Chemosphere 209 (2018) 156–162

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Influence of salinity on the toxicity of silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) in halophilic microalgae, *Dunaliella salina*

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## HIGHLIGHTS

- Increased salinity reduced toxic effects of AgNPs and $AgNO_3$ on *D. salina.*
- Toxic effects of AgNPs and $AgNO_3$ on *D. salina* were time and concentration-dependent.
- $AgNO_3$ was more toxic than AgNPs to *D. salina* in all salinities tested.

## ARTICLE INFO

*Article history:*
Received 2 April 2018
Received in revised form
10 June 2018
Accepted 12 June 2018
Available online 13 June 2018

Handling Editor: Tamara S. Galloway

*Keywords:*
Aquatic nanotoxicology
Biomass
Microalgae
*Dunaliella salina*
Salinity
Silver ions
Silver nanoparticles

## ABSTRACT

This study aim to evaluate the potential toxic effects of citrate coated silver nanoparticles (AgNPs) and ionic silver ($AgNO_3$) on marine microalgae *Dunaliella salina* under three different salinities (35, 70, and 140 g/L). The toxicity was investigated according to modified OECD guideline (No. 201) by 72 h exposure of microalgae to various concentrations of each of the chemicals in Walne's saline media. According to the results, the growth inhibitory effects of AgNPs and $AgNO_3$ increased significantly coincidence with increasing time and concentration compared to control ($P < 0.05$). The values of median inhibitory concentrations ($IC_{50}$) of AgNPs and $AgNO_3$ based on average specific growth rate and yield for *D. salina* increased significantly with elevation of water salinity from 35 to 140 g/L ($P < 0.05$). Toxicity of $AgNO_3$ based on $IC_{50}$ to *D. salina* was significantly higher than AgNPs at all salinities ($P < 0.05$). In conclusion, both AgNPs and $AgNO_3$ inhibited the growth of *D. salina* at different saltwater medium.



## 1. Introduction

Nowadays, nanotechnology is considered as a fast-expanding field of science and technology (Wan et al., 2018). Metal and metal oxide nanomaterials have unique physicochemical characteristics that can influence material properties and enable them utilize in many consumer products and industrial technologies (Montes et al., 2012; Rana and Kalaichelvan, 2013).

Silver nanoparticles (AgNPs) due to their antimicrobial properties are used in a wide range of applications, including pharmaceuticals, cosmetics, medical devices, textiles, and water purification (Choi et al., 2008). The increase utilization of products containing AgNPs can lead to release of metallic silver nanoparticles and silver ions ($Ag^+$) into marine, estuarine, and coastal environments (Becaro et al., 2015; Macken et al., 2012). In aquatic environment, it is likely that AgNPs surface will be oxidized by dissolved oxygen in water and silver ions will be released (Zhong, 2013) and perhaps exert toxic effects on aquatic organisms (Blaser et al., 2006). The predicted environmental concentrations (PECs) for Ag-NPs in aquatic environments are at the range of 0.03–0.08 μg/L (Mueller and Nowack, 2008).

Several environmental parameters such as pH, dissolved oxygen, temperature, and ionic strength affect the dissolution of AgNPs (Li et al., 2010). It is well known that the suspension of NPs in ion-rich aqueous solution such as brackish and saltwater ecosystems

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https://doi.org/10.1016/j.chemosphere.2018.06.098
0045-6535/

often agglomerates resulting in the changes in their surface area, charge, and size compared to the as-synthesized particles (Truong et al., 2012). Therefore, these conditions influence on the toxicity, behavior, fate and bioaccumulation of NPs in aquatic environments. It is reported that larger silver nanoparticles aggregates exhibit lower toxicity on bacteria and human cells compared to smaller AgNPs (Gao et al., 2009; Jin et al., 2010). The finding of different studies have been demonstrated the potential toxicity effects of AgNPs on marine species at different trophic levels including algae (*Skeletonema costatum* and *Dunaliella tertiolecta*), cnidaria (*Aurelia aurita*), crustaceans (*Artemia salina* and *Amphibaanus amphitrite*), echinoderms (*Paracentrotus lividus*) (Gambardella et al., 2015), sea urchin) *Paracentrotus lividus* ((Šiller et al., 2013) and oysters (McCarthy et al., 2013).

The microalgae species are very important in primary production of marine ecosystems (Macken et al., 2012) and have the potential to transfer metals and other pollutants along the marine food chain (Quigg, 2013). Moreover, most engineered nanomaterials ecotoxicological assessments are limited to freshwater species used in regulatory testing (i.e. OECD, ISO) including fish, crustaceans, and algae (Miao et al., 2010; Macken et al., 2012; Sohn et al., 2015). *Dunaliella salina* as hypersaline unicellular green algae is widely distributed in open seawater and hypersaline lakes (Shirazi et al., 2015). Marine algae due to their high sensitivity to engineered nanomaterials and high bioaccumulation capability can be utilized as a pollution indicator in marine ecosystem (Barhoumi and Dewez, 2013).

The objective of the present study was to evaluate the effect of different salinities on the toxicity of AgNPs compare to $Ag^+$ ions in marine microalgae *Dunaliella salina*. Algal biomass and growth inhibition rates of average specific growth and yield were employed to examine the toxic effects and determine median inhibitory concentration ($IC_{50}$) and $IC_{10}$ of AgNPs and $AgNO_3$.

