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On how environmental and experimental conditions affect the results of aquatic nanotoxicology on brine shrimp (*Artemia salina*): A case of silver nanoparticles toxicity☆

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ABSTRACT

The genus *Artemia* sp. has been accepted as a reliable model organism for aquatic toxicity and nanotoxicity experiments, as far as the ISO TS 20787 has recently been published to standardize nanotoxicity test with this organism. Experimental and environmental conditions may affect the toxicity of nanomaterials on aquatic organisms including *Artemia* sp. nauplii. In this study, acute toxicity effects of silver nanoparticles (AgNPs) on the nauplii of *Artemia salina* was investigated under various conditions (e.g. different lights, salinities, temperatures, volume and agitation of exposure media and instar stages of nauplii). The EC values were calculated using Probit program and all data were analyzed statistically by SPSS software. At all test conditions, the immobilization rate of *Artemia* nauplii increased in a concentration-dependent manner ($P < 0.05$). The sensitivity of instar stage II to different concentrations of AgNPs was significantly higher than instar I ($P < 0.05$). The toxicity effect of AgNPs was affected by alteration of environmental conditions, so that the effective concentration (EC) values for instar I of *A. salina* decreased with increasing water temperature, decreasing water salinity and in continuous darkness condition. The EC_{50} value of AgNPs was significantly lower in 100 mL beakers (21.35 ± 5.67 mg L^{-1}) than 10 mL well plates (42.44 ± 11.30 mg L^{-1}). Agitation of exposure media did not affect the toxicity of AgNPs. The results indicated that the experimental and environmental conditions influence on the toxicity of AgNPs in the nauplii of *A. salina*.

1. Introduction

In the past decades, advances in nanotechnology have led to the production and application of metal and metal oxide nanoparticles (NPs) with specific physicochemical characteristics in many household and healthcare products and industrial technologies (Cho et al., 2009; Montes et al., 2012). Silver nanoparticles (AgNPs) especially because of their antimicrobial properties are widely used as one of the most popular manufactured nanomaterials (MNMs) in medical devices, textiles, cosmetic products and water disinfection devices (Cho et al., 2009). Due to vast utilization of MNMs, their discharge into water bodies and ultimate impact on biota is inevitable (Batley et al., 2013). Production volumes of NPs may give appropriate perspective on their release to aquatic environments (Bundschuh et al., 2018). Piccinno et al. (2012) estimated the production volume of AgNPs approximately 55 t/year worldwide in 2010. It is estimated that aquatic environment receive about 7% of the production volume of NPs (Keller et al., 2013). Therefore, it is important to evaluate the potential toxicological impacts of released NPs on aquatic organisms. For example, maintenance of zebrafish (*Danio rerio*) spawn in water containing AgNPs increased the rates of mortality and morphological abnormalities in their larvae (Lee et al., 2007). Also several papers revealed negative effects of MNMs even at molecular and cellular levels of the aquatic organisms (Chupani et al., 2017 and 2018a,b). The ecotoxicological effects of AgNPs on aquatic organisms belonging to different trophic level such as green algae (Oukarroum et al., 2012a), aquatic plant *Spirodela polyrhiza* (Jiang et al., 2014), crustaceans like *Daphnia magna* (Newton et al., 2013) and *Artemia salina*

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(Gambardella et al., 2015) as well as fish (Yue et al., 2017; Lacave et al., 2018) have been well documented. AgNPs exert their toxicity on different organisms by nano-form of silver or free-silver ions (Ag^+) released from AgNPs (Jiang et al., 2014; McShan et al., 2014). Wang et al. (2012) found that the free Ag^+ contributed to the toxicity of AgNPs colloids to three aquatic organisms including an alga (*Raphidocelis subcapitata*), a cladoceran (*Chydorus sphaericus*), and a freshwater fish larva (*Danio rerio*). The stability of AgNPs and release of silver ions are influences by agglomeration or aggregation, dispersion, sedimentation, and dissolution of NPs in aqueous conditions (Arulvasu et al., 2014; McShan et al., 2014). These phenomena dependent on several environmental parameters such as ionic strength, pH, temperature, light, dissolved oxygen and presence of natural organic matters (Li et al., 2010; Fabrega et al., 2011; Odzak et al., 2017). These physicochemical properties affect on surface area/volume ratio, charge, and size of NPs and consequently their toxicity on aquatic animals (Truong et al., 2012; Angel et al., 2013). Yi and Cheng (2017) showed that AgNPs highly aggregated in estuarine water and hydrodynamic diameter of NPs and subsequently their toxicity to bacteria *Bacillus subtilis* increased. The results of another study revealed that the presence of humic substances (HS) alleviated the toxicity of the AgNP colloids to *R. subcapitata*, *C. sphaericus*, and *D. rerio* (Wang et al., 2015). Another factor that influences on NPs toxicity is the exposure concentration. An et al. (2019) reported the higher toxic effects of AgNPs and silver nanowires (AgNWs) to *A. salina* at higher exposure concentrations. However, there is no enough data about the effects of different aqueous and exposure conditions on the toxicity of different NPs in aquatic organisms like crustaceans.

