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Cobalt tungstate nanoparticles ($CoWO_4$ NPs) affect the photosynthetic performance of the green microalga *Raphidocelis subcapitata*

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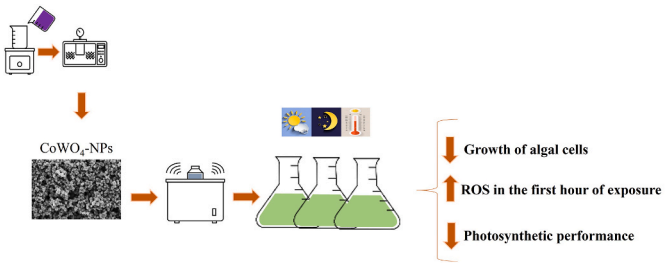
HIGHLIGHTS

- $CoWO_4$ inhibited the growth of algal cells from 42.37 mg L^{-1}.
- $CoWO_4$ induced ROS production in the first hour of exposure (1 h).
- Oxygen evolving complex (OEC) is one of the main targets of NPs.
- $CoWO_4$ affected the ability of the microalgae to tolerate high light intensities.

GRAPHICAL ABSTRACT



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ABSTRACT

Innovative applications of cobalt tungstate nanoparticles ($CoWO_4$ NPs) are directly linked to their increased production and consumption, which can consequently increase their release into aquatic ecosystems and the exposure of organisms. Microalgae are autotrophic organisms that contribute directly to global primary productivity and provide oxygen for maintaining many organisms on Earth. In this paper, we assessed the toxicity of $CoWO_4$ NPs when in contact with the freshwater microalga *Raphidocelis subcapitata* (Chlorophyceae). To this end, we assessed algal growth, reactive oxygen species (ROS) production and photosynthetic performance. Our results show that the NPs inhibited the growth of algal cells from 42.37 mg L^{-1}, significantly induced ROS production in the first hours of exposure (1 h) at all concentrations and directly compromised the photosynthetic activity, reinforcing that the oxygen-evolving complex (OEC) is one of the main targets of NPs and that the light curve parameters were the most sensitive endpoints. We observed reductions in the maximum relative electron

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transport rate (rETRmax) of around 53% and decreases in the saturating irradiance (Ek) of around 40%, which demonstrated that the NPs not only compromised the photosynthetic activity, but also decreased the ability of algal cells to tolerate high light intensities. Our results also highlight that Co was mostly particulate and that the dissolved fraction represented only ~8% at the lowest concentration and ~0.70% at the highest concentration of CoWO4 NPs, suggesting that the toxicity observed was caused by the NPs. $CoWO_4$ NPs exhibited a high negative surface charge of −33mV in L.C. Oligo medium. The polydispersity index (PdI) varied from 0.22 to 0.45, while the hydrodynamic size ranged from 217.8 ± 11.36 to 503.43 ± 32.78 nm, indicating greater aggregation in this medium. This study elucidates how the $CoWO_4$ NPs interact with *R. subcapitata*, resulting in changes mainly in its photosynthetic performance.

1. Introduction

Advances in the synthesis and manufacturing of nanoparticles (NPs) can bring social and economic benefits through their varied applications, particularly in medicine, electronics, energy, personal care, and water treatment, among others (McClellan-Green and Oberdo, 2007). Among the NPs, cobalt tungstate ($CoWO_4$-NPs) has been used in supercapacitors (Xing et al., 2015), batteries (Wang et al., 2008), solar cells and sensors (Ahmed et al., 2016; Subramanian et al., 2013; Cui et al., 2018), and catalysts (Han et al., 2018), as well as for the degradation of waste in polluted waters (Zhang et al., 2018). Since one of the applications of $CoWO_4$ NPs is precisely the decontamination of water bodies, aquatic organisms can be exposed to them and suffer toxic effects.

Considering that the freshwater ecosystem is an important target of contaminants, including NPs, it becomes essential to understand their effects on aquatic organisms for their safe use and the establishment of thresholds for NPs in order to protect aquatic ecosystems and mitigate their negative effects. Among the organisms present in freshwater ecosystem, the microalgae are fundamental for maintaining the balance of aquatic ecosystems since they are primary producers, i.e., the base of the trophic web (Nogueira et al., 2015). They convert inorganic carbon (CO_2) into biomass, consequently providing energy and organic matter for higher trophic levels, such as zooplankton and fish (Thoré et al., 2023). Indeed, these microorganisms are responsible for more than 50% of the Earth's primary productivity, being directly related to global oxygen production (Gigova and Marinova, 2016). In this work, we chose a species of unicellular green alga, *Raphidocelis subcapita*, to be used as a test organism. This species belongs to the class Chlorophyceae and are fast-growing and highly sensitive to different compounds, being widely used to identify the negative effects caused by nanoparticles and tungstate based materials (Sousa et al., 2018; Alho et al., 2020; Abreu et al., 2022; Gebara et al., 2024). Furthermore, regarding tungsten toxicity, Strigul et al. (2009) reported that a concentration of approximately 2.42 g L^{-1} was required to inhibit 75% of the growth of *Selenastrum capricornutum* (now *Raphidocelis subcapitata*). However, for plants, tungsten (W) exhibited adverse effects at the morphological, cytological, and gene expression levels (Adamakis et al., 2012).

