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Acute toxicity, uptake, and elimination of zinc oxide nanoparticles (ZnO NPs) using saltwater microcrustacean, *Artemia franciscana*

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ABSTRACT

This study aims to evaluate the potential toxic effects of ZnO nanoparticles on *Artemia franciscana* nauplii. The ZnO NPs suspension was characterized by TEM, EDS and DLS techniques. Acute toxicity was investigated by exposure of nauplii to concentrations of 1, 5, 7.5, 10, 15, 20, 25 and 30 mg/L of ZnO NPs for 48 h and 96 h. The 96-h EC_{10} and EC_{50} values of ZnO NPs were found to be 1.39 mg/L and 4.86 mg/L respectively. The ZnO NPs suspensions did not cause any significant acute toxicity after 48 h of exposure, but the immobilization rate increase significantly compare to control group after 96 h ($P < 0.05$). The results showed that the uptake, accumulation, and elimination of NPs in nauplii depends on the concentration of NPs and time. The elimination rates of 46.66% and 83.85% were recorded at 1 and 10 mg/L of NPs after 24 h of depuration period, respectively.

1. Introduction

Nowadays, nanoscience and nanotechnology are considered as rapidly growing fields (Zhu et al., 2017). With development of nanotechnology, the application of variety of nanomaterials in household products, clothing, electronic devices, cosmetics, pharmaceuticals, water filters, and biomedical products has been increased (Jin et al., 2010).

Metal and metal oxide nanomaterials possess specific physico-chemical properties, which enable them utilize in many consumer products and industrial technologies (Montes et al., 2012). Zinc oxide (ZnO) nanoparticles due to antimicrobial and optical properties are used in various areas such as food preservation, sunscreens, cosmetic products, paint pigments, semiconductors, catalysts, and polishers (Fan and Lu 2005; Becheri et al., 2008). Because of vast use of these products, ZnO nanoparticles may inevitably release into aquatic environment (Bhuvaneshwari et al., 2016), especially into marine ecosystems (Wong et al., 2010). Keller et al. (2013) estimated the release of approximately 3700 tons of ZnO NPs into aquatic environment per year.

The toxic effects of ZnO nanoparticles have been studied towards marine organisms such as marine diatoms *Skeletonema costatum* and *Thalassiosia pseudonana*, the crustaceans *Tigriopus japonicus* and *Elasmopus rapax*, and the medaka fish *Oryzias melastigma* (Wong et al., 2010), protozoa (Mortimer et al., 2010) and algae (Aruoja et al., 2009). Several studies reported that other than ZnO NPs (Poynton et al., 2011; Bhuvaneshwari et al., 2016), the release of Zn^{2+} ions from these nanoparticles (Ates et al., 2013; Heinlaan et al., 2008) and the generation of oxidative stress due to NPs interaction (Bhuvaneshwari et al., 2016; Wong et al., 2010) are responsible for the toxicity of ZnO NPs for aquatic organisms. Heinlaan et al. (2008) showed that the toxicity of ZnO NPs for crustaceans *Daphnia magna* and *Thamnocephalus platyurus* was due to solubilized Zn^{2+} ions. They also emphasized that intimate contact between cells (crustacean gut environment) and particles is much more important than entrance of metal oxide particles into cells to cause the toxicity.

Brine shrimp, *Artemia* spp., as an invertebrate zooplankton are found nearly worldwide in saline lakes and pools (Lavens and Sorgeloos, 1996). *Artemia* spp. involve in the energy flow of the food chain in many of saltwater lake ecosystems (Ates et al., 2013). This aquatic invertebrate is widely used as a live food source to feed larval of aquatic organisms in aquaculture farming. *Artemia* spp. is a species of the non-selective filter feeders, which can directly ingest particles smaller than 50 μm in size (Hund-Rinke and Simon 2006). Moreover, Artemia possess distinct advantages including short life cycle, ready

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availability, small body size, high offspring production and ease of culture (Libralato, 2014). Because of these features, nowadays, Artemia species are used as one of the most valuable test organisms for medical toxicology and nanoecotoxicology studies (Rajabi et al., 2015; Libralato, 2014). Also recent efforts of TC 229 (Nanotechnologies) experts in the International Organization for Standardization (ISO) organization have led to the development of an international standard on the use of *Artemia* sp. Nauplii in the toxicity assessment of manufactured nanomaterials (ISO TS 20787).

In few studies, the toxicity effects and accumulation of ZnO NPs on Artemia larvae have been investigated. Ates et al. (2013) reported that the toxicity of nanoparticulate zinc oxide on the brine shrimp *A. Salina* was dependent on concentration and time of exposure. Bhuvaneshwari et al. (2016) found the toxicity and accumulation of ZnO NPs under pre-UV-A-irradiation and visible light condition on *A. salina* depends on the concentration, type irradiation, and the dissolved Zn^{2+} ions. The exposure of *A. salina* to different sizes and concentrations of ZnO nanoparticles demonstrated that these nanoparticles were not acutely toxic to Artemia larvae during 24 h of exposure and immobilization rate clearly depends on size and concentration of nanoparticles (Ates et al., 2013).

The aim of the present study was to evaluate the toxicity effects of ZnO NPs on *Artemia franciscana* larvae in saltwater medium. The acute toxicity of different concentrations of ZnO NPs were studied for 48 h and 96 h of exposure. Also, uptake and elimination patterns of different concentrations of ZnO NPs in Artemia larvae were investigated at different time intervals in a 24 h period.

