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Toxic Effect of Aluminum Oxide Nanoparticles on Green Micro-Algae *dunaliella salina*

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ABSTRACT: Aluminum oxide nanoparticles are the most widely used nanoparticles in various industries.The increasing use of nanoparticles in the past two decades and their entry into the industrial and non-industrial waste water necessitates the assessment of potential effects of these substances in aquatic ecosystems. OECD standard method was applied to determine the toxicity of this substance. After performing the detection range testing, the cells of 7 treatments and 2 controls were counted every 24 hours for 72 hours in three replicates for each concentration. After extraction, chlorophyll a and carotenoid were measured using spectrophotometry. Scanning electron microscopy (SEM) was used to image the exposure of the algae cells to nanoparticles. The 72-hour levels of EC_{10}, EC_{50}, EC_{90}, and NOEC, specific growth rate (μ), doubling time (G), and percent inhibition (I%) were also calculated. The obtained 72-hour levels were $EC_{10}=1.66\times10^{-3}$, $EC_{50}=0.162$, $EC_{90}=15.31$, and NOEC=16.2×10^{-2}mg/L. The control and treatment algae had a significant difference in terms of cell density and growth inhibition rate ($p<0.05$). Aluminum oxide nanoparticles had a significant impact on the shape and topography of *Dunaliella salina* cells and resulted in their swelling and enlargement. A significant difference existed in chlorophyll a and carotenoid concentrations between the treatment and control groups and the levels of carotenoid decreased following increase in concentration of treatments ($p<0.05$). Aluminum oxide nanoparticles have a significant toxic effect on *Dunaliella salina*. With increasing nanoparticles concentration, *Dunaliella salina* chlorophyll and carotenoid concentration reduced significantly ($p<0.05$).

Key words: Nanoparticles, Toxicity, Aluminum oxide, *Dunaliella salina*

INTRODUCTION

Nanoparticles are widely used due to their magnetic, electrical, chemical, mechanical, and optical properties (Oberdorster *et al* , 2005-; Lee *et al*., 2008). 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 (--Howard, 2004;Daughton, 2004) The behavior of nanoparticles depends on their average size, elemental composition, contact area, prosity, surface ionic charge, and hydrodynamic diameter (Videa *et al*., 2011). Physicochemical properties of nanoparticles affect on how organisms respond biologically to them (MonteiroRiriere *et al* ., 2009) . Nanoparticles are highly mobile in water and can easily enter vast aquatic ecosystems (Oukarroum *et al*., 2012). Nanotoxiology is one the important topics of nanotechnology. Safety and toxicity of nanomaterials are closely related. The boundary between toxicity and safety of nanoparticles must be identified using tests and technologies. Among these tests and technologies, determination of EC (Effective concentration) which is an indicator of effect and toxicity, is the most important one. Algae are the first link in the aquatic food chain. They also have an important role in aquatic ecosystems and an essential role in self-purification of polluted waters. Therefore, they are considered as model organisms in testing toxicity of nanoparticles (Jing *et al*., 2011; Jiangxin *et al*., 2008) Any change in the density, biomass and algae population affects the food chain. Therefore, studying the response of algae to nanoparticles is of special importance. The unicellular green algae Dunaliella salina is widely distributed in seawater. Aside from food applications,

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D. salina is used to produce biofuel or biodiesel due to its potential for production of lipids (Brennan &Owende, 2010). The first impact of nanoparticles on algae is cell compression (Oukarroum *et al*., 2012). Aggregation and compression of algae cells may reduce its accessibility to light which in turn could inhibit algae growth (Perreault *et al*., 2011) and reduce the absorption of essential nutrients from the environment through blocking the pores of the cell wall (Wei *et al*., 2010). One of the most widely used particles, is aluminum oxide nanoparticle that is increasingly applied in various industries. Few studies have been carried out on the effects of ecological toxicity of aluminum nanoparticles on aquatic species and algae. For example, Fujiwara, 2008 studied the toxicity of aluminum nanoparticles on Chorella kesslari (Fujiwara *et al*., 2008). Mohammed Sadiq*et al*. investigated the toxicity of aluminum nanoparticles in two species of algae (Scenedesmus spp and Chlorella spp)(Sadiq *et al*., 2011).

Therefore, it seems 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(Botanical Monograph, 2006). In the present study, the impact of aluminum oxide nanoparticles was investigated on growth inhibition of *Dunaliella salina* and calculated its NOEC, EC10, EC50, and EC90.

MATERIALS & METHODS

This study was conducted in the laboratory of Kavoshgaran Tabiat Pak in Rasht in 2012, in order to assess the toxic effects of aluminum oxide 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). The algae were purified using solid linear and liquid culture. 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. with a lux meter (model TES-1336A). To evaluate the algae growth during the 28-day phase, algae from the main stock (with a density of 29.5×10^4 (cell/mL) 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 size 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 (Fogg & Thake,1987) (Equation 1).

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

Al_2O_3 nanoparticles were obtained from Pishgaman Nanomavad Iran Company (USA, 2011). Its characteristics are depicted in Table 1 and its Scanning Electronic Microcopy images (SEM) were offered by Pishgaman Nanomavad Iran Company (Fig. 1).

