EFFECT OF ZINC-SELENIUM NANOPARTICLES ON MICROALGAE *SCENEDESMUS QUADRICAUDA*

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Abstract: The increasing industrial use of nanomaterials in recent years poses a potential risk to the environment. The first organisms that come into contact with these substances include aquatic organisms, and therefore this study focuses on microalgae that are at the beginning of the food chain. In this study, the toxicity of ZnSe nanoparticles in the freshwater green microalga *Scenedesmus quadricauda* was investigated. The effect of zinc in the form of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and the combination of zinc and nanoparticles were also monitored. The microalgae were exposed to these nanoparticles at concentrations of 10, 50, 100 and 250 µM for 7 days. The microalgae responses were analysed at the level of chlorophyll-*a*, chlorophyll-*b*, carotenoids, flavonoids and total antioxidant capacity. For chlorophyll-*a*, -*b* and carotenoids, similar results were obtained. The most significant effect was found in the sample with a combination of zinc and ZnSe nanoparticles. This sample also affected the most the flavonoid content, especially at concentrations of 50, 100 and 250 µM, where increased synthesis of these compounds was observed. Similar results were obtained in the total antioxidant capacity assay, where a sample with combination of zinc and ZnSe nanoparticles showing an increasing trend, particularly at a concentration of 250 µM.

Key Words: microalgae, nanoparticles, zinc nanoparticles, secondary metabolism, flavonoids

INTRODUCTION

With the rapid development of nanotechnology and the widespread use of nanomaterials, there is an increasing risk of environmental contamination by these particles. Nanoparticles are defined as particles with at least two dimensions between 1–100 nm (Bhatt and Tripathi 2011). They are a natural part of the environment. However they are also artificially synthesized for the needs of the industry. Their increased production and use can lead to release into the environment where they can interact with biotic and abiotic components. In spite of their great advantages, the presence of nanoparticles in nature can have a dangerous biological effect (Bhatt and Tripathi 2011). In particular, heavy metal nanoparticles may have a negative impact on the environment. The potential consequences of such contamination are currently difficult to assess as the toxicity of nanoparticles is not well known (da Costa et al. 2016). Heavy metal nanoparticles are one of the most commonly used nanoparticles in the industry (Nagajyoti et al. 2010). Due to their unique physical and chemical properties, nanoparticles of metals are increasingly being used in various commercial products, leading to concerns about their potential toxicity. In the industry, it has a wide range of applications including catalysis, sensors and environmental remediation, personal care products (e.g. sunscreen creams), coatings or paints (Franklin et al. 2007). ZnSe can be used as a material for n-type semiconductors. Due to the ability to emit fluorescent light can be used as quantum labels for biological use (Iwahori et al. 2005). It can also be used as a semiconductor material, which is potentially used in the diodes of blue-green light, laser diodes and solar cells (Shakir et al. 2009).

Zinc is an essential micronutrient, which is important for normal growth of algae. Its deficiency leads to poor growth and low dry weight (Li et al. 2006). Zinc plays an important role in maintaining the stability of cell membranes in the activation of more than 300 enzymes in protein and nucleic acid metabolism (Soto et al. 2011). However, it can also be toxic when applied in higher amounts. It was found that zinc affects chlorophyll content due to the peroxidation of chloroplast membranes (Li et al. 2006). It has also been found that zinc in the form of zinc oxide (ZnO) and zinc oxide nanoparticles (ZnO NPs) show algal toxicity in the form of growth inhibition (Aruoja et al. 2009). Nanoparticles of zinc (ZnO NPS) substantially reduces the viability of the cells, increases the activity of antioxidant enzyme superoxide dismutase (SOD), increases the level of lipid peroxidation and causes substantial morphological changes and damage to the cell wall of microalgae (Suman et al. 2015) In the study Chen et al. 2012 was also found distortions of the morphology, viability and integrity of the microsurface membrane resulting from the dissolution of zinc ions.

It is generally assumed that nanoparticles will persist in aquatic systems and that their bioavailability may be significantly higher than for larger particles. There are scientific concerns that these nanoparticles may pose an increased health and environment risks. A small size of nanoparticles results in both greater mobility and potentially increased permeability through biological membranes. This can be reflected by cell-level responses (Franklin et al. 2007).