Brine shrimp, *Artemia* sp., as an aquatic microcrustacean are found abundantly in many of saltwater ecosystems. This invertebrate is a non-selective filter feeder capable of ingesting particles smaller than 50 μm in size (Hund-rinke and Simon, 2006). This feature makes them one of the first candidates to absorb many pollutants such as NPs (Ates et al., 2015). In addition, this zooplankton due to distinct features such as high offspring production, rapid hatching and easy accessibility of nauplii, small body size, simplicity of culture and cost-effectiveness (Manfra et al., 2012; Libralato, 2014) is used as a model organism in toxicity assessment of MNMs as far as the International Organization for Standardization (ISO) has recently developed and published a Technical Specification (TS) to standardize toxicity test with *Artemia* sp. nauplii (ISO/TS 20787, 2017, Johari et al., 2019).

The toxicity of MNMs on aquatic organisms including *Artemia* sp. nauplii may be affected by environmental conditions such as water pH, salinity, temperature, etc, all of which are influenced by natural conditions, especially climate changes. On the other hand, the life stages of organism as well as the laboratory test conditions (e.g. agitation and volume of exposure media, light regime, etc) may affect the toxicity results. Therefore the responses of *Artemia* sp. to MNMs under various exposure conditions need to be studied.

In the present study, we evaluated the effect of various conditions (e.g. different lights, salinities, agitation and volume of exposure media, and life stage of nauplii) on the toxicity of AgNPs as a high consumption MNMs against *Artemia salina*. In addition, the stability and silver ions content of AgNPs suspension at different salinities were examined.

2. Materials and methods

2.1. Silver nanoparticles and characterizations

A water-based colloidal suspension of citrate-capped AgNPs with commercial name of Nanocid® that contained 4000 mg L^{-1} of metallic silver was purchased from Nano Nasb Pars Co. (Tehran, Iran). A transmission electron microscopy (TEM) analysis was performed using Carl Zeiss AG - Zeiss EM900 transmission electron microscope. The diameters of 200 randomly selected particles were measured using AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany). The AgNPs observed by TEM were spherical in shape with an average diameter of 8.80 ± 5.13 nm and maximum diameter of 29.1 nm (Fig. S1 and a). Scanning the absorption spectra of AgNPs suspension using a UV–Vis spectrometer (SECOMAM UVIKON SCHOTT Instruments) within a range of 300–600 nm showed a maximum peak of absorbance at 415 nm (Fig. S1 and b). Dynamic light scattering (DLS) method was applied using a nanoparticle size and zeta potential analyzer (SZ-100, Horiba, Japan). When the temperature of the instrument holder was 25.2 °C, the zeta potential, Z-average, and polydispersity index were −28.3 mV, 106.4 nm, and 0.426, respectively (Fig. S1, c and d).

2.2. Release of silver ions to AgNPs suspension in different salinities

The percentages of silver ions in the AgNPs dispersions in different salinities were determined by centrifugation technique (modified method of An et al., 2019). For this purpose, salinities of 0 (distilled water), 25, 30 and 35‰ were prepared in glass beakers. Then, a concentration of 5 mg L^{-1} of AgNPs was prepared in each beaker and kept under photoperiod of 16:8 (light: dark) for 48 h. Afterwards, 1.5 mL of water was sampled from the middle part of the water column in each beaker, and centrifuged through an Amicon Ultra centrifugal filter (3-kD nominal cut-off value, Amicon, Millipore, Germany) at 7000 rpm for 60 min. The silver ions content in each centrifuged sample as well as total silver content in the un-filtrated AgNPs suspensions was measured using graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle™ 900T, USA) after acid digestion of samples in 2 mL of concentrated HNO_3 (65% Suprapur®, Sigma-Aldrich). The percentage of Ag^+ ions in the each salinity was determined using following equation; where Ag_i is the silver ions content in filtrated sample and Ag_t is the total silver in un-filtrated sample.

$$\text{Silver ion content } (\%) = (Ag_i / Ag_t) \times 100$$

2.3. Stability of AgNPs suspension in different salinities

An experiment was conducted to determine the process of sedimentation of various concentrations of AgNPs in different salinities over a period of 24 h. At first, 400 mL of each AgNPs concentration (10, 100 and 200 mg L^{-1}) at four levels of salinity (0, 25, 30 and 35‰) were prepared in glass beakers and kept in fixed location for 24 h. After 0.5, 2, and 24 h, 3 mL of water was sampled from the mid-water column in each beaker and transferred to the cuvettes of the spectrophotometer and the absorbance of each sample was read at 415 nm using a UV–vis spectrophotometer (SECOMAM UVIKON SCHOTT Instruments).

2.4. Test organism

The cysts of *Artemia salina* were donated by Binzhou Evergreen Aquaculture Co., Ltd. (China) and used for hatching. To prepare ASW (25, 30 and 35‰), 25, 30 and 35 g of synthetic sea salt (Delta Marine®, Iran) were dissolved into 1 L of double-distilled water and aerated continuously for 24 h. The prepared seawater (1 L) was transferred into V–bottomed glass incubators and then dried cysts (0.5 g) were added. The nauplii (instar I) were hatched after 24 h at 30 ± 1 °C under condition of continuous light illumination of 1500

lx using a fluorescent lamp and constant aeration from the bottom of the hatching incubators. If needed, the hatched nauplii were kept in incubator for another 24 h to turn to instar II.