## 2. Materials and methods

### 2.1. Nanoparticles and test chemicals

In the present study, a stock suspension of a well-characterized citrate-capped AgNPs (commercial name: Nanocid®) containing 4000 mg/L metallic silver in deionized water were purchased from Nano Nasb Pars Co., Ltd (Tehran, Iran). According to Johari et al. (2013) and in a nutshell, concentration of this colloid based on ICP-MS analysis is 3980 mg/L, mean diameter of NPs based on TEM images is 12.65 nm, and their zeta potential is $-53.33 \pm 7.86$ mV. We also measured the hydrodynamic diameter distribution of silver particles in this colloid by using nanoparticle tracking analysis system (NanoSight NTA, LM10-HS) according to the method described by Sivashanmugan et al. (2017) and it was $63 \pm 24$ nm. In addition, a stock solution of 1000 mg/L $Ag^+$ prepared by dissolving the appropriate amount of $AgNO_3$ (Merck, Germany) in deionized water and stored in dark bottle at 4 °C until utilization. Test solutions of AgNPs and $Ag^+$ ions for use in the toxicity tests were prepared by diluting the stock suspension/solution in the appropriate saline media.

The percentage of $Ag^+$ ions in the AgNPs suspension (100 mg/L) were determined by centrifugation through a Amicon Ultra Centrifugal filter (3-kD nominal cut-off value, Amicon, Millipore, Germany). After 60 min centrifugation at 7000 rpm, the total $Ag^+$ content in the unfiltered AgNPs suspension, as well as its respective filtrates, was measured using graphite furnace atomic absorption spectroscopy (Perkin Elmer PinAAcle™ 900 T, USA). The percentage of soluble Ag in the AgNPs suspension was calculated by dividing the $Ag^+$ content in the filtrates by the $Ag^+$ content in the unfiltered AgNPs suspension multiplied by 100 (van der Zande et al., 2012). Accordingly, the percentage of $Ag^+$ ions in the AgNPs suspension was $0.72 \pm 0.18$%.

### 2.2. Test species

The microalgae *Dunaliella salina* was used as the test organism in this study. The microalgae were cultured in our laboratory for more than three years in accordance with modified standard OECD guideline number 201 to provide ideal conditions for marine microalgae (Lavens and Sorgeloos, 1996). Briefly, 5 ml of microalgae (1000 cells/ml) inoculated in 2 L glass vessels containing Walne's medium and sterilized artificial seawater (35 g/L) at $27 \pm 1$ °C under continuous light illumination of 2000 lux and constant aeration from the bottom. Based on salinity treatment, different concentrations of artificial seawater (35, 70 and 140 g/L) were used for experiments which prepared by dissolving appropriate amount of synthetic seawater salt (Delta Marine®, Iran) in deionized water followed by continues aeration for 24 h. Since the main stocks of microalgae were raised in salinity of 35 g/L, acclimation to higher salinities (70 and 140 g/L) was done when needed. Briefly, for acclimation to 70 g/L, microalgae were transferred from 35 to 70 g/L and were harvested in the exponential growth phase and were again cultivated at salinity of 70 g/L; this was done twice and the third generation was used for toxicology tests in 70 g/L. As the same way, for acclimation to 140 g/L, acclimated microalgae with 70 g/L were transferred to 140 g/L and after two generations were used for toxicology tests in 140 g/L.

### 2.3. Microalgae toxicity test

The acute (72 h) toxicity tests were generally conducted in accordance with standard OECD guideline number 201, however, since the species studied in here was a saline water microalgae, necessary changes in terms of temperature, salinity and culture medium were considered. Microalga cells in the exponential growth phase were used for all the experiments. After conducting a series of pre-tests (data not shown), concentrations of 0 (control), 0.1, 0.2, 0.5 and 1 mg/L of AgNPs and concentrations of 0 (control), 0.025, 0.05, 0.1, 0.25, 0.5 and 1 mg/L of $Ag^+$ ions (prepared from $AgNO_3$) were selected for conducting acute toxicity tests at three water salinities of 35, 70 and 140 g/L in triplicate. In all acute tests, aliquots of 1000 ml of the algal culture (initial biomass ≈25 cells/ml) in growing media were exposed to tested concentrations and incubated in the condition as described above for 72 h. The aliquots of 100 µl of algal cells were sampled every 24 h in triplicate to determine the algal biomass based on cell counting using a hemocytometer under a microscope.

The average specific growth rate was determined as the biomass increase after 72 h using following equation (OECD, 2011); where $\mu_{i-j}$ is the average specific growth rate from time $i$ to time $j$, $t_i$ is the initial time of exposure period, $t_j$ is the final time of exposure, $C_i$ is the biomass at time $i$, and $C_j$ is the biomass at time $j$.

$$\mu_{i-j} = \frac{(\mathbf{lnCj} - \mathbf{lnCi})}{\mathbf{tj} - \mathbf{ti}}$$

The percent inhibition of growth was also calculated using following equation (OECD, 2011); where % $I_r$ is the percentage inhibition of the average specific growth rate, $\mu_c$ is the mean value of the average specific growth rate in the control group, and $\mu_t$ is the average specific growth rate after treatment.

$$\%I_r = \left\{\frac{(\mu c - \mu t)}{\mu c}\right\} \times 100$$

The percent inhibition of yield ($I_y$) was obtained using following equation (OECD, 2011); where $y_C$ is mean value for yield in control group, $y_T$ is mean value for yield in treatment group.

$$\%I_y = \left\{\frac{(yc - yt)}{yc}\right\} \times 100$$

The inhibitory concentrations (ICs) including the median inhibitory concentration ($IC_{50}$) also were calculated based on the average specific growth rate and percentage inhibition of average specific growth rate using EPA Probit analysis program (Version 1.5).