In order to determine the range of original concentrations of the tests, several range finding steps were performed as pre-test on nanoparticles to determine the range of toxicity. Finally, 5 treatments in 3 replications and two controls were selected. The final logarithmic concentrations were 0.00, 0.005, 0.026, 0.14, 0.7, and 3.8 mg/L. OECD (201) method was used to expose the algae to nanoparticle (OECD (201).

Based on calculations, the mentioned concentrations of nanoparticles solutions were added to culture medium in test tubes to obtain a volume of 10 mL. Then, 5×10^3 cells from the original stock of *Dunaliella salina* were added to 10 mL of the treatments and controls.

Table 1. Al_2O_3 nanoparticle characteristics (Pishgaman Nanomavad Iran Company, 2012)

Chemical formula	**γ-Al_2O_3(gamma)**
Purity (%)	99
Particle size (nm)	20
Specific area (m^2/g)	>138m^2/g

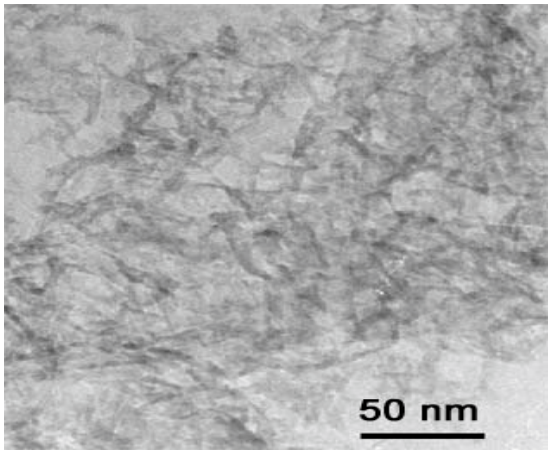

Fig 1. SEM of Aluminum oxide nanoparticles (PishgamanNanomavad Iran Company, 2012)

Test tubes were then placed at 25 ± 1 °C and exposed to 12 hours darkness and 12 hours light. The temperature and lighting conditions were regulated by a thermostat and an electric chronometer (TS-MD20), respectively. These conditions were kept constant during the test period (*i.e.* 72 hours). The solutions in test tubes were sampled at 24, 48, and 72 hours with Pasteur pipette and were counted using Thoma slides under an optical microscope (Japan, Microphot-fxt, Nikon) with lens 40.

After counting the algae cells, 24, 48, and 72-hour levels of EC_{10}, EC_{50}, and EC_{90} were derived from the Probit analysis table and NOEC was calculated (Equation 2) (Finny,1971) One-way ANOVA was used to determine the significance of differences among treatments at various concentrations of algae cells and control samples. The Tukey's test was used to identify differences between each level of treatment.

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

Values of μ (growth rate per hour) are expressed d, h, or min. G (doubling time per hour) and I (percent inhibition) were calculated from the proposed equations (Fogg & Thake,1987). (Eqs. 3, 4, and 5).

$$\mu = \ln x_1 - \ln x_0 (t_1 - t_0)^{-1} \quad (3)$$

$$G = \ln 2 \mu^{-1} \quad (4)$$

$$I\% = (\mu_c - \mu_t)/\mu_c \quad (5)$$

Scanning electron microscopy (SEM, LEO 1430VP, Germany) was used to investigate the effect of nanoparticles on the shape and size of cells in microscopic tissues of *Dunaliella salina* and to take image of the surfaces. Imaging of the control and treatments surfaces at the concentration of 0.2 mg/L which had affected 50% of the cells was performed with magnification of 3-10 micron (Fig. 4).

Chlorophyll was measured to investigate the effect of nanoparticles on the chlorophyll content of *Dunaliella salina* (ASTM, 1996). To measure aluminum nanoparticles, a specified amount of four treatments at concentrations zero (control), 0.2, 0.4, and 2 mg/L was prepared in three replicates in 50 ml erlenmeyer flasks and sampled periodically.

The chlorophyll measurement method (ASTM, 1996) was used to study the effect of the nanoparticle on the levels of chlorophyll in *Dunaliella* algae. To extract chlorophyll and â-carotene, a number of centrifuge tubes were prepared and 4 mL algae suspension was poured into each one. Then they were vortexed and centrifuged with a microprocessor centrifuge (co-w300, Para-Azma, Iran) at 5500 rpm for 10 min to separate the culture medium from algae solution. Centrifugation was repeated for another 10 min. Then the supernatant, containing the pigments, were isolated and 4 mL 90% acetone was added to the extract yielded from algae precipitation. The precipitate was transferred into a falcon and was frozen.

The obtained solution (almost green-colored) was poured into the cuvette of a spectrophotometer (Apada, UV-6300Pc) and the absorbance was read at 630, 647, and 664 nm. Finally, the concentration of chlorophyll a was calculated in ìg/mL. (Equations6)

$$Ca = 11.85\ A664 - 1.54\ A647 - 0.08\ A630 \quad (6)$$

To obtain the amount of algae carotenoid, the absorbance was read at 470 nm (Equations7 and 8). One-way ANOVA was used to evaluate the effect of each nano-aluminum treatment. The significance level was considered 95% in all calculations. Tukey's test was used in the case that a significant difference existed between treatments. All experiments for each treatment were performed in three replicates and statistical calculations were carried out by SPSS-21.

$$Caa = Ca \times V_{aceton}/V_{water} \times 1000\ \mu g/mL \quad (7)$$

$$Cc = 10 \times A(480) \times V_{aceton}/V_{water} \times 1000\ \mu g/mL \quad (8)$$

RESULTS & DISCUSSION

Specific growth rate curve at the 28-day growth phase was calculated and then was plotted by Excel-2007 (Fig.2).

