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Short Communication

Toxicity Effects of SiO_2 Nanoparticles on Green Micro-Algae Dunaliella Salina

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Abstract

New extension of nanoparticles used in the last two decades and hence, entrance of them to industrial and non-industrial sewage necessitate study of probable effects of these materials in aquatic ecosystems. This research was performed in order to determine the toxicity effect of silica dioxide (SiO_2) nano particles on Dunaliella salina green algae in laboratory conditions. SiO_2 nanoparticle is one of the best full-used nano particles which have application in industries like production of ceramics, plastics, glass, cosmetics, medicine and paper. Dunaliella algae because of having economic value and different biochemical composition is used as complements with natural origin in food and pharmacology industries. For toxicity determination of this material, the experiment was performed according to O.E.C.D standard method. Experiments on Dunaliella were performed for 72 hours with 7 treatments, two controls and three replicates in each treatment and daily counting of cells in each tube. Counting cell algae population was done by microscope on a Thoma counting slide. For data analysis, probit analysis, Excel software and SPSS21 were used. The 72 hours NOEC, EC_{90}, EC_{50} and EC_{10} were calculated. The amounts of 72 hours are $EC10 = 5.37.10^{-5}$, EC50 = 0.169, EC90 = 512.86, NOEC = 1.6×10^{-2} mg/l. Cell compression noticeably decreased ($P < 0.05$) by increasing nanoparticle concentration and silica oxide nanoparticle caused to inhibit growth in Dunaliella species.

Key words: *Algae Dunaliella salina, EC_{50}, SiO_2, Toxicity, Nanoparticles, Nanotechnology.*

1. INRODUCTION

Nanoparticles are widely used due to their magnetic, electrical, chemical, mechanical, and optical properties [1, 2]. Increased large-scale production and diverse application of these particles inevitably lead to their accidental release and dissemination in the environment through municipal, industrial, and agricultural waste and sewage that may exert extensive environmental hazards [3]. The behavior of nanoparticles depends on their average size, elemental composition, contact area, porosity, surface ionic charge, and hydrodynamic diameter [4].

As one of the most widely used particles, silica nanoparticle is increasingly applied in various industries. Physicochemical properties of nanoparticles effect on how organisms respond biologically to them [5]; therefore, the study of the biological response to nanoparticles is of particular importance. Nanoparticles are very dynamic in water and hence, easily enter large aquatic ecosystems [6]. Algae, as the first link in the food chain, play an important role in aquatic ecosystems and in purification of polluted waters. They have also been considered as a model organism for toxicity testing of nanoparticles [7-8]. Any change in density, biomass, and population of algae affects the food chain.

The unicellular green algae *Dunaliella salina* is widely distributed in seawater. Aside from food applications, *D.salina* is used to produce biofuel or biodiesel due to its potential for production of lipids [9]

The first impact of nanoparticles on algae is cell compression [6]. Aggregation and compression of algae cells may reduce its accessibility to light which in turn could inhibit algae growth [10] and reduce the absorption of essential nutrients from the environment through blocking the pores of the cell wall [11]. Few studies have been carried out on the effects of ecological toxicity of silica nanoparticles on aquatic species and algae. For example, Fujiwara et al. [12] studied the toxicity of silica nanoparticles on *Chorella kesslari.* (Fujiwara, 2008) and Van Hoecke (2009) investigated the toxicity of silica nanoparticles on *Pseudokirchneriella subcapitata* green algae [13].

Therefore, it is necessary to perform further research on toxic effects of nanoparticles on aquatic species and algae, in particular *Dunaliella* species with its nutritional and economic value. In the present study, the impact of silicon oxide nanoparticles was investigated on growth inhibition of *Dunaliella salina* and calculated its NOEC, EC_{50}, and EC_{90}.

2. MATERIALSAND METHODS

2.1. Stocks of Algae Culture

This study was conducted in the laboratory of Kavoshgaran Tabiat Pak in Rasht in 2012, in order to assess the toxic effects of silica nanoparticles on *Dunaliella* algae. To do so, *D. salina* Teodoresco seawater algae were isolated from the Urmia Lake and after identification by Artemia Research School in Urmia, they were transferred to the Ecology Laboratory of Dr Dadman International Sturgeon Research Institute in Rasht for culture (Fig. 1 and 2). The algae were purified using solid linear and liquid culture.