### 2.4. Dissolution of AgNPs in seawater medium

In order to evaluate the extent of dissolution of AgNPs to $Ag^+$ ions in different salinities, microalgae culture media containing 1 mg/L of AgNPs and Walne's medium prepared in sterilized artificial seawater (35, 70 and 140 g/L) and kept under aeration and light illumination for 72 h. Then, 1.5 ml of each suspension was sampled and the percentage of $Ag^+$ ions in AgNPs suspension were determined based on the procedure described in section 2.1.

### 2.5. Statistical analysis

All data of this study were presented as mean ± SD. The statistical analyses were performed using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). All percentage data was transformed using the arcsine method. Normality assumptions of data were determined using the Kolmogrov-Smirnov test. Differences between the means were analyzed using One-Way analysis of variance (ANOVA). The Duncan test was also used to compare significant differences among the means. The Independent-Sample T test was employed to compare the differences between two independent samples. Statistical significance was accepted at the level of $P < 0.05$.

## 3. Results

The algal biomass exposed to AgNPs increased about 19.90, 9.75 and 9.00 folds after 72 h in control groups (0 mg/L AgNPs or $Ag^+$ ions) at salinities of 35, 70 and 140 g/L respectively. The incremental trends in biomass of algae exposed to different concentrations of AgNPs were slower than control groups during 72 h at different salinities (Fig. 1a–c). After 72 h of exposure, the algal biomass inversely decreased coincident with increasing the concentration of AgNPs at salinity of 35 and 70 g/L ($P < 0.05$), but this trend of decreasing was not observed at salinity of 140 g/L (Fig. 2). The highest toxicity effect on algal biomass was recorded at 1 mg/L of AgNPs at different salinities ($P < 0.05$).

The biomass values of algae exposed to different $Ag^+$ concentrations were ascending during 72 h at all salinities. This trend was slower coincident with increasing the concentration of Ag at different salinities. At salinity of 35 g/L, the growth of algae was inhibited at 1 mg/L Ag (Fig. 3 a–c). After 72 h of exposure, the algal

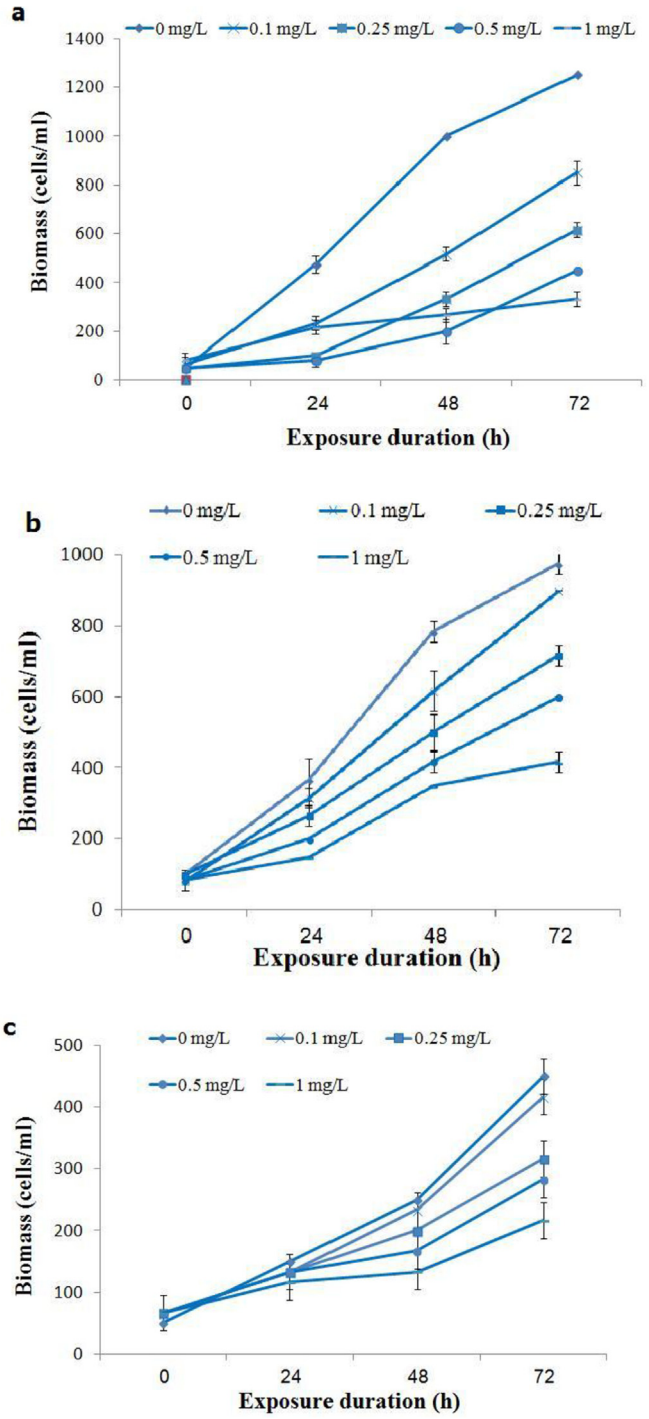


**Fig. 1.** Algal growth curves of *Dunaliella salina* exposed to different concentrations of AgNPs in different salinities (35 (a), 70 (b) and 140 g/L (c)) at different time intervals (0, 24, 48 and 72 h). Values are mean ± SD.

