Int. J. Aquat. Biol. (2021) 9(5): 333-343
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Long-term effect of zinc oxide nanoparticles on population growth, reproductive characteristics and zinc accumulation of marine rotifer, *Brachionus plicatilis*

Shilan Mohammadi[1], Nasrollah Ahmadifard[*1], Behrooz Atashbar[2], Abbas Nikoo[3], Ramin Manaffar[1]

[1]Department of Fisheries, Faculty of Natural Resources, Urmia University, P.O. Box: 57153-165, Urmia, Iran.
[2]Department of Artemia, Artemia and Aquaculture Research Institute, Urmia University, P.O. Box: 57153-165, Urmia, Iran.
[3]Department of Analytical and Physics and Applide Chemistry, Faculty of Chemistry, Urmia University, Urmia, Iran.

Abstract: In the present study, the effects of ZnO nanoparticles (NPs) on marine rotifer, *Brachionus plicatilis,* was investigated in three separate experiments. Firstly, the sensitivity and reproductive characteristics of *B. plicatilis* were studied at concentrations of 0, 0.1, 0.5, 1, 3, 5 and 10 mg L^{-1} of ZnO-NPs for 10 days. Based on the results, the total number of rotifers (TNR) significantly decreased at 5 and 10 mg L^{-1} of ZnO-NPs. In addition, the specific growth rate (SGR) of animals was negative at two of the concentrations of ZnO-NPs. In the second experiment, the TNR at 4 concentrations of ZnO-NPs (0, 10, 13, 17, and 19 mg L^{-1}) during 72 h were tested and the 24-72 h LC_{50} of ZnO-NPs was calculated. After three days, the entire population of rotifers was generally lost at 19 mg L^{-1} of ZnO NPs. The LC_{50} of ZnO-NPs in animals at 24, 48, and 72 h intervals was registered as 18.2±1.34, 12.43±0.08, and 9.63±0.26 mg L^{-1}, respectively. Finally, the zinc accumulation in rotifers was measured at different concentrations (0, 0.1, 0.5, and 1.3 mg L^{-1}) of ZnO-NPs and maximum zinc (123 μg g^{-1} of rotifer DW) uptake by rotifers was observed in treatment 3 mg L^{-1} of ZnO-NPs. In sum, it can be concluded that the *B. plicatilis* can be used as a biological model for studying marine water contaminants with nanoparticles, especially ZnO-NPs.

Article history:
Received 7 July 2021
Accepted 5 October 2021
Available online 25 October 2021

Keywords:
Biological model
Nanoparticles
Rotifer
Zinc bioaccumulation

Introduction

In recent years, the release of nano-materials into the environment has been a source of concern. ZnO nanoparticles (ZnO-NPs) are a kind of metal oxide used in various applications of catalysts, semiconductors, and paints (Bhuvaneshwari et al., 2017). Besides, zinc is required in low amounts as a nutrient for the activation of some enzymes and proteins in the body of organisms, for instance, phytoplankton; however, its high concentrations are toxic to many aquatic species (Hogstrand, 2011). The zinc particle size is highly effective in its toxicity (Bhuvaneshwari et al., 2017; Sarkheil et al., 2018) and releasing toxic ions of ZnO-NPs caused ecotoxicity risks (Ates et al., 2013; Ma et al., 2013). In addition, cross-sectional area and environmental factors, including pH, temperature, and organic matter affect the solubility of nanoparticles (Ma et al., 2013; Bhuvaneshwari et al., 2017). In general, the smaller particles have higher cross-sections; hence, the nanoparticles are more soluble than powdered (ZnO bulk). Particle-solubility is one of the highest toxicity mechanisms of nanoparticles, compared to powdery states. Nanoparticles produce toxicity by producing hydroxyl radicals and active oxygen. In addition, the photo-catalytic properties of the nanoparticles play an important role in their toxicity (Ma et al., 2013). The ultimate destination of this kind of environmental pollutant is aquatic environment, especially the marine environment (Sarkheil et al., 2018), which is necessary to examine their effects on marine organisms.

Rotifers are well-suited organisms for ecological relationships, biological properties, and practical

*Correspondence: Nasrollah Ahmadifard
E-mail: nasrollah@gmail.com
DOI: https://doi.org/10.22034/ijab.v9i5.1292

capability to check the toxicity of materials in aquatic organisms (Gama-Flores et al., 2004). In general, rotifers are one of the main components of freshwater and marine ecosystems frequently used in monitoring heavy metals (Luna-Andrade et al., 2002). *Brachionus* genus of rotifers is particularly important as a laboratory organism for environmental studies due to its global distribution, rapid reproduction, short reproductive periods, easy cultivation, and easy access to their latent eggs. These creatures, due to their relatively short lifespan and high growth rates, are also favorable for chronic short-term toxicity studies (Gama-Flores et al., 2004; Hagiwara and Yoshinaga, 2017). This genus is used to determine the standard of contamination based on the standard biochemical standard (Luna-Andrade et al., 2002).

The ecotoxicological studies of ZnO-NPs on large aquatic organisms, including fish, common carp (Hao and Chen, 2012), Zebra fish (Ma et al., 2013), crustaceans (Wong et al., 2010), and small creatures such as phytoplankton (Aruoja et al., 2009) and zooplanktons, including *Daphnia* (Ma et al., 2013) and *Artemia franciscana* nauplii (Ates et al., 2013; Bhuvaneshwari et al., 2017; Sarkheil et al., 2018) have been conducted. Moreover, the effects of different sizes of nanoparticles on the Salt Lake rotifer *Brachionus manjavacas* were investigated by Snell and Hicks (2011). The effect of TiO_2 and CuO nanoparticles on *B. plicatilis* and *B. calyciflorus* was studied by Clément et al. (2013) and Manusadžianas et al. (2012), respectively.

