INTI JOURNAL | eISSN:2600-7320
Vol.2022:36

Photosynthetic Microbes as Bioindicator for Nanoparticles in Aquatic Environment: A Review

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Abstract

The intensive usage of nanoparticles such as Ag, ZnO, and TiO_2 in industries inevitably leads to environmental pollution, especially in the water ecosystem. The bio-uptake and accumulation of nanoparticles in aquatic environments can lead to dire consequences to the flora and fauna, thereby affecting the whole food chain. Photosynthetic microorganisms such as microalgae and cyanobacteria are found abundantly in the natural environment which may be affected by the presence of nanoparticles in the environment, due to their high sensitivity to the presence of pollutants. The present review highlighted the photosynthetic responses after exposure of microalgae and cyanobacteria to these nanoparticles. The different parameters studied on the photosynthetic responses are algal growth, chlorophyll fluorescence emission, algal biomass, primary metabolic content (carbohydrates, lipids, and protein), and the microscopic examination of algae after exposure were summarized and discussed. The effective concentration of microalgae and cyanobacteria was also further investigated in determining the algae with the highest sensitivity as the best potential bioindicator for nanoparticle toxicity in an aquatic environment.

Keywords

Nanoparticles, Bioindicators, Microalgae, Cyanobacteria, Aquatic toxicity

Introduction

Nanotechnology enables nanometre-scale engineering and manufacturing in particle characterization, device, and system design, as well as structure production and application. (Emerich & Thanos, 2005; SCENIHR, 2006). Nanoparticles (NPs) are particulate substances with a length scale between 1 to 100 nanometers in size. These NPs can be engineered to perform a specific function, produced as by-products of combustion reactions, or by occurring naturally (Khan et al., 2019). Metallic nanoparticles have been widely used in a wide range of industries since centuries ago and continue to be so today. Metal and metal oxides are among the most

Submission: 28 June 2022; **Acceptance:** 27 September 2022

commonly used nanoparticles in various consumer products, with titanium dioxide nanoparticles (TiO_2 NPs) and zinc oxide nanoparticles (ZnO NPs) being produced the most abundantly worldwide (Piccinno et al., 2012). Silver nanoparticles (Ag NPs) are also the most popular nanomaterial as their presence in consumer products is more widely advertised with a greater marketing value than the other NPs (Calderón-Jiménez et al., 2017). The presence of nanoparticles in many consumer products has no doubt improved the quality of life and provided countless benefits and economic utility (Pulit-Prociak & Banach, 2016). Nevertheless, it is inevitable for NPs contamination in the environment, especially the aquatic environment due to the growing demand for the manufacturing of nanomaterial products. This has become a matter of concern due to the potential risk and health effects of these NPs that enter the environment through air, soil, and water during various human activities (Khan et al., 2019).

To date, it has been observed that the toxicity of NPs to microbes is due to the release of toxic metallic ions, formation of reactive oxygen species (ROS) which contributes to oxidative stress, internalization of NPs, surface contact, and cell membrane damage (Pulit-Prociak & Banach, 2016; Leareng et al., 2019). Due to the stress of NPs, ROS production will increase to a level that is toxic to the cell, leading to cell damage or cell death (Slavin et al., 2017). In some other cases, NPs can also be retained in the living body and the environment for a longer period because of their greater stability which can lead to bioaccumulation in animals further up the food chain. (Sahu & Hayes, 2017). Photosynthetic microorganisms such as microalgae and cyanobacteria are found abundantly in the natural environment and can be used as a potential bioindicator to detect the presence of NPs due to their high sensitivity toward NPs and high build-up ability (Hazeem et al., 2019). They are also the primary producers in the aquatic systems which have a rapid response to environmental changes and a high reproduction rate (Gökçe, 2016). Microalgae contain photosynthetic pigments, such as chlorophyll which is responsible for capturing light energy and its content. Štefanić et al. (2021) have reported on the impact of Ag NPs on photosynthesis where the most significant change was in the content of chlorophyll.

In this study, the literature research has been done on the different photosynthetic responses such as algal growth, chlorophyll fluorescence emission, algal biomass, the content of carbohydrates, lipids, and proteins of photosynthetic microbes to short-term exposure of zinc oxide nanoparticles, silver nanoparticles, and titanium dioxide nanoparticles in a water-based environment. Algae microscopic examination and effective concentration (EC_{50}) were also studied. The potential of the photosynthetic microbes as a bioindicator is evaluated.