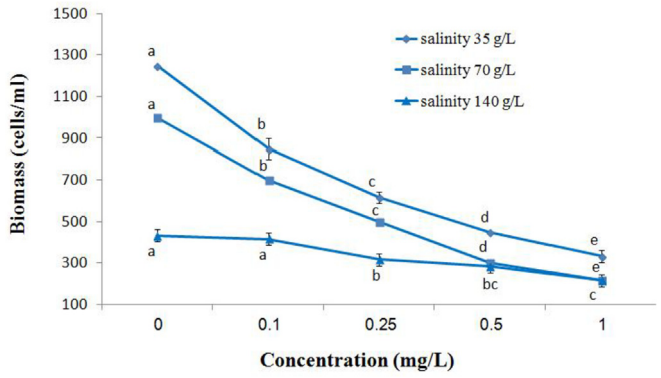


**Fig. 2.** Comparison between algal growth rates of *Dunaliella salina* after 72 h exposure to different concentrations of AgNPs in different salinities (35, 70 and 140 g/L). Values with different letters in each line are significantly different (mean ± SD, $P < 0.05$).

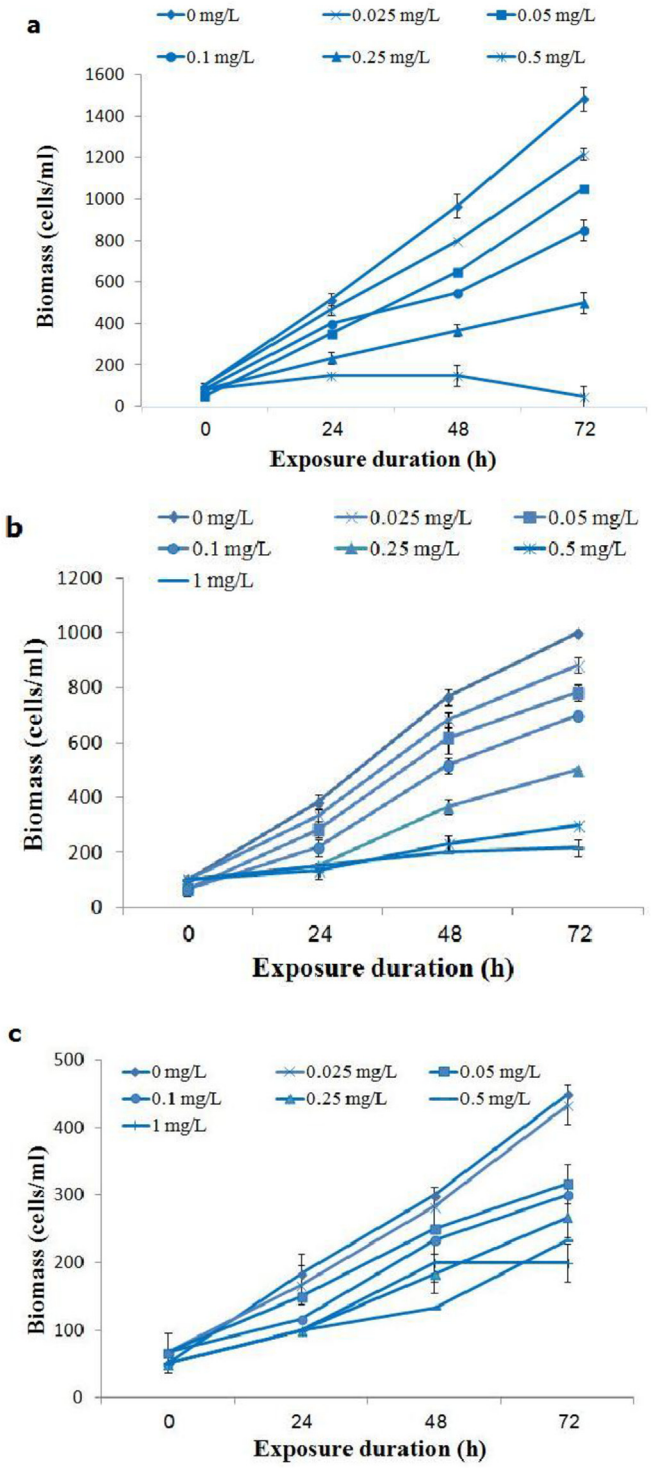


**Fig. 3.** Algal growth curves of *Dunaliella salina* exposed to different concentrations of $Ag^+$ ions (derived from $AgNO_3$) in different salinities (35 (a), 70 (b) and 140 g/L (c)) at different time intervals (0, 24, 48 and 72 h). Values are mean ± SD.

biomass inversely decreased with increasing the Ag concentration at salinity of 35 and 70 g/L ($P < 0.05$) (Fig. 4). At salinity of 140 g/L, the algal biomass did not decrease significantly with increasing concentrations from 0 (control) to 0.025, from 0.05 to 0.25 and from 0.5 to 1 mg/L Ag after 72 h of exposure ($P > 0.05$) (Fig. 3).

