Cytotoxicological evaluation of copper oxide nanoparticles on green algae, bacteria and crustacean systems

B. Janani¹ · Dunia A. Al Farraj² · Lija L. Raju³ · Mohamed S. Elshikh² · Noorah A. Alkubaisi² · Ajith M. Thomas⁴ · Arunava Das¹ · S. Sudheer Khan¹

Received: 4 September 2019 / Revised: 9 September 2020 / Accepted: 4 October 2020 / Published online: 10 October 2020
© Springer Nature Switzerland AG 2020

Abstract

Purpose Copper oxide (CuO) nanoparticles (NPs) have been utilized in several industries including textile, consumer products, medical, automobiles etc. The discharge of industrial effluents in environment increased the probability of CuO NPs contamination in the ecosystem.

Methods The present investigation used CuO NPs to determine the toxic effect on *Lyngbya* species, fresh water algae isolated from natural pond, bacterial species *Pseudomonas aeruginosa* and *Staphylococcus aureus* and a crustacean species *Daphnia magna*.

Results The NPs average diameter and zeta potential was estimated to be 45 ± 3 nm and 29 ± 1.78 mV respectively. The results showed that 0.1 $\mu\text{g/mL}$ CuO NPs showed the growth inhibition of $47 \pm 2\%$ on *Lyngbya* sp. after 5 days of incubation. The CuO NPs also showed toxic effect to bacterial systems such as *P. aeruginosa* and *S. aureus* and crustacean system *D. magna*. Further, there was an increased lipid peroxidation and generation of reactive oxygen species (ROS) in algal cells observed up on NPs exposure. The exposure of NPs suppressed the antioxidant defense system. The amount of glutathione was reduced after the exposure of NPs.

Conclusion The study suggested the role of ROS in toxicity of algal and bacterial systems. The present study pointed out the potent toxicity of CuO NPs to the organisms present in the aquatic environment.

Keywords Copper oxide nanoparticles · Toxicity · Oxidative stress · Green algae · Bacteria · Crustaceans

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s40201-020-00561-1>) contains supplementary material, which is available to authorized users.

✉ S. Sudheer Khan
sudheerkhan@bitsathy.ac.in

- ¹ Nanobiotechnology Laboratory, Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Tamil Nadu, India
- ² Department of Botany and Microbiology, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia
- ³ Department of Zoology, Mar Ivanios College, Nalanchira, Thiruvananthapuram, India
- ⁴ Department of Botany and Biotechnology, St Xavier's College, Thumba, Thiruvananthapuram, India

Introduction

Nanoscience attempts to study the nanoscale level objects and nanotechnology seeks to combine, alter and modify materials at this level [1]. Nanotechnology and nanoscience branch act as a bridge and connect in many fields including manufacturing technologies, medicine, electronics, pharmaceuticals and telecommunications [1, 2]. Metal oxide nanoparticles (NPs) were widely used in semiconductors, fillers, microelectronics, catalysts and cosmetics [3–6] and it increases the possibility of reaching these particles to reach the environment. Copper oxide (CuO) is found to cause toxic effects towards algae, crustaceans and bacteria [7, 8]. Though the living organisms require copper, an essential trace element, cellular damage can be caused by Cu when its concentration superfluous [9].

The attention towards cyanobacteria or blue-green algae is increased in the recent decades due to the diverse production of biologically active or toxic compounds, which is majorly applied in environmental health and biomedicine [10]. In

globe, one of the most wide spreader fresh water algae is *Lyngbya* species. and it is a rich source for bioactive metabolites producers [10]. CuO NPs are frequently used as antifouling agent. The extensive use of CuO NPs can lead to the contamination of aquatic ecosystem and it can collapse the ecosystem balance [11]. However, the environmental toxicity caused by these NPs is not well understood. Hence, it is very necessary to estimate the potential toxicity of CuO NPs to the organisms present in the environment. Therefore, the present study focused to understand the CuO NPs toxicity on *Lyngbya* sp. For evaluating CuO toxicity on bacteria, *Pseudomonas aeruginosa* (MTCC 1688) and *Staphylococcus aureus* (MTCC 3160) were used and *Daphnia magna* sp. was chosen as the crustacean system.