Despite the fact that Clément et al. (2013) used a maximum of 48 hours to investigate of nanoparticles effect on rotifers; in natural environments, rotifers are involved with entering pollutants on a daily basis. In aquatic water bodies, rotifers can also be fed when faced with any type of contaminant, and this may also be an antagonist to food. In this case, its impact will be different from that used by Clément et al. (2013). Therefore, in this study to simulate with the environment, we tried to use a long-term study along with the nutrition of rotifers with algae. In this regard, we studied some of the life-related and historical characteristics of *B. plicatilis* in response to different concentrations of zinc oxide nanoparticles (ZnO-NPs). In addition, the toxicity threshold value and accumulation rate of ZnO nanoparticles in rotifer were investigated.

Materials and Methods

Preparation of ZnO-NPs solution: The ZnO nanoparticle powder with a purity of 99.98% and a particle size of 20-25 nm (2-13-314Cas ≠) was obtained from the Spanish company with the commercial name of TENAN. To prepare the stock solution (1000 mg L^{-1}), 1 g of ZnO-NPs was dissolved in 10 ml of deionized water. Then, it was placed in an ultrasonic bath (Intersonic, IS-2, 300W, 35 kHz) for 10 min and eventually reached 1000 ml volume. The prepared solution was stored in a dark container and room temperature (Sarkheil et al., 2018). The particle size of ZnO-NPs suspension was measured using a Transmission Electron Microscopy (TEM) (Philips BioTwin, the Netherlands). The electron micrographs were taken via electron microscopy section, Urmia University, Urmia, Iran. The percentage of the Zn-ions dissolved in the solution was determined by centrifuging at 3200 g for 20 min and the zinc content in the filtrate suspension was measured using atomic absorption (Nov AA 400, Analytic Jena, Germany). The soluble zinc was calculated by dividing the measured zinc (as Zn) per the total used ZnO-NPs (1 g) and multiplying by 100 (Lowry and Lopez, 1946).

General experimental procedures: The marine water rotifer, *B. plicatilis,* Müller cysts obtained from the Shrimp Research Institute in Bushehr, Iran, and incubated in standard seawater at 20 ppt. The juveniles were scaled to mass density in a wet-lab using *Nannochloropsis oculta.* The saltwater for rotifer culture was prepared with synthetic salt in accordance with OECD Guideline 201 (OECD, 1984), passed through a 2 μm filter, and autoclaved at 121°C for 15 min to avoid any pathogens. The sterilized distilled water was also used to dilute saltwater. A glass vessel with a working volume of 100 ml was used to test the steps. To each test vessel, 50 rotifers per mL of water with 30 g L^{-1} were introduced. To control the temperature, all containers were performed in a

temperature-controlled glass aquarium. Light intensity (1000 lx) and temperature (27°C) were maintained during the test.

Test steps: This research was carried out in 3 separated tests, including (1) low concentration test (2) high concentration test, and (3) bioaccumulation of Zn in the rotifer body.

Low concentration test: In this experiment, tests were set up in triplicate at nominal concentrations of 0, 0.1, 0.5, 1, 3, 5 and 10 ZnO NPs mg L^{-1} for 10 days. During the experiment, the culture water daily renewed with the mentioned concentration of ZnO NPs. Rotifers were daily fed with 2.5×10^6 cell mL^{-1} of *N. oculta* algae. Water quality parameters, including salinity (30±1 g L^{-1}), pH (7.6-8.3) and temperature (24±1°C) were regularly checked. Gentle and continuous aeration was performed to meet the needs of rotifers. The growth and reproductive characteristics of rotifers were evaluated at 24-h intervals.

High concentration test: After determining the results of the first experiment, which showed chronic effects on the population of rotifers, at this stage, higher concentrations of ZnO-NPs were used to investigate the possible acute effects on rotifers. For this purpose, the four concentrations of ZnO-NPs including 10, 13, 17, and 19 mg L^{-1} along with a control treatment (with zero ZnO-NPs) at 3 replicates were selected. The culture conditions were the same as in the first experiment. In this experiment, the number of rotifers for 72 hours was counted along with the control group (with a normal growth rate).

Zinc bioaccumulation: In this experiment, to determine Zn uptake by rotifers, 50 ind mL^{-1} of rotifers were placed in saline water at 30 g L^{-1} and temperature of 27°C. Then, these rotifers were exposed to 0.1, 0.5, 1, and 3 mg L^{-1} of ZnO-NPs along with a control treatment (with zero ZnO-NPs) at triplicate for 5 days. After 5 days, the rotifers were filtered using a 50 μm mesh, rinsed with filtered and sterilized saline water, and finally the rotifers as paste were kept in -20°C for Zn content analysis.

Measuring factors

Total number and growth rate of rotifers: To monitor the growth rate of the rotifer, 1 to 2 mL of rotifer's water samples were daily taken using a micro-pipette. Then, 2 drops of Lugol's solution were added to fix the specimens and counted by microscope using the hemocytometer (Neubauer) chamber. Through the following equation, specific growth rate (SGR) was calculated (Krebs, 1995). $SGR = (LnN_t - LnN_0) /t$, where N_0 and N_t respectively shows the initial and final population of rotifer and t represents the experiment period (days). SGR value was calculated in the exponential phase of the population (Kennari et al., 2008).

Biometric factors of rotifers: At the end of the first experiment, the effect of ZnO NPs on possible changes in length and width of rotifers and eggs were also investigated. For this purpose, 15 adult amictic rotifers were randomly selected from each replicate and fixed by 6.5% HCl. Then, the length and width of the lorica and the large and small diameter of the egg were measured under a microscope using a calibrated scale. From those measured factors, the lorica bio-volume and egg volume were calculated as follows (Kennari et al., 2008): Lorica bio-volume (μm^3) = 0.52 × a × b × c, where a= length, b= width and c= body height (is considered to be 0.4 a). Egg volume (μm^3) = 4/3 π ($a^2b + ab^2$) / 16, where a, and b are two dimensions of eggs diameter (μm).

