Journal of Experimental Biology and Agricultural Sciences, August - 2022; Volume – 10(4) page 767 – 772

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Effect of Titanium, Silver and Zinc Nanoparticles on Microalgae in the Aquatic Environment

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Received – November 01, 2021; Revision – January 14, 2022; Accepted – March 28, 2022
Available Online – August 30, 2022

DOI: http://dx.doi.org/10.18006/2022.10(4).767.772

KEYWORDS

Metal nanoparticles

Microalgae

Aquatic ecosystem

Toxicity

ABSTRACT

Metallic nanoparticles (MNPs) are commonly incorporated in products found in households, industries, and agriculture. The presence of MNPs in the aquatic environment causes damage to living organisms and pollutes the water body rendering it harmful for human consumption. Several studies have been made on the toxicity of MNPs toward microalgae. Most of these studies reported changes in the cellular structure, growth rate, pigments, proteins, and enzymatic activity of microalgae. This review paper focuses on the toxic effects of titanium, zinc, and silver nanoparticles on microalgae in the aquatic environment. A better understanding of the behavior of MNPs in the ecosystem will allow scientists to produce environmentally safe MNPs.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Nanoparticles (NPs), with a size range of fewer than 100 nanometers (Strambeanu et al. 2014) can be classified into different categories based on their shape, size, chemical or physical properties (Khan et al. 2017). These nanoparticles are released into the environment, causing serious environmental implications (Wang et al. 2019). Metallic nanoparticles (MNPs) are nano-sized metals that are known to cause inconsiderable damage to the organisms in aquatic habitats (Krysanov et al. 2010). Water must be easily accessible, sufficient, and clean for the consumption of living beings (Hunter et al. 2010). The MNPs bring a potential threat to aquatic organisms as these pollutants cause bioaccumulation due to their size and unique properties (Kahlon et al. 2018). Contaminants in water are a threat to the aquatic ecosystem and will indirectly affect human health (Madhav et al. 2020). The unprecedented use of NPs leads to harmful effects on soil and water when NPs make their way into the environment. The NPs enter the environment during the production, usage, and disposal of the products made of NPs (Bundschuh et al. 2018). There are an abundance of previous studies evidence the potential of engineered nanoparticles to harm aquatic organisms if present in enough high concentrations (Liang et al. 2020). The most common MNPs that are easily found in water bodies are titanium dioxide, zinc oxide, and silver nanoparticles (Wang et al. 2019).

There are difficulties in the development of a highly reliable method to observe the effect of MNPs on the environment. Frequently, hefty and costly equipment such as aerosal-mass spectrometry systems delayed detection through inductively coupled plasma atomic emission spectroscopy, size-exclusion chromatography, microfiltration, field-flow fractionation, and capillary electrophoresis are required to detect the presence of contaminants in the water body. These detection methods are impractical for detection at the location of the exposure due to the high cost, time-consuming, and requisition of an expert to carry out the testing (Lenaghan et al. 2013).

Primary producers, especially photosynthetic microorganisms such as algae, are efficient bioindicators of NPs toxicity due to their high bioaccumulation ability, sensitivity, rapid growth phase, ease of culturing, and observation at the cellular level (Wang et al. 2019). These microbes are often used in environmental studies because of their sensitivity and the ability to accumulate pollutants (Gadzała-Kopciuch et al. 2004).

This review paper is aimed at analyzing the previously published studies from 2010 to 2020 on the toxicity effects of three MNPs namely titanium dioxide (TiO_2), zinc oxide (ZnO), and silver (Ag) nanoparticles towards the growth, photosynthetic fluorescence, and cell structure of the microalgae.

2 Effect of Titanium Nanoparticle Exposure on Microalgae

Titanium dioxide nanoparticles (TiO_2NPs) are present in three forms such as rutile, anatase, and brookite (Cho et al. 2013). TiO_2NPs are used to remove ethylene from the air for the longer shelf life of fruits, vegetables, and cut flowers, and are also used for the production of toothpaste and paints to give them opaque features (Frazer 2001). Cho et al. (2013) further stated that TiO_2 increases its transparency when the particle size decreases. The smaller TiO_2 particles possess higher UV-blocking properties and prevent microbial growth which is useful for food storage applications (Frazer 2001). They are also used as a potent photocatalyst to break down nearly any organic compound (Frazer 2001). Due to the restricted toxicity, biocompatibility, and inertness, TiO_2 is identified as “the environmental white knight”. The food and Drug Administration (FDA) approved it as a food additive in 1996 (Shah et al. 2017). Karakoti et al. (2006) reported that TiO_2NPs are more reactive due to their physiochemical properties of larger surface area and smaller size.

