BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2024. Vol. **21**(2), p. 779-788

Impact of CuO nanoparticles on *Dunaliella* spp. BDUG10113 Growth, Photosynthesis, and Cellular Processes

Rajnandini Srivastava and R. C. Mishra*

Department of Botany, Mahakaushal University, Bhopal, M.P, India.

http://dx.doi.org/10.13005/bbra/3264

(Received: 10 October 2023; accepted: 06 April 2024)

This study investigates the impact of Copper Oxide (CuO) nanoparticles on Dunaliella sp., assessing relative growth rate (RGR), growth rate coefficient (K), and physiological parameters. Increasing CuO nanoparticle concentrations led to a decline in RGR, reflecting negative growth effects, but the lowest concentration (25μl) exhibited the highest RGR and K values. Pigment estimation revealed decreasing chlorophyll a, chlorophyll b, and carotenoid concentrations, indicating disrupted photosynthetic activity. Protein concentration decreased with increasing CuO nanoparticle treatment, signalling interference with synthesis and metabolic processes. Conversely, glutathione superoxide transferases and lipid peroxidase concentrations increased, suggesting activated defence mechanisms against nanoparticle-induced oxidative stress. These findings enhance our understanding of CuO nanoparticles' adverse effects on Dunaliella sppp., emphasizing the need for further research to ensure the safe application of nanoparticles in aquatic environments. The study underscores the importance of sustainable nanoparticle use and its implications for aquatic organisms.

Keywords: Aquatic organisms; Copper Oxide NP; Dunaliella; Growth inhibition; Nanoparticles; Toxicity.

The extensive proliferation and utilization of nanoparticles (NPs) across various sectors, including agriculture, biomedicine, environmental science, electronics, and textile industry, has surged significantly[1]. This surge is attributable to the distinctive properties of NPs, such as catalytic activity, conductivity, and optical and transport characteristics, which distinguish them from conventional materials[2]. These unique properties contribute to heightened reactivity with organisms upon environmental release, particularly in natural aquatic ecosystems. The toxicity of NPs has been documented in numerous aquatic organisms, with reported direct effects and consequential indirect effects through dissolution and release of constituent elements[3]. Oxidative stress and inflammation are currently recognized as the predominant mechanisms underlying NP toxicity[4].

Copper nanoparticles (CuNPs) exhibit controlled release of metal species, demonstrating the ability to impede the growth of microorganisms. Consequently, they have been incorporated or coated with various materials for antibacterial applications[5], introducing biological toxicity[6,7,8]. Recent years have seen the demonstration of CuNP toxicity in a range of organisms, including arthropods[9], water fleas[10,11], barnacle larvae, *Dunaliella spp. BDUG10113*sorokiniana;[12], ciliates, *Euplotes aediculatus*;[13], and intestinal microbiota of broiler chickens[14]. Notably, CuNPs exhibit higher toxicity to smaller organisms compared to most other NPs, inducing toxic effects

*Corresponding author E-mail: vc@mku.ac.in

on plankton at concentrations as low as 10^{-1}- 10^{-3}mg/L [15,16]. The potential for biomagnification in the food chain, especially in keystone organisms like freshwater algae, cannot be ignored[17].

To investigate the effects of Copper II Oxide nanoparticles (CuO NPs), *Dunaliella spp. BDUG10113*was selected due to its position in the upper echelons of the food chain and its resilience to diverse environmental stresses. *Dunaliella* BDDUG10113, classified under the phylum Chlorophyta, order Volvocales, and family Polyblepharidaceae, are unicellular, photosynthetic, and motile biflagellate microalgae characterized by the absence of a rigid cell wall [18]. Prominent species include *Dunaliella salina, Dunaliella tertiolecta, Dunaliella primolecta, Dunaliella viridis, Dunaliella bioculata, Dunaliella acidophyla, Dunaliella parva, and Dunaliella media*.

Dunaliella possesses remarkable halotolerance, with *Dunaliella acidophila* thriving in highly acidic environments (pH 0–1). *Dunaliella* Antarctica can flourish at subzero temperatures, and certain strains of *D. salina* xhibit tolerance to high light intensities. Additionally, *Dunaliella* displays heightened resistance to fuel oil contamination compared to other planktonic algae[19]. Hence, these organisms exhibit unique adaptive capabilities to endure some of the most extreme global habitat conditions.

The objective of this study is to assess the impact of Copper II Oxide nanoparticles (CuO NPs) with a particle size below 50 nm at varying concentrations (0, 25, 100, 500, 1000, 2000 µl) on the physiochemical behaviour of *Dunaliella spp. BDUG10113*Parameters evaluated include chlorophyll a and b concentrations, viable cell concentration, reactive oxygen species (ROS) formation through enzyme antioxidant assays encompassing superoxide dismutase, catalase, glutathione-S-transferase, lipid peroxidase, protein, total phenolic content, and extra- and intracellular alterations in the microalga.