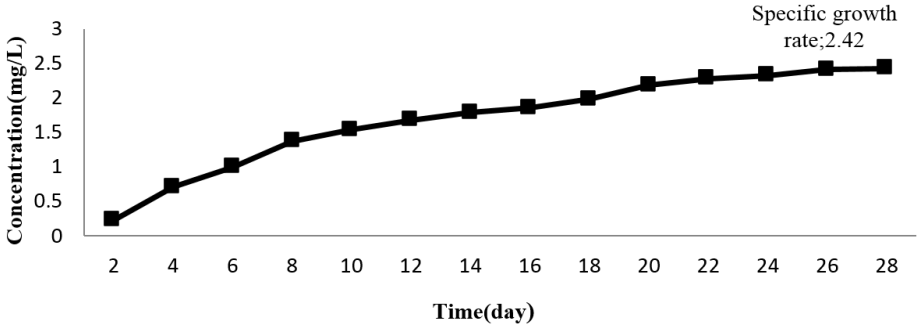

Fig 2. Specific growth rate curve of *Dunaliella salina* (mean cell density ± SD)

Cells were in lag phase during early days, which is necessary before cell division. By the day fourteen, cells entered the exponential phase (log phase) and grew and divided at maximum possible speed. The results of *Dunaliella salina* growth curve showed that the growth reached its peak at the sixteenth day and this cycle continued to the day twenty-eight. Finally, the growth stopped. The results showed that an increase in light duration can increase cell density of *Dunaliella salina*. According to the diagram of 3, the number of cells decreased with increasing concentrations at 24-hour exposure. The highest number of cells was observed in the control.

The number of treatments' cells had a constant trend up to a concentration of 0.7 mg/L. A significant decrease in the number of cells was recorded only at 3.8 mg/L. According to one-way ANOVA and Tukey's test, the number of cells exposed to aluminum nanoparticles are different at 24 and 72 hours, but according to SIG=0.488, which is greater than 5%, it can be concluded that the concentration and time in combination, did not affect the number of cells exposed to aluminum oxide nanoparticles. Control cells had an increasing trend over time and reached to 4×10^4 cells after 72 hours (Fig. 3).

According to Fig 4, we conclude that increased concentration resulted in decreased cell density. The highest cell density was seen in the controls.

Comparison between the means (of three replications) performed according to Tukey HSD test ($p<0.05$). Treatments without common letters have statistically significant difference ($p<0.05$). Each column represents the mean ± SD.

When determining the relationship between toxicity (decreased cell number), one-way ANOVA was used to estimate the significance of differences among treatments and Tukey's test was used to identify differences between each level of treatments at confidence level of 95%. According to ANOVA, the Fisher statistical value was equal to 81.431, Sig<0.05; therefore, increased concentration resulted in a significant difference in various concentrations ($p<0.05$). According to *t*-test ($p<0.05$), the number of cells in aluminum oxide nanoparticles-containing treatments had significant differences at every 24-hour exposure in comparison with the control (Fig. 5). This study showed that aluminum oxide nanoparticles had toxic effects and reduced the specific growth rate (μ) of *Dunaliella salina*. According to one-way ANOVA

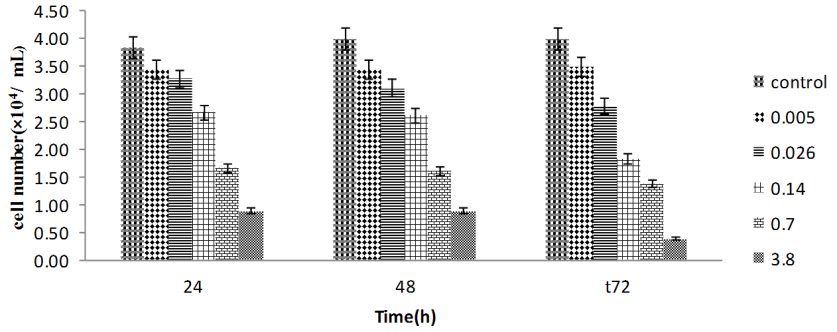

Fig 3. Number of *Dunaliela salina* cells at different times and concentrations of aluminum oxide nanoparticles

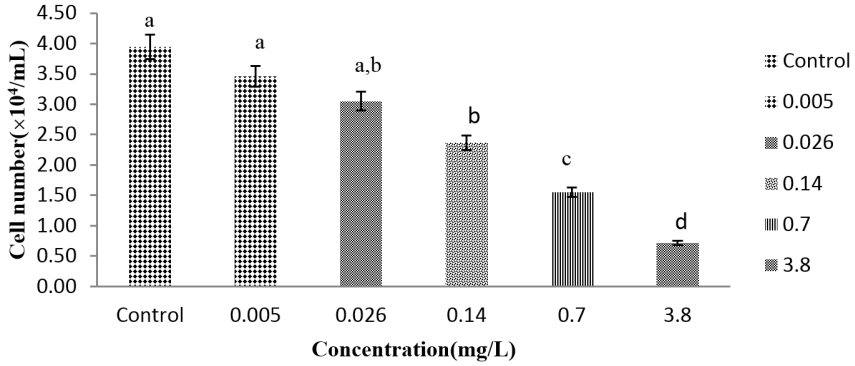

Fig 4. Effect of aluminum nanoparticles concentration on *Dunaliella salina* cells Comparison between the means (of three replications) performed according to Tukey HSD test ($p<0.05$). Treatments without common letters have statistically significant difference ($p<0.05$). Each column represents the mean ± SD.

