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# Comparative toxicity of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) on saltwater microcrustacean, *Artemia salina*

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ABSTRACT

This study evaluated the potential toxic effects of silver nanoparticles (AgNPs) and silver nanowires (AgNWs) on saltwater microcrustacean *Artemia salina* nauplii under ISO TS 20787 guideline. To investigate the acute toxicity of these nanomaterials, the nauplii were exposed to different concentrations of 0 (control), 0.39, 1.56, 6.25, 25 and 100 mg/L AgNPs and concentrations of 0 (control), 0.01, 0.1, 1, 10, 50 and 100 mg/L AgNWs for 72 h. Immobilization rate of *A. salina* exposed to both AgNPs and AgNWs for 72 h increased significantly in a concentration-dependent manner ($P < 0.05$). The 72 h $EC_{10}$ and $EC_{50}$ were found to be 1.48 ± 0.6 and 10.70 ± 1.3 mg/L for AgNPs, respectively, and 0.03 ± 0.02 and 0.43 ± 0.04 mg/L for AgNWs, respectively. Based on the $EC_{10}$ and $EC_{50}$ values, the toxicity of AgNWs was significantly higher than AgNPs ($P < 0.05$). Oxidative stress resulted from 48 h exposure to both AgNPs and AgNWs in *A. salina* was assessed by measuring reactive oxygen species (ROS) production and superoxide dismutase (SOD) activity. The results revealed that both AgNPs and AgNWs could induce ROS production. The SOD activity decreased significantly with the increase of exposure concentration ($P < 0.05$). In conclusion, the present results show that both nanomaterials have toxic effects on *A. salina* nauplii and thus, more effort should be made to prevent their release into saltwater ecosystems and trophic transfer in the aquatic food chain.

## 1. Introduction

Manufactured nanomaterials (MNMs) as materials in the range of 1–100 nm have specific physical and chemical characteristics differ from that of the bulk (Baalousha et al., 2010) which enable them to be employed in various fields, including medicine, electronics, food industry, energy and environmental remediation (Massarsky et al., 2013; Sund et al., 2011). Therefore, it is expected that large amounts of MNMs release to the environment. It was estimated that about 0.4–7% of over 260,000–309,000 metric tons of MNMs produced globally in 2010 was discharged into aquatic environments (Keller et al., 2013). Therefore, study on toxicological effects of MNMs on aquatic animals is essential in order to better understand the destructive effects of these materials. For example, the effects of dietary ZnO-NPs on the intestinal folds and the muscular parts of juvenile common carp (*Cyprinus carpio* L.) intestine revealed the changes in abundance of 32 proteins in the intestinal folds and 28 proteins in the muscular parts (Chupani et al., 2017, 2018a, 2018b). It has been reported that Ag-NPs reduce the photosynthetic performance of green algae (Oukarroum et al., 2012) and increase the mortality of brine shrimp (Arulvasu et al., 2014). In addition to direct toxicity effects of MNMs on aquatic organisms, trophic transfer of them in the aquatic food chain has been reported (Ates et al., 2014; Dalai et al., 2014; Wang et al., 2016).

The MNMs are existed in shapes of nanoparticles, nanowires, nanotubes, nanorods, and nanoplates (Chae and An, 2016). They may be defined as zero-dimensional (0D), one dimensional (1D), two dimensional (2D), or three dimensional (3D) (Kwak and An, 2015). Silver nanomaterials (AgNMs) as one of the most important nanomaterials are synthesized in different forms including silver nanoparticles, silver nanowires, silver nanorods, silver nanocubes and silver nanocoils (Bachenheimer et al., 2017; Hoop et al., 2016). Silver nanoparticles (AgNPs) due to their unique antimicrobial characteristics are widely used in consumer health products (Choi et al., 2008; Prabhu and Poulose, 2012). Silver nanowires (AgNWs) due to their one-dimension structure are high aspect-ratio MNMs and have specific physicochemical properties (Jones et al., 2018). The high electrical and thermal

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conductivity of AgNWs has made them widely used in transparent films, conductive products and coating agents (Langley et al., 2013; Sohn et al., 2015). The effective bactericidal activity of AgNWs also has been reported in literature (Cui and Liu, 2015; Hong et al., 2016; Visnapuu et al., 2013). The potential toxicity effects of AgNPs on freshwater and saltwater species at different trophic levels including algae such as *Skeletonema costatum* (Gambardella et al., 2015) and *Dunaliella salina* (Johari et al., 2018a), crustaceans like *Daphnia magna* (Asghari et al., 2012; Sohn et al., 2015), *Artemia salina* (Gambardella et al., 2015), and *Artemia franciscana* (Kos et al., 2016) as well as fish (Johari et al., 2013; Sohn et al., 2015) have been reported. However, information about the ecotoxicological effects of AgNWs on aquatic organisms is limited. George et al. (2012) investigated the toxicity effects of different shapes of silver nanomaterials including nanospheres, nanowires, and nanoplates on *Danio rerio* embryos and *Oncorhynchus mykiss* gill cells. The results revealed that mortality of embryos induced at 120 hpf at 5 mg/L of AgNWs. The potential oxidative stress of AgNWs on the rainbow trout gill epithelial cell line was also reported. Chae and An (2016) evaluated the toxicity of 10 and 20 μm-long polyvinylpyrrolidone-coated AgNWs to the microalga (*Chlamydomonas reinhardtii*), water flea (*D. magna*), and the zebrafish (*D. rerio*). According to results, longer AgNWs (20 μm) were more toxic than shorter ones (10 μm) to both algae and daphnia but shorter AgNWs were accumulated more than longer ones in the body of the fish. In addition, the toxicity effects of silver vanadate nanowires decorated with AgNPs on *D. similis* (Artal et al., 2013), and AgNWs coated with silica dioxide ($SiO_2$) and polyvinylpyrrolidone (PVP) on *D. magna* (Scanlan et al., 2013) have been reported.

Biochemical responses have been recently considered as valid sensitive biomarkers for the assessment of the potential toxic impacts of MNMs on aquatic species (Falugi et al., 2012; Gambardella et al., 2013). Reactive oxygen species (ROS) as a group of short-lived chemically reactive oxygen including superoxide radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$), and hydrogen peroxide ($H_2O_2$) (He et al., 2011) are considered as a biomarker to clarify the toxic effects of nanomaterials (Lee et al., 2008). The levels of antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) have been widely used as an indicator of oxidative stress (Ates et al., 2015: Zhu et al., 2016). Antioxidant enzymes can protect an organism from oxidative stress through catalyzing the decomposition of ROS (Cazenave et al., 2006). Ulm et al. (2015) measured five biomarkers including ROS content, GSH level, catalase (CAT), Acetylcholinesterase (AChE) and SOD activities in *D. magna* to evaluate the toxicity of AgNPs and $AgNO_3$. Kumar et al. (2017) also examined the intracellular oxidative stress of zero-valent iron nanoparticles (nZVI) and $Fe^{+2}$ ions in *A. salina* by measuring ROS generation, SOD, GSH, and catalase activities. To the best of our knowledge, the information on biochemical responses such as ROS production and SOD activity in *A. salina* following exposure to AgNPs and AgNWs is limited. Some studies have reported the AgNPs-generated ROS in zebrafish embryos (Massarsky et al., 2013) and nematode, *Caenorhabditis elegans* (Lim et al., 2012).