Algal Growth Inhibition

The algal cell viability after exposure to ZnO NPs, NiO NPs, Ag NPs, and TiO_2 NPs shows a decrease in number, which cause concentration-dependent growth inhibition of microalgae and cyanobacteria (Gong et al., 2011; Bhuvaneshwari et al., 2015; Djearamane et al., 2019; Pham, 2019a). In a time-dependent experiment on the exposure of Ag NPs to *C. vulgaris*, cell density was shown to decrease with increasing NPs concentration of 57% at 90 µg/L, 92% at 360 µg/L, and 89% at 1440 µg/L after 96 h. There was increased linear growth inhibition of algae till it reached a plateau with substantial cell lysis (Romero et al., 2020). Bhuvaneshwari et al. (2015)

have shown that algal cells treated with 1 mg/L ZnO NPs have shown membrane damages, cell wall breakage, aggregation of algal cells, and nanoparticles agglomerates encapsulated on algae.

Reduction In Algal Biomass

The algal biomass concentrations had been observed to decrease with increasing time due to the presence of nanoparticles. (Hartmann et al., 2012; Aravantinou et al., 2017; Djearamane et al., 2018; Djearamane et al., 2019) have shown experimental results of concentration and time-dependent inhibitory effects of TiO_2 NPs and ZnO NPs on microalgae. The algal cells which interacted with NPs for the longest period of time had the maximum reduction in biomass. The biomass yield of both *C. vulgaris* and *D. splendida* after Ag NPs treatment was also shown to decrease progressively with increasing Ag NPs concentration when compared to control (Shanab et al., 2021). Aravantinou et al. (2017) have shown that a low concentration of ZnO NPs does not affect the growth of microalgae compared to higher concentrations which resulted in almost zero microalgae growth. The reduction in algal biomass is likely due to the aggregation and agglomeration of the nanoparticles in microalgae.

Chlorophyll Fluorescence Emission

The analysis of chlorophyll fluorescence has been used to monitor the photosynthetic performance of plants non-invasively (Baker, 2004). Chlorophyll can be used as an indicator of toxicity as it is an important primary photosynthetic pigment in the function of algal cells (Cepoi et al., 2020). The fluorescence emission of chlorophyll was shown to decrease with the increasing exposure of time to NPs and concentration of NPs that has been shown using ZnO NPs on algal cells (Djearamane et al., 2019). Fazelian et al. (2020) have also reported a significant decrease in the concentration of chlorophyll *a* in algal cells with increasing the concentration of NPs, with the highest reduction after exposure of 200mg/L NPs. Total chlorophyll was also observed to decrease when *Chlorococcum* sp. algal cells were exposed to ZnO-NPs for 96 h due to destabilization of the cell membranes and induction of oxidative stress (Oukarroum et al., 2018). In contrast, chlorophyll fluorescence emission can also be increased which is shown in the exposure of 1–100 mg of NiO NPs to *C. vulgaris* (Oukarroum et al., 2017). This can be due to nanoparticles which can either hinder the ETC (Electron Transport Chain) in photosynthesis or boost up the photosynthesis process by improving LHC (Light Harvesting Complex) (Shweta et al., 2016). Dewez & Oukarroum (2012) showed that after 3h of exposure to Ag NP, the Chl *a* fluorescence emission rose rapidly, where the algal cells were observed to show a photoinibitory effect.

Protein Content

After exposure of metal nanoparticles, protein content was observed to increase compared to untreated algal cells (Gong et al., 2011; Mahfooz et al., 2016; Fazelian et al., 2020). Studies on the exposure of ZnO NPs to *N. oculata* have reported similar results, where the increase in protein content may be considered an active defense mechanism against abiotic stresses to prevent the destruction of algae cells (Fazelian et al., 2020). An increase in protein content has also been

reported by Elrefaey et al. (2021) on *S. opoliensis* cells after ZnO NPs treatment. *Chlorella vulgaris* showed that the accumulation of intracellular proteins along with growth rate reduction was targeted to its survival and irrelevant to cell division. Thus, this can relate to the inability of cell division, leading to an increase in cell size (Romero et al., 2020). Saxena et al. (2021) reported using proline to showcase the protein content in algal cells when exposed to ZnO NPs as it plays a role of antioxidant by mitigating ROS. In response to nanoparticle stress, increased levels of proline content in a dose-dependent manner may participate in ROS scavenging.