Iswarya et al.(2015) measured the toxic effects of anatase and rutile forms of TiO_2NPs using *Chlorella* sp. at three concentrations from 0.25 to 1 mg/L under UV radiation for 72 hours. Authors deduced that the cell viability and chlorophyll content were reduced to a great extent in the TiO_2NPs treated cells. It was reported that the rutile form had a significant effect on the reduction of chlorophyll content as compared to the anatase form. The size of NPs correlates to reactive oxygen species (ROS) produced per surface area. The ROS was also observed to have a direct relationship with the decline in the chlorophyll in the NPs treated cell. All cells were observed under a transmission electron microscope to detect the impairment in the cell membrane and nucleus of the microalgae due to the exposure of NPs. Anatase treated cells showed altered morphology, cellular uptake of NPs, and damaged cell membrane while the rutile treatment resulted in altered structure and caused damage to chloroplast and internal organelle. The authors inferred that the different crystalline structures of NPs can cause different impacts on the cell.

In another study by Chen et al. (2012), the harmful effects of TiO_2NPs on *Chlamydomonas reinhardtii* were investigated. The nanoparticle used was a combination of anatase and rutile nanoparticles. The microalgae were cultured in SE medium (Bristol Medium and Soil–water supernatant), followed by exposure to four varying concentrations from 0 to 100 mg/L of TiO_2NPs for 8 hours to 72 hours. Upon exposure, the cells were then observed. The authors found that the TiO_2NPs affected the photosynthetic activity and cell growth. After 8 hours of exposure, carotenoid, chlorophyll *b*, and malondialdehyde (MDA) content increased. However, after 8 hours, the MDA content decreased to a very low amount at 72 hours. The observation indicated that when

Table 1 Toxic effect of TiO_2 nanoparticles on microalgae

Microalgae	Effects	Reference
Dunaliella tertiolecta	Inhibition of cell growth	Manzo et al. 2013
Phaeodactylum tricornutum	Inhibition of protein synthesis (reduction in soluble protein)	Deng et al. 2017
Raphidocelis subcapitata	Increased lipid peroxidation of the cell membrane leads to deformation of the membrane structure	Ozkaleli and Erdem 2018
Chlorella vulgaris	Deformation of the cell wall and irregular morphology	Xia et al. 2018
Chaetoceros gracilis	Increase in polyunsaturated and monounsaturated fatty acids, and a decrease in saturated fatty acids	Baharlooeian et al. 2021

Table 2 Toxic Effects of ZnO nanoparticles on microalgae

Microalgae	Effects	Reference
Pseudokirchneriella subcapitata	Inhibition of cell growth	Aruoja et al. 2009
Chlorella vulgaris	Morphological changes and cell wall damage	Ji et al.2011; Suman et al. 2015
Thalassiosira pseudonana *Chaetoceros gracilis* *Phaeodactylum tricornutum*	Decrease in cell division rates	Peng et al. 2011
Coelastrella terrestris	Cell organelle damage, cell wall breakage, and cytoplasm shrinkage	Sendra et al. 2017
Chlorella sp.	Increased lipid production ability, chlorophyll pigmentation, carotenoid, and starch accumulation	Kaliamurthi et al. 2019
Chlorella vulgaris	Decrease in the chlorophyll content, algal biomass, and cell viability. Cell rupture and aggregation were observed in treated cells	Djearamane et al. 2019a, b
Haematococcus pluvialis	Induced oxidative stress through an increase in reactive oxygen species and lipid peroxidation levels.	Djearamane et al. 2020

the concentrations of TiO_2NPs increased, more cells were disrupted with decreased chlorophyll content and degradation of organelles. The toxic effects of TiO_2NPs on other microalgae are listed in Table 1.

3 Effect of Zinc Oxide Nanoparticle Exposure on Microalgae

Zinc oxide nanoparticle (ZnO NP) is the world's third largest manufactured NPs with 550 tonnes produced yearly (Piccinno et al. 2012). ZnO NPs are known to have high exciton binding energy of 60 meV, piezoelectric and pyroelectric properties, a wide band gap of 3.37ev, and a wurtzite structure lacking the centre of symmetry (Sendra et al. 2017). Zinc is important for the human body's metabolism as it's an essential trace element needed for the activation of numerous metabolic enzymes. This element is also important for protein and nucleic acid synthesis, hematopoiesis, and neurogenesis. ZnO NP can be easily absorbed by the human body and thus used as a food additive. The FDA graded ZnO as a "GRAS" (generally recognized as safe) substance (Jiang et al. 2018).

The authors of a project studied the toxicity effects of different sized and shaped ZnONPs, bulk ZnO, and Zn^{2+} towards freshwater microalgae, *Raphidocelis subcapitata*. Spherical and rod-shaped ZnO NPs of varying sizes and lengths were used for toxicity testing. The microalgae were exposed to various concentrations of each type of ZnO NP from 0.01 to 0.7 mg/L. After 96 hours, it was observed that the highest concentration of ZnO NP completely inhibited the growth of microalgae. The size of spherical-shaped particles did not show any effect on the toxicity. However, the toxicity decreased as the surface area of rod-shaped particles increased. In terms of toxicity toward *R. subcapitata*, spherical ZnO NPs were more damaging than rod ZnO NPs. The exposure of the highest concentration of ZnO nanorods caused 30% cell death, while 50% cell death was detected by treating with nanospherical ZnO on *R. subcapitata* (Samei et al. 2018).