Many factors influence the growth and metabolism of microalgae. An essential metal such as cobalt (Co) is required at low concentrations by cells to synthesize cyanocobalamin (vitamin B12). However, depending on the concentration, Co can cause harmful effects on microalgae, similar to those exerted by metallic nanoparticles, e.g., protein damage, oxidative DNA damage, and increased production of reactive oxygen species (ROS) (Novak et al., 2013). Moreover, as the concentration of Co increases, the synthesis of chlorophyll decreases, affecting the composition of biomolecules like lipids and negatively impacting their photosynthetic activity (El-Sheekh et al., 2003; Fathi et al., 2008; Reis et al., 2021, 2024; Reis et al., 2022; Rocha and Melão, 2024). Environmental concentrations of Co in water bodies vary. For example, in Brazil concentrations of Co range from 1.9 μg L^{-1} (Weber et al., 2013) to 250 μg L^{-1} (Dourado et al., 2017), exceeding the maximum limit of 200 μg L^{-1} recommended for surface waters (Brazil, 2005). On the other hand, in China concentrations range from less than 0.1 μg L^{-1} (Xiao et al., 2014). Furthermore, according to Masoud et al. (2005), concentrations of 4.41 × 10^{-7} M were recorded on Lake Edku, in Egypt and according to Aziz et al. (2012) 4.05 × 10^{-5} M Co on the Tanjero River, in Iraq.

To evaluate the negative effects of these NPs on microalgae, robust and sensitive techniques are used, such as flow cytometry, used to identify reactive oxygen species and algal growth (Sousa et al., 2018; Alho et al., 2020; Abreu et al., 2022; Gebara et al., 2024), and pulse-amplitude-modulation fluorescence (Phyto-PAM), adopted to evaluate parameters related to photosynthetic activity (Alho et al., 2019; Gebara et al., 2020, 2023; Moreira et al., 2020; Reis et al., 2022; Reis et al., 2024; Rocha and Melão, 2024; Abreu et al., 2022). Our study aimed to analyze the toxic effects of $CoWO_4$ NPs on a freshwater microalga, *R. subcapitata*, through the assessment of algal growth, intracellular ROS production, and photosynthetic activity. To the best of our knowledge, this is one of the first studies on the potential toxicity of $CoWO_4$ NPs and their toxic effects on freshwater microalgae. We also determined the concentration of dissolved Co (<0.45 μm) in all $CoWO_4$ treatments in order to understand whether the toxicity is caused by the NPs or the dissolved metal and we carried out the characterization of the nanoparticles at all the concentrations tested.

2. Materials and methods

2.1. Synthesis and characterization of $CoWO_4$ nanoparticles

Various synthesis methods can be applied for the synthesis of $CoWO_4$ nanoparticles, such as coprecipitation, solvothermal, hydrothermal, microwave, solid-state reaction, among others (Song et al., 2009; Rajagopal et al., 2010; Sagadevan et al., 2016; Kumar and Karuppuchamy, 2016; Thilagavathi et al., 2021). Among these, we highlight the coprecipitation method in an aqueous medium followed by microwave hydrothermal treatment due to its simplicity and cost, as well as using water as the synthesis solvent, which generates fewer toxic residues (Lima et al., 2022; Patrocínio et al., 2024). Furthermore, the successive processes of crystallization and redissolution allow for fine-tuning of the morphology and size of the obtained nanoparticles. Firstly, two solutions were prepared: the first containing 1x10^{-3} mol of $Co(NO_3)_2.6H_2O$ (Alfa-Aesar, 99.8%) in 50 mL of distilled water and the second containing 1x10^{-3} mol of $Na_2WO_4.2H_2O$ (Sigma Aldrich, 99.9%) in 50 mL of water. After the complete dissolution of reagents, the solution of $Co(NO_3)_2.6H_2O$ was added to $Na_2WO_4.2H_2O$, resulting in a purple precipitate. The suspension was then transferred to a sealed Teflon reactor and placed in a microwave hydrothermal system (2.45 GHz, maximum power of 800 W) for various periods of time, specifically for 32 min at 160 °C. Subsequently, the suspension was centrifuged, and the precipitate was washed once with water and dried at 60 °C for 12 h. Afterwards, the resulting powder underwent thermal treatment in a conventional muffle furnace at a ramp of 10 °C/min up to 500 °C for 2 h.

The $CoWO_4$ NPs were characterized by X-ray diffraction (XRD) using a D/Max-2500PC diffractometer (Rigaku) with Cu Kα radiation (λ = 1.5406 Å), field emission scanning electronic microscopy (FE-SEM), with the microscope operated at 10 kV (Supra 35-VP, Carl Zeiss), and Raman spectroscopy using a Horiba iHR550 with a red laser at a wavelength of 633 nm. The characterization analyses were carried out

over time in L.C. Oligo medium (AFNOR, 1980) and in ultrapure water. The hydrodynamic size, polydispersity index (PdI) and zeta potential by dynamic light scattering (DLS) were obtained using Zetasizer Nano ZS90, Malvern.

2.2. Cobalt determination and dissolved metal

First, we washed the cellulose acetate membrane filters (pore size of 0.45 μm, Sartorius, Germany) with HNO_3 1 mol L^{-1}, and then rinsed them thoroughly with ultrapure water. Subsequently, a volume of 46 mL of the $CoWO_4$ samples was filtered. The filtered volume was acidified with 1% HNO_3 and quantified to determine the dissolved Co (<0.45 μm). The filter was then digested with aqua regia at a ratio of 3 M HNO_3: 1 M HCl, according to Lombardi et al. (2002) to determine the concentrations of NPs and particulate metal. Co was quantified on an HR-CS MAS ContrAA model 300 high-resolution molecular absorption spectrometer with a continuous source (Analytik Jena, Jena, Germany), with limits of quantification and detection of 0.020 and 0.006 mg L^{-1}, respectively.

