

Toxicity Mechanisms of ZnO Nanoparticles in *Nanochloropsis Oculata* with a Comparative Approach with CuO and Ag Nanoparticles

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Research Article

Keywords: Nannochloropsis, ZnO nanoparticles, Zinc ion release, Antioxidant responses, EDX

Posted Date: November 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1001406/v1>

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Abstract

The purpose of present work was the investigation of different concentrations of zinc oxide nanoparticles on the marine microalga *Nannochloropsis oculata* and compare the results of this study with previous studies. Dissolution of ZnO NPs in nanopure water was 0.378-3.12 mg/L and the rate solubility decreased with increasing the concentrations of ZnO NPs. ZnO NPs were toxic to this microalga with EC50 of 153/72 mg/L. The toxicity of 200 mg/L ZnO NPs was 59.36% for the cell number, 61.27% for MTT test, and 57.34% for the chlorophyll content. Increase the content of malondialdehyde and hydrogen peroxide in response to increasing the concentration of ZnO NPs was indicated the induction of oxidative stress in *N. oculata*. The activity of catalase and lactate dehydrogenase increased in the treated cells, while the activity of ascorbate peroxidase was decreased. Concurrently, an increase in the content of carotenoids and phenolic compounds was observed in the treated cells. SEM and TEM analyses confirmed the aggregation of algal cells, damages in cell membrane and atypical changes in morphology of cell wall after NPs treatments. The FTIR results confirmed the interaction of ZnO NPs with C-H, C-O and C=O groups on the cell surface. All of these changes were indicated the significant toxic impacts of ZnO NPs on the *N. oculata* cells. Comparison between the results obtained in previous studies with our results showed that the defensive mechanisms of *N. oculata* probably was not effective against the oxidative stress by >10 mg/L of ZnO NPs, > 5 mg/L of CuO NPs and > 1 mg/L of Ag NPs. Therefore, *N. oculata* is sensitive to such concentrations of these NPs.

Highlights

- The concentrations of more than 5 mg/L of ZnO NPs cause significant toxicity in the marine microalga *N. oculata*.
- The solubility of ZnO NPs decreased with increasing the concentrations of this NP.
- The Formation of MDA, SEM and TEM images and LDH activity confirmed the damage of cell membrane and cell wall in high concentrations of ZnO NPs.
- Increase of the content of carotenoids and phenolic compounds as well as the activity of CAT and PPO represented defense mechanisms have been activated through ZnO NPs in response to oxidative stress.
- Defensive mechanisms of *N. oculata* probably was not effective against the oxidative stress by >10 mg/L of ZnO NPs, > 5 mg/L of CuO NPs and > 1 mg/L of Ag NPs.

1. Introduction

Nanoparticles (NPs) as atomic or molecular aggregates with a diameter of 1 - 100 nm possess different physical and chemical properties compared to the bulk materials (Dolatabadi et al. 2015). The usage of engineered metal oxide NPs has increased in recent years in material science and commercial products (Aravantinou et al. 2013). ZnO NPs have wide applications in different products such as paints, coatings, cosmetics and household appliances because of their bactericidal and fungicidal properties (Manzo et al.

2011; Choi et al. 2016; Zafar et al. 2016). According to an estimation, the production of ZnO NPs is around 55-528 tons/year and there is an increase in production and utilization with time (Choudhary et al. 2018). Large-scale production and consumption are resulted in discharge of the nanomaterials-containing products to aquatic ecosystems and agricultural lands (Lead et al. 2008). Thus, the risk of natural water contamination by synthetic NPs was continuously enhanced (Lead et al. 2008). Nonetheless, the potential toxic effects of NPs on living organisms are poorly studied. This is mainly because of the complexity of factors that influence the toxicological characteristics of nanomaterials, including size, surface/area ratio, morphology, surface coatings and their other physicochemical properties (Ates et al. 2013; Zaka et al. 2016). The toxicity of metal oxide NPs on living organisms was reported to depend on several parameters including: (1) the internalization of NPs; (2) the shading effect; (3) surface modification and interaction with media; (4) the generation of reactive oxygen species (ROS); (5) release of metal ion (Wang et al. 2012; Aruoja et al. 2009; Zafar et al. 2016; Chen et al. 2012 c; Aravantinou et al. 2013; Suman et al. 2015).

In a number of previous works, toxicity issues have been recognized mainly as a consequence of the released zinc ions from the NPs (Ates et al. 2013; Song et al. 2016). It was also suggested that toxic effects on living organisms were induced by not only zinc ions but also tiny NPs (Wong et al. 2010; Song et al. 2016). Taken together, biotoxicity studies of ZnO NPs revealed that zinc ions released from NPs can intensify the production of ROS and cause oxidative damage in cells (Sharifi et al. 2012; Suman et al. 2015). Investigations on algal cells showed that different metal oxide NPs like titanium oxide, zinc oxide, cerium oxide, and silver oxide could be absorbed by the algal cells and induce oxidative stress through the production of ROS (Xiao et al. 2008). Overproduction of ROS is believed to be a main mechanism of the NPs toxicity (Suman et al. 2015). Algal species contain enzymatic and non-enzymatic antioxidant defense systems to protect cells from oxidative damages (El-Baky et al. 2007). The primary scavenging enzymatic defense systems include superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Dolatabadi et al., 2015). Non-enzymatic defense compounds are composed of carotenoids, phenols, ascorbic acid and chlorophyll derivatives (El-Baky et al. 2007; Zafar et al. 2016).

The toxicity of ZnO NPs on plants (Zafar et al. 2016), bacteria (Hsueh et al. 2015), zebrafish (Choi et al. 2016), nematodes (Wang et al. 2009) and soil organisms (Manzo et al. 2011) have been formerly investigated. At the base of ecotoxicity indicator organisms, algae are considered as representatives of primary producers that play an essential role in the aquatic ecosystems (Skjoldung et al. 2016). However, there are only limited studies on the toxicity of ZnO NPs on microalgae. The biotoxicity of ZnO NPs and bulk ZnO on *Pseudokirchneriella subcapitata* was previously confirmed (Franklin et al. 2007; Aruoja et al. 2009). Nano ZnO (EC50 1.94 mg/L) was more toxic than its bulk counterpart (EC50 3.57 mg Zn/L) for *Dunaliella salina* (Manzo et al. 2013). Also, the evaluation of toxicity of ZnO NPs on *Chlorella vulgaris* by flow-cytometric and cytotoxicity assays showed a substantial reduction in the viability of cells on a dose dependent manner (Suman et al. 2015).

The unicellular algae *Nannochloropsis oculata* (Eustigmatophyta) was considered as one of the most promising marine microalgae for eicosapentaenoic acid (20:5) production, an important polyunsaturated

fatty acid for human consumption for prevention of several diseases (Forján et al. 2011). Biomass productivity of this microalgae can be 50 times higher than that of the fast growing terrestrial plants (Li et al. 2008). Thus, *N. oculata* was selected as a model marine biosystem, on the basis of its presence in municipal wastewater, its potential use for biofuel, and its extensive usage as a source for commercially valuable compounds (Forján et al. 2011; Taylor et al. 2012; Aravantinou et al. 2013). The effect of CuO NPs and Ag NPs investigated on *N. oculata* (Fazelian et al. 2019, 2020b). Fazelian et al. (2020a) have also compared the toxicity of ZnO NPs with Fe₂O₃ and CuO NPs in *N. oculata*, but did not determine whether this toxicity was due to ZnO NPs, Zn²⁺ release, and / or induction of oxidative stress. Therefore, the main aim of this study was to examine the toxicity of ZnO NPs to *N. oculata* by measuring ionic dissolution and some oxidative stress indices. The content of hydrogen peroxide (H₂O₂) as well as malondialdehyde (MDA) and the activity of some antioxidant enzymes (CAT, APX and PPO) were assessed. Moreover, the interaction of cell wall with ZnO NPs were investigated using FTIR and TEM techniques. Here we focus on the comparative aspects of the results of our research with previous studies (Fazelian et al. 2019, 2020a, 2020b).

2. Materials And Methodss

2.1. Algal culture

The strain of *N. oculata* was obtained from Ecological Research Institute of Persian Gulf and was cultivated in f/2 medium. F/2 growing media was prepared ahead of time for algal growth. To prepare this media, seawater was filtered and autoclaved for 20 min at 121 °C to sterilize the water. Subsequently, macronutrients, trace metals, and essential vitamins were added to the seawater. 50 mL of a mother culture of *N. oculata* was inoculated into the 500 ml Erlenmeyer flasks containing 350 mL of liquid f/2 medium. The cultures were then uniformly mixed and an initial count of cell number in solution was performed using a Neubauer counting chamber. The cultures were exposed to 26-28 °C and a 12:12 light-dark cycle of 5000 lux illumination (Fazelian et al. 2019).

2.2. Nanoparticles characterization

ZnO NPs were purchased from the nanomaterials pioneers company (Houston, TX, USA). The ZnO NPs had particle size smaller than 100 nm and a specific area of 20-60 m²/g. The size and shape of ZnO NPs were assessed using the conventional methods for NPs characterization such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-ray diffraction (XRD) (Table. 1). A stock solution of 10 g/L ZnO NPs was prepared by dispersing the NPs in Milli-Q water with a ultrasonic bath for 30 min to separate the suspending particulates and NP aggregates. Different concentrations of ZnO NPs including 0, 5, 10, 50, 100 and 200 mg/L were prepared by stock solution.