There is little information about the environmental fate of nanomaterials and their possible toxicity to aquatic biota. There are many studies on algal zinc toxicity, but in the form of NPs their toxicity is scarcely explored.

MATERIAL AND METHODS

Biological material

Green freshwater microalgae *Scenedesmus quadricauda* (Turp.) Breb. (Chlorophyta, Chlorophyceae) tribe UTEX 76 was obtained from the University of Texas, Austin.

Microalgae cultivation

Scenedesmus quadricauda was cultivated in vitro on Petri dishes in a cultured room with controlled conditions (23 ± 1 °C, 70 µmol m^2/s of light intensity, light/dark cycle 12:12 h) for several weeks until a significant percentage increase in biomass. Microlgae were then transferred to liquid TAP (Tris Acetate Phosphate) media with varying concentrations of ZnSe nanoparticles, zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and combinations thereof. The concentrations were chosen in the range of 10–250 µM (10, 50, 100 and 250 µM). Samples were taken after 7 days of culture, then lyophilized and analysed.

Chlorophylls and carotenoids determination

The chlorophyll content was determined according to the method of Lichtenthaler and Wellburn (Lichtenthaler and Wellburn 1983). The ethanolic extract was centrifuged and measured at wavelengths of 470, 649 and 665 nm. The calculation was carried out according to the above method.

Spectrophotometric determination of flavonoids

The sample of lyophilized algae was extracted with 80% methanol in an automatic homogenizer. The extract thus prepared was analysed using a 5% $NaNO_2$, 10% $AlCl_3.6H_2O$ and 1 ml of NaOH. The reaction mixture was then measured at 415 nm. The rutin was used as the reference standard.

Spectrophotometric determination of TAC

Total antioxidant capacity (TAC) was determined in a lyophilized sample extracted with 80% methanol according to phosphomolybdate assay system with some modifications (Shabbir et al. 2013). The prepared extract was placed in an ultrasonic bath at room temperature in the dark for 45 minutes. The samples were then centrifuged. The analysis is based on reaction with special reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 60 minutes and then measured at 695 nm against water as a blank. The trolox was used as the reference standard.

Statistical analysis

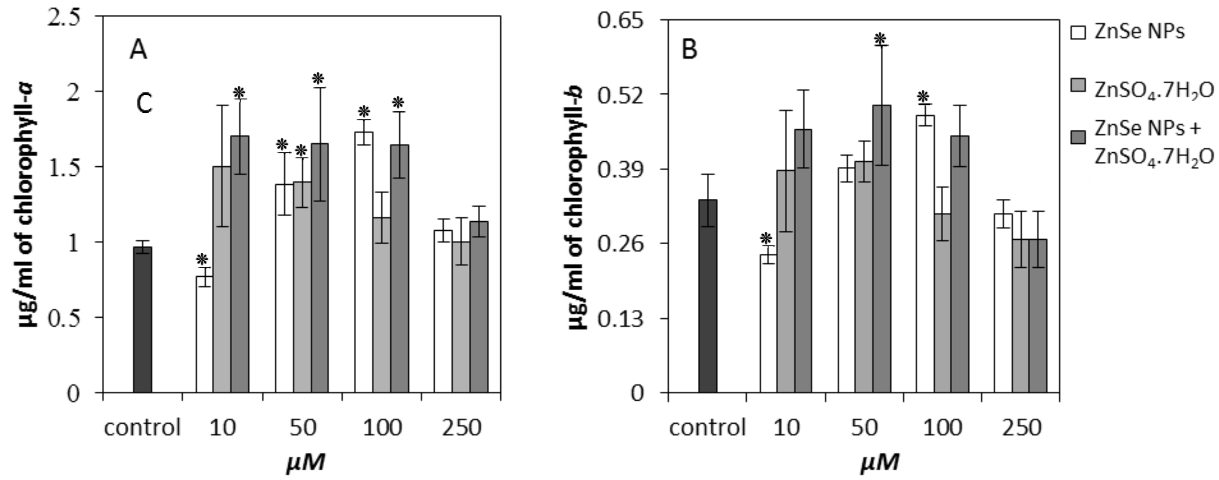
The data were statistically analysed by using software R, version 3.4.0 for windows (www.r project.org). Significant differences compared to the control samples are shown as the asterisk and represent statistically significant differences compared to the control samples ($p < 0.05$, $n = 3$). Statistical analysis was carried using student's *t*-test.