Lipid Content

The lipid content of algae was observed to increase after exposure to NPs. A study showed that after ZnO-NPs treatment, the lipid contents of *S. opoliensis* were progressively increased compared to the control condition and the highest growth was observed at 200 mg/L by 116.22% (Elrefaey et al., 2021). In AgNPs treatment to *C. vulgaris*, there was a significant increase in the concentration of lipid content per cell at 90 to 1440 µg/L Ag NPs from 87% to 439% compared to control in a concentration-dependent way (Romero et al., 2020). Some studies reveal that stress conditions can generate the production of stress proteins that would alter starch production to lipid synthesis. The treatment of NPs on algal cells that induces the rise in lipid production was caused by the generation of oxidative stress (Elrefaey et al., 2021). As lipid content is the first response to any environmental change, the change in it could be the trigger of the algae's protective mechanism in protecting itself from the oxidative stress caused by NPs (Huang et al., 2016). This can also be seen as lipid peroxidation which indicates oxidative stress, as it is triggered by a single ROS. When *P. subcapitata* was introduced to increasing ENP concentration of TiO_2, Al_2O_3, and SiO_2, it causes an increase in normalized specific MDA (Metzler et al., 2012). Similarly, elevated MDA concentrations have been observed in *C. reinhardtii* after 8 h exposure to TiO_2 ENPs (0.1–100 mg/L), along with signs of regeneration and acclimatization after 72 h (Chen et al. 2012). Lipid peroxidation has been reported to be a highly damaging process as it can increase membrane permeability, leading to the loss of membrane selectivity, fluidity, and integrity (Moos & Slaveykova, 2013).

Carbohydrate Content

Carbohydrates are the main photosynthetic product of microalgal photosynthesis as they are energy and carbon storage components stored as starch grains in chloroplasts (Huang et al., 2016). Romero et al. (2020) have shown that there was an increase in the algal carbohydrate content of *C. vulgaris* to Ag NPs of 22, 29, and 80% at the concentration of 360, 720, and 1440 µg/L Ag NPs, respectively. This could be due to an elicit for an increase in respiration, a signal of cellular stress, or energy production that require a higher carbohydrate utilization (Romero et al., 2020). According to He et al. (2017), improved carbohydrate production is a response to environmental stress (oxidative stress) by acting as a protective mechanism that could also trigger the production of specific ROS scavengers. *Chlorella vulgaris* also showed increased carbohydrate content with a maximum yield of 13.8% with increasing concentration of Ag NPs from 50 µg/g to 150 µg/g, but was constant once beyond 150 µg/g. This might be due to the availability of more silver nanoparticles that greatly rupture the cell membrane of microalgae (Razack et al., 2016). In

contrast, following ZnO NPs exposure to *S. opoliensis*, carbohydrate content was observed to decrease significantly in a concentration-dependent manner. Interestingly, it was shown to not affect carbohydrate content at 10 mg/L, but showed a significant decrease of 29.64% and 43.56 % in carbohydrate content when at high ZnO NPs concentrations of 50 and 100 mg/L respectively (Elrefaey et al., 2021). Carbohydrate yield in *C. vulgaris* and *D. splendida* also showed progressive and significant reduction with increasing Ag NPs concentrations (Shanab et al., 2021). This is due to the high metal concentration that causes cell membrane damage, leading to intake and uncontrolled release of electrolytes (Anusha et al., 2017).

Microscopic Examination

Electron microscopy images of NPs treated microalgae culture revealed several morphological changes in the algal cell, depending on the concentration of nanoparticles. The untreated control cells showed uniform distribution of cell chloroplast, intact cell wall, compact thylakoids, a very thin capsule, and a high number of carboxysomes (Cepoi et al., 2020). Under the impact of nanoparticles, the cells were completely damaged with distorted structure along with complete cell wall lysis. Based on Elrefaey et al. (2021), algal cells after ZnO-NPs treatment showed deformed and swollen cells, irregular cell outlines, colourless cells with no chlorophyll content, cell shrinkage, and stretching. This is due to oxidative stress generated by ZnO-NP, causing disruption to the lipid bilayer and cellulose composition of the microalgae cell wall, leading to cell wall degradation and leakage of cellular material (Razack et al., 2016; Elrefaey et al., 2021). It was also observed that TiO_2 particles were surrounding the cell surface which could become an obstacle in the exchange of substances (e.g. nutrients) between algal cells and their surrounding environment, interrupting the growth of algae cells (Chen et al. 2012, Saxena et al., 2021). The metal nanoparticles are small in size compared to the maximal pore size of algae which allows them to easily pass through the cell wall. The adhered NPs to algae cell surface will enhance the membrane damage and metal accumulation in algae cells, leading to excessive generation of ROS, cell wall depression, increase in cell membrane permeability, resulting in cell death (Li et al., 2020).

Dose-Response of Algae Cell Expose to Nanoparticles

From previously conducted studies, different inherent sensitivity of microalgae towards nanoparticles can be shown through toxicity testing of various algae by comparing their EC_{50} values. In this context, the EC_{50} is the effective concentration (or dose) of nanoparticles that produce 50% of the maximal response that results in algal growth inhibition, relative to control, at a given duration of time. The comparison of EC_{50} on different algae after exposure to silver, zinc oxide, and titanium nanoparticles are stated in Table 1, 2, and 3. EC_{50} is a parameter that reflects the overall physiological state of the algal cell, as nanoparticles in low concentration that inhibit cell growth significantly indicate a highly toxic effect on cell growth (Romero et al., 2020). The differences in the values of effective concentrations are probably due to the different parameters that affect the toxicity of NPs to the algal cell such as the mode of preparation of the colloidal suspension of the nanoparticles, their size, morphology, state of aggregation, medium, exposure time and exposed concentration among others (Romero et al., 2020).