The aim of the present study was to evaluate the aquatic toxicity of AgNPs (as 0D silver nanostructure) and AgNWs (as 1D silver nanostructure) comparatively on brine shrimp (*A. salina*) in saltwater medium with respect to the newly published ISO test specification (ISO TS 20787). The acute toxicities of different concentrations of AgNPs and AgNWs on *A. salina* were assessed during 72 h of exposure. To investigate the oxidative stress induced by these silver nanostructures in *Artemia* nauplii, the biochemical responses including ROS production and SOD activity were also determined.

## 2. Materials and methods

### 2.1. Nanomaterials and characterization

The AgNPs powder contained polyvinylpyrrolidone (PVP) as a capping agent with a particle size of < 100 nm and purity of 99.5% was purchased from Sigma Aldrich (Lot # MKCB0361V). A suspension of AgNPs (100 mg/L) was prepared in ultrapure water (Milli-Q®, Merck Millipore Inc., Germany) with tip sonication (40 kHz, 15 min) (Q700, Qsonica Inc., USA). The AgNWs dispersion of 0.5 wt% in isopropanol (IPA), < 60 ± 10 nm diameter size, < 10 ± 5 μm length size was purchased from Sigma Aldrich (Lot # MKBQ8287V).

To characterize the AgNMs, scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX) analyzes were performed using a LYRA 3XMH, (TESCAN Inc., Czech Republic) field emission scanning electron microscope (FESEM) equipped with energy dispersive X-ray spectroscopy (EDX). The dimensions of randomly selected 50 particles/wires were measured using AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany).

### 2.2. Test organism

The cysts of *A. salina* were purchased from INVE Aquaculture N.V./S.A., Belgium, and stored at 4 °C. Dried cysts were hatched in transparent "V"–bottomed glass incubators filled with 1 L of sterilized artificial seawater (ASW) at 30 g/L and 30 ± 1 °C. ASW used in all experiments were prepared with dissolving 30 g of synthetic seawater salt (13045 Process®, Aqua Craft®, Inc., USA) to 1 L of deionized water followed by continues aeration for 24 h. The nauplii were hatched after 24 h by providing of continuous light illumination of 1500 lx using a fluorescent lamp and constant aeration from the bottom of the hatching incubators. The newly hatched nauplii (instar I) were transferred into freshly prepared seawater medium for use in toxicity studies.

### 2.3. Acute toxicity test

The acute toxicity tests were conducted on instar I in accordance with ISO TS 20787 (2017) with minor changes (test duration prolonged for up to 72 h and water salinity was reduced to 30 g/L). Here, concentrations of 0 (control), 0.39, 1.56, 6.25, 25 and 100 mg/L AgNPs and concentrations of 0 (control), 0.01, 0.1, 1, 10, 50 and 100 mg/L AgNWs were selected for acute toxicity test after conducting a series of pre-tests (data not shown). For nanomaterial exposure, every ten nauplii were moved into each 100 mL exposure glass beakers which contained tested concentration in ASW (50 ml) under water temperature of 29 ± 1 °C and 16 h light/8 h dark regime. During the exposure period, there was no aeration and the nauplii were not fed. Each concentration carried out by three replicate. The immobilized *Artemia* were removed at 24 h intervals and counted under a stereoscopic microscope. The immobilization was considered as an 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 (Johari et al., 2018b). The median effective concentration ($EC_{50}$) value of nanomaterials was calculated using Probit analysis. After 72 h of exposure, the ingested nanomaterials inside the gut of live *Artemia* were visualized using a phase contrast microscope (Olympus CKX41 inverted Phase Contrast microscope, UK).

### 2.4. Oxidative stress assay

To determine the production of reactive oxygen species (ROS) and superoxide dismutase (SOD) enzyme, the nauplii of *A. salina* hatched from 1 g cysts were exposed to concentrations of 0 (control), 6.25, 25 and 100 mg/L AgNPs as well as concentrations of 0 (control), 0.1, 1and 10 mg/L AgNWs, in triplicate. The exposure was conducted in

transparent "V"–bottomed glass incubators containing 500 ml of each exposure concentration in ASW (30 g/L) with constant aeration from the bottom of the incubators. After 24 h and 48 h of exposure, the nauplii were harvested and rinsed with DW and used for the following biochemical assays.

The production of ROS was determined using a non-fluorescent 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) dye according to the method described by Bhuvaneshwari et al. (2018). Briefly, *A. salina* nauplii exposed to the test substance were washed with DW. Then, 10 nauplii were transferred to 24-well plates and treated with 10-μM DCFH-DA dye for 30 min at 37 °C. The nauplii were washed twice with PBS pH 7.4, and homogenized using a handy vortex mixer (Labkorea Inc., Korea). The fluorescence intensity of DCFH-DA was measured at an excitation and emission wavelength of 485 and 535 nm, respectively, using a fluorescence spectrophotometer (TriStar$^2$ LB 942, Berthold technologies Inc., Germany).

SOD activity of the nauplii cells was quantified according to the kit protocol (BioVision K335-100). Briefly, the cells were homogenized in ice-cold 0.1 M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM β-ME, 0.1 mg/mL PMSF. The homogenate cells were centrifuged at 14000 x g for 5 min at 4 °C and the cell debris was discarded. Then, the reagent of the SOD activity assay kit was added to the collected supernatant based on the standard protocol. The reaction mixture was incubated at 37 °C for 20 min, and the SOD activity was measured by recording the absorbance values at 450 nm using a microplate reader (ELx808 ™*, Associate of Cape Cod Inc., USA).

### 2.5. Statistical analysis

Data of all experiments were presented as mean ± SD. The EC values were calculated by SPSS Probit analysis. The Kolmogrov-Smirnov test was conducted to determine the normality assumption of data. Significant differences between the means were analyzed using one- way analysis of variance (ANOVA) followed by the Duncan multiple range test. Comparison of significant differences between two independent and paired samples was performed using the Independent-Sample *t*-test and the Paired-Sample *t*-test, respectively. All statistical analyses were performed using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). Statistical significance was accepted at a level of $P < 0.05$.

## 3. Results

### 3.1. Characterization of AgNMs

Examples of SEM micrographs of experimental AgNMs are shown in Fig. 1. SEM revealed that the AgNPs have an average diameter of 81.6 ± 11.0 nm. Particle agglomerates were also observed in suspensions of 100 mg/L AgNPs (Fig. 1 A). In the case of AgNWs, SEM revealed that they have an average diameter of 85.1 ± 5.4 nm and an average length of 11.7 ± 4.1 μm. The EDX data demonstrated that silver was the main element in both tested AgNMs.

### 3.2. Acute toxicity of AgNMs to A. salina

The results of the immobilization rate of *A. salina* exposed to different concentrations of AgNMs at different time intervals are shown in Fig. 2. After 72 h of exposure, no immobilization rate was observed in control groups. Immobilization rate ranged from 0% to 93.33% and 0% to 100% at different concentrations of AgNPs and AgNWs, respectively. For 72 h of exposure, the immobilization rate increased coincident with increasing the concentration of AgNPs ($P < 0.05$). The highest immobilization rate was observed at 100 mg/L of AgNPs ($P < 0.05$) (Fig. 2 A). As shown in Fig. 2B, with further increasing AgNWs concentration from 0.01 to 0.1 mg/L, the immobilization rate increased significantly after 72 h of exposure ($P < 0.05$).