2.5. Toxicity of AgNPs on A. salina in different experimental conditions

Toxicity effects of AgNPs on *Artemia* nauplii under different conditions (Table 1) were examined in separate experiments and in accordance with the general principles of ISO/TS 20787 (2017). Here, different concentrations of AgNPs (e.g. 0, 5, 20, 40, 60, 80, 100 and 200 mg L^{-1}) were tested based on the results obtained from the pre-tests and the range finding tests (data not shown). Each exposure carried out by triplicate and the density was ten nauplii per vessel in each replicate. The nauplii were not fed during exposure period. Physico-chemical parameters of water including dissolved oxygen, pH and electrical conductivity (EC) were measured daily. Shaking was not used except for the treatment that effect of agitation of exposure media was the goal, where the FTSK-350 Shaker (SCI FINETECH, Republic of Korea) was used at 90 rpm. To understand the effect of different light conditions, four light treatments including continuous UV-A (PHILIPS, BLB F8T5), permanent visible light (continuous light), full darkness, and a photoperiod regime (8 h of darkness and 16 h of visible light) were tested. The immobility rate of *Artemia* nauplii was evaluated within 48 h of exposure. The median effective concentration (EC_{50}) of AgNPs was calculated using Probit analysis. The visual uptake of NPs by *Artemia* nauplii after 48 h of exposure was also visualized using a E200 biological microscope (ECLIPSE, Nikon) equipped with a digital camera (DS-FI1, Nikon).

2.6. Statistical analysis

Data were reported as mean ± standard deviation. SPSS software version 16 was used to compare the effects of variables on toxicity of AgNPs on *A. salina*. The data were examined to be normalized using Kolmogorov-Smirnov test. No data transformation was performed since the data were homoscedastic and had a normal distribution. One-way ANOVA followed by the Duncan multiple range test was used to compare the significant differences between means. Comparison of significant differences between two samples was done using the Independent-Sample T test. In all experiments, the significance level of the test was considered as $P < 0.05$.

3. Results

3.1. Properties of artificial seawater (ASW) used in exposure experiments

Measurements of dissolved oxygen and pH of artificial seawater used in different experiments revealed no significant effect of experimental conditions (the averages of DO and pH were 6.49 ± 0.05 mg L^{-1} and 8.19 ± 0.03, respectively). In the case of electrical conductivity (EC), just salinity affected this parameter and EC values were 24.33 ± 0.21, 29.08 ± 0.17, and 34.08 ± 0.16 μS cm^{-1} in water salinities of 25, 30, and 35‰.

3.2. Release of silver ions from AgNPs in different salinities

The measurement of the portion of silver ions in the AgNPs suspension in different salinities after 48 h showed that increasing salinity from 0 to 25, 30, and 35‰ resulted to the elevation of the percentage of Ag^{+} ions in the AgNPs suspension from 7.3 to 12.8, 19.8, and 21.8%, respectively.

3.3. Stability of AgNPs suspension in different salinities

The results of stability of 10, 100 and 200 mg L^{-1} AgNPs suspensions at salinities of 0, 25, 30 and 35‰ after different time intervals were shown in Fig. S2 and S3 (a–c). The higher absorbance values were observed in the higher AgNPs concentrations at all salinities and all times. The absorbance value of different concentrations of AgNPs decreased coincident with increasing salinity at all time intervals. The decreasing trend of absorbance value was more intense in the higher concentrations of AgNPs at 2 h and 24 h time intervals (Fig. S2).

3.4. Toxicity effect of AgNPs on different life stages of A. salina

No immobilization rate of instar I and II was recorded in 0 mg L^{-1} (Fig. 1 a). The immobilization rate of instar I exposed to different concentrations of AgNPs increased significantly with increasing concentration from 5 to 200 mg L^{-1} ($P < 0.05$). The elevation of AgNPs concentration from 5 to 40 mg L^{-1} resulted to increasing the immobilization rate of instar II to 94.4% ($P < 0.05$) and this value did not increased significantly in higher concentrations ($P > 0.05$). In all exposure concentrations, the immobilization rate of instar II was significantly higher than instar I ($P < 0.05$), except in 200 mg L^{-1} which the immobilization rate was 100%. Also the lower 48 h EC_{50} of AgNPs for instar II (Fig. 1 b) revealed the higher sensitivity of this naupliar stage to AgNPs.

Table 1
Different conditions at which the toxicity of silver nanoparticles was assessed using *A. salina* nauplii according to the purpose of the test.

