Nanomed Res J 4(3):164-175, Summer 2019

RESEARCH ARTICLE

Effect of SiO_2 Nanoparticles on Chlorophyll, Carotenoid and Growth of Green Micro-Algae *Dunaliella salina*

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ARTICLE INFO

Article History:
Received 23 May 2019
Accepted 18 June 2019
Published 15 September 2019

Keywords:
Algae Dunaliella Salina
Carotenoid
Chlorophyll
Nanomaterials
Nano Toxicity
Pigment

ABSTRACT

As a rapidly-evolving global technology, nanotechnology has presumably brought drastic changes to our lives in the past two decades using engineered nanoparticles, whose penetration into industrial and non-industrial wastewater requires examination of their probable effects in aquatic ecosystems. The main aim of this work is to determine the toxicological and biological effects of nanomaterials. Experiments on exposure of *Dunaliella salina* to SiO_2 nanoparticles were performed for 72 hours with 7 treatments, two controls and three replicates were in each treatment and daily counting of cells was done in each tube. Chlorophyll a and carotenoid were determined through spectrophotometry method after extraction. Imaging of nanoparticles encountering algae cells was performed using cell imaging method by scanning electron microscope (SEM). The population growth rate alterations were evaluated. Probit analysis and softwares such as Excel and SPSS21 were used for data analysis. After exposure to SiO_2 NPs, a significant difference was observed between chlorophyll a and carotenoid compared with control ($p<0.05$) and also carotenoid content was decreased with increasing the concentration in treatments and a significant difference was observed ($P<0.05$). Also, SiO_2 NPs caused to inhibit growth in Dunaliella species.

How to cite this article
Shariati F, Ayatallahzadeh Shirazi M. Effect of SiO_2 Nanoparticles on Chlorophyll, Carotenoid and Growth of Green Micro-Algae Dunaliella salina. Nanomed Res J, 2019; 4(3): 164-175. DOI: 10.22034/nmrj.2019.03.005

INTRODUCTION

Nanoparticles are extensively used due to their physicochemical, magnetic, optical (1) electrical, thermal resistance, radiation and mechanical properties that can enhance its performance (2). Various industrial applications are such as textiles, laser imaging system and biosensors (3, 4). Production, rapid growth, and widespread application of nanoparticles (NPs) has resulted in their direct and indirect release in the environment leading to abundant environmental hazards.

They can also be a threat to the health of the aquatic environment, associated with potential consequences, especially for aquatic organisms, plants, and algae (5-7).

Biflagellate unicellular green algae, *Dunaliella Salina* grows in salty water (8). They are wall-less flagellate (9). The presence of chlorophyll a for photosynthesis, β-carotene antioxidant, and precursors of vitamin A in this algae have made it commercially very advantageous (10, 11). Currently, it is considered as the richest natural source of carotenoids (12) and one of the most important algae in terms of aquaculture and biodiesel fuel production (12, 13). According to statistics, 10000 metric tons/year the maximum annual amount of silica nanomaterials waste is produced in the world. Also, 2100 tons waste from these nanomaterials is released into the water (14).

NPs physicochemical properties such as particle size, surface area, and dissolution rate are important determinants of behavioral response of aquatic organisms (15, 16). NPs are absorbed and accumulated in algae (17, 18). They can also result

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in cell compression and cell membrane damage (17, 19), leading to less light absorption by algae and hence their poor growth (20). Their short and long term reactions to NPs vary depending on various factors (21, 22).

In the nature, NPs are used due to their different applications (23). Extensive use of various NPs has resulted to their deliberate or accidental release to the environment (24, 25). As one of the most widely used particles, Silica nanoparticles)SiO_2(are increasingly applied in reforming cement mortar as a surface protection matter. (26).

It is used in industries such as ceramics, cosmetic, rubber, glass, cosmetic, pharmaceutical and paper (27, 28). Spread of NPs in different ecosystems, especially water, has resulted in huge damages to aquatic organisms living in the water and has become one of the biggest environmental problems (29, 30). NPs can ultimately cause damage, inflammation, and weaken human body, and be absorbed through the skin, lungs, and digestive system (25). Therefore, it is better to examine their impact on all valuable species in aquatic ecosystems before their use (24). Algae are at the top of energy pyramid and are producers of food chain. (18, 31). Very few investigations have been conducted about the toxic effect of SiO_2 NPs on aquatic species. But, there are many reports about the toxicity of SiO_2 NPs using human, mammals, and fish models. For example, in a previous work (32), the researchers studied the toxicity of Al_2O_3 NPs on *Dunaliella* algae and showed their inhibitory effect on the algae growth, and the decrease in chlorophyll content with increasing levels of Al_2O_3 NPs.

Researchers have revealed that SiO2 NPs can inhibit algae growth (33). In a study conducted, after collision of *Chlorella kessleri* with SiO_2 NPs, the chlorophyll content decreased in comparison with the control (34). Some works showed that 1000 $mg.L^{-1}$ SiO_2 NPs stopped algae *Chlorella sp* growth on the second day by 20% (31). Other researchers also found that after exposure of *Scenedesmus obliquus* to SiO_2 nanoparticles, chlorophyll function index and its photosynthetic pigments significantly decreased (35).