2.2. Preparation of Culture Media

The algae were cultured in JW medium which was prepared by adding 50 g water-purified rock salt to one liter of water and dissolving with a magnetic mixer (Iran, Fanavaran Sahand Azar, model HMS-300). Water salinity was adjusted on 80 ppt through measuring by Optech (Germany, model K7117). Then 1 ml of each 9 chemicals was added to salty water and the culture medium was sterilized. The bottles were then stored at 6 °C. The temperature of the incubator (Iran, Fanavaran Sahand Azar, model IN55F) with in-wall fluorescent lamp, was adjusted on 25 ± 1 °C. The light was continuously set on 50 µmol photon.$m^{-2}.s^{-1}$ with a lux meter (model TES-1336A).

To evaluate the algae growth during the 28-dayphase, algae from the main stock (with a density of 29.5×10^4 cells.ml^{-1}) were added to 10 ml medium in a test tube. The growth cycle of algae was examined with a Thoma counting slide (with a depth of 0.1 mm and small square shape with 0.0025 mm^2size of 0.0025 mm^2 under a light microscope (Japan, Microphot-fxt, Nikon) with lens 40. The growth curve of *D. salina* stock was drawn during the 28-day growth phase. The Excel software was used to draw *D. salina* population change curve [14]. (Equation1).

$$\mu = \ln x_1 - \ln x_0 (t_1 - t_0)^{-1} \quad (1)$$

2.3. Preparing of nanoparticle powder:

Silicon oxide nanoparticles were obtained from Pishgaman Nanomavad Iran Company (USA, 2011). Its characteristics are depicted in Table 3 and its Scanning Electronic Microcopy images (SEM) were offered by Pishgaman Nanomavad Iran Company (Figure. 1).

Table 1. *Nanoparticle characteristics (Pishgaman Nanomavad Iran Company, 2012)*

Chemical formula	SiO_2
Purity (%)	99
Particle size (nm)	11-14
Specific area (m^2/g)	190-685
Mass density	<0.11

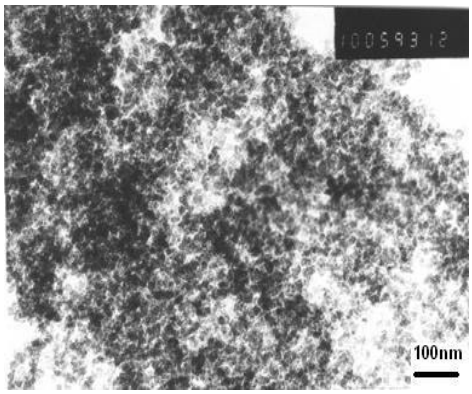

Figure1. *SEM of silicon oxide nanoparticles (Pishgaman Nanomavad Iran Company, 2012)*

2.4. Preparation of the Main Test Treatments

To determine the main concentration of the experiment, 7 treatments and 2 control samples were selected after several steps of range finding tests had been performed in triplicate. Finally, the determined logarithmic concentrations were 0, 0.1, 0.3, 0.85, 2.4, 7, 20, and 50mg/L. OECD 201 (Organization for Economic Cooperation and Development) method was used to evaluate the algae growth inhibition [15].

According to calculation of the mentioned concentrations, certain amounts of the nanoparticle solutions were added to the culture medium in the test tubes to reach volume of 10 ml. Then 5×10^3 cells of *D. salina* from original stock were added to10 ml of treatment and control samples.

The test tubes were then placed at 25± 1 °C which has been regulated by a thermostat. The samples were exposed to 12h light and 12 h dark. These conditions were maintained for 72 hours throughout the experiment.

The solutions in test tubes were sampled with Pasteur pipettes after 24, 48, and 72 hours from the start of the experiment and the algae cells were counted by a Thoma counting slide under a light microscope (Japan, Microphot-fxt, Nikon) with lens40.