The values of inhibition of average specific growth ranged between 19.1 and 55.5%, 0.044–26.7% and 12.3–43.14%, the inhibition

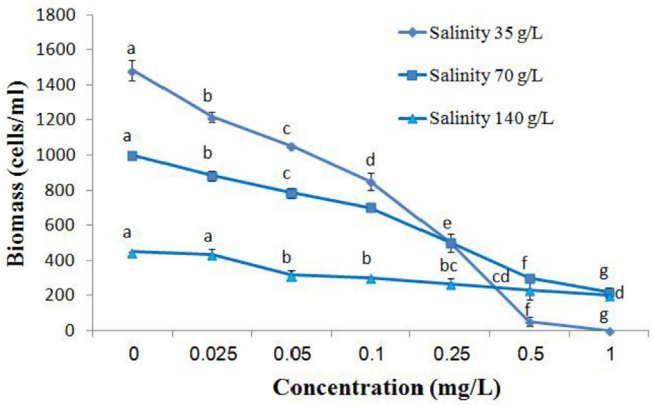


**Fig. 4.** Comparison between algal growth rates of *Dunaliella salina* after 72 h exposure to different concentrations of $Ag^+$ ions (derived from $AgNO_3$) in different salinities (35, 70 and 140 g/L). Values with different letters in each line are significantly different (mean ± SD, $P < 0.05$).

rates of yield also were found to be 36.5–79.1%, 6.66–69.9%, and 8.7–60.8% in algae exposed to 0.1–1 mg/L of AgNPs at salinity of 35, 70 and 140 g/L, respectively. The values of average specific growth and yield of algae significantly increased with increasing of AgNPs concentrations at different salinities ($P < 0.05$). The highest inhibitory rates was measured at concentration of 1 mg/L of AgNPs ($P < 0.05$) (Fig. 5 a–c).

The inhibition rates of 7.34–100%, 3.2–67.7% and 12.4–37% for average specific growth and 19.3–100%, 12.9–87% and 8.3–62.5% for yield of algae exposed to 0.025–1 mg/L of ionic silver were measured at salinity of 35, 70 and 140 g/L, respectively (Fig. 6). Significant increase in inhibition rates was observed with increasing exposure concentration of $Ag^+$ ions from 0 to 1 mg/L at all salinities ($P < 0.05$).

The highest values of $IC_{10}$ and $IC_{50}$ based on average specific growth rate and yield for *D. salina* was calculated for AgNPs at salinity of 140 g/L ($P < 0.05$). The $IC_{10}$ and $IC_{50}$ values were similar in $Ag^+$ ions in 35 and 70 g/L as well as in AgNPs in 35 g/L as highest toxicity for this algae ($P > 0.05$) (Table 1). The values of $IC_{50}$ of $Ag^+$ ions based on average specific growth rate and yield were significantly lower than AgNPs at all salinities ($P < 0.05$). The $IC_{10}$ of $Ag^+$ ions also decreased significantly compared to AgNPs at salinity of 70 and 140 g/L ($P < 0.05$) (Table 2).

The percentage of $Ag^+$ ions in the AgNPs suspension at salinities of 35, 70 and 140 g/L were 11.98 ± 1.13%, 15.6 ± 0.8% and 20.04 ± 1.85%, respectively.

## 4. Discussion

The purpose of this study was to investigate the potential toxicity of both silver nanoparticles and silver ions on marine water microalgae *Dunaliella salina* and influence of different salinities on observed toxicity. The results indicate a time-dependent effect of both AgNPs and $Ag^+$ ions towards microalgae at all salinities. In this regards, the differences between growths of algae in exposed groups increased compared to control groups during 72 h of exposure period. Biomass of algae exposed to 0.1–1 mg/L of AgNPs for 72 h decreased approximately 1.4–3.7, 1.4–4.6 and 0–2 times compared to control group at salinities of 35, 70 and 140 g/L, respectively. The reduction of about 1.2–100, 1.2–4.6 and 0–1.71 times also was recorded in algal biomass exposed to 0.025–1 mg/L of $Ag^+$ ions. Different studies have shown the potential toxicity effects of AgNPs (Gambardella et al., 2015; Miao et al., 2009; Navarro et al., 2008) and silver ions (Lee et al., 2005) on various