ZnO NPs uptake by rotifers: Freeze dried samples were weighed and wet-digested with nitric acid (65% HNO3 Suprapur®, Merck, Germany) using MLS-1200 MEGA Microwave for 30 minutes under cold water and were then diluted with distilled water to reach the required volume. The zinc content was obtained using an atomic absorption device (Nov AA 400, Analytic Jena, Germainy) (Lowry and Lopez, 1946).

Statistical analysis: After ensuring the normalization of the data by Shapiro-Wilk test, the homogeneity of variances was investigated via Levene's test. One-way ANOVA was used to investigate the effects of different concentrations of probiotic and Duncan's test was used to find the differences among meanings. The Probit analysis of SPSS software is used to calculate the LC50 of ZnO NPs. The minimum significance level of the test was considered as

Table 1. Lorica biovolume (LB) and egg volume (EW) of the rotifers exposed to different amount of ZnO NPs in culture media (first experiment) (mean±SD, n=3).

	Amount of ZnO NPs in culture vessel (mg L^{-1})						
Factors	Zero	0.1	0.5	1	3	5	10
LB ($\times 10^3$ µm^3)	1994±115	2271±227	2476±202	2570±185	2378±169	2172±316	2269±140
EV ($\times 10^3$ µm^3)	301±45^{c}	480±117b^{c}	793±117^{ab}	854±155^{a}	860±138^{a}	617±96^{bc}	805±78^{ab}

Different letters in each row indicate a significant difference (P<0.05).

Table 2. Lethal concentration (10, 30, 50 and 90) (mg L-1) of ZnO NPs on *Brachionus plicatilis* at different exposition times (hours), (mean±SD, n=3).

	Lethal concentration (mg L^{-1}) in different expose time (hours)		
	24	48	72
LC_{10}	7.21±2.17	7.06±0.05	6.09±0.16
LC_{30}	13.26±0.97	9.95±0.06	7.98±0.21
LC_{50}	18.21±1.34	12.43±0.08	9.63±0.26
LC_{90}	39.58±2.90	21.45±0.14	15.23±0.40

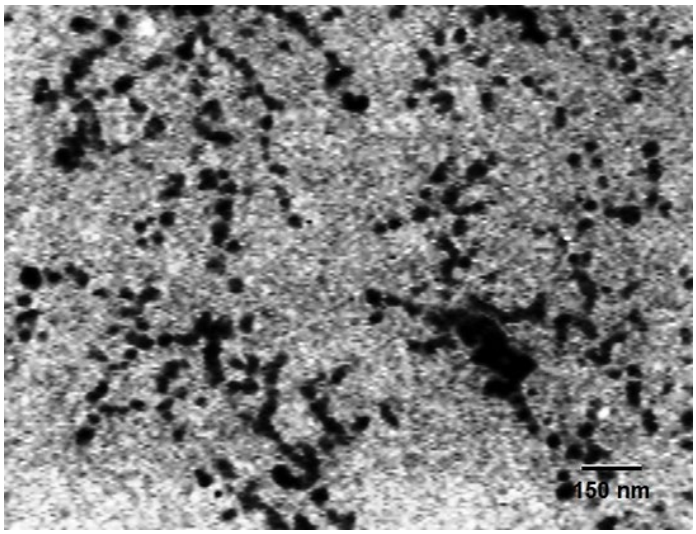

Figure 1. TEM image of ZnO NPs from stock suspension.

P<0.05. SPSS software version 21 was used to check the data and Excel software to draw charts.

Results

Characterization of ZnO nanoparticles suspension: The TEM image of ZnO NPs from stock suspension is shown in Figure 1. Based on the electron micrograph, the particles of ZnO included electron dense oval and round shapes. The mean diameter of relative uniform ZnO NPs was 24.28±12.3 nm. The percentage of the Zn-ions dissolved in the stock solution was 56.84%.

First experiment

Total number of rotifers: There is no report on the use of *B. plicatilis* as a biological model for ZnO NPs toxicity testing. Figure 2 shows the effect of ZnO NPs on population growth of rotifer during 10 days. On the 10th day, the highest number of rotifers was significantly related to the control treatment, while, in the other groups, the rotifer number significantly decreased (P<0.05); thus, the total number of rotifers in the treatment with 10 mg L^{-1} of ZnO NPs was less than other groups. In the treatment with 0.1 and 0.5 mg L^{-1} of ZnO NPs, the number of rotifers showed an increasing trend; however, it was significantly lower than the control treatment.

Specific growth rate: The effect of ZnO NPs on SGR of *B. plicatilis* is shown in Figure 3. The changing trend of SGR was related to the amount of ZnO NPs. The lowest SGR was observed at two concentrations of 5 and 10 mg L^{-1} of ZnO NPs which was found to be negative (-0.823 and -0.1866, respectively). Up to 3 mg L^{-1} of ZnO NPs, the SGR of rotifers was not shown to be negative; however, it was significantly lower than the control treatment (P<0.05).