After cell counting, the values of 24, 48 and 72 hours of EC_{90}, EC_{50}, EC_{10} were calculated based on Probit Analysis [16]. The value of NOEC was obtained (Equation 2), too. In order to estimate the significance of differences between treatments in different concentrations of nanoparticle in algae cells and the control, one- way ANOVA and Tukey test were used to identify the differences between each treatment's level.

$$NOEC = EC_{50}/10 \qquad (2)$$

3. RESULTS

3.1. Specific Growth Rate

During the early days of culture, cells are in the late phase. The presence of the late phase before the start of cell division is essential. In the fourteenth day, cells enter the log phase and grow with highest possible speed and divide. Instead of having a sharp (discontinuous) increase, the growth curve goes up gradually. Results from the Dunaliella Algae growth curve showed that the growth in the sixteenth day reached to its peak and continued for 28 days. Finally the population growth stopped (Figure2).

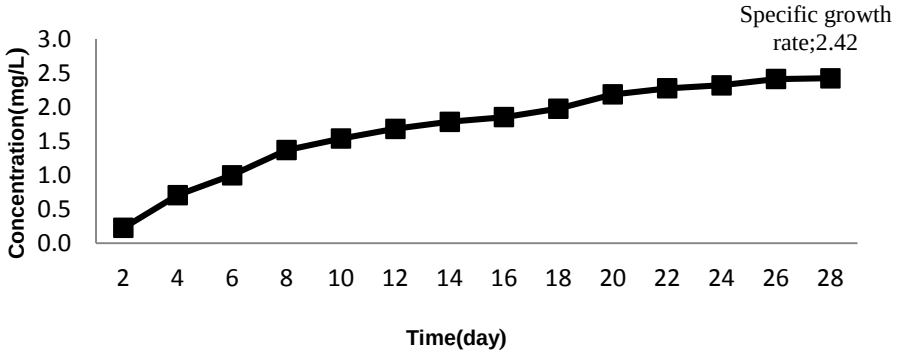

Figure 2. *Specific growth rate curve of Dunaliella salina (mean cell density ± SD)*

3.2. Results of Cell Count

3.2.1.The effects of exposure concentration and time of nanoSiO_2 on *Dunaliella salina* cell number

Figure3 shows SiO_2 nanoparticle effect on cell number of *Dunaliella salina* Algae. With increasing the concentration, the number of cells shows a decreasing trend in 24 hours exposure; but after 48 or 72 hours, there is no significant effect ($p>0.05$). After 72 hours the control cells became 2.66 x 10^4 (Figure3).

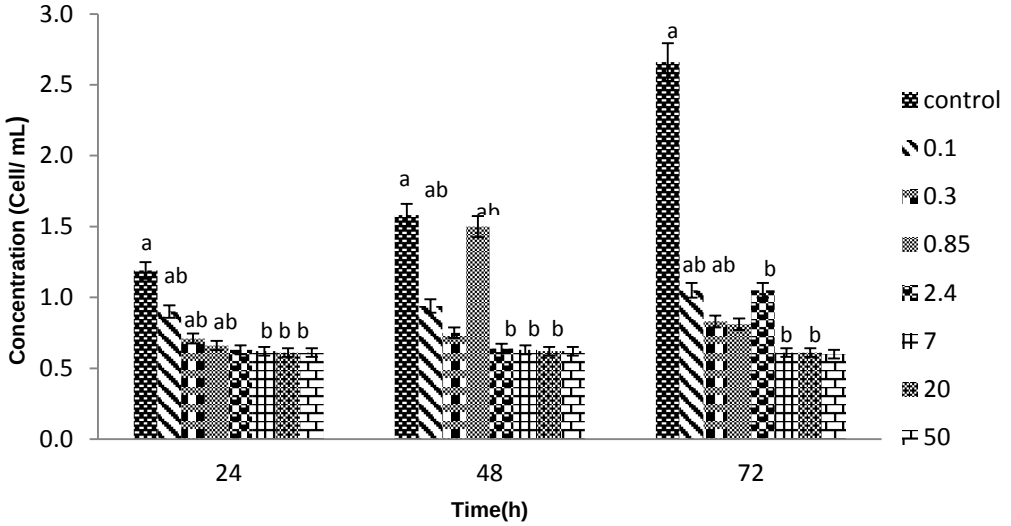

Figure 3. *Number of cells in various concentrations of SiO_2 nanoparticles for Dunaliella salina species.*

3.2.2. The effect of exposure concentration on *Dunaliella salina* cell number

Figure 4 shows the concentration of silica nanoparticles effects on the cell count. According to ANOVA test, the statistical value of Fischer was 11.312, Sig<0.05; thus a significant difference was observed between various concentrations when compared with the control (p<0.05), but there was not any significant difference among treatments (p>0.05).