The 72 h $EC_{10}$ and $EC_{50}$ of AgNPs for *A. salina* were 1.48 ± 0.6 and 10.70 ± 1.3 mg/L respectively, and values of 0.036 ± 0.02 and 0.43 ± 0.04 mg/L were recorded for AgNWs respectively (Table 1). Both $EC_{10}$ and $EC_{50}$ values for AgNPs were significantly higher than AgNWs ($P < 0.05$).

The visual accumulation of silver nanomaterials in the whole body and inside the gut of *A. salina* exposed to different concentrations of AgNPs and AgNWs for 72 h are shown in Fig. 3. The guts are empty in controls and low concentrations, whereas ingested nanomaterials are visible as a dark strip inside the guts of exposed *Artemia* to the high and middle concentrations.

### 3.3. ROS production

The production of ROS in *A. salina* nauplii exposed to all concentrations of AgNPs for 24 h increased significantly compared to the control ($P < 0.05$), but this increase only was observed at concentrations of 25 and 100 mg/L of AgNPs after 48 h of exposure ($P < 0.05$) (Fig. 4 A and B). For AgNWs, the generation of ROS increased

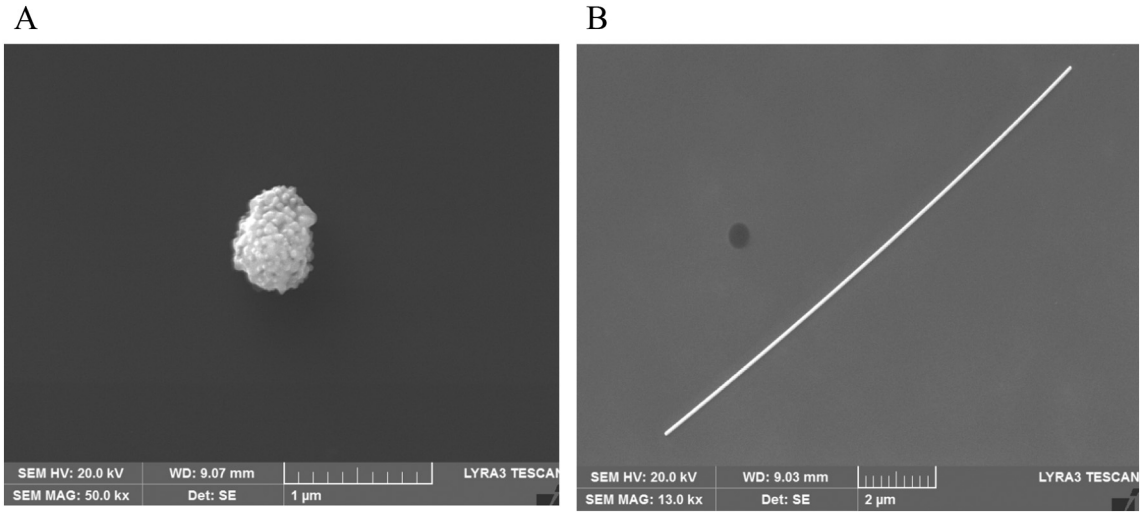


**Fig. 1.** SEM micrograph of AgNPs (A) and AgNWs (B) from 100 mg/L stock suspensions.

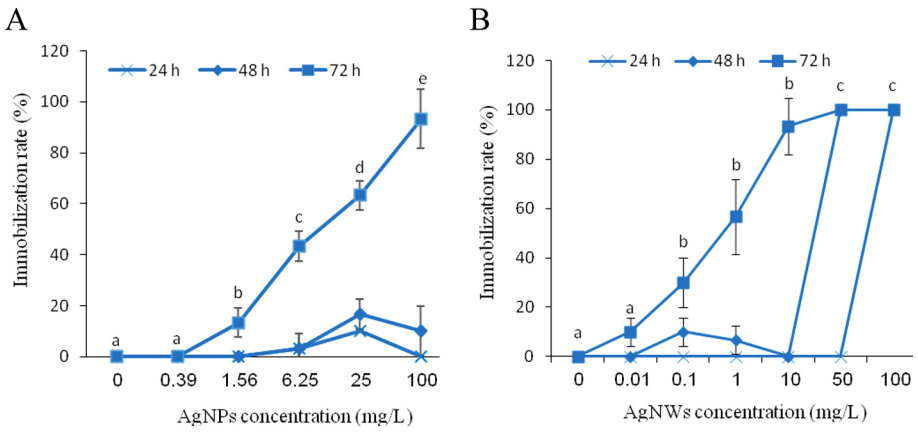


**Fig. 2.** Immobilization rate of nauplii (instar I) of *A. salina* exposed to different concentrations of AgNPs (A) and AgNWs (B) for 24, 48, and 72 h. Values are mean ± SD. Means with different letters (i.e. a, b, c, d, e) in each line are significantly different (ANOVA, $P < 0.05$).

**Table 1**
$EC_{10}$ and $EC_{50}$ for *A. salina* exposed to AgNPs and AgNWs for 72 h (mean ± SD).

| Silver NM types | $EC_{10}$ (mg/L) | $EC_{50}$ (mg/L) |
|---|---|---|
| AgNPs | 1.48 ± 0.63[a] | 10.70 ± 1.30[a] |
| AgNWs | 0.036 ± 0.02[b] | 0.43 ± 0.041[b] |

Means with different letters in the same column are significantly different (Independent-Sample T test, $P < 0.05$).

significantly in all tested concentrations (0.1, 1 and 10 mg/L) compared to the control after 24 and 48 h of exposure ($P < 0.05$) (Fig. 4 C and D). The production of ROS in nauplii exposed to different concentrations of AgNPs did not alter significantly along with further extended exposure time from 24 to 48 h ($P > 0.05$) (Fig. 5 A). There were also no significant differences between the production of ROS in *Artemia* exposed to different concentrations of AgNWs except 1 mg/L, for 24 and 48 h ($P > 0.05$) (Fig. 5 B).

### 3.4. Superoxide dismutase (SOD) activity

The SOD activity in *Artemia* nauplii exposed to 100 mg/L AgNPs for 48 h decreased significantly compared to the control ($P < 0.05$), but the activity of this enzyme at the concentration of 6.25 mg/L was significantly higher than control ($P < 0.05$). There was no significant difference in the SOD activity for 25 mg/L of AgNPs and control ($P > 0.05$) (Fig. 6 A). In nauplii exposed to AgNWs for 48 h, the SOD activity at concentrations of 10 mg/L decreased significantly compared to the control ($P < 0.05$), while the higher activity of this enzyme was observed at the concentration of 0.1 mg/L. The SOD activity in nauplii exposed to 1 mg/L AgNWs did not change significantly compared to the control ($P > 0.05$) (Fig. 6 B). The nauplii exposed to 100 mg/L AgNPs and 10 mg/L AgNWs showed the lowest SOD activity with respect to increasing exposure concentration.