Hazeem et al. (36) studied the negative graphene oxide (GO) effect on chlorophyll and photosynthetic pigment content of *Picochlorum* Sp, *Chlorophyta*. Several studies also showed the adverse effects of NPs on plants. For example, in the study of Lee et al. (37), longevity of metal oxide nanoparticles to *Arabidopsis thaliana* roots decreased significantly at different concentrations in comparison with the control. Furthermore, some other studied the effect on Elodea plant and found that chlorophyll fluorescence (ChlF) decreased more than the control at 685 and 720-740 nm, resulting subsequently in reduced photosynthesis (38). Vo et al. (39) found that effect of SiO_2 NPs on rainbow trout (fish) NPs at a smaller size, higher concentrations, and longer exposure to all sizes resulted in severe cellular changes and decreased cell longevity. Morphologically, there were significant changes in stress-induced *in situ* cell vibration, cellular contraction, and nuclear condensation in the first 12 hours of exposure. Adams et al. (40) reported the decreasing effect of SiO_2 NPs on bacterial growth. In addition, Napierska et al. (41) studied the effect of SiO_2 NPs on human and revealed exposure to at least 0.5-10 µm can affect DNA, the cardiovascular system, and the respiratory system in human, and damage the lungs. The sensitivity of *Dunaliella* algae to metals was also studied. Shariati and Yahyaabadi (42) showed the effect of heavy metals on this algae.

Thus, evaluation of the specific growth rate and growth inhibition percent of algae in dealing with NPs are practical ways to realize the damage level, which is the most important factor to assess changes in the environmental conditions. Measuring the amount of chlorophyll and carotenoid in order to analyze their compatibility and behavioral response are among affecting factors, and chlorophyll a is an important indicator of phytoplankton biomass (43). So, since the effects of NP SiO_2 on the micro-marine algae *Dunaliella salina* and its biological toxicity have not yet been investigated, in this research, we studied the effect of different concentrations of this NP on cell number and algae pigments to know whether it has inhibition effects on growth, pigments and other parameters or not.

MATERIALS AND METHODS

Preparation of the main test treatments

Some steps of range finding tests had been performed in triplicate with 7 treatments and 2 control samples to determine the ultimate concentrations of the experiment. Consequently, the specified logarithmic concentrations were 0, 0.1, 0.3, 0.85, 2.4, 7, 20, and 50 mg/L^{-1}. The exposure method was based on OECD 201 (Organization for Economic Cooperation and Development) to determine the algae growth inhibition (44).

According to the calculations, the prepared concentrations of NPs solutions were added to

culture medium in test tubes to reach a volume of 10 mL. Then, 5×10^3 cells from the original stock of *Dunaliella salina* were added to 10 mL of each treatments and controls (45).

Afterwards experimental tubes were placed at 25 ± 1 °C and exposed to 12 hours darkness and 12 hours light, alternatively. A thermostat and an electric chronometer (TS-MD20) were used for the temperature and lighting conditions regulation, respectively. During the test period (i.e. 72 hours), these conditions were kept stable. The solutions in experimental tubes were sampled at 24, 48, and 72 hours with Pasteur pipette and the enumeration was performed using Thoma slides under an optical microscope (Japan, Microphot-fxt, Nikon) with lens 40. The average number of cells in the up and down squares was calculated after counting and recording data and cells quantity was obtained as follows (equation 1).

$$\text{Cell density in ml} = \text{Total cells counted in the large square} \times 10^4 \quad (1)$$

To examine the significance of differences among treatments at different concentrations of algae cells and control samples, one way ANOVA test was used. To determine differences between each level of treatments the Tukey's test was applied.

Growth inhibition in the algae Dunaliella Salina

The amount of μ (growth rate per hour) are expressed in d^{-1}, h^{-1}, or min^{-1}. G (doubling time per hour) and I (percent inhibition) were calculated from the following equations (46) (Equations 2, 3, and 4).

$$\mu = \ln x1 - \ln x0\ (t1-t0)-1 \quad (2)$$

$$G = \ln 2\mu - 1 \quad (3)$$

$$I\% = (\mu c - \mu t)/\mu c \quad (4)$$

Observation of cell shape under scanning electron microscopy (SEM)

To study the effect of NPs on the shape and size of cells in microscopic tissues of *Dunaliella salina* and to take image of the surfaces, scanning electron microscopy (SEM, LEO 1430VP, Germany) was used. The control and treatments surfaces were imaged at the concentration of 0.2 $mg.L^{-1}$ which had affected 50% of the cells with magnification of 3-10 micron.

Measurement of chlorophyll and carotenoid

Chlorophyll a was determined to investigate the effect of NPs on the chlorophyll concentration in *Dunaliella salina* (47). The reason for measuring this chlorophyll is that the main pigment in photosynthesis is in all algae and plants. For determination the range of concentrations of the tests, some range finding stages were conducted as pre-test on nanoparticles to determine the range of toxicity. Finally, three treatments at concentrations 0.017, 0.034, and 0.170 mg. L^{-1} in three replications for each sample and two controls (zero) were selected, was prepared in 50 ml flasks and sampled during the determined time.

The chlorophyll determination method (47) was used to study the NPs effect on the concentration of chlorophyll in *Dunaliella* algae. To extract chlorophyll and β-carotene, some centrifuge tubes were prepared and 4 mL algae suspension was poured into each one. Then they were shaked in a vortex and centrifuged with a microprocessor centrifuge (co-w300, Para-Azma, Iran) for 10 min at 5500 rpm in order to separate the culture medium from algae solution. Centrifugation was repeated for 10 min once again. Then the supernatant was isolated that contained the pigments and 4 mL 90% acetone was added to the extract yielded from algae precipitation. The precipitate was moved into a falcon and was frozen.