Table 2. The values of EC_{10}, EC_{50}, EC_{90}, and NOEC for *Dunaliella salina*

Time (h) / EC(mg/L)	24	48	72
EC_{10}	8.71×10^{3}	3.09×10^{-3}	1.66×10^{-3}
EC_{50}	0.54	0.398	0.162
EC_{90}	33.88	50.81	15.31
NOEC	5.4×10^{-2}	39.8×10^{-3}	16.2×10^{-2}

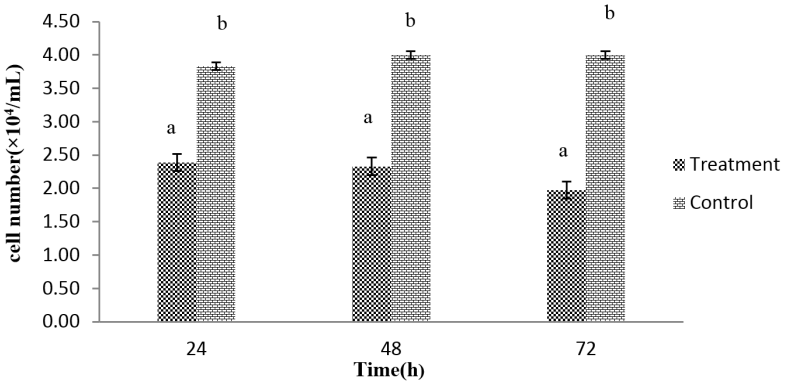

Fig. 5. Effect of time on the mean number of *Dunaliella salina* cells exposed to aluminum nano-oxide compared with control at every 24 hours

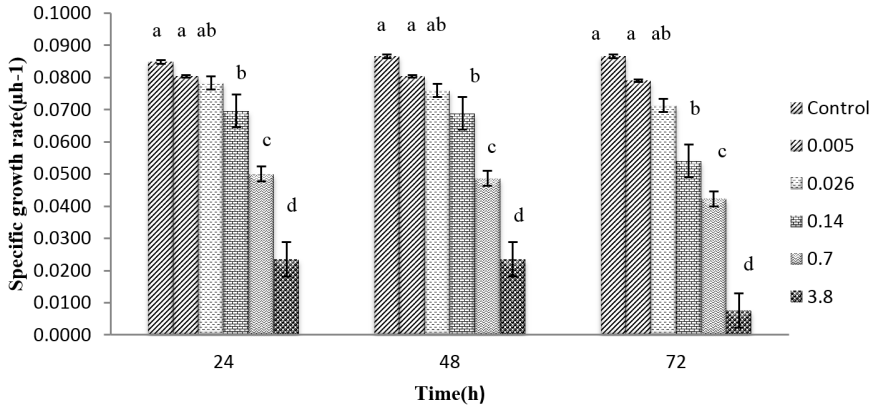

Fig. 6. Specific growth rate of *Dunaliella salina* in contact with aluminum oxide nanoparticles

and Tukey's test, the Fisher statistical value was 59.969 and Sig<0.05; the maximum specific growth rate (μ_{max}) had a significant difference between various treatments and a decreasing trend can be observed from lower to higher concentrations. This trend was always increasing in the controls (p<0.05). No significant differences were observed between various treatments after 24, 48, and 72 h (Fig. 6).

Doubling time (G) parameter showed an increasing trend of doubling rate over time in treatments. According to one-way ANOVA and Tukey's test, the statistical amount of Fisher was 0.000 and Sig<0.05; and increased concentration of nanoparticles during a period resulted in an increase in cell doubling time and a significant difference was observed (p<0.05). The lowest cell doubling time was found in the control (Fig.7). Growth percent inhibition (I) of *Dunaliella salina* increased with increase in exposure time and concentration. This value is always zero in the control (Fig. 8).