## 4. Discussion

In the present study, the potential toxicity effects of direct exposure of *A. salina* nauplii to both AgNPs and AgNWs in the saltwater medium were investigated. The immobilization rate of *A. salina* nauplii which is one of the most important endpoints in acute toxicity assessments of nanomaterials (ISO TS 20787, 2017) was zero in the control groups and did not alter during 72 h of the exposure period. This result indicates that deprivation of food during 72 h of exposure period did not have an adverse effect on the viability of *Artemia* nauplii. Our results showed a concentration-dependent effect of both AgNMs on immobilization rate of *A. salina* after 72 h of the exposure period. In this regards, the immobilization rate of nauplii exposed to 1.56, 6.25, 25, and 100 mg/L AgNPs increased to 13.3, 43.3, 63.3, and 93.3% respectively. The values of 10, 30, 56.66, 93.33, and 100% of immobilization rate also recorded for nauplii exposed to 0.01, 0.1, 1, 10, 50, and 100 mg/L AgNWs, respectively. Arulvasu et al. (2014) showed that as exposure concentration of silver nanoparticles (spherical shape of 30–40 nm) to *Artemia* nauplii for 48 h increased from 2 nM to 4, 6, and 8 nM, the mortality rate increased from 60 to 83.3, 86.6, and 90%, respectively.

In the present study, the 72 h $EC_{10}$ and $EC_{50}$ of AgNPs were found to be 1.48 ± 0.6 mg/L and 10.70 ± 1.3 mg/L, respectively. Becaro et al. (2015) reported the 48 h $EC_{50}$ value of 0.05 mg/L of AgNPs for *A. salina*. Falugi et al. (2013) also obtained the 48 h $LC_{50}$ value of 0.007 mg/L of AgNPs for *A. salina*. Numerous reports have been demonstrated the ecotoxicity effects of nanoparticles on aquatic organisms but few studies have been investigated the ecotoxicological effects of nanowires (Kwak and An, 2015). The toxicity effects of Ag-based NWs have been evaluated in three crustacean species including *Hyalella azteca*, *Daphnia similis* and *D. magna* (Kwak and An, 2015). To our knowledge, no information is available about the impacts of AgNWs in *Artemia* species. Chae and An (2016) assessed the toxicity of 10 and 20 μm long AgNWs to the water flea *D. magna* and found the 48 h $EC_{50}$ of 256.16 μg/L and 247.08 μg/L for 10 and 20 μm of AgNWs, respectively. Artal et al. (2013) estimated the 48 h $EC_{50}$ of silver vanadate nanowires ($AgVO_3$ NWs) at 1 μg/L for *D. similis*. Sohn et al. (2015) also calculated the 48 h $EC_{50}$ of AgNWs (diameter: 57 nm; length: 2.1 μm) as 0.139 mg/L for *D. magna*. In this study, 72 h $EC_{10}$ and $EC_{50}$ of AgNWs were found to be 0.03 ± 0.02 and 0.4 ± 0.04 mg/L for *A. salina*, respectively. Based on the 72 h EC values, the $EC_{10}$ and $EC_{50}$ proportion of AgNPs to AgNWs were 41.1 and 24.8 times, respectively, indicating AgNWs were more toxic than the AgNPs for *A. salina*. In contrast to the present results, Sohn et al. (2015) reported the higher toxicity of AgNPs than AgNWs for *D. magna*.

ROS production plays a major role in nanomaterial induced toxicity (Manke et al., 2013). ROS are highly toxic and able to damage cells and biomolecules (Clemente et al., 2014) and their production and consequent oxidative stress could induce the toxicity of nanomaterials (Li et al., 2008). The ROS responses are affected by the physicochemical characterization of nanomaterials including particle size, surface charge, and chemical composition (Shvedova et al., 2012). Some studies revealed that AgNPs generated ROS were toxic to zebrafish embryos (Massarsky et al., 2013), nitrifying bacteria (Choi and Hu, 2008), nematode, *Caenorhabditis elegans* (Lim et al., 2012) and aquatic plant, *Spirodela polyrhiza* (Jiang et al., 2014). In the present study, ROS content in *Artemia* nauplii exposed to all concentration of AgNPs for 24 and 48 h was significantly higher than the control group ($P < 0.05$). The highest ROS content was observed at 100 mg/L of AgNPs for 24 h of

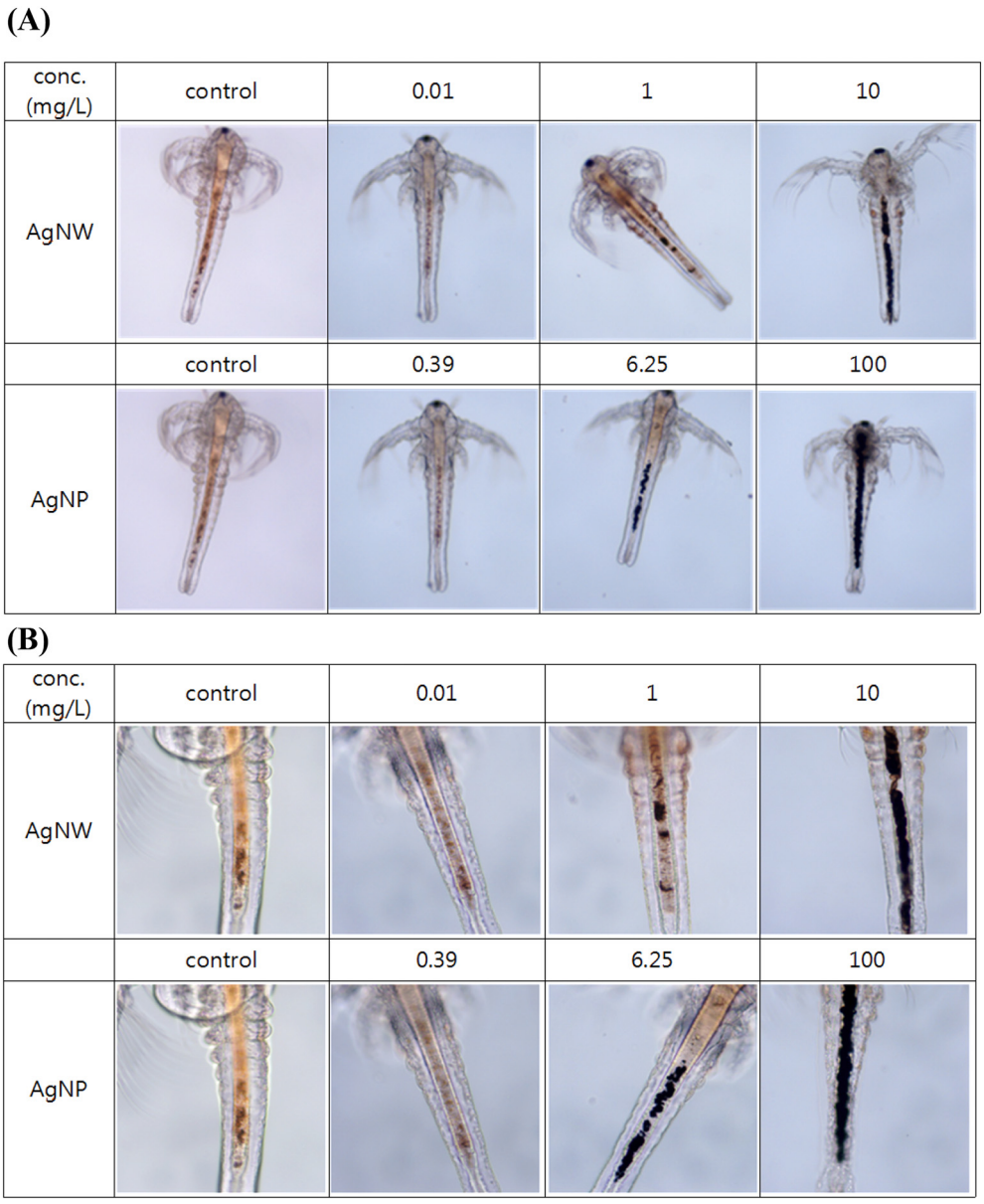


**Fig. 3.** Phase contrast microscope images of live *Artemia* nauplii showing the accumulated AgNPs and AgNWs in the whole body (A) and inside the gut (B). The guts are empty in controls and low treatments. Ingested AgNMs are visible as a dark strip inside the guts in the high and middle treatments.