Purpose of the test	Life stage	Exposure vessel type/Volume	Lighting condition	Temperature (°C)	Salinity (‰)	Shaking
Effect of life stage on AgNPs toxicity	a) Instar I b) Instar II	6-well plates/10 mL	16 h:8 h light: dark	30	35	No
Effect of salinity on AgNPs toxicity	Instar I	6-well plates/10 mL	16 h:8 h light: dark	30	a) 25 b) 30 c) 35	No
Effect of temperature on AgNPs toxicity	Instar I	6-well plates/10 mL	16 h:8 h light: dark	a) 25 b) 30	35	No
Effect of light on AgNPs toxicity	Instar I	6-well plates/10 mL	a) 16 h:8 h light: dark, b) Continuous light (24 h light), c) Full darkness (no light), d) Continuous UV-A radiation	30	35	No
Effect of volume of exposure media on AgNPs toxicity	Instar I	a) 6-well plates/10 mL, b) Glass beakers/100 mL	16 h:8 h light: dark	30	35	No
Effect of agitation (shaking) on AgNPs toxicity	Instar I	6-well plates/10 mL	16 h:8 h light: dark	30	35	90 rpm

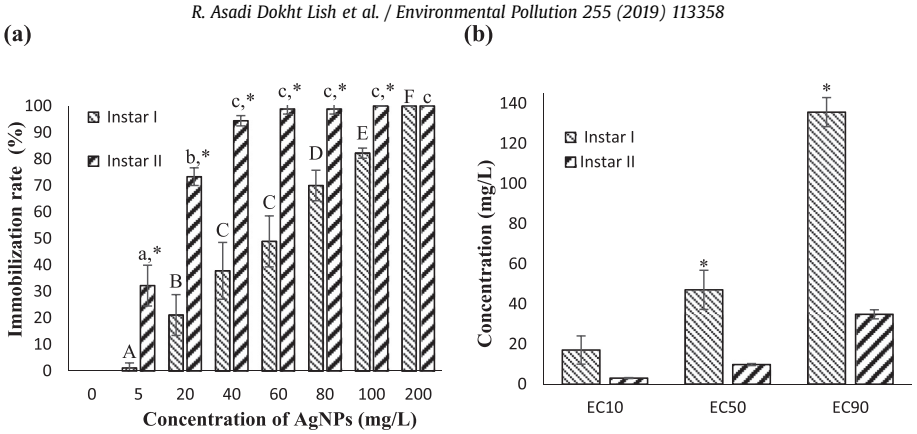

Fig. 1. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) of instar I and II of *A. salina* exposed to different concentrations of AgNPs for 48 h. The bars with different letters in each life stage of nauplii are significantly different (mean ± SD, ANOVA, $P < 0.05$). Asterisk (*) indicate a significant difference between instar I and II in constant concentrations of AgNPs as well as between ECs (mean ± SD, the Independent-Sample T test, $P < 0.05$).

3.5. Effect of salinity on the toxicity of AgNPs in A. salina

In all AgNP concentrations (except 200 mg L^{-1}), the immobilization rate of nauplii decreased significantly with increasing salinity from 25 to 35‰ ($P < 0.05$) (Fig. 2 a). Also the highest EC values were observed at salinity of 35‰ ($P < 0.05$) (Fig. 2 b) which suggest alleviation effect of higher salinities on the toxicity of AgNPs.

3.6. Effect of temperature on the toxicity of AgNPs in A. salina

The comparison between the temperatures of 25 and 30 °C showed higher immobilization rate at 30 °C in all AgNPs concentrations ($P < 0.05$) (Fig. 3 a). The values of EC_{50} and EC_{90} were lower at 30 °C compare to 25 °C ($P < 0.05$) revealed that toxicity of AgNPs elevated by increasing the temperature (Fig. 3 b).

3.7. Effect of type and duration of light exposure on the toxicity of AgNPs in A. salina

The results of exposure of *Artemia* nauplii to various concentrations of AgNPs in four different light conditions showed that the immobilization rate of the nauplii increased significantly coincident with increasing exposure concentration in all light conditions ($P < 0.05$) (Fig. 4 a). The immobilization rate of nauplii was higher in continuous visible lighting and in full darkness compared to other light conditions in concentrations of 20, 40 and 60 mg L^{-1} ($P < 0.05$). In 100 mg L^{-1} of AgNPs, the immobilization rate of nauplii showed no significant difference in continuous UV-A radiation, continuous visible lighting and full darkness conditions. The lowest immobilization rate was observed in photoperiod condition (16 h: 8 h light: dark). There was no significant difference in the immobilization rate of nauplii in different light conditions in concentrations of 5, 80 and 200 mg L^{-1}.

3.8. Effect of volume of the exposure media on the toxicity of AgNPs in A. salina

The immobilization rate of *Artemia* nauplii increased significantly along with elevation of exposure concentrations of AgNPs from 10 mg L^{-1} to 200 mg L^{-1} in both volumes of the exposure vessel ($P < 0.05$). The immobilization rate of the nauplii exposed to 30 and 60 mg L^{-1} of AgNPs was significantly higher in the 100 mL beakers than 10 mL 6-well plates ($P < 0.05$), while this difference was not observed in other concentrations ($P > 0.05$) (Fig. 5 a). The EC_{50} of AgNPs in the 10 mL 6-well plates increased significantly