2.3. Algae culture and toxicity tests

The freshwater microalga *Raphidocelis subcapitata* (Chlorophyceae) was obtained from stock cultures of the Ecotoxicology Laboratory at the Federal University of São Carlos, SP, Brazil (Mansano et al., 2017). Stock cultures were kept in L.C. Oligo culture medium (AFNOR, 1980) (Table S1, Suplementary Material) at initial pH 7.0 and under controlled conditions of light intensity (4200 lux), photoperiod (12:12 h light: dark) and temperature (25 ± 1 °C, according to ABNT (2018)). The cultures were gently shaken (manual shaking) three times per day. In the toxicity tests, the $CoWO_4$ NPs were dispersed in ultrapure water using a bath sonicator (Ultra cleaner 1400 Unique) for 30 min. The algal cultures in the exponential growth phase were inoculated at a concentration of 1×10^5 cells mL^{-1} and exposed for 72 h to the following measured concentrations: control (i.e., no NPs added), 13.08, 22.62, 30.44, 42.37, and 65.51 mg $CoWO_4$ NPs L^{-1}, in 500 mL polycarbonate Erlenmeyers containing 250 mL of test solutions, in triplicate and the toxicity tests followed the same culture conditions.

2.4. Growth and ROS production

The algal growth and ROS determination were assessed daily, during 72h of exposure. The samples were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) with a 15 mW argon-ion laser (488 nm excitation) using 6-μm fluorescent beads as an internal standard (Fluoresbrite carboxylate microspheres; Polysciences, Warrington, Pennsylvania, USA). For cell counting we used 1.8 mL of the sample fixed with 1% formaldehyde, frozen and stored at −20 °C until analysis. The cell density of microalga were identified using the parameters FL3-H (red fluorescence) *versus* SSC-H (side scatter), as described by Sarmento et al. (2008). For ROS determination, we use 495 μL of each replicate *in vivo* and 10 μM of DCFH-DA (2′,7′-Dichlorofluorescein diacetate, Sigma Aldrich). Afterwards, the samples were kept in the dark for 60 min and immediately analyzed (Alho et al., 2019; Gebara et al., 2020, 2023, 2024). To identify the relative ROS, we used the parameters FL3-H *versus* FL1-H and it was calculated adopting the equations described by Hong et al. (2009).

2.5. Evaluation of photosynthetic performance by Phyto-PAM

To evaluate chlorophyll fluorescence, we used a pulse amplitude modulation fluorometer (Phyto-PAM, Walz, Germany). A total of 3 mL of algal culture, which was left in the dark for 15 min to allow the oxidation of PSII reaction centers, was sampled daily. From the minimum fluorescence (F_0), maximum fluorescence (F_M) and maximum quantum yield (ΦM) values provided by the equipment (Schreiber, 1986; Schreiber et al., 1986), it was possible to determine the efficiency of the oxygen evolving complex (OEC) following the equation: F_0/F_V, where $F_V = F_M - F_0$ (Kriedemann et al., 1985).

After 72 h, the samples were exposed to continuous actinic light, followed by the application of a saturation pulse every 20 s to obtain the light-adapted parameters. Using fluorescence (F_S) and the maximum fluorescence of light-adapted cells (F_M'), the effective efficiency of PSII was calculated by the equation: (F_M'- F_S)/F_M' (Baker, 2008; Cosgrove and Borowitzka, 2006; Genty et al., 1989). The equations in Table S1 were applied to establish the photochemical (qP) and non-photochemical (qN, NPQ, Y(NPQ) and Y(NO)) quenchings. Also after 72 h, as preconized by Rocha et al. (2021), rapid light saturation curves were obtained from the increase in the intensity of photosynthetically active radiation (PAR) up to 1780 μmol photons $m^{-2}s^{-1}$, with light pulses every 20 s. Then, the light saturation curve was adjusted with the relative electron transport rate (rETR; μmol electrons $m^{-2}s^{-1}$) and PAR data using the Jassby and Platt (1976) equation. This allowed us to determine the initial slope (α) and the maximum relative electron transport rate (rETRmax; μmol electrons $m^{-2}s^{-1}$) and calculate the saturating irradiance (Ek), given by: Ek = rETRmax/α; μmol photons $m^{-2}s^{-1}$ (Rocha et al., 2021).

2.6. Statistical analysis

To evaluate statistical differences between treatments, normal distributed data were analyzed with one-way ANOVA, followed by Dunnett's post-hoc multiple comparison test, while non-normal distribution values were investigated by Kruskal-Wallis test and multiple comparisons using Dunn's test. The statistical significance level was defined as $p < 0.05$.

3. Results and discussion

3.1. Characterization of nanoparticles and dissolved metal (<0.45 μm)

For the structural analysis of the synthesized $CoWO_4$ NPs, XRD measurements, Raman spectra, and FE-SEM micrographs were obtained (Fig. 1). Both XRD and Raman spectroscopy serve as complementary analyses, revealing structural parameters of long- and short-range orders, respectively. In this work, both analyses indicated that the synthesized material possesses a monoclinic structure belonging to the space group *P*2/*c*. Additionally, no diffraction peaks or Raman modes related to other materials were observed, confirming its high purity. The analysis of morphology and particle size revealed that the material is composed of irregular nanometric polyhedra, with an average particle size of 33.7 ± 8.7 nm.