The absorbance of the resultant solution (almost green-colored) was read at 630, 647, and 664 nm using a spectrophotometer (Apada, UV-6300Pc). The content of chlorophyll a was calculated in μg/mL. (Equations 5)

$$Ca = 11.85\ A_{664} - 1.54\ A_{647} - 0.08\ A_{630} \quad (5)$$

To determine the concentration of algae carotenoid, the absorbance reading was performed at 470 nm (Equations 6 and 7). One-way ANOVA test was used to determine the effect of each SiO_2 NPs treatment. In all calculations the significance level was considered 95%. If there was a significant difference between treatments, Tukey's test was used. All experiments for each treatment were conducted in three replicates and statistical calculations were done by SPSS-21.

$$Caa = Ca \times Vaceton/Vwater \times 1000\ \mu g/mL \quad (6)$$

$$Cc = 10 \times A\ (480) \times V_{aceton}/V_{water} \times 1000\ \mu g/mL \quad (7)$$

RESULTS AND DISCUSSION

Experimental data of cell count (Cell.mL^{-1}) in Dunaliella salina

Effect of concentration on Dunaliella salina cell number

According to Table 1, we concluded that increased concentration led in reduced cell density. The highest cell density was observed in the controls.

Specific growth rate of Dunaliella salina after exposure to SiO_2 NPs

Regarding the specific growth rate (μ) of *Dunaliella salina*, based on one-way ANOVA and Tukey tests, the statistical value of Fisher was 6.476 (Sig<0.05), and the toxicity effect of SiO_2 NPs on growth rates was observed following increasing concentrations of NPs (p<0.05). After 48 and 72 hours, there was no significant difference (p>0.05), but the number of cells was decreased after 72 hours compared with 24 and 48 hours, except for the concentrations 0.85 and 2.4 mg.L^{-1} at 48 and 72 hours. In the controls, the trend was always increasing (Fig. 1).

Doubling time of Dunaliella salina cells after exposure to SiO_2 NPs

The parameter G in the studied treatments showed that the doubling rate was increased (with increase in concentration), except for the concentrations 0.85 and 2.4 mg .L^{-1} at 48 and 72 hours. The statistical value of Fisher was 12.033 and Sig<0.05 according to one-way ANOVA and Tukey's test. With increasing concentration of nanoparticles during a specified period, an increase in cell doubling time and a significant difference was observed (p<0.05). This trend was always negative in the controls (p<0.05) (Fig. 2).

Table 1. Effect of SiO_2 NPs concentration on Dunaliella salina cells

Concentration (mg L^{-1})	control	0.1	0.3	0.85	2.4	7	20	50
Cell number (×10^{4}mL^{-1})	1.81±0.44	0.96±0.05	0.76±0.03	0.99±0.26	0.77±0.14	0.62±0.01	0.61±0.01	0.61±0.01

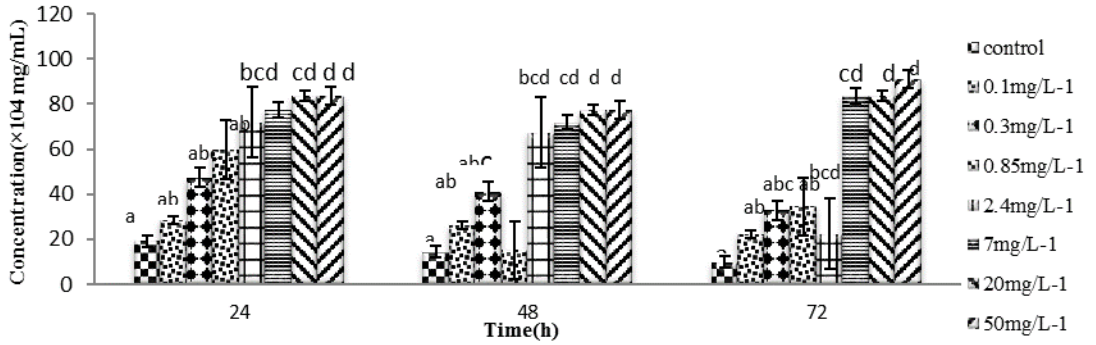

Fig. 1. Specific growth rate of Dunaliella salina after exposure to SiO_2 NPs
Notes: Treatments with at least one commonality are statistically significant (p<0.05).

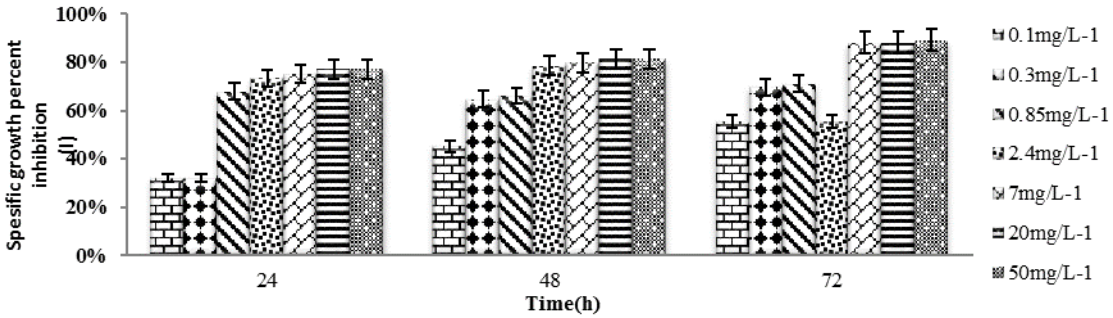

Fig. 2. Doubling time Growth Rate (G) of Dunaliella salina during the Experiment at different concentrations of SiO_2 NPs

Growth percent inhibition of Dunaliella salina after exposure to SiO_2 NPs

With increase in exposure concentration and time, growth percent inhibition (I) of *Dunaliella salina* raised. In the control, this value was always zero (Fig. 3).

