Toxicity of Zinc Oxide Nanoparticles to *Chlorella vulgaris* and *Scenedesmus dimorphus* Algae Species

Hamide Pendashte, Fatemeh Shariati, Abdolkarim Keshavarz and Zohreh Ramzanpour

1Department of Environmental Engineering, Natural Resources Faculty, Islamic Azad University, Lahijan Branch, Lahijan, Iran
2Department of Environment, Islamic Azad University, Lahijan Branch, Lahijan, Iran
3Dr. Dadman International Sturgeon Research Institute, Rasht, Iran

**Abstract:** Taking into consideration the increasing application of nanoparticles resulting to their entry into industrial and non-industrial wastes makes necessary to investigate on potential effects of these materials on aqueous environments. One of these common compounds is zinc oxide nanoparticle which as a ceramic absorbent material has different applications. In this research the effect of this nanoparticle is investigated on two algae species *Chlorella vulgaris* and *Scenedesmus dimorphus*. The appropriate experimental concentrations were determined by performing range-finding tests and the effect of each concentration (5 treatments and three replicates for each) of ZnO nanoparticle was studied during three time periods of 24, 48 and 72 hours against the control. The experiment was performed based on Organization for Economic Cooperation and Development (OECD) method. The number of algae cells was daily counted. Data analysis was performed using Probit analysis. The amounts of EC, EC, EC, No Observed Effect Concentration (NOEC), specific growth rate, doubling time and percentage of growth inhibition were calculated. The amounts of cell density and growth inhibition in control were significantly different (p<0.05) from those in treatments of nanoparticle. 72h EC value is 0.01 mg/l for *Chlorella* and 0.09 mg/l for *Scenedesmus*. The sensitivity of *Chlorella* species to ZnO nanoparticle is much more than *Scenedesmus* species.

**Key words:** *Chlorella vulgaris* • EC, • Growth Inhibition • *Scenedesmus dimorphus* • ZnO Nanoparticle

**INTRODUCTION**

Nanotechnology is the science of particles in atomic scale. As particles size reduces to 0.1 nm or less, quantum effects appear. These effects can change optical, magnetic and electrical properties of materials to a large extent. By studying the structure of materials in nano scale, it is possible to design and manufacture new materials with completely new properties [1-4].

Metal oxide nanoparticles have been recently manufactured at industrial level and have tremendous applications in water treatment, medicine, cosmetics and engineering. For example, zinc oxide nanoparticles are starting material for electronics applications, transparent UV-protection films and chemical sensors [4-6] as well as UV-filters in sunscreens [7-11].

Bioassays are used to determine the toxicity of chemical substances and to indicate which organisms are the most sensitive to such chemicals which are done on a wide range of organisms [12-19]. However, only few studies have investigated nanoparticle toxicity to algae. Hund-Rinke and Simon [20] have studied 25 nm and 100 nm TiO₂ particles in regard to their toxicity to the green algae *Desmodesmus subspicatus* and found that smaller particles were more toxic (72 h EC₅₀ of 25 nm particles was 44 mg/l, for 100 nm particles the EC₅₀ was beyond the tested range, >50 mg/l). TiO₂ particles have also been tested with the alage *P. subcapitata*. In a study with “fine” (~99% TiO₂ core with ~1% Al surface coating, median particle size ~380 nm according to dynamic light scattering) and “ultrafine” particles (median particle sizes of ~140 nm; 90 wt.% TiO₂, 7% alumina and 1% amorphous...
silica) there was little difference between toxicities: EC<sub>50</sub> values were determined to be 16 mg/l for “fine” and 21 mg/l for “ultrafine” particles [21].

Natasha et al. [22] have studied the toxicity of ZnO nanoparticles to P. subcapitata while also determined the concentration of dissolved Zn ions derived from ZnO. The toxicity of ZnO particles as well as ZnCl<sub>2</sub> was found to be essentially due to dissolved Zn. Navarro et al. [23] and Lubick [24] suggested the toxicity of nanoAg could not be solely resulted from the release of Ag<sup>+</sup> ions; the interaction of nanoparticles with algae also played an important role. Likewise, the toxicity of nanoCeO<sub>2</sub> was not ascribed to the dissolved Ce<sup>2+</sup> ions, but to the entrapment of algal cells into the aggregates of nanoparticles [25].

Sadiq et al. [26] showed that the growth inhibitory effect of alumina nanoparticles occurred for both (72 h EC<sub>50</sub> value, 45.4 mg/l for Chlorella sp.; 39.35 mg/l for Scenedesmus sp.). Bulk alumina also showed toxicity though to a lesser extent (72 h EC<sub>50</sub> value, 110.2 mg/l for Chlorella sp. and 100.4 mg/l for Scenedesmus sp.) [26].

