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Short- and long-terms stimulatory impacts of calcium oxide nanoparticles (CaONPs) on the growth, photosynthesis and antioxidant enzymes of *Chlorella* sp.

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ABSTRACT

Calcium is an essential macro element for growth of plants and algae; it regulates an impressive and diverse number of physiological processes. Research on the impact of calcium oxide nanoparticles (CaO NPs) is very little in the literature. In this work, responses of growth, photosynthesis, and antioxidant enzymes activities in *Chlorella* sp to short term treatment (5 hours) and to long-term treatment (5 days) by CaO NPs (0, 20, 40, 60, 80 and 100 ppm) were investigated. The results revealed that, the highest *Chlorella* sp growth and Chlorophyll content were recorded at 100 ppm CaO NPs. The highest photosynthetic oxygen evolution (P_N) was recorded in the *Chlorella* cultures treated with 100 ppm CaO NPs during both long- and short-term experiments. Protein content was enhanced by treatment with CaO NPs up to 60 ppm CaO NPs. Catalase and guaiacol peroxidase activities recorded their highest values at 80 ppm CaO NPs. Total antioxidants increased in all CaO NPs treatments. In general, overall results of this research indicated that, treatment by CaO NPs improves growth, photosynthesis and modulates antioxidant responses of *Chlorella* sp. by stimulating antioxidation system.

INTRODUCTION

Calcium plays central roles in many physiological and biochemical processes in plants, where it is essential for growth, photosynthesis, stress alleviation [1-3], and mediation of responses to hormones [4] and microorganismal interaction [4, 5]. For instance, treatment of cucumber seedlings with 10 mM $CaCl_2$ increased chlorophyll content, photosynthetic rate, catalase (CAT) activity, and peroxidase (POD), under low light intensity [1]. Calcium is also essential to many signalling processes [6,7].

Nanotechnology is very exciting science and technology field with the probability to open a new agricultural and biotechnology applications [8]. Nanoparticles have different physicochemical properties and can improve plant metabolism [9]. Because of the prevalent usage of engineered nanoparticles, it is predictable that large quantities of them will finally spread in the atmospheric, aquatic, and terrestrial environments [10]. Nowadays, the investigation of the biological positive and negative effects of engineered nanoparticles on living organisms urgently needed. Metal oxide nanoparticles are more beneficial resources than the metal oxide salt form to develop the biological roles of many organisms [11,12].

In natural environments, the microalgae are main producers for food and have a significant role in the food chains. Microalgae participate in the economy and society in addition to biodiesel production and water purification system. Several studies reported the impact of nanoparticles on microalgae from few hours [13] to some days [14]. Nanoparticles have been used to enhance growth of microalgal and produce valuable products. The creation of oxidative stress by nanoparticles may be a potential for promoting algal growth and the accumulation of secondary metabolites [15]. For example, the exposure of *Chlorella vulgaris* to metal NP induces biochemical, physiological, or molecular modifications, thus stimulating the growth [16].

Numerous investigations have focused on the toxic impacts of NPs on various microalgae species [17–20]. AgNPs have been shown to limit the development of freshwater green microalgae in previous investigations [21]; short-term exposure of the green alga *Chlamydomonas reinhardtii* to AgNPs, decreased photosynthesis activity [22] and stimulate the antioxidant enzymes activities in *Chattonella marina* [23]. The decreased in chlorophyll content, viable algal cells, formation of reactive oxygen species (ROS), and lipid peroxidation in the freshwater microalga *Chlorella vulgaris* and marine microalga

Dunaliella tertiolecta were observed after exposure to AgNPs for 24 h [24]. The growth inhibition of the green algae *Desmodesmus* sp. by Titanium oxide nanoparticles was also reported [25].

Nevertheless, the positive effects of NPs on different algae species at proper concentrations were studies. For example, Zn NPs at 1.17×10^{-5} M concentration increased growth rate of three microalgae (*Pavlova lutheri, Isochrysis galbana and Tetraselmis suecica*) [26]. Also, enhancement in the cell density and chlorophyll content of *Scenedesmus obliquus*, was observed by adding 20 mg/L of Fe_2O_3 NPs at in the growth medium [27]. Rastar et al [28] showed that the growth performance and chlorophyll biosynthesis of *Haematococcus pluvialis* were significantly improved by using Fe and Zn in nano forms. Moreover, Deng et al [29] found that the growth of a marine diatom *Phaeodactylum tricornutum* in 2.5 and 5 mg/ L nano-CeO_2 was higher than the control. While, after 2 days exposure to CeO_2 nano at 20 and 40mg/ L, growth significantly reduced. A similar result was reported by [30] on *Scenedesmus obliquus* exposed to nano-CeO_2 (5 and 10 mg/ L) where this is caused growth enhancement, as nano-CeO_2 concentrations increased, the inhibition increased. In higher plants, promotion of shoot and root growth in rice and maize (Zea mays) seedlings was also observed by application of calcium phosphate nanoparticles [31,32].

So far, few reports on calcium nanoparticles influencing photosynthesis and the antioxidant enzymes activity, and no studies have been reported on the stimulatory impact of calcium oxide nanoparticles on algae. Hence, the current research was carried out to know the impact of CaO NPs on growth, photosynthesis and antioxidant enzymes of the green microalga *Chlorella* sp.

MATERIALS AND METHODS

Calcium oxide nanoparticles (CaO NPs)

Calcium oxide nanoparticles CaO-NPs were prepared from calcium nitrate ($CaNO_3$) by mechanical milling as described by [33].

Short- and long-term exposure of *Chlorella* sp to CaO NPs

Chlorella sp. cultures were grown under sterile conditions in modified BG11 medium [34], where ferric ammonium citrate was used instead of iron III citrate, sodium EDTA was used instead EDTA of, and cobalt nitrate was used instead of cobalt chloride, at a temperature of 25± 2^{o}C, 7.5 pH and light intensity 48.4 μmol m^{-2} s^{-1}; the cultures were agitated at 130 rpm using orbital shaker. The following Calcium oxide nanoparticles treatments were used separately for algal cultures: 0, 20, 40, 60, 80 and 100 ppm. The cultures were grown for five hours in short- term experiment and for 5 days in long-term experiments.