2.3. Dissolution of ZnO NPs

Different concentrations of ZnO NPs (5-200 mg/L) were obtained by dispersing ZnO NPs in f/2 medium, and 10 mL of each treatment was centrifuged at 10,000 rpm for 15 min and the supernatants were

collected. The concentrations of Zn^{2+} in the supernatants were measured using atomic absorption spectrometry (Suman et al. 2015).

2.4. Nanoparticle exposure and algal growth evaluation

For assessing the effects of ZnO NPs on the growth of *N. oculata*, the media containing 4×10^4 cells mL^{-1} of alga were incubated for 4 days. Then, the algal cultures were exposed to different concentrations of ZnO NPs (5, 10, 50, 100, and 200 mg/L) for 72 h. The toxicity tests for algae were performed for NPs according to the Organization for Economic Cooperation and Development testing guidelines. After 72 h, cell density as a growth indicator was counted using a Neubauer chamber. After counting the algal cells, cell density, cell viability and cytotoxicity were calculated using the following formula (Fazelian et al. 2019):

Cell density per ml = Average number of cells in squares $\times 10^4$

% Viability = Number of viable cells / Total number of cells counted $\times 100$

% Cytotoxicity = $100 - \% \text{ Viable cell}$

2.5. Assessing the photosynthetic pigments

The concentration of chlorophyll a and carotenoids were determined by Jeffrey and Humphrey (1975). The samples were centrifuged and immersed in acetone (85% V/V) in darkness and dark. After 24 h, the absorption of the solvent was investigated in 664 nm, 647 nm, and 470 nm.

2.6. Total phenol content

To determine the total phenol contents, algal samples were homogenized in methanol for 24 h in the dark. The homogenate was centrifuged at 10000 g for 15 min and then 2.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL of 7.5% $NaHCO_3$ were added to the 0.5 ml of methanolic extract. The content of phenolic compounds was estimated using absorbance at 765 nm. The results were expressed as mg of GA/g of extract (Singleton et al. 1999).

2.7. Cytotoxicity assay

Tetrazolium reduction assay (MTT test), lipid peroxidation, hydrogen peroxide (H_2O_2) content, and the activity of antioxidant enzymes including catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) were measured according to the methods which described in our previous work (Fazelian et al. 2019)

2.8. Determining the activity lactate dehydrogenase (LDH)

LDH activity was measured spectrophotometrically in the pyruvate/lactate direction by recording the decrease of absorbance at 340 nm. 1 ml of the cell suspensions was centrifuged at 6000 rpm for 15 min and the supernatant was harvested. The assay mixture contained 100 μ l of 30 mM sodium pyruvate, 2.8 mL of 0.2 M Tris-HCl and 100 μ l of 6.6 mM NADH (Suman et al. 2015; Fazelian et al. 2019).

2.9. Microscopic analysis

Field emission scanning electron microscopy (FESEM) was used to examine surface morphology. The samples were freeze-dried, prepared and analyzed with FESEM (Zeiss-Sigma VP-500, Germany).

For Transmission electron microscopy (TEM) analysis, the primary-fixation of algal samples was performed with 4% glutaraldehyde diluted with 0.1 M cacodylate buffer. By 1% osmium tetroxide in the same buffer was carried the secondary fixation. Embedding in resin and sectioning was conducted after dehydration. The sections were post-stained and observed by a with TEM (Zeiss-EM10C-100 KV, Germany) (Fazelian et al. 2019, 2020b).

2.10. Surface chemical analysis through Fourier-transform infrared spectroscopy (FTIR)

After treatment of cells for 72 h with 100 mg/L of ZnO NPs, 10 ml of suspension was collected by centrifugation for 15 min at 6000 rpm. The algal samples were dried by freeze dryer and the dried samples were investigated with a potassium bromide technique using a Perkin-Elmer FTIR spectrometer. Finally, the samples were scanned from 400-4000/cm (Fazelian et al. 2019).

2.11. Statistical analysis

All experiments were performed in 3 replicates. The data were shown as mean \pm standard error (SE), and the means were compared by Duncan's test. Statistical significance was considered at $P \leq 0.05$.

3. Results

3.1. Dissolution of ZnO NPs

According to Fig. 1.A., Zn^{2+} ion concentration was slightly raised with the increase of ZnO NPs concentration. A slight increase of the released zinc ions was observed, likely due to the availability of free ZnO NPs. The concentration of the dissolved Zn^{2+} at the concentration of 5 mg/L of NPs was 0.378 mg/L, while at 200 mg/L of ZnO NPs only a quantity of 3.12 mg/L of Zn^{2+} ion was produced.

3.2. Growth Parameters

The results of this work revealed that ZnO NPs were toxic to this microalga with EC₅₀ of 153/72 mg/L. The MTT test and cell number analysis confirmed the toxic effect of 10-200 mg/L of ZnO NPs on *N. oculata*. Actually, a decrease of algal cell viability was found after 72 h exposure to ZnO NPs (Fig. 2.A). According to the Fig. 2.B, 10 mg/L of ZnO NPs (0.386 mg/L Zn^{2+}) and 200 mg/L of NPs (2.2 mg/L of Zn^{2+}) respectively displayed 9.99% and 61.27% toxicity for MTT test and 15.24% and 59.36% for cell number. Also, 5 mg/L of NPs (0.378 mg/L Zn^{2+}) did not cause any toxicity on the algal cells. Based on the results of cell counts, ZnO NPs showed toxicity in a concentration-dependent manner (Fig. 2.B).

3.3. Photosynthetic Pigments

Treatments of *N. oculata* with ZnO NPs resulted in significant changes in chlorophyll a content (Fig. 3.A). Although 5 mg/L of ZnO NPs did not show remarkable changes in the amount of chlorophyll a, 10-200 mg/L concentrations of ZnO NPs caused a significant decrease in the content of chlorophyll a compared to the control. The content of carotenoids was increased in algal cells after exposure to 5-100 mg/L of ZnO NPs, but was not observed the significant change in 200 mg/L (Fig. 3.B).

3.4. Phenolic Compounds and PPO Activity

The content of phenolic compounds in *N. oculata* was increased by 10-200 mg/L of ZnO NPs (Fig. 3.C). In contrast, the PPO activity in *N. oculata* increased significantly under 50 mg/L of ZnO NPs treatment and decreased in higher concentrations (Table 2).

3.5. Oxidative Stress

Our results revealed that exposure of *N. oculata* cells to 50-200 mg/L of ZnO NPs increased MDA content as a marker for oxidative stress (Fig. 4.A). These findings showed that ZnO NPs established oxidative damages, possibly by generating ROS (Fig. 4.B). The effect of different concentrations ZnO NPs (10-200 mg/L) on H₂O₂ content of *N. oculata* was also significant at the level of $P < 0.05$. (Fig. 4.B).

The activity of CAT was considerably promoted by treatment of ZnO NPs ($P < 0.05$) and the highest CAT activity was observed at the concentration of 200 mg/L of NPs. The enzyme activity did not show significant changes in presence of 5 and 10 mg/L of ZnO NPs (Table 2). The application of ZnO NPs significantly decreased APX activity in the algal cells ($P < 0.05$), however, 5 mg/L of ZnO NPs did not cause remarkable change in the APX activity (Table 2).

3.6. LDH Activity

Enhanced LDH activity in *N. oculata* cells was observed after the treatment of algal cells with the increasing concentrations (50-200 mg/L) of ZnO NPs. The significant changes of LDH activity was not observed in response to 5-10 mg/L of ZnO NPs (Table 2).

3.7. SEM and TEM Analysis

EDX graph showed the presence of ZnO NPs in *N. oculata* cells (Fig. 1.B). SEM images confirmed that the particle size of ZnO NPs was ranging from 20 to 75 nm (Fig. 5.A-C). Study of the algal cells revealed the membrane damages in the treated *N. oculata* cells with 100 mg/L ZnO NPs (Fig. 5.D). The aggregation of ZnO NPs and the algal cell aggregation also observed in Fig. 5.A-D and Fig. 6.A and D. respectively.

TEM images of control and treated algal cell demonstrated that after exposure to ZnO NPs, the algal cell have shrunken and formed some wrinkles (Fig. 5.E-H). The entry of ZnO NPs into the *N. oculata* cells was

also observed (Fig. 5.H). The entry of ZnO NPs into the algal cell can also be demonstrated according to the XRD image (Fig. 1.).

3.8. Surface Characterization by FTIR

FTIR analysis of control sample showed the existence of reactive groups on the algal surface (Fig. 6.), while the treated sample showed a variation in the number of peaks. A control spectrum represented peaks at 3412.23 cm^{-1} : O-H group, 2926.24 cm^{-1} and 614.37 cm^{-1} : C-H group, 1639.91 cm^{-1} : C=O group and 1020.30 cm^{-1} : C-O group (Table 3) (Crist et al. 1994; Wahab et al. 2008; Phukan et al. 2011; Suman et al. 2015; Sukarni et al. 2015). Exposure of *N. oculata* to ZnO NPs resulted in peaks at 2925.10 cm^{-1} : C-H group, 1637.28 cm^{-1} and 1617.06 cm^{-1} : C=O group, 1023.46 cm^{-1} C-O and C-O-C groups, and new peak characteristic of 480.15 cm^{-1} stretching band, that represents the presence of ZnO NPs on the algal cell surface (Fig. 6.) (Wahab et al. 2008; Suman et al. 2015).