According to Aruoja et al. [27], EC<sub>50</sub> of TiO<sub>2</sub> nanoparticle was 44 mg/l for Scenedesmus species. Tsai et al. observed no differences among toxicity of nanoparticulate ZnO, bulk ZnO and ZnCl<sub>2</sub> and these three materials had the same toxicity with LC<sub>50</sub> value equal to 0.068 [28].

Jing et al. [29] have studied toxicity of oxide nanoparticles to the green algae Chlorella sp. No significant toxicity was observed for nano-SiO<sub>2</sub> with concentration up to 1000mg/L during the 6 days inhibited the algal growth by ca. 20% (p<0.05) at the 2nd day. Both nano-ZnO and bulk-ZnO significantly inhibited the algal growth with a percent survival compared to the control of 48.8±8.7% and 59.0±11.4% at 1000mg/L, respectively.

According to Persoone and Manusad-ianas et al. cupric ions toxic effects were observed on unicellular algae Selenastrum capricornutum (growth inhibition, 72h EC<sub>50</sub>= 0.04 mg/l), macrophytic algae Nitellopsis obtusa (96h LC<sub>50</sub> = 0.13 mg/l) [30-31].

Manusad-ianas et al. [32] reported that among the three test organisms, macrophytic algae cells Nitellopsis obtusa was substantially less susceptible to nanoZnO particle toxicity (effect concentrations ranging 500-1000 mg/l) than shrimps Thamnocephalus platyurus (0.09-0.21 mg/l) and rotifers Brachionus calyciflorus (0.34-2.11 mg/l).

In this research, ZnO nanoparticle, one of the most applicable nanoparticles [5, 22, 27, 29, 32] which is used in cosmetic industries and possibly enters to aquatic environments through the sewages of the mentioned industriens tested [3, 33-37]. Because of growing trend in the use of ZnO nanoparticle [37-41] investigating its effects on different levels of the food chain specially phytoplanktons as producers at basic level of aquatic food chain sounds essential.

**Chlorella vulgaris and Scenedesmus dimorphus** algae which largely distribute in fresh waters are good species for biological toxicity tests [4, 9, 22, 23, 26, 27, 29]. Since algae are located in the base of food chain, any changes in their density, biomass and population affect the whole food chain. So, studying the response of these organisms to ZnO nanoparticle is very important. Therefore, the effect of this nanoparticle on growth inhibition and also EC<sub>50</sub> and EC<sub>90</sub> were determined for Chlorella vulgaris and Scenedesmus dimorphus algae.

**MATERIALS AND METHODS**

**The Research Method:** All steps of range-finding and main experiment were performed according to OECD 201 method [42, 43].

**Nanoparticles:** ZnO nanoparticles produced by Iranian nanomaterials Pioneers Company, were used (Iran nanomaterials pioneers, 2011). The size of used ZnO nanoparticles was 20nm. A 250 mg/l stock solution was used for preparing appropriate concentrations.

**Preparation of Culture Medium and Cultivation of Algae:** Four main stock solutions were used for preparing Zander culture medium. pH of culture medium was adjusted to 6.8. The culture medium was sterilized in autoclave at 121°C (model 121 A, Iran production company, Iran) for 15 min. Then the bottles were refrigerated at 6°C.

In order to cultivate the algae, Chlorella vulgaris and Scenedesmus dimorphus algae were initially purified. For this purpose, water sample from a natural environment passed through a 20 micron net and was transferred to the culture chamber, which was sterilized by ultraviolet ray and purified under inverted microscope and semi-dense cultivation was conducted in Zander culture medium at 23 to 25°C, under light intensity of 3500±300 Lux and light program of 12:12, 12 hourslight and 12 hours darkness [42].

**Preparation of Main Treatments for Testing Chlorella vulgaris and Scenedesmus dimorphus:** For Chlorella algae, three range-fining experiments whose conditions were the same as the main experiment were performed and then suitable concentrations were determined based on...
RESULTS

The data pertaining to reduction of algae cells, which were obtained by continuous 24-hour recording during 72-hour test period, were compared to the control results. Regression curves were plotted for each 24 hour period using the amount of reduction in cells number comparing to the control and its respective Probit value from Probit values table. The values of EC_{90}, EC_{50}, EC_{30} and NOEC for both Chlorella vulgaris and Scenedesmus dimorphus species were calculated per 24 hour. Specific growth rate, doubling time and growth inhibition percentage were derived from the calculated values. The calculations were performed based on confidence level of 95%.

Cell Count Test (cell.ml^{-1}) for Chlorella vulgaris Algae: Fig. 1 showed toxicity of ZnO nanoparticle on Chlorella vulgaris algae cells and it is observed that at 24 hours after the start of the experiment the number of cells reduced as the concentration increased. But this reduction was more significant at concentration of 1 mg/l.

Cell Count Test (cell.ml^{-1}) for Scenedesmus dimorphus algae: The comparison of the control with each treatment revealed that in Scenedesmus dimorphus species, an obvious reduction in algae cells was observed after 24, 48 and 72 hours (Fig. 2). This reduction in cell density during 72 hours indicates the acute toxicity of ZnO nanoparticle and its effect on Scenedesmus algae growth. The results showed that growth inhibitory effects and reduction in cell density appear after 24 hours.