Results of chlorophyll measurements

Based on the hypothesis that pigments synthesis may be affected by SiO_2 NPs, this experiment was performed. Since the data were independent and their distribution was normal in both carotenoid and chlorophyll and also, it was intended to compare between different concentrations, ANOVA analysis was applied.

Based on ANOVA statistical analysis and graph, SIG=0.000 is less than 5%, so it shows that chlorophyll concentration has varied at various concentrations of silica NPs and the concentration of SiO_2 NPs had a significant effect on chlorophyll content, so that the concentration of chlorophyll was higher in the control group than other treatments and a significant decrease in comparison with the control was observed ($p<0.05$) (Fig. 4).

Results of carotenoid measurements

According to SIG=0.000, it can be concluded that the carotenoid content of treatments has reduced following increasing concentration of silica and a significant reduction in comparison with the control was observed ($p<0.05$) A significant difference existed between NPs treatments ($p<0.05$) (Fig. 5).

Results of microscopic examination of the effect of SiO_2 NPs on Dunaliella salina

Fig. 6.b shows the collision moment of NPs with algae cell. In Fig. 6.c, cells in contact with SiO_2 NPs were wrinkled and had smaller size compared with the control (Fig 6.a). The cells exposed to NPs had lost their flagella. Also, the cells had local sliding movements (Figs. 6 ; b, c, d).

Scanning electron microscopy (SEM) images of the effect of SiO_2 NPs on Dunaliella salina

According to size, shape, form and number of cells covering the surface of the samples, the toxicity was determined after 72 hours of exposure. The image of NPs free sample (control) shows a

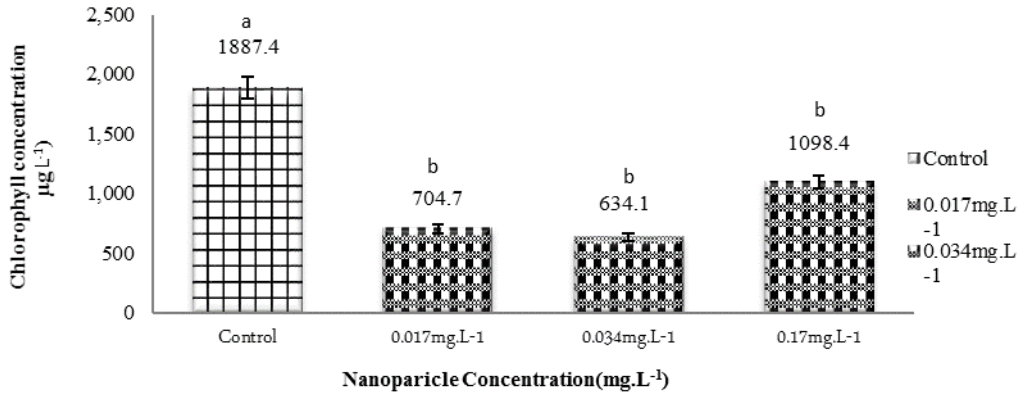

Fig. 3. Growth percent inhibition (I) of Dunaliella salina in contact with SiO_2 NPs
Notes: Treatments with at least one commonality are statistically significant ($p<0.05$).

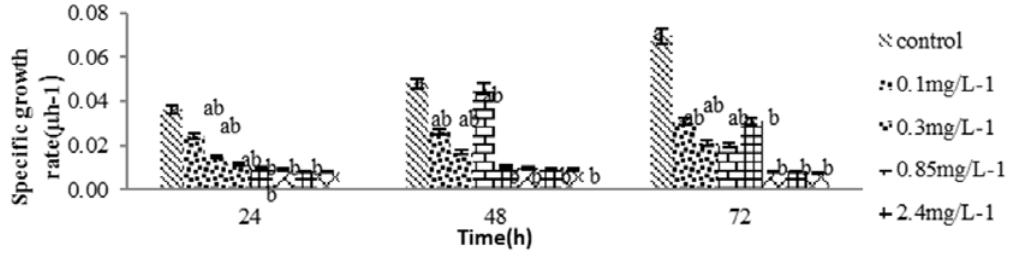

Fig. 4. Mean chlorophyll a level at different concentrations of SiO_2 NPs
Notes: Treatments with at least one commonality are statistically significant ($p<0.05$).

nearly uniform distribution of particles size in all directions with a roughly elliptical shape with normal shape and size (Figs. 7; a, b). Because of nano-sized particles presence, they were agglomerated. Agglomeration (accumulation and adherence) of fine particles with coarse ones results to binding of NPs and their aggregation (Fig. 7-c). Compared with the controls, the shape of algae cell was not significantly changed due to exposure to SiO_2 NPs, only the loss of cell flagella is observed in Fig d (Figs. 7; c, d).

DISCUSSIONS

The study the effect of these nanomaterials on metabolism and physiological indices (48) is an important case. Growth rate, cell division, and algae pigmentation are considered important factors for investigation the tensile response of NPs in plants and especially in microalgae (49).