Biometric factors of rotifers: Based on the results, the lorica biovolume was insignificantly affected by different concentrations of ZnO NPs. However, the highest lorica biovolume of rotifer was observed at a concentration of 1 mg L^{-1}. By increasing the ZnO NPs concentration, the lorica biovolume of rotifer decreased; nevertheless, it was still more than the control group. However, ZnO NPs significantly affected the egg volume of rotifer which was larger than those observed at 1 and 3 mg L^{-1} of ZnO NPs

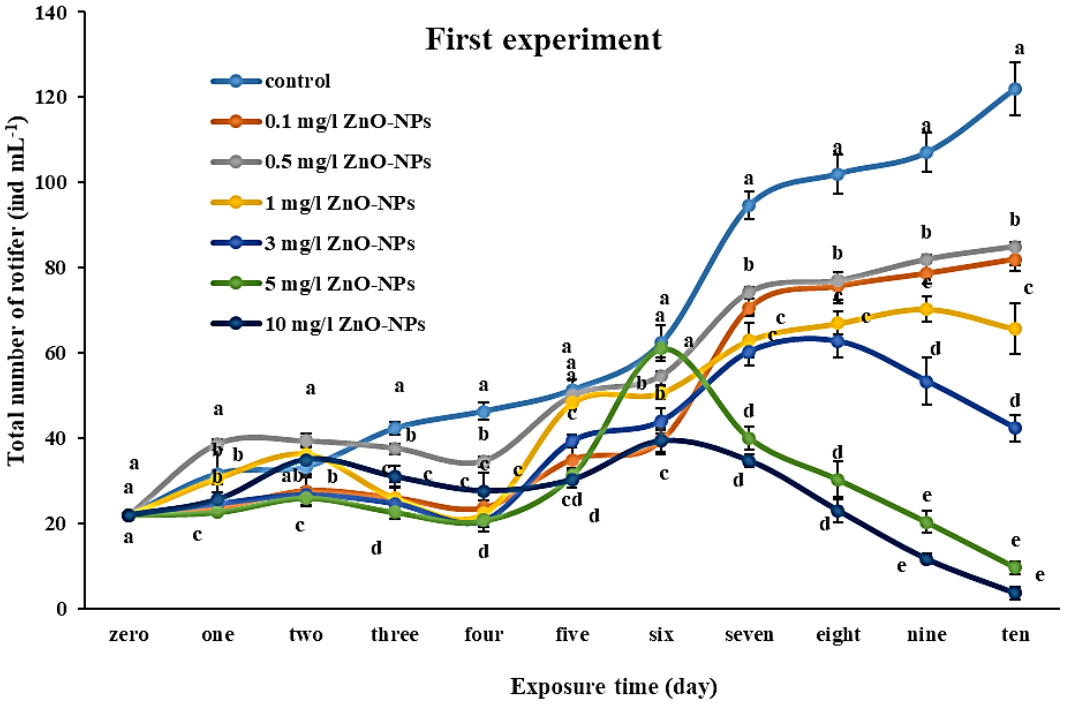

Figure 2. The total number of rotifers (ind mL^{-1}) during 10 days of exposition to low concentrations of ZnO nanoparticles, Bars with different letters are significantly different (mean±SD, n=3, ANOVA, $P<0.05$).

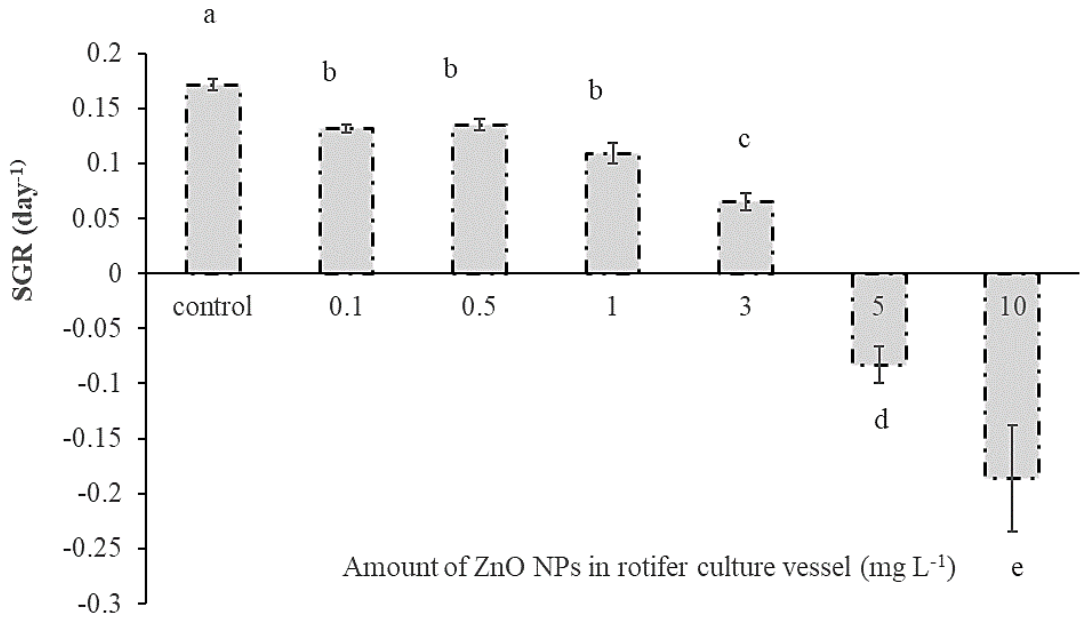

Figure 3. Specific growth rate (SGR) of rotifers (day^{-1}) during 10 days of exposition to low concentrations of ZnO nanoparticles, Bars with different letters are significantly different (mean±SD, n=3, ANOVA, $P<0.05$).

compared to other groups (Table 1).