2. Materials and methods

2.1. ZnO nanoparticles and characterization

The ZnO nanoparticles dispersion of 50 wt.% in water, < 100 nm particle size (DLS), < 35 nm average particle size (APS), product of Buhler, inc was purchased from Aldrich (SKU 721077). Particles size distribution and morphology of ZnO nanoparticles suspension were characterized by transmission electron microscopy (TEM) using an Ultra Corrected Energy Filtering Transmission Electron Microscopy (UC-EF-TEM, Germany) instrument providing a lattice resolution of 0.9–1.4 Å (with 2 µm MC-slit) at an accelerating voltage of 60–200 KV. We measured randomly the diameters of 100 individual nanoparticles from three images to estimate the mean size distribution of particles by Microstructure Distance Measurement Software (Nahamin Pardazan Asia Co.). Elemental analysis of ZnO NPs suspension was determined by energy dispersive x-ray spectrometer (EDS) using a UC-EF-TEM attached to an EDS detector (X-Max 80 T, Oxford, U.K.) instrument. Hydrodynamic size distribution of nanoparticles in stock solution was measured by dynamic light scattering (DLS) method using a photon cross-correlation spectroscopy (PCCS) (NANOPHOX, Germany) instrument. The percentage of Zn ions in the ZnO nanoparticle dispersion was determined by centrifugation, through a Amicon Ultra Centrifugal filter (3-kD nominal cut-off value, Amicon, Millipore, Germany). After 60 min centrifugation at 4000 RPM, the total Zn content in the unfiltered ZnO NPs suspension, as well as its respective filtrates, was measured using graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle™ 900T, USA). The percentage of soluble zinc in the zinc oxide nanoparticle suspension was calculated by dividing the zinc content in the filtrates by the zinc content in the unfiltered ZnO NPs suspension multiplied by 100 (van der Zande et al., 2012).

2.2. Test organism

Cysts of *Artemia franciscana* were purchased from INVE Aquaculture N.V./S.A., Belgium, and stored at 4 °C. For all experiments, dried cysts were hatched in transparent "V"–bottomed glass incubators containing 1 l of sterilized artificial seawater at 35 g/L and 30 ± 1 °C. Artificial seawater were used for all experiments which prepared by dissolving 140 g of synthetic seawater salt (13045 Process®, Aqua Craft®, Inc., USA) to 4 l deionized water followed by continues aeration for 24 h. After providing of continuous light illumination of 1500 lx and constant aeration from the bottom of the hatching incubators, the nauplii hatched in 24 h. The hatched nauplii were transferred into fresh seawater medium and were used for toxicity studies. The newly hatched (Instar I) and 24-h old (Instar II) *A. franciscana* nauplii were used for acute toxicity test and bioaccumulation experiments, respectively.

2.3. Acute toxicity test

After conducting a series of pre-tests (data not shown), concentrations of 0 (control), 1, 5, 7.5, 10, 15, 20, 25 and 30 mg/L of zinc oxide nanoparticles were selected for acute toxicity test. All acute tests were conducted in fully aerated artificial seawater in 100 ml glass vessels filled with 100 ml of exposure concentrations under 12 h dark/12 h light regime. During the exposure period, there was no aeration and the nauplii were not fed. Each concentration carried out by ten replicate and each replication contained ten newly hatched nauplii (Instar I). The water temperature was adjusted at 30 °C by immersing exposure vessels in a water bath with automatic temperature adjustment. Acute tests were continued for 96 h and the immobilized Artemia were removed and counted under a stereoscopic microscope at 24 h intervals. According to ISO TS 20787, immobilization was considered as inability of the nauplii to swim during the 15 s following gentle agitation of the test and control solutions, even if the nauplii can still move their appendages. After 96 h exposure, the apparent uptake of nanoparticles inside the gut of live Artemia was visualized using a phase contrast microscope (Olympus CKX41 inverted Phase Contrast microscope, UK).

2.4. Uptake and elimination of ZnO NPs

The newly hatched nauplii (50 larvae/ml) were maintained in 1000 ml glass vessels containing artificial seawater and aerated from the bottom for 24 h at 30 °C to transform from instar I into instar II. Then the instar II nauplii were exposed in triplicate to 1 and 10 mg/L ZnO NPs in the same vessels for 24 h. Constant aeration from the bottom of the exposure vessels was used to provide sufficient oxygen levels, and to keep both nauplii and nanoparticles in suspension. After 1, 2, 4, 6, and 24 h of exposure, approximately 1gr (wet weight) of exposed nauplii were randomly collected from each replicate for further determination of the body burden of Zn by the nauplii. In addition, to investigate the ability of nauplii to eliminate the assimilated nanoparticles, remained nauplii of each treatment were first gently washed with artificial seawater on plankton net and then transferred to 1000 ml glass vessels newly filled with clean and freshly prepared artificial seawater. Again, approximately 1gr (wet weight) Artemia nauplii were sampled at 1, 2, 4, 6, and 24 h intervals for further determination of the remained Zn in the body.

The sampled nauplii were thoroughly washed with deionized water on plankton net and then filtered on 0.45 mm Whatman® filter paper and were dried at 60 °C for 12 h in an oven. Dried nauplii were carefully weighted and then digested with concentrated nitric acid (Suprapur grade, Merck, Germany). The Zn contents in digested samples were measured using graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle™ 900T, USA). The device was first calibrated with standard zinc solution (TraceCERT®, Sigma-Aldrich).