Growth monitoring

Throughout the experimental period (5 days), growth of *Chlorella* sp. cultures were spectrophotometrically monitored using Unico UV -2100 spectrophotometer, by measuring optical density (OD) at 750 nm.

Chlorophyll (*a* and *b*) content was determined in methanolic extracts using the method of Marker [35]; assessed as μg. mL^{-1} algal suspensions.

Estimation of net photosynthetic oxygen evolution (P_N) and respiratory oxygen uptake (R_D)

P_N and R_D were monitored using a Clark type electrode (OMS, Hansatech Instruments Inc.). Two mL of algal culture was followed under light intensity of 100 μmol m-2 sec-1 at 25±2 °C for 10 min; the rate of P_N was calculated as nmole O_2↑mg Chl^{-1} h^{-1}. The rate of R_D was calculated as nmole O2↓mg Chl^{-1} h^{-1}.

Determination of protein content

The algal cells were collected for protein analysis at 5 days old cells using the method described by Lowry [36].

Antioxidation capacity

For the preparation of antioxidant enzymes extract, 100 mL of algal culture were centrifuged at 2000 Xg; the pellet was sonicated (using Sonicator HD 3200 homogenizer) in 5 ml K-P buffer (pH 7.8) containing EDTA and polyvinylpyrrolidone under cooling; the homogenate was centrifuged, the supernatants were used for assays of catalase, ascorbate peroxidase and guaiacol peroxidase. Catalase activity was assessed by

monitoring the consumption of H_2O_2 for 1 min at 240 nm [37,38]. Guaiacol peroxidase activity was evaluated spectrophotometrically at 470 nm [39]. Ascorbate peroxidase

activity was by measuring the oxidation of ascorbate as substrate at 290 nm in [40]. Enzymatic activity in each case was expressed as µmol μg^{-1} protein min^{-1}.

Total antioxidation capacity of the harvested cells following 5 days of CaO NPs exposure as described by Prieto et al [41].

Short term experiments

In these experiments, *Chlorella* culture was treated for only 5 hours with 0, 20, 40, 60, 80 and 100 ppm CaN OPs, only P_N and R_D were measured at (0, 1, 2, 3, 4 and 5) hours.

Each experiment was repeated three times and the mean values of three replicates ± standard error (SE) is presented.

RESULTS

1. Growth and Chlorophyll contents

Figure (1) presents growth as the absorbance (OD 750 nm) of *Chlorella* sp. grown in BG11 medium contain (0, 20, 40, 60, 80, 100 ppm CaO NPs) for 5 days. The highest optical density of *Chlorella* sp. has been recorded at 100 ppm CaO NPs, followed by 60 ppm; other concentrations of calcium oxide nanoparticles showed values close to that in case of the control.

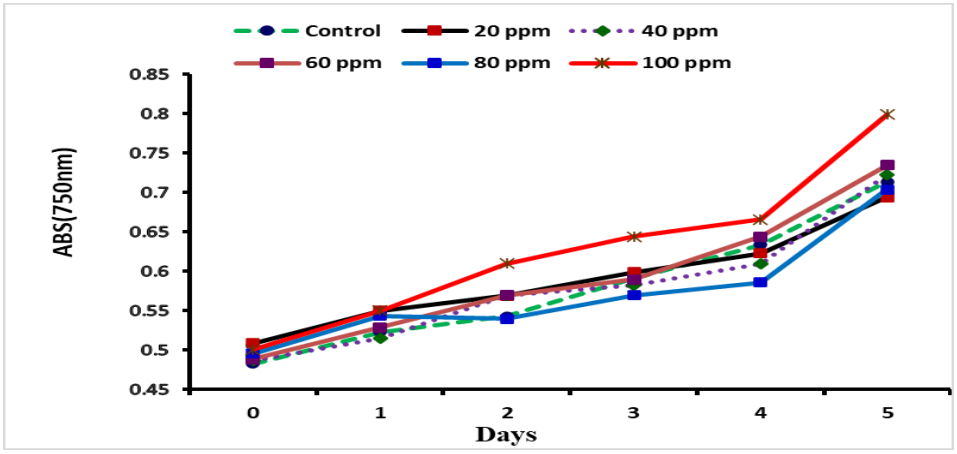

Figure (1): Absorbance (750 nm) in *Chlorella* sp. grown for 5 days at various concentrations of CaO NPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

Chlorophyll (*a* + *b*) contents of *Chlorella* sp. is presented in Figure (2). The highest chlorophyll content of *Chlorella* sp. was recorded at 100 ppm CaO NPs. After two days of growth, the cultures grown in 60 ppm CaO NPs was given chlorophyll value close to the control, while chlorophyll content decreased at 20 and 40 ppm CaO NPs.

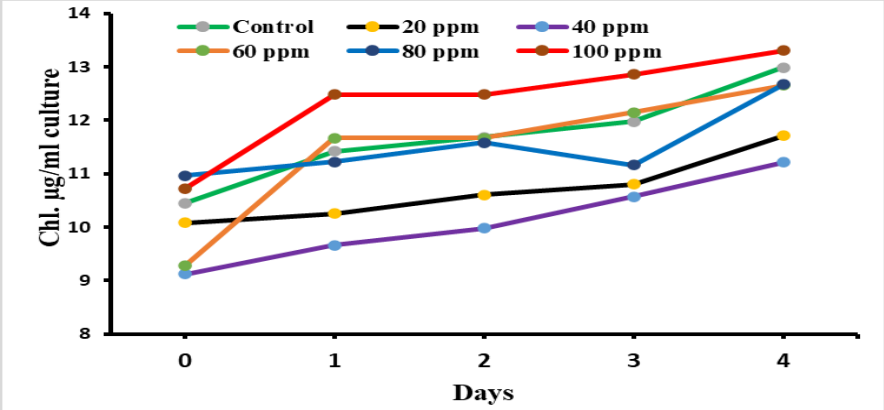

Figure (2): Chlorophyll (*a* + *b*) contents (µg.ml^{-1}culture) in *Chlorella* sp. grown for 5 days at various concentrations of CaO NPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

2. Photosynthetic oxygen evolution and respiratory oxygen uptake:

Net photosynthetic oxygen evolution (P_N) of *Chlorella* sp, in long term experiments (grown for 5 days), exhibited higher values than the control in all concentrations of CaO NPs, P_N was enhanced with increasing CaO NPs concentrations, the highest activity noted at 100 ppm CaO NPs (Figure 3 a). While dark respiratory oxygen uptake (R_D) recorded lower values in all cultures grown in CaO NPs, it decreased with increasing CaO NPs concentrations, the lowest was documented at 100 ppm CaO NPs (Figure 3 b).