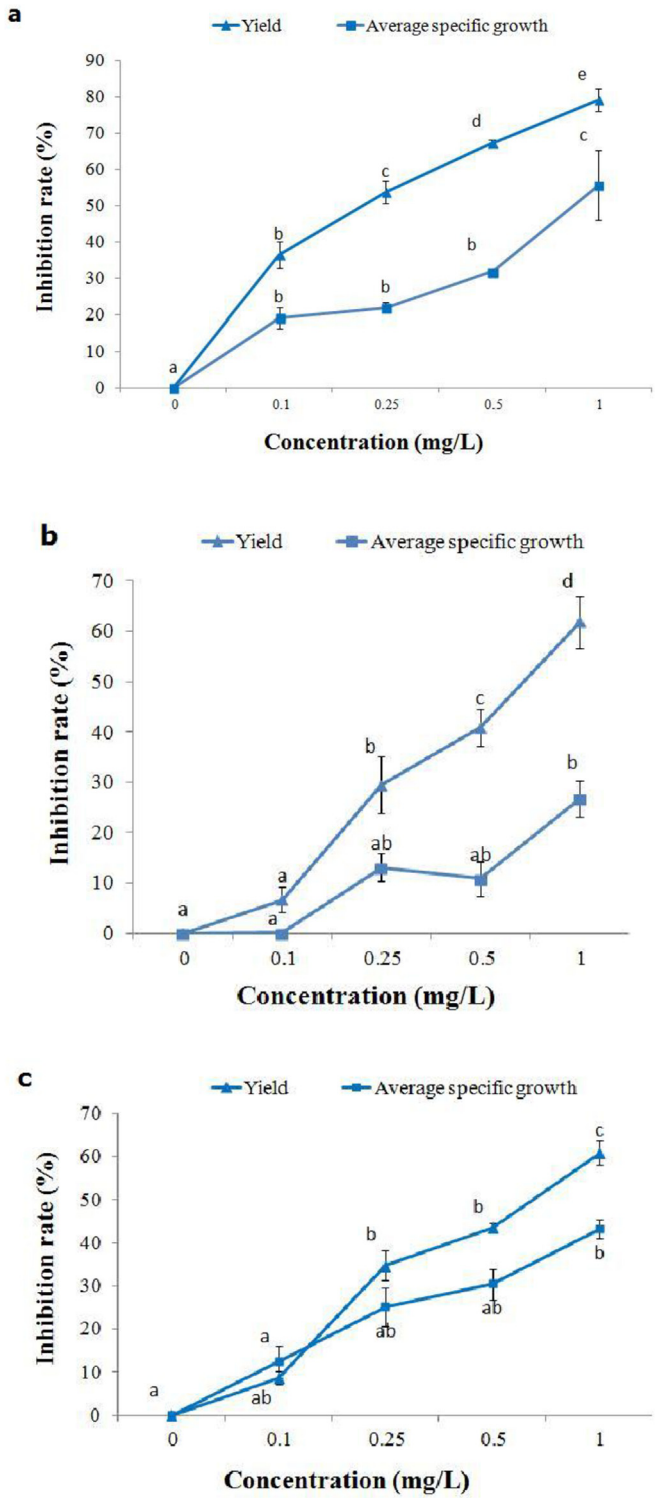


**Fig. 5.** The percent inhibition of growth rate and yield of *Dunaliella salina* after 72 h exposure to different concentrations of AgNPs in different salinity (35 (a), 70 (b) and 140 g/L (c)). Values with different letters in each line are significantly different (mean ± SD, $P < 0.05$).