exposure ($P < 0.05$), whereas this value was not significantly different in *Artemia* exposed to different concentrations of AgNPs for 48 h ($P > 0.05$). For AgNWs, exposure to all tested concentrations also significantly induced ROS production after 24 and 48 h. These results indicate that both AgNPs and AgNWs could induce oxidative stress to *A. salina* nauplii. However, the ROS production was not concentration- or time-dependent for either AgNPs or AgNWs (Figs. 4 and 5). In contrast to our finding, Jiang et al. (2014) showed a concentration-dependent increase in ROS in the tissue of an aquatic plant, *Spirodela polyrhiza*, after 72 h exposure to 6-nm AgNPs. Bhuvaneshwari et al. (2018) also found a concentration-dependent increase in ROS generation in *A. salina* exposed to $TiO_2$ NPs with respect to the control.

The level and action of harmful ROS in cells are controlled by several enzymatic defense mechanisms such as SOD (Ulm et al., 2015). SOD plays a role in the conversion of the superoxide radical to hydrogen peroxide and molecular oxygen (Liochev and Fridovich, 2000). Increase in SOD activity in *A. salina* exposed to oxidized multi-walled carbon nanotubes (O-MWCNTs) (Zhu et al., 2017a), graphene oxide (GO) (Zhu et al., 2017b) and α-$Fe_2O_3$ NPs (Wang et al., 2017) has been reported. In contrast to these observations, Bhuvaneshwari et al. (2018) showed that the SOD activity in *A. salina* exposed to $TiO_2$ NPs decreased with respect to increasing exposure concentration. The results of a study also showed that the SOD activity in the liver of the AgNPs treated Medaka (*Oryzias latipes*) was significantly inhibited in a dose-dependent manner but the activity of this enzyme in the gills did not significantly change (Wu and Zhou, 2013). Ulm et al. (2015) also reported that the SOD activity in *D. magna* exposed to different concentrations of AgNPs for 48 h did not significantly alter when comparing with the control group. Based on the results of the present study, the SOD activity in *A. salina* nauplii exposed to low concentration of AgNPs (6.25 mg/L) for 48 h increased significantly compared to the control group, but the SOD level decreased with increasing concentration of AgNPs to 100 mg/L. A significant increase in the SOD level was observed at 0.1 mg/L AgNWs, while a further increase of exposure concentration to 10 mg/L resulted in a significant reduction in the SOD activity. Inhibition of antioxidant enzymes such as SOD and CAT may be related to high concentrations of ROS, such as $^{\bullet}O_2$ and $H_2O_2$ (Ostman and Bohmer, 2001; Gottfredsen et al., 2013).

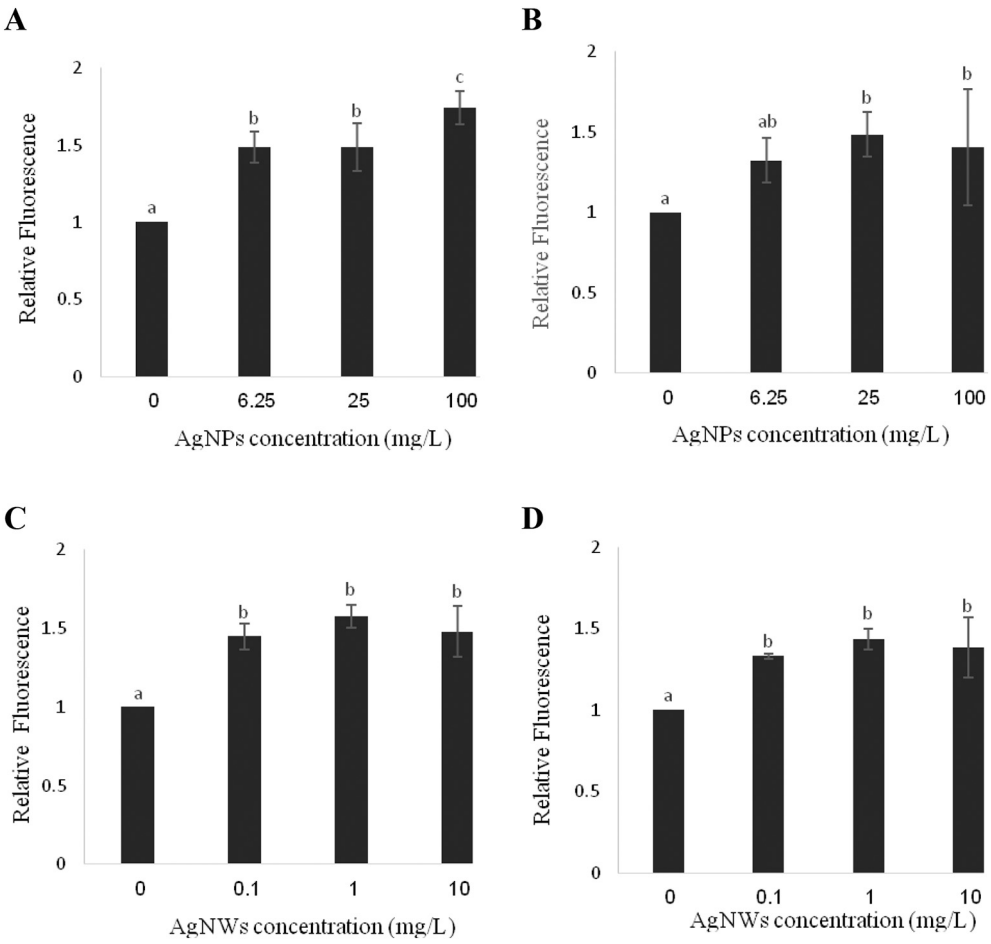


**Fig. 4.** ROS production in *A. salina* nauplii exposed to different concentrations of AgNPs for 24 h (A), 48 h (B) and AgNWs for 24 h (C) and 48 h (D). Bars with different letters (i.e. a, b, c) are significantly different (mean ± SD, $n = 3$, ANOVA, $P < 0.05$).