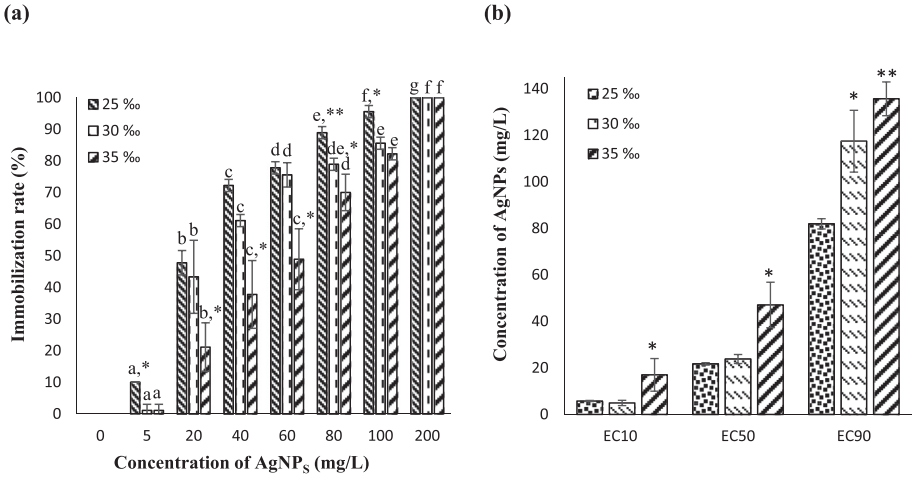

Fig. 2. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) for *A. salina* (instar I) exposed to various concentrations of AgNPs for 48 h at different salinities. The bars with different letters at each salinity concentration are significantly different. Asterisks indicate a significant difference between salinities of 25, 30 and 35‰ in constant concentration of AgNPs as well as between ECs (mean ± SD, ANOVA, $P < 0.05$).

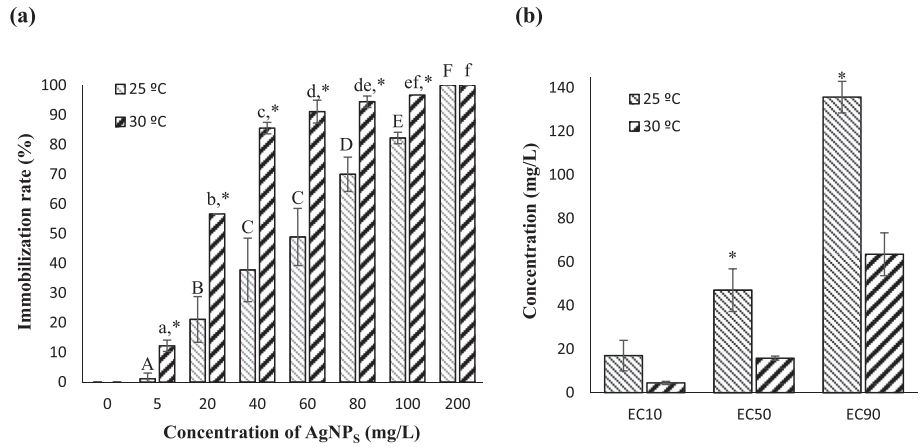

Fig. 3. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) for *A. salina* (instar I) exposed to different concentrations of AgNPs for 48 h at temperatures of 25 and 30 °C. The data with different letters at each temperature are significantly different (mean ± SD, ANOVA, $P < 0.05$). Asterisk (*) indicate a significant difference between two temperatures in equal concentration of AgNPs as well as between ECs (mean ± SD, the Independent-Sample T test, $P < 0.05$).