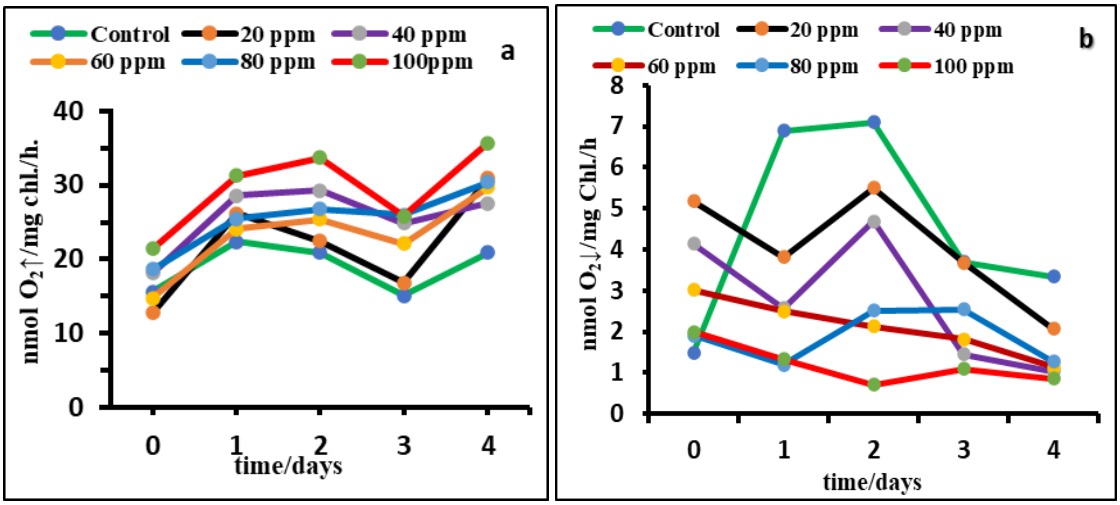

Figure (3): Photosynthetic oxygen evolution (a) and Respiratory oxygen uptake (b) as nmole O_2.mg Chl^{-1}.h^{-1} in *Chlorella* sp. grown for 5 days at various concentrations of CaO NPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

Figure (4 a) presents the photosynthetic oxygen evolution of *Chlorella* sp. in short term experiments (grown for 5 hours), P_N was higher than the control at 80 and 100 ppm CaO NPs, the highest at 100 ppm CaO NPs after 3 h, while the other concentrations (20, 40 and 60 ppm) were lower than the control. Respiratory oxygen uptake was higher than the control in all calcium oxide nanoparticles, 20 and 80 ppm exhibited higher values at the beginning, then decreased, while 60 ppm exhibited the highest value after 4 hours treatments (Figure 4 b) .

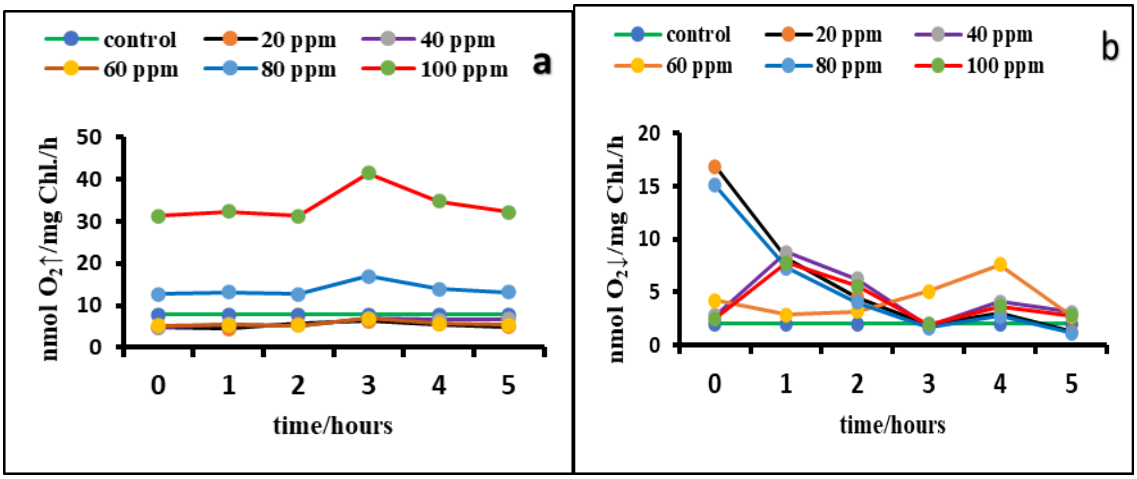

Figure (4): Photosynthetic oxygen evolution (a) and Respiratory oxygen uptake (b) as nmole O_2.mg $Chl^{-1}.h^{-1}$ of in *Chlorella* sp. grown for 5 hours at various concentrations of CaO NPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

3. Total Soluble Proteins

Figure (5) shows soluble proteins contents of *Chlorella* sp.as µg ml^{-1} cultures. Protein contents was increased gradually up to 60 ppm CaO NPs (higher values than the control culture), then decreased at 80 and 100 ppm CaO NPs.