Table S3 (A, B, C and D) summarizes the results of the characterization of $CoWO_4$ NPs in culture medium and ultrapure water, over time. At the beginning of the experiment the zeta potential in L.C. Oligo medium was −33 mV. The polydispersity index (PdI) varied from 0.22 to 0.45, demonstrating that the NPs were homogeneously dispersed at all tested concentrations. This is in agreement with Danaei et al. (2018), who proposed that PdI corresponds to the particle size distribution, and Lemarchand et al. (2003), who stated that values around 0.3 indicate a homogeneous size distribution of particles in the solution. The hydrodynamic size of the samples ranged from 217.8 ± 11.36 to 503.43 ± 32.78 nm, revealing a greater degree of NP aggregation in L.C. Oligo medium. The quantification data are listed in Table S4. Regarding the dissolved metal, there was a tendency towards a more significant release of Co at low concentrations of NPs (8.79% of dissolved cobalt at the lowest concentration of $CoWO_4$ NPs (13.08 mg L^{-1}) against 0.70% of the dissolved fraction at the highest concentration (65.51 mg L^{-1}). Thus, it can be inferred that the largest amount of Co was in the form of particulate metal, which suggests that the toxicity observed was caused by the presence of $CoWO_4$ NPs, mainly at high concentrations.

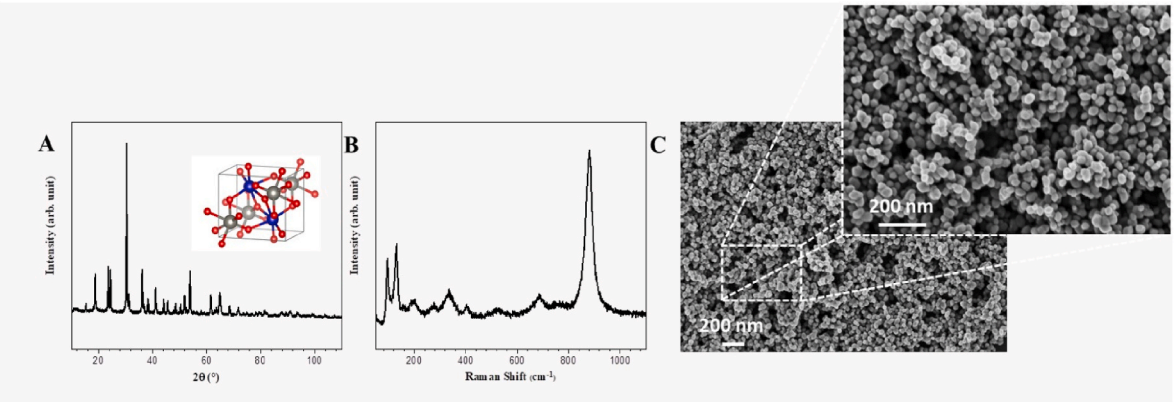

Fig. 1. XRD (A), Raman spectrum (B) and FE-SEM of $CoWO_4$ NPs (C).

3.2. Growth and ROS production

$CoWO_4$ NPs at 13.08 mg L^{-1} favored by ~12% (Dunnett's test; $p < 0.05$) the growth of algal cells after 24 h of exposure (Fig. 2A). After 48 and 72 h, negative effects on cell growth were observed at concentrations of 42.37 (decreased ~ 19%) and 65.510 mg L^{-1} (decreased ~ 30%) (Dunnett's test; $p < 0.05$).

As far as we know, there is only one study assessing the toxicity of $CoWO_4$ NPs using the microalgae species *Dunaliella salina* from saline environments (Hassanpour et al., 2021). In the referred study, the authors found that at concentrations of 15 and 30 mg L^{-1} there was an increase in cell growth, while at high concentrations it was inhibited (Hassanpour et al., 2021). This corroborates our results, which pointed a slight increase in cell growth at the lowest concentration (13.08 mg L^{-1}) and growth inhibition at high concentrations of $CoWO_4$ NPs (42.37 and 65.51 mg L^{-1}). This finding highlights the importance of using freshwater species to identify the negative effects and possible damage of metal NPs to the ecosystem.

Many factors can be considered responsible for causing negative effects on cell growth, such as the release of metal from the NPs (Navarro et al., 2008; Aravantinou et al., 2015; Suman et al., 2015), intracellular ROS (Li et al., 2015; Sendra et al., 2017; Lekamge et al., 2019) and even the light shading effect (Sadiq et al., 2011; Hassanpour et al., 2021). As noted, $CoWO_4$ NPs release Co in the dissolved fraction (<0.45), and at high concentrations they can affect luminosity. An important and curious point is that at the lowest concentration of $CoWO_4$ NPs (13.08 mg L^{-1}), cell growth increased, with a proportion of dissolved fraction of 8.79% – a value higher than that (around 0.70%) found at the highest concentration (65.51 mg L^{-1}) (Table S3).