4. Discussion

The results of zinc ion solubility showed that the concentration of Zn^{2+} was enhanced with increasing ZnO NPs treatment but the percentage of dissolved ions actually was decreased. In other words, the percentage of the total Zn^{2+} ion released from the ZnO NPs were actually lower at 200 mg/L (= 1.6%) compared to 5 mg/L (= 7.5%), which is a common phenomenon described by other investigations, e.g. for CuO NPs (Sørensen et al. 2016). ZnO Particles and Zn^{2+} ions are observed in algal suspensions, and the toxicity of ZnO NPs can be related to the particles, ionic, or the total effect of both. The toxicity of Zn^{2+} ions confirmed by available studies (Chen et al. 2012b; Suman et al. 2015; Wang et al. 2016a). The released Zn^{2+} ion from ZnO NPs can easily penetrate into the algal cells by small pores in algal cell wall (Melegari et al. 2013). Lee (2018) reported that Zn^{2+} can not permeate across the cellular membrane and the membranes of intracellular structures because it is a hydrophilic ion. Zn^{2+} transporters (ZnTs) family facilitates the mobilization of Zn^{2+} in the opposite direction of Zn^{2+} importers (ZIPs) family (Lee 2018). Further, the other membrane transporter proteins are involved in mobilization of Zn^{2+} across the cell membrane (Lee 2018). Most researchers have found that there is the significant links between the dissolution rate of ZnO NPs and their toxic effects (Suman et al. 2015; Skjolding et al. 2017). It is well-established that the toxicity of ZnO NPs partly caused by the dissolved zinc ions which can increase the ROS production and induce oxidative damage in cells (Sharifi et al. 2012; Suman et al. 2015). Furthermore, the regulatory role of zinc in mitochondrial homeostasis is imported because mitochondria are the main source of ROS production (Lee 2018). On the other hand, zinc is known as a redox-inert metal which play as an antioxidant by the catalytic action of copper/zinc-superoxide dismutase, conservation of the protein sulfhydryl groups, fixation of membrane structure, and upregulation of the expression of metallothionein, which has a metal-connection capacity as well as antioxidant functions (Lee 2018).

The decreased growth of *N. oculata* was observed in response to 10-200 mg/L of ZnO NPs. Study of the growth of *Clorella vulgaris* after exposure to 50-300 mg/L of ZnO NPs using MTT test shows that the cell viability was significantly decreased (Suman et al. 2015). Likewise, the investigation of long-term toxic effect of ZnO NPs (0.81-810 mg/L) on the growth of *S. rubescens* in BG11 and BBM media were showed that algal growth is affected by the exposure time, NPs concentrations, and mainly type of culture medium (Aravantinou et al. 2017). Interestingly, 0.081-0.810 mg/L concentrations of ZnO NPs had toxic effects on *P. subcapita*, while 0.81 mg/L of ZnO NPs did not restrict the viability of *Chlorella sp.* after 24 h in Algo-Gro medium (Aruoja et al. 2009). Taken all together, the effects of ZnO NPs on microalgae were dependent on the algal species, the culture medium, concentration of NPs, solubility of NPs and time of exposure (Arouja et al. 2009; Suman et al. 2015; Aravantinou et al. 2017; Fazelian et al. 2020a). It should be noted that the different NPs cause different toxicity in *N. oculata*. Fazelian et al. (2020a) reported the order of toxicity of metal oxide NPs in *N. oculata* as CuO-NPs > ZnO-NPs > Fe₂O₃-NPs. On the other hand, the EC50 of Ag NPs for *N. oculata* was 20.88 mg/L, while the EC50 of CuO-, ZnO-, and Fe₂O₃-NPs was 116.98, 153.72, and 202.92 respectively (Fazelian et al. 2019, 2020a, 2020b). The growth inhibition by ZnO NPs could be associated with the inhibition of photosynthetic processes. In any case, decrease of chlorophyll content might be responsible for the reduction of *N. oculata* growth. In agreement with our results, the negative effects of CuO NPs, Ag NPs and TiO₂ NPs on the chlorophyll content and growth of *N. oculata*, *Chlorella sp.* and *Chlamydomonas reinhardtii* were reported (Chen et al. 2012a; Wang et al. 2013; Fazelian et al. 2019, 2020b). In the presence of ZnO NPs, a decrease in chlorophyll content might induce oxidative stress or inversely an increase in oxidative stress may cause a decrease in chlorophyll content.

Oxidative damage is a common mechanism responsible for the NPs-induced cell disorders (Sharifi et al. 2012). Lipid peroxidation leads to the destruction of biomembranes and has been used extensively as a sign for *in vivo* oxidative stress (Sayeed et al. 2003). The produced free radicals by cellular exposure to NPs are extremely reactive and rapidly disrupt normal cell metabolism and increase lipid peroxidation (Dawes 2000). Significant effects of different NPs on generation of MDA in a number of microalgal species were formerly reported (Chen et al. 2012a; Fazelian et al. 2019, 2020b). At high concentrations of ZnO NPs occurs a decrease in the level of some antioxidant structures, which results in rapid accumulation of ROS in algal cells (Sayeed et al. 2003; Suman et al. 2015). The uncontrolled production of H₂O₂ (as a ROS), may destroy cellular components and oxidative stress can be caused by excess H₂O₂ accumulation (Sayeed et al. 2003). Accordingly, high lipid peroxidation coupled with high H₂O₂ content might cause damage to chloroplast, decreased algae biomass and inhibited chlorophyll synthesis, leading to lower chlorophyll concentration in *N. oculata*.

Many attempts have been made to enhance the tolerance to oxidative stress by modifying the antioxidant defense system (Liu et al. 2008). Carotenoids (canthaxanthin and β -carotene) play an important role in defense against oxidative stress by scavenges of single oxygen and suppressing lipid peroxidation in all photosynthetic organisms and (Salguero et al. 2003). Also, these compounds protect the photosynthetic apparatus from excess photons through xanthophylls cycle (El-Bakey et al. 2007). Similarly, the increase of

carotenoids was observed in response to TiO₂ NPs in the alga *Phaeodactylum tricornutum* and Ag NPs in *N. oculata* (Wang et al. 2016a; Fazelian et al. 2020b). It has been also reported that the increase of zeaxanthin and β-carotene in *Nannochloropsis* is the antioxidant response against UV-A radiation (Forján et al. 2011). Contrary to our results, 5-200 mg/L of CuO NPs significantly decreased the content of carotenoids in *N. oculata* (Fazelian et al. 2019).

Phenolic compounds have also been studied due to their secondary ecological functions (e.g., reproductive role in algal reproduction and protective mechanism against biotic factors) (Machu et al. 2015). These compounds possess antioxidant activity through inhibition of lipid peroxidation, scavenging molecular species of active oxygen, and chelation of metal ions (Michalak 2006). The similar to our findings, CuO NPs (100-200 mg/L) significantly increased the content of phenolic compounds in *N. oculata* (Fazelian et al. 2019), while 5-50 mg/L of Ag NPs decreased these compounds (Fazelian et al. 2020b). It has also been reported that photosynthetic microorganisms can produce phenolic compounds in order to prevent cell damages in response to TiO₂ NPs stress (Comotto et al. 2014). It seems that changes in PPO activity are directly related to the amount of phenolic compounds, because the enhanced activity of PPO showed a direct correlation with the contents of phenolic compounds of *N. oculata*. Increase of phenolic compounds and PPO activity was also observed in *N. oculata* exposed to 50-200 mg/L of CuO NPs (Fazelian et al. 2019). Ruiz et al. (2003) also reported that induction of PPO activity is possibly due to its role in phenolic compound synthesis, which has a function in detoxification in microalgae.

It is well known that antioxidant enzymes are the most sensitive indices in response of algae to environmental stress (Fazelian et al. 2019). In fact, the increased activity of antioxidant enzymes in living cells is as an early warning indicator of pollution in the environment (Song et al. 2008). The potential of ZnO NPs to induce oxidative stress was assessed by measuring the activities of a number of antioxidant enzymes including CAT, APX and PPO (Suman et al. 2015). The activity of CAT was considerably promoted by treatment of ZnO NPs, while APX activity decreased in response to ZnO NPs. Therefore, CAT plays an important role in protection of cells against oxidative damage caused by ZnO NPs. The increase of CAT activity was reported for *C. vulgaris* by exposure of algal cells to ZnO NPs (Chen et al. 2012b) and for *N. oculata* in response to 50-200 mg/L of CuO NPs (Fazelian et al. 2019) and 5-50 mg/L of Ag NPs (Fazelian et al. 2020b). Contrary to present results, 5-200 mg/L of CuO NPs and 10-25 mg/L of Ag NPs increased the activity of APX enzyme in *N. oculata* (Fazelian et al. 2019). On the other hand, the reduction of APX activity in response to 50 mg/L of Ag NPs was similar to our study. APX was reported to be involved in H₂O₂ removal using ascorbate as a specific electron donor, particularly in chloroplast (Donahue et al. 1997). A reduction in the activity of APX under ZnO NPs treatment established that APX enzyme is not an effective scavenger of ROS in *N. oculata* and this microalgae is unable to decompose the excess H₂O₂ radicals by this enzyme. Furthermore, the destruction of *N. oculata* chloroplast in response to oxidative stress induced by ZnO NPs was probably another reason of the reduction of APX activity.