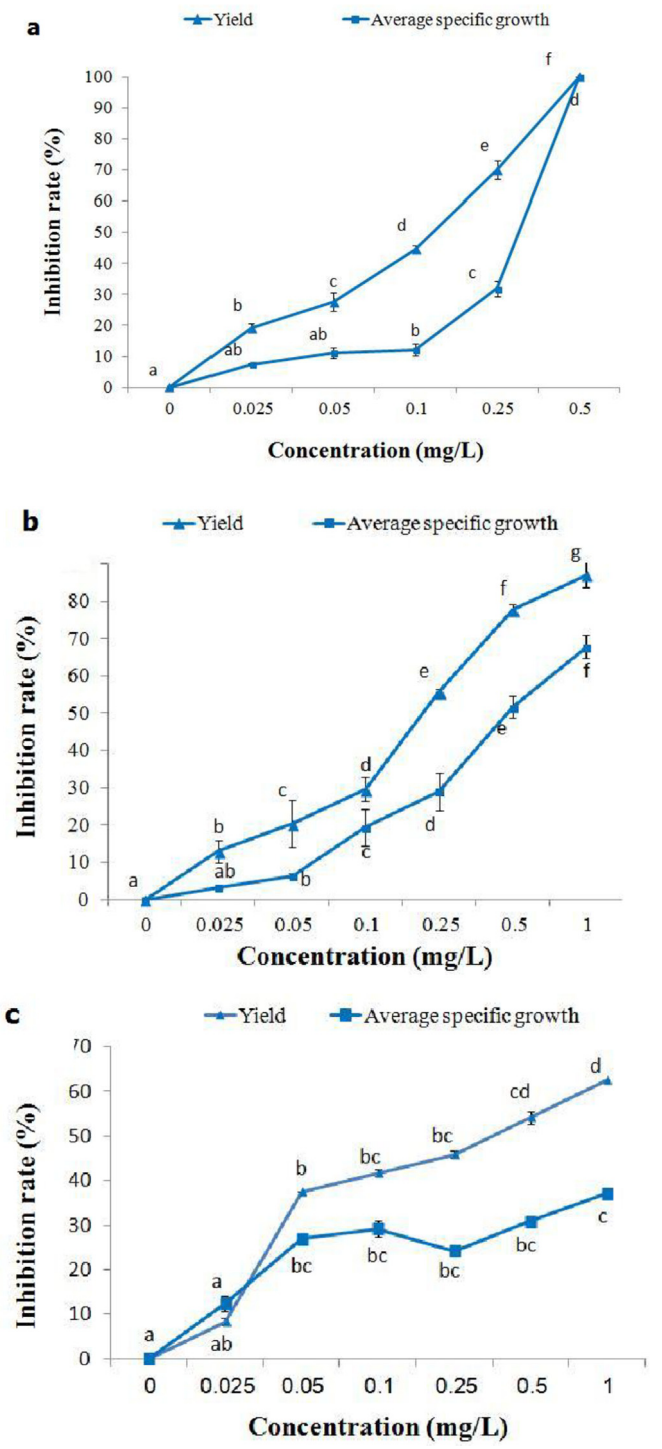


**Fig. 6.** The percent inhibition of growth rate and yield of *Dunaliella salina* following 72 h exposure to different concentrations of $Ag^+$ ions (derived from $AgNO_3$) in different salinity (35 (a), 70 (b) and 140 g/L (c)) (mean ± SD, $P < 0.05$). Values with different letters in each line are significantly different (mean ± SD, $P < 0.05$).

microalgae. Toxicity of AgNPs to microalgae is mainly related to their direct interaction with the algal cells' surface and formation of cell aggregates (Oukarroum et al., 2012), the release of $Ag^+$ (Miao et al., 2009; Navarro et al., 2008), reactive oxygen species (ROS) formation and lipids peroxidation injury (Oukarroum et al., 2012).

Algal cells' aggregation due to exposure to nanoparticles might reduce its accessibility to light and thereby inhibit algal growth and reduce the absorption of essential nutrients from the environment (Perreault et al., 2012; Wei et al., 2010). Such effect was reported in freshwater microalga *Chlorellavulgaris* and marine microalga *Dunaliella tertiolecta* exposed to 0–10 mg/L of 50 nm AgNPs for 24 h (Oukarroum et al., 2012). The results of this study showed a

**Table 1**
Inhibitory concentrations ($IC_{10}$ and $IC_{50}$) of AgNPs and $Ag^+$ ions (derived from $AgNO_3$) based on average specific growth rate and yield for *Dunaliella salina* after 72 h exposure in different salinities (mean + SD).

| mg/L | | Salinity (g/L) | | | | | |
|---|---|---|---|---|---|---|---|
| | | AgNPs | | | $Ag^+$ ions | | |
| | | 35 | 70 | 140 | 35 | 70 | 140 |
| $IC_{50}$ | Average specific growth rate | $0.480 \pm 0.022^a$ | $1.231 \pm 0.110^b$ | $2.922 \pm 0.197^d$ | $0.218 \pm 0.23^a$ | $0.489 \pm 0.097^a$ | $2.154 \pm 0.147^c$ |
| | Yield | $0.206 \pm 0.021^a$ | $0.554 \pm 0.105^c$ | $0.759 \pm 0.111^d$ | $0.092 \pm 0.010^a$ | $0.184 \pm 0.018^a$ | $0.332 \pm 0.033^b$ |
| $IC_{10}$ | Average specific growth rate | $0.059 \pm 0.020^a$ | $0.35 \pm 0.076^b$ | $0.81 \pm 0.440^c$ | $0.049 \pm 0.018^a$ | $0.064 \pm 0.019^a$ | $0.30 \pm 0.095^b$ |
| | Yield | $0.031 \pm 0.016^a$ | $0.134 \pm 0.017^b$ | $0.197 \pm 0.060^c$ | $0.020 \pm 0.006^a$ | $0.025 \pm 0.008^a$ | $0.060 \pm 0.009^a$ |

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

**Table 2**
Comparison of $IC_{10}$ and $IC_{50}$ between AgNPs and $Ag^+$ ions (derived from $AgNO_3$) based on average specific growth rate (a) and yield (b) for *Dunaliella salina* after 72 h exposure in different salinities (mean + SD).

| mg/L | | Average specific growth rate | | | Yield | | |
|---|---|---|---|---|---|---|---|
| | | Salinity (g/L) | | | | | |
| | | 35 | 70 | 140 | 35 | 70 | 140 |
| $IC_{10}$ | AgNPs | $0.059 \pm 0.020^a$ | $0.35 \pm 0.076^a$ | $0.81 \pm 0.44^a$ | $0.031 \pm 0.016^a$ | $0.134 \pm 0.017^a$ | $0.197 \pm 0.060^a$ |
| | $Ag^+$ ions | $0.049 \pm 0.018^a$ | $0.064 \pm 0.019^b$ | $0.3 \pm 0.095^b$ | $0.020 \pm 0.006^b$ | $0.025 \pm 0.008^b$ | $0.060 \pm 0.009^b$ |
| $IC_{50}$ | AgNPs | $0.48 \pm 0.022^a$ | $1.23 \pm 0.110^a$ | $2.92 \pm 0.197^a$ | $0.206 \pm 0.021^a$ | $0.554 \pm 0.105^a$ | $0.759 \pm 0.111^a$ |
| | $Ag^+$ ions | $0.218 \pm 0.23^b$ | $0.48 \pm 0.097^b$ | $2.15 \pm 0.147^b$ | $0.09 \pm 0.010^b$ | $0.184 \pm 0.018^b$ | $0.332 \pm 0.033^b$ |