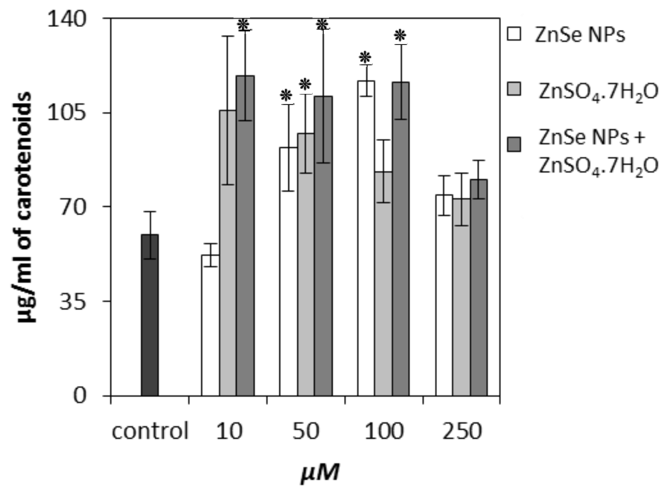
RESULTS AND DISCUSSION

Chlorophylls and carotenoids

For ZnSe nanoparticles, a slight decrease of chlorophyll-*a* (Figure 1A) was first observed compared to control. Then there was a gradual increase up to a concentration of 250 µM, in which we observed a decrease. On the other hand, the samples with zinc ($ZnSO_4.7H_2O$) and a combination of zinc and nanoparticles have increased since the concentration of 10 µM. We observed a stagnation or a slight decrease at the other concentrations, which is most pronounced at a concentration of 250 µM (Figure 1A), when all the samples return to the control value. Similar results were found for chlorophyll-*b* (Figure 1B) and for carotenoids (Figure 1C). This is in contradiction with the outcome of the study Soto et al. 2011, where concentration of chlorophyll-*a* decreased significantly at zinc ($ZnSO_4.7H_2O$) concentration of 0.075 mg/l. Differences could have been caused by a different type of tested algae (*Pseudokirchneriella subcapitata*) that could otherwise react to the presence of zinc (Soto et al. 2011).

Figure 1 The concentration of chlorophyll-a (A), chlorophyll-b (B) and carotenoids (C) in Scenedesmus quadricauda depending on different concentrations of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), ZnSe nanoparticles and combination thereof. Error bars correspond to standard error of mean (n=3).

