.05$); one-way ANOVA followed by Tukey or Kruskal Wallis post-hoc test

Group	GLU (U/L)	GPT (U/L)	GOT (U/L)	LDH (U/L)
Initial	48.0 ± 2.9	8.8 ± 0.2	53.7 ± 2.5	544 ± 10
Control	48.3 ± 2.5 ^{ab}	8.8 ± 0.3	53.2 ± 2.1 ^{ab}	532 ± 6 ^b
SS-ZnNPs (1 mg/L)	52.6 ± 2.0 ^{ab}	9.2 ± 0.4	48.7 ± 1.6 ^b	517 ± 18 ^b
SS-ZnNPs (10 mg/L)	65.5 ± 5.0 ^a	9.7 ± 0.8	66.3 ± 3.7 ^a	523 ± 9 ^b
LS-ZnNPs (1 mg/L)	51.3 ± 2.4 ^{ab}	9.1 ± 0.9	61.9 ± 3.8 ^{ab}	550 ± 12 ^b
LS-ZnNPs (10 mg/L)	42.3 ± 1.7 ^b	9.6 ± 1.1	60.4 ± 3.7 ^{ab}	658 ± 14 ^a
Control	47.3 ± 2.1 ^{bc}	8.6 ± 0.4 ^{ab}	70.5 ± 1.7 ^a	472 ± 29 ^{ab}
SS-ZnNPs (1 mg/L)	61.1 ± 3.6 ^{ab}	9.1 ± 0.5 ^a	75.0 ± 2.0 ^a	466 ± 22 ^{ab}
SS-ZnNPs (10 mg/L)	69.1 ± 3.2 ^a	9.8 ± 0.9 ^a	72.0 ± 3.5 ^a	408 ± 18 ^b
LS-ZnNPs (1 mg/L)	34.3 ± 3.8 ^c	5.7 ± 0.49 ^b	43.1 ± 3.7 ^b	521 ± 23 ^a
LS-ZnNPs (10 mg/L)	52.4 ± 3.0 ^{ab}	6.9 ± 1.1 ^{ab}	47.7 ± 3.0 ^b	536 ± 12 ^a

Histopathological changes in the kidney, liver and gill of *O. niloticus* exposed to 1 and 10 mg/L suspensions of SS-ZnNPs (40–60 nm) and LS-ZnNPs (80–100 nm) and control fish

Table III

Group	Kidney			Liver		Gill	
	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day	14 th day
Control	Normal histology	Normal histology	Normal histology	Normal histology	Normal histology	Normal histology	Normal histology
1 mg/L SS-ZnNPs	Tubular deformations, glomerular expansions, mononuclear cell infiltrations	Tubular deformations, melanomacrophages aggregations	Mononuclear cell infiltrations, fatty changes	Hepatocellular vacuolations	Basal hyperplasia, capillary dilations, clubbing of tip of secondary lamellae	Basal hyperplasia, clubbing of tip of secondary lamellae	Basal hyperplasia, clubbing of tip of secondary lamellae
10 mg/L SS-ZnNPs	Tubular deformations, glomerular expansions mononuclear cell infiltrations	Glomerular deformations, tubular deformations	Mononuclear cell infiltrations, fatty changes, hepatocellular vacuolations	Sinusoidal dilations, hepatocellular deformations	Basal hyperplasia, lamellar fusions, clubbing of tip of secondary lamellae	Epithelial liftings, clubbing of tip of secondary lamellae	Epithelial liftings, clubbing of tip of secondary lamellae
1 mg/L LS-ZnNPs	Tubular deformations, Glomerular deformations, increasing of melanomacrophages aggregations, necrosis	Glomerular deformations, tubular deformations	Mononuclear cell infiltrations, fatty changes, sinusoidal dilations	Fatty changes, cytoplasmic vacuolations, mononuclear cell infiltrations	Basal hyperplasia, clubbing of tip of secondary lamellae	Epithelial liftings	Epithelial liftings
10 mg/L LS-ZnNPs	Tubular deformations, Glomerular deformations, increasing of melanomacrophages aggregations, necrosis, hemorrhage	Tubular deformations, mononuclear cell infiltrations	Mononuclear cell infiltrations, fatty changes	Cytoplasmic vacuolations, mononuclear cell infiltrations	Basal hyperplasia, lamellar fusions, clubbing of tip of secondary lamellae	Epithelial liftings, clubbing of tip of secondary lamellae	Epithelial liftings, clubbing of tip of secondary lamellae