Second experiment

Total number of rotifers: The effect of high concentrations of ZnO NPs on the number of *B. plicatilis* is illustrated in Figure 4. In the control treatment, an increase in density was observed, while, in the treated groups, the total number significantly decreased on the basis of nanoparticle content over three days ($P<0.05$). Thus, three days after adding 19 mg L^{-1} of ZnO NPs, the entire population of rotifers was generally lost. In the other treatments, the rotifer population reached its minimum level according to the

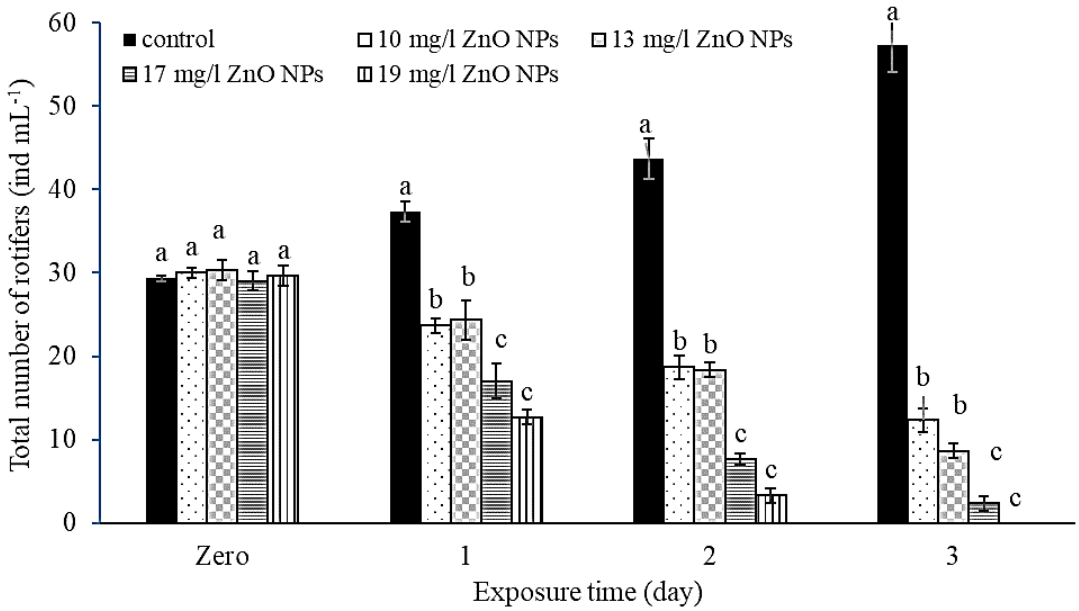

Figure 4. Total number of rotifers (ind mL^{-1}) within 72 h exposition to high concentrations of ZnO nanoparticles (second experiment), Bars with different letters are significantly different (mean±SD, n=3, ANOVA, P<0.05).

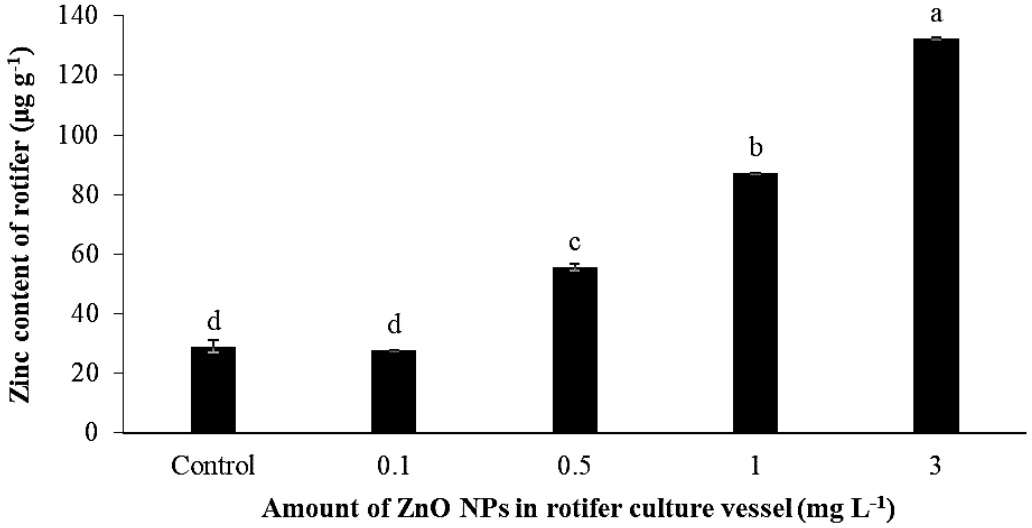

Figure 5. Zinc content in the rotifer *Brachionus plicatilis* exposed to 0 (control), 0.1, 0.5, 1, and 3 mg L-1 of ZnO NPs for 5 days. Bars with different letters are significantly different (mean±SD. ANOVA, P<0.05) (third experiment).

concentration of ZnO NPs.

***Lethal concentration (LC) of ZnO NPs on rotifer B. plicatilis*:** In Table 2, the results of the LC_{10}, LC_{30}, LC_{50}, and LC_{90} of ZnO NPs are given at 24, 48, and 72 hours after being added to the culture media. The 24h and 72h LC_{50} of ZnO NPs were 18.21 and 9.63 mg L^{-1} and the 24 and 72 h LC_{90} were 39.58 and 15.23 mg L^{-1}, respectively.

***ZnO NPs uptake by rotifers*:** Figure 5 illustrates the uptake of ZnO NPs by *B. plicatilis* after 5 days' exposure to low concentrations of ZnO NPs in seawater medium. In the control group, the rotifer samples showed 25.95±2.11 µg g^{-1} of Zn which was accumulated from algae and seawater medium. Exposure of ZnO NPs to rotifer medium culture caused the accumulation of about 132.47±0.37 µg g^{-1} of Zn at 3 mg L^{-1} of ZnO NPs. The highest zinc uptake and the lowest zinc content were obtained in the group with 0.1 g of ZnO NPs without any significant difference with the control treatment.

Discussions

Brachionus plicatilis Müller 1786 is a common inhabitant of the Persian Gulf or seasonal water in southern part of Iran. Growth and survival characteristics and chronic and acute mortality of these species were studied at different concentrations of ZnO NPs (size < 30 nm) in the laboratory. In the case of the chronic effects, the total number of rotifers decreased with increasing the concentrations of ZnO NPs. On the early days, ZnO NPs had no negative effect on the rotifer population and positive effects were observed. The chronic toxicity effect of nanoparticle on the population growth of rotifers was observed after the 6th day. Based on the results, the specific growth rate of the rotifers exposed to nanoparticles decreased with increasing ZnO NPs concentration. At higher concentrations, the effect of nanoparticles caused a negative effect on specific growth rate. In previous studies on rotifer, the maximum population density and specific growth rate are two important bio-indicators (Dahms et al., 2011).