To calculate the eliminated zinc at each sampling time of depuration stage, the amount of accumulated Zn after 24 h of exposure to each concentration was subtracted from remained Zn after relevant depuration time of same exposure concentration. Also the percentage of elimination rates of zinc were calculated using following equation; where E_t is elimination rate at time *t*, A_{24} is the accumulated zinc following 24 h exposure to ZnO NPs, and R_t is remained Zn in the body at time *t*.

$$E_t = (A_{24} - R_t)/A_{24} \times 100$$

2.5. Statistical analysis

All data of this study were presented as mean ± SD. The EC_{10} and the EC_{50} values were calculated by EPA Probit analysis program (Version 1.5). The statistical analysis were performed using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). Normality assumption of data were determined using the Kolmogrov-Smirnov test. Differences between the means were analyzed using One-Way analysis of variance (ANOVA) and One-Way repeated measures analysis of variance (ANOVA with repeated measures). The significant differences between the means were determined using Tukey test. The Independent-Sample T test and the Paired- Sample T test were employed to compare the differences between two independent and paired samples, respectively. Statistical significance was accepted at the level of $P < 0.05$.

3. Results

3.1. Characterization of ZnO NPs suspension

The TEM image and histogram for particles size distribution of nanoparticles in ZnO NPs stock suspension are shown in Fig. 1a, b. This micrograph shows that the ZnO nanoparticles had a spherical shape and relative uniform size. A mean diameter of 32.28 ± 13.30 nm and a size distribution that ranged from 10.87 to 80.85 nm were determined. Fig. 1c shows the EDS analysis of the ZnO NPs suspension, which confirmed the presence of zinc and oxygen as main elemental composition of this stock suspension. The mean hydrodynamic diameter and hydrodynamic size distribution of ZnO NPs in stock suspension were measured 79.31 and 50–100 nm, respectively (Fig. 1d). The percentage of zinc ions in the ZnO nanoparticle suspension was 12.43%.

Table 1
Immobilization rate (%) of *A. franciscana* exposed to ZnO NPs (mean ± SD).

Concentration (mg L^{-1})	Exposure Time	
	48 h	96 h
Control	0	6.66 ± 5.77
1	0	13.33 ± 5.77
5	3.33 ± 5.77	36.66 ± 5.77*
7.5	3.33 ± 5.77	60.00 ± 10.0*
10	3.33 ± 5.77	70.00 ± 10.0*
15	3.33 ± 5.77	93.33 ± 5.77*
20	0	96.66 ± 5.77*
25	0	96.66 ± 5.77*
30	3.33 ± 5.77	100.00 ± 0.00*

The significant differences between control and ZnO nanoparticles-exposed groups is symbolized by asterisks (ANOVA, $P < 0.05$).