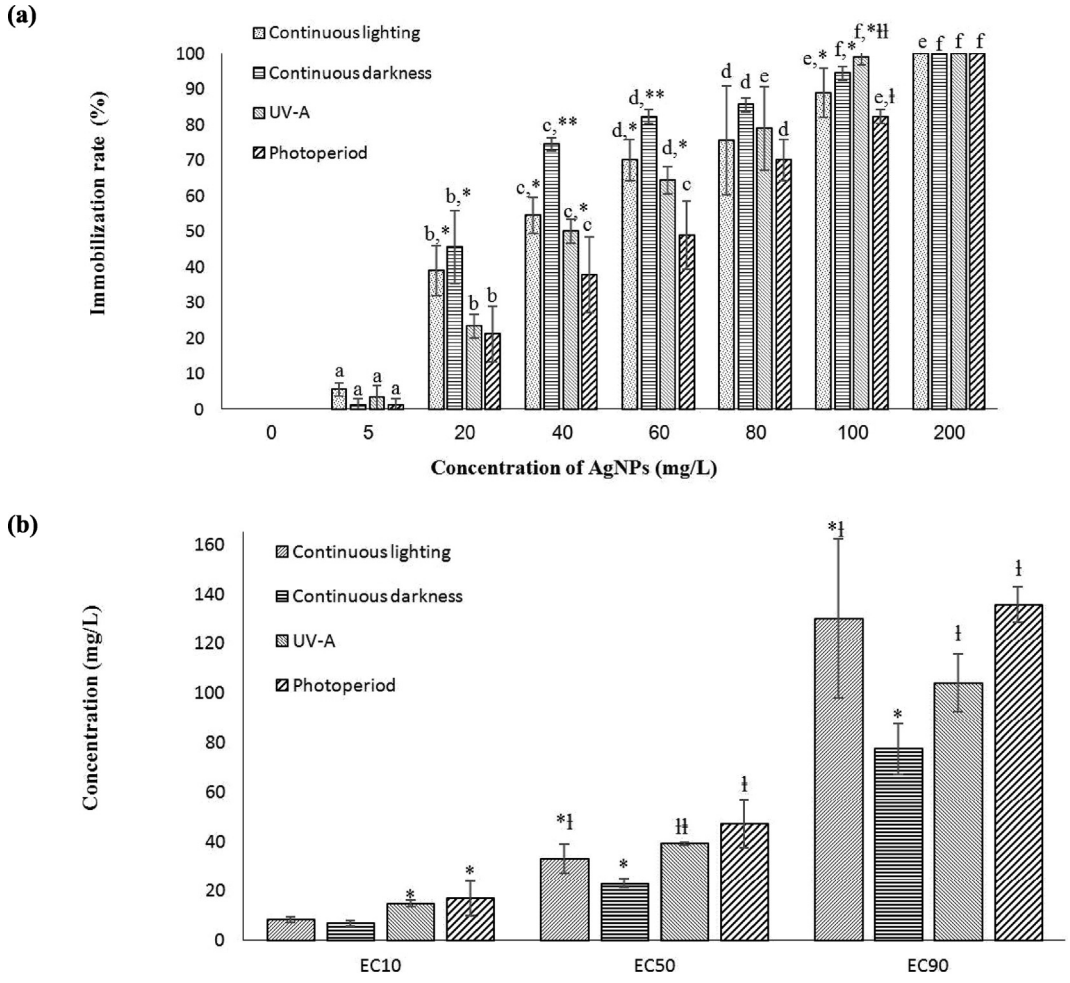

Fig. 4. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) for *A. salina* (instar I) exposed to different concentrations of AgNPs for 48 h in four different light conditions. The data with different letters in each light condition are significantly different (mean ± SD, ANOVA, $P < 0.05$). Different symbols (*, etc) indicate a significant difference between different light conditions in the constant concentration of AgNPs as well as between ECs (mean ± SD, the Independent-Sample T test, $P < 0.05$).

compared to the 100 mL beakers ($P < 0.05$). There were no significant differences in EC_{10} and EC_{90} values between two exposure vessels ($P > 0.05$) (Fig. 5 b).

3.9. Agitating effect on the toxicity of AgNPs in A. salina

The results showed that the immobilization rate increased

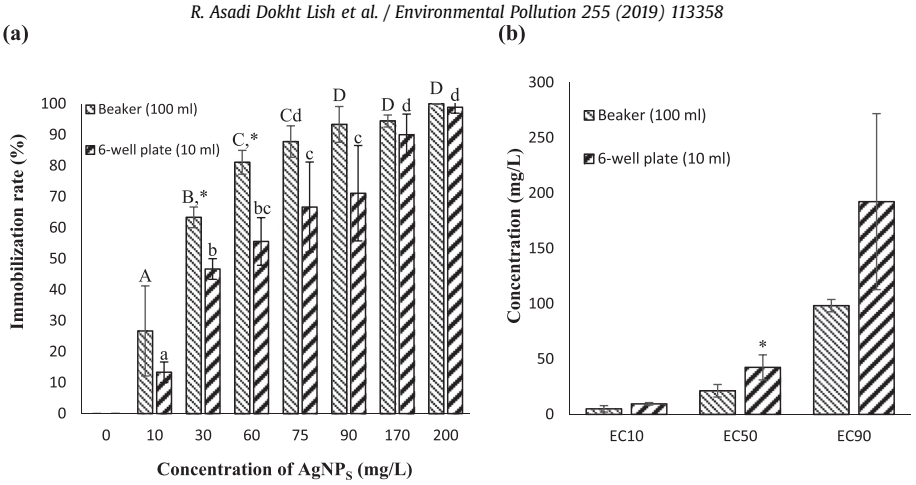

Fig. 5. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) for *A. salina* (instar I) exposed to different concentrations of AgNPs for 48 h in two different volumes of exposure media (100 mL beakers vs. 10 mL 6-well plates). The bars with different letters for each exposure vessel are significantly different (mean ± SD, ANOVA, $P < 0.05$). Asterisk (*) indicate a significant difference between the two volumes of exposure media in equal concentrations of AgNPs as well as between ECs (mean ± SD, the Independent-Sample T test, $P < 0.05$).

significantly with increasing AgNPs concentration in both shaking and no shaking conditions ($P < 0.05$). The agitation of exposure media increased the immobilization rate compared to the no shaking condition only in 10 mg L^{-1} of AgNPs ($P < 0.05$) (Fig. 6 a). There were no significant differences in the EC_{10}, EC_{50} and EC_{90} values for *A. salina* between shaking and no shaking conditions (Fig. 6 b).

3.10. Visual uptake of AgNPs by A. salina

Figure S4 shows the visual uptake of AgNPs in whole body cavity and inside the gut of *A. salina* exposed to different concentrations of AgNPs for 48 h. In control group (0 mg L^{-1}), the gut of the nauplii is devoid of NPs but the visual uptake of particles increased with elevation of exposure concentration.