Materials and methods

Materials

CuSO₄, NaOH and polyvinylpyrrolidone (PVP) were obtained from Merck, India. The particles were dispersed using an ultrasonic processor (Sonics, USA). The experiments were performed in six replicates.

Collection and culturing of algae

The algal species was isolated and purified as per the method described by Rippka [12]. The algae were collected from a natural fresh water pond (Kumbakonam, Tamil Nadu, India – 10.9617° N, 79.3881° E), and identified as *Lyngbya* sp., and Bold's 3N modified medium was used to culture the algae. The algal samples weighed equally of 1 g and transfer was made to separate flasks with Bold's 3N medium. At room temperature (25 °C), the flasks were incubated in a shaker under fluorescent light in the rhythm of day (8 h) and night (16 h).

Synthesis of nanoparticles

CuSO₄ (0.1 M) and NaOH (0.4 M) were prepared in separate conical flask. CuSO₄ solution was heated on a magnetic stirrer till the solution attains a temperature of 80° C. Further, NaOH was added drop by drop. PVP was added to stabilize the particles. After 3 h of stirring, the mixture was centrifuged at 4 °C (10000 x g, 30 min) and the pellet was lyophilized.

Characterization of nanoparticles

The NPs size distribution was estimated with particle size analyzer (Malvern, UK). The NPs structure, surface state, and morphology were found using high resolution transmission electron microscope (HRTEM) (JEOL JEM 2100, Japan)

with an acceleration voltage of 200 keV. The energy-dispersive X-ray spectroscopy (EDS) (AMETEK EDAX Octane series) was carried out for elemental analysis. The particle zeta potential was determined by using Malvern zeta analyzer, UK. X-ray photoelectron spectra (XPS) was performed with monochromatized Al K α excitation for NPs to find chemical states using Thermo-VG Scientific Escalab 250 spectrometer.

Cytotoxic evaluation of CuO NPs on algal cells

The toxicity of CuO NPs on *Lyngbya* sp. was evaluated based on the reduction in chlorophyll content due to NPs exposure. The amount of chlorophyll content is directly proportional to the live cells number. Briefly, the algae (1 gm) was freshly grown and transferred to Bold's 3N modified medium, and the OD at 663 nm was adjusted to 0.5. Various studies reported the toxicity of CuO NPs to algae with EC50 value in-between 0.54 to 37.55 mg Cu/L [13]. Depending on the results we have designed the concentration of CuO NPs (0.1–25 μ g/mL). The algal cells were made to interact with CuO and incubated under fluorescent light for 10 days in the rhythm of day (8 h) and night (16 h). To extract the chlorophyll pigment from the above grown algae, 80% acetone (10 ml) was added to the sample. The overnight incubated (4 °C) samples were centrifuged at 10000 x g (10 min, 4 °C). The absorbance of supernatant was measured using UV-visible spectrophotometer at 652 nm. The toxicity was estimated by measuring the total chlorophyll content's absorbance in the control and the test samples.

Lipid peroxidation assessment

To determine the peroxidation of lipid, thiobarbituric acid reactive species (TBARS) formed after NPs exposure was measured. To the flasks containing different concentration of NPs in culture medium, algae of 0.5 gm was suspended, and incubated for 24 h. Centrifugation (10000 rpm for 5 min at 4 °C) was done to collect pellets and homogenized the pellet using trichloroacetic acid (5% TCA). The samples again centrifuged (4 °C, 10 min, 10000 x g) and collected the supernatant. Thiobarbituric acid (0.5% TBA) in TCA (20%) was added at equal volume to the above supernatant and incubated in water bath (96 °C, 30 min). Thereafter, the supernatant was taken by centrifuging solutions for 10 min at 10000 x g (4 °C) and measured the absorbance at 532 nm.