Table 1. Toxic effects of silver nanoparticles on algae.

Tested algae species	Exposure Period	Exposure concentration	Toxic Effect	References
Chlorella vulgaris	96 h	90 – 1440 µg/L	EC_{50}: 110 µg/L	(Romero et al., 2020)
Chlorella vulgaris	72 h	1 – 100 µg/L	N-Ag NPs and P-Ag NPs EC_{50}: 70 µg/L; 50 µg/L EC_{10}: 12 µg/L; 5 µg/L	(Zhang et al., 2020)
Scenedesmus acuminatus	96 h	1 – 100 µg/L	EC_{50}: 38.5 µg/L	(Pham, 2019b)
Scenedesmus sp.	72 h	5 - 200 µg/L	EC_{50}: 89.92 ± 9.68 µg/L	(Pham, 2019c)
Thalassiosira sp.			EC_{50}: 107.21 ± 7.43 µg/L	
Pseudokirchneriella subcapitata	72 h	1 – 125 µg/L	EC_{50}: 32.40 µg/L	(Ribeiro et al., 2014)

Table 2. Toxic effects of zinc nanoparticles on algae.

Tested algae species	Exposure Period	Exposure concentration	Toxic Effect	References
Chlorella vulgaris	35 days	1–5 mg/L	25^{th} day – IC_{50}: 0.258 mg/L bulk-form: 1.255 mg/L	(Saxena et al., 2021)
Scenedesmus opoliensis	96 h	0.1–$1e^5$ µg/L	EC_{50}: 180 µg/L	(Ye et al., 2017)
Arthrospira platensis	96 h	10–200 mg/L	72h – EC_{10}: 1.29 mg/L; EC_{50}: 31.56 mg/L 96h – EC_{10}: 0.83 mg/L; EC_{50}: 13.97 mg/L	(Djearamane et al., 2018)
H. pluvialis			72h – EC_{10}: 6.8 mg/L; EC_{50}: 241.21 mg/L 96h – EC_{10}: 2.37 mg/L; EC_{50}: 186.67 mg/L	
Anabaena sp.	96 h	1–50 mg/L	EC_{50}: 0.38 ± 0.004 mg/L.	(Tang et al., 2013)
Pseudokirchneriella subcapitata	72 h	0.01–100 mg/L	EC50: 0.10 mg/L	(Aruoja et al., 2015)

Table 3. Toxic effects of titanium dioxide nanoparticles on algae.

Tested algae species	Exposure Period	Exposure concentration	Toxic Effect	References
Chlorella vulgaris	120 h	150–1200 mg/L	24 h – EC_{50}: 289.338 mg/L 48 h – EC_{50}: 366.97 mg//L 72 h – EC_{50}: 769.029 mg/L 96 h – EC_{50}: 1126.75 mg/L 120 h – EC_{50}: 598.42 mg/L	(Andronic et al., 2021)
Chlorella vulgaris	72 h	3–192 mg/L	EC_{50}: 16.12 mg/L	(Sadiq et al., 2011)
Scenedesmus sp.			EC_{50}: 21.2 mg/L	
Scenedesmus bijugus	96 h	5.00×10^{-9} – 5.00×10^{-6} mol/L	EC_{20}: 1.20×10^{-7} mol/L	(Barreto & Lombardi, 2016)
Scenedesmus obliquus	72 h	25 – 800 µM	EC_{10}: 6.83±0.53 µM EC_{50}: 136.88±2.30 µM EC_{90}: 2770.09±189.99 µM	(Iswarya et al., 2017)
Pseudokirchneriella subcapitata	72 h	0.01–100 mg/L	EC_{50}: 1.5 mg/L	(Aruoja et al., 2015)

Conclusions

In conclusion, microalgae & cyanobacteria are potential to be used as a bioindicator in identifying NPs in an aquatic environment due to the cytotoxic effects exhibited including reduction of cell viability, algal biomass, chlorophyll fluorescence emission, increasing levels of protein, lipid, and carbohydrate, and major adverse morphological changes. Among the algae test species recorded, *P. subcapitata is* the most sensitive algae with the lowest EC_{50} towards all Ag, ZnO, and TiO_2NPs, showing it to be the best potential bioindicator in identifying NPs contamination in the nutrient microalgae and also for testing the toxic effects of metal NPs on the aquatic organisms.

Acknowledgements

This project is supported by research fund provided by the Ministry of Higher Education Malaysia (Grant No. FRGS/1/2020/STG03/INTI/01/1).

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