With respect to growth inhibition, the main factor was intracellular ROS production by algal cells. According to Fig. 2B, in the initial hours of exposure the amount of intracellular ROS increased significantly (Dunnett's test $p < 0.05$) in all treatments, with a more pronounced increase at 65.51 mg L^{-1}. High levels of ROS can affect biological molecules, causing oxidative stress and cellular damage (Halliwell and Gutteridge, 1999), damage to proteins, lipids and nucleic acids (Okamoto et al., 2003), and even drastic effects, such as cell disruption and death (Von Moos and Slaveykova, 2014; Vale et al., 2016). Specifically for autotrophic organisms, Pinto et al. (2003) highlight that ROS production can be harmful since one source of the $O_2^{\cdot-}$ radical is the reduction of a single electron from molecular oxygen by the electron transport chain. Furthermore, organelles such as chloroplasts and mitochondria may also be susceptible to oxidative damage. According to our results, we believe that the production of intracellular ROS in the first hours of exposure to NPs is directly related to the impacts of photosynthetic activity, discussed in detail in the topic "*Photosynthetic performance*".

After 24 h, the amount of ROS was significantly reduced in all $CoWO_4$ treatments (around 58% at 13.08; 61% at 22.62; 72% at 30.44; 49% at 42.37 and 53% at 65.510 mg L^{-1}), and after 48 h there were decreases (Dunn's test; $p < 0.05$) of around 65% at 22.62 and 42.37 mg L^{-1}. At the end of exposure, significant reductions were observed at 22.62, 30.44 and 42.37 mg L^{-1}. Therefore, we hypothesize that the algal cells possibly activated their antioxidant mechanisms due to the stress caused by the $CoWO_4$ NPs, with values lower than the control. It is established that when faced with excessive ROS production, algal cells

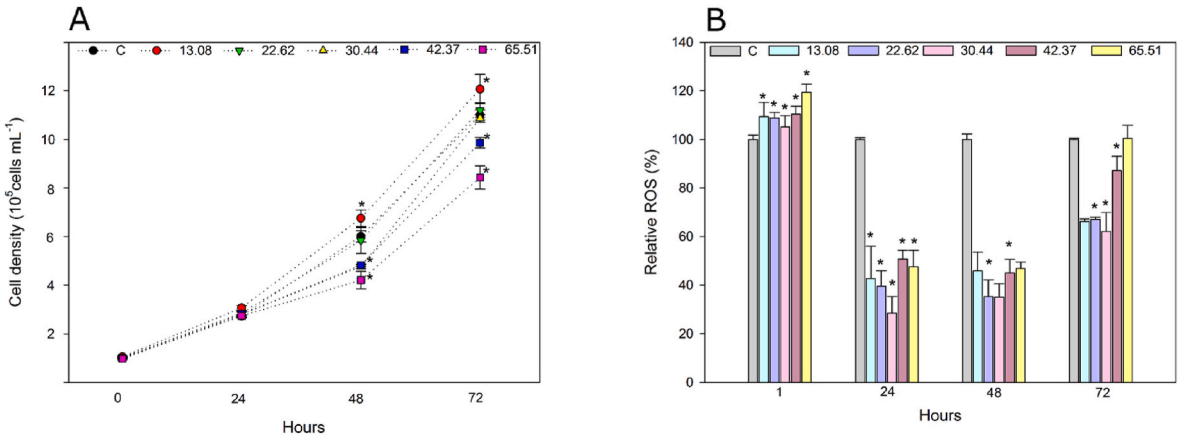

Fig. 2. Cell growth (A) and relative ROS (B) of *Raphidocelis subcapitata* exposed to $CoWO_4$ NPs for 72 h. C represents the control group, the symbol * means a significant difference ($p < 0.05$) when compared to the control group and concentrations are expressed in mg L^{-1}. Separate statistical analyses were performed for each day of exposure.

activate their antioxidant mechanisms to detoxify reactive oxygen species, thus maintaining their physiology (Wang et al., 2008; Qian et al., 2016; Lekamge et al., 2019; Xu et al., 2022; Hamed et al., 2024). This may explain the reduced relative ROS production at some concentrations after 24, 48 and 72 h of exposure, which is corroborated by our cell growth results, where complete growth inhibition was not observed.

3.3. Photosynthetic performance

According to Juneau et al. (2005), the parameters obtained in Phyto-PAM are rapid and relatively simple methods that can indicate the physiological health in autotrophic organisms, being reliable and important to determine possible stresses (Juneau and Popovic, 2000; Rocha et al., 2021). In this present study, slight negative effects on maximum quantum yield were observed daily (Fig. 3A) at the highest NP concentrations. In general, reductions in these values remained at around 6% (Dunn's test). After 24 h of exposure, there was a subtle decrease of 4.34% at 42.37 mg L^{-1} and 5.79% at 65.51 mg L^{-1} (Dunn's test). Likewise, reductions were also identified after 48 h (5.88% at 42.37 mg L^{-1} and 7.35% at 65.51 mg L^{-1}) and 72 h of exposure (around 6%; Dunn's test). This decreased maximum yield indicates that the algal cells exposed to $CoWO_4$ NPs had a slightly reduced PSII ability to carry out primary photochemical reactions (Dewes and Oukarroum, 2012). This parameter is associated with the amount of light used in photosynthesis and an indicator of the physiological state of microalgae (Herlory et al., 2013).