ROS determination

The amount of ROS formation was evaluated indirectly by measurements of dichlorofluorescein content formed from dichlorofluorescein diacetate (DCFH-DA) oxidation, a non-fluorescent product. After the algae-NPs interaction for 24 h,

cells were centrifuged and resuspended in pH 7.8 PBS containing DCFH-DA (30 $\mu\text{g/mL}$) and kept in a shaker for 30 min. The excitation and emission wavelength were set at 485 nm and 520 nm and the fluorescence values were recorded.

Toxicity evaluation by esterase activity

Fluorescein diacetate (FDA) was used to estimate the esterase activity of algal cells [14, 15]. The responses in the esterase activity on algae *Lyngbya* sp. by CuO NPs interaction was evaluated by quantifying the mean conversion of FDA rates. Here, 10 μL of 10 μM FDA was added with 500 μL of cell suspension. The mean fluorescence intensity was calculated since the activity of esterase continuously changes in cell and the data are reported based on the percentage difference from control [15].

Antioxidant enzyme assays

The oxidative stress was evaluated based on different parameters including lipid peroxidation, ROS formation and antioxidant enzyme activity after exposure of 0.1–25 mg/L NPs to algal cells. The catalase activity was calculated using the amount of H_2O_2 reduction in the cell. To the algal sample, cell lysates (0.5 mL) and H_2O_2 (0.1 M) in phosphate buffer (0.1 M) was added. Then, A240 was recorded for every 1 min interval for 5 min. To estimate the activity of superoxide dismutase (SOD), nitro blue Tetrazolium (NBT) (1.5 mM) with MgSO_4 (10 mM) and 2-mercaptoethanol (0.1 mM) were added to the cell lysate. The absorbance of above solution was measured at 350 nm, for every 1 min for 5 min. To estimate the activity of antioxidant glutathione reductase (GR), DTNB (400 μM) and NADPH (300 μM) was added to the cell lysate. The A405 of above solution was recorded for every 1 min. To estimate the amount of reduced glutathione (GSH), 5-thio(2-nitrobenzoic acid) (TNB) formed from oxidation of GR by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was measured by taking the absorbance at 405 nm.

Bacterial toxicity testing

The evaluation of bacterial toxicity by CuO NPs was done by dilution plate count method to count the number of live cells present compared to control. The present study used two bacterial cultures, *S. aureus* and *P. aeruginosa* to test the antibacterial property of CuO NPs. Briefly, 10 mL overnight cultures were centrifuged and the pellets were re-suspended 0.75 mM saline. Before interaction with CuO NPs, the OD value of the bacterial suspension at 600 nm was adjusted to 0.1 that corresponding to 1×10^8 cells per mL. The toxic effect of CuO NPs towards a wide range of gram -ve and + ve bacteria is reported previously at 10 to 100 mg/L [16]. Based on their

experimental results, the present study chosen the concentrations of CuO NPs (0.1–25 $\mu\text{g/mL}$). Then the interaction was continued for 4 h and the dilution plate count method was used to count the number of live cells left the interaction solution. Lipid peroxidation assay, ROS generation and antioxidant activity were also evaluated for bacterial culture after CuO NPs exposure as described earlier.

Crustacean toxicity testing

Here, the CuO NPs toxicity on crustacean system was tested on *Daphnia* sp. isolated from a natural pond located in Kumbakonam, Tamil Nadu (10.9617° N, 79.3881° E). A light microscope was used for the morphological identification of *Daphnia* sp. and the toxicity studies were performed using ElenMtM7 media. Briefly, in ElenMt M7 media, 24 h old *Daphnia* neonates (30 numbers) were exposed to CuO NPs for 24 h. The CuO NPs toxicity to crustacean was estimated in various reports with EC_{50} values up to 9.8 mg Cu/L [13]. Based on this we extended the CuO NPs concentration from 0.1–25 mg/L. The toxicity was calculated based on the number of motile daphnids.

Statistical analysis

The tests were done with six replicates and standard errors were calculated. Data were collected from samples and the data were studied for normal distribution and variance homogeneity. The statistical significance was tested using paired t-test followed by signed rank test using Sigma Plot 11 (Systat Software Inc.). The accepted level of significance was $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*), and the insignificant value was $P > 0.05$.

