

Defense responses of the green microalgae *Chlorella vulgaris* to the vanadium pentoxide nanoparticles

by

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Abstract

Although vanadium-based nanomaterials have found extensive use in industry, their influence on ecosystems and living organisms is not yet well investigated. In this study, hydrothermal methods were utilized for the synthesis of vanadium pentoxide nanoparticles (V_2O_5 NPs). The gained NPs were characterized using XRD, FT-IR, EDS, DLS, SEM and TEM techniques. Subsequently, the toxic effects of V_2O_5 NPs on the model green microalgae *Chlorella vulgaris* were evaluated. According to the obtained results, V_2O_5 NPs caused a significant reduction in cell number and biomass production of algae in a dose and time dependent manner. Moreover, flow cytometric analysis confirmed a reduction in the quantity of living cells. Scanning electron microscopy showed plasmolysis and deformation of the cells after exposure to nanoparticles. The photosynthetic pigments and phenolics content exhibited a decrease in comparison with the control sample. Although, non-enzymatic antioxidant system in *C. vulgaris* displayed an average action, antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) showed a dose dependent increasing trend. These intercellular reactions designated the activation of the antioxidant defense system in response to the induced oxidative stress by V_2O_5 NPs.

Key words: *Chlorella vulgaris*, Algae, V_2O_5 nanoparticles, Oxidative stress, Antioxidant enzymes

1. Introduction

The improving applications of different types of nanoparticles (NPs) in many industrial products give rise to their undesirable discharge into the ecosystems. Thus, the hazardous impacts of NPs on the biosphere and living organisms have to be regularly assessed (Abbas et al. 2020). In recent times, inquiry into the results and performance of manufactured NMs in the ecosystem has gained growing attention (Malakar et al. 2021). Indeed, it is uncertain whether the advantages of nanotechnologies exceed the threats related to the environmental discharge of NPs. Thus, supplementary toxicological research is needed to identify crucial knowledge gaps in the current understanding of the harmful influences of NPs on the environment (Klaine et al. 2008).

Transition metal oxides have been frequently a research issue because of their variable valence states and broad technological applications (Yuan et al. 2021). Vanadium as a transition metal has the potential to assume multiple oxidation states. It can create different compounds with oxygen such as VO, VO₂, V₂O₃, and V₂O₅ in various configurations, which determines their chemical properties (Sieradzka et al. 2011). The toxic impact of vanadium oxides on living organisms is influenced by different issues such as their solubility, concentration, length of treatment, and the type of treated cells (Xi et al. 2019; Xi et al. 2021). Vanadium pentoxide-based materials are broadly utilized in industrial products such as gas sensors (Niu et al. 2021), solar cells (Costals et al. 2021), smart glasses (Ma et al. 2008), and smart windows (Li et al. 2023). Notably, *in situ* experiments have demonstrated that paints containing V₂O₅ NPs might inhibit the biofouling of surfaces in seawater (Natalio et al. 2012). Moreover, therapeutic use of nanoscaled V₂O₅ against cancer cells has been recently reported (Das et al. 2020).

Aquatic ecosystems as the terminal destination of domestic and industrial wastewater have a specific importance for the evaluation of NP toxicity. Accordingly, aquatic organisms were subjected to studies of the unsafe effects of released contaminants into the ecosystems (Walters et al. 2014). However, only a few studies have been performed on the effects of nanoscaled vanadium on algae and cyanobacteria. Recently, the capability of four microalgae in the bioremoval of V₂O₅ has been assessed, and only *Nannochloropsis oculata* showed high absorption efficiency (Kim et al. 2022). Moreover, the negative impacts of vanadium oxide nanoparticles on the protein and pigment content of the cyanobacterium *Spirulina platensis* have been recognized (Padash et al. 2023). Thus, a substantial approach is necessary

to contribute more information towards the harmful effects of V₂O₅ NPs on algal cells.

Because of having simple cultivation requirements, a short life cycle, and sensitivity to different classes of contaminants, unicellular green microalgae were widely used as model microorganisms in the risk assessment of pollutants (DeLorenzo et al. 2002; Karimi et al. 2017a,b; Nazari et al. 2018). In this context, the use of the *Chlorella* species in nanotoxicology investigations has been described by some previous studies (Adochite & Andronic 2021; Feizi et al. 2022; Hamed et al. 2017; Saxena et al. 2021). *Chlorella vulgaris* (*C. vulgaris*) is a photosynthetic microalga with a noteworthy dispersal in the sea and freshwater. It is a model organism for toxicity investigations at the cellular level. Furthermore, this microalga was recognized for the bioremoval of many environmental pollutants (Asghari et al. 2020; Hernández-Zamora et al. 2015; Xaaldi et al. 2017; Movafeghi et al. 2019).

Here, we examined some of the physiological and cytological criteria of *C. vulgaris* after treatment with V₂O₅ NPs. The growth of microalgae throughout the experiments was analyzed by counting the cells supported by spectrophotometric measurements at 680 nm as the maximum absorbance wavelength of algal cells. Additionally, the impacts of the V₂O₅ NPs on cell viability, and the non-enzymatic and enzymatic antioxidant defense systems of algal cells were investigated. The acquired results might shed light on the risk factors of synthetic NMs and their hazardous effects on biosystems.

2. Materials and methods

2.1. Synthesis and description of V₂O₅ NPs

V₂O₅ NPs as nanospheres were synthesized by the previously published method (Wu et al. 2013). A mixture of 1 ml H₂O₂ (30%) and 4 ml H₂O was added into a methanol solution of VO(acac)₂ (0.7 mmol; 30 ml). After shaking for 1 h, the solution was transferred into an autoclave and subsequently was positioned in an oven at 150°C for 24 h. The obtained precipitates were centrifuged and washed with a small amount of ethanol, and the final product was calcined under air at 400°C for 2 h to acquire spherical V₂O₅ NPs. The crystal structure and the vibrational spectra analysis of the synthesized NPs was examined by XRD (Tongda, TD-3700, China) and FT-IR (SENSOR 27, Bruker, Germany), respectively. The size distribution and zeta potential of the prepared NPs were demonstrated using DLS (Nanotracer Wave, Microtrac, USA). High resolution images of the V₂O₅ NPs, indicating their

size, morphology and elemental composition, were acquired using TEM (Zeiss LEO 906, kv 100, Germany) and SEM (MIRA3 FEG-SEM, Tescan, Czech Republic), equipped with EDS (Dağlıoğlu et al. 2023).