Regarding the F_0/F_v shown in Fig. 3B, after 24 h there was an increase of 15 and 17% at 42.37 and 65.51 mg L^{-1} (Dunnett's test; $p < 0.05$), respectively. After 48 h, this parameter increased at all concentrations, especially higher ones, with increases of approximately 21 and 26% at 42.37 and 65.51 mg L^{-1} (Dunnett's test $p < 0.05$), respectively. At the end of exposure, from 22.62 mg L^{-1} the F_0/F_v remained higher than the control. F_0/F_v indicates the efficiency of the OEC of PSII (Kalaji et al., 2011), where the water molecule breaks down in the presence of light producing oxygen (Mattoo et al., 1999). An increase in F_0/F_v suggests damage to the water separation apparatus, which appears to be a target for $CoWO_4$ NPs. This is not surprising if we consider the excellent photocatlytic property of the composite and the ROS produced by algal cells, especially in the first hours of exposure. Moreover, it is established that OEC is an important target and can be compromised by metals (Herlory et al., 2013), mainly bivalent ones, e.g., zinc, cadmium, copper, chromium, nickel and cobalt (Mallick and Mohn (2003). This is because the OEC is composed of proteins and manganese atoms, which can be replaced by these metals, causing the OEC to become inefficient (Herlory et al., 2013; Juneau et al., 2002). Our results are similar to those reported by Reis et al. (2021), who exposed *Raphidocelis*

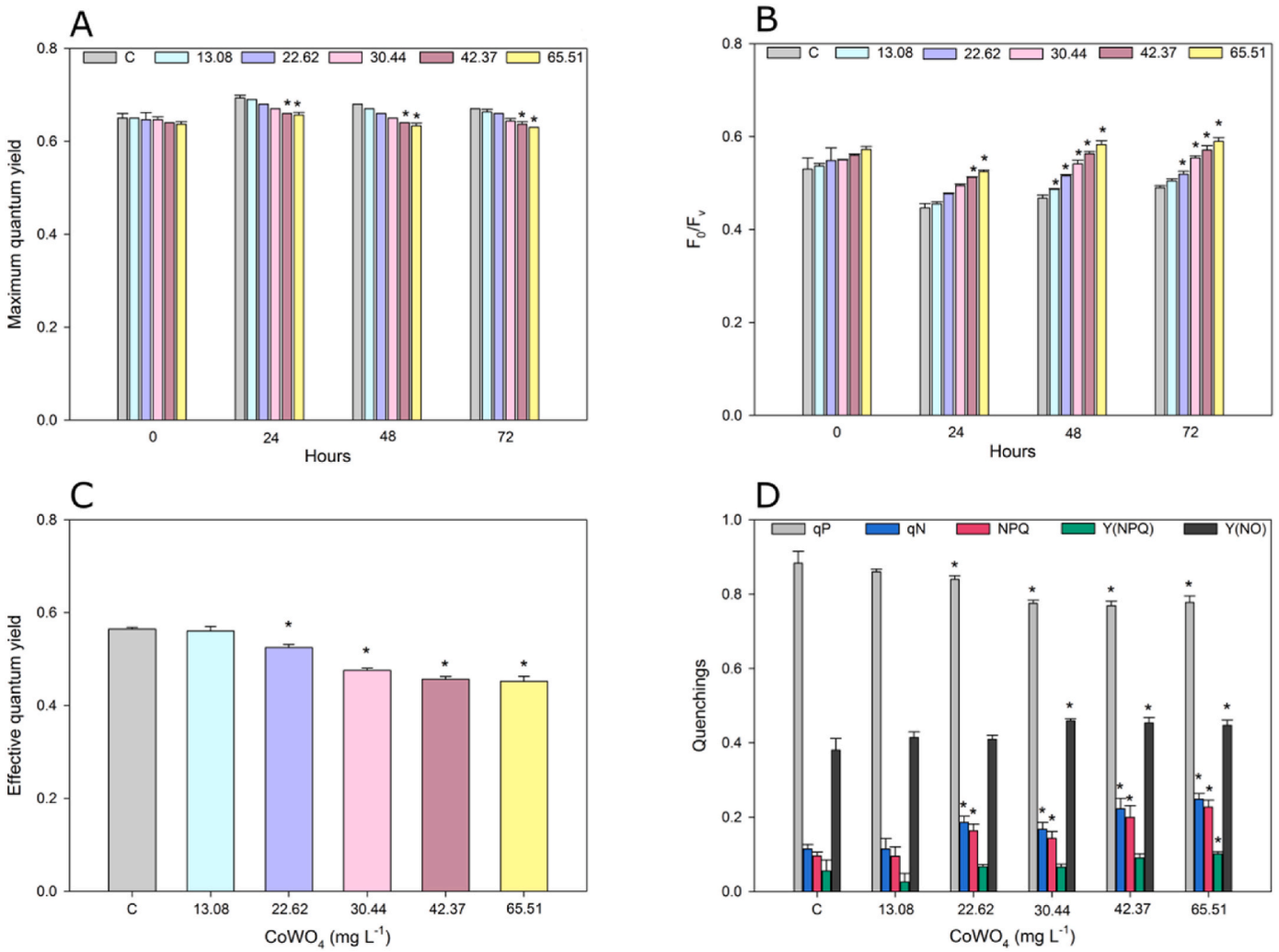

Fig. 3. Maximum quantum yield (A); measurement of the efficiency of oxygen evolving complex (F_0/F_V) (B); effective quantum yield (C); and photochemical (qP) and non-photochemical (qN, NPQ, Y(NPQ) and Y(NO)) quenchings (D) of *Raphidocelis subcapitata* exposed to $CoWO_4$ NPs for 72 h. C = control group, the symbol * means a significant difference ($p < 0.05$) when compared to the control group and concentrations are expressed in mg L^{-1}. Separate statistical analyses were performed for each day of exposure.

subcapitata to Co and found an increased F_0/F_v. As previously mentioned, in this work Co was released into the medium in which the algal cells were exposed, which could explain their toxicity. Therefore, the water splitting apparatus was one of the targets of the $CoWO_4$ NPs, and the small decrease in the maximum quantum yield was probably a consequence of the affected OEC.