There is no study on the effects of ZnO NPs on population growth rate of rotifer *B. plicatilis*, while chronic Pb toxicity to the population growth rate of *B. calyciflorus* (Grosell et al., 2006) and *Philodina rapida* (Esbaugh et al., 2012) was studied. In other invertebrates such as copepod *Amphiascus tenuiremis*, the chorionic toxicity of carbon nanotubes was investigated by Templeton et al. (2006) who obtained an increase in mortality and a decrease in molting success and fertilization rates. In another study, chronic toxicity of $ZnSO_4$ and ZnO NPs in *Daphnia magna* (Bacchetta et al., 2017) was studied. After 21 days of exposure, the organisms exposed to low concentrations of ZnO NPs showed complete improvement and full potential reproduction, while the organisms exposed to $ZnSO_4$ showed a dose-dependent reduction and a reduced life span (Bacchetta et al., 2017).

Planktons including phytoplankton and zooplankton are more sensitive to the surrounding environments, especially in a wide range of pollutants, than larger animals, including fish and crustaceans (Suthers and Rissik, 2009). They are often used as model organisms in the standard toxicity test to evaluate the environmental impact of chemical substances (Lam and Wang, 2008; Snell and Hicks, 2011; Manusadžianas et al., 2012; Clément et al., 2013; Manfra et al., 2017; Sarkheil et al., 2018). Rotifers are one of the marine filter-feeding animals and constantly filter food particles, including nanoparticles. Therefore, nanoparticles may become toxic in the ionic form or by bonding with the detritus. In this study, it seems that the ZnO nanoparticle had effects on life history of rotifer by bonding with algae. The study of Esbaugh et al. (2012) showed that the Pb bound to food items are more toxic than the case it was exposed only the rotifer *P. rapida.* In the study of ZnO nanoparticles toxicity on the *Acartia tunsa* transmitted through a phytoplankton regimen, its dose-dependence over time and in decreasing survival and reproduction in copepod was obtained (Jarvis et al., 2013).

It was shown that nanoparticle via absorption into tissues depresses the population growth rate of rotifers (Snell and Hicks, 2011). The mechanism of nanoparticle effect could be due to a decrease in food intake which consequently decreased reproduction in animals. Most likely, the reduction of food intake is an adaptation mechanism to minimize the contact with toxic substances. Such response behavior of the rotifer *B. manjavacas* is also reported by Snell and Hicks (2011) where it was exposed to a composition of 37 nm polystyrene nanoparticle. In their studies, it was stated that the nanoparticles with dimensions of 37 nm can be absorbed by rotifer *B. manjavacas*, while larger particles pass the intestine without absorption. They also indicated that nanoparticles are able to pass through the intestinal wall or up taken by epithelial phagocytosis. It was also found that SGR of *B. manjavacas* with increasing the nanoparticle concentration from 1 to 2 mg L^{-1} significantly decreased, while, in 0.5 mg L^{-1} of 37-nm nanoparticle or in 100- and 200 nm particles, no significant difference was found in SGR of rotifer (Snell and Hicks, 2011).

The body size of rotifers strongly correlated with physiological and ecological conditions (Kirk, 1997;

Hotos, 2002). The body size in rotifers and its plasticity in nature have been suggested to be an evolutionary response to predation (Allan, 1976) as well as environmental factors (Kennari et al., 2008). Released nano-materials, as environmental factors, can lead to evolutionary responses. In the present study, the size-change of the rotifers in term of lorica biovolume and egg volume in response to chronic toxicity of ZnO NPs was significantly indicated. This may suggest that the rotifers fed with ZnO NPs physiologically responded to changing environmental conditions. As an emerging field, the effect of nano-materials on body change need to be investigated in further research, since small organisms such as rotifers have high surface to volume ratio and are susceptible to contaminant exposure.

In the present study, the 48 h LC_{50} value of ZnO NPs on *B. plicatilis* was found to be 12.43 mg L^{-1}. The 48 h LC_{50} of zinc metal on the freshwater rotifers *P acutiformis* species at 25 and 5°C was detected to be 500 and 1550 µg L^{-1}, respectively (EPA, 1987). The effect of TiO_2 nanoparticle on immobility of *B. plicatilis* showed that 48 h EC_{50} of 25 nm TiO_2 nanoparticle was 10.4 mg L^{-1}; however, the toxicity was reduced using larger particles (Clément et al., 2013). Manusadžianas et al. (2012) investigated the acute toxicity of CuO nanoparticles to the freshwater rotifer *B. calyciflorus*. They showed that 24 h LC_{50} of CuO nanoparticles was 0.24 mg L^{-1} that is more sensitive than algae *Nitellopsis obtuse* and shrimp *Thamnocephalus platyurus* with 24h LC_{50} 4.3 and 9.8 mg L^{-1}, respectively. The ecotoxicity of polystyrene NPs to *B. plicatilis* was demonstrated by Manfra et al. (2017). In their study, the neonoate rotifers were exposed to 24-48 h polystyrene NPs in the range of 0.5-50 mg L^{-1}. Two forms, namely anionic and cationic polystyrene NPs, had different effects on the rotifer. They observed no mortality for anionic PS-COOH vs. a concentration-dependent increase in mortality for cationic PS-NH_2. In natural seawater, the 48 h LC_{50} value of cationic PS-NH_2 was 6.62 mg L^{-1}. There is a difference in the toxicity of nanoparticles in fresh and salt waters as well as the marine and fresh water species. Bhuvaneshwari et al. (2017) stated that the electrostatic repulsion between nanoparticles can reduce in higher salinity of seawater medium. However, in the *B. plicatilis*, the toxicity of ZnO nanoparticles is much higher than anionic polystyrene NP and is lower than TiO_2 nanoparticle and cationic polystyrene nanoparticle. More comparative studies are still needed to determine the sensitivity of various species to the toxicity of nanoparticles including ZnO NPs. In comparison, with *D. magna*, the LC_{50} nanoparticle was one third of that of *B. plicatilis.* In another study, for *D. magan,* the 48h LC_{50} ZnO NPs was reported at 3.2 mg L^{-1} (Heinlaan et al., 2008) and 2.6 mg L^{-1} (Blinova et al., 2010). In *A. salina,* at 30 mg L^{-1}, ZnO NPs did not show any significant acute toxicity after 48 hours of exposure (Sarkheil et al., 2018). Khoshnood et al. (2017) reported that 24 h exposition of *A. franciscana* nauplii to 200 mg L^{-1} of ZnO NPs had only 26.6 % mortality.