2.2. Cultivation condition

C. vulgaris strain CCAP 211/11B was cultured under sterile circumstance in BG-11 medium, with constant shaking under 5000 lux fluorescent light and a 16/8 h light/dark photoperiod at 25°C. The number of cells was determined every day by means of a hemocytometer. Cell counting for each medium was conducted three times. The regression equivalence between cell number ($x \times 10^4$ ml) and OD_{680} (y) was found to be $y = .0072x - .0275$ ($R^2 = .9973$). Changes in OD_{680} were measured using a spectrophotometer (UV/VIS, S2000, England).

Algal cells were exposed to V_2O_5 NPs in the exponential growth stage on the day between 6 to 8 of culture. For this purpose, V_2O_5 NPs were added to 250 ml flasks holding 200 ml of cell containing media with the final quantities of 50, 100, 150, and 200 mg l^{-1} . Treated cells were collected every 24 h for 4 days by centrifugation at $5000 \times g$ for 10 min and saved at $-80^\circ C$. The determination of treatment concentration was based on initial experiments and in correlation with the mean range of vanadium quantity in soil, which was reported to be around 150 mg kg^{-1} and also excess density of its environmental existence because of human activities (Aihemaiti et al. 2020).

2.3. Growth analysis

The growth of algae was estimated every day, not only by assessing the cell number but also by determining the absorption at 680 nm. A preliminary cell number of 30×10^4 was used for all examinations. Changes in fresh weight were also evaluated after gathering the cells by centrifugation at $5000 \times g$ for 10 min.

2.4 Flow cytometric examination

The viability of algae after the application of 200 mg l^{-1} of V_2O_5 NPs for 96 h was measured by flow cytometric tests. Nearly 1.0×10^6 cells were harvested by centrifugation at $5000 \times g$ for 10 min, rinsed with phosphate buffer (PBS, pH 7.4), and treated with 5 μl of propidium iodide (PI) in the dark for 20 min. The fluorescence emission of the cells was obtained from $\sim 10\,000$ events per sample in the FACSCalibur FL2 channel (Becton Dickinson on Immunocytometry Systems, San Jose, CA, USA). The produced chlorophyll emissions were assembled in the FL3 channel.

2.5. Morphological observation of cells

Exposed algae to 100 and 200 mg l^{-1} of V_2O_5 NPs for 96 h were precipitated at $5000 \times g$ for 5 min using a centrifuge (Eppendorf, 5810R, Germany). Subsequently, the cells were washed three times with phosphate buffered saline (PBS), fixed with formaldehyde and dehydrated sequentially in 20, 40, 60, 80 and 100% ethanol. After freeze-drying and subsequent gold coating, the structure of the treated and control cells was examined under a scanning electron microscope (MIRA3 FEG-SEM, Tescan, Czech Republic) (Ozturk et al. 2019).

2.6. Examination of pigment content

The content of chlorophyll *a*, *b*, and total carotenoids as photosynthetic pigments was quantified using 100% methanolic extract of algae by an earlier stated standard practice, employing a double-beam UV-visible spectrophotometer (UV/VIS, S2000, England) (Wellburn 1994). The following formulae were applied for the calculation of the pigments' content:

$$C_a = [15.65 \times A_{666}] - [7.34 \times A_{653}]$$

$$C_b = [27.05 \times A_{653}] - [11.21 \times A_{666}]$$

$$C_{x+c} = [1000 A_{470} - 2.86 C_a - 129.2 C_b] / 221$$

where C_a , C_b , and C_{x+c} indicate the content of chlorophyll *a*, *b* and carotenoids in mg g^{-1} FW.

2.7. Estimation of phenol and flavonoid level

The cells were crushed in 100% methanol prior to be kept at $4^\circ C$ for 24 h in the dark. After centrifugation of the homogenate at $10\,000 \times g$ for 10 min, the supernatant was employed for the experiments. The total phenol quantity was assessed based on the Folin-Ciocalteu technique (Meda et al. 2005). Accordingly, 2.5 ml of distilled water was added to 100 μl of the methanolic algal extract, and then 100 μl of Folin-Ciocalteu was mixed with the solution. After 6 min, 150 μl of sodium carbonate (20%) was mixed and the solution was retained in the dark for 30 min. Subsequently, the absorbance of the solution was read at 760 nm. The overall phenol quantity was expressed as equivalent to a milligram of gallic acid per fresh weight of algae. The assay of total flavonoid was conducted through an adapted aluminum chloride colorimetric process (Chang et al. 2002). A volume of 500 μl aluminum chloride (2%) was added to 500 μl of methanolic extract. Afterwards, the mixture was placed



at 4°C for 1 h in the dark, and its absorbance was read at 415 nm. The total flavonoid level of the extracts was reported as equivalent to a milligram quercetin per fresh weight of microalgae.

2.8. Assay of antioxidant enzyme activities

The attained cells from media were macerated in 2 ml of phosphate buffer (100 mM, pH 7) after adding in liquid nitrogen. The achieved homogenate was centrifuged, and the supernatant was immediately employed for the assessment of the amount of protein content (Bradford 1976), as well as the measurement of antioxidative enzymes activities, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX).

The action of superoxide dismutase (SOD) was calculated by determining the inhibiting the photoreduction of nitro-blue-tetrazolium (NBT) by the extract. This presented the basic quantity of the enzyme for a 50% inhibition of NBT photoreduction as one unit of SOD activity (Winterbourn et al. 1976). The CAT function was noted at 240 nm by the subsequent breakdown of H_2O_2 for 1 min, where one μmol of reduced H_2O_2 per minute displayed one unit of enzyme activity (Chance & Maehly 1955). The

activity of ascorbate peroxidase (APX) was determined in absorbance at 290 nm regarding the oxidation of ascorbate with the algal extract. The amount of enzyme needed for the oxidation of 1 μmol ascorbic acid in one minute was considered as one unit of enzyme activity (Nakano & Asada 1981).