Results and discussion

Characterization of nanoparticles

CuO NPs were primarily characterized with HRTEM and it is shown in Fig. 1a. Figure 1b shows the typical EDS spectra of CuO NPs. EDS analysis indicated that the synthesized products were composed of only copper and oxygen elements. The particles exhibited the mean diameter of 45 ± 3 nm (Fig. 1c). The particles zeta potential was found to be 29 ± 1.78 mV. The particles found to be positively charged and hence the possible interaction with the microorganisms will be more. The microorganisms possess negative surface charge due to the peptidoglycan content in the cell wall, hence the electrostatic force of attraction favors the interaction between the microbes and the particles [17] and thereby increase the potential toxicity of CuO NPs.

Fig. 1 Characterization of copper oxide nanoparticles: (a) transmission electron microscopic image, (b) energy-dispersive X-ray spectroscopy, (c) particle size distribution of CuO NPs, (d) full-scan X-ray photoelectron spectrum of CuO-NPs and corresponding deconvoluted peaks in the high resolution spectra for (e) Cu-2p and (f) O-1 s elements

XPS was performed to determine chemical states present in synthesized CuO NPs (Fig. 1d). As expected, for CuO NPs, the major components present were Cu and O with no trace of unwanted contaminants. Further (Fig. 1e), Cu2p core level scan spectra observed with doublet peaks centered at ~ 953.5 eV and ~ 933.6 eV attributed to Cu2p_{1/2} and Cu2p_{3/2}, respectively. Also, peaks at 943.7 eV, 940.9 eV and 962.3 eV were noted as set of satellites peaks attributed to Cu²⁺ state in CuO [18]. In O-1 s spectra (Fig. 1f), two components were noted at 529.54 eV and 531.20 eV. This result attributed to the O²⁻ ion in the Cu-O bonds and oxygen vacancies in the CuO lattice [18].

Algal toxicity

According to Kahru and Dubourguier [19] classification, CuO NPs was included in the category of ‘very toxic’. Here, the toxic effect of CuO NPs was tested against *Lyngbya* sp. and the results depicted in Fig. 2. The toxic potential of the particles increases with increase in NPs concentrations. Here, 0.1 $\mu\text{g/mL}$ CuO NPs showed the growth inhibition of $27 \pm 2\%$ and 25 $\mu\text{g/mL}$ showed the toxicity of $62 \pm 5\%$ on *Lyngbya* sp. after 5 day of incubation (Fig. 2). Similar trend was noted in investigation of Melegari et al. [20] where carotenoids levels in *Chlamydomonas reinhardtii* had significant reduction, as a response of increased CuO NPs concentration. The present study, we have evaluated the toxicity based on the reduction in total chlorophyll and a significant reduction in chlorophyll content observed with increase in CuO NPs exposure.

Esterase activity

One of the key indicators demonstrated to determine algal toxicity by exposure to Cu ions and CuO NPs was the esterase activity [15, 20–22]. The reduction of FDA fluorescence showed a loss of integrity of cell membrane or activity of enzyme and toxicity which is expressed in terms of percentage difference from control (algae without NPs interaction). Algal cells were interacted with various dosages from 0.1 to 25 mg/L of CuO NPs. The algae showed decreased esterase activity of $-12 \pm 2\%$ for 0.1 mg/L and $-34 \pm 3\%$ for 25 mg/L (Supplementary material Fig. 1). The results showed that the enzyme activity decreases with increase in concentrations of

Fig. 2 Toxicity of CuO NPs on *Lyngbya* species. Values are the mean of $n = 6$ (mean $\pm$ standard error). Level of significance $P < 0.001$ (***)

NPs. The decrease of esterase activity indicated loss of cell membrane integrity or deficit in enzyme activity. Similar trend in the reduction of enzyme activity was observed in the study of Melegari et al. [20] where they studied the toxicity of CuO NPs in green algae *Chlamydomonas reinhardtii*.