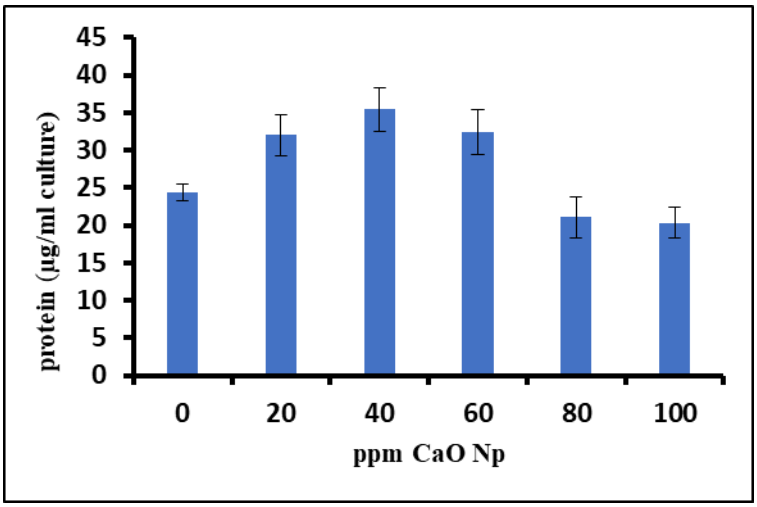

Figure (5): Soluble protein content (µg.ml^{-1}culture) in *Chlorella* sp. grown at various concentrations of CaONPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

4. Antioxidant enzymes' activity

Figure 6 (a-d) presents the antioxidant enzymes activities of catalase (CAT) ascorbate peroxidase (APX) guaiacol peroxidase (POD) and total antioxidant of *Chlorella* sp. grown for 5 days at various concentrations of CaONPs (0, 20, 40, 60, 80, 100 ppm).

Catalase activity was lower than control in 20 ppm CaO NPs and increased with increasing CaO NPs treatment up to 80 ppm it reach to the highest value (also higher than the control), then decreased at 100 ppm (Figure 6 a).

Guaiacol peroxidase activity, exhibited as catalase activity with the highest value at 80 ppm (58% increased than the control) (Figure 6 b). Ascorbate peroxidase demonstrated lower activity than control in all CaO NPs treatments the lowest value was at 40 ppm (Figure 6 c).

Figure (6 d) shows that all calcium nanoparticle treatments increased the total antioxidant activity from 2 to 4 times folders than the control culture however, however, it decreased with increasing CaO NPs.

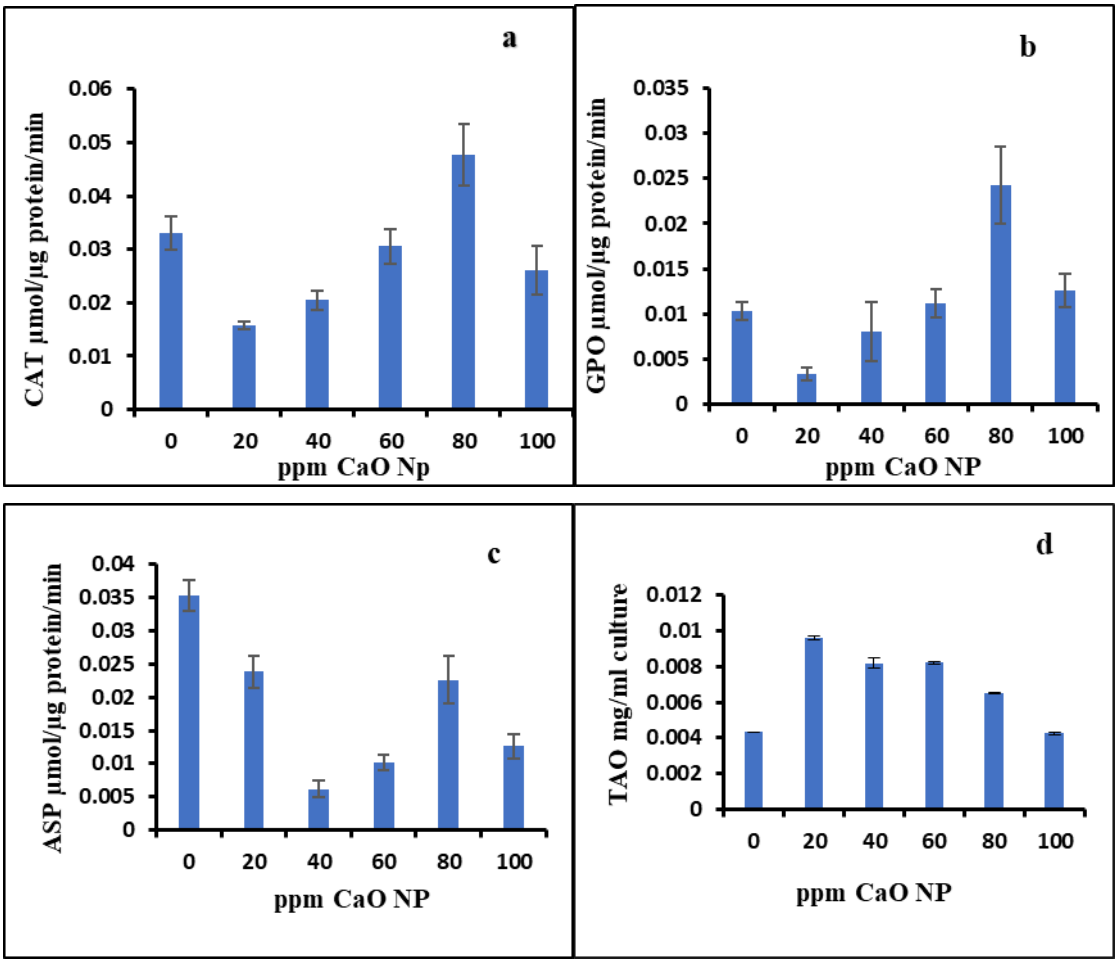

Figure 6 (a-d) : Antioxidant enzymes Catalase (a), Guaiacol Peroxidase (b), Ascorbate Peroxidase (c) activity (µmol· μg^{-1} protein min^{-1}) and total antioxidants (d) in *Chlorella* sp. grown for 5 days at various concentrations of CaO NPs (0, 20, 40, 60, 80, 100 ppm). The values are represented as means ±SE (n=3).

DISCUSSION

In this research, the impact of calcium oxide nanoparticles on *Chlorella* sp. growth was followed by daily recording of optical density ($OD_{750\ nm}$) at various nanoparticles treated cultures (0, 20, 40, 60, 80, 100 ppm CaO NPs). Optical density exhibits a good correlation with algal density and time saving in rapid follow up studies. The highest optical density of *Chlorella* sp. has been recorded at 100 ppm CaO NPs. With the same approach, using metal-based NPs instead of metals salts in algae culture increased the growth rate, biomass, pigments content, and other bioactive content of *Chlorella vulgaris* [42]. The exposure of microalgae such as *Scenedesmus obliquus, S. rubescens, C.vulgaris, C. pyrenoidosa, Parachlorella kessleri, Trachydiscus minutus, , and Tetraselmis suecica,* to metal nanoparticles under different environmental conditions affects on several physiological or molecular changes, resulting in increasing the growth rate, biomass and lipid production [16]. However, the appropriate application of nanoparticles to assist algal growth is still nascent and the mechanisms, for the most part, are not well unknown [16].