According to Table 2, the effective concentration on *Dunaliella salina* for 72-hEC_{50} and 72-h EC_{90} was calculated 0.162 and 15.31mg/L, respectively. According to ANOVA statistical analysis and graph, SIG=0.000 is smaller than5%, so it can be concluded that chlorophyll concentration has varied at different

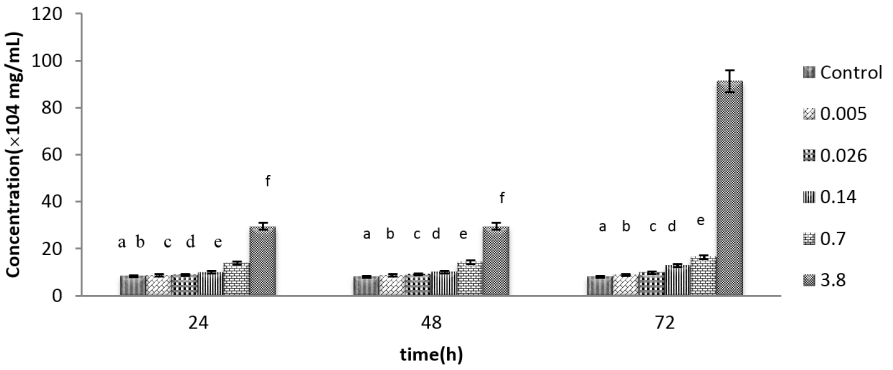

Fig. 7. Doubling time (G) during the experiment at different concentrations of *Dunaliella salina*

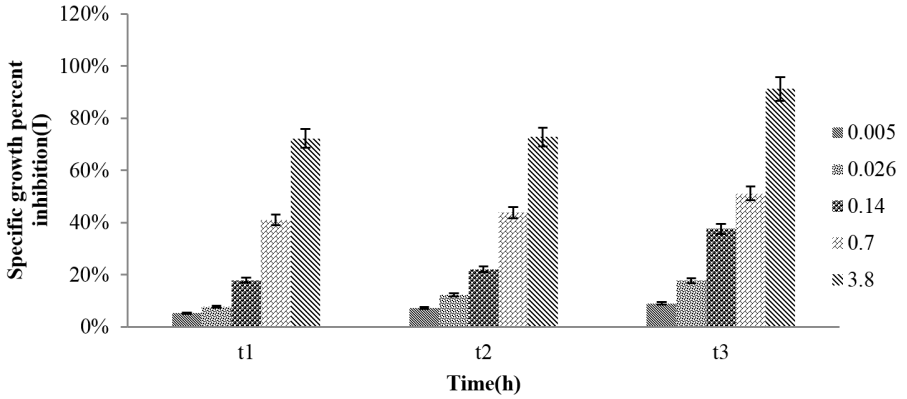

Fig. 8. Growth percent inhibition (I) of *Dunaliella salina* in contact with aluminum oxide nanoparticles

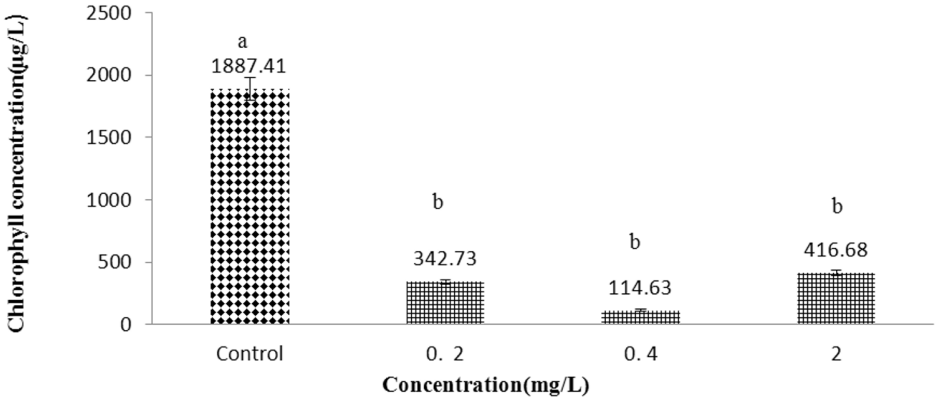

Fig 9. Mean chlorophyll a level at different concentrations of aluminum oxide nanoparticles. Treatments with at least one commonality are not statistically significant (p<0.05).

concentrations of aluminum and the levels of aluminum nanoparticles had a significant effect on chlorophyll concentration, so that the concentration of chlorophyll was higher in the control group than the other groups (p<0.05). However, no significant differences were found between the treatments (p>0.05) (Fig.9).

According to SIG=0.000 which is smaller than5%, it can be concluded that the carotenoid level of treatments has decreased following increasing concentration of aluminum and showed a significant reduction in comparison with the control (p<0.05) (Fig.10).

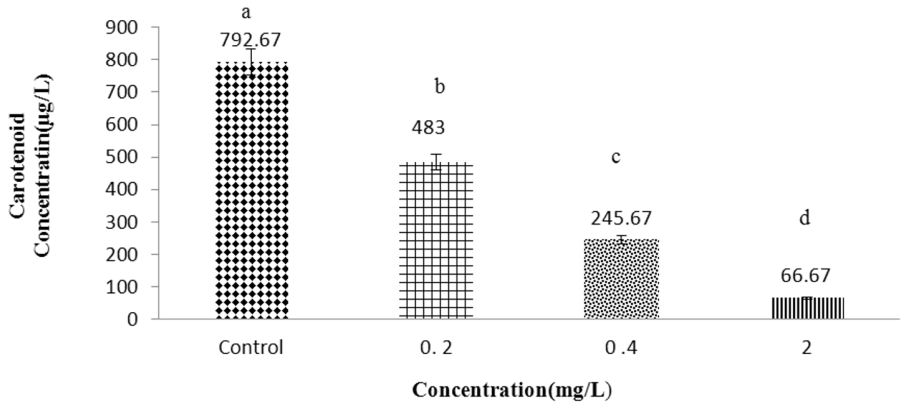

Fig. 10. Mean carotenoid concentration in various levels of aluminum oxide nanoparticles