Control cells had an increasing trend, and the number of cells reached 1.81×10^4 in the controls and 0.61×10^4 in the treated group at the level of 50 mg. L^{-1}. Toxicity of SiO_2 NPs was increased with

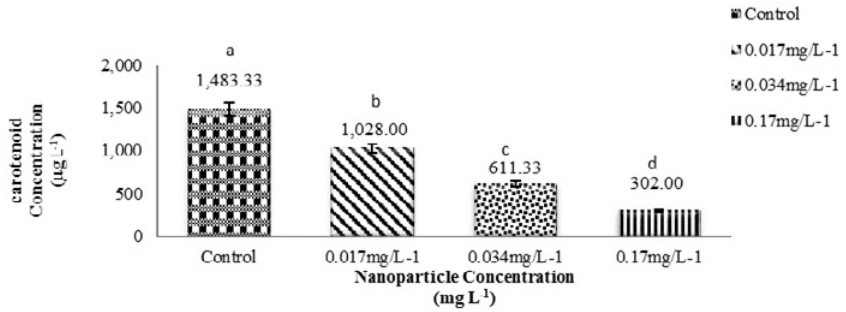

Fig. 5. Mean carotenoid level at different concentrations of SiO_2 NPs.
Notes: Treatments with at least one commonality are statistically significant ($p<0.05$).

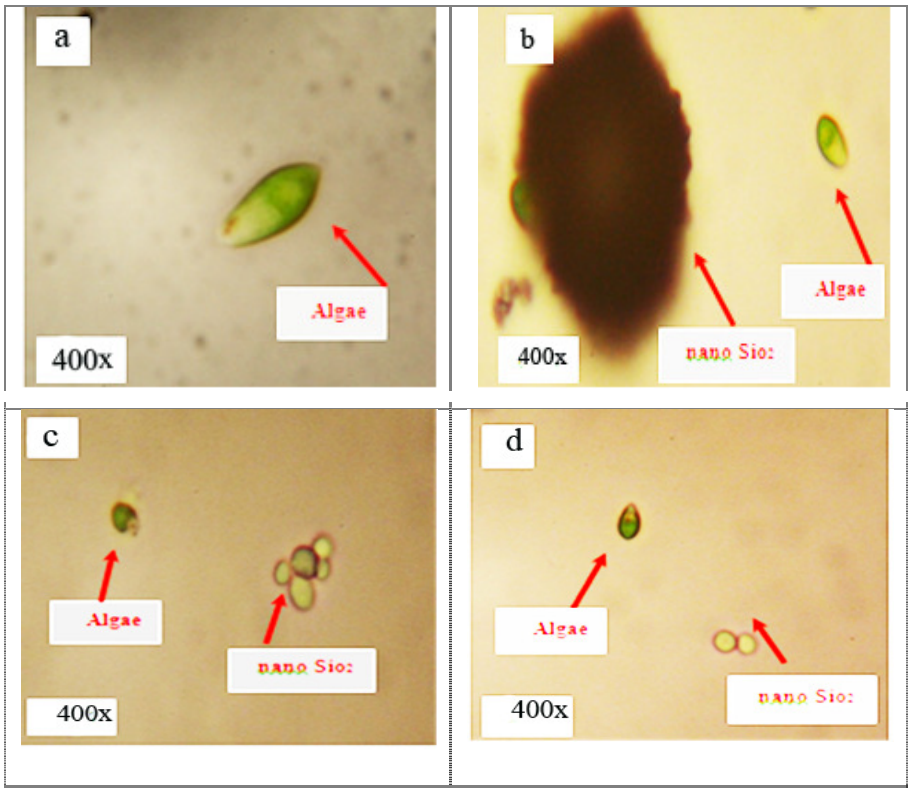

Fig. 6. a,b: Dunaliella salina before contact with SiO_2 NPs; c and d: accumulation of NPs on Dunaliella salina after 72 hours contact with SiO_2

increasing their levels. In a study by Ayatallahzadeh Shirazi et al. (32) increasing of Al_2O_3 NPs exposure time to 72 hours

decreased the number of cells to 2.66×10^4, and increasing the concentration decreased cell number Compared with the control ($p<0.05$); that are similar to our findings. This can indicate the sensitivity of *Dunaliella* algae species to Al_2O_3 and SiO_2 NPs. Similar findings were also reported for SiO_2 NPs. Van Hoecke et al. (33) found that increasing SiO_2 NPs level increases their surface reactivity with *Pseudokirchneriella* alga cells. These results are close to our research findings. In the present study, from collision with SiO_2 NPs, the growth inhibitory effect of *Dunaliella* alga cells was also increased. *Dunaliella* algae reacts rapidly to changes in external and internal osmotic pressures due to the simplicity of cell structure and the lack of cellulosic wall. In this study, the highest number of cells, the specific growth rate,

the lowest doubling time, and the lowest inhibitory levels belonged to the control group. Several other researchers have reported growth inhibition by these NPs. Ji et al. (31) showed that 1000 mg. L^{-1} SiO_2 NPs stopped algae growth by 20% on the second day. Manzo et al. (50) and Fujiwara et al. (34) also found that algae doubling (cell division) due to collision with SiO_2 NPs had a significant difference with the control and caused cell wall destruction. Wei et al. (51), growth inhibition and photosynthetic pigment contents decrease were observed in 96 hours at silica levels of 50, 100, and 200 mg.L^{-1}. Lee et al. (37) confirmed the growth inhibitory effect of SiO_2 NPs on plants. In this study, scanning electron microscopy images) SEM) showed that the NPs were strongly