The synthesized calcium carbonate nanoparticles have positive impacts on seed germination of *Vigna mungo (L.) Hepper* [43]. Shoot biomass of *Hordeum vulgare* increased by 331% with application of CeO2 nanoparticles; but at higher concentration (500 mg/kg), the grain was not produced which is a vast loss [44]. Cell walls of plants, algae, and fungi represent a primary site for interaction and a barrier for the entrance of NPs. Nanoparticles might directly cause changes of cell membranes and other structures and particles, along with protective mechanisms [45].

Biotransformation of nanomaterials may either have positive or negative impacts on the living cells [46]. These biotransformations are correlated to oxidation reduction reactions, sulphur addition, phosphorylation and molecular alteration [47]. Some plants may absorb and translocate nanoparticles in different tissues. For example, maize plants can reduce CuO nanoparticles to Cu_2O and Cu_2S [48]. It has been reported that plant cells can

protect themselves against adverse effects of ROS by antioxidant enzymes as catalase, superoxide dismutase and peroxidase POD [49]. The exposure of some plants to nanoparticles could regulate diverse physiological morphological, and metabolic processes in plants, by improving free-radical hunting potential, antioxidant enzymes activity and micro RNAs expression [50].

In this study, the peak of chlorophyll contents coincided with those of the optical density at ١٠٠ ppm CaO NPs. In this respect, Eroglu et al [51] showed that the presence of metal nanoparticles in solutions increased the pigments content of microalgae. Addition of low concentrations of Cu nano carboxylates (20 to 40 mg/ L) and Ce nano carboxylates (0.07 to 0.2 mg /L), increased biomass of *Chlorella* and accompanied by enhanced of chlorophyll content [52]. Also, different concentrations of TiO_2 nanoparticles concurrently increased the content of photosynthetic pigments of *Chlorella pyrenoidosa* [53], and copper oxide nanoparticles caused enhanced of *Chlamydomonas reinhardstii* pigments [54]. There is one explanation about the elicitation of chlorophyll contents of algae by NPs, is that induction of ROS which can attack some pigments, these could convert to chlorophyll *a* under NPs and cause higher Chl *a* in the cells [55].

Photosynthetic oxygen evolution of *Chlorella* sp exhibited higher values than the control in all concentrations of CaO NPs, the highest activity recorded at 100 ppm CaO NPs. Also, there was a clear increase in the amount of oxygen released from photosynthesis when CaO NPs was applied at 100 ppm in short-term experiments. The efficiency of production of chemical energy in photosynthesis can improve by using metal NPs [56]. The foliar treatments of metal NPs on plants significantly increases the chlorophyll content, which produce additional light harvesting complexes to capture a greater amount of light energy and increase photosynthesis [57]. In this line, Hong et al [58, 59] studied the effects of TiO_2NPs on the light reaction of spinach chloroplasts and verified that TiO_2NPs applications induced the increase of chloroplasts activity and Hill reaction, which enhanced Fe-Cy reduction and oxygen evolution. Spinach thylakoid membranes were affected by TiO_2NPs and the light- harvesting complexes (LHCII) content were increased [58-60], this stimulates energy transfer and oxygen evolution in PSII [60-62]. Another explanation for increased photosynthetic quantum yield centres after treatment of spinach with titanium oxide nanoparticles, is an increase in Rubisco activity [63].

The present study showed that soluble protein contents were increased in *Chlorella* sp.in the cultures treated with 20, 40 and 60 ppm CaO NPs than the control. Similarly, the soluble protein content of *Chlamydomonas reinhardtii*at imposed to low dosage TiO_2 NPs was increased than the control and decreased in higher concentration [64]; they suggest that extra soluble protein was existing in algal cells treated with lower concentration TiO_2 NPs, may relate to synthesis of a new protein, which may play a significant role in algal cell adaptability to NMs treatment [64]. Increased soluble protein concentration is thought to be an active defence mechanism to keep algal cells from destructive by abiotic stress [65].

Several nanoparticles have been shown to interact with plant cells, resulting in increased antioxidant enzyme activity [66,67]. According to several research [68,69], nanoparticle exposure alters the expression of the superoxide dismutase (SOD) gene in plants, as well as the expression of other enzymes. Plants can protect their cells from the toxic effects of reactive oxygen species by regulation of antioxidant enzymes (SOD, CAT, GPO and ASP, etc.) and non-enzymatic components as carotenoids, ascorbate and tocopherol, etc. [70,71]. In the current study, catalase (CAT) and guaiacol peroxidase (GPO) activity decreased than the control by application of CaO NPs however, enhanced by increasing CaO NPs concentration up to 80 ppm it reached the highest value. Also, calcium oxide nanoparticle treatments increased the total antioxidant activity to 4 times folders than the control culture. In this respect, the CAT content in rice shoots was increased by 32%, 38%, and 60% at 10, 20 and 50 mg/L calcium phosphate NP respectively, comparatively to control while GPOx activity increased in rice roots by 4.4%, 0.39% at 10 and 20 mg/L and declined by 5.3% at 50 mg/L calcium phosphate NP respectively, in comparison to the control [31].

Nanoparticles also have uneven and randomized impacts on enzyme activity, according to certain researches. After treatment with titanium oxide nanoparticles, higher SOD, CAT, APOX, and GPOX activities were observed in spinach and *Lemna minor* [72,73]. However, some authors reported contradictory findings, such as reduced GR and APOX activity in *Vici faba.* treated with TiO2 NPs [74]. Antioxidant enzymes are activated by

plants as part of their detoxifying systems [74]. Catalase (CAT) activity rose in lettuce roots exposed to Cu/CuO NPs, but ascorbate peroxidase (APX) activity decreased. [75]. APX enhanced in lettuce roots and alfalfa roots exposed to Cu/CuO NPs, but CAT decreased in both shoots and roots [76]. As a result, the impact of nanoparticles on the diversity of antioxidant defence mechanisms in plants and algae is currently understudied.