Cell membrane damage is also known as one of the causes of NP-toxicity and changes in LDH activity is an important parameter for determining cellular toxicity by metal oxide NPs (Zhang et al. 2012; Suman et al. 2015). LDH is known as an important enzyme that is released from damaged cells (Bergmeyer and Bernt 1965). Pathakoti et al. (2013) reported that the ROS production results in the degradation of cell membrane by LDH release. These findings were similar to the previous reports showing that the treatment of ZnO NPs, CuO NPs and Ag NPs increased LDH activity in *C. vulgaris* and *N. oculata* respectively (Suman et al. 2015; Fazelian et al. 2019, 2020b). Interestingly, 200 mg/L of ZnO NPs and CuO NPs increased the activity of this enzyme by 2.38 and 5.7 fold respectively, as compared to the control ($P < 0.05$). Haung et al. (2008) reported that the released Zn^{2+} ions from ZnO NPs could be the reason for cell toxicity and cell membrane-damages by NPs.

Our data for viability, lipid peroxidation, LDH activity and SEM images showed that ZnO NPs was associated with algal cell surfaces and induced the cell membrane damages. These results are in accordance with the previous results on *C. vulgaris*, *P. subcapitata*, and *N. oculata* showing that ZnO NPs, TiO_2 NPs, CuO NPs and Ag NPs caused membrane damages (Metzler et al. 2011; Suman et al. 2015; Fazelian et al. 2019, 2020b). The aggregation of ZnO NPs also observed in SEM images and confirmed the shading effect as well as decreasing the light availability which ultimately reduce the photosynthesis rate and the cell growth. Similar to our results, the aggregation of TiO_2 NPs on the cell wall of *Chlorella* sp. and *Scenedesmus* sp. were reported by SEM images (Mohammed Sadiq et al. 2011). The algal cell aggregation after treatment with ZnO NPs is probably a defensive mechanism of *N. oculata* against the physical interaction with NPs. The other researchers reported that the aggregation of algal cells acts as a barrier against CuO NPs (Zhao et al. 2016; Fazelian et al. 2019).

TEM images of *N. oculata* were identical with the results obtained by Lee and An (2013) on *P. subcapitata*. and Fazelian et al. (2019) on *N. oculat*. Unlike CuO NPs, the aggregation of ZnO NPs was not observed in TEM images of *N. oculata* and the wrinkling of *N. oculata* cell wall in response to ZnO NPs was more than CuO NPs. Likewise, it was reported that the cell wall damages play an important role in the toxicity of TiO_2 NPs to the marine microalga *Phaeodactylum tricorutum* (Wang et al. 2016b). The cell wall of algae acts as a selective and semipermeable barrier with pores ranging from 5-20 nm (Melegari et al. 2013). The interaction of cell wall with ZnO NPs can change the size of pores and/or can create new pores with higher size rather than typical pores which allow the entry of ZnO NPs through the cell wall structure (Navarro et al. 2008). The change of cell wall structure and internalization of NPs into microalgae has been observed by other researchers (Perreault et al. 2012; Melegari et al. 2013). Similarly, the TiO_2 NPs were localized around the cell membrane of *C. reihardtii* while the entering the NPs into the cells was limited even at high concentrations of TiO_2 NPs (Al-awady et al. 2015). The entry of ZnO NPs into the *N. oculata* cell can also be demonstrated according to the XRD image. On the other hand, the entry of ZnO NPs into *N. oculata* can release Zn^{2+} ions into the cell. Zinc is a divalent cation and does not directly undergo redox reactions and interact with ROS (Haas and Franz 2009). This property of zinc is unlike other bioactive metals such as iron and copper, and thereby Zn^{2+} can act as an efficient Lewis acid and it is often interacted with side chains of some amino acids (Haas and Franz 2009; Lee 2018). Also,

zinc known as an antioxidant or prooxidant, which its antioxidant function was investigated previously (Lee 2018). Numerous studies have linked the induction of oxidative stress in response to high levels of Zn^{2+} , which owing to its role as a prooxidant (Suman et al. 2015; Lee 2018).

In addition to the TEM image, the FTIR analysis is also a useful tool for the study of NPs interaction with algal cell wall (Liu et al. 2014; Mohammed Sadiq et al. 2011). FTIR results indicated strong interactions between the C-O, C=O and C-H groups on the surface of the algal cells with ZnO NPs (Table 3). Similarly, the interaction of CuO NPs with C-O, C=O and C-H groups as well as Ag NPs with C=O, C-H and O-H groups of *N. oculata* was observed (Fazelian et al. 2019, 2020b). In actual fact, cellulose, polysaccharides and glycoproteins of the algal cell walls act as binding sites for NPs (Chen et al. 2012b). The uptake of NPs by cells through crossing the cell wall and plasmamembrane were facilitated by the high specific area and tiny size of NPs (Chen et al. 2012b). On the other hand, a number of functional groups with a net negative charge and high affinity for metal ions located on the surfaces of algal cells. These functional groups interact with the ions and help them in entering into the algal cells (Crist et al. 1994).

5. Conclusion

Our data revealed that concentrations of 10-200 mg/L of ZnO NPs induced oxidative stress in *N. oculata* cells. As a result, the activity of a number of antioxidant enzymes and the content of some non-enzymatic antioxidant components such as carotenoids and phenols were changes. In addition, the contents of MDA and H_2O_2 were elevated that may be a consequence of induced oxidative stress. The growth parameters as well as chlorophyll a content were reduced in *N. oculata* exposed to ZnO NPs. SEM and TEM surveys showed aggregation of algal cells and uncommon changes in their morphology after treatment with NPs. The presence of ZnO NPs on the surface of algal cells was confirmed by the SEM and TEM images. Concordance between our results with previous studies showed that *N. oculata* as one of the sensitive species to NP-toxicity and the defense mechanisms of this microalgae against oxidative stress induced by ZnO, CuO, and Ag NPs may not have been strong enough (Table.4). Therefore, the entry of these nanoparticles into aquatic ecosystems causes toxicity in *N. oculata*.

Declarations

Ethical Approval and Consent to Participate: Not applicable

Consent to Publish: Not applicable

Authors' Contributions: Movafeghi & Fazelian studied conception and designed; Fazelian, Movafeghi, & Yousefzadi analyzed and interpreted the data; Fazelian, Movafeghi, & Yousefzadi wrote the manuscript and Fazelian & Movafeghi supervised and advised the entire research work. All authors read and approved the final manuscript.

Competing Interests: The authors declare that they have no competing interests.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Funding: No funding was received.

References

1. Al-awady MJ, Greenway GM, Paunov VN (2015) Nanotoxicity of polyelectrolyte-functionalized titania nanoparticles towards microalgae and yeast: role of the particle concentration, size and surface charge. *The Royal Society of Chemistry* 5:37044–37059
2. Aravantinou AF, Theodorakopoulos MA, Manariotis ID (2013) Selection of microalgae for wastewater treatment and potential lipids production. *Bioresour Technology* 147:130–134
3. Aruoja V, Dubourguier HC, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae *Pseudokircehneriella subcapitata*. *Sci Total Environ* 407:1461–1468
4. Ates M, Daniels J, Arslan Z, Farah IO, Rivera HF (2013) Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. *Environmental Science: Processes & Impacts* 1:225–233
5. Bergmeyer HU, Bernt E (1965) Enzymatic determination of ketone bodies in blood. *Advanced Techniques in Biology & Medicine* 19:65–76
6. Chen LZ, Zhou LN, Liu YD, Deng SQ, Wu H, Wang GH (2012a) Toxicological effects of nanometer titanium dioxide (nano-TiO₂) on *Chlamydomonas reinhardtii*. *Ecotoxicol Environ Saf* 84:155–162
7. Chen P, Powell BA, Mortimer M, Ke PCh (2012b) Adaptive interactions between zinc oxide nanoparticles and *Chlorella* Sp. *Environ Sci Technol* 46:12178–12185
8. Chen XX, Zhu X, Li R, Yao H, Lu Z, Yan X (2012c) Photosynthetic toxicity and oxidative damage induced by nano-Fe₃O₄ on *Chlorella vulgaris* in the aquatic environment. *Open Journal of Ecology* 1:21–28
9. Choi JS, Kim RO, Yoon S, Kim WK (2016) Developmental toxicity of zinc oxide nanoparticles to Zebrafish (*Danio rerio*): A transcriptomic analysis. *Public library of science* 11:1–15
10. Choudhary P, Vishal Khandelwal V, Bhatia AK (2018) Effects of zinc oxide nanoparticles on callus induction of banana under salt stress condition. *International Journal of Engineering Technology Science and Research* 5:2394–3386
11. Comotto M, Casazza AA, Aliakbarian B, Caratto V, Farretti M, Perego P (2014) Influence of TiO₂ nanoparticles on growth and phenolic compounds production in photosynthetic microorganisms. *The Scientific World Journal* 10:1–9
12. Crist RH, Martin JR, Carr D, Watson JR, Clarke HJ, Crist DR (1994) Interaction of metals and protons with algae. 4. ion –exchange vs adsorption models and a reassessment of scatchard plots; ion-exchange rates and equilibria compared with calcium alginate. *Environ Sci Technol* 28:1859–1866