2.9. Statistical analysis

The measurements used for statistical examinations were conducted in three repetitions. One-way analysis of variance (ANOVA) was conducted with Duncan's multiple comparison tests by SPSS 18 software. The normality of the data was checked using the Kolmogorov-Smirnov test. The differences were documented as significant when the p -value was less than 0.05.

3. Results

3.1. Characterization of V_2O_5 NPs

The structure, purity, and phase composition of the nanospheres were further studied in detail. The XRD chart of V_2O_5 NPs is presented in Fig. 1A. All

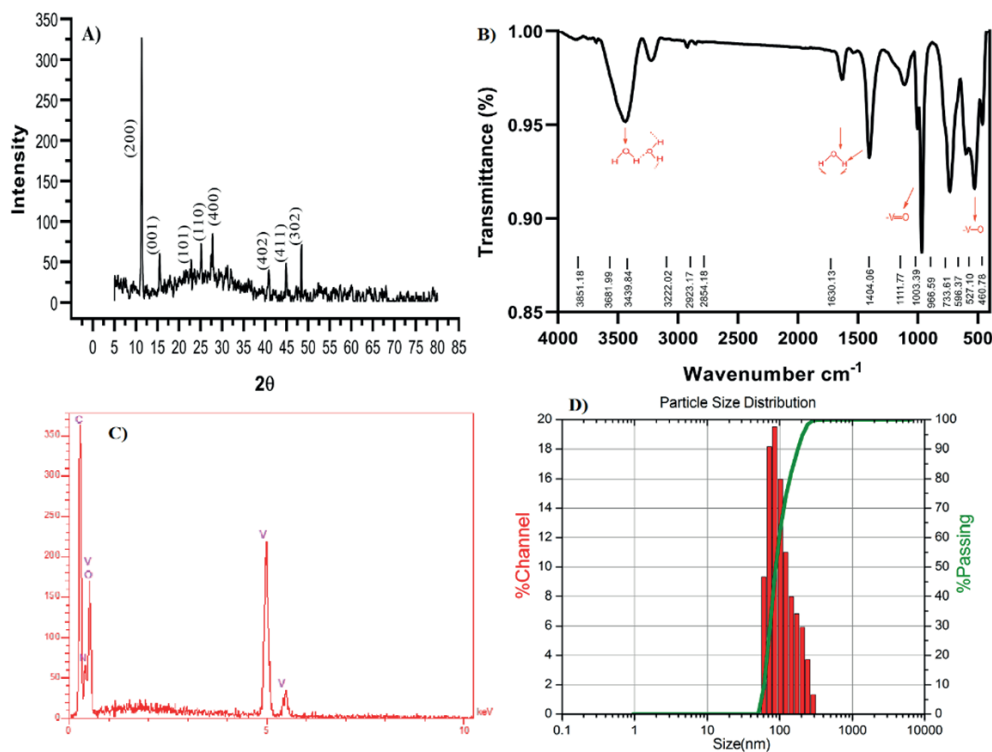


Figure 1

(A) XRD graph, (B) FT-IR data, (C) EDS and (D) DLS spectrum of the synthesized V_2O_5 NPs approving their precise chemical structure

patterns at $2\theta = 11.5^\circ, 16.2^\circ, 23.0^\circ, 26.1^\circ, 28.0^\circ, 40.5^\circ, 45.1^\circ,$ and 48.3° corresponding to the (200), (001), (101), (110), (400), (402), (411) and (302) planes can be signed to the orthorhombic phase of V_2O_5 (JCPDS no. 41-1426) (Wu et al. 2013) without any extra peaks related to impurities. FT-IR spectra of the vanadium pentoxide particles are demonstrated in Fig. 1B. The V = O stretching of the V_2O_5 nanospheres appeared at 980 cm^{-1} (Kera et al. 1967). The bands at 1411 and 1590 cm^{-1} are caused by the bending vibration of H_2O molecules on vanadium oxides, and the broad absorption bands at $3200\text{--}3400\text{ cm}^{-1}$ fit the hydrogen bonds of intermolecular water molecules and the stretching vibration of H_2O molecules. An EDS examination confirmed the existence of vanadium and oxygen atoms in the V_2O_5 NPs (Fig. 1C). To determine the behavior of the synthesized NPs in the aqueous phase, the particle size and zeta potential of the NPs were measured in distilled water. According to the obtained size distribution histogram, hydrodynamic sizes of V_2O_5 NPs were determined to be $70\text{--}289\text{ nm}$ (with a peak below 100 nm), and zeta potentials were

measured as $+9.8\text{ mV}$ (Fig. 1D). The amount of zeta potential supported the necessary stability of V_2O_5 NPs in the suspension medium. The structural information of the V_2O_5 nanospheres was also examined by SEM and TEM observation. The micrographs showed that the V_2O_5 particles are nanospheres with approximately 100% morphological yields and particle diameter in the range of $60\text{--}100\text{ nm}$ (Fig. 2A, B).

3.2. The influence of V_2O_5 NPs on the growth of *C. vulgaris*

The first evaluated growth factor was cell number. The concentration and time dependent influence of V_2O_5 NPs on the changes in cell number of *C. vulgaris* over 96 h was observed (Fig. 3A). Although an elevating trend in curves even after the exposure to V_2O_5 NPs was recognized, prevention of usual growth particularly by time was evident. Further measurement of growth using fresh weight confirmed the concentration-dependent restrictive impact of V_2O_5 NPs on the growth of algae (Fig. 3B).