MATERIAL METHODOLOGY

Procurement of *Dunaliella* species

The vials containing *Dunaliella* BDUG10113 algae cells were procured from the National facility for marine Cyanobacteria, Bharathidasan University, Palkalaiperur Tiruchiraoalli- 620024. This study was was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines. Further, the BBM medium (Bold's Basal Medium) was meticulously prepared and employed for the cultivation and development of *Dunaliella* spp.

Estimation of Growth kinetics

An algal growth rate test was conducted using bath cultures in separate flasks following standard procedures outlined in the OECD guidelines from 2006. Freshwater *Dunaliella spp. BDUG10113*was utilized for the experiments. A laminar air flow cabinet was pre-sterilized using ultraviolet light, and all necessary materials for preparing the culture medium underwent sterilization at 0.1 MPa for 1200 seconds control flasks were employed as a reference standard. The experiments commenced during the exponential growth phase, with an initial algal cell count of approximately 1 × 105 cells/ml in each culture.[40]

The growth response of *Dunaliella* sp. exposed to the tested substances was evaluated by determining the relative growth rate (G) and measuring reactive oxygen species (ROS). Cell counts were performed using a hemocytometer, and the number of algae per ml was calculated following OECD guidelines. Algal cell counts were conducted at 24-hour intervals, and the relative growth rate (G) was calculated using the formula G = [N/N0] * 100, where G represents the relative growth rate, N is the number of cells counted, and N0 is the primary cell number. [40]

The growth constant coefficients (K) of algae for each group were computed using the first-order kinetic model equation at various time intervals, and the mean values of K were reported as K = Ln (N/N0)/t, where K denotes the growth constant coefficient. The values of K were obtained by plotting Ln (N/N0) against time, and the linearity of the plot was assessed using the regression coefficient (R2).

Total Protein Content

Protein analysis was conducted based on the method outlined by[24], involving the reaction of 300 ìL supernatant with 300 ìL of Lowry D reagent. Absorbance was measured at ë 750 nm after incubation.

Enzyme Activity

Superoxide dismutase (SOD) activity analysis followed the method of[25]. This method involved reacting 1 mL of Tris-HCl buffer (pH 8.2) with 1 mL of aquabidest and 15 ìL of algae extract supernatant, followed by the addition of 10 ìL of pyrogallol 2 mM. Absorbance measurements were taken at a wavelength of 470 nm with 120 seconds intervals.

Catalase activity was measured according to a modified method[27]. A mixture of 1.99 mL phosphate buffer solution 50 mM pH 7.0 and 10 ìL supernatant sample was combined with 1 mL H2O2 solution (3% concentration). Catalase enzyme activity was measured at a wavelength of 240 nm for 120 seconds and enzyme activity was expressed in ìmol H_2O_2 gram-1 fresh weight.

Ascorbate peroxidase (APOX) activity was determined following the method of[28], involving a mixture of potassium phosphate buffer, ascorbate, and H2O2. The decrease in absorbance was measured at ë 290 nm for 120 seconds.

Glutathione s transferase activity

In this study, Dunaliella spp. microalgae (strain BDUG10113) were cultivated in a mineral medium under controlled conditions. Glutathione S-transferase (GST) activity was evaluated by treating the cultures with various concentrations of GST inhibitors, such as diethyl maleate. Microalgal extracts were prepared and subjected to spectrophotometric analysis to measure GST activity using a model substrate, 1-chloro-2,4-dinitrobenzene (CDNB). Protein quantification was performed to normalize GST activity. Results were statistically analyzed, with experiments conducted in triplicate. This study sheds light on the GST activity of Dunaliella microalgae under different experimental conditions, offering insights into their metabolic and stress response mechanisms.

Photochemical Quenching

Microalgae Cultivation

Dunaliella spp. BDUG10113 microalgae were cultivated in sterile conditions using a liquid mineral medium. The medium composition included essential nutrients and trace elements necessary for algal growth. Cultures were maintained under controlled environmental conditions, including temperature, light intensity, and photoperiod.

Nanoparticle Treatments

Cu nanoparticles carboxylated with citric acid (nCu-Citr) and selenium nanoparticles carboxylated with citric acid (nSe-Citr) were added to the microalgae cultures. Concentrations of nCu-Citr ranged from 0.67 to 40 mg L”1, while concentrations of nSe-Citr ranged from 0.07 to 4 mg L”1. Nanoparticle stock solutions were prepared using appropriate solvents and thoroughly mixed with the culture medium to achieve the desired concentrations.

Measurement Techniques

Chlorophyll fluorescence parameters were measured using the XE-PAM fluorometer. This device allows for precise and non-invasive monitoring of photosynthetic activity in photosystem II. Various parameters, including but not limited to, Fv/Fm (maximum quantum yield of photosystem II), ÖPSII (effective quantum yield of photosystem II), and NPQ (non-photochemical quenching) were measured under different experimental conditions.