13. Dawes IW (2000) Response of eukaryotic cells to oxidative stress. *Agriculture Chemistry & Biotechnology* 43:211–217
14. Dolatabadi A, Sani B, Moaveni P (2015) Impact of nanosized titanium dioxide on agronomical and physiological characteristics of annual medic (*Medicago scutellata L.*). *Cercetari Agronomice in Moldova* 3:53–61
15. Donahue JL, Okpodu CM, Gramer CL, Grabau EA, Alscher R (1997) Responses of antioxidant to paraquat in pea leaves. *J Plant Physiol* 113:249–257
16. El-Baky HH, El Baz FK, Gamal S (2007) Enhancement of antioxidant production in *Spirulina plantensis* under oxidative stress. *American-Eurasian Journal of Scientific Research* 2:170–179
17. Fazelian N, Movafeghi A, Yousefzadi M, Rahimzadeh M (2019) Cytotoxic impacts of CuO nanoparticles on the marine microalga *Nannochloropsis oculata*. *Environ Sci Pollut Res* 26:17499–17511
18. Fazelian N, Yousefzadi M, Movafeghi A (2020a) Algal response to metal oxide nanoparticles: Analysis of growth, protein content and fatty acid composition. *Bioenergy Res* 13:944–954
19. Fazelian N, Movafeghi A, Yousefzadi M, Rahimzadeh M, Zarei M (2020b) Impact of silver nanoparticles on the growth, fatty acid profile, and antioxidative response of *Nannochloropsis oculata*. *Acta Physiol Plant* 42:126
20. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Gasey PS (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl₂ to a freshwater microalgae (*Pseudokirchneriella subcapitata*): The importance of particles solubility. *Environ Sci Technol* 41:8484–8490
21. Forján E, Garbayo I, Henriques M, Rocha J, Vega JM, Vilchez C (2011) UV-A mediated modulation of photosynthetic efficiency, xanthophyll cycle and fatty acid production of *Nannochloropsis* Marine *Biotechnology* 13:366–375
22. Haas KL, Franz KJ (2009) Application of metal coordination chemistry to explore and manipulate cell biology. *Chem Rev* 109:4921–4960
23. Hsueh YH, Ke WJ, Hsieh CT, Lin KS, Tzou DY, Chiang CL (2015) ZnO nanoparticles affect *Bacillus subtilis* cell growth and biofilm formation. *Public Library of Science* 10:1–23
24. Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c₁ and c₂ in higher plants, algae, and natural phytoplankton. *Biochemie und physiologie der Pflanzen* 167:191–194
25. Lee SR (2018) Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. *Hindawi Oxidative Medicine and Cellular Longevity* 15:1–11
26. Lee WM, An YJ (2013) Effects of zinc oxide and titanium dioxide nanoparticles on green algae under visible, UVA, and UVB irradiations: No evidence of enhanced algal toxicity under UV pre-irradiation. *Chemosphere* 91:536–544
27. Li Y, Harsman M, Wu N, Lan CQ, Dubois-Calero N (2008) Biofuels from microalgae. *Biotechnol Prog* 24:815–820

28. Liu D, Zou J, Wang M, Jiang W (2008) Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. *Bioresour Technol* 99:2628–2636
29. Liu G, Dong X, Liu L, Wua L, Peng, Sh, Jiang C (2014) Boron deficiency is correlated with changes in cell wall structure that lead to growth defects in the leaves of navel orange plants. *Sci Hort* 176:54–62
30. Machu L, Misurcova L, Ambrozova JV, Orsavova J, Micek J, Sochor J, Jurikova T (2015) Phenolic contents and antioxidant capacity in algal food products. *Molecules* 20:1118–1133
31. Manzo S, Miglietta ML, Rametta G (2013) Toxic effects of ZnO nanoparticles towards marine algae *Dunaliella tertiolecta*. *Sci Total Environ* 445:371–376
32. Manzo S, Rocco A, Carotenuto R, Picione FL, Miglietta ML, Rametta G, Di Francia G (2011) Investigation of ZnO nanoparticles ecotoxicological effects towards different soil organisms. *Environ Sci Pollut Res* 18:756–763
33. Melegari SP, Perreault F, Costa RHR, Popovic R, Matias WG (2013) Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. *Aquat Toxicol* 142:431–440
34. Metzler DM, Li M, Erdem A, Huang C (2011) Responses of algae to photocatalytic nano-TiO₂ particles with an emphasis on the effect of particle size. *Chem Eng J* 170:538–546
35. Miller G, Shulaev V, Mitter R (2008) Reactive oxygen signaling and abiotic stress. *Physiol Plant* 133:481–489
36. Mohammed Sadiq I, Dalai S, Chandrasekaran N, Mukherjee A (2011) Ecotoxicity study of titania (TiO₂) NPs on two microalgae species: *Scenedesmus* sp. and *Chlorella* sp. *Ecotoxicology and Environmental Safety* 74: 1180-1187
37. Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, Sigg L, Behra R (2008) Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environmental Science and Technology* 42:8959–8964
38. Pathakoti K, Morrow S, Han C, Pelaez M, He X, Dionysiou DD, Hwang HM (2013) Photoinactivation of *Escherichia coli* by sulfur-doped and nitrogen-fluorine-codoped TiO₂ nanoparticles under solar simulated light and visible light irradiation. *Environ Sci Technol* 47:9988–9996
39. Phukan MM, Chua RS, Konwar BK, Kataki R (2011) Microalgae *Chlorella* as a potential bio-energy feedstock. *Appl Energy* 88:3307–3312
40. Rodolfi L, Chini-Zittelli G, Bassi N, Padovani G, Biondi N, Bionini G, Tredici MR (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol Bioeng* 102:100–112
41. Ruiz JM, Rivero RM, López-Cantarero I, Romero L (2003) Role of Ca²⁺ in the metabolism of phenolic compounds in tobacco leaves (*Nicotiana tabacum* L.). *Plant Growth Regul* 41:173–177

42. Salguero AM, Benito J, Vigarra JM, Vega C, Vilchez Leon R (2003) Carotenoids as protective response against oxidative damage in *Dunaliella bardawil*. *Biomol Eng* 20:249–253
43. Sato M, Murata Y, Mizusawa M (2004) A simple and rapid dual fluorescence viability assay. *Microbiology and Culture Collection* 20:53–59
44. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S (2003) Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish *channa punctatus* Bloch. *Ecotoxicological and Environmental Safety* 56:295–301
45. Sharifi I, Shokrollahi H, Doroodmand MM, Safi R (2012) Magnetic and structural studies on CoFe_2O_4 nanoparticles synthesized by co-precipitation, normal micelles, and reverse micelles methods. *J Magn Magn Mater* 324:1854–1861
46. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178
47. Skjolding LM, Sørensen SN, Hartmann NB, Hjorth R, Hansen SF, Baun A (2016) Aquatic ecotoxicity testing of nanoparticles-The quest to disclose nanoparticle effects. *Angewandte Chemie (International Edition)* 55:15224–15239
48. Song U, Lee S (2016) Phytotoxicity and accumulation of zinc oxide nanoparticles on the aquatic plants *Hydrilla verticillata* and *Phragmites Australis*: leaf-type-dependent responses. *Environ Sci Pollut Res* 23:8539–8545
49. Song Y, Zhu L, Wang S, Wang J, Liu JH, Xie H (2008) DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia fetida*) induced by atrazine. *Soil Biol Biochem* 41:905–909
50. Sørensen SN, Holten Lützhøft HC, Rasmussen R, Baun A (2016) Acute and chronic effects from pulse exposure of *D. magna* to silver and copper oxide nanoparticles. *Aquat Toxicol* 180:209–217
51. Stehfest K, Toepel J, Wilhelm C (2005) The application of micro FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. *Plant Physiol Biochem* 43(7):717–726
52. Suman TY, Radhika Rajasree SR, Kirubakaran R (2015) Evaluation of zinc oxide nanoparticles toxicity on marine algae *Chlorella vulgaris* through flow cytometric, cytotoxicity and oxidative stress analysis. *Ecotoxicol Environ Saf* 113:23–30
53. Taylor RL, Rand JD, Caldwell GS (2012) Treatment with algae extracts promotes flocculation, and enhances growth and neutral lipid content in *Nannochloropsis oculata*—a candidate for biofuel production. *Mar Biotechnol* 14:774–781
54. Wahab R, Ansari SG, Kim YS, Dar MA, Shin HS (2008) Synthesis and characterization of hydrozincite and its conversion into zinc oxide nanoparticles. *J Alloys Compd* 461:66–71
55. Wang H, Wick RL, Xing B (2009) Toxicity of nanoparticulate and bulk ZnO , Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. *Environ Pollut* 157:1171–1177
56. Wang L, Luo Y, Jing L (2012) Effect and toxic mechanism of nanoparticles to algae. *Adv Mater Res* 343:81–84