The effective quantum yield decreased (Dunnett's test; $p < 0.05$) in the presence of $CoWO_4$ NPs from 22.62 mg L^{-1} (Fig. 3C). At 65.51 mg L^{-1} (highest concentration), this value decreased dramatically by around 19.64%. With respect to quenchings, the presence of $CoWO_4$ significantly reduced (Dunnett's test; $p < 0.05$) the qP (photochemical quenching) from 22.62 mg L^{-1}. The opposite occurred with the qN and NPQ (non-photochemical quenchings), which showed higher values than the control from 22.62 mg L^{-1}. The Y (NO) increased from 30.44 mg L^{-1}, whereas the Y (NPQ) only increased from 65.51 mg L^{-1}. Based on these parameters, we can infer that the algal cells activated the photoprotection mechanisms and that these mechanisms were not damaged, which is corroborated by the increase in qN, NPQ and Y (NPQ). Higher Y (NPQ) values indicate high photoprotective capacity (Sheng et al., 2017), and according to Kromkamp et al. (2008), the increase in Y (NPQ) shows the activation of PSII photoprotective mechanism at higher light intensities. On the other hand, there was an increase in the dissipation of unregulated energy in the form of heat and fluorescence (increase in Y(NO)) (Klughammer and Schreiber, 2008), in addition to the closure of some reaction centers, as indicated by the reduction in qP. Reduced qP implies that the energy used for photochemistry is related to the redox state of QA, suggesting that the PSII is not functioning properly (Baracho et al., 2019). From 22.62 mg L^{-1} of NPs, it was possible to observe that the functioning of PSII was affected. As reported by Rocha et al. (2021), qP corresponds to the proportion of open PSII reaction centers; therefore, its decrease indicates the closure of some reaction centers, which in turn can compromise carbon assimilation (Krause and Jahns, 2003; Rocha and Espíndola, 2021). In summary, photoprotective mechanisms were activated, evidencing protection against photodamage rather than photoinhibition.

Fig. 4 shows the rapid light curve parameters, which were the most sensitive photosynthetic ones. As it can be noted, $CoWO_4$ decreased (Dunnettt's test; $p < 0.05$) the $rETR_{max}$ (Fig. 4A) and Ek (Fig. 4B) from 13.08 mg L^{-1}. When compared to the control, the reduction in $rETR_{max}$ indicates inhibition of the electron transport rate from the lowest concentration of $CoWO_4$ NPs, while at the highest concentrations, the reductions were around 53%. Previous studies have shown that this effect is commonly reported for different species of autotrophic organisms exposed to metallic nanoparticles, as reported by Barreto et al. (2021) when exposing *Ankistrodesmus densus* to copper NPs (Barreto et al., 2021), *C. reinhardtii* to chromium NPs (Costa et al., 2016), *Scenedesmus obliquus* to iron NPs, and *Spirodela polyrhiza* to silver NPs (Sheng et al., 2017). Regarding the Ek, it was reduced by 43 and 41% at 42.37 and 65.51 mg L^{-1}, respectively. Another parameter that decreased was the alpha (Dunnettt's test; $p < 0.05$) from 22.62 mg L^{-1} (Fig. 4C). According to Ralph and Gademann (2005), this parameter reveals the efficiency of light use by cells. Therefore, the light capture efficiency was compromised from this concentration, which is in congruence with the impairment of PSII also at concentrations above 22.62 mg L^{-1}. Furthermore, the light curve parameters demonstrate that the algal cells had practically the same response to NPs, even at very different concentrations (30.44, 42.37 and 65.51 mg L^{-1}).

We emphasize that the light curve parameters are very sensitive for assessing the toxicity of metallic NPs, being the endpoint that showed the greatest decline when compared to the control and that confirmed the stress imposed by the $CoWO_4$ NPs on the algal cells, which not only compromised their photosynthetic activity, but also reduced their ability to tolerate high light intensities.

In summary, some of our results related to photosynthetic performance indicated slight decreases. We highlight that the biological responses of algal cells exposed to contaminants can be rapid, even in cases that are slightly impacted (McCormick and Cairns, 1994). According to McCormick and Cairns (1994), algae are capable of signaling the likelihood of more severe damage to the ecosystem, as they are considered environmental indicators, responding promptly to changes.