Dry Algal Mass Determination

After nanoparticle treatments and incubation periods, algal cultures were harvested for dry mass determination. This involved centrifugation of culture samples to separate algal biomass from the culture medium. Subsequently, the collected biomass was dried using a suitable method, such as oven drying or freeze-drying. The dry mass of the algae was then measured to assess growth rates and biomass yields.

RESULTS AND DISCUSSION

Relative Growth Rate (RGR %) and Growth Rate Coefficient (K) of *Dunaliella* spp.

Relative Growth Rate (RGR) of *Dunaliella spp. BDUG10113*was studied in control and CuO nanoparticles (25µl, 50µl, 250µl, 500µl, 1000µl, 2500µl) treated samples. A decrease in the RGR (0.248%) with increase in the metal concentration (2500µl) was observed. At 25µl CuO Nanoparticles treatment highest RGR value of 37.47% was observed (Figure 1). The results suggest that there is a negative impact of increasing the metal concentration on the growth of the *Dunaliella* spp.

Growth Rate Coefficient (K) of *Dunaliella spp. BDUG10113*was studied in control and CuO nanoparticle treated samples. Sample D1 (25µl) showed the highest value of K (1.82). This result suggests that the D1 had the highest growth rate of

37.47% which has decreased to -3.18 on increasing the metal concentration.

According to a study, the growth rate of microalgae increased due to presence of copper concentration in the sample and as the concentration of copper increased there is no evidence in the increment of growth of the microalgae.[1] Another study by[7], found that copper has the ability to affect the growth rate of *D. tertiolecta* by 15% on the first day and 34% by the 8th day. This research shows the effect of CuO nanoparticles on the growth of *Dunaliella spp. BDUG10113*by observing the optical density (OD 750 nm) At every 24 hour interval at various treatment concentrations (Control, 20, 40, 60, 80, 100µl CuO NPs). Optical density reveals a worthy correlation with algal density and also a time tradable in rapid follow up of the studies. The highest optical density of *Dunaliella spp. BDUG10113*has been recorded at 25µl CuO NPs. Similar results were obtained when metal-based NPs were introduced into microalgae which has increases the growth rate, biomass, lipids pigment contents and content of bioactive compounds[13].

In the cell wall of algae, fungi etc., there is a major site for the interaction with nanoparticles and also presence of a barrier. Along with the protection mechanism of nanoparticles, sometimes the side effects of nanoparticles might cause cell membrane disruption [16] Introduction

Table 1. Relative Growth Rate (RGR %) and Growth Rate Coefficient (K) of *Dunaliella spp. BDUG10113*Values are means ± SE of three replicates

S. No.	Sample concentration(µl)	Relative Growth Rate(RGR %)	Growth Constant Coefficient(K)
1	Control	31.83	1.66
2	25	37.47	1.82
3	50	28.64	1.56
4	100	21.72	1.2
5	250	16.98	1.03
6	500	14.11	0.85
7	1000	10.01	0.51
8	2500	0.248	-3.18

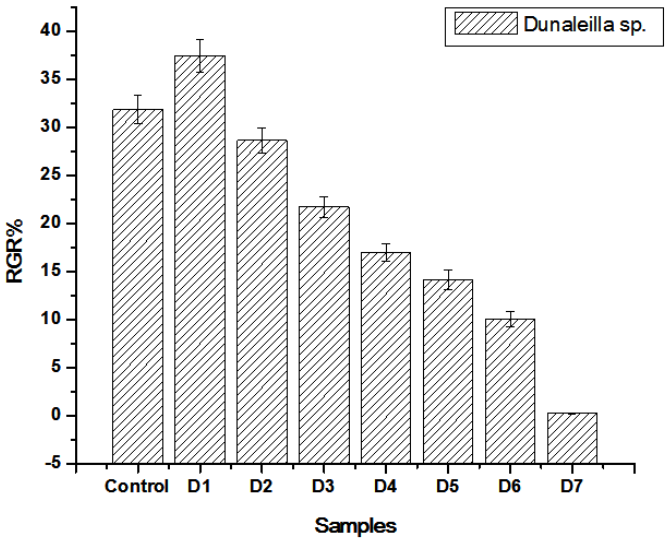

Fig. 1. Relative Growth Rate (RGR) of *Dunaliella spp. BDUG10113*in control and treated samples. Values are means ± SE of three replicates.

of nanoparticles in biotic compounds may have positive or negative impact on them. These biotransformations are directly related to the redox reaction, addition of sulphur, phosphorylation and change in molecular components [12].