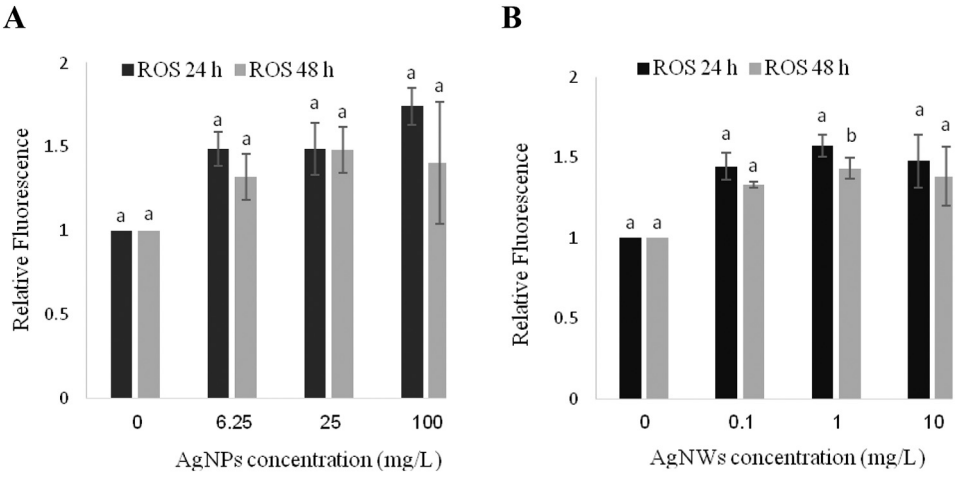


**Fig. 5.** Time-dependence comparison between ROS production in *A. salina* nauplii exposed to different concentrations of AgNPs (A) and AgNWs (B) for 24 h and 48 h. Bars with different letters (i.e. a, b) are significantly different (mean ± SD, n = 3, Paired-Sample *t*-test, $P < 0.05$).

## 5. Conclusion

The results of the present study revealed that immobilization rate of instar I nauplii exposed to both AgNPs and AgNWs for 72 h significantly increased in a concentration-dependent manner. The visual accumulation of AgNMs in the whole body and inside the gut of *Artemia* nauplii was observed after 72 h of exposure for both AgNPs and AgNWs. The lesser $EC_{10}$ and $EC_{50}$ values of AgNWs for *A. salina* indicated their higher toxicity. Exposure to both AgNPs and AgNWs for 48 h led to ROS production. The SOD activity in exposed *Artemia* to both AgNPs and AgNWs decreased significantly with the increasing concentration. Based on these results, AgNPs and AgNWs would appear to have toxic effects on *A. salina* nauplii, thus, more effort is needed to manage their release into saltwater aquatic ecosystems.

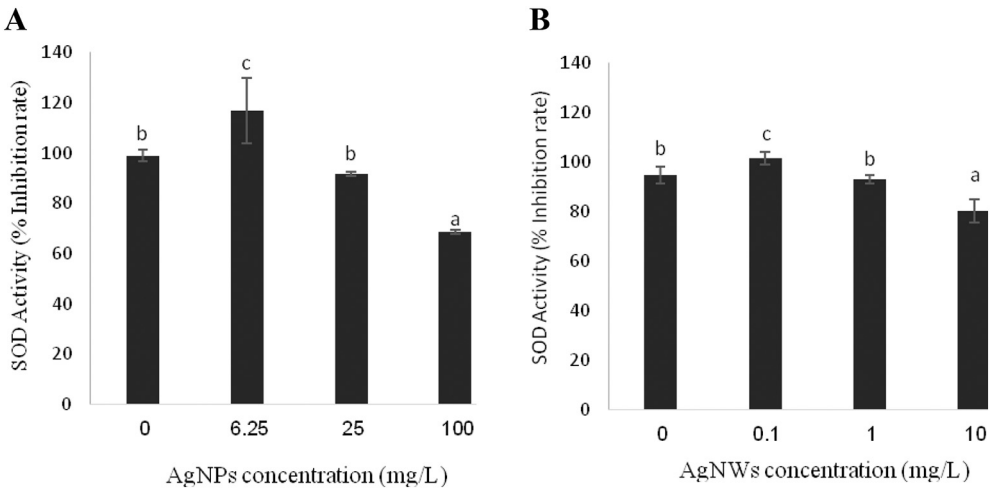


**Fig. 6.** Superoxide dismutase (SOD) activity of *A. salina* upon exposure to different concentrations of AgNPs (A) and AgNWs (B) for 48 h. Bars with different letters (i.e. a, b, c) are significantly different (mean ± SD, n = 3, ANOVA, $P < 0.05$).

## Experimental guideline

This work was conducted using the ISO/TS 20787: 2017, Nanotechnologies - Aquatic toxicity assessment of manufactured nanomaterials in saltwater lakes using *Artemia* sp. Nauplii, https://www.iso.org/standard/69087.html

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## Conflict of interests

The authors have no conflict of interest to declare.

## References

Artal, M.C., Holtz, R.D., Kummrow, F., Alves, O.L., Umbuzeiro, G. de A., 2013. The role of silver and vanadium release in the toxicity of silver vanadate nanowires toward *Daphnia similis*. Environ. Toxicol. Chem. 32 (4), 908–912. https://doi.org/10.1002/etc.2128.

Arulvasu, C., Jennifer, S.M., Prabhu, D., Chandhirasekar, D., 2014. Toxicity effect of silver nanoparticles in brine shrimp Artemia. Sci. World J. 2014, 1–11. https://doi.org/10.1155/2014/256919.

Asghari, S., Johari, S.A., Lee, J.H., Kim, Y.S., Jeon, Y.B., Choi, H.J., ... Yu, I.J., 2012. Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. Beilstein J. Nanotechnol. 10 (1), 14. https://doi.org/10.1186/1477-3155-10-14.

Ates, M., Arslan, Z., Demir, V., Daniels, J., Farah, I.O., 2014. Accumulation and toxicity of CuO and ZnO nanoparticles through waterborne and dietary exposure of goldfish (*Carassius auratus*). Environ. Toxicol. 30 (1), 119–128. https://doi.org/10.1002/tox.22002.

Ates, V., Demir, Z., Arslan, J., Daniels, I.O., Farah, C.B., 2015. Evaluation of alpha and gamma aluminum oxide nanoparticle accumulation, toxicity, and depuration in *Artemia salina* larvae. Environ. Toxicol. 24 (3), 296–303. https://doi.org/10.1002/tox.

Baalousha, M., Lead, J.R., Ju-Nam, Y., 2010. Natural colloids and manufactured nanoparticles in aquatic and terrestrial systems. In: Treatise on Water Science. 3. pp. 89–129. https://doi.org/10.1016/B978-0-444-53199-5.00053-1.

Bachenheimer, L., Scherzer, R., Elliott, P., Stagon, S., Gasparov, L., Huang, H., 2017. Degradation mechanism of Ag nanorods for surface enhanced raman spectroscopy. Sci. Rep. 7 (1), 4–7. https://doi.org/10.1038/s41598-017-16580-2.

Becaro, A.A., Jonsson, C.M., Puti, F.C., Siqueira, M.C., Mattoso, L.H.C., Correa, D.S., Ferreira, M.D., 2015. Toxicity of PVA-stabilized silver nanoparticles to algae and microcrustaceans. Environ. Nanotechnol. Monit. Manag. 3, 22–29. https://doi.org/10.1016/j.enmm.2014.11.002.

Bhuvaneshwari, M., Thiagarajan, V., Nemade, P., Chandrasekaran, N., Mukherjee, A., 2018. Toxicity and trophic transfer of P25 $TiO_2$NPs from *Dunaliella salina* to *Artemia salina*: effect of dietary and waterborne exposure. Environ. Res. 160, 39–46. https://doi.org/10.1016/j.envres.2017.09.022.