In each column, AgNPs and $Ag^+$ ions are compared in each salinity and means with different letters are significantly different (ANOVA, $P < 0.05$).

concentration-dependent of toxicity effect of both AgNPs and $Ag^+$ ions on growth of algae at all salinities. The growth inhibitory effects of AgNPs and $Ag^+$ ions based on average specific growth rate and yield increased significantly with increasing exposure concentrations. Similarly, the results of a study revealed a dose-dependent effect of AgNPs towards green algae *Dunaliella tertiolecta* and diatom *Skeletonema costatum* (Gambardella et al., 2015). He et al. (2012) also observed the increasing in toxicity of AgNPs and Ag (I) on marine raphidophyte *Chattonella marina* as total silver concentration increases.

Our study demonstrated that toxicity of AgNPs and $Ag^+$ ions toward *D. salina* depend on salinity of medium, increasing the $IC_{50}$ of AgNPs and $Ag^+$ ions based on average specific growth rate and yield in a concentration-dependent manner. The test of dissolution of AgNPs to $Ag^+$ ions in seawater medium revealed an increase in the values of $Ag^+$ ions in seawater from $11.98 \pm 1.13\%$, to $20.04 \pm 1.85\%$, with increasing salinity from 35 g/L to 140 g/L. These finding suggest that the reduction of silver toxicity toward *D. salina* with increasing salinity may depend on its bioavailability. The toxicity of silver to aquatic organism is more influenced by the bioavailable fractions in water rather than the concentration of total metal (Ward and Kramer, 2002). It is known that the toxicity of $Ag^+$ ions strongly depends on the ionic strength and amount of the ligands such as chloride and thiosulfate present in the medium (Ratte, 1999; Lee et al., 2005). In seawater, the ligands that complex with $Ag^+$ ions with negligible organic matter are $Cl^-$, $Br^-$ and $I^-$. The formation of AgCl species is more predominant than other species, such as bound to $Br^-$and $I^-$ (Ward and Kramer, 2002). Insoluble AgCl complex will be formed by reaction of $Cl^-$ with silver ions (Li et al., 2010), but in higher salinities, excessive $Cl^-$ could react with the AgCl precipitate and form the soluble forms of $AgCl^{m-1}{}_m$ complexes (Zhong, 2013).

Recently, light irradiation was found to have important roles in toxicity of AgNPs and dissolution of AgNPs to $Ag^+$ ions (Grillet et al., 2013; Shi et al., 2013). Morphology change, aggregation, and further sedimentation of polyvinylpyrrolidone (PVP) coated AgNPs under 3 h sunlight irradiation was observed by Yin et al. (2015) in typical environmental water samples with different ionic strengths, hardness, and dissolved organic matter (DOM) concentrations. Agglomeration of NPs influence on toxicological outcome by altering surface area, charge and sizes parameters compared with the as-synthesized particle (Truong et al., 2012). Aggregation of nanoparticles in seawater is more likely than in freshwater (Handy et al., 2008). The formation of large agglomerates (>400 nm) of AgNPs in the seawater growth medium used for cultivation of marine microalga *D. tertiolecta* was reported by Oukarroum et al. (2012). Therefore, nanoparticles aggregation in seawater medium may have participated in the toxicological effects of AgNPs on *D. salina*. However, further study is needed to elucidate the effects of other physicochemical parameters as well as light irradiation on toxicity of AgNPs to *D. salina*.

Based on the $IC_{50}$ values of AgNPs and $Ag^+$ ion, the toxicity of $Ag^+$ ions was significantly higher than AgNPs at different salinities. The $IC_{10}$ values also revealed the higher toxicity of $Ag^+$ ions at salinity of 70 and 140 g/L (Table 2). It was found that soluble $Ag^+$ ions at salinity of 35 g/L had higher toxicity to marine raphidophyte *C. marina* than AgNPs and suggested that this effect most likely associated with rapid intracellular accumulation of dissolved silver ions (He et al., 2012).

## 5. Conclusion

While AgNPs and $Ag^+$ ions were found to be toxic to marine microalgae *D. salina,* their toxicity was dependent on time and concentration of exposure and salinity of media. The growth inhibitory effects of AgNPs and $Ag^+$ ions increased significantly coincidence with increasing time and concentration compared to control ($P < 0.05$). The results revealed the reduction of the $IC_{50}$ of AgNPs and $Ag^+$ ions based on average specific growth rate and yield with decreasing in salinity in a concentration-dependent manner. *D. salina,* were found to be more sensitive to $Ag^+$ ions as compared to AgNPs. Study on ecotoxicological effects of ENPs on microalgae is very important because of their roles in primary production of marine ecosystems and transfer of metals along the marine food chain. Therefore, the potential of *D. salina* as marine microalgae, to transfer AgNPs to aquatic food chain should be consider in future

studies.

## Conflicts of interest

The authors have no conflict of interest to declare.

## Acknowledgment

This research was undertaken with supported of University of Kurdistan (UOK, Iran) under the research grant No. 4.1367/1395-12-15.

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