Bacterial toxicity testing

Our study reported that CuO NPs showed toxic effect on both *S. aureus* and *P. aeruginosa* (Fig. 3). The toxicity was studied under various NPs concentration (0.1 to 25 mg/L) and the toxicity increased when NPs concentration increased. *P. aeruginosa* showed higher toxicity after NPs interaction compared to *S. aureus*. *P. aeruginosa* showed 69 ± 2 for 0.1 mg/L and 99 ± 3 for 25 mg/L NPs concentration. *S. aureus* showed the toxicity of $62 \pm 2\%$ for 0.1 mg/L and $98 \pm 4\%$ for 25 mg/L NPs concentration. Sun et al. [23] investigated the toxicity of different metal oxide NPs on A549, CNE-2Z and H1650 cell lines and they identified CuO NPs as most toxic. Microbial toxicity caused by different NPs including CuO, Sb_2O_3 , ZnO and NiO were tested on *E. coli*, *S. aureus* and *B. subtilis* and the result showed that CuO NPs showed higher toxicity among the tested NPs (CuO, Sb_2O_3 , ZnO and NiO) [24]. In another work CuO NPs toxicity was investigated in presence of dissolved organic matter on *E. coli* and it was found that the bacterial membrane damaged severely by CuO NPs [25]. Various reports suggested the dissolution of CuO NPs in biological systems as an important factor that causes harmful effects [19]. Xu et al. [26] investigated the toxicity on soil microbes by TiO_2 and CuO and they reported that CuO NPs showed stronger negative effects on soil microbes than that of TiO_2 NPs and exposure of CuO NPs reduced the soil microbial biomass than TiO_2 .

Fig. 3 Toxicity for *Pseudomonas aeruginosa* and *Staphylococcus aureus* after CuO NPs treatment. Values are the mean of $n = 6$ (mean $\pm$ standard error). Level of significance $P < 0.001$ (***)

Lipid peroxidation, ROS formation and oxidative stress on algal and bacterial cells

The evaluation of free radicals and antioxidant are important as the imbalance of free radicals and antioxidant in body causes oxidative stress. The lipid peroxidation study suggested that 0.1 $\mu\text{g/mL}$ of CuO NPs able to generate 67% higher TBARS in algal cells compared to control. The treatment of algal cells with increasing concentrations of CuO NPs showed a progressive increase in TBARS formation and it indicated increased lipid peroxidation (Supplementary material Fig. 2). Supplementary material Fig. 2 shows the ROS formation in algal cells up on the exposure of different concentrations of CuO NPs. The ROS evaluation result supported the analysis of lipid peroxidation. Excessive ROS generation can induce damage in algal membrane, which may cause cell lysis and finally cell death [27]. Due to abundance of ROS generation, CuO NPs can cause oxidative stress and a range of biological responses [28]. Figure 4 represents the antioxidant defense enzyme activity of algal cells. The activity of these enzymes decreased after the interaction with CuO NPs, compared to control. Reduced form of glutathione content was measured. A decreased level of reduced glutathione content was observed after algal- NPs interaction (Fig. 4). According to Melegari et al. [20] the formation of ROS and lipid peroxidation was induced in an algae *Chlamydomonas reinhardtii* at CuO NPs concentration of 1000 $\mu\text{g/mL}$. On contrary, the study observed that CuO NPs induced the formation of ROS and peroxidation of lipid in *Lyngbya* sp. at 0.1 $\mu\text{g/mL}$. The toxic effect may vary with different genera of algal species.

In bacterial systems, *P. aeruginosa* showed increased lipid peroxidation after NPs exposure compared to *S. aureus* (Supplementary material Fig. 3). The lipid peroxidation was

Fig. 4 Effect of NPs on antioxidant activity in *Lyngbya* sp. Data are plotted by comparison with control samples, without NPs treatment. Values are the mean of $n = 6$ (mean $\pm$ standard error). Level of significance $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*)