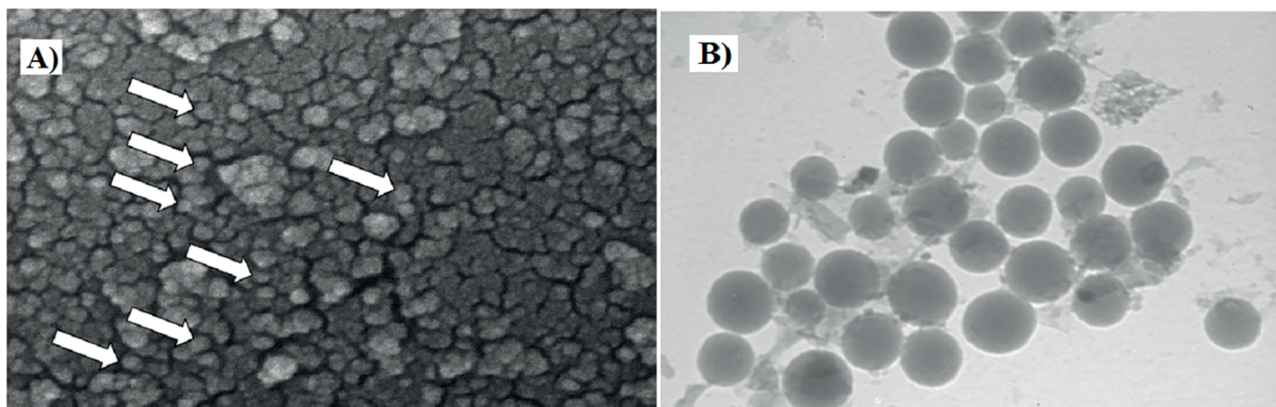


Figure 2

(A) SEM and (B) TEM micrographs displaying the morphology and size of the V_2O_5

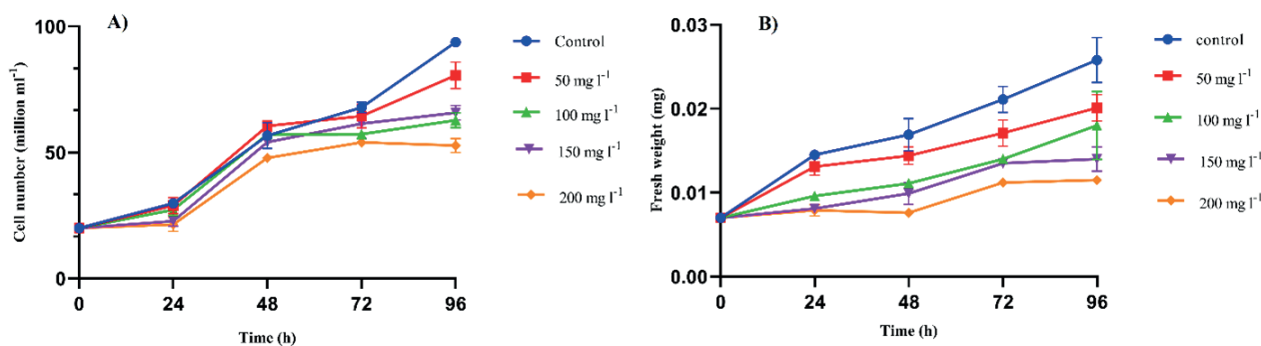


Figure 3

The concentration-dependent negative influences of V_2O_5 NPs on (A) cell number, and (B) fresh weight of *C. vulgaris*. The results are represented as mean averages \pm SE ($n = 3$)



3.3. The influence of V_2O_5 NPs on cell viability

We managed to explore the extent of cells' viability by flow cytometry. In this method, propidium iodide (PI) was used as a nucleic acid dye. PI can enter into the perished cells and attach to DNA molecules and produce red fluorescence. In this case, dead fluorescent cells could be distinguished from unstained live cells (Suman et al. 2015). In the graphs, the upper left and right parts expose the ratio of lifeless cells. The lower left and right quadrants specify the number of intact cells and the lower right quadrant signifies the chlorophyll emission (Fig. 4). Cell viability analysis was performed after the treatment of *C. vulgaris* to 200 mg l⁻¹ of V_2O_5 NPs. Nearly 99% of the untreated sample was alive after 96 h of incubation, and all the cells showed chlorophyll emission (Fig. 4A). By contrast, the percentage of intact live cells in the samples exposed to 200 mg l⁻¹ of V_2O_5 NPs has lessened to 91%. Furthermore, 7% of the cells didn't display chlorophyll emission (Fig. 4B).

3.4. Morphological changes of the cells influenced by V_2O_5 NPs

SEM analysis revealed morphological modification of algal cells after exposure to 100 and 200 mg l⁻¹ of V_2O_5 NPs as median and final applied concentrations, respectively. Untreated cells displayed the intact

turgescence structure in the SEM micrographs (Fig. 5A, B). In comparison, slight plasmolysis of treated cells after treatment with 100 mg l⁻¹ (Fig. 5C, D) and remarkable shrinkage and contraction of cells at 200 mg l⁻¹ V_2O_5 NPs (Fig. 5E, F) were observed.

3.5. The influence of V_2O_5 NPs on the photosynthetic pigments

For an exploration of the influence of diverse concentrations of V_2O_5 NPs on algal cells, their photosynthetic pigment content was assessed after 96-hour exposure to NPs. As a result of vanadium oxide treatment, the level of chlorophyll *a*, *b* and carotenoids significantly declined in a dose-dependent manner ($p \leq 0.05$) (Fig. 6).

3.6. Changes in the phenol and flavonoid content

In the current survey, the number of phenols and flavonoids rose in a similar outline at low concentrations of V_2O_5 NP up to 100 mg l⁻¹ and then lessened intriguingly with the rising concentration of NPs (Fig. 7A, B).