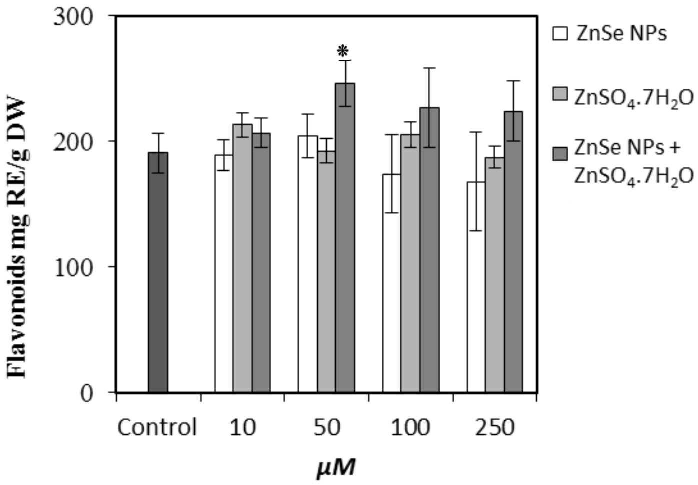




Flavonoids

Flavonoids belong to a large heterogeneous group of substances that are important secondary metabolites of plants (Goiris et al. 2014). They are an important part of the plant's antioxidant mechanism that prevents oxidative stress. Plants under strong stress conditions accumulate flavonoids that are effective reactive oxygen species (ROS) separators. It was assumed that changes in cellular redox homeostasis due to stress activate the flavonoid biosynthesis (Agati et al. 2012). In the sample with ZnSe nanoparticles, we did not notice significant differences in flavonoids content compared to control at concentrations of 10 and 50 µM. At a concentration of 100 and 250 µM, there was a slight downward trend. On the other hand, the zinc ($ZnSO_4.7H_2O$) sample did not show significant differences compare to control at any of these concentrations. The most significant effect on flavonoid content was found in a sample of both nanoparticles and zinc, in which we recorded a slight increase in concentrations of 50, 100 and 250 µM. At these concentrations, the microalgae attempted to prevent oxidative stress by increasing the synthesis of these substances.

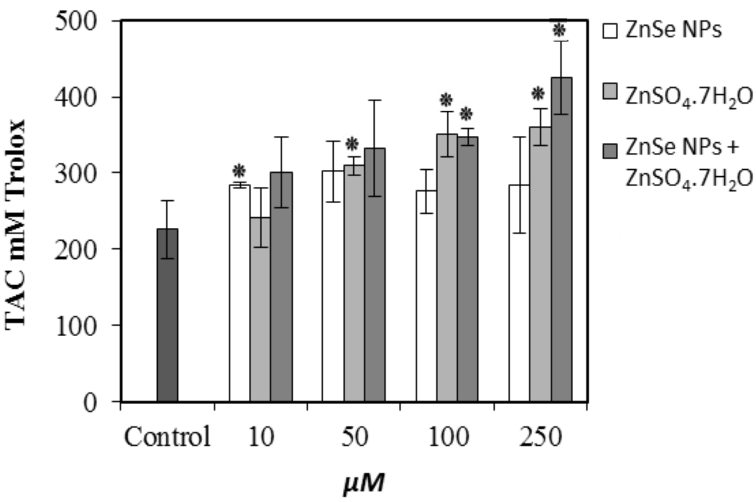
Figure 2 The concentration of flavonoids in Scenedesmus quadricauda depending on different concentrations of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), ZnSe nanoparticles and combination thereof. Error bars correspond to standard error of mean (n=3).



Total Antioxidant Capacity – TAC

The ZnSe nanoparticle sample did not have a significant effect on total antioxidant capacity. On the other hand, a significant increase in concentration was observed for the zinc sample and the zinc-nanoparticle combination. The most significant increase was observed at concentrations of 50, 100 and 250 µM, consistent with results in flavonoid assays. This confirms the hypothesis that the algae at these higher concentrations increased the activity of the antioxidant system to inhibit oxidative stress (Miazek et al. 2015), especially in the sample with a combination of zinc nanoparticles and zinc sulphate heptahydrate.

Figure 3 Total antioxidant capacity in Scenedesmus quadricauda depending on different concentrations of zinc sulphate heptahydrate ($ZnSO_4.7H_2O$, ZnSe nanoparticles and combination thereof. Error bars correspond to standard error of mean (n=3).



One explanation for this result is the possibility of releasing zinc ions from ZnSe nanoparticles, which could increase the concentration of Zn^{2+} ions themselves together with $ZnSO_4$. Therefore, the combined effect of zinc and ZnSe nanoparticles could have these significant effects.

CONCLUSION

Toxicity of ZnSe nanoparticles on green freshwater microalgae *Scenedesmus quadricauda* was investigated. Zinc can be toxic in both bulk and nano forms. The results of this study compare the effect of zinc in the form of bulk, nano and combination of both. It has been shown that zinc in bulk and nanoparticles form can affect chlorophyll-*a*, chlorophyll-*b* and carotenoid levels, resulting in an increase in the content of these pigments, especially at concentrations of 10, 50 and 100 µM. The most significant effect on flavonoid content and total antioxidant capacity was shown by a combination of zinc and nanoparticles. In this sample, increased flavonoid synthesis and increasing concentration of antioxidant capacity were observed. This combination could have the greatest effect due to the release of Zn^{2+} ions from ZnSe nanoparticles, which together with zinc produced a significant effect.

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