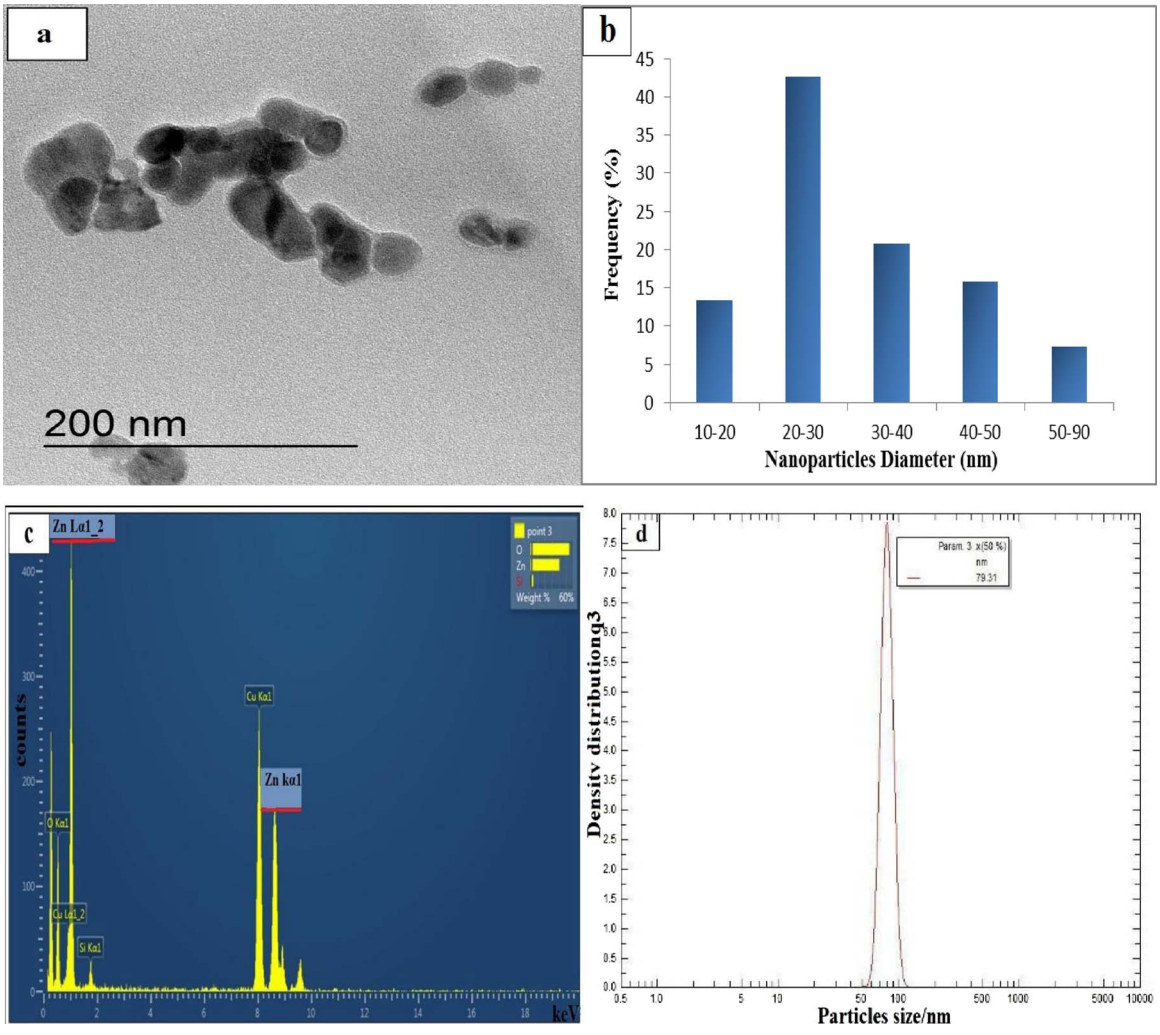

Fig. 1. TEM image of ZnO NPs from stock suspension of 100 mg/L (a), size distribution of ZnO NPs measured using TEM (b), EDS analysis of ZnO NPs (c) and hydrodynamic size distribution of ZnO NPs determined using DLS (d).

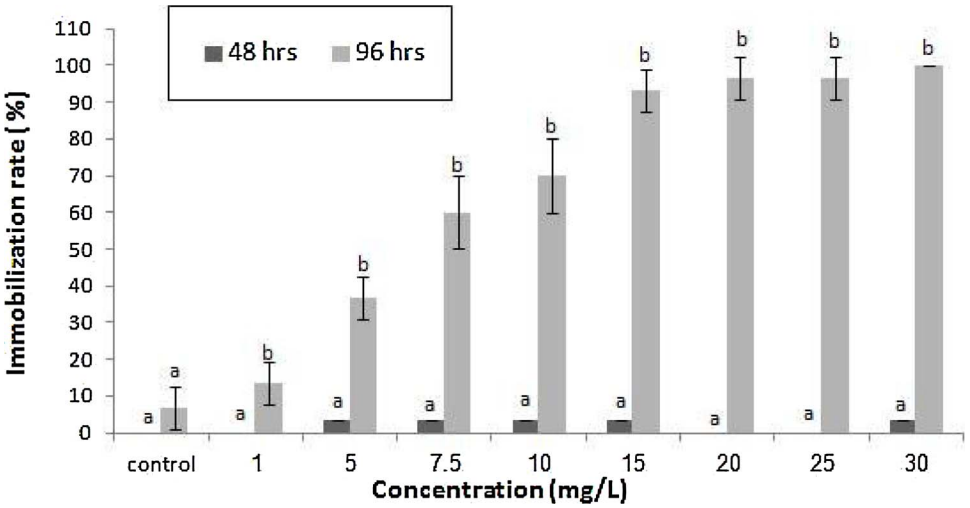

Fig. 2. Comparison between immobilization rate of instar I nauplii of *A. franciscana* exposed to different concentrations of ZnO NPs for 48 and 96 h. Bars with different letters in each concentration are significantly different (mean ± SD, $P < 0.05$).

3.2. Acute toxicity of ZnO NPs on A. franciscana

The results of immobilization rate of *A. franciscana* nauplii after 48 h and 96 h of exposure to different concentrations of ZnO NPs are shown in Table 1. After 48 h of exposure, no immobilization was observed in the control group, while the immobilization rate ranged from 0% to 3.33% at different concentrations of ZnO NPs. After 96 h of exposure, the immobilization rate was measured between 6.66-100% at different concentrations. In fact, the mortalities increased coincident with increasing the concentration of ZnO NPs ($P < 0.05$). The control group showed 6.66% immobilization rate and the minimum and maximum immobilization rates of exposed Artemia were recorded at concentrations of 1 and 30 mg/L of ZnO NPs, respectively.

As shown in Fig. 2, with further extended exposure time from 48 h to 96 h, the immobilization rate increased significantly ($P < 0.05$) at all of the concentrations, except the control group. The immobilization rate increased from 0% to 13.3% at 1 mg/L, whereas an increase from 3.3% to 100% was observed at 30 mg/L of ZnO NPs (Fig. 2).

The appearance of nanoparticles inside the gut of live *A. franciscana* nauplii exposed to concentrations of 10, 20, and 30 mg/L of ZnO NPs is shown in Fig. 3. The guts of nauplii were empty in control group, whereas the aggregated nanoparticles were visible throughout the guts of exposed nauplii.

The 96-h EC_{10} and EC_{50} values of ZnO NPs were found to be 1.39 mg/L (with 95% confidence limits of 0.46–2.41 mg/L) and 4.86 mg/L (with 95% CI of 2.99–6.63 mg/L) respectively.

3.3. Uptake of ZnO NPs by A. franciscana

The trend of Zn body burdens in *A. franciscana* exposed to different concentrations of ZnO NPs at different time intervals (1, 2, 4, 6 and 24 h) are illustrated in Fig. 4. The background zinc in the control sample ranged from 0.21 ± 0.04 to 0.23 ± 0.05 mg g^{-1} dry weight during 24 h. For 1 mg/L ZnO NPs, Zn content in nauplii increased significantly during 24 h ($P < 0.05$). The uptake of Zn increased significantly to 0.63 ± 0.01 mg g^{-1} after 6 h ($P < 0.05$) but the increase did not continue significantly up to 24 h ($P > 0.05$). The exposure to 10 mg/L ZnO NPs resulted in a significant increase of Zn body burden of about 6.2 ± 0.052 mg g^{-1} in 4 h ($P < 0.05$) but this value did not increased significantly with more exposure time ($P > 0.05$). The results demonstrated that a 10-fold increase in exposure concentration of ZnO NPs has led to approximately 10-fold increase in the Zn body burden.