In another study by Manzo et al. (2013), the toxic effects of ZnO NPs and bulk ZnO on green microalgae *Dunaliella tertiolecta* were investigated. The growth rate was measured daily and the study inferred that the nanosized ZnO caused higher toxicity than the bulk ZnO. The increased toxicity of ZnO NPs directly correlates to the physicochemical properties of the nano state compared with the bulk ZnO. The toxic effects of ZnONPs on other microalgae are summarized in Table 2.

4 Effect of Silver Nanoparticle Exposure on Microalgae

Silver nanoparticles (AgNPs) are leading as solutions to the issues of food security and diseases. This nanoparticle is also useful in

Table 3 Toxic effects of Ag nanoparticles on microalgae

Algae Species	Effects	Reference
Pseudokirchneriella subcapitata; *Chlamydomonas reinhardtii*	Reduced chlorophyll content and cell growth inhibition	Wang and Wang 2014; Wang et al. 2016
Dunaliella tertiolecta; *Chlorella vulgaris*	Deterioration of photosynthetic system; *D. tertiolecta* showed a strong decrease in fluorescence indicating more toxicity than *C. vulgaris*	Oukarroum et al. 2012
Thalassiosira weissflogii	Reduction in cell growth, photosynthesis and chlorophyll production; showed secretion of polysaccharide-rich Algal exopolymeric substances (EPS) for Ag+ detoxification	Miao et al. 2009

solar energy applications. The antimicrobial properties and low toxicity toward mammalian cells allowed the vast usage of AgNPs in many household products such as shampoo, soap, toothpaste, and biocidal coatings.

The AgNPs are potentially released into the environment through improper disposal as there are at least 250 products in which these nanoparticles were used and these vary from medical devices, electronic devices, and clothes to disinfectants. Over 2500 tonnes of AgNPs were produced in the United States every year where 80 tonnes were disposed of in surface waters and 150 tonnes in sewage sludge (Tripathi et al. 2017).

Silver nanoparticles were used to control infections in ancient times due to their physicochemical features and biological peculiarities. AgNPs are found to kill the bacteria present in the wound exudates. Thus, FDA approved the use of these MNPs for treating wound infections (Burdușel et al. 2018).

Nam and An (2019) tested the effects of AgNPs, nanowires (AgNWs), and nanoplates (AgPLs) treated with polyvinyl pyrrolidone on the growth and photosynthetic activity of *Chlorococcumin fusionum*. The test solutions were diluted with Bold's Basal Medium (BBM) and were placed into a microplate in triplicates. Algae in the exponential phase were inoculated in each well and incubated. Chlorophyll fluorescence from the algal cells was measured at excitation at 420 nm and emission at 671 nm. The authors reported that the toxicity of AgNPs in *C. infusionum* decreased in the order of AgPLs, AgNWs, and AgNPs based on the inhibition of photosynthesis and growth. The mechanisms for the toxicity of silver nanomaterials depend on the different structures that contain many atoms that allow for more contact surface due to the high surface area of the nanoparticle. This further allowed for a conclusion that the toxicity of nanoparticles is dependent on the diameter and shape.

Li et al. (2015) demonstrated the effect of AgNPs on *Euglena gracilis*, a green alga with a pellicle by showing a decrease in the photosynthetic yield as the concentration of AgNPs increased. The cells showed an irregular round morphology indicating an algal stress response induced by AgNPs. The toxic effects of AgNPs on other microalgae are stated in Table 3.

Conclusion

Upon reviewing studies on the effect of nanoparticle exposures on microalgal growth as above, it raises concerns that the properties, size, and shape of a MNP may influence the adsorption rate of the MNPs into the microalgal cells. As microalgal cells can accumulate these toxicants into their cells, the different sizes and shapes of nanoparticles are crucial to determine the toxicity level of the said nanoparticles in microalgal cells and thus in the environment. More studies should be done on the relationship between the morphological character of MNPs and the toxicity level of the MNPs in the aquatic environment. Apart from that, the properties of MNPs may influence different behaviors in different types of aquatic habitats wherein volume of water, presence of other toxicants, a combination of more than one MNPs, and concentrations of MNP also play a role in determining the pollution level of this environment. Smaller nanoparticles have a higher surface area that provides an attachment site for the interaction with the cellular components and causes cellular damage. More studies and investigations are needed to study the relationship between the toxicity of nanoparticles to microalgal cells and the fate of nanoparticles such as biosorption, uptake, and bioaccumulation in these cells. It is also crucial to determine if the type, shape, and size of the nanoparticle influence the toxicity level of the nanoparticle in the aquatic environment. Scientists will be able to design environmentally safe MNPs through a better understanding of MNP behaviors in biologically active environments.

Acknowledgment

The author would like to acknowledge the Ministry of Higher Education Malaysia, (MOHE)(Grant No. FRGS/1/2020/STG03/INTI/01/1)for supporting the project and publication of this paper.

Conflict of Interest

The author would like to declare that there is no conflict of interest.

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