Cazenave, J., Bistoni, M.D.L.A., Pesce, S.F., Wunderlin, D.A., 2006. Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR. Aquat. Toxicol. 76 (1), 1–12. https://doi.org/10.1016/j.aquatox.2005.08.011.

Chae, Y., An, Y., 2016. Toxicity and transfer of polyvinylpyrrolidone-coated silver nanowires in an aquatic food chain consisting of algae, water fleas, and zebrafish. Aquat. Toxicol. 173, 94–104. https://doi.org/10.1016/j.aquatox.2016.01.011.

Choi, O., Hu, Z., 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ. Sci. Technol. 42 (12), 4583–4588. https://doi.org/10.1021/es703238h.

Choi, O., Deng, K.K., Kim, N.J., Ross, L., Surampalli, R.Y., Hu, Z., 2008. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. Water Res. 42 (12), 3066–3074. https://doi.org/10.1016/j.watres.2008.02.021.

Chupani, L., Zusková, E., Niksirat, H., Panáček, A., Lünsmann, V., Haange, S.-B., von Bergen, M., Jehmlich, N., 2017. Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (*Cyprinus carpio* L.). Sci. Total Environ. 579, 1504–1511.

Chupani, L., Niksirat, H., Lünsmann, V., Haange, S.B., Bergen, M.V., Jehmlich, N., Zuskova, E., 2018a. Insight into the modulation of intestinal proteome of juvenile common carp (*Cyprinus carpio* L.) after dietary exposure to ZnO nanoparticles. Sci. Total Environ. 613–614, 62–71.

Chupani, L., Niksirat, H., Velíšek, J., Stará, A., Hradilová, Š., Kolařík, J., Panáček, A., Zusková, E., 2018b. Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio* L.): tissue accumulation and physiological responses. Ecotoxicol. Environ. Saf. 147, 110–116.

Clemente, Z., Castro, V.L., Jonsson, C.M., Fraceto, L.F., 2014. Minimal levels of ultraviolet light enhance the toxicity of $TiO_2$ nanoparticles to two representative organisms of aquatic systems. J. Nanopart. Res. 16 (8). https://doi.org/10.1007/s11051-014-2559-z.

Cui, J., Liu, Y., 2015. Preparation of graphene oxide with silver nanowires to enhance antibacterial properties and cell compatibility. RSC Adv. 5 (104), 85748–85755. https://doi.org/10.1039/c5ra16371d.

Dalai, S., Iswarya, V., Bhuvaneshwari, M., Pakrashi, S., Chandrasekaran, N., Mukherjee, A., 2014. Different modes of $TiO_2$ uptake by *Ceriodaphnia dubia*: relevance to toxicity and bioaccumulation. Aquat. Toxicol. 152, 139–146. https://doi.org/10.1016/j.aquatox.2014.04.002.

Falugi, C., Aluigi, M.G., Chiantore, M.C., Privitera, D., Ramoino, P., Gatti, M.A., ... Matranga, V., 2012. Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. Mar. Environ. Res. 76, 114–121. https://doi.org/10.1016/j.marenvres.2011.10.003.

Falugi, C., Aluigi, M.G.A., Faimali, M., Ferrando, S., Gambardella, C., Gatti, A.M., Ramoino, P., 2013. Dose dependent effects of silver nanoparticles on reproduction and development of different biological models. EQA - international. J. Environ. Qual. 8 (8), 61–65. https://doi.org/10.6092/issn.2281-4485/3828.

Gambardella, C., Aluigi, M.G., Ferrando, S., Gallus, L., Ramoino, P., Gatti, A.M., ... Falugi, C., 2013. Developmental abnormalities and changes in cholinesterase activity in sea urchin embryos and larvae from sperm exposed to engineered nanoparticles. Aquat. Toxicol. 130–131, 77–85. https://doi.org/10.1016/j.aquatox.2012.12.025.

Gambardella, C., Costa, E., Piazza, V., Fabbrocini, A., Magi, E., Faimali, M., Garaventa, F., 2015. Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar. Environ. Res. 111, 41–49. https://doi.org/10.1016/j.marenvres.2015.06.001.

George, S., Lin, S., Ji, Z., Thomas, C.R., Li, L., Mecklenburg, M., ... Nel, A.E., 2012. Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. ACS Nano 6 (5), 3745–3759. https://doi.org/10.1021/nn204671v.

Gottfredsen, R.H., Larsen, U.G., Enghild, J.J., Petersen, S.V., 2013. Hydrogen peroxide