Fig. 5 shows the differences between Zn body burdens in *A. franciscana* exposed to different concentrations of ZnO NPs for 1, 2, 4, 6, and 24 h. At all sampling times, the Zn content in nauplii exposed to ZnO NPs, increased significantly compare to the control ($P < 0.05$). Artemia nauplii exposed to 10 mg/L showed higher burden of Zn than 1 mg/L ($P < 0.05$).

3.4. Elimination of ingested ZnO NPs from A. franciscana

The trend of body burden and elimination of zinc at different time intervals during the transfer of exposed Artemia nauplii to clean water are shown in Table 2. The values of remained Zn and eliminated Zn were descending and ascending during 24 h, respectively, at both 1 and 10 mg/L ZnO NPs ($P < 0.05$). The amount of remained Zn in nauplii that exposed to 1 and 10 mg/L ranged from 0.55 ± 0.016 to 0.36 ± 0.016 and from 3.74 ± 0.124 to 1.15 ± 0.126 mg g^{-1} dry weight during 24 h, respectively. When exposed Artemia to 1 and 10 mg/L, were transferred to nanoparticle-free water, the elimination of Zn increased significantly after 4 h at both exposure concentrations and this increase continued with time ($P < 0.05$). During 24 h of depuration phase, the elimination rate ranged from 17.71% to 46.66% and from 47.95% to 83.85% at 1 and 10 mg/L, respectively. The values of eliminated Zn at 1 and 10 mg/L were found to be in the range of 0.11 ± 0.015 to 0.31 ± 0.005 and 3.46 ± 0.488 to 6.07 ± 0.389 mg g^{-1} dry weight, respectively.

The values of remained body burden of zinc (after elimination) at different time intervals of depuration phase are illustrated in Fig. 6. The remained body burdens of zinc in nauplii previously exposed to 10 mg/L ZnO NPs were higher significantly than 1 mg/L in all depuration times ($P < 0.05$).

4. Discussion

The results of this study demonstrated that the immobilization rate in control group ranged from 0% to 6.6% for 48 h and 96 h of exposure, which were not statistically significant ($P > 0.05$) and was acceptable according to ISO TS 20787 (less than 10%). In fact, deprivation of food during 96 h exposure period did not result in significant immobilization of Artemia nauplii. Although even at the highest concentration (30 mg/L) the ZnO NPs did not cause any significant acute toxicity after 48 h of exposure, with prolonging exposure time to 96 h, these NPs showed their toxicity effects, so that, the mortalities increased with increasing NPs concentration. The immobilization rate of 100% was recorded at 30 mg/L of ZnO NPs. Ates et al. (2013) reported that the suspensions of ZnO NPs were not acutely toxic to *Artemia salina* during 24 h of exposure in seawater medium, but the mortalities increased remarkably with increasing concentration after 96 h. Exposure of *A.salina* larvae to different metal oxide NPs up to a concentration of 1000 mg/L in seawater did not induce any significant lethal effect after 48 h

Fig. 3. Microscope images of the accumulated nanoparticles inside the gut of instar I nauplii of *A. franciscana* after 96 h exposure to 0 (control, a), 10 (b), 20 (c) and 30 mg/L of ZnO NPs (d).

(Gambardella et al., 2014). Khoshnood et al. (2017) showed that the mortalities of *A. franciscana* larvae exposed to 100–200 mg/L of ZnO NPs ranged from 0% to 26.67% after 48 h of exposure and these values increased significantly to 15% and 63.33% after 96 h. Several studies have reported the appearance of ZnO NPs inside the gut of brine shrimp larvae (Ates et al., 2013; Bhuvaneshwari et al., 2016), but this accumulation does not cause lethal effects after 24 h of exposure (Cornejo-Garrido et al., 2011).

In the present study, the 96-h EC_{10} and EC_{50} values of ZnO NPs with a mean particles diameter of 32.28 nm were found to be 1.39 and 4.86 mg/L, respectively, which were much lower than the previous reports. Ates et al. (2013) reported that 96-h EC_{50} value of ZnO NPs (10–30 nm) in *A.salina* was above 100 mg/L. Another report showed that the 96-h EC_{10} and EC_{50} values of ZnO NPs (10–30 nm) in *A. franciscana* were 91 and 173.2 mg/L, respectively (Khoshnood et al., 2017). In comparison to Artemia, the EC_{50} values of ZnO NPs reported

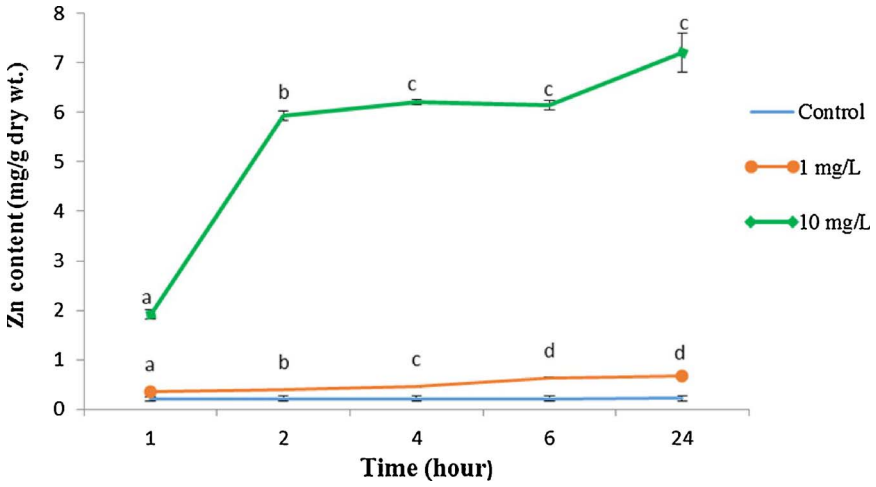

Fig. 4. Zn body burdens in *A. franciscana* nauplii exposed to different concentrations of ZnO NPs at different time intervals (1, 2, 4, 6, and 24 h). Values are mean ± SD. Means with different letters in each line are significantly different (ANOVA with Repeated Measures, $P < 0.05$).