3.7. Response of the enzymatic antioxidant system

In this section, the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate

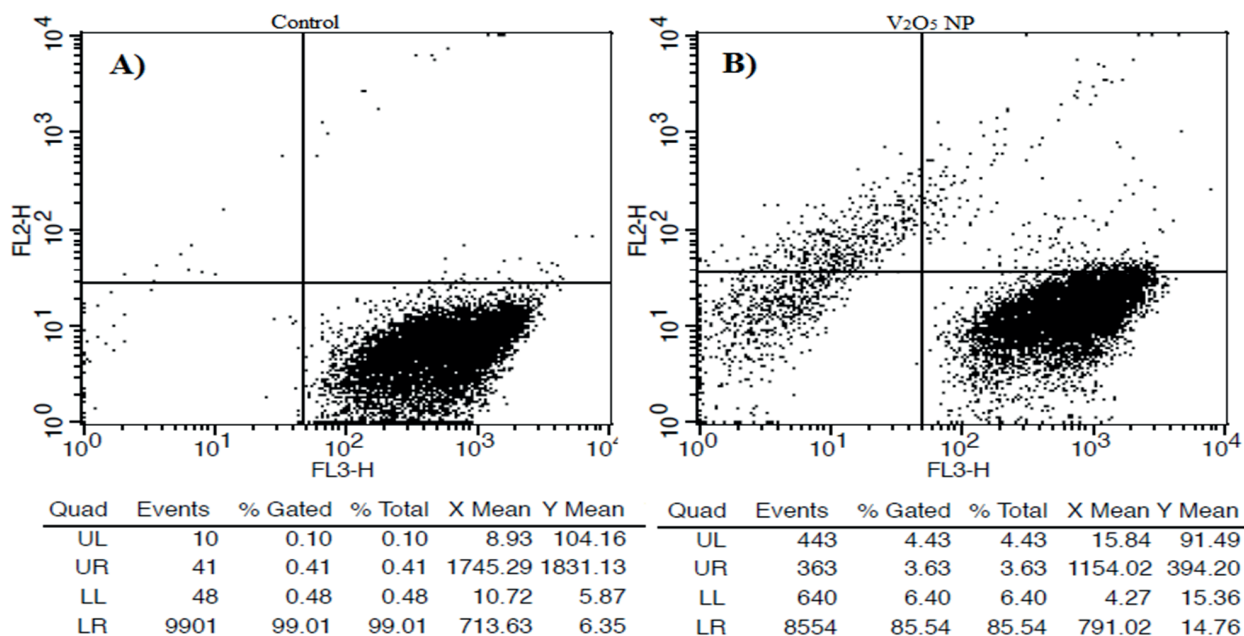
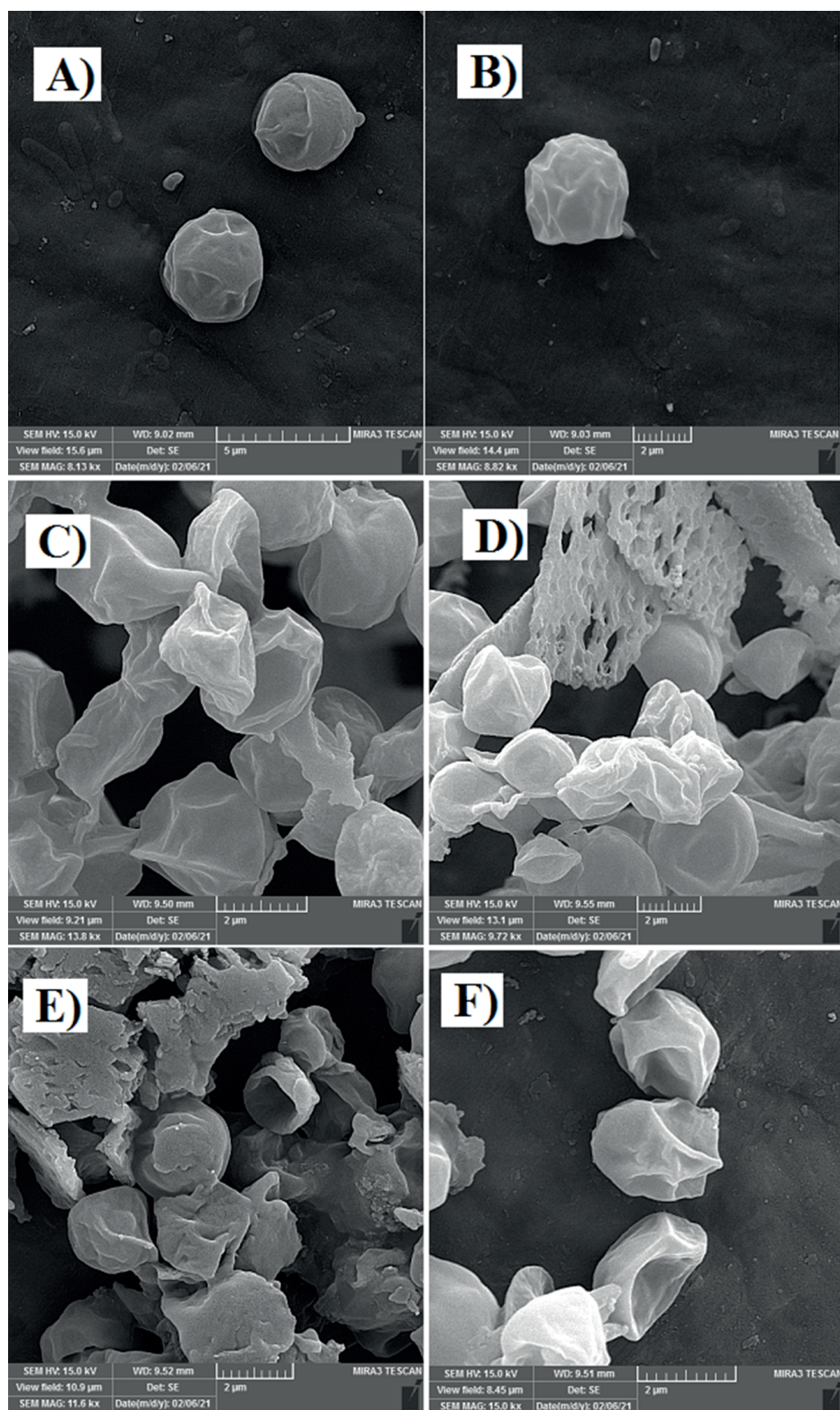