57. Wang L, Wang M, Peng Ch, Pan J (2013) Toxic effects of Nano-CuO, micro-CuO and Cu²⁺ on *Chlorella sp.* Journal of Environmental Protection 4:86–91
58. Wang X, Yang X, Chen S, Li Q, Wang W, Hou Ch, Gao X, Wang L, Wang Sh (2016a) Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in *Arabidopsis*. Front Plant Sci 6:1–9
59. Wang Y, Zhu X, Lao Y, Lv X, Tao Y, Huang B, Wang J, Zhou J, Cai Z (2016b) TiO₂ nanoparticles in the marine environment: Physical effects responsible for the toxicity on algae *Phaeodactylum tricorutum*. Sci Total Environ 565:818–826
60. Xiao T, Kovoichich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nei AE (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2:2121–2134
61. Zafar H, Ali A, Ali JS, Haq IU, Zia M (2016) Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: Growth dynamics and antioxidative response. Frontier in Plant Science 20:535–543
62. Zhang H, Ji Z, Xia T, Meng H, Low-Kam CR, Liu S, Pokhrel S, Lin X, Wang YP et al (2012) Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. ACS Nano 6:4349–4368
63. Zhao J, Cao X, Liu X, Wang Z, Zhang C, White JC, Xing B (2016) Interactions of CuO nanoparticles with the algae *Chlorella pyrenoidosa*: Adhesion, uptake and toxicity. Nanotoxicology 10:1–31
64. Zaka M, Abbasi BH, Rahman L, Shah A, Zia M (2016) Synthesis and characterization of metal nanoparticles and their effects on seed germination and seedling growth in commercially important *Eruca sativa*. IET Nanobiotechnol 10:134–140

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Table 2

Effects of ZnO NPs on the activity of CAT, APX, PPO and LDH enzymes. Values in a group followed by the same letter are not statistically different at $P \leq 0.05$ level as determined by Duncan's test.

ZnO NPs conc (mg/L)	CAT (unit/mg.protein)	APX (unit/mg.protein)	PPO (unit/mg.protein)	LDH (unit/10 ⁵ cells)
0	0.05 ± 0.08 ^d	1.55 ± 0.03 ^a	2.24 ± 0.04 ^d	5.87 ± 0.01 ^d
5	0.06 ± 0.06 ^d	1.43 ± 0.09 ^a	2.17 ± 0.09 ^d	5.7 ± 0.17 ^d
10	0.05 ± 0.06 ^d	0.97 ± 0.17 ^b	3.3 ± 0.03 ^a	5.92 ± 0.03 ^d
50	0.07 ± 0.05 ^c	0.86 ± 0.14 ^{bc}	3.42 ± 0.02 ^a	8.76 ± 0.01 ^c
100	0.08 ± 0.02 ^b	0.51 ± 0.06 ^{cd}	2.8 ± 0.12 ^b	12.82 ± 0.02 ^b
200	0.12 ± 0.09 ^a	0.54 ± 0.03 ^d	2.5 ± 0.07 ^c	13.99 ± 0.03 ^a

Table 3: Assignments of bands found in the FTIR spectra of *N. oculata* sample.

Band	Main peak (/cm) of control	Main peak (/cm) of treated sample	Frequency range (/cm)	Assignment	References
1	3412.22	-	3512-3003	Water: (O-H) stretching Protein: (N-H) stretching (amide A)	Sukarni et al. (2015)
2	2926.24	2925.10	2940-2897	Lipid: (CH ₂) asymmetric stretching of methylene	Sukarni et al. (2015)
3	1639.91	1637.28 1617.06	1692-1599	Protein: (C=O) stretching (Amid I band mainly)	Phukan et al. (2011)
4	1384.27	-		Acidic group of polygalacturonic acids: (C-H) stretching	Crist et al. (1994)
5	1020.30 1079.88 1154.33	1023.46	1160-970	Carbohydrates: (C-O) and (C-O-C) stretching of polysaccharides (The spectral envelope of the band depends on the crystallinity and conformation of the polysaccharide). Phospholipids, DNA and RNA: (P=O) symmetric stretching Siloxane, silicate frustules: (Si-O) stretching	Crist et al. (1994) Suman et al. (2015)
6	-	480.15		Probably (ZnO NPs) stretching	Suman et al. (2015) Wahab et al. (2008)

Table 4

Comparison between results of this study with previous studies. The changes of physiological parameters of *N. oculata* is in response to the highest concentrations of ZnO NPs and CuO NPs (200 mg/L) and Ag NPs (50 mg/L) in compared with control (Fazelian et al. 2019, 2020a, 2020b).

	ZnO NPs	CuO NPs	Ag NPs
Diameter (nm)	10-30	10-40	44
Hydrodynamic diameter (nm)	745.9±49	493±43	163.5±35
EC50 (mg/L)	153.72	116.98	20.88
GR	96% decrease	97.9% decrease	93.6% decrease
Chl.a	57% decrease	52.7% decrease	48% decrease
Car	non-significant change	54% decrease	2-fold increase
phenol	Increase	1.8- fold increase	69% decrease
MDA	2- fold increase	6 -fold increase	1.7-fold increase
H ₂ O ₂	6.4-fold increase	2.6-fold increase	3.2-fold increase
CAT	2-fold increase	2.4-fold increase	4.3-fold increase
APX	35% decrease	2.5-fold increase	52% decrease
PPO	1.1-fold increase	4.8-fold increase	61% decrease
LDH	2.3-fold increase	5.7-fold increase	8.9-fold increase
FTIR	Interaction with C-O, C=O and C-H	Interaction with C-O, C=O and C-H	Interaction with C=O, C-H and O-H
SEM	-NPs aggregation -membrane damage -algal cell aggregation	-NPs aggregation -membrane damage -algal cell aggregation	-NPs aggregation
TEM	-cell shrinkage -NPs entry	-cell Shrinkage -NPs aggregation -NPs entry	-cell shrinkage -NPs entry

Figures

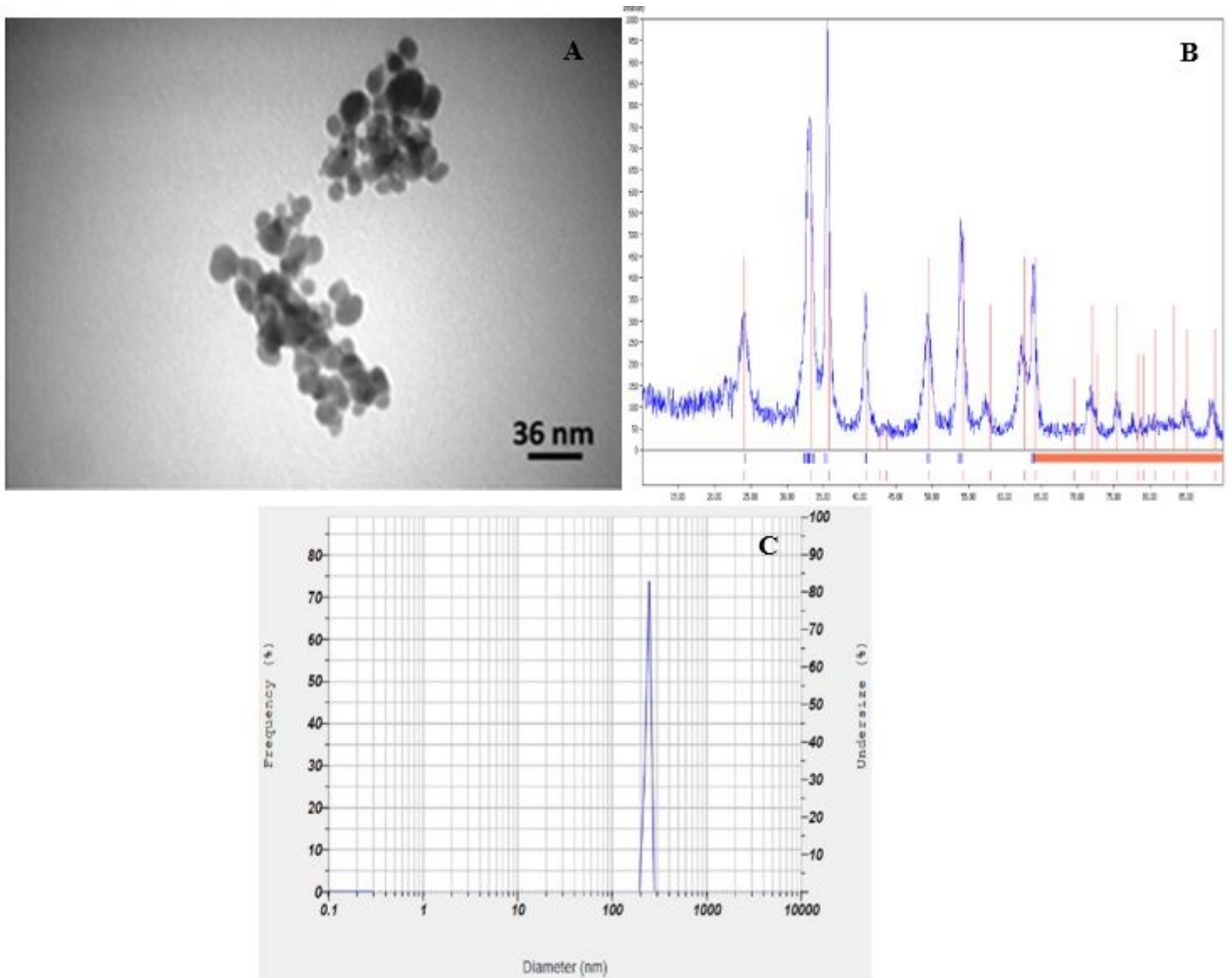


Figure 1

Dissolved Zn²⁺ concentrations in different concentrations of ZnO NPs (A) and EDX of *N. oculata* treated with ZnO NPs (B).