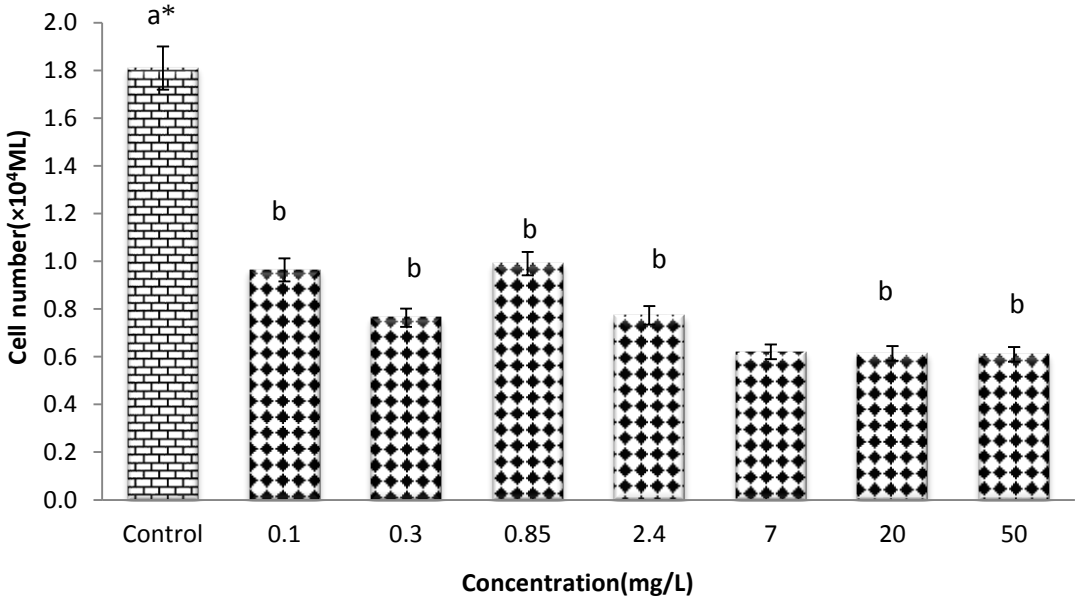

Figure 4. *The impact of silica nanoparticles on Dunaliella salina cell.*

* Comparison of the means (mean of three replications) based on Tukey HSD test (p<0.05). Those treatments without common letters have a significant statistical difference (p<0.05).

3.2.3. Effect of exposure time on *Dunaliella salina* cell number

According to *t*-test, during every 24 hours exposure, the number of treatment cells containing nanoSiO_2 had a significant difference with the control (p<0.05), (Figure 5).

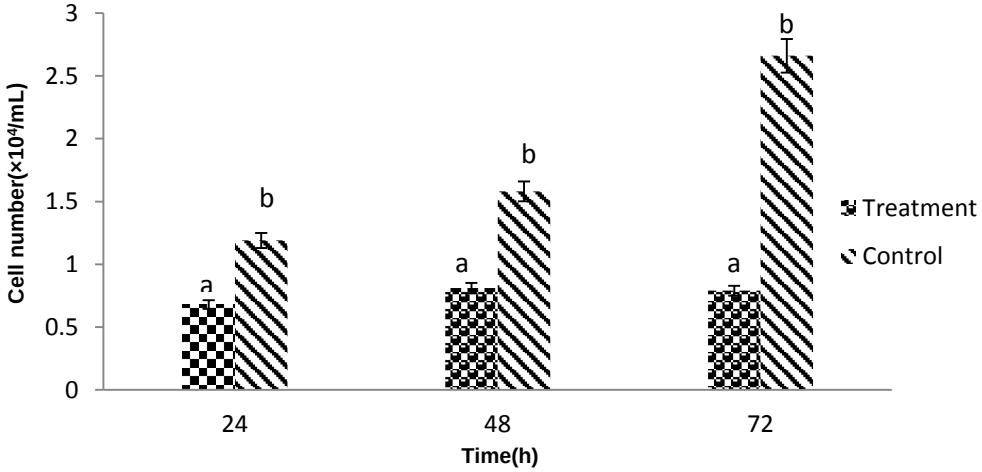

Figure 5. *The effect of time on the mean of Dunaliella salina cell numbers exposed to nanoSiO_2*

3.3. Effective concentration (EC) and non-observed-effect concentration (NOEC) of silicon oxide nanoparticles on *Dunaliella salina*

Table 2 shows regression equations of silica nanoparticles in different times.