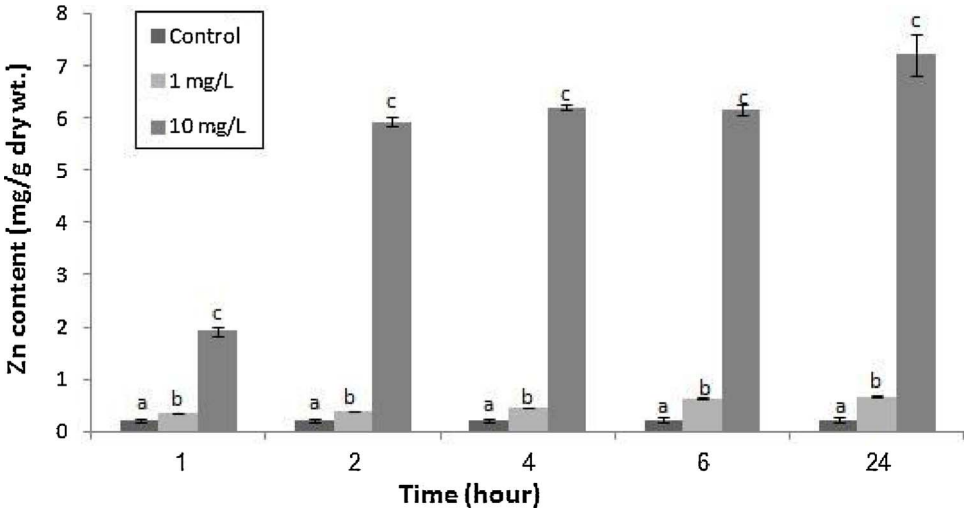

Fig. 5. Comparison between Zn body burdens in *A. franciscana* nauplii exposed to 0 (control), 1 and 10 mg/L of ZnO NPs at different time intervals (1, 2, 4, 6, and 24 h). Bars with different letters in each sampling time are significantly different (mean ± SD. ANOVA, $P < 0.05$).

for *Daphnia magna* as a freshwater crustacean, range from 2.1 (Blinova et al., 2010) to 22 mg/L (Poynton et al., 2011). Several factors can alter reliability of results obtained from the use of *Artemia* spp. in nanoecotoxicity testing such as the environmental conditions (.e.g. chemical composition of seawater, oxygen, hatching temperature and photoperiod duration) and the origin of cysts and their maintenance conditions (Libralato, 2014). Some characteristics of nanoparticles including preparation method, kind of capping agent, size, and shape may change their effect on living organisms (Asghari et al., 2012). Different toxic effects of one kind of nanoparticles observed in different reports may be derived from different experimental conditions as well as varied properties of nanoparticles and therefore, it is suggested that even in the case of nanoparticles with the same chemical formula, their toxicity investigate case by case.

Brine shrimp (Artemia) can filter a large amount of water per hour and thus face a higher risk of exposure to different pollutants (Zhu et al., 2017). This species is a non-selective filter feeder and can readily ingest particles of up to 50 µm in diameter (Hund-Rinke and Simon 2006). Metal oxide nanoparticles suspended in seawater tend to form aggregates that ranged from 400 nm up to several microns in diameter. However, the nanoparticles aggregate sizes are lesser than the sizes that Artemia can assimilate (Bhuvaneshwari et al., 2016). Several study have reported that the uptake of ZnO NPs by Artemia larvae is only influenced by the NPs concentration and the time of the exposure and that the size of the NPs was not a major factor (Ates et al., 2013; Bhuvaneshwari et al., 2016). The results of this study showed that Artemia larvae were able to uptake ZnO NPs from saltwater at the first hours of exposure, so that, the Zn concentration in Artemia exposed to both tested concentrations increased significantly compare to the control ($P < 0.05$). The uptake of Zn tended to increase significantly up to 6 h and 4 h after exposure to 1 and 10 mg/L of ZnO NPs, respectively ($P < 0.05$), while, this value did not influenced by extending exposure time to 24 h. In addition, the uptake of Zn was found to be concentration dependent, and exposure to 10 mg/L of ZnO NPs Obviously resulted in higher uptake of Zn than 1 mg/L. Approximately 10-fold increase in uptake of Zn was observed with increasing exposure concentration from 1 to 10 mg/L after 4 h of exposure.

The elimination of ingested Zn in *A. franciscana* after 24 h of depuration period were dependent on exposure concentration of ZnO NPs and time. The elimination values increased significantly ($P < 0.05$) after 4 h of depuration phase in both exposure concentration of ZnO NPs and these increasing continued up to 24 h. Ates et al. (2013) mentioned that the elimination value of ingested ZnO NPs (10–30 nm) increased from 32 to 126 µg g^{-1} dry weight during 96 h of depuration period.

For a 24 h depuration period, the elimination values of 0.11 ± 0.015 to 0.31 ± 0.005 and 3.46 ± 0.488 to 6.07 ± 0.389 mg g^{-1} dry weight were recorded for 1 and 10 mg/L of ZnO NPs, respectively. In fact, the highest elimination occurred for Artemia exposed to higher concentration due to more uptake of Zn. Bhuvaneshwari et al. (2016) showed that the elimination amount of Zn after 24 h in *A. salina* exposed to 10 and 160 mg/L ZnO NPs under visible light condition were 0.004 to1.5 mg g^{-1} dry weight, respectively. The results of another study demonstrated that the highest elimination of Zn occurred for *A. salina* exposed to higher concentration of ZnO NPs (Ates et al., 2013).