calculated compared to control cells and it was found that 53% and 88% on *P. aeruginosa* and 48% and 85% on *S. aureus* for 0.1 and 25 mg/L respectively. The ROS formation results showed that *P. aeruginosa* produced ROS slightly higher than *S. aureus* compared to control (Supplementary material Fig. 3). The slight variation might be due to the difference in cell wall composition. The ROS formed was identified calculated based on the percentage difference from control and it was 90% and 130% on *P. aeruginosa* and 78% and 125% on *S. aureus* for 0.1 and 25 mg/L NPs respectively. The ROS were considered as a toxic by-product of aerobic metabolism and these ROS were disposed with help of antioxidants produced by the cell [29]. Hence the formation of antioxidant enzyme up on interaction with NPs is an important area to study since it can cause oxidative stress. In low dosage of NPs the enzyme SOD, catalase and total GSH + GSSG show increased activity of 15%, 19% and 8.53% (0.1 mg/L) respectively. For further increase in dosage of NPs, the enzyme SOD, catalase and total GSH + GSSG level decreased with respect to control (Fig. 5). Glutathione reductase activity and GSH level was found to be reducing up on exposure of CuO NPs from 0.1 to 25 mg/L. Bondarenko et al. [16] studied the toxicity of nano-CuO on *E. coli* and it was found that hydrogen peroxide and superoxide anions formation were triggered by CuO NPs at low sub-toxic level (0.1 mg Cu/L). Shaw et al. [30] demonstrated the CuO NPs induces lipid peroxidation in gills and tissue of rainbow trout.

The toxicity of the organisms can be directly correlated with the oxidative stress caused by CuO NPs. The two important responses of NPs interaction with algal and bacterial systems were lipid peroxidation and ROS generations. The oxidative damage of polyunsaturated fatty acids leads to the formation malondialdehyde in organisms, which is used as a biomarker for lipid peroxidation [31]. It is considered as an indirect method to evaluate the lipid membrane damage by ROS. The stress generated by the NPs interaction leads to the production of oxidative radicals [32]. Further, the membrane selectivity, integrity and fluidity was lost by the damage of lipid membrane by the oxidative radicals which increased cell membrane permeability and increase CuO entry and uptake [33]. Higher levels of ROS observed in present investigation could be attributed to antioxidant enzyme activity inhibition resulted in oxidative stress and eventually cellular toxicity [34]. Thus, individual ROS measurements have a direct correlation to the measured total ROS in the system. Also, the quantity of radicals produced also depends on the particles' concentration.

Crustacean toxicity testing

An established model species for monitoring environmental toxicity by contaminants in the world is *Daphnia*. This have key role in regulatory criteria by government agency [35].

Fig. 5 Effect of NPs on antioxidant activity in (a) *P. aeruginosa* and (b) *S. aureus* after CuO NPs treatment. Values are the mean of $n = 6$ (mean $\pm$ standard error). Level of significance $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*)

In this study, *Daphnia* sp. was used to study crustacean toxicity of CuO NPs. The number of motile *Daphnia* sp. count was used to determine the toxicity up on exposure to NPs (0.1 to 25 mg/L). At 0.1 mg/L, *Daphnia* sp. showed 22% toxicity and for 25 mg/L it was around 74% toxicity (Fig. 6). The result denotes the CuO NPs toxicity on *Daphnia* sp. increases with increase in NPs exposure. Similar trend was noted in investigations of Fan et al. [36] where CuO toxicity on crustacean system increased with respect to gradual increase in concentration of micro/nano CuO. Researchers reported that the toxic effect of NPs on *Daphnia* sp. arises due to its dissolved fraction [37] and due to ROS formation [38]. ROS generation triggered by CuO NP will vary depending on specific mode of toxic action in different cellular sites [20, 38]. However, further investigation required to understand the underlying mechanism behind the toxicity of CuO NPs on *Daphnia magna*.

Fig. 6 Toxicity on *Daphnia magna* after CuO NPs exposure. Values are the mean of $n = 6$ (mean $\pm$ standard error). Level of significance $P < 0.001$ (***)

Conclusions

In summary, this study demonstrates that CuO NPs induces toxicological effects which may be mediated through oxidative stress and peroxidation of lipid in algal cells and bacterial cells. It suppressed the catalase, SOD and glutathione reductase and it caused the depletion of glutathione which are anti-oxidant defense system of living organisms. The study warrants the need of a thorough toxicological evaluation of CuO NPs on different living organisms. The present study also indicates the need of regulatory authorities to monitor the use of CuO NPs in various products.