Specific Growth Rate ($\mu$) of Chlorella vulgaris and Scenedesmus dimorphus Algae after exposure to ZnO nanoparticle: When studying specific growth rate ($\mu$) in Chlorella vulgaris, it was observed that ZnO nanoparticle reduced specific growth rate. In Scenedesmus dimorphus species, a reduction in specific growth rate was observed at all concentrations after 72 hours. This trend is continuously increasing in control (Figs. 3 and 4).

Doubling Time ($G$) in Chlorella vulgaris and Scenedesmus dimorphus algae after exposure to ZnO nanoparticle: In both Chlorella vulgaris and Scenedesmus dimorphus species, G parameter in control had a decreasing trend over time while this trend was increasing at all concentrations of the nanoparticle (Figs. 5 and 6).
Growth Inhibition Percentage (1%) in *Chlorella vulgaris* and *Scenedesmus dimorphus* Algae Afterexposure to ZnO Nanoparticle: For both *Chlorella vulgaris* and *Scenedesmus dimorphus* species, an increase in growth inhibition percentage was observed as time and concentration increased. This value was continuously zero in the control (Figs.7 and 8).

Effective Concentration (EC) and No-Observed Effective concentration (NOEC) of ZnO nanoparticle in *Chlorella vulgaris* and *Scenedesmus dimorphus* species: In Chlorella species, the amount of EC$_{50}$ during 72 hours
Fig. 8: Growth inhibition percentage (I%) versus time for \emph{Scenedesmus dimorphus} at different concentrations of ZnO nanoparticle.

Table 1: EC and NOEC of ZnO nanoparticle to \emph{Chlorella vulgaris} and \emph{Scenedesmus dimorphus} species

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<thead>
<tr>
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<th>Chlorella vulgaris</th>
<th>Scenedesmus dimorphus</th>
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<tbody>
<tr>
<td>72h EC_{50} (mg/l)</td>
<td>$10^{-2}$-2.000</td>
<td>$9.908 \times 10^{-4}$</td>
</tr>
<tr>
<td>72h EC_{50} (mg/l)</td>
<td>0.013</td>
<td>0.090</td>
</tr>
<tr>
<td>72h EC_{50} (mg/l)</td>
<td>7.663</td>
<td>8.227</td>
</tr>
<tr>
<td>NOEC (mg/l)</td>
<td>$1.300 \times 10^{-3}$</td>
<td>$0.900 \times 10^{-2}$</td>
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was less than that in Scenedesmus species. This indicates that ZnO nanoparticle is more toxic to Chlorella species (Table 1).

**DISCUSSION AND CONCLUSION**

This research showed that acute toxicity of ZnO nanoparticle to Chlorella species is much more than Scenedesmus species. The difference between effective concentrations (EC_{50}) in both algae indicated that the amount of the nanoparticles toxicity is also dependent on algae species. In this regard, based on our results Chlorella is nine times more sensitive than Scenedesmus. This is in agreement with findings by Franklin et al. [44] who studied the effect of ZnO nanoparticle on Scenedesmus algae.

According to their results, EC_{50} of ZnO nanoparticle was calculated to be 60 mg/l for \emph{Pseudokirchneriella subcapitata} species. So the sensitivity of Chlorella and Scenedesmus species to ZnO nanoparticle is much more than of \emph{Pseudokirchneriella subcapitata} algae [4].

According to Aruoja et al. [27] in which EC_{50} of TiO\textsubscript{2} nanoparticle was 44 mg/l for Scenedesmus species and our results, the toxicity of ZnO nanoparticle to Scenedesmus species is 7333 times more than the toxicity of TiO\textsubscript{2} nanoparticle to this species (Table 1).

These considerable differences between results of mentioned studies and present research can be due to genetic differences of the studied algae species as well as differences in nanoparticles and experimental methods [4, 27]. The results of present research also indicated that metal oxide nanoparticles can have different toxicity effects and their toxicity depends on their nano structures and high surface to mass ratio as well as the nature of their constitutive element [27].

Taking these results into the consideration, it can be stated that setting and applying standards for disposal of ZnO nanoparticle need more precaution in comparison to TiO\textsubscript{2} nanoparticles.

Van Hoecke et al. [25] investigated the toxicity of CeO\textsubscript{2} nanoparticle to \emph{Pseudokirchneriella subcapitata} species and found that LC_{50} is 56 to 100 mg/l. Based on the investigations conducted on these algae species, ZnO nanoparticle has the highest toxicity followed by aluminum oxide, titanium dioxide and cesium oxidenanoparticles, respectively. Therefore, disposal of ZnO nanoparticles needs more attention and precaution and more strict laws must be regulated for disposal of ZnO nanoparticles in aquatic environments.

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