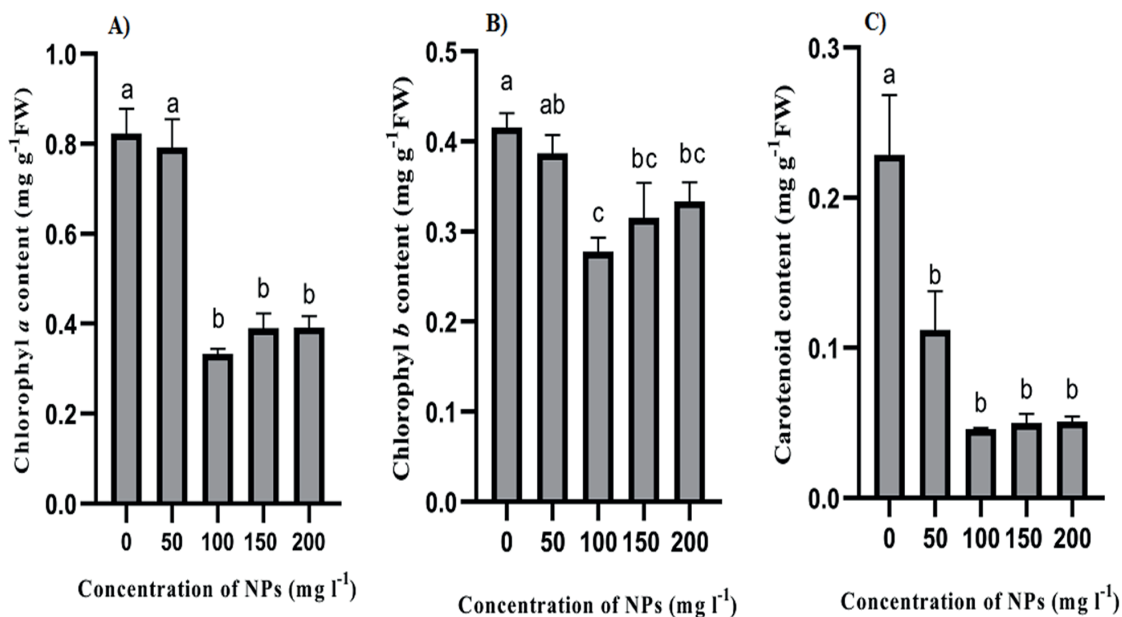
Figure 4

(A) Flow cytometry of untreated medium showed nearly 99% cell viability. (B) Application of 200 mg l⁻¹ V_2O_5 NPs for 96 h resulted in viability lessening of *C. vulgaris* to ~91%.

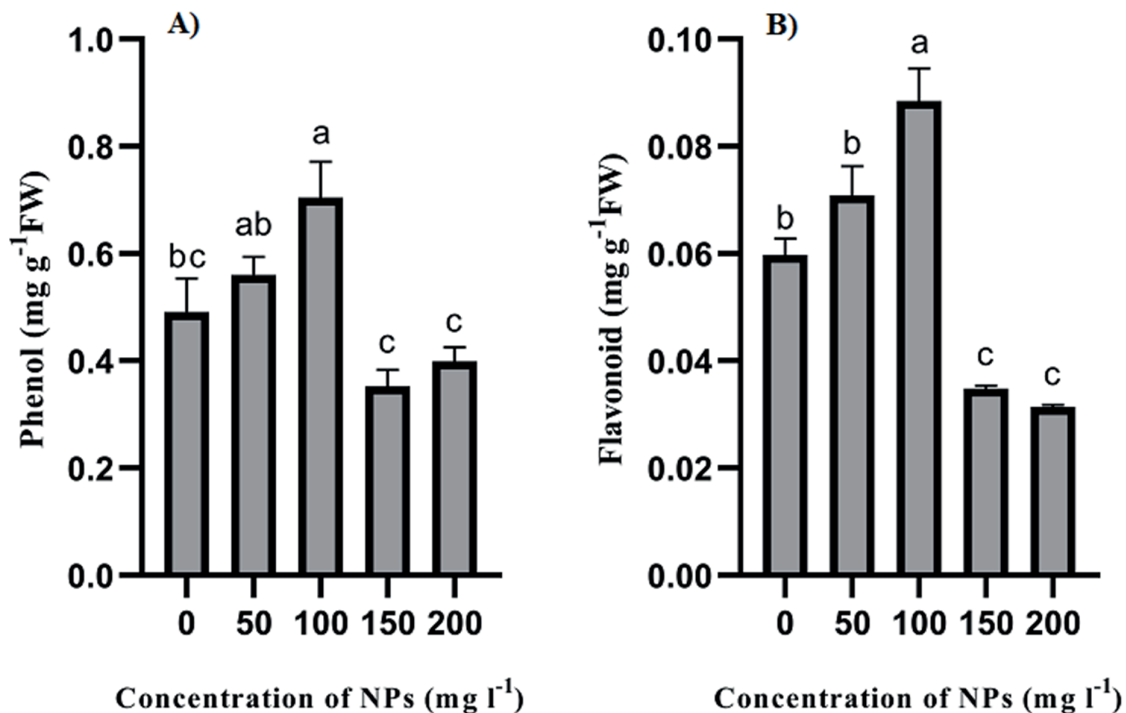
**Figure 5**

SEM micrographs of control and treated algal cells. (A, B) Intact turgescient globular control cells. (C, D) Slight plasmolysis of cells after treatment with 100 mg l⁻¹ of NPs and (E, F) notable shrinkage of cells after using 200 mg l⁻¹ V₂O₅ NPs



**Figure 6**

Effects of different concentrations of V_2O_5 NPs on photosynthetic pigment contents in *C. vulgaris* after 96 h of exposure. Different letters show significant differences according to Duncan's test at $p < 0.05$. The results are shown as mean averages \pm SE ($n = 3$).

**Figure 7**

Influence of different concentrations of V_2O_5 NPs on the (A) phenol and (B) flavonoid content of *C. vulgaris* after 96 h of exposure. Different letters indicate significant differences according to Duncan's test at $p < 0.05$. The results are presented as mean averages \pm SE ($n = 3$).

peroxidase (APX), as main antioxidant enzymes of cells, were evaluated after exposure of microalgae to different concentrations of V_2O_5 NP for 96 h. Based on our results, an increase in the activity of SOD occurred with the elevating concentration of nanoparticles up to 150 and 200 $mg\ l^{-1}$ (Fig. 8A). Although the CAT activity did not show significant change at 50 and 100 $mg\ l^{-1}$ NP, the enzyme activity rose at concentrations of 150 and 200 $mg\ l^{-1}$ of V_2O_5 NPs (Fig. 8B). The significantly elevated activity of APX was also detected at 150 and 200 $mg\ l^{-1}$ of NPs (Fig. 8C).