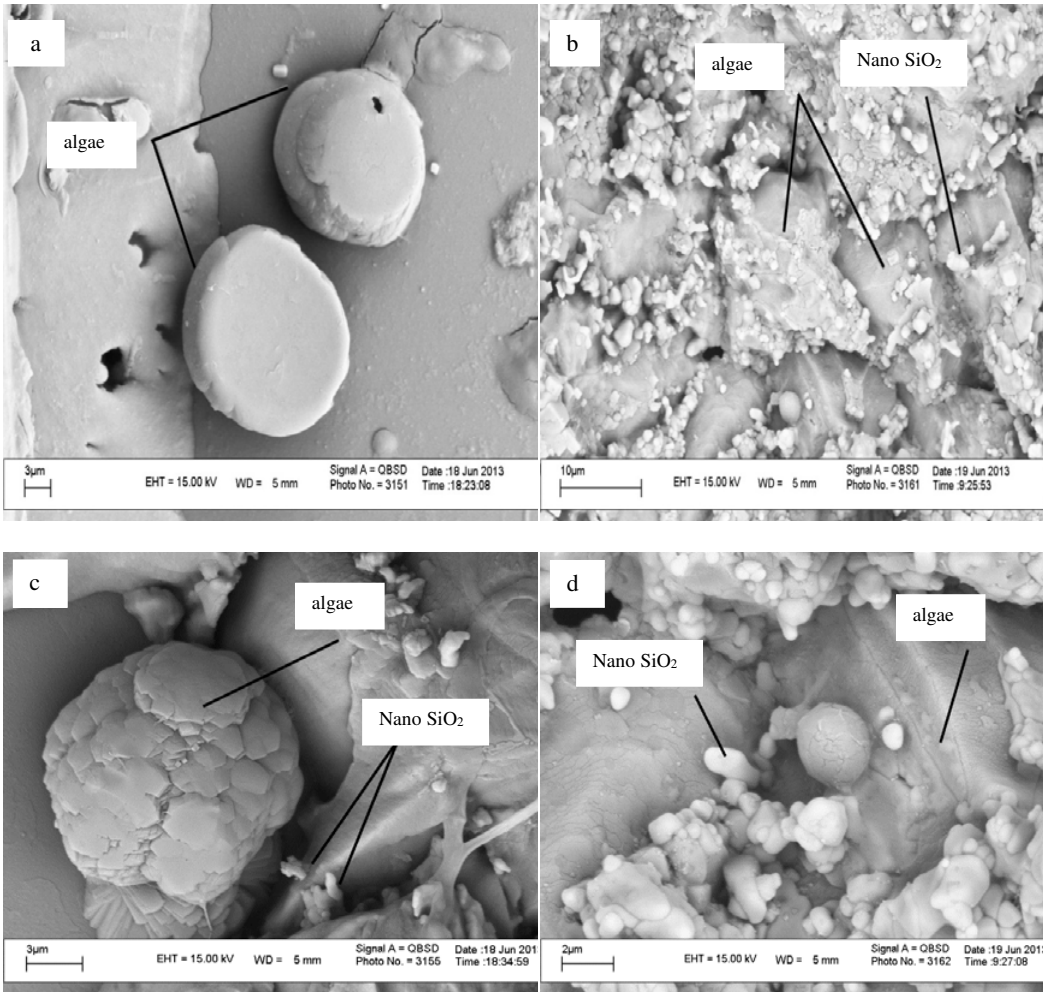

Fig. 7. Scanning electron microscopy (SEM) a-e: 72 hours exposure of Dunaliella salina to SiO_2 NPs

agglomerated because of decreased surface area to volume ratio. The morphological effects of silica NPs on *Dunaliella* algae were determined by cellular shrinkage and loss of flagella. These results are consistent with those of (34, 50, 52). Other researchers revealed the adsorption of NPs at cell surface (33, 48, 53).

Van Hoecke et al (33) observed morphological changes in the cell at a concentration of 100 mg. L^{-1}. Microscopic examination of algae by Fujiwara et al. (34) revealed the enlargement and accumulation of overlying cells and cellular deformation resulted from collision with silica NPs, as well as the increased cell division and growth before colliding with NPs. Based on microscopic findings, of Manzo et al. (50) some smaller silica NPs were penetrated into algae cells and prevented the absorption of nutrients, and higher concentrations of larger particles accumulated and led to their damage.

Ma et al. (54) found that the cumulative effect and adhesion of silica NPs to *Chlorella pyrenoidosa* algae has damaged the cells. According to the results of electron microscopy in the present study, adhesion and accumulation of SiO_2 NPs on *Dunaliella* algae cells resulted in their agglomeration. Collision with SiO_2 NPs has also led to the loss of flagella and shrinkage of the cells. Accumulation of NPs can be due to their high uptake by the algae.

The level of SiO_2 NPs has a significant effect on the concentration of chlorophyll and carotenoids. The level of chlorophyll a differed significantly with that of the control (p<0.05). The level of carotenoids in the treatments decreased with increasing concentrations of silica NPs, and had a significant decrease in comparison to the control (p<0.05). A study by Oukarroum et al. (55) reported that exposure of two algal species of *Chlorella vulgaris* and *Dunaliella tertiolecta* to 50 nm Ag NPs at the levels of 0-10 mg. L^{-1} for 24 hours resulted in a widespread compaction of algae cells. In addition, a significant reduction was observed in the chlorophyll pigment in the studied algae. Ag NPs inhibited the growth in two algae species. Ag NPs decreased the growth of algae cells and sharply declined chlorophyll content due to their accumulation in this species. Chlorophyll and carotenoids were decreased in the present study, which is consistent with this research.