In rotifers like other zooplanktons, nanoparticles with bioaccumulation can be transferred to higher trophic levels (Holbrook et al., 2008). In the present study (bioaccumulation test/ third experiment), the Zn uptake by the rotifers exposed to ZnO NPs was assayed and indicated that, via increasing the nanoparticle in saltwater, the uptake by rotifers significantly increased and exposition to 3 mg L^{-1} of ZnO NPs resulted in higher bioaccumulation compare to 0.1 mg L^{-1}. Marine rotifer is a non-selective filter feeder and can ingest particles up to 20 µm (Sarma et al., 2011). In seawater, nanoparticle materials tend to aggregate (Manfra et al., 2017), despite the fact that it can still be consumed by rotifers. Nonetheless, the uptake of nanomaterial by rotifer have been reported by other researchers. Snell and Hicks (2011) stated that the nanoparticle of 37 nm is strongly absorbed by the exposed rotifer and subsequently transferred to amictic eggs. They also found that polystyrene nanoparticle with a size < 50 nm affect feeding rate and reproduction of rotifer through entering tissues and passing from mother to produced eggs. Rubio-Franchini and Rico-Martínez (2011) and Alvarado-Flores et al. (2012) indicated that diet is the main route of Pb accumulation in rotifers such as *B. calyciflorus* and *Asplanchna brightwellii*. The bioaccumulation of

Zn can be due to the passing of the ZnO nanoparticles through intestinal duct and being absorbed by epithelial phagocytosis. Alvarado-Flores et al. (2012) showed that accumulated metal had physiological effects on the freshwater rotifer *Brachionus calyciflorus*. In this study, as shown in the first experiment of the present study, via increasing the concentrations of ZnO NPs, its effects on the population growth of rotifers also increased. The bioaccumulation of ZnO NPs has been reported for other marine species. Della Torre et al. (2014) showed a significant retention of anionic polystyrene NP in sea urchin embryos. Sarkheil et al. (2018) indicated that *Artemia* nauplli can uptake the zinc oxide nanoparticle from saltwater in a concentration-dependent manner. Additionally, Ates et al. (2013) found a time and concentration-dependence of Zn bioaccumulation by *Artemia salina* nauplli after being exposed to 96 h of nanoparticle up to 100 mg L^{-1}.

As our conclusion, the ZnO NPs can lead to physiological effects on fresh and marine creature. In this study, the concentration dependent of ZnO NPs on the rotifers was significantly indicated. The rotifers fed with ZnO NPs physiologically responded to ZnO NPs in term of total population, specific growth rate, mortality and zinc uptake from water. As an emerging field, the other effect of this common nanomaterial need to be investigated in further research, since small organisms such as rotifers have high surface to volume ratio and are susceptible to contaminant exposure. Here, we obtained the first report of ZnO NPs effect on morphology and physiology of rotifer which the change mechanism should be further studied.

References

Allan J.D. (1976). Life history patterns in zooplankton. The American Naturalist, 110(971): 165-180.

Alvarado-Flores J., Rico-Martínez R., Ventura-Juárez J., Silva-Briano M., Rubio-Franchini I. (2012). Bioconcentration and localization of lead in the freshwater rotifer *Brachionus calyciflorus* Pallas 1677 (Rotifera: Monogononta). Aquatic Toxicology, 109: 127-132.

Aruoja V., Dubourguier H.-C., Kasemets K., Kahru A. (2009). Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Science of the Total Environment, 407(4): 1461-1468.

Ates M., Daniels J., Arslan Z., Farah I.O., Rivera H.F. (2013). Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. Environmental Science: Processes and Impacts, 15(1): 225-233.

Bacchetta R., Santo N., Marelli M., Nosengo G., Tremolada P. (2017). Chronic toxicity effects of $ZnSO_4$ and ZnO nanoparticles in *Daphnia magna*. Environmental Research, 152: 128-140.

Bhuvaneshwari M., Sagar B., Doshi S., Chandrasekaran N., Mukherjee A. (2017). Comparative study on toxicity of ZnO and TiO_2 nanoparticles on *Artemia salina*: effect of pre-UV-A and visible light irradiation. Environmental Science and Pollution Research, 24(6): 5633-5646.

Blinova I., Ivask A., Heinlaan M., Mortimer M., Kahru A. (2010). Ecotoxicity of nanoparticles of CuO and ZnO in natural water. Environmental Pollution, 158(1): 41-47.

Clément L., Hurel C., Marmier N. (2013). Toxicity of TiO_2 nanoparticles to cladocerans, algae, rotifers and plants–effects of size and crystalline structure. Chemosphere, 90(3): 1083-1090.

Dahms H.-U., Hagiwara A., Lee J.-S. (2011). Ecotoxicology, ecophysiology, and mechanistic studies with rotifers. Aquatic Toxicology, 101(1): 1-12.