The restrictive influence of V_2O_5 NPs on the growth of alga in a concentration and time dependent manner was in agreement with some previous findings. The undesirable impact of different transition metal oxide NPs on the growth criteria of plants and algae has previously been reported (Aravantinou et al. 2020; Cardinale et al. 2012; Khan et al. 2021). The lessening in cell number and fresh weight of the algal cell after treatment with metal oxide NPs, such as V_2O_5 NPs, may occur due to their harm to the cell membrane (Ozkaleli et al. 2018; Xia et al. 2015), inhibiting the

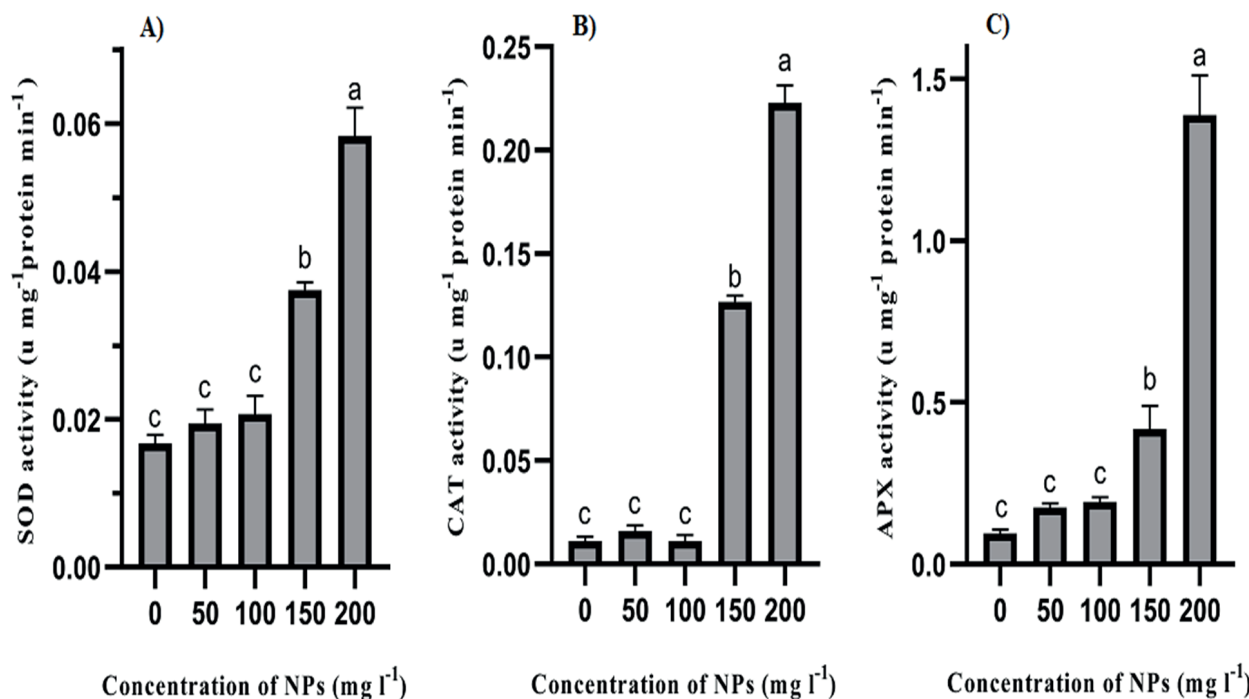


Figure 8

Activity of (A) SOD, (B) CAT and (C) APX in algal cells after 96 h exposure to V_2O_5 NPs. Different letters indicate significant differences according to Duncan's test at $p < 0.05$. The results are displayed as mean averages \pm SE ($n = 3$).

4. Discussion

The XRD, FT-IR and EDX examinations established the correct structure, purity, and phase composition of the synthesized V_2O_5 NPs. The acquired TEM and SEM micrographs revealed the appropriate shape and size of V_2O_5 NPs, which appeared to fit for the goals of the current investigation. The obtained results of DLS and zeta potential analysis confirmed the suitable size and surface charge of the synthesized NPs in solution that are necessary for their uptake from culture media by cells.

photosynthesis by adsorption of NPs on the algal cell wall and shading effect (Chen et al. 2018; Navarro et al. 2008) and intracellular damages by the formation of reactive oxygen species (ROS) caused by NPs (Movafeghi et al. 2019).

Flow cytometry outcome indicated that V_2O_5 NPs treatment has only a slight effect on *C. vulgaris* cells' death. This means that the cells of *C. vulgaris* have effective mechanisms to tolerate cytotoxicity of V_2O_5 NPs and stay alive. The defensive response and repair mechanisms of the algal cells may prevent durable oxidative damages of NPs to the cell wall, cell membrane, and organelles (Wang et al. 2019). This



observation revealed the need for extra morphological and biochemical investigations of treated cells for probable cellular strategies against toxicity.

SEM analysis illustrated a modification of the algal cells in NPs exposure. Similar deformation of cells after exposure to metal oxide NPs was formerly affirmed (Li et al. 2020; Movafeghi et al. 2019). The shapes and turgescence of cells are controlled by osmotic force and the function of cell membrane. The first intercourse of NPs with cells occurs in the cell membrane of the target cell. This interaction could cause disruption of membrane integrity and alteration in its hydrophobicity, cationic charge, and permeability (Rai & Biswas. 2018). Furthermore, the exposure of cells to NPs may result in the formation of ROS and resulting changes in metabolic reactions and interruption in the cell membrane function (Abdal et al. 2017; Wei et al. 2017). Subsequently, cell plasmolysis may occur, causing changes in cell morphology (Saxena et al. 2021).