Caretenoid Estimation (Chlorophyll a and b)

The Amount of chlorophyll a was studied in control and CuO nanoparticles treated samples which reflect that D1 has the highest amount of chlorophyll a amongst all treated samples. These findings indicated that increase in the metal concentration has declined the chlorophyll a pigment content in the *Dunaliella spp. BDUG10113*

Similarly the Concentration of Chlorophyll b was investigated in control and treated samples which also showed the highest

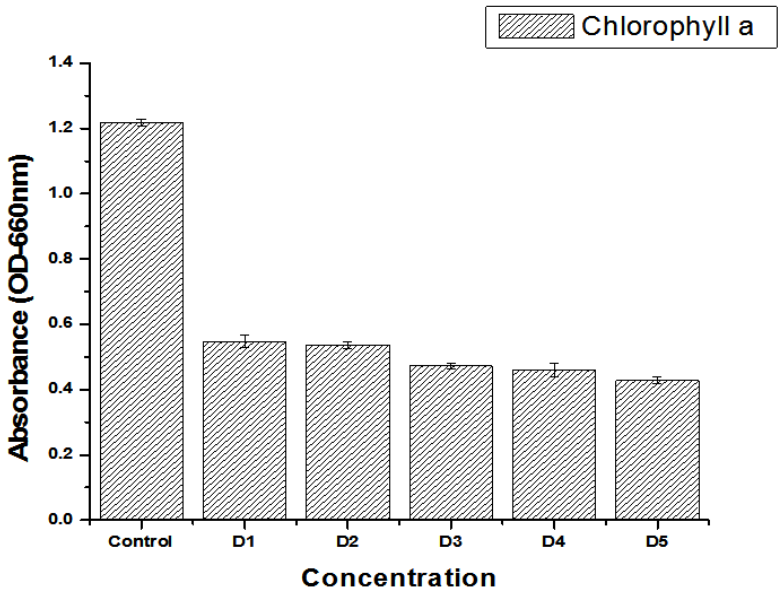

Fig. 2. Chlorophyll a concentration of *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates.

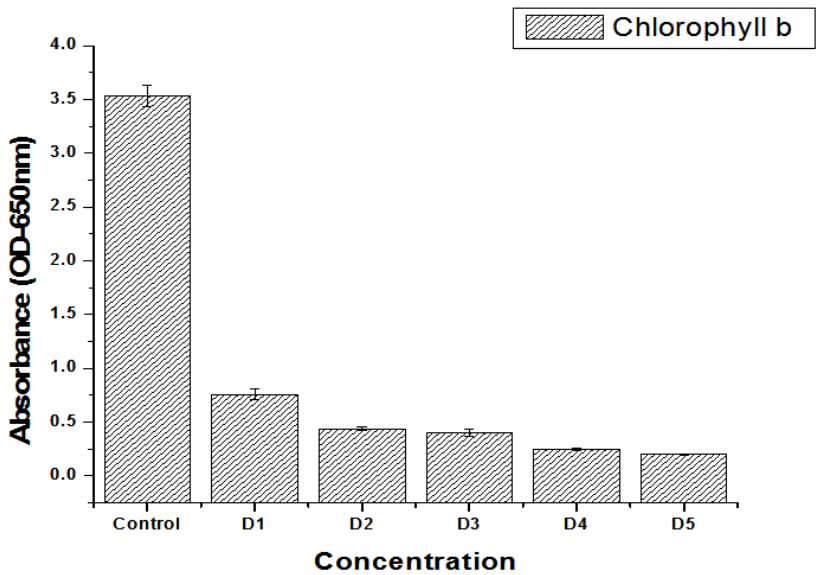

Fig. 3. Chlorophyll b concentration of *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates.

pigment concentration in sample D1. This result suggests that the amount of chlorophyll b also decreased while increasing the metal concentration.

Another study on microalgae (*Chlorella* vulgaris, shows that the chlorophyll content in the cell gets increases in 100ppm (0.1µl) metal nanoparticle treatment concentration[28,29] also reported that the metal nanoparticles have the ability to increases the microalgae pigment content. According to a study, microalgae like *Chlamydomonas reinhardstii* and *Chlorella pyrenoidosa* when treated with different concentrations of CuO and TiO_2 nanoparticles respectively might increase the photosynthetic pigments of these alga [30]. One theory on the induction of ROS, which may attack certain pigments, might cause them to convert

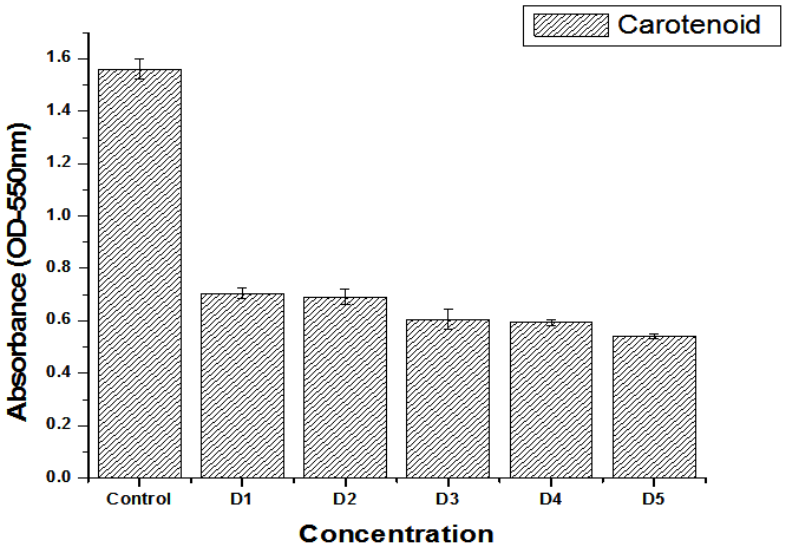

Fig. 4. Carotenoid concentration in *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates.