The findings of several studies have showed that Artemia larvae are unable to eliminate the accumulated Zn because of the formation

Table 2
Accumulated and eliminated Zn at different time intervals of depuration phase in *A. franciscana* nauplii exposed previously to 1 and 10 mg/L of ZnO NPs for 24 h (mean ± SD, $P < 0.05$).

Depuration time	Exposure concentration of ZnO NPs (mg/L)					
	1			10		
	Zn body burden (mg/g dry wt.)	Eliminated Zn (mg/g dry wt.)	Elimination rate of Zn (%)	Zn body burden (mg/g dry wt.)	Eliminated Zn (mg/g dry wt.)	Elimination rate of Zn (%)
1 h	0.55 ± 0.016^{a}	0.11 ± 0.015^{a}	17.71	3.74 ± 0.124^{a}	3.46 ± 0.488^{a}	47.95
2 h	0.53 ± 0.005^{a}	0.14 ± 0.006^{a}	21.4	3.36 ± 0.084^{ab}	3.85 ± 0.314^{ab}	53.36
4 h	0.45 ± 0.011^{b}	0.22 ± 0.006^{b}	33.03	3.20 ± 0.177^{b}	4.01 ± 0.369^{b}	55.44
6 h	0.44 ± 0.010^{bc}	0.23 ± 0.008^{bc}	34.52	2.23 ± 0.110^{c}	4.97 ± 0.312^{c}	68.88
24 h	0.36 ± 0.016^{d}	0.31 ± 0.005^{d}	46.66	1.15 ± 0.126^{d}	6.07 ± 0.389^{d}	83.85

Means with different letters in the same column are significantly different (ANOVA with Repeated Measures, $P < 0.05$).

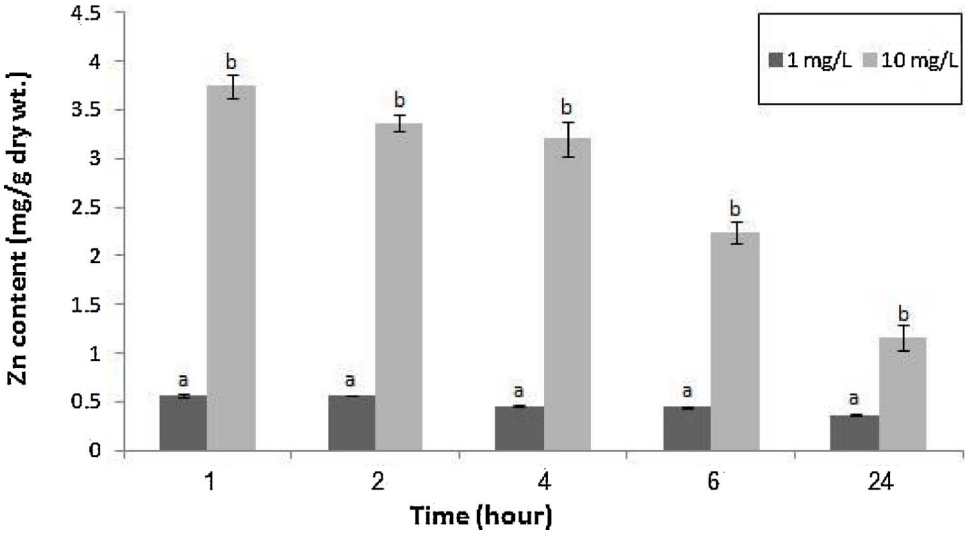

Fig. 6. Comparison between accumulated Zn in *A. franciscana* nauplii previously exposed to 1 and 10 mg/L ZnO NPs at different depuration time intervals (1, 2, 4, 6, and 24 h). Bars with different letters in each sampling time are significantly different (mean ± SD, $P < 0.05$).

massive aggregates of ingested ZnO NPs inside their guts (Ates et al., 2013; Bhuvaneshwari et al., 2016; Soniya et al., 2015). In contrast, the results of this study showed that *A. franciscana* exposed to 1 and 10 mg/L of ZnO NPs were able to eliminate about 46.66% and 83.85% of ingested NPs during 24 h of depuration period, respectively. Ates et al. (2013) reported a reduction of about 9.7% of ingested Zn in *A. salina* exposed to ZnO NPs (10–30 nm) in 96 h. The amounts of remained Zn body burden after the depuration period in exposed Artemia to 1 and 10 mg/L of ZnO NPs were found to be 0.36 ± 0.016 and 1.15 ± 0.126 mg g^{-1} dry weight, respectively. Different depuration capabilities of accumulated NPs from Artemia may be derived from different properties of tested nanoparticles, depuration time, and life stage of tested organisms.

5. Conclusion

The exposure of *A. franciscana* to different concentrations of ZnO NPs did not induce significant mortalities after 48 h. These nanoparticles showed their lethal effects after 96 h, so that the low 96-h EC_{10} and EC_{50} values of ZnO NPs show their toxicity. According to results of uptake of ZnO NPs test, the Artemia nauplii were able to uptake NPs from saltwater and this uptake were concentration and time dependent. The results confirmed that Artemia nauplii are not able to eliminate all of the ingested NPs and part of these NPs accumulates inside their bodies after depuration period. Therefore, their potential to transfer nanoparticles to aquatic food chain should be consider in future studies.

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References

Aruoja, V., Dubourguier, H.C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci. Total. Environ. 407, 1461–1468.