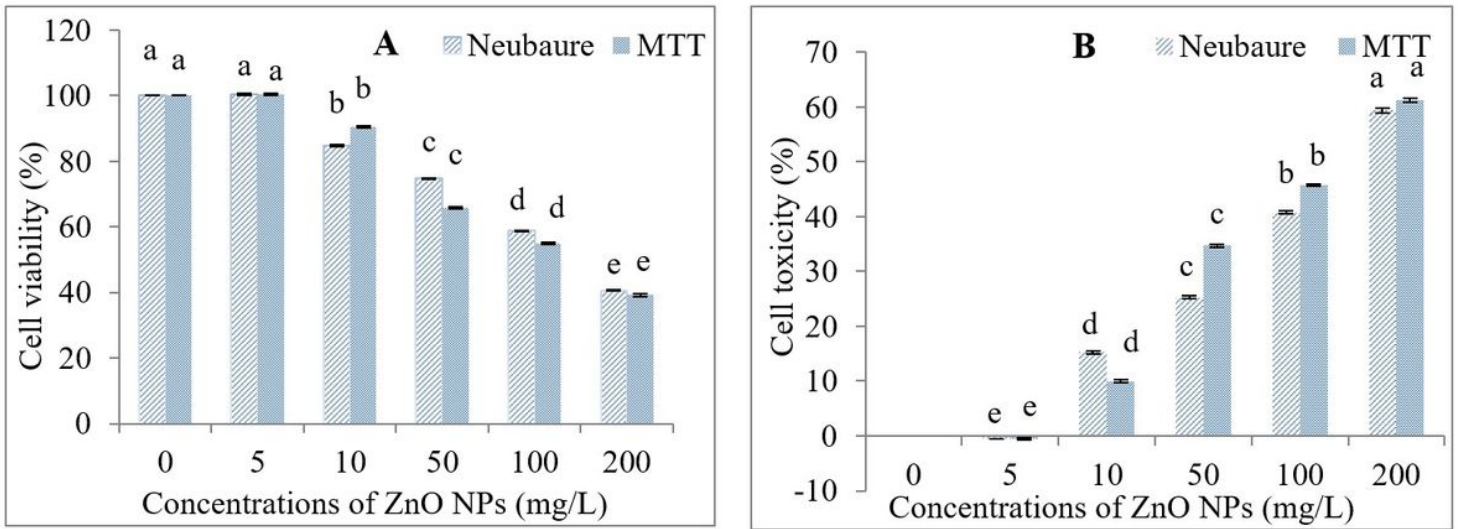


Figure 2

Cell viability (A) and cell toxicity (B) of *N. oculata* after exposure to different concentrations of ZnO NPs. Means in a group followed by the same letter are not significantly different at $P \leq 0.05$. Experimental conditions: initial algae concentration = 4×10^4 cell/ml, test duration = 72 h, and initial test volume = 400 ml.

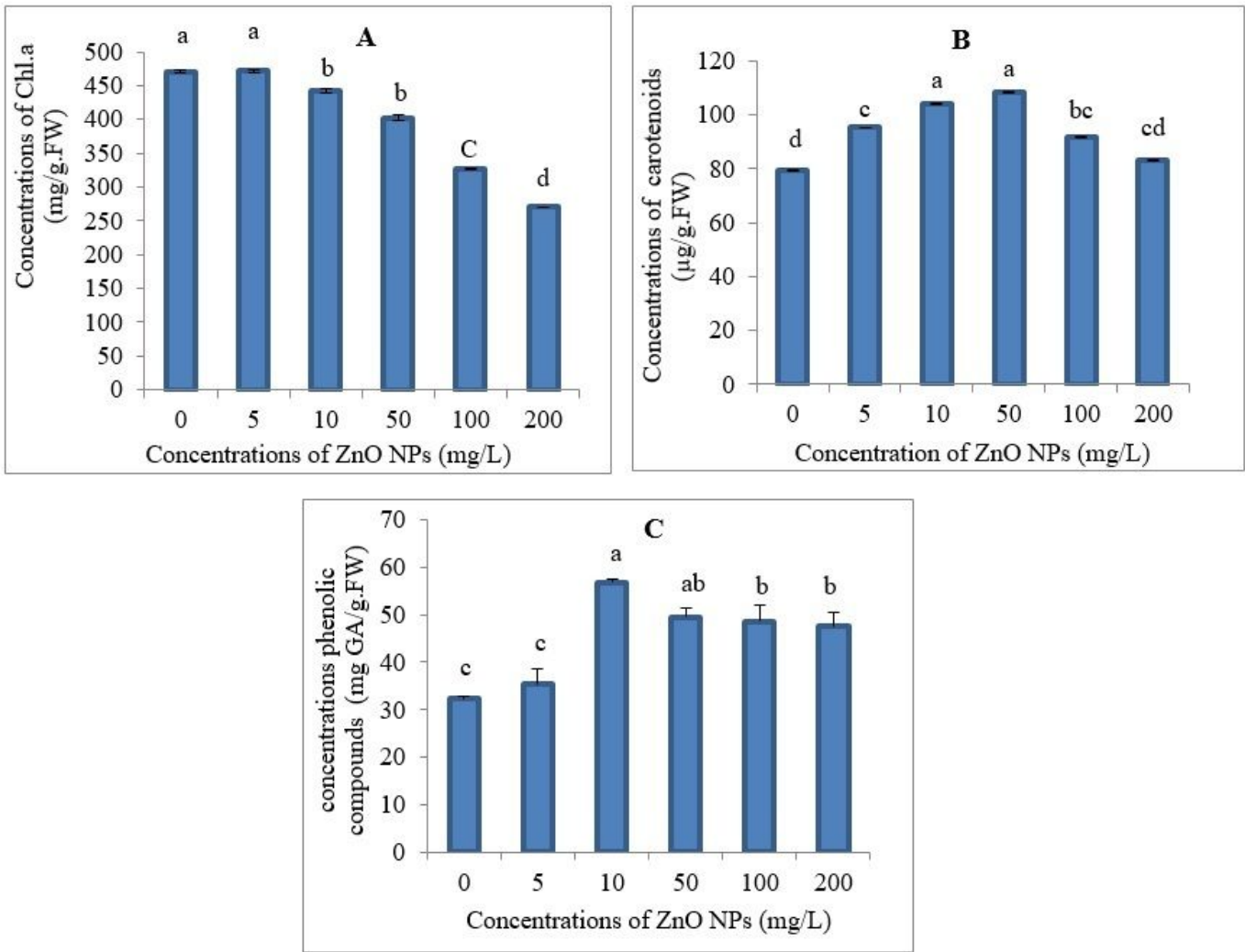


Figure 3

Effects of ZnO NPs treatments on the content of chlorophyll a (A), carotenoids (B) and phenolic compounds (C) in *N. oculata*. Means with the same letter are not statistically different at $P \leq 0.05$ level.

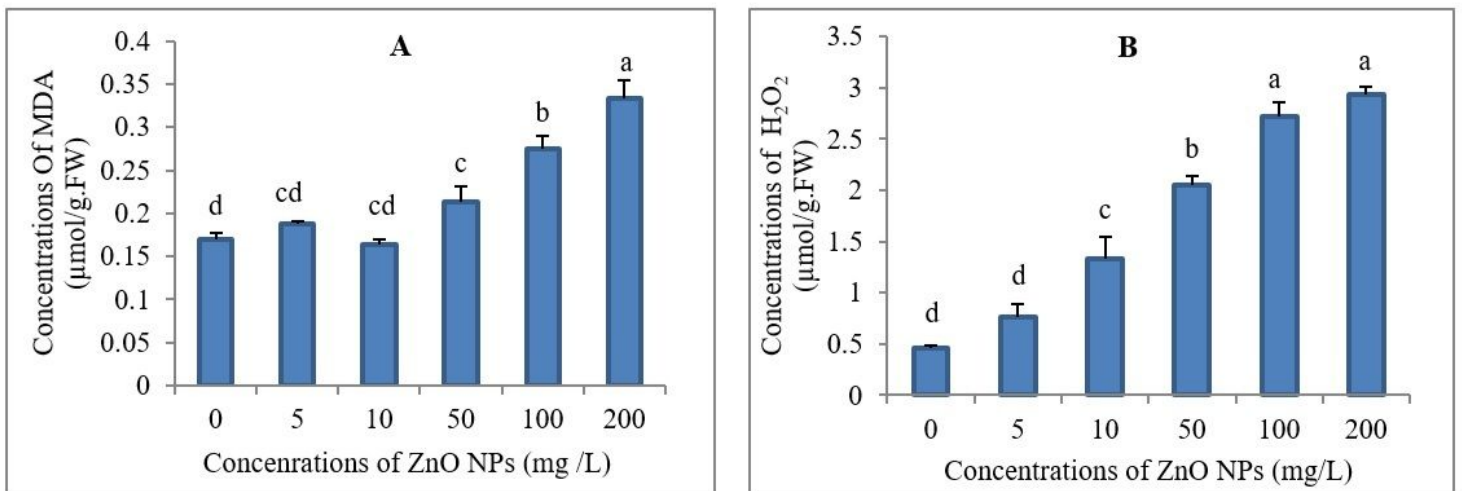


Figure 4

Effects of ZnO NPs on MDA (A) and H₂O₂ (B) content in *N. oculata*. Means with the same letter are not statistically different at $P \leq 0.05$.