According to the table3, effective concentration of *Dunaliella salina* (EC_{50}) in 72 hours was 0.169 mg/L, while EC_{90} was calculated as 15.31 mg/L in 72 hours.

Table 2. *Regression equations of silica nanoparticles in different times*

Time (hour)	24	48	72
Linear equation	y = 0.787x + 5.057	y = 0.394x + 5.375	y = 0.367x + 5.286
R^2	0.964	0.868	0.827

Table 3. *Effective concentration of nanoSiO_2 on D. salina*

Time (hours)	*24*	*48*	*72*
mg/L (EC)			
EC_{10}	0.02	6.31×10^{-5}	5.37×10^{-5}
EC_{50}	0.851	0.112	0.169
EC_{90}	35.48	199.53	512.86
NOEC	8.5×10^{-2}	1.1×10^{-2}	1.6×10^{-2}

4. DISCUSION AND CONCLUSION

According to the results, silicon oxide nanoparticles inhibited *D. salina* growth and cell density decreased significantly with increasing of nanoparticles concentration (p<0.05). The growth of *Dunaliella salina* declined following increment of the concentration and passing

the time. The highest number of the cells in the algae belonged to the control.

Nelson *et al*. studied the effect of silica nanoparticles on zebra fish and concluded that smaller particles would have more toxicity [17].

The toxicity of silica nanoparticles on *Pseudokirchneriella subcapitata* green algae was investigated and accordingly, EC_{10} was calculated as 55 mg/L [13]. In our study, EC_{10} was calculated as 5.37×10^{-5} mg/L. This difference may indicate the sensitivity of Dunaliella sp. (algae) to nano silica. In addition, the toxicity of the same nanoparticles is different due to their size and nanostructure.

Fujiwara (2008) studied the toxicity of silica nanoparticles on *Chorella kesslari* algae and found the LC_{90} of nanoparticles as 0.6 mg/L for 5 nm particles, 2.8 mg/L for 26 nm particles, and 4.7 mg/L for 78 nm particles and concluded that the toxicity of these nanoparticles increased when their size decreased [18]. The obtained LC_{90} results are not consistent with the results of this study where 72 h EC_{90} value was 512.86 mg/L. This difference can be attributed to the different dimensions of silicon oxide nanoparticles and the type of algae.

Manzo *et al*. (2013) studied the toxicity of zinc oxide and zinc bulk on *Dunaliella tertiolecta* and reported 72 h EC_{50} of zinc nano oxide, zinc bulk, and zinc chloride as 3.57, 1.94, and 0.65 mg/l, respectively. The results showed that nano zinc oxide had the highest toxicity which increased following increment time of exposure [19]. In this study, EC_{50} was calculated as 0.169 mg/L.

Regarding to the results of the present research and comparing them with the results of other studies, it is concluded that silica nanoparticles have significant toxic effect on *Dunaliella salina* algae.

Unique properties of nanoparticles such as high surface are due to their small size and their mobility make them hazardous to the environment. Therefore, proper management is required to prevent irrepaiable contamination and the consequences of these new compounds.

Based on the results of this study and the correspondence between these results and the findings reported by other researchers, it can be concluded that nanoparticle has significant toxic effect on *Dunaliella salina* algae species and, it hinders the growth of this species. Data analysis indicated that the growth of *Dunaliella salina* algae cells is reduced with the increase of density and exposure time. Due to their unique properties such as high surface area, small size, and high dynamicity, nanoparticles have the potential to seriously damage the environment. Hence, it is necessary to determine and indentify the actual effects of nanotechnology before nano residues appear in the environment and before the introduction of new nano products to market. In case of taking proper actions concerning the management of these new compositions, it will be possible to prevent from the irretrievable pollutions and their consequences.

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