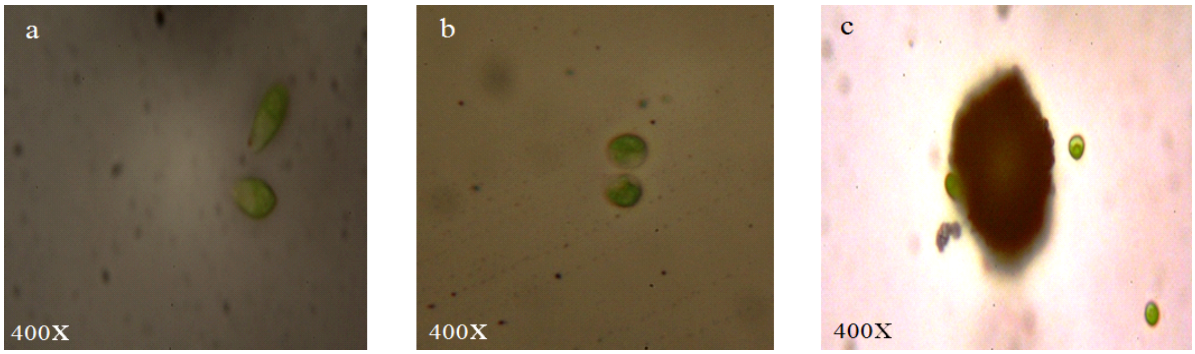

Fig 11. a: *Dunaliella salina* before contact with aluminum oxide nanoparticles; b and c: accumulation of nanoparticles on *Dunaliella salina* after 72 hours contact with aluminum oxide nanoparticles.

Figures b and c show that cells exposed to nanoparticles were shrunk and larger than the control cells (Fig.11; a, b, c).

According to form, shape, size, and number of cells covering the surface of the samples, the toxicity was evaluated after 72 hours of exposure. The image of nanoparticle-free control sample shows a nearly uniform distribution of particles size in all directions with a roughly elliptical shape with normal shape and size (Fig. 12; a, b). Due to the

presence of nano-sized particles, they were agglomerated. Agglomeration (accumulation and adherence) of fine particles with coarse ones leads to binding of nanoparticles and their aggregation (Fig. 12-c). In fact, the surface to volume ratio increased with decreasing particles size and thus increased the attraction force between particles, which in turn resulted in formation of strong agglomerates which leads to larger and more swollen cells of *Dunaliella salina* compared to the control samples, as in images (Fig. 12; d, e). According to Tables and Figures and the results of EC_{50} and NOEC, cell density decreased significantly with increase in concentration of nanoparticles (p<0.05) and toxic effects of aluminum oxide nanoparticles increased significantly after 48 hours of exposure to 0.026 mg/L and after 72 hours of exposure to 0.14 and 3.8 mg/L aluminum oxide nanoparticles. Increased concentration and contact time during a period led to an increase in growth percent inhibition of *Dunaliella salina* cells. The highest cell number, chlorophyll, carotenoid, specific growthrate, the lowest doubling time and the inhibitory concentration were observed in the control group.

Scanning electron microscopy revealed that the nanoparticles were highly agglomerated due to decreased surface to volume ratio. The toxicity of aluminum nanoparticles on *Dunaliella salina* suggests the changes in morphology and dimensions. Fujiwara (2008) studied the toxicity of aluminum nanoparticles on *Chorella kesslari* and found that $LC_{90}$was 0.6, 8.2, and 7.4 mg/L for 5, 26, and 78 nm nanoparticles, respectively, representing decreased nanoparticles toxicity by increasing their sizes(Fujiwara *et al*., 2008).

Manzo *et al.* (2013) studied the toxicity of zinc nano-oxide and zinc bulk on the *Dunaliella tertiolecta.* According to their findings, the 72-hour EC_{50} for zinc