Hazeem et al. (56) investigated the effects of Fe_3O_4 and TiO_2 NPs on two types of algae and showed that exposure to 200 mg. L^{-1} of both sizes of NPs for 24 hours decreased chlorophyll, and each one had a significant difference in comparison with the control (p<0.05). The results of this study proved that increased concentration in this species can lead to an increase in cell growth. According to literature, increased levels decrease the growth rate and chlorophyll content, and lower concentrations exhibit toxic effects earlier; this is inconsistent with the present study. Different toxicological responses can arise from the surface area of NPs and the difference in their toxicity and type of species studied. With increasing concentrations, NPs are accumulated and attached together, and their low motility and low energy in these conditions reduce the toxicity of NPs in higher concentrations (17, 57). In addition, they achieved the same results with GO NPs Hazeem et al. (36). Other researchers such as Metzler et al. (58) showed the negative effect of SiO_2 NPs on chlorophyll content of *Pseudokirchneriella subcapitata* algae, even at low concentrations. These results are close to our research findings. Several researchers also reported the effect of SiO_2 NPs on chlorophyll content and chlorophyll pigments of other algae. In a study regarding the effect of SiO_2 NPs on *Scenedesmus obliquus*, Wei et al. (35) showed that the chlorophyll function index and its photosynthetic pigments were significantly decreased after 96 hours at high concentrations (p<0.05). The results regarding chlorophyll are consistent with the findings of this study. But insignificance of the carotenoid results may arise from the lack of light, reducing chlorophyll content, while carotenoids are more stable, and light cannot affect them. In this research, both of these parameters had a significant difference with the control. There are also some other studies which results are different from ours. SiO_2 NPs had positive effects on tree species and increased the growth rate and chlorophyll index per leaf area. Zarafshar et al. (30), studied the effects of 10,100, 500 and 1000 mg. L^{-1} NPs. Avestan and Naseri (59) NPs levels of 25, 50,100 and 200 mg. L^{-1}, also Ashkavand et al. (60) at the level of 10, 50 and 100 mg. L^{-1} NPs on pear, hawthorn, and apple trees, respectively, and showed that the addition of SiO_2 NPs increased the growth rate compared with the control. Photosynthesis and chlorophyll content have also increased, which is not consistent with the results of this research. The present study is about an algae species while the mentioned study pertains to a tree species. This difference can be attributed to the type of species studied, the sensitivity of algae to NPs, the biological characteristics, and

Table 2. Comparison between our results and those reported in the literature listing the toxic effects of SiO_2 NP and other NPs on different organisms

Test organism	Toxicity causing NP or Metal	Concentration ($mg.L^{-1}$) (Efficiency) time/h Days /h	Summary of findings (Major NP effects)	Ref.
Dunaliella Salina	SiO_2	72h 0.85, 0.3, 0.0, 1 50mg.l^{-1} 2,7,20.4	Cell density considerably declined with increases in concentration of NPs.($P<0.05$)	(45)
Pseudokirchneriella subcapitata	SiO_2	72h	The algae adsorbed silica NPs. The degree of surface reactivity in algal cells enhanced with increases in concentration. Growth suppression of the algae was reported at 20-28.8 mg. L^{-1}. Growth was suppressed by 50 percent at 100-460 mg. L^{-1}. Morphological changes were observed at 100 mg. L^{-1}	(33)
cultured human bronchoalveolar carcinoma-derived cells	SiO_2	72h Concentration of SiO_2 : 10,50,100 µg/mL	SiO_2 NPs damaged cells and reduced their viability.. Exposure to them damages membranes and is toxic to human cells. Damages to DNA and cell membranes were observed at higher doses and longer exposure durations.	(63)
Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli*	SiO_2	Dark and Light conditions	Nanoparticles of SiO_2 caused an increase in the growth of *B. subtilis* and *E. coli* by 99±1.8% and 48±8.5%, respectively. The toxicity effect of SiO_2 nanoparticles has been reported to be greater in the dark than in the light. The size of nanoparticles did not affect the toxicity. In addition, the effect of SiO_2 nanoparticles in both algae led to the disruption of membrane integrity.	(40)
Porphyridium aerugineum Geitler	SiO_2 Al_2O_3	10 Days 0, 1,10,100,500,1000 mg/L^{-1}	At concentrations 1-1000 mg. L^{-1}, SiO_2 nanoparticles showed no toxic effect. However, they had the greatest effect on chlorophyll content at concentration 500 mg/ L^{-1}. They increased chlorophyll content by 0.3 µg/l at concentration 1000 mg. L^{-1}. The highest toxicity of Al_2O_3 nanoparticles was observed at concentration 1 mg. L^{-1}. Al_2O_3 nanoparticles decreased the growth and chlorophyll content of this alga at concentrations 100-500 mg. L^{-1}.	(62)
animal models (Example Rats) or the human body	SiO_2	48 h 0.5-10µm	Nanosilica toxicity was found during in vivo and in vitro studies about toxic effects of silica on human and the laboratory sample (mouse). Exposure to at least 0.5-10 µm can affect the DNA, cardiovascular system, and respiratory system of human and cause injuries to the lungs. It can also have more severe inflammatory responses at higher concentrations. In addition, the toxicity of particles was highly depended on their size; smaller particles of silica more easily entered the cell cytoplasm and accumulated there.	(41)
rainbow trout (fish)	SiO_2	24 h 100-200µg/mL^{-1}	They found that the fish cell lines have been severely damaged. Silica nanoparticles in smaller sizes, in exposure to higher concentrations, and in longer exposures in all sizes caused severe cell changes and reduced cells survival ($p<0.05$).	(39)
Daphnia Magna (Zooplankton)	SiO_2	96h 0.5,1.6, 5, 15.8, 50 and 100 mg. L^{-1}	At concentrations 50 and 100 mg. L^{-1}, the effect of SiO_2 was higher than other concentrations. The accumulation of nanoparticles in the digestive tract of *Daphnia* was observed. With the increase in exposure time at lower concentrations, this species showed higher sensitivity to SiO_2 nanoparticles.	(64)
Pseudokirchneriella subcapitata	Al_2O_3 coating on of SiO_2 NP	72h mg. L^{-1} -1)(Efficiency)) 46 and 220 mg. L^{-1}	highest toxicity was observed at pH 7.6, with 48 h-ErC10 of 6.1 mg. L^{-1} Compared to the control, effects of NP SO2 NPs at ≤ 46 mg. L^{-1} without aluminum coating on chlorophyll content were significant through adsorption on algal cell walls (except at pH6). Algal cells that were impregnated with silica nanoparticles without alumina coating were poisoned and 52% of them died. After surface coating of silica with alumina, no toxicity effects were observed up to 1000 mg. L^{-1}. Smaller nanoparticles penetrated more deeply into the algal cells.	(52)
Chlorella vulgaris	CuO	6 Days 0.01, 1. 10 , 100 mg. L^{-1}	The number of cells decreased with increasing concentrations. The amount of chlorophyll a, chlorophyll b, total chlorophyll, and beta-carotene also reduced with the increase in the concentration of CuO nanoparticles, compared to the control. CuO nanoparticles have inhibited the growth of algae. The growth inhibitory rate increased with increasing NP concentration.	(49)
Chlamydomonas reinhardtii	TiO_2	48 h (0, 6, 12, 24) 0.001, 0.01, 0.1, 1, 10,100(mg. L^{-1})	The results of chlorophyll measurement at concentrations 1, 10, and 100 (mg L-1) and within 48h did not show much difference in their content reduction. Microscopic images showed NP accumulation at higher concentrations.	(4)
seeds of soybean (Glycine max) plants))	Ag	0-200µm chlorophyll absorption measurements: 300-800 nm carotenoids absorption measurements:600-700 nm	The resulting changes in the absorption of chlorophyll and carotenoid between 350 and 550 nm sharply reduced.	(7)