Therefore, calcium oxide nanoparticles have been proven to stimulate photosynthesis and growth and modulating antioxidant enzymes.

CONCLUSION

The current study noticeably demonstrated the stimulatory effect of CaO NPs on *Chlorella* sp. as shown by the increase in algal growth, chlorophyll contents, photosynthesis. In addition, enhanced the total antioxidants and induced the activity of antioxidant enzymes (especially at 80 ppm CaO NPs). More research is needed to determine the effect of calcium oxide nanoparticles on algae and plants under a variety of environmental stresses, as well as to increase the production of high-value microalgae products.

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REFERENCES

[1] W.J. Liang, M.L. Wang, X.Z. Ai, The role of calcium in regulating photosynthesis and related physiological indexes of cucumber seedlings under low light intensity and suboptimal temperature stress, Sci. Hortic., 123 (2009) 34.

[2] J.J. Brand, D.W Becker, Evidence for direct roles of calcium in photosynthesis, J. Bioenerg. Biomembr. 16 (1984) 239.

[3] H. Knight, A.J. Trewavas, M.R. Knight, Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity, Plant J. Cell Mol. Bio., 12 (2010) 1067.

[4] P. Shi, F. Zeng, W. Song, M. Zhang, R. Deng, Effects of calcium and lanthanum on ABA biosynthesis in Cucumber Leaves, Russ. J. Plant Physiol., 49 (2002) 696.

[5] J. Vadassery, R. Oelmüller, Calcium signalling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between *Piriformo spora* indica and *Arabidopsis thaliana*, Plant Signal. Behav., 4 (2009) 1024.

[6] A. Falciatore, M.R. D'alcala, P. Croot, C. Bowler, Perception of environmental signal by a marine diatom, Science, 288 (2000) 2363.

[7] G.L. Wheeler, I. Joint, C. Brownlee, Rapid spatiotemporal patterning of cytosolic Ca^{2+} underlies flagellar excision in *Chlamydomonas reinhardtii*, Plant J, 53 (2008) 401.

[8] M.H. Siddiqui, M.H. Al-Whaibi, Role of nano-SiO2 in germination of tomato (*Lycopersicum esculentumseeds* Mill.), Saudi J BiolSci, 21 (2014) 13.

[9] J.P. Giraldo, M.P. Landry, S.M. Faltermeier, T.P. McNicholas, N.M. Iverson, Plant nanobionics approach to augment photosynthesis and biochemical sensing, Nature materials, 13 (2014) 400.

[10] B. Nowack, T.D. Bucheli, Occurrence, behaviour and effects of nanoparticles in the environment, Environ. Pollut., 150 (2007) 5.

[11] B.D. Johnston, T.M. Scown, J. Moger, S.A. Cumberland, M. Baalousha, K. Linge, R. Aerle, K. Jarvis, J.R. Lead, C.R. Tyler, Bioavailability of Nanoscale Metal Oxides TiO2, CeO2, and ZnO to Fish Environmental, Science & Technology, 44 (3) (2010) 1144.

[12] E. Kadar, P. Rooks, C. Lakey, D. A. White, The effect of engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures, Science of The Total Environment, 439 (2012) 8.

[13] J. Kalman, B.P. Kai, F.R. Khan, V. Stone, T.F. Fernandes, Characterisation of bioaccumulation dynamics of three differently coated silver nanoparticles and aqueous silver in a simple freshwater food chain, Environ. Chem., 12 (2015) 662.

[14] F. Ribeiro, J.A. Gallegourrea, R.M. Goodhead, C.A. Gestel, J. Moger, A.M. Soares, S. Loureiro, Uptake and elimination kinetics of silver nanoparticles and silver nitrate by *Raphidocelis subcapitata:* The influence of silver behaviour in solution, Nanotoxicology, 9 (2015) 686.

[15] N.K. Kang, B. Lee, G.G. Choi, M. Moon, M. S. Park, J. K. Lim., Enhancing lipid productivity of Chlorella vulgaris using oxidative stress by TiO2 nanoparticles, Korean J. Chem. Eng., 31 (2014) 861.

[16] R.D. Sarkar, H.B. Singh, M.C. Kalita, Enhanced lipid accumulation in microalgae through nanoparticle-mediated approach, for biodiesel production, Heliyon, 7 (2021) e08057.

[17] A.F. Aravantinou, V. Tsarpali, S. Dailianis, I.D. Manariotis, Effect of cultivation media on the toxicity of ZnO nanoparticles to freshwater and marine microalgae, Ecotoxicol. Environ. Saf., 114 (2015) 109.

[18] F. Wang, W. Guan, L. Xu, Z. Ding, H. Ma, A. Ma, N. Terry, Effects of Nanoparticles on Algae: Adsorption, Distribution, Ecotoxicity and Fate,Appl. Sci., 9 (2019) 1534.

[19] P. Saxena, V. Saharan, P.K. Baroliya, V.S. Gour, M.K. Rai, Harish, Mechanism of nanotoxicity in Chlorella vulgaris exposed to zinc and iron oxide, Toxicology reports, 8 (2021) 724.

[20] K. Miazek, W. Iwanek, C. Remacle, A. Richel, D. Goffin, Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis, International Journal of Molecular Sciences, 16 (2015) 23929.

[21] F. J. Ribeiro, A. Gallego-Urrea, K. Jurkschat , Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Daniorerio, Science of the Total Environment, 466-467 (2014) 232.

[22] Navarro, B. Wagner, N. Odzak, L. Sigg, R. Behra, Effects of differently coated silver nanoparticles on the photosynthesis of *Chlamydomonas reinhardtii*, Environmental Science & Technology, 49 (2015) 8041.

[23] D. He, J.J. Dorantes-Aranda, T.D. Waite, Silver nanoparticle—algae interactions: oxidative, dissolution, reactive oxygen species generation and synergistic toxic effects, Environmental Science & Technology, 46 (2012) 8731.

[24] A. Oukarroum, S. Bras, F. Perreault, R. Popovic, Inhibitory effects of silver nanoparticles in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, Ecotoxicology and Environmental Safety, 78 (2012) 80.