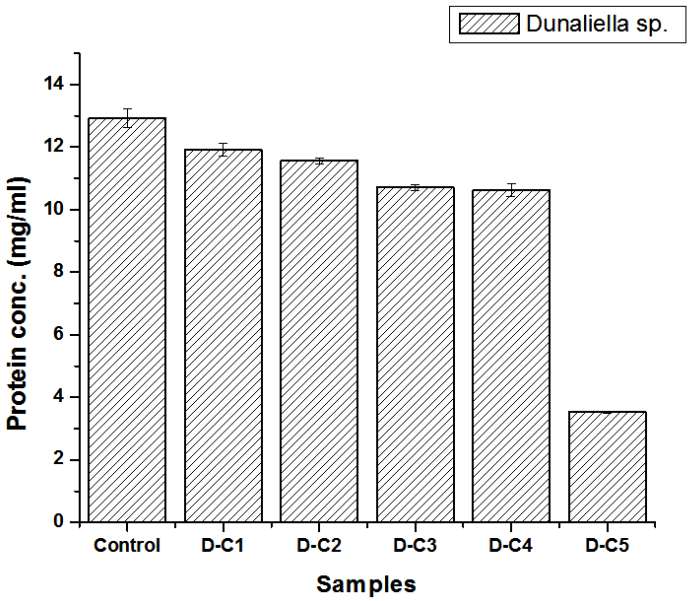

Fig. 5. Protein concentration (mg/ml) of *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates

to chlorophyll pigment under NPs and produce increased Chlorophyll pigment in the cells, is one explanation for the elicitation of chlorophyll contents of algae by NPs[31].

The Amount of carotenoid was studied in control and CuO nanoparticles treated samples which depicted that treatment at 25µl (D1) had the highest amount of carotenoid (0.70563 OD) amongst all treated samples. This result implies that carotenoid concentration in the *Dunaliella spp. BDUG10113*narrows down by increasing the CuO nanoparticles treatment. According to a research, the carotenoid (an antioxidant pigment) gets increased on treating with Cu (II) concentrations in microalgal cells[9].

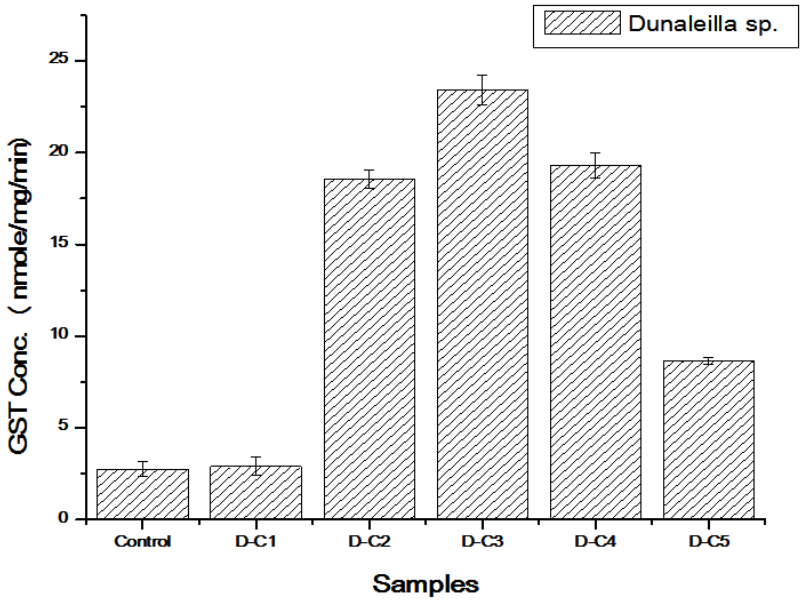

Fig. 6. Glutathione Superoxide Transferase Concentration (nmol/mg/min) of *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates

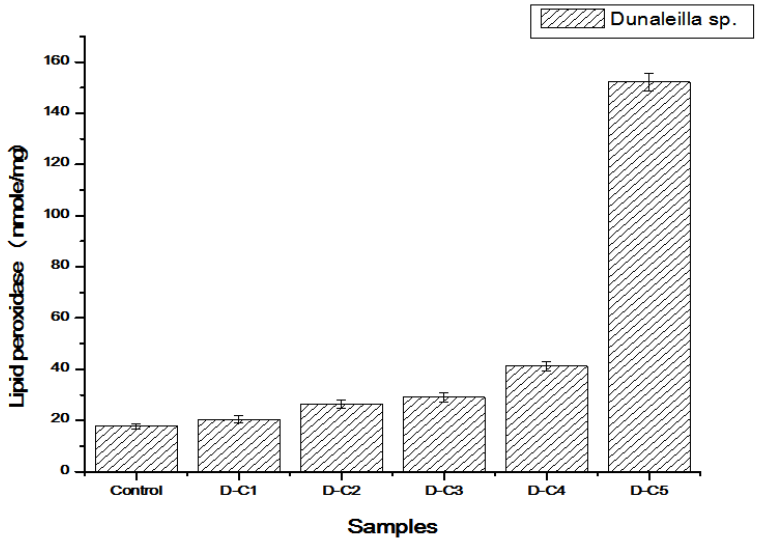

Fig. 7. Concentration of Lipid peroxidase (nmol/mg/min) of *Dunaliella spp. BDUG10113*in control and CuO Nanoparticles treated samples. Values are means ± SE of three replicates.

Protein concentration (mg/ml) was studied in control and CuO nanoparticles treated samples of *Dunaliella* spp.. D-C1 with 25µl treatment had the highest amount of protein concentration which was about 11.91mg/ml among all treated samples. This result indicated that the higher the treatment concentration the lower the protein concentration in the samples.

In a research performed earlier showed that the protein concentration had increased in *Chlorella sp.* at different concentration of metal nanoparticles[17]. Expanded dissolvable protein fixation is believed to be a functioning guard system to keep algal cells from damaging by abiotic stress[26].

Amount of Glutathione Superoxide Transferases (nmol/mg/min) was studied in control and treated samples of *Dunaliella* spp.. This result shows that lowest concentration of treatment (D1) had the lowest amount of Glutathione Superoxide Transferase (2.89 nmol/mg/min) amongst the treated samples. As the concentration increases the amount of Glutathione Superoxide Transferase increased.

The results of a research shows that on addition of copper to microalgae (*Chlamydomonas reinhardstii*), the GST (Glutathione Superoxide Transferase) level increased from lower to higher concentration by several folds[31].

Lipid peroxidase concentration (nmol/mg) was studied in control and CuO nanoparticles treated samples. The lowest concentration was 26.42nmol/mg of DC-1 (25µl) treated sample with this result it was depicted that the higher the CuO nanoparticles treatment concentration the higher the value of Lipid peroxidase in the samples. In a research by[15,] the lipid peroxidation concentration in cells of microalgae (S. vacuolatus) increased by increased copper concentration[23]. Mallick (2004) also shows parallel results while working with *Chlorella vulgaris*. Lipid peroxidation is typically is an indicator of (ROS) Reactive Oxygen Species caused by the cell's oxidative stress[27]. Cell lysis and membrane damage are the key results of surge in MDA levels[32].

Photochemical Quenching

*Dunaliella spp. BDUG10113*microalgae were subjected to the addition of copper nanoparticles to assess their impact on various chlorophyll fluorescence parameters. The concentrations of copper nanoparticles ranged from 2 to 4 mg L"1. The study found that the addition of copper nanoparticles initially led to an increase in the Fv/Fm and Fv'/Fm' parameters, indicating the ability of both dark-adapted and light-adapted algal cells to convert light energy into chemical energy.

However, as the experiment progressed, the difference between the copper nanoparticle-treated samples and the control samples decreased. By day 24, only samples treated with 4 mg L"1 copper nanoparticles displayed elevated FV/Fm and FV2 /Fm2 values compared to the control. In contrast, the addition of 2 mg L"1 copper nanoparticles did not result in significant differences in these parameters compared to the control.

The photochemical quenching coefficients, qP and qL, which represent the fraction of open photosystem II reaction centers and the proportion of light excitation energy used for electron transport, were not influenced by the concentration of copper nanoparticles. At the end of the experiment, their values decreased by 9–15% compared to the control.

These findings contribute to our understanding of the physiological responses of *Dunaliella spp. BDUG10113*microalgae to copper nanoparticle exposure and highlight the importance of considering nanoparticle concentrations in assessing their impact on algal photosynthetic processes. Although with these functions no previous researches has been performed but in Chlorella vulgaris citric acid complex of copper nanoparticles and selenium nanoparticles were studied by[33] they concluded in their research that Cu nanocarboxylates (0.67-4 mg L"1) promoted 20% Chlorella biomass growth, while higher concentrations (20-40 mg L"1) inhibited it. Se nanocarboxylates (0.4-4 mg L"1) increased C. vulgaris biomass by 40-45%, with lower concentrations causing temporary growth retardation. Cu nanocarboxylates (2-4 mg L"1) and Se nanocarboxylates (0.4-4 mgL"1) initially enhanced chlorophyll a fluorescence parameter (Fv/Fm and Fv'/Fm'), but fluorescence quenching coefficients changed over time, decreasing with Cu nanocarboxylates and increasing with Se nanocarboxylates. These changes influenced the overall quantum yield of photosynthetic electron transport in photosystem II[34,35].