the different biochemical, physiological, and genetic parameters (51). Moreover, according to the research, the effect on plant species growth can be due to biocompatibility, as well as antimicrobial and antibacterial effects of NPs. After placing them on the surface of the desired tree species, they provided nutrients (61). Karunakaran et al. (62) also showed the effect of SiO_2 and Al_2O_3 NPs on *Porphyridium aerugineum Geitler* algae. According to the results, at SiO_2 NPs concentration of 1-1000 mg. L^{-1} no toxicity was observed. The level of 500 mg. L^{-1} had the greatest effect on the chlorophyll content. Reports indicate that the toxicity of Al_2O_3 NPs is higher than SiO_2 NPs.The results of SiO_2 NPs

experiments on the algae are not consistent with the research carried out on *Dunaliella*. In the present study, algae reacted even at low concentrations. Absorption, translocation, and accumulation of NPs vary depending on the plant species and the size, type, structure, chemical composition, and strength of NPs. The most important reason for different absorptions of NPs by algae cells to be pointed out are the level and nature of NPs, the nature of environment, the contact method, and the active biology of the organism (61, 65).

Table 2 presents a comparison between our results and those reported in the literature.

ACKNOWLEDGMENT

The authors would like to thank Iran Nanotechnology Initiative Council (INIC), for the financial support of this research. Also, the authors are thankful to the manager of laboratory Kavoshgaran Tabiat Pak in Rasht, (Dr. Zohreh Ramzanpour) for providing necessary facilities, assistance with the algal culture and constant encouragement to carry out this study. We also appreciate the Centre for Nanostructure Imaging, Iran Nanotechnology Laboratory Network University of Mohaghegh Ardabili for Electron Microscopy facilities and Mr. Khodaei for his technical assistance.

CONCLUSIONS

Comparison of the findings with the relevant literature showed that aquatic organisms react negatively to NPs compared with terrestrial species. As a factor that influences the toxicity of NPs, their solubility in aqueous media can play an important role in this regard. On the other hand, algae are more sensitive to NPs than other organisms, such as plants, fish, and invertebrates. Beyond the damage to the food chain of algae, increased use of NPs can even harm human health after entering the cells of human body. Therefore, identifying surface characteristics of NPs, their infiltration in the aquatic environment according to various concentrations, and control and management of NPs –containing waste are necessary. It is concluded that SiO_2 NPs have significant toxic effect on *Dunaliella salina* algae. Data analysis showed a direct relationship between the concentration of NPs and their toxicity on this organism. As NPs concentration increased, chlorophyll and carotenoid of *Dunaliella salina* reduced significantly ($p<0.05$). In case of infiltration of SiO_2 NPs in the aquatic environment within the defined limits, the results of this research are acceptable for Dunaliella. The consequences and potential damage of NPs in aquatic environments require further research. The irreparable pollution with these new compounds and its consequences can be prevented through proper management. Finally, with the sustainable development of nanomaterials and taking into account their appropriate concentration, we can definitely promise the beneficial role of nanotechnology in our life, without adversely impacts of the development of nanotechnology.

CONFLICT OF INTEREST

None declared.

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