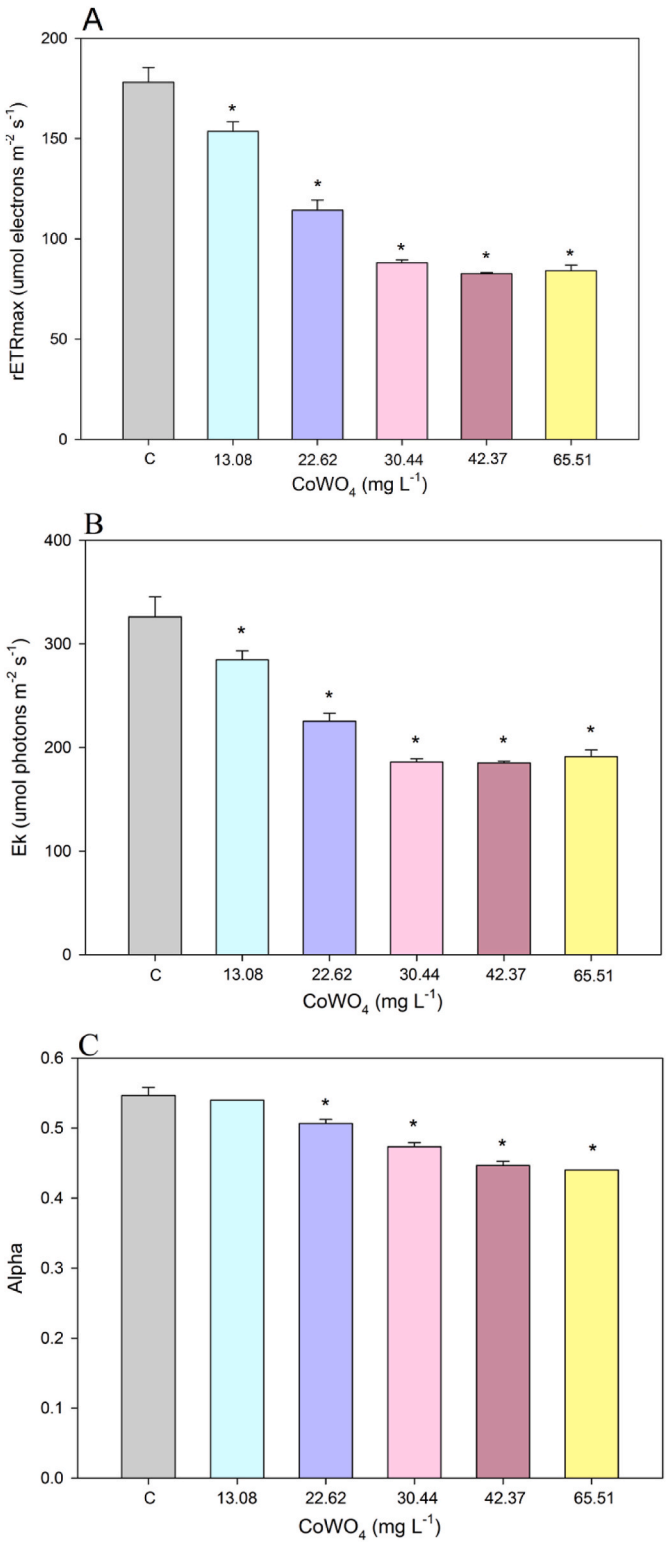

Fig. 4. Light curve parameters of *R. subcapitata* exposed to $CoWO_4$ NPs. Electron transport rate - rETRmax (μmol electrons m^{-2} s^{-1}) (A), saturation irradiance – Ek (μmol electrons m^{-2} s^{-1}) (B) and alpha (C). C represents the control group, the symbol * means a significant difference ($p < 0.05$) when compared to the control group and concentrations are expressed in mg L^{-1}.

Consequently, they can predict impacts on organisms at higher trophic levels, which may take some time to respond to environmental disturbances. Furthermore, these authors point out that photosynthetic performance corresponds to one of the components of ecosystem functioning, and the measurement of *in vivo* fluorescence corresponds to an indicator of this component. Nalewajko and Olaveson (2018) also highlight that changes in photosynthetic responses can be a reliable indicator of toxicity. Therefore, even though some of our results contain slight decreases in photosynthetic performance under $CoWO_4$ NPs exposure, there is biological and ecological relevance.

4. Conclusion

We assessed for the first time the toxicity of $CoWO_4$ NPs when in contact with the freshwater microalga *Raphidocelis subcapitata*. The exposure to CoWO4 NPs significantly decreased the cell density of *R. subcapitata* from 42.37 mg L^{-1} and induced ROS production in the first hours of exposure. The analysis of photosynthetic parameters allowed the identification of the main targets of $CoWO_4$ on the species evaluated, especially the $rETR_{max}$, showing drastic reductions of ~53% in the electron transport rate at the highest concentrations of NPs. These results were strongly corroborated by the loss of efficiency of the oxygen-evolving complex, which showed significant increases in F_0/F_v. The identification of the main targets of $CoWO_4$ NPs on primary producers, together with the determination of ROS production by algal cells and the quantification of the metal dissolved in the solution, is crucial to understand the mechanisms of action of this semiconductor on Chlorophyceae. Especially considering that microalgae are directly related to the cycling of matter and energy in the aquatic ecosystem, alterations to this trophic level can result in alterations to other trophic levels.

CRediT authorship contribution statement

Cínthia Bruno de Abreu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Renan Castelhano Gebara:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Giseli Swerts Rocha:** Writing – review & editing, Methodology, Formal analysis. **Adrislaine da Silva Mansano:** Writing – review & editing, Methodology, Formal analysis. **Marcelo Assis:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Thalles Maranesi Pereira:** Writing – review & editing, Investigation, Formal analysis. **Luciano Sindra Virtuoso:** Writing – review & editing, Investigation, Formal analysis. **Ailton José Moreira:** Writing – review & editing, Investigation, Formal analysis. **Patrícia Franklin Mayrink Nogueira:** Writing – review & editing, Investigation, Formal analysis. **Valtencir Zucolotto:** Writing – review & editing, Investigation, Formal analysis. **Maria da Graça Gama Melão:** Writing – review & editing, Resources, Methodology, Conceptualization. **Elson Longo:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2025.144085.

Data availability

Data will be made available on request.

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