4. Discussion

The purpose of the present study was to investigate the effect of various experimental and environmental conditions (e.g. different lights, salinities, agitation and volume of exposure media, and life stage of nauplii) on the toxicity of AgNPs in *Artemia salina* as a model aquatic organism. Release of silver ions from nanoparticle suspension is an important mechanism in AgNPs toxicity (Auffan et al., 2014; Liu and Hurt, 2010). It is well known that silver ions exert their toxicity by interaction with different biomolecules within a cell of organism like cell wall components, sulfur-containing cell components and nucleic acids (Greulich et al., 2012). Yang et al. (2012) reported that there was a linear correlation between dissolved silver and AgNPs toxicity to *Caenorhabditis elegans*. Many studies have investigated the dissolution and release of silver ions under different conditions (Kittler et al., 2010; Cao et al., 2010; Yang et al., 2012). In the present study, AgNPs suspension (5 mg L^{-1}) was incubated at different salinities for 48 h and the percentage of released silver ions in AgNPs suspension were found to increase from 12.8 to 19.8 and 21.84% with increasing water salinity from 25 to 30 and 35‰, respectively. It has been shown that the dissolution of citrate coated AgNPs incubated in abiotic 10% ASW for 48 h increased compared to 0% ASW (Auffan et al., 2014). Levard et al. (2013) also showed that the release of silver ions into AgNPs suspension increased with elevation Cl/Ag molar ratio from 5 to 26750. The stability of AgNPs also influenced

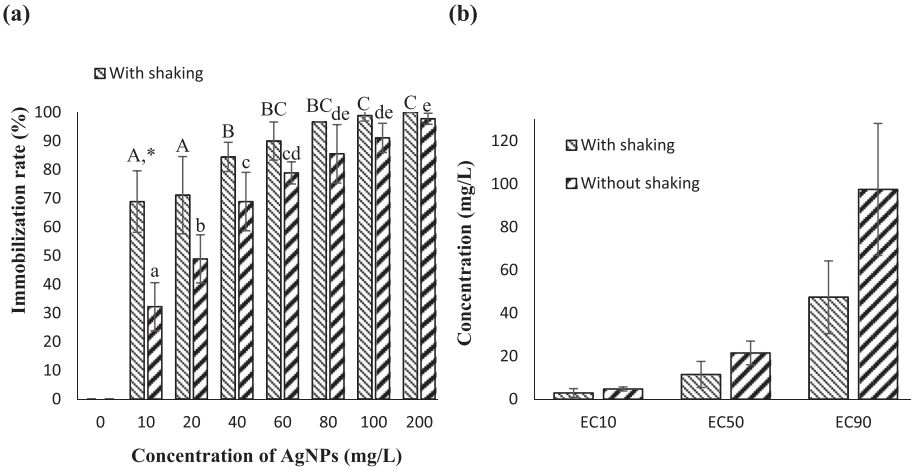

Fig. 6. Comparison of the immobilization rates (a) and effective concentrations (ECs) (b) for *A salina* (instar I) exposed to different concentrations of AgNPs for 48 h in shaking and no shaking conditions. The bars with different letters in each agitation condition are significantly different (mean ± SD, ANOVA, $P < 0.05$). The value with asterisk (*) shows the significant difference between two agitation conditions in 10 mg L-1 AgNPs (mean ± SD, the Independent-Sample T test, $P < 0.05$).

on their toxicity in various environments (Suresh et al., 2012; Yang et al., 2012). It is well known that ionic strength and composition of solution such as sulfur groups and chloride ions (Cl^-) strongly affected on the stability of AgNPs and therefore their toxicity (Lee et al., 2012; Salari-Joo et al., 2012; Wang et al., 2012; Levard et al., 2013; Joo et al., 2018). Li et al. (2010) have explored the effects of Cl^- on the stability of the AgNPs. They found that the formation of an AgCl layer on the AgNPs decreased their dissolution. The results of a study also showed that AgNPs in a solution contained 10 mM of NaCl agglomerated slowly (MacCuspie, 2011). The agglomeration of NPs in brackish and seawater environments can alter their size, surface area and charge and ultimately their toxicity (Truong et al., 2012). In the current study, the values of absorbance spectra of 10, 100 and 200 mg L^{-1} of AgNPs suspensions decreased with increasing salinity from 0 to 35‰, after 0.5, 2 and 24 h. The higher decreasing of absorbance value was observed in 200 mg L^{-1} of AgNPs after 24 h. These results indicate that instability and sedimentation of AgNPs increase with increasing concentration of NPs, water salinity and incubation time.