CONCLUSION

This study provides crucial insights into the impact of CuO nanoparticles on *Dunaliella* spp.'s growth and physiological parameters. Elevated concentrations of CuO nanoparticles adversely affect the relative growth rate (RGR) and growth rate coefficient (K), indicating an inhibitory effect on overall microalgae growth. Pigment estimation reveals decreasing concentrations of chlorophyll a, chlorophyll b, and carotenoid, disrupting photosynthetic activity. Protein concentration shows a declining trend, suggesting interference with synthesis and metabolic processes, while concentrations of glutathione superoxide transferases and lipid peroxidase increase, signifying defense mechanisms against oxidative stress induced by CuO nanoparticles. These findings highlight potential adverse effects on *Dunaliella* spp.'s growth, photosynthesis, and cellular processes, emphasizing the need for further research to ensure the safety and sustainability of nanoparticle applications in aquatic environments.

REFERENCES

1. Vaquero I, Ruiz-Domínguez MC, Márquez M, Vílchez C. Cu-mediated biomass productivity enhancement and lutein enrichment of the novel microalga Coccomyxa onubensis. *Process Biochemistry*. 2012;47(5):694-700.
2. López A, Rico M, Santana-Casiano JM, González AG, González-Dávila M. Phenolic profile of *Dunaliella* tertiolecta growing under high levels of copper and iron. *Environmental Science and Pollution Research*. 2015;22:14820-14828.
3. Sibi GAKD, Kumar DA, Gopal T, Harinath K, Banupriya S, Chaitra SJ. Metal nanoparticle triggered growth and lipid production in Chlorella vulgaris. *Int J Scientific Res Environ Sci Toxicol*. 2017;2(1):1-8.
4. Sarkar RD, Singh HB, Kalita MC. Enhanced lipid accumulation in microalgae through nanoparticle-mediated approach, for biodiesel production: A mini-review. *Heliyon*. 2021;7(9):e08057.
5. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, et al. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology*. 2008;17:372-386.
6. Lowry GV, Gregory KB, Apte SC, Lead JR. Transformations of nanomaterials in the environment. 2012.
7. Abdel-Kader HA. Short-and long-term stimulatory impacts of calcium oxide nanoparticles (CaONPs) on the growth, photosynthesis and antioxidant enzymes of Chlorella sp. *Assiut University Journal of Multidisciplinary Scientific Research*. 2022;1(1):242-262.
8. Eroglu E, Eggers PK, Winslade M, Smith SM, Raston CL. Enhanced accumulation of microalgal pigments using metal nanoparticle solutions as light filtering devices. *Green Chemistry*. 2013;15(11):3155-3159.
9. Middepogu A, Hou J, Gao X, Lin D. Effect and mechanism of TiO2 nanoparticles on the photosynthesis of Chlorella pyrenoidosa. *Ecotoxicology and Environmental Safety*. 2018;161:497-506.
10. Melegari SP, Perreault F, Costa RHR, Popovic R, Matias WG. Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga Chlamydomonas reinhardtii. *Aquatic Toxicology*. 2013;142:431-440.
11. Xiaoxiao C, Xing Z, Rui L, Hanchao Y, Zhisong L, Xu Y. Photosynthetic toxicity and oxidative damage induced by nano-Fe3O4 on Chlorella vulgaris in aquatic environment. *Open Journal of Ecology*. 2012.
12. Sabatini SE, Juarez AB, Eppis MR, Bianchi L, Luquet CM, de Molina MDCR. Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. *Ecotoxicology and Environmental Safety*. 2009;72(4):1200-1206.
13. Jiang Y, Zhu Y, Hu Z, Lei A, Wang J. Towards elucidation of the toxic mechanism of copper on the model green alga Chlamydomonas reinhardtii. *Ecotoxicology*. 2016;25(7):1417-1425.
14. Mallick N. Copper-induced oxidative stress in the chlorophycean microalga Chlorella vulgaris: response of the antioxidant system. *J. Plant Physiol*. 2004;161:591–597.
15. Sandman G, Bo¨ger P. Copper deficiency and toxicity in Scenedesmus. *Z. Plant. Physiol*. 1980;98:53–59.
16. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free radical biology and medicine*. 1990;9(6):515-540.
17. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. *Current science*. 1999;658-666.
18. Kiran B, Thanasekaran K. Metal tolerance of an indigenous cyanobacterial strain, Lyngbya putealis. *Int Biodeterior Biodegradation*. 2011;65:1128–1132.
19. Li X, Yang WL, He H, Wu S, Zhou Q, Yang

C, Zeng G, Luo L, Lou W. Responses of microalgae Coelastrella sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. *Bioresource Technology*. 2018;251:274–279.