Asghari, S., Johari, S.A., Lee, J.H., Kim, Y.S., Jeon, Y.B., Choi, H.J., Moon, M.C., Yu, I.J., 2012. Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. J. Nanobiotechnol. 10 (14), 1–11. http://dx.doi.org/10.1186/1477-3155-10-14.

Ates, M., Daniels, J., Arslan, Z., Farahb, I.O., Riverac, H.F.E., 2013. 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. Process. Impact 15, 225–233.

Becheri, A., Durr, M., Nostro, P.L., Baglioni, P., 2008. Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. J. Nanopart. Res. 10, 679–689.

Bhuvaneshwari, M., Sagar, B., Doshi1, S., Chandrasekaran, N., Mukherjee, A., 2016. Comparative study on toxicity of ZnO and TiO_2 nanoparticles on *Artemia salina*: effect of pre-UV-A and visible light irradiation. Environ. Sci. Pollut. Res. 24, 5633–5646.

Blinova, I., Ivask, A., Heinlaan, M., Mortimer, M., Kahru, A., 2010. Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environ. Pollut. 158 (1), 41–47.

Cornejo-Garrido, H., Kibanova, D., Nieto-Camacho, A., Guzmàn, J., Ramirez-Apan, T., Fernandez-Lomelin, P., 2011. Oxidative stress, cytotoxicity, and cell mortality induced by nano-sized lead in aqueous suspensions. Chemosphere 84, 1329–1335.

Fan, Z., Lu, J.G., 2005. Zinc oxide nanostructures: synthesis and properties. J. Nanosci. Nanotechnol. 5, 1561–1573.

Gambardella, C., Mesaric, T., Milivojevic, T., Sepcic, K., Gallus, L., Carbone, S., Ferrando, S., Faimali, M., 2014. Effects of selected metal oxide nanoparticles on Artemia salina larvae: evaluation of mortality and behavioural and biochemical responses. Environ. Monit. Assess. 186 (7), 4249–4259.

Heinlaan, M., Ivask, A., Blimova, I., Dubourguier, H.C., Kahru, A., 2008. Toxicity of nanosized and bulk ZnO: CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71, 1308–1316.

Hund-Rinke, K., Simon, M., 2006. Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids. Environ. Sci. Pollut. Res. 13, 225–232.

ISO TS 20787, International Organization for Standardization, 2017. Nanotechnologies – Aquatic toxicity assessment of manufactured nanomaterials in saltwater lakes using *Artemia sp.* Nauplii. Also is accessible at: https://www.iso.org/standard/69087.html.

Jin, X., Li, M., Wang, J., Marambio-Jones, C., Peng, F., Huang, X., Damoiseaux, R., Hoek, E.M.V., 2010. High-throughput screening of silver nanoparticle stability and bacterial inactivation in aquatic media: influence of specific ions. Environ. Sci. Technol. 44, 7321–7328.

Keller, A.A., McFerran, S., Lazareva, A., Suh, S., 2013. Global life cycle releases of engineered nanomaterials. J. Nanopart. Res. 15 (1692), 1–17.

Khoshnood, R., Jaafarzadeh, N., Jamili Sh Farshchi, P., Taghavi, L., 2017. Acute toxicity of TiO_2, CuO and ZnO nanoparticles in brine shrimp, *Artemia franciscana*. Iran. J. Fish Sci. 16 (4), 1287–1296.

Lavens, P., Sorgeloos, P., 1996. Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper 361. pp. 90–100.

Libralato, G., 2014. The case of *Artemia* spp. in nanoecotoxicology. Mar. Environ. Res. 101, 38–43.

Montes, M.O., Hanna, S.K., Lenihan, H.S., Keller, A.A., 2012. Uptake, accumulation, and biotransformation of metal oxide nanoparticles by a marine suspension-feeder. J. Hazard. Mater. 225–226, 139–145.

Mortimer, M., Kasamets, K., Kahru, A., 2010. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophile*. Toxicology 269 (2–3), 182–189.

Poynton, H.C., Lazorchak, J.M., Impellitteri, C.A., Smith, M.E., Rogers, K., Patra, M., Hammer, K.A., Allen, H.J., Vulpe, C.D., 2011. Differential gene expression in *Daphnia magna* suggests distinct modes of action and bioavailability for ZnO nanoparticles and Zn ions. Environ. Sci. Technol. 45 (2), 762–768.

Rajabi, S., Ramazani, A., Hamidi, M., Naji, Tahereh., 2015. *Artemia salina* as a model organism in toxicity assessment of nanoparticles. J. Pharm. Sci. 23, 20–26.

Soniya, P., Kumaresn, K., Parthiban, D., Arun, N., Kumaravel, P., 2015. Effect of zinc oxide nanoparticles on *Artemia salina*. Res. J. Pharm. Dosage Forms Technol. 7 (2), 104–111.

van der Zande, M., Vandebriel, R.J., Van Doren, E., Kramer, E., Herrera Rivera, Z., Serrano-Rojero, C.S., Gremmer, E.R., Mast, J., Peters, R.J., Hollman, P.C.,

Hendriksen, P.J., Marvin, H.J., Peijnenburg, A.A., Bouwmeester, H., 2012. Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS Nano 6 (8), 7427–7442.
Wong, S.W., Leung, P.T., Djurisic, A.B., Leung, K.M., 2010. Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. Anal. Bioanal. Chem. 396 (2), 609–618.
Zhu, S., Xue, M.Y., Luo, F., Chen, W.C., Zhu, B., Wang, G.X., 2017. Developmental toxicity of Fe_3O_4 nanoparticles on cysts and three larval stages of *Artemia salina*. Environ. Pollut. 230, 683–691.