Acknowledgment Authors sincerely thank the management of Bannari Amman Institute of Technology, Tamil Nadu for providing the necessary facilities and financial support to carry out this research work. The authors extend their appreciation to the Researchers supporting project number (RSP-2020/190) King Saud University, Riyadh, Saudi Arabia.

Compliance with ethical standards

Conflict of interest None.

References

- Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z. Chap. 1 – An introduction to nanotechnology. *Interface Sci Technol*. 2019;28:1–27.
- Samal SS, Manohara SR. Nanoscience and nanotechnology in India: A broad perspective. *Mater Today Proc*. 2019;10:151–8.
- Falcaro P, Ricco R, Yazdi A, Imaz I, Furukawa S, Maspoch D, Ameloot R, Evans JD, Doonan CJ. Application of metal and metal oxide nanoparticles@MOFs. *Coord Chem Rev*. 2016;307:237–54.
- Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. *Science*. 2006;311:622–7.

- Jagadish K, Shiralgi Y, Chandrashekar BN, Dhananjaya BL, Srikantasamy S. Chap. 8 – Ecofriendly synthesis of metal/metal oxide nanoparticles and their application in food packaging and food preservation. *Impact of Nanoscience in Food Industry*; 2018. pp. 197–216.
- Serpone N, Dondi D. Inorganic and organic UV filters: Their role and efficacy in sunscreens and sun care products. *Inorg Chim Acta*. 2007;360:794–802.
- Muna M, Blinova I, Kahru A, Vrček IV, Pem B, Orupöld K, Heinlaan M. Combined effects of test media and dietary algae on the toxicity of CuO and ZnO nanoparticles to freshwater microcrustaceans *Daphnia magna* and *Heterocypris incongruens*: food for thought. *Nanomaterials*. 2019;9:23.
- Liu X, Tang J, Wang L, Giesy JP. Mechanisms of oxidative stress caused by CuO nanoparticles to membranes of the bacterium *Streptomyces coelicolor* M145. *Ecotoxicol Environ Saf*. 2018;158:123–30.
- Vardhan KH, Kumar PS, Panda RC. A review on heavy metal pollution, toxicity and remedial measures: Current trends and future perspectives. *J Mol Liq*. 2019;290:111197.
- Williams PG, Yoshida WY, Quon MK, Moore RE, Paul VJ. Ulongapeptin, a cytotoxic cyclic depsipeptide from a Palauan marine cyanobacterium *Lyngbya* sp. *J Nat Prod*. 2003;66:651–4.
- Joonas E, Aruoja V, Olli K, Kahru A. Environmental safety data on CuO and TiO₂ nanoparticles for multiple algal species in natural water: Filling the data gaps for risk assessment. *Sci Total Environ*. 2019;647:973–80.
- Rippka R. Isolation and purification of cyanobacteria. *Methods Enzymol*. 1988;167:3–27.
- Wu F, Bortvedt A, Harper BJ, Crandon LE, Harperac SL. Uptake and toxicity of CuO nanoparticles to *Daphnia magna* varies between indirect dietary and direct waterborne exposures. *Aquat Toxicol*. 2017;190:78–86.
- Xi X, Ying HZ, Xu CY, Qiang LX, Hua L, Chao QY. Optimization of FDA-PI method using flow cytometry to measure metabolic activity of the cyanobacteria, *Microcystis aeruginosa*. *Phys Chem Earth A/B/C*. 2011;36:424–9.
- Yu Y, Kong F, Wang M, Qian L, Shi X. Determination of short-term copper toxicity in a multispecies microalgal population using flow cytometry. *Ecotoxicol Environ Saf*. 2007;66:49–56.
- Bondarenko O, Ivask A, Kärkinen A, Kahru A. Sub-toxic effects of CuO nanoparticles on bacteria: Kinetics, role of Cu ions and possible mechanisms of action. *Environ Pollut*. 2012;169:81–9.
- Khan SS, Mukherjee A, Chandrasekaran N. Studies on interaction of colloidal silver nanoparticles (SNPs) with five different bacterial species. *Colloids Surf B*. 2011;87:129–38.
- Siddiqui H, Parra MR, Pandey P, Qureshi MS, Haque FZ. Utility of copper oxide nanoparticles (CuO-NPs) as efficient electron donor material in bulk-heterojunction solar cells with enhanced power conversion efficiency. *J Sci Adv Mater Devices*. <https://doi.org/10.1016/j.jsamd.2020.01.004>.
- Kahru A, Dubourguier H-C. From ecotoxicology to nanoecotoxicology. *Toxicology*. 2010;269:105–19.
- Melegari SP, Perreault F, Helena R, Costa R, Popovic R, Matias WG. Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. *Aquat Toxicol*. 2013;142–143:431–40.
- Franklin NM, Adams MS, Stauber JL, Lim RP. Development of an improved rapid enzyme inhibition bioassay with marine and freshwater microalgae using flow cytometry. *Arch Environ Contam Toxicol*. 2001;40:469–80.
- Stauber JL, Franklin NM, Adams MS. Applications of flow cytometry to ecotoxicity testing using microalgae. *Trends Biotechnol*. 2002;20:141–3.