20. Morelli E, Scarano G. Copper-induced changes of non-protein thiols and antioxidant enzymes in the marine microalga Phaeodactylum tricornutum. *Plant Science*. 2004;167(2):289–296.
21. Mallick N. Copper-induced oxidative stress in the chlorophycean microalga Chlorella vulgaris: response of the antioxidant system. *J Plant Physiol*. 2004;161(5):591–597.
22. Li M, Hu C, Zhu Q, Chen L, Kong Z, Liu Z. Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga Pavlova viridis (Prymnesiophyceae). *Chemosphere*. 2006;62(4):565–572.
23. Shamim A, Tripathi G, Ansari JA, Mahfooz S, Mahdi AA, Khan AR, Farooqui A, Mishra V. Effect of pH on aluminum uptake and differential aluminum tolerance in cyanobacterial strains: A bioresource for agricultural and environmental sustainability. *Bioresour Technol Rep*. 2022;18:100999.
24. Abbasi R, Shineh G, Mobaraki M, Doughty S, Tayebi L. Structural parameters of nanoparticles affecting their toxicity for biomedical applications: a review. *J Nanoparticle Res*. 2023;25(3):43. doi:10.1007/s11051-023-05690-w.
25. Altammar KA. A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Front Microbiol*. 2023;14:1155622. doi:10.3389/fmicb.2023.1155622.
26. Anreddy RNR. Copper oxide nanoparticles induces oxidative stress and liver toxicity in rats following oral exposure. *Toxicol Rep*. 2018;5:903–904. doi:10.1016/j.toxrep.2018.08.022.
27. Briffa J, Sinagra E, Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 2020;6(9):e04691. doi:10.1016/j.heliyon.2020.e04691.
28. Chen H, Qi H, Xiong P. Phycobiliproteins-A Family of Algae-Derived Biliproteins: Productions, Characterization and Pharmaceutical Potentials. *Mar Drugs*. 2022;20(7):450. doi:10.3390/md20070450.
29. Crisan MC, Teodora M, Lucian M. Copper Nanoparticles: Synthesis and Characterization, Physiology, Toxicity and Antimicrobial Applications. *Appl Sci*. 2022;12(1):141. doi:10.3390/app12010141.
30. Eisler R. *Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals, Three Volume Set*. 2000.
31. Franco-Morgado M, Amador-Espejo GG, Pérez-Cortés M, Gutiérrez-Uribe JA. Microalgae and cyanobacteria polysaccharides: Important link for nutrient recycling and revalorization of agro-industrial wastewater. *Appl Food Res*. 2023;3(1):100296. doi:https://doi.org/10.1016/j.afres.2023.100296.
32. Hassan A, Sayed-ElAhl R, El-Hamaky A, Mansour M, Oraby N. Copper nanoparticles: Synthesis, characterization, and its veterinary applications. In: *Nanostructures for Antimicrobial Therapy*. Elsevier; 2022:507–534. doi:10.1016/B978-0-12-823833-2.00016-7.
33. Irfan S, Alatawi AMM. Aquatic Ecosystem and Biodiversity: A Review. *Open J Ecol*. 2019;09(01):1–13. doi:10.4236/oje.2019.91001.
34. Jaiswal D, Sengupta A, Sengupta S, Madhu S, Pakrasi HB, Wangikar PP. A Novel Cyanobacterium Synechococcus elongatus PCC 11802 has Distinct Genomic and Metabolomic Characteristics Compared to its Neighbor PCC 11801. *Sci Rep*. 2020;10(1):191. doi:10.1038/s41598-019-57051-0.
35. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *J Nanobiotechnology*. 2022;20(1):262. doi:10.1186/s12951-022-01477-8.
36. Latifi A, Ruiz M, Zhang C-C. Oxidative stress in cyanobacteria. *FEMS Microbiol Rev*. 2009;33(2):258–278. doi:10.1111/j.1574-6976.2008.00134.x.
37. Liao W, Zhu Z, Feng C, et al. Toxicity mechanisms and bioavailability of copper to fish based on an adverse outcome pathway analysis. *J Environ Sci*. 2023;127:495–507. doi:https://doi.org/10.1016/j.jes.2022.06.002.
38. Lin Y, Cao Y, Yao Q, Chai OJH, Xie J. Engineering Noble Metal Nanomaterials for Pollutant Decomposition. *Ind Eng Chem Res*. 2020;59(47):20561–20581. doi:10.1021/acs.iecr.0c04258.
39. Malhotra N, Ger TR, Uapipatanakul B, Huang JC, Chen KH. Review of copper and copper nanoparticle toxicity in fish. *Nanomaterials*. 2020;10(6):1–28. doi:10.3390/n.
40. Hund-Rinke, K., Schlinkert, R. & Schlich, K. Testing particles using the algal growth inhibition test (OECD 201): the suitability of in vivo chlorophyll fluorescence measurements. *Environ Sci Eur* 34, 41 2022. https://doi.org/10.1186/s12302-022-00623-1