Della Torre C., Bergami E., Salvati A., Faleri C., Cirino P., Dawson K., Corsi I. (2014). Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus*. Environmental Science and Technology, 48(20): 12302-12311.

EPA. (1987). *A*mbient water quality criteria for zinc. U.S. Environmental Protection Agency Report 440/5-87-003.

Esbaugh A., Brix K., Mager E., De Schamphelaere K., Grosell M. (2012). Multi-linear regression analysis, preliminary biotic ligand modeling, and cross species comparison of the effects of water chemistry on chronic lead toxicity in invertebrates. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 155(2): 423-431.

Gama-Flores J.L., Sarma S., Nandini S. (2004). Acute and chronic toxicity of the pesticide methyl parathion to the rotifer *Brachionus angularis* (Rotifera) at different algal (*Chlorella vulgaris*) food densities. Aquatic Ecology,

38(1): 27-36.

Grosell M., Gerdes R.M., Brix K.V. (2006). Chronic toxicity of lead to three freshwater invertebrates-*Brachionus calyciflorus*, *Chironomus tentans,* and *Lymnaea stagnalis*. Environmental Toxicology and Chemistry, 25(1): 97-104.

Hagiwara A., Yoshinaga T. (2017). Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology: Springer. 180 p.

Hao L., Chen L. (2012). Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. Ecotoxicology and Environmental Safety, 80: 103-110.

Heinlaan M., Ivask A., Blinova I., Dubourguier H.-C., Kahru A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere, 71(7): 1308-1316.

Hogstrand C. (2011). Zinc: Homeostasis and Toxicology of Essencial Metals. Academic Press San Diego. pp: 135-200.

Holbrook R.D., Murphy K.E., Morrow J.B., Cole K.D. (2008). Trophic transfer of nanoparticles in a simplified invertebrate food web. Nature Nanotechnology, 3(6): 352.

Hotos G.N. (2002). Selectivity of the rotifer *Brachionus plicatilis* fed mixtures of algal species with various cell volumes and cell densities. Aquaculture Research, 33(12): 949-957.

Jarvis T.A., Miller R.J., Lenihan H.S., Bielmyer G.K. (2013). Toxicity of ZnO nanoparticles to the copepod *Acartia tonsa*, exposed through a phytoplankton diet. Environmental Toxicology and Chemistry, 32(6): 1264-1269.

Kennari A., Ahmadifard N., Kapourchali M., Seyfabadi J. (2008). Effect of two microalgae concentrations on body size and egg size of the rotifer *Brachionus calyciflorus*. Biologia, 63(3): 407-411.

Khoshnood R., Jaafarzadeh N., Jamili S., Farshchi P., Taghavi L. (2017). Acute toxicity of TiO_2, CuO and ZnO nanoparticles in brine shrimp, *Artemia franciscana*. Iranian Journal of Fisheries Sciences, 16(4): 1287-1296.

Kirk K.L. (1997). Life-history responses to variable environments: starvation and reproduction in planktonic rotifers. Ecology, 78(2): 434-441.

Krebs C.J. (1995). Two paradigms of population regulation. Wildlife Research, 22(1): 1-10.

Lam I.K., Wang, W.-X. (2008). Trace element deficiency in freshwater cladoceran *Daphnia magna*. Aquatic Biology, 1(3): 217-224.

Lowry O.H., Lopez J.A. (1946). The determination of inorganic phosphate in the presence of labile phosphate esters. Journal of Biological Chemistry, 162: 421-428.

Luna-Andrade A., Aguilar-Duran R., Nandini S., Sarma S. (2002). Combined effects of copper and microalgal (*Tetraselmis suecica*) concentrations on the population growth of *Brachionus plicatilis* Müller (Rotifera). Water, Air, and Soil Pollution, 141(1-4): 143-153.

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

Manfra L., Rotini A., Bergami E., Grassi G., Faleri C., Corsi I. (2017). Comparative ecotoxicity of polystyrene nanoparticles in natural seawater and reconstituted seawater using the rotifer *Brachionus plicatilis*. Ecotoxicology and Environmental Safety, 145: 557-563.

Manusadžianas L., Caillet C., Fachetti L., Gylytė B., Grigutytė R., Jurkonien S., Vitkus R. (2012). Toxicity of copper oxide nanoparticle suspensions to aquatic biota. Environmental Toxicology and Chemistry, 31(1): 108-114.

Rubio-Franchini I., Rico-Martínez R. (2011). Evidence of lead biomagnification in invertebrate predators from laboratory and field experiments. Environmental Pollution, 159(7): 1831-1835.

Sarkheil M., Johari S.A., An H.J., Asghari S., Park H.S., Sohn E.K., Yu I.J. (2018). Acute toxicity, uptake, and elimination of zinc oxide nanoparticles (ZnO NPs) using saltwater microcrustacean, *Artemia franciscana*. Environmental Toxicology and Pharmacology, 57: 181-188.

Sarma S., Resendiz R.A.L., Nandini S. (2011). Morphometric and demographic responses of brachionid prey (*Brachionus calyciflorus* Pallas and *Plationus macracanthus* (Daday)) in the presence of different densities of the predator *Asplanchna brightwellii* (Rotifera: Asplanchnidae). Hydrobiologia, 662(1): 179-187.

Snell T.W., Hicks D.G. (2011). Assessing toxicity of nanoparticles using *Brachionus manjavacas* (Rotifera). Environmental Toxicology, 26(2): 146-152.

Suthers I.M., Rissik D. (2009). Plankton: A guide to their ecology and monitoring for water quality. CSIRO publishing. 273 p.

Templeton R.C., Ferguson P.L., Washburn K.M., Scrivens W.A., Chandler G.T. (2006). Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. Environmental Science and Technology, 40(23): 7387-7393.

Wong S.W., Leung P.T., Djurišić A., Leung K.M. (2010). Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. Analytical and Bioanalytical Chemistry, 396(2): 609-618.