23. Sun T, Yan Y, Zhao Y, Guo F, Jiang C. Copper oxide nanoparticles induce autophagic cell death in A549 cells. *PLoS One*. 2012;7: 43442.
24. Baek YW, Joo Y. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb₂O₃) to *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus aureus*. *Sci Total Environ*. 2011;409:1603–8.
25. Zhao J, Wang Z, Dai Y, Xing B. Mitigation of CuO nanoparticle-induced bacterial membrane damage by dissolved organic matter. *Water Res*. 2013;47:4169–78.
26. Xu C, Peng C, Sun L, Zhang S, Huang H, Chen Y, Shi J. Distinctive effects of TiO₂ and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. *Soil Biol Biochem*. 2015;86:24–33.
27. Wahab R, Mishra A, Yun SI, Kim YS, Shin H-S. Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. *Appl Microbiol Biotechnol*. 2010;87:1917–25.
28. Xia T, Kovochich M, Liong M, Madler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *ACS Nano*. 2008;2:2121–34.
29. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. 2002;7:405–10.
30. Shaw BJ, Al-Bairuty G, Hand RD. Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): physiology and accumulation. *Aquat Toxicol*. 2012;116: 90–101.
31. Dauda S, Chia MA, Bako SP. Toxicity of titanium dioxide nanoparticles to *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 (*Trebouxiophyceae*, *Chlorophyta*) under changing nitrogen conditions. *Aquat Toxicol*. 2017;187:108–14.
32. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci*. 2014;2:53.
33. Lei C, Zhang L, Yang K, Zhu L, Lin D. Toxicity of iron-based nanoparticles to green algae: Effects of particle size, crystal phase, oxidation state and environmental aging. *Environ Pollut*. 2016;218: 505–12.
34. Dubey G, Prasad SM. Differential display of antioxidants in mitigating adverse effects of UV-B radiation in *Nostoc muscorum* and *Phormidium foveolarum* photoacclimated to different irradiances. *Appl Biochem Biotechnol*. 2015;175:2703–28.
35. Shaw JR, Pfreder ME, Eads BD. *Daphnia* as an emerging model for toxicological genomics. *Adv Exp Biol*. 2008;2:327–8.
36. Fan WH, Shi ZW, Yang XP, Cui MM, Wang XL, Zhang DF, et al. Bioaccumulation and biomarker responses of cubic and octahedral Cu₂O micro/nanocrystals in *Daphnia magna*. *Water Res*. 2012;46: 5981–8.
37. Jo HJ, Choi JW, Lee SH, Hong SW. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: the importance of their dissolved fraction varying with preparation methods. *J Hazard Mater*. 2012;227:301–8.
38. Wang Y, Qin S, Li Y, Wu G, Sun Y, Zhang L, Huang Y, Lyu K, Chen Y, Yang Z. Combined effects of ZnO nanoparticles and toxic *Microcystis* on life-history traits of *Daphnia magna*. *Chemosphere*. 2019;233:482–92.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.