[25] K. Hund-Rinke, M. Simon, Ecotoxic effect of photocatalytic active nanoparticles (TiO2) on algae and Daphnids, Environ. Sci. Pollut. Res., 13 (2006) 225.

[26] E. Kadar, P. Rooks, C. Lakey, D.A. White, The effect of engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures, Sci. Total Environ., 439 (2012) 8.

[27] H. Meilin, Y. Yongquan, P. Feng, W. Mingzhu, G. Temesgen, Z. Shanmei, W. Changhai, Improvement on lipid production by *Scenedesmus obliquus* triggered by low dose exposure to nanoparticles, Sci. Rep., 7 (2017) 15526.

[28] M. Rastar, S. Pezhman, H. Shekarabi, S.M. Mehrgan, S. Sabzi, Effects of iron and zinc concentrations on growth performance and biochemical composition of *Haematococcus pluvialis*: a comparison between nanoparticles and their corresponding metals bulks, J. Algal Biomass Utln., 9(2) (2018) 59.

[29] X.Y. Deng, J. Cheng, X.L. Hu, L. Wang, D. Li, K. Gao, Biological effects of TiO2 and CeO2 nanoparticles on the growth, photosynthetic activity, and cellular components of a marine diatom *Phaeodactylum tricornutum*, Science of the Total Environment, 575 (2017) 87.

[30] Q. Zhong, Z. He, A.Q. Dai, C. Zhang, X. Yang, X.J. Hu, J. Guo, L. Xiong, D.L. Liu, Toxicities of nanoparticulate CeO2 on *Scenedesmus obliquus* Environ. Sci., 31 (2) (2012) 299.

[31] H. Upadhyaya, L. Begum, B. Dey, P.K. Nath, S.K. Panda, Impact of Calcium Phosphate Nanoparticles on Rice Plant, J. Plant Sci Phyto pathol., 1 (2017) 001.

[32] M. Rane, M. Bawskar, D. Rathod, D. Nagaonkar, M. Rai, Infuence of calcium phosphate nanoparticles, *Piriformo sporaindica* and *Glomusmos seaeon* on growth of Zea mays, Advances in Natural Sciences: Nanoscience and Nanotechnology, 6 (2015) 045014.

[33] D.M. Abd El-Aziz, N.M.H. Yousef, Antimicrobial Effects of Calcium Oxide Nanoparticles and Some Spices in Minced Meat, ARC Journal of Animal and Veterinary Sciences, 3 (2017) 38.

[34] R. Rippka and M. Herdman, "Catalogue of Strains. Pasteur Culture Collection of Cyanobacterial Strains in Axenic Culture," Institut Pasteur, Paris, 1992.

[35] A.F.H. Marker, The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin, Fresh Wat Biol., 2 (1972) 361.

[36] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with Folin phenol reagent, J. Biol. Chem., 193 (1951) 265.

[37] H.J.C.I.V. AEBI, Methods in enzymology, 105 (1984) 121.

[38] T. Matsumura, N. Tabayashi, Y. Kamagata, C. Souma, H. Saruyama, Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress, Physiol Plant., 116 (2002) 317.

[39] Z. Tatiana, K. Yamashita, H. Matsumoto, Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber root, Plant Cell Physiol., 40 (1999) 273.

[40] NAKANO, K.Y. ASADA, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol., 22 (1981) 867.

[41] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E, Anal. Biochem., 269 (1999) 337.

[42] G.Sibi, D. Ananda Kumar, T. Gopal, K. Harinath, S. Banupriya, S. Chaitra, Metal Nanoparticle Triggered Growth and Lipid Production in Chlorella Vulgaris, Int. J. Sci. Res. Environ. Sci. Toxicol., 2(1) (2017) 1.

[43] P. Yugandhar, N. Savithramma, Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of *Vigna mungo* (L.). Hepper., International Journal of Advanced Research, 8 (2013) 89.

[44] C.M. Rico, A.C. Barrios, W. Tan, R. Rubenecia, S.C. Lee, A. Varela-Ramirez, J.R. Peralta- Videa, J.L. Gardea-Torresdey, Physiological and biochemical response of soil-grown barley (*Hordeum vulgare L.*) to cerium oxide nanoparticles, Environ. Sci. Pollut. Res., 22 (2015)10551.

[45] E. Navarro, A. Baun, R. Behra, N.B. Hartmann, J. Filser, A.J. Miao, A. Quigg, P.H. Santschi, L. Sigg, Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi, Ecotoxicology, 17 (2008) 372.

[46] D.A. Abramowicz, Aerobic and anaerobic biodegradation of PCBs, Crit. Rev. Biotechnol., 10 (1990) 241.

[47] G.V. Lowry, K.B. Gregory, S.C. Apte, J.R Lead, Transformations of nanomaterials in the environment, Environ. Sci.Technol., 46 (2012) 6893.

[48] Z. Wang, X. Xie, J. Zhao, X. Liu, W. Feng, J.C. White, B. Xing, Xylem- and Phloem-based transport of CuO nanoparticles in maize (Zea mays L.), Environ. Sci. Technol., 46 (2012) 4434.

[49] H. Wang, X. Kou, Z. Pei, J.Q. Xiao, X. Shan, B. Xing, Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (Loliumperenne L.) and pumpkin (Cucurbitamixta) plants, Nanotoxicology, 5 (2011) 30.

[50] K.S. Siddiqi, A. Husen, Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects, Nano Res. Lett., 11 (2016) 363

[51] E. Eroglu, P.K. Eggers, M. Winslade, S.M. Smith, C.L. Raston, Enhanced accumulation of microalgal pigments using metal nanoparticle solutions as light filtering devices, Green Chem., 15 (2013) 3155.

[52] N.F. Mykhaylenko, E.K. Zolotareva, The Effect of Copper and Selenium Nanocarboxylates on Biomass Accumulation and Photosynthetic Energy Transduction Efficiency of the Green Algae *Chlorella Vulgaris*, Nanoscale Res. Lett., 12(1) (2017) 147.

[53] A. Middepogua, J. Houa, X. Gaoa, D. Lina, Effect and mechanism of TiO2 nanoparticles on the photosynthesis of *Chlorella pyrenoidosa*, Ecotoxicology and Environmental Safety, 161 (2018) 497.