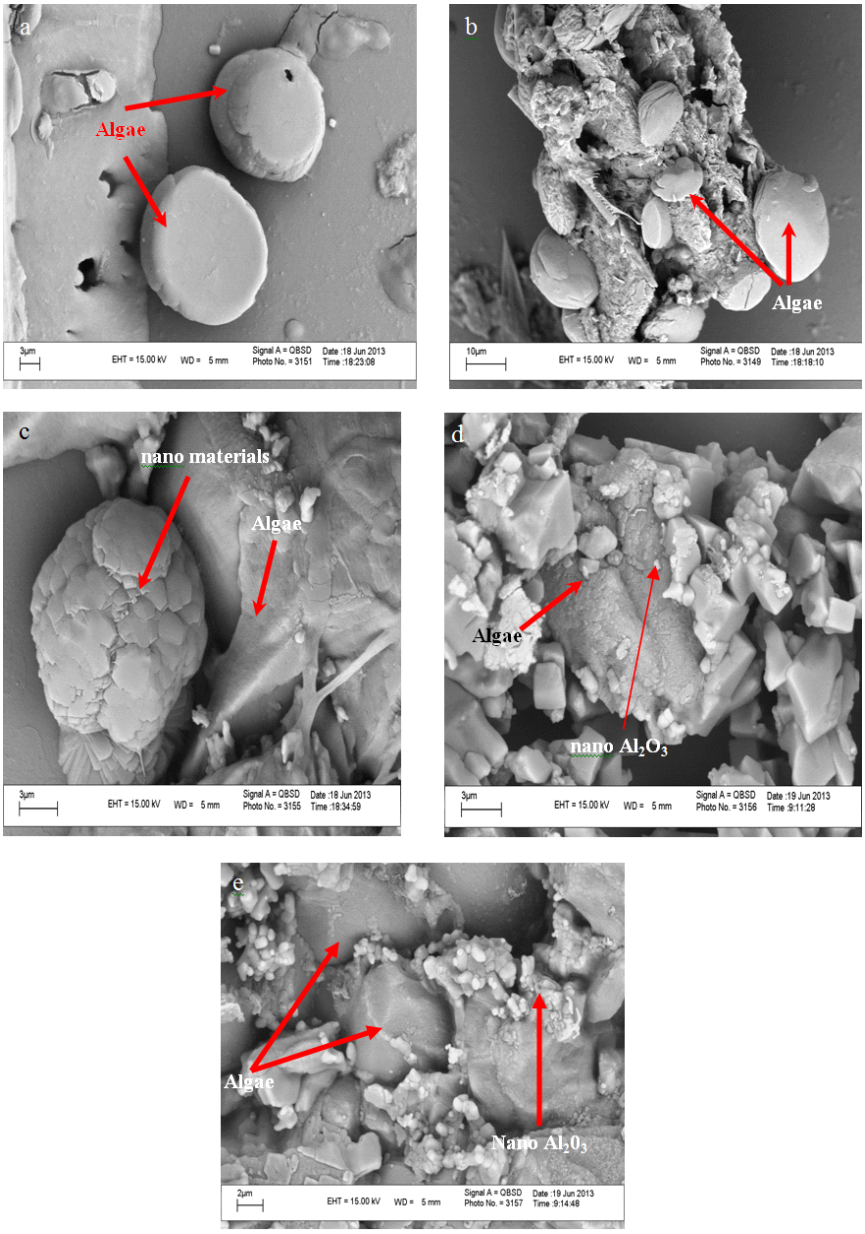

Fig. 12. Scanning electron microscopy (SEM); a-e: 72 hours exposure of *Dunaliella salina* to aluminum oxide nanoparticles

nano-oxide, zinc bulk, and zinc chloride was 3.57, 1.94, and 065, respectively. The results showed that zinc nano-oxide has the highest toxicity and the toxicity of nanoparticles is increased with increasing exposure time (Manzo *et al*., 2013).

Oukarroum *et al.* (2012) studied algae *Chlorella vulgaris* and *Dunaliella tertiolecta* and concluded that the effect of 50 nm silver nanoparticles for 24 hours at 0-10 mg/L concentration resulted in extensive compression of algal cells. In addition, algae chlorophyll was severely reduced. Silver nanoparticles had growth inhibitory effects in both species of algae. They also dramatically decreased cell survival (Oukarroum *et al*., 2013).

Mohammed Sadiq *et al.* studied the toxicity of aluminum (titanium) oxide nanoparticles on algae *Scenedesmus* and *Chlorella*. Growth was inhibited in both species and EC_{50} was 45.4 mg/L for *Chlorella* and 39.35 mg/L for *Scenedesmus* (Sadiq *et al.*, 2011).The results of this research also revealed the inhibiting effect of aluminum nano-oxide on *Dunaliella salina* growth. According to SEM images, aluminum oxide nanoparticles release Al^{3+} ions in culture medium that lead to changes in morphology and to shrinkage

of algae cells. Aluminum nano-oxide EC_{50} was 0.162 mg/L for *Dunaliella salina*. Marked difference in EC_{50} of aluminum oxide nanoparticles in various algae shows that *Dunaliella salina* is more sensitive than *Chlorella* and *Scenedesmus* and demonstrates higher toxicity. *Scenedesmus* is less sensitive than other two algae species (Sadiq *et al*., 2011). The effect of silica nanoparticles on zebra fish was investigated in 2010 and researchers concluded that smaller particles led to higher toxicity (Nelson *et al*., 2010). In 2009, the toxicity of silica nanoparticles on green algae *Pseudokirchneriella subcapitata* was studied and EC_{10} was calculated as 55 mg/L(Van Hoecke *et al*., 2009).

According to the results of this research, the findings are consistent with the study of Mohammed Sadiq *et al.* in terms of growth inhibitory effect of aluminum nano-oxide and its toxicity on morphology and size of algal cells, as well as the study of Fujiwara regarding the toxic effects of aluminum nano-oxide on algae. The acute toxicity of aluminum oxide nanoparticles in the present study was higher than the toxicity estimated by Fujiwara *et al.* This difference can be due to differences in the types of algae and properties of nanoparticles (Kahru *et al*., 2008).

Nanoparticles can highly damage the environment due to their unique properties such as small size and hence high surface and their mobility. Therefore, it is essential to identify the actual impact of nanotechnology before their appearance in environment as nano-waste and prior to introduction of new nano-products to the market. Proper measures can be taken to manage these new compounds and prevent irreparable pollution and its consequences.