In the following, the toxicity effects of different concentrations of AgNPs on various life stages of *A. salina* were examined at salinity of 35‰. According to results, the immobilization rate of *Artemia* nauplii increased in a concentration-dependent manner. The immobilization rate of instar I and II increased to 100% in concentration of 200 mg L^{-1} after 48 h. An et al. (2019) also reported a concentration-dependent effect of AgNPs on immobilization rate of *A. salina* (instar I) at salinity of 30 g L^{-1} after 72 h. The comparison of toxicity effect of AgNPs on different life stages of nauplii showed that the sensitivity of instar II to AgNPs was higher than instar I. Kos et al. (2016) also reported increased mortality rate of 48 h old nauplii of *Artemia franciscana* exposed to AgNPs for 48 h compared to 24 h old nauplii. Brine shrimp nauplii ingest particles smaller than 50 μm in size starting from instar II onwards (Van Stappen, 1996) and higher sensitivity of instar II to AgNPs which was observed in our study and in some previous studies is likely to be related to further water filtration resulted from starting filter-feeder behavior by this microcrustacean in instar II that leads to more uptake of particles.

In this study we attempted to investigate the alteration of environmental conditions including salinity, temperature and type and duration of light on toxicity of different concentrations of AgNPs on instar stage I of *A. salina*. The immobilization rate of nauplii decreased with increasing salinity from 25 to 35‰. The highest EC values were observed at salinity of 35‰. Johari et al. (2018) also showed that median inhibitory concentrations (IC_{50}) of AgNPs for marine microalgae *Dunaliella salina* increased with elevation of water salinity from 35 to 140‰. Also the bioavailability of 80 nm citrate-coated AgNPs to marine medaka (*Oryzias melastigma*) decreased at high salinities (15 and 30‰) because of the aggregation of NPs (Wang and Wang, 2014). The NP sedimentation rate is one of the main parameters influence their behavior in environmental media. The reduction of CuO NPs toxicity against *Vibrio anguillarum* has been observed at high salinity (2.0–3.5% NaCl) because of the presence of salt ions and reducing the repulsive effect among NPs which promote their agglomeration (Rotini et al., 2017).

The results of present study showed that increase of water temperature induced the toxicity effect of different concentrations of AgNPs on the nauplii of *A. salina*. The 48 h EC_{50} and EC_{90} values for the nauplii were lower in temperature of 30 °C in comparison to 25 °C. Oukarroum et al. (2012b) reported that high temperature (31 °C) enhanced the toxic effects of AgNPs on the photosynthetic performance of two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. It is reported that elevated toxicity of metals at higher temperatures may be related to the their higher uptake by organism (Sokolova and Lanning, 2008). It is found that the toxicity of oxide NPs such as ZnO and CuO is related to their solubility which is changed by temperature and pH (Chang et al., 2012). Aggregation of some NPs suspensions can be induced by lowering the temperature (Powell et al., 2016). Therefore, increasing the toxicity of AgNPs at higher temperature could be related to their higher solubility.

Some studies have revealed that the light irradiation can influence on the toxicity of AgNPs in aquatic environment (Dewez and Oukarroum, 2012; Shi et al., 2013). Pu et al. (2019) showed that 12 h artificial lights at night alleviated the toxicity effect of AgNPs on *Pterocarya stenoptera* leaf litter decomposition by fungal community. Shi et al. (2013) also examined the toxicity effects of two sizes of AgNPs on *Tetrahymena pyriformis* under dark and light condition. They found that small AgNPs (5–10 nm) toxicity was decreased under light condition for 24 h because of the reduction of released silver ions, the increase of particle size and aggregation of AgNP, whereas the release of silver ions increased under dark condition which induced the toxicity of small AgNPs. Also in the present study the EC values of AgNPs for the nauplii of *A. salina* under continuous darkness condition were lower than visible light photoperiod and continuous UV-A radiation. In contrast to the results of mentioned studies, the results of present study revealed that toxicity of AgNPs did not decreased significantly under continuous light condition compared to continuous darkness condition.

In this study, we made an effort to examine the effects of experimental conditions in terms of the volume and agitation of exposure media on toxicity of AgNPs in *A. salina*. The EC_{50} value of AgNPs was significantly lower in 100 mL beakers compared to 10 mL 6-well plates. In fact, the exposure of nauplii to a larger volume of AgNPs may provide access to a greater number of particles and influence their toxicity. The agitation of exposure media had no significant effect on the toxicity of different concentrations of AgNPs except 10 mg L^{-1}. The immobilization rate of nauplii exposed to 10 mg L^{-1} of AgNPs in shaking condition (90 rpm) increased significantly compared to no shaking condition. It may be is associated with increased contact of the nauplii with AgNPs in shaking condition and subsequently higher uptake of NPs at low exposure concentration. However, further studies are recommended in this regard.

5. Conclusion

The results of this study indicated increasing release of silver ions into AgNPs suspension with increasing water salinity from 25 to 35‰. The stability of AgNPs was affected by concentration of NPs, salinity and incubation duration. Based on the results, a concentration-dependent effect of AgNPs was observed on immobilization rate of *A. salina* (instar I and II) at salinity of 35‰ after 48 h. The finding of the present study highlighted the importance of environmental conditions such as salinity, temperature and type and duration of light on the toxicity of AgNPs in *A. salina*. Therefore, the harmonization of experimental and environmental conditions for the use of *Artemia* species in ecotoxicology and nano-ecotoxicology studies requires more attention. Further studies on the potential effects of type and duration of light exposure as well as agitation of exposure media on the toxicity of NPs in aquatic organisms are suggested.

Declaration of competing interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113358.

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