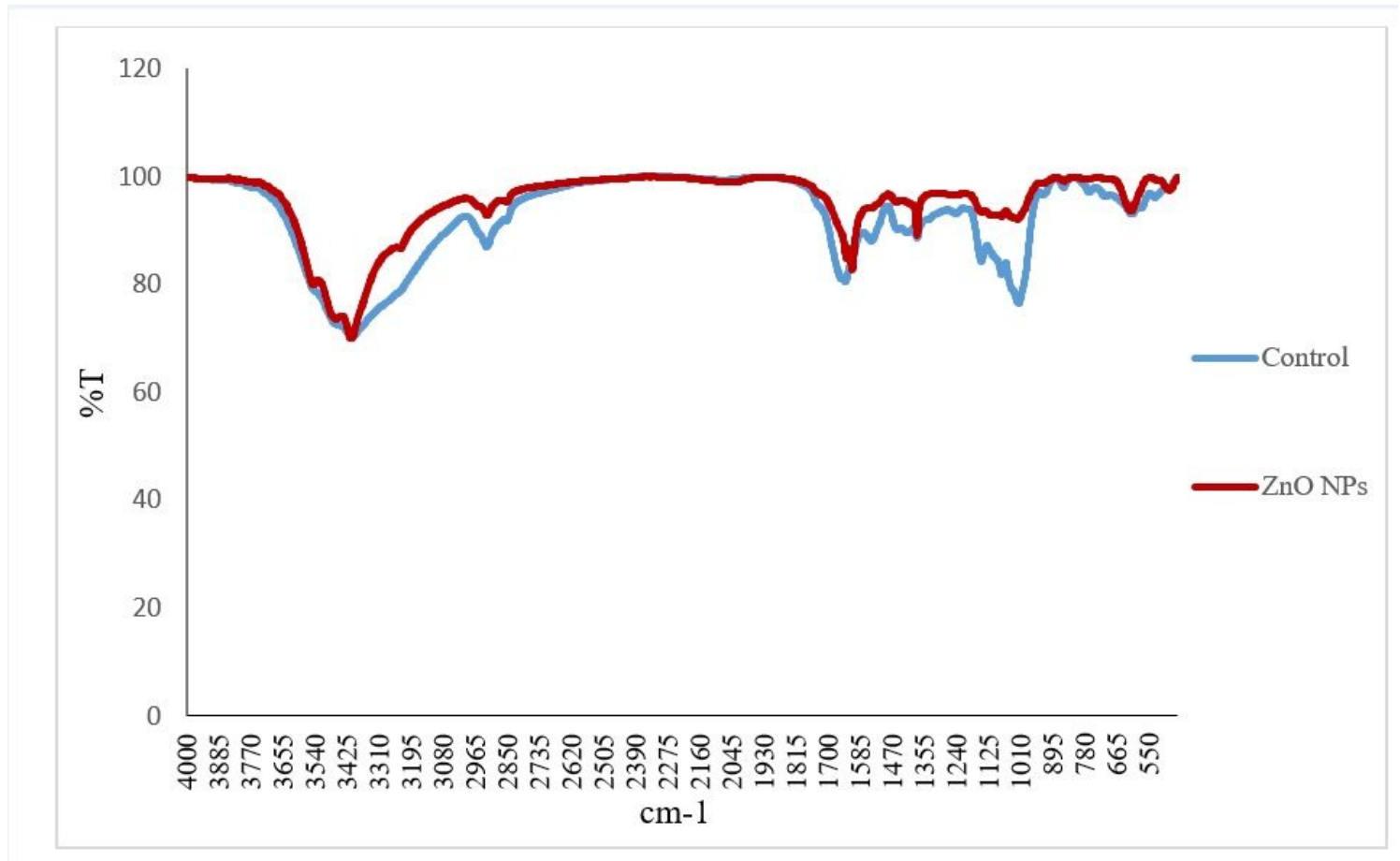


Figure 5

SEM images: *N. oculata* cells exposed to ZnO NPs displays aggregation of NPs on the cell surface (A-D: black arrows), membrane damage (D: red arrow) and cell aggregation (A and D: green arrows) as a result of NP treatment. Scale bar: A=200 nm; B=200 nm; C=200 nm; D=1 μ . ZnO NPs have been shown in the images by black arrows (A-D). TEM image of the control sample (E), morphological changes in the structure of cell wall (red arrows) (F-H) and NPs in the algal cells (H: green arrows) after exposure to 100 mg/L of ZnO NP. Scale bar: A=500 nm; B=500 nm; C=300 nm; D=250 nm.

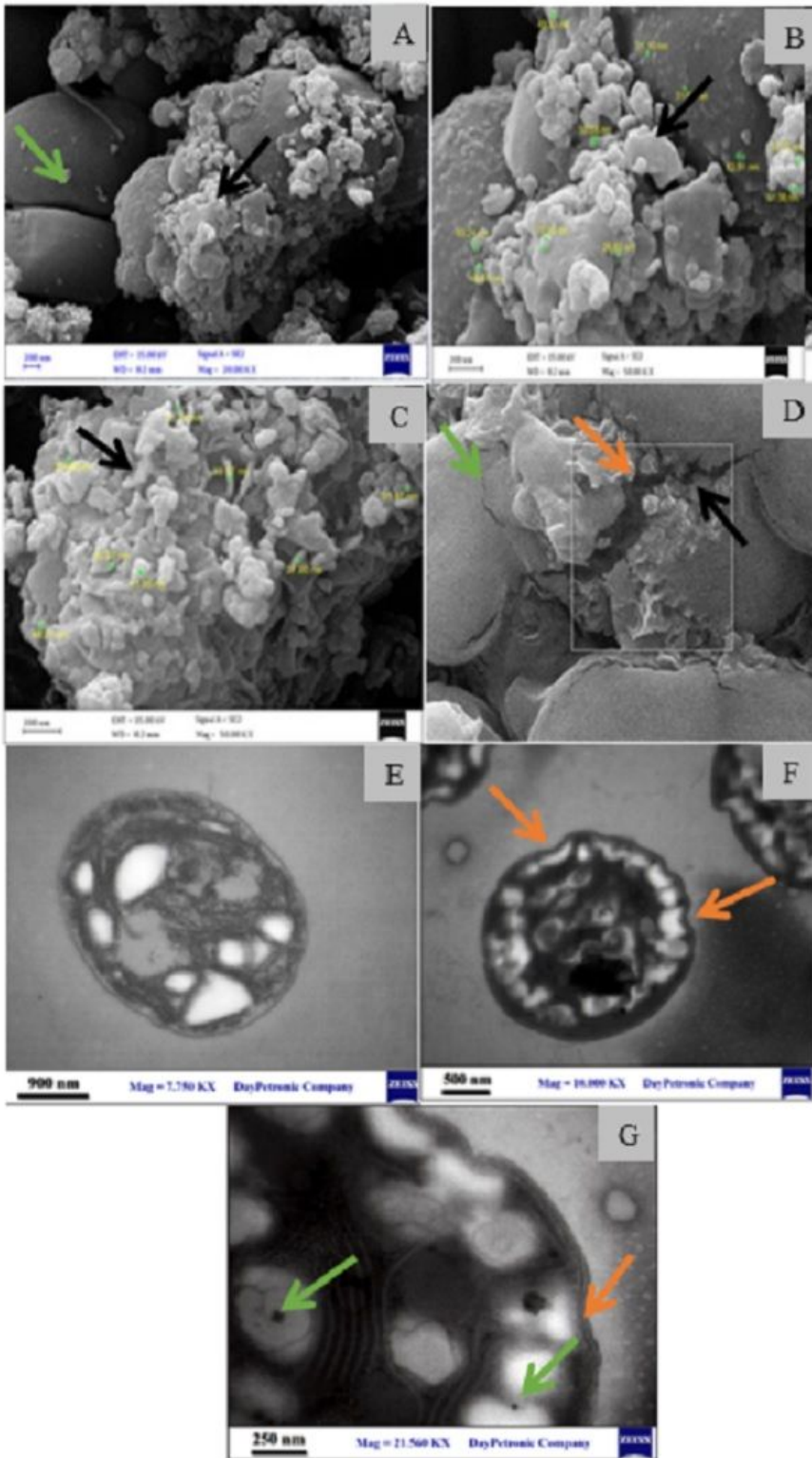


Figure 6

FTIR of control and treated cells of *N. oculata* with ZnO NPs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.jpg](#)