induce modifications of human extracellular superoxide dismutase that results in enzyme inhibition. Redox Biol. 1 (1), 24–31. https://doi.org/10.1016/j.redox.2012.12.004.
He, D., Jones, A.M., Garg, S., Pharm, A.N., Waite, T.D., 2011. Silver nanoparticle – reactive oxygen species interactions: application of a charging – discharging model. J. Phys. Chem. C 115, 5461–5468. https://doi.org/10.1021/jp111275a.
Hong, X., Wen, J., Xiong, X., Hu, Y., 2016. Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. Environ. Sci. Pollut. Res. 23 (5), 4489–4497. https://doi.org/10.1007/s11356-015-5668-z.
Hoop, M., Shen, Y., Chen, X.Z., Mushtaq, F., Iuliano, L.M., Sakar, M.S., ... Pané, S., 2016. Magnetically driven silver-coated nanocoils for efficient bacterial contact killing. Adv. Funct. Mater. 26 (7), 1063–1069. https://doi.org/10.1002/adfm.201504463.
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.
Jiang, H.S., Qiu, X.N., Li, G.B., Li, W., Yin, L.Y., 2014. Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. Environ. Toxicol. Chem. 33 (6), 1398–1405. https://doi.org/10.1002/etc.2577.
Johari, S.A., Kalbassi, M.R., Soltani, M., Yu, I.J., 2013. Toxicity comparison of colloidal silver nanoparticles in various life stages of rainbow trout (*Oncorhynchus mykiss*). Iran. J. Fish. Sci. 12 (1), 76–95.
Johari, S.A., Sarkheil, M., Behzadi Tayemeh, M., Veisi, S., 2018a. Influence of salinity on the toxicity of silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) in halophilic microalgae, *Dunaliella salina*. Chemosphere 209, 156–162. https://doi.org/10.1016/j.chemosphere.2018.06.098.
Johari, S.A., Rasmussen, K., Gulumian, M., Ghazi-Khansari, M., Tetarazako, N., Kashiwada, S., ... Je Yu, I., 2018b. Toxicology Mechanisms and Methods Introducing a New Standardized Nanomaterial Environmental Toxicity Screening Testing Procedure, ISO/TS 20787: Aquatic Toxicity Assessment of Manufactured Nanomaterials in Saltwater Lakes Using *Artemia* sp. nauplii. pp. 6516. https://doi.org/10.1080/15376516.2018.1512695.
Jones, R., Draheim, R., Roldo, M., 2018. Silver nanowires: synthesis, antibacterial activity and biomedical applications. Appl. Sci. 8 (5), 673. https://doi.org/10.3390/app8050673.
Keller, A.A., McFerran, S., Lazareva, A., Suh, S., 2013. Global life cycle releases of engineered nanomaterials. J. Nanopart. Res. 15 (6). https://doi.org/10.1007/s11051-013-1692-4.
Kos, M., Kahru, A., Drobne, D., Singh, S., Kalčíková, G., Kühnel, D., Rohit, R., Gotvajn, A.Ž., Jemec, A., 2016. A case study to optimise and validate the brine shrimp *Artemia franciscana* immobilization assay with silver nanoparticles: the role of harmonisation. Environ. Pollut. 213, 173–183. https://doi.org/10.1016/j.envpol.2016.02.015.
Kumar, D., Roy, R., Parashar, A., Raichur, A.M., Chandrasekaran, N., Mukherjee, A., Mukherjee, A., 2017. Toxicity assessment of zero valent iron nanoparticles on *Artemia salina*. Environ. Toxicol. 32 (5), 1617–1627. https://doi.org/10.1002/tox.22389.
Kwak, J.I., An, Y.J., 2015. A review of the ecotoxicological effects of nanowires. Int. J. Environ. Sci. Technol. 12 (3), 1163–1172. https://doi.org/10.1007/s13762-014-0727-4.
Langley, D., Giusti, G., Mayousse, C., Celle, C., Bellet, D., Simonato, J.P., 2013. Flexible transparent conductive materials based on silver nanowire networks: a review. Nanotechnology 24 (45). https://doi.org/10.1088/0957-4484/24/45/452001.
Lee, C., Kim, J.Y., Lee, W. Il, Nelson, K.L., Yoon, J., Sedlak, D.L., 2008. Bactericidal effect of zero-valent iron nanoparticles on *Escherichia coli*. Environ. Sci. Technol. 42 (13), 4927–4933. https://doi.org/10.1021/es800408u.
Li, N., Xia, T., Nel, A.E., 2008. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. Free Radic. Biol. Med. 44 (9), 1689–1699. https://doi.org/10.1016/j.freeradbiomed.2008.01.028.
Lim, D., Roh, J.Y., Eom, H.J., Choi, J.Y., Hyun, J., Choi, J., 2012. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. Environ. Toxicol. Chem. 31 (3), 585–592. https://doi.org/10.1002/etc.1706.
Liochev, S.I., Fridovich, I., 2000. Copper- and zinc-containing superoxide dismutase can act as a superoxide reductase and a superoxide oxidase. J. Biol. Chem. 275 (49), 38482–38485. https://doi.org/10.1074/jbc.M007891200.
Manke, A., Wang, L., Rojanasakul, Y., 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed. Res. Int. 2013. https://doi.org/10.1155/2013/942916.
Massarsky, A., Dupuis, L., Taylor, J., Eisa-beygi, S., Strek, L., Trudeau, V.L., Moon, T.W., 2013. Chemo sphere assessment of nanosilver toxicity during zebrafish (*Danio rerio*) development. Chemosphere 92 (1), 59–66. https://doi.org/10.1016/j.chemosphere.2013.02.060.
Ostman, A., Bohmer, F.D., 2001. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol. 11 (6), 258–266 (doi:S0962-8924(01)01990-0 [pii]).
Oukarroum, A., Polchtchikov, S., Perreault, F., Popovic, R., 2012. Temperature influence on silver nanoparticles inhibitory effect on photosystem II photochemistry in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. Environ. Sci. Pollut. Res. 19, 1755–1762.
Prabhu, S., Poulose, E.K., 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int. Nano Lett. 2 (1), 32. https://doi.org/10.1186/2228-5326-2-32.
Scanlan, L.D., Reed, R.B., Loguinov, A.V., Antczak, P., Tagmount, A., Aloni, S., ... Gilbert, B., 2013. Silver nanowire exposure results in internalization and toxicity to *Daphnia magna*. ACS Nano 7 (12), 10681–10694. https://doi.org/10.1021/nn4034103.
Shvedova, A.A., Pietroiusti, Antonio, Bengt Fadeel, V.E.K., 2012. Mechanisms of carbon nanotube-induced toxicity: focus on pulmonary inflammation. Toxicol. Appl. Pharmacol. 261 (15), 121–133. https://doi.org/10.1016/j.taap.2012.03.023.
Sohn, E.K., Johari, S.A., Kim, T.G., Kim, J.K., Kim, E., Lee, J.H., ... Yu, I.J., 2015. Aquatic toxicity comparison of silver nanoparticles and silver nanowires. Biomed. Res. Int. 2015. https://doi.org/10.1155/2015/893049.
Sund, J., Alenius, H., Vippola, M., Savolainen, K., Puustinen, A., 2011. Proteomic characterization of engineered nanomaterial-protein interactions in relation to surface reactivity. ACS Nano 5 (6), 4300–4309. https://doi.org/10.1021/nn101492k.
Ulm, L., Krivohlavek, A., Jurašin, D., Ljubojević, M., Šinko, G., Crnković, T., ... Vinković Vrček, I., 2015. Response of biochemical biomarkers in the aquatic crustacean *Daphnia magna* exposed to silver nanoparticles. Environ. Sci. Pollut. Res. 22 (24), 19990–19999. https://doi.org/10.1007/s11356-015-5201-4.
Visnapuu, M., Joost, U., Juganson, K., Künnis-Beres, K., Kahru, A., Kisand, V., Ivask, A., 2013. Dissolution of silver nanowires and nanospheres dictates their toxicity to *Escherichia coli*. Biomed. Res. Int. 2013 (i). https://doi.org/10.1155/2013/819252.
Wang, Z., Yin, L., Zhao, J., Xing, B., 2016. Trophic transfer and accumulation of $TiO_2$ nanoparticles from clamworm (*Perinereis aibuhitensis*) to juvenile turbot (*Scophthalmus maximus*) along a marine benthic food chain. Water Res. 95, 250–259. https://doi.org/10.1016/j.watres.2016.03.027.
Wang, C., Jia, H., Zhu, L., Zhang, H., Wang, Y., 2017. Toxicity of α-$Fe_2O_3$ nanoparticles to *Artemia salina* cysts and three stages of larvae. Sci. Total Environ. 598, 847–855. https://doi.org/10.1016/j.scitotenv.2017.04.183.
Wu, Y., Zhou, Q., 2013. Silver nanoparticles cause oxidative damage and histological changes in medaka (*Oryzias latipes*) after 14 days of exposure. Environ. Toxicol. Chem. 32 (1), 165–173. https://doi.org/10.1002/etc.2038.
Zhu, S., Zhu, B., Huang, A., Hu, Y., Wang, G., Ling, F., 2016. Toxicological effects of multi-walled carbon nanotubes on *Saccharomyces cerevisiae*: the uptake kinetics and mechanisms and the toxic responses. J. Hazard. Mater. 318, 650–662. https://doi.org/10.1016/j.jhazmat.2016.07.049.
Zhu, S., Luo, F., Tu, X., Chen, W.C., Zhu, B., Wang, G.X., 2017a. Developmental toxicity of oxidized multi-walled carbon nanotubes on *Artemia salina* cysts and larvae: uptake, accumulation, excretion and toxic responses. Environ. Pollut. 229, 679–687. https://doi.org/10.1016/j.envpol.2017.07.020.
Zhu, S., Luo, F., Chen, W., Zhu, B., Wang, G., 2017b. Toxicity evaluation of graphene oxide on cysts and three larval stages of *Artemia salina*. Sci. Total Environ. 595, 101–109. https://doi.org/10.1016/j.scitotenv.2017.03.224.