CONCLUSIONS

Aluminum oxide nanoparticles have significant toxic and growth inhibition effects on *Dunaliella salina* algae. Data analysis showed a direct relationship between the concentration of nanoparticles and their toxicity on the algae. With increasing nanoparticles concentration, chlorophyll and carotenoid of *Dunaliella salina* decreased significantly (p<0.05).

REFERENCES

ASTM, (-1996). Annual book of ASTM standards, Water and Environmental Technology, Biologycal Effects and Environmental fate, Biotechnology, Pesticides, Printed in Easton, MD, USA, American society for testing and materials,Volume **11**/05.

Brennan, L. and Owende., P. (2010). Biofuels from microalgae- a review of technologies for production, processing, and extractions of biofuels and co-products. Journal of Renewable and Sustainable Energy Reviews, **14**, 557 – 577.

Botanical Monograph, Australian Whole dried *Dunaliella salina* .(2006). The new treasure in natural medicine. Report by International Clinical Laboratories**.4,** 10 .

Oberdorster, G., Sharp, Z., Atudorei,V., Elde, RA., Gelein, R., Kreyling,W. and Cox, C. (-2004). Translocation of inhaled ultrafine particles to the brain.Inhal Toxicologica, **16**,437-45.

Daughton, C.G.(2004). Non-regulated water contaminant : emerging research. Environmental Impact Assess Reviews, **24**, 711-320.

Finny, D. (-1971). Probit analysis Cambridge.Cambridge University Press, 1-333.

Fogg, G.E. and Thake, B. (1987). Algal culture and phytoplankton ecology (3rd edition). The University of Wisconsin Press, 12-56.

Fujiwara, K., Suematsu, H., Kiyomira, E., Aoki, M. and Moritoki, N. (2008). Size-dependent toxicity of silica nano particles to *chlorella kessleri*. Journal Environmental,Science,Health A Tox Hazard Subst Environmental Engineering, **43(10)**, 1167-1173.

Howard, C.V. (-2004). Small particles-big problem. International Laboratory News, **34(2),** 28-9.

Jiangxing, W., Zhang, X., Chen,Y., Sommerfeld-, M. and Qiang Hu. (-2008). Toxicity assessment of manufactured nanomaterials using the unicellular green alga *Chlamydomonas reinhardtti*. Chemospher, **73**, 1121-1128.

Jing, J., Zhifeng, L. and Daohui-, L. (2011). Toxicity of oxide nanoparticles to the algae *Chlorella sp*., Chemical Engineering Journal, **170**, 525-530.

Kahru, A., Dubourguier, H.C., Blinova, I., Ivask, A. and Kasemets, K. (2008). Biotests and biosensors for ecotoxicology of metal oxide nanoparticles. Aminirreview, Sensors, **8**, 5153- 5170.

Manzo, S., Miglietta, M.L., Rametta, G., Buono, S., Francia., G.D. (2013). Toxic effects of ZnO nanoparticles towards marine algae *Dunaliella tertiolecta*. Science of the Total Environment, **15**, 445-446.

Monteiro Ririere, NA., Lang Tran, C. (-2009). Nanotoxicology (-Characterization, Dosing and- Health Effects). University of Tehran Press, pp, 248.

Nelson, SM., Mahmoud, T., Beaux, M., Shapiro, P., Mcllroy, DN. and Stenkamp, D.-L. (2010). Toxic and teratogenic silica nanowires indeveloping vertebrate embryos. Nanomedicine, **6**, 93-102.

Lee, W. M., An, Y.J., Yoon, H. and Kweon, H.S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants Mung bean *(phaseolus radiatus)* and Wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. Environmental toxicology and chemistry, **27 (9),** 1948-1957.

OECD GUIDELINES FOR THE TESTING OF CHEMICALS, Test No. 201,- (-1984). Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

Oukarroum, A., Bras, S., Perreault, F. and Popovic, R. (-2012). Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, Ecotoxicology and Environmental safety, **78**, 80-85.

Perreault, F., Bogdan, N. and Morin, M. (2011).- Interaction- of gold nanoglycodendrimers with algal cells. Nanotoxicology, 1-12.

Sadiq, I.M.,- Dalai, S. and Chandrasekaran, N. (2011). Ecotoxicology study of TiO_2 nanoparticles on *Scenedesmus sp*.and *Chlorella sp*. Ecotoxicological and Environmental. Safety, **74**, 1180-1187.

Van Hoecke, K., De Schamphelaere, K., Vandermeeren-, P., Lcucas, S. and Janssen, C. (2009). The ecotoxicity of silica nanoparticles to the algae *Pseudokirchneriella subcapitata:* importance of surface area. Environmental and Toxicological Chemistry, **27**, 127-136.

Videa, J. R., Zhao, L. and Lopez-Moreno, L.(2011). Nanomaterials and the environment: A review for the biennium 2008-2010, Journal of Hazardous materials, **186**, 1-15.

Wei, C., Zhang, Y., Guo, Han, B., Yang, X. and Yuan, J. (2010). Effects of silica *n*anoparticles on growth and photosynthesis pigments contents of *Scenedesmus obliquus*, Journal of Environmental Science, **22**, 155-160.