[54] S.P. Melegari, F. Perreault, R.H.R. Costa, R. Popovic, W. G. Matias, Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii,* Aquat.Toxicol., 142 (2013) 431.

[55] X. Chen, X. Zhu, R. Li, H. Yao, Z. Lu, X. Yang, Photosynthetic toxicity and Oxidative Damage Induced by nano-Fe3O4 on *Chlorella vulgaris* in Aquatic Environment, Open Journal of Ecology, 02(01) (2012) 21.

[56] A.O. Govorov, I. Carmeli, Hybrid structures composed of photosynthetic system and metal nanoparticles: plasmon enhancement effect, Nano Lett., 7(3) (2007) 620.

[57] M. Kolenčík, D. Ernst, M. Urík, Ľ. Ďurišová, M. Bujdoš,M. Šebesta , E. Dobročka, S.Kšiňan, R. Illa, Y.Qian, H. Feng,I. Černý, V. Holišová ,G. Kratošová ,Foliar application of low concentrations of titanium dioxide and zinc oxide nanoparticles to the common sunflower under field conditions, Nanomaterials 2020, 10, 1619.

[58] F.S. Hong, P. Yang, F.Q. Gao, L. Zheng, F. Yang, J. Zhou, Effect of nano TIO_2 spectral characterization of photosystem II particles from spinach, Chem.Res.Chin.Univ., 12 (2005) 196.

[59] F.S. Hong, J. Zhou, C. Liu, F. Yang, C. Wu, P. Zheng, Effect of nano-Tio_2 on photochemical reaction of chloroplasts of spinch.Biol., Trace Elem.Res.,105 (2005) 296.

[60] Z. Lei, S. Mingyu, L. Choo, Effect of nanoanatase TiO_2 on photosynthesis of spinach chloroplasts under different light illumination, Biol.Trace. Elem.Res., 119 (2007) 68.

[61] M.Y. Su, X. Wu, C. Liu, C.X. Qu, Q. X. Liu, L. Chen, H. Huang, F.S. Hon, Effect of nano-anatase Tio2 on absorption, distribution of light photochemical activities of chroplast of spinach, Bio. Trace Elem. Res., 118 (2007) 120.

[62] E. Morteza, P. Moaveni, H.A. Farahani, M. Kiyani, Study of photosynthetic pigments changes of maize (Zea mays L.) under nano Tio2 spraying at various growth stages, Springer plus 2 (2013) 1.

[63] F. Gao, C. Liu, C. Qu, L. Zheng, F. Yang, M. Su, F. Hong, Was improvement of spinach growth by nano-TiO2 treatment related to the changes of rubiscoactivase?, Biometals, 21 (2008) 211.

[64] I. Chen, L. Zhou, Y. Liu, S. Deng, H. Wub, G. Wang, Toxicological effects of nanometer titanium dioxide (nano-TiO2) on Chlamydomonas reinhardtii, Ecotoxicology and Environmental Safety, 84 (2012) 155.

[65] S.E. Sabatini, A.B. Juárez, M.R. Eppis, L. Bianchi, C.M. Luquet, C. Ríos de Molina Mdel, Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. Ecotoxicol Environ Saf. 72(4): (2009) 1200.

[66] M.N. Khan, Nano-titanium dioxide (nano-TiO2) mitigates NaCl stress by enhancing antioxidant enzymes and accumulation of compatible solute in Tomato (*Lycopersicom esculentum* Mill), J.Plant Sci., 11 (2016) 1.

[67] A.A.H. Abdel Latef, M.F. Abu Alhmad, K.E. Abdelfattah, The Possible Roles of Priming with ZnO Nanoparticles in Mitigation of Salinity Stress in Lupine (Lupinus termis) Plants, J. Plant Growth Regul., 36 (2017) 60.

[68] H.F. Alharby, E.M.R. Metwali, M.P. Fuller, A.Y. Aldhebiani, The alteration of mRNA expression of SOD and GPX genes, and proteins in tomato (Lycopersicon esculentum Mill) under stress of NaCl and/or ZnO nanoparticles, Saudi Journal of Biological Sciences, 23 (2016) 773.

[69] Z. Hossain, G. Mustafa, S. Komatsu, Plant Responses to Nanoparticle Stress, Int. J. Mol. Sci., 16 (2015) 26644.

[70] A. Husen, Growth characteristics, physiological and metabolic responses of teak (*Tectona grandis* Linn.f.) clones differencing in rejuvenation capacity subjected to drought stress, Silvae Gene., 59 (2010) 124.

[71] I.I. Ozyigit, E. Filiz, R. Vatansever, K.Y. Kurtoglu, I. Koc, M.X. Öztürk, N.A. Anjum, Identification and comparative analysis of H2O2-scavenging enzymes (ascorbate peroxidase and glutathione peroxidase) in selected plants employing bioinformatics approaches, Front Plant Sci., 7 (2016) 301.

[72] Z. Lei, S. Mingyu, W. Xiao, et al., Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation, Biol. Trace Elem. Res., 121 (2008) 69.

[73] G. Song, Y. Gao, H. Wu, et al., Physiological effect of anatase TiO2 nanoparticles on Lemna minor, Environ. Toxicol. Chem., 31 (2012) 2147.

[74] A.S. Foltete, J.F. Masfaraud, E. Bigorgne, Environmental impact of sunscreen nanomaterials: ecotoxicity and gentoxicity of altered TiO2 nanocomposites on Vicia faba, Environ. Pollut, 159 (2011) 2515.

[75] A.K. Shaw, S. Ghosh, H.M. Kalaji, K. Bosa, M. Brestic, M. Zivcak, Z. Hossain, Nano-CuO stress induced modulation of antioxidativedefense and photosynthetic performance of Syrian barley (Hordeumvulgare L.), Environ. Exp. Bot., 102 (2014) 37.

[76] J. Hong, C.M. Rico, L. Zhao, A.S. Adeleye, A.A. Keller, J.R. Peralta-Videa, J.L. Gardea-Torresdey, Toxic effects of copper-based nanoparticles or compoundstolettuce (Lactucasativa) and alfalfa (Medicagosativa), Environ. Sci. Process.Impact, 17 (2016) 177.