Photosynthetic pigments are recognized as trustworthy indicators for algal cell toxicity (Liu et al. 2009). The cellular metabolic status of algae can be evaluated by determining the content of chlorophylls and carotenoids. Our findings designated the reduction of photosynthetic pigments content. Different studies indicated adverse impacts of metal-based nanoparticles on photosynthetic pigments in algae. Comparable to our finding, the number of photosynthetic pigments in *Nannochloropsis oculata* and *Chaetoceros muelleri* have diminished after exposure to CuO and TiO₂ NPs, respectively (Bameri et al. 2022; Fazelian et al. 2019). Lessening the chlorophyll and carotenoid level may be a consequence of the enhanced production of ROS by NPs, which might have successively oxidized the pigment molecules (Foyer 2018). Moreover, decreasing the content of photosynthetic pigments may possibly be one of the substantial factors for the declined growth of *C. vulgaris*. On the other hand, some NPs such as SiO₂ and Au NPs, increased the chlorophyll content in some algal species (Karunakaran. 2015; Li et al. 2020). NPs may bind to the cell surface and prevent light harvesting in algae, which is referred to the 'shading effect'. In this case, the photosynthetic organism starts to produce more pigments for improvement of the light harvest (Middepogu et al. 2018). These dissimilar outcomes may be due to the diverse features of NPs, dissimilar study species, and different experimental conditions (Barhoumi & Dewez 2013; Dağlıoğlu, & Yılmaz Öztürk 2018).

Exposure to nanoparticles may result in the formation of an excessive amount of ROS that damage cellular structures and inhibit biochemical

reactions (Yu et al. 2020). Phenols and flavonoids as non-enzymatic antioxidant molecules in plants and algae scavenge different free radicals or ROS due to their chemical structure (Alghazeer et al. 2013; Ferdous & Balia 2021; Kumar et al. 2008; Melato et al. 2012). Thus, alteration of their content could be considered as a non-enzymatic tolerance mechanism against NP toxicity and induced oxidative stress. Monitoring of phenol and flavonoid content exhibited a rise at a low concentration of NPs and then a drop at high amounts of it. These changes in the content of non-enzymatic defense metabolites in *C. vulgaris* were in agreement with the findings of preceding studies, confirming alteration in their content under oxidative stress (Çelekli et al. 2016; Fazelian et al. 2019; Janova et al. 2021). The upturn in the biosynthesis of phenols and flavonoids at low concentrations of V₂O₅ NP might be a defense reaction against the damaging impacts of the free radicals. However, by heightening the concentration of V₂O₅ NP to 150 and 200 mg l⁻¹ of V₂O₅ NP, the level of these phytochemicals has reduced. The diminished level of phenols and flavonoids at high concentrations of V₂O₅ NP could be related to the decrease in their biosynthesis as a result of NP toxicity. It seems that the non-enzymatic antioxidant system in *C. vulgaris* was not strong enough to overcome the forceful oxidative condition induced by high quantities of NPs.

Beside non-enzymatic antioxidants, cells have developed antioxidant enzymes as powerful ROS scavenging systems to balance the stress-induced ROS (Dvořák et al. 2021; Wang et al. 2019). Superoxide dismutase (SOD) is an essential antioxidant enzyme, which catalyzes superoxide breakdown into oxygen and H₂O₂, and therefore has a significant function in the tolerance of cells to oxidative stress. In this study, increased activity of the SOD was observed at high concentration of nanoparticles. The role of SOD in the defensive response of algal species to metal oxide nanoparticle toxicity has been previously reported. For instance, it was shown that titanium dioxide and zinc oxide NPs increase the SOD activity in *Chlorella ellipsoidea* and *C. vulgaris*, respectively (Matouke et al. 2018; Feizi et al. 2022). Furthermore, treatment with TiO₂ NPs raised the activity of SOD in *Spirodela polyrrhiza* (Movafeghi et al. 2018). Catalase (CAT) is another fundamental antioxidant enzyme, which causes the detoxification of the overproduced hydrogen peroxide to water and oxygen. Our study showed increasing activity of the CAT in high exposure to nanoparticles. Similarly, the heightened activity of CAT in the presence of SiO₂ and TiO₂ in *Scenedesmus obliquus* was reported (Liu et al. 2018). Moreover, up-regulation of CAT activity after ZnO

and Fe_2O_3 treatment was shown in *C. vulgaris* in a time dependent manner (Saxena et al. 2021). Thus, microalgae could enhance the activity of CAT as one of the crucial antioxidant enzymes in stress conditions induced by NPs. Ascorbate peroxidase (APX) is the most abundant antioxidant enzyme, which regulates the levels of ROS in different subcellular compartments. APX uses ascorbic acid as a particular electron donor and reduces H_2O_2 to water (Caverzan et al. 2012; Omoarelojie et al. 2021). We determined comparable enhancing tendency in the activity of APX with the activities of SOD and CAT after treatment of the cells with the used concentrations of V_2O_5 NPs. In agreement with our data, we witnessed improved activity of APX in *Anabaena* sp. and *Nannochloropsis oculata* treated with CuO NPs (Karimi et al. 2017b; Fazelian et al. 2019). Taken together, these results suggest that only at concentrations of 150 and 200 mg l^{-1} of V_2O_5 NPs the enzymatic antioxidant system was significantly induced as a cellular defensive response to the encouraged oxidative stress. The upsurge in activities of SOD, CAT and APX in *C. vulgaris* acts as a forceful protective mechanism against the intracellular accumulation of ROS.

5. Conclusion

This research was directed to explore the encouraged cellular toxicity of V_2O_5 NPs on *C. vulgaris*. The characterization of the produced nanoparticles exhibited their appropriate nano size for the entrance into the microalgae cells. Further assessments revealed the cytotoxicity of the synthesized NPs in a concentration and time-dependent trend. Enhancing concentrations of V_2O_5 NPs led to a decline in the growth and content of photosynthetic pigments of algal cells. Algal cells battled to manage the destructive impacts of V_2O_5 NPs by enzymatic and non-enzymatic antioxidant systems. The content of phenols and flavonoids as non-enzymatic defense molecules increased at low concentrations of V_2O_5 NPs and then decreased at higher doses of NPs. This means that the non-enzymatic defense system of *C. vulgaris* could not withstand the exceeded oxidative condition induced by high concentration of NPs. By contrast, treated cells with high concentrations of NPs showed elevated antioxidant enzyme activity in a similar outline. Further examination is required to survey the toxicity mechanisms of V_2O_5 NPs on microalgae and to explore the resistance mechanisms of the algal cells to handle the induced stress.

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