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SUB-LETHAL TITANIUM DIOXIDE NANOPARTICLES (TiO_2 NPs) INDUCES DISRUPTION OF CHLOROPHYLLS AND SELECTED ANTIOXIDANTS ACTIVITIES ON *CHLOROIDIUM ELLIPSOIDEUM* (GERNECK)

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Abstract

The increasing production of manufactured product containing TiO_2 NPs is a global threat that raises concern for freshwater biodiversity and human health. The objective of this study was to examine the chronic effects of TiO_2 NPs on the biomass (chlorophyll *a*, *b*) and antioxidant activities (catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GRx) and malondialdehyde (MDA) of *C. ellipsoideum* exposed to sublethal doses of TiO_2 NPs. The initial, exposure of *C. ellipsoideum* to acute doses of TiO_2 NPs demonstrated toxicological response with EC_{50} 69.90 mg L^{-1}. However, the effects of sub-lethal concentrations on the microalgae showed significant reduction ($p < 0.05$) of Chlorophyll a and b with the increase of sub-lethal concentrations of TiO_2 NPs. The percentages of increment on selected markers of oxidative stress in this study increased compared to the control; Catalase (18.30–58.66%), SOD (2.68–16.85%), GRx (26.50–86.67%) and MDA (40.00–106.00%). This study suggests that sub-lethal exogenous concentrations are disruptive to the physiology of *C. ellipsoideum*. Therefore, care should be taken when handling and disposing of manufactured products containing TiO_2 NPs. This study is useful for understanding the potential harmful effects of TiO_2 NPs bioaccumulated in aquatic ecosystems. Further studies are recommended on other commonly used nanomaterials and their physiological influence on microalgae.

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Introduction

Engineered nanoparticles (ENPs) are increasingly found in manufactured agricultural, pharmaceutical, cosmetics, sunscreens, paints, drugs, medical, recreational industrial and house hold products (CONTADO 2015, KESSLER 2011). The presence of nanomaterials in a large number of manufactured products likely leads to their increasing release into the environment. Therefore nanomaterials entering the aquatic environments through wastewater and effluents have certainly increased in bottoms and sediments of freshwater ecosystems (WU et al. 2015). The presence and impact of large amount of nanomaterials in aquatic ecosystems have become a great concern for the welfare of aquatic biodiversity, more especially the primary consumers inhabiting freshwater ecosystems.

Approximately five million tons of titanium dioxide were consumed in 2009; 1.5 million tons are produced annually in the European Union and it is expected to continue to increase further globally (LANDSIEDEL et al. 2010, ORTLIEB 2010). Titanium dioxide (TiO_2) has become part of our everyday lives. It is found in various consumer goods and products of daily use such as cosmetics, paints, dyes and varnishes, textiles, paper and plastics, food and drugs (WEIR et al. 2012).

Nanoparticles have wide applications in various fields due to their small size (LAURENT et al. 2010). TiO_2 NPs are bright with high refractive index ($n=2.4$) which makes them suitable for industry dealing with toothpaste, pharmaceuticals, coatings, papers, inks, plastics, food products, cosmetics and textile (WEIR et al. 2012). Three crystalline phases of titanium dioxide, are anatase (tetragonal), rutile (tetragonal), and brookite (orthorhombic) in which brookite has no commercial value (PAOLA et al. 2013).

TiO_2 NPs has a potential role in photocatalytic degradation of pollutant in water, and also their catalytic activity could be attributed to a larger area per unit mass (CHEN and MAO 2007). The TiO_2 NPs have wide applications, viz., reducing toxicity of dyes and pharmaceutical drugs; waste water treatment; reproduction of silkworm; space applications; food industries; etc., and so have immense industrial importance. Due to their self-cleaning and antifogging property, they are used in the preparation of cloths, windows, tiles and anti-fogging car mirrors (EPIFANI et al. 2008).

The careless handling of TiO_2 NPs result in their unintended release into industrial and non-industrial waste streams discharged inadvertently into natural aquatic environments and becoming a menace for aquatic biodiversity including human (OZAKI 2013).

The presence of TiO_2 nanoparticles into the environment affects phytoplankton and coastal ecosystems that support fishing and recreational

activities (*Titanium dioxide*… 2012). Algae are primarily used as biological marker in aquatic system to study biological toxicity of pollutants. TiO_2 nanoparticles inhibit algae by causing membrane structure deformation due to increased lipid peroxidation (OZKALELI and ERDEM 2018). TiO_2 NPs exhibit more cellular toxicity in anatase due to increased amount of intracellular reactive oxygen species (ROS). Growth rate of freshwater green algae predominant in North America viz., *Scenedesmus quadricauda*, *Chlamydomonas moewusii* and *Chlorella vulgaris*, was found to be inhibited due to the presence of TiO_2 NPs in freshwater microcosms (CARDINALE et al. 2012). Combination of anatase and rutile showed more toxicity and antagonistic effect on freshwater algae Chlorella (MUKHERJEE et al. 2015). Elsewhere, some authors demonstrated the adverse effects of TiO_2 NPs on the growth rate, biomass and antioxidant of some Chlorella species (MATOUKE et al. 2018, MUKHERJEE et al. 2015, KULACKI and CARDINALE 2012). In Natural aquatic ecosystems TiO_2 nanoparticulate are toxic and disruptive with negative impact on aquatic ecosystems (WU et al. 2015). This nanomaterial has been reported in United Kingdom (UK) rural, agricultural and urban industrial rivers with an average concentration of 2.10 $\mu g\ L^{-1}$ (NEAL et al. 2011). In freshwater surface and sediments from Xiamen Bay in China with a concentration of 2.74 $g\ kg^{-1}$ (LUO et al. 2011).

In natural environment TiO_2 NPs may interact with organisms, natural organic matter and other naturally occurring geogenic and biogenic colloids because of its peculiar properties such as size, shape (ADELEYE and KELLER 2016). However, with the increasing production of TiO_2 NPs it is expected that many natural aquatic ecosystems may be polluted through water drainages into sewages, lakes, ponds, rivers, estuaries and the fate of aquatic organisms compromised.

Despite the suspected galloping growth of TiO_2 NPs in our environment and coupled with the fact that it could be harmful to freshwater aquatic biodiversity; studies on the impact of TiO_2 NPs on primary producers in freshwater ecosystems are sparse. Moreover, in many countries where TiO_2 NPs is highly consumed, there are scanty legislative measures for the monitoring of this metallic compound in aquatic environment. Therefore, the study of the impact of TiO_2 NPs on primary producer (microalgae) is of great concern for aquatic food web because it provides oxygen and nutrient for consumers (MUKHERJEE et al. 2015, BAJGUZ 2012).

Some studies on microalgae exposed to TiO_2 NPs showed a wide range of toxicological effects on cells growth, but focused only on the acute effects (OZKALELI and ERDEM 2018, KULACKI and CARDINALE 2012). However, studies on the sub-lethal effects of TiO_2 NPs on growth rate, photosynthe-

sis are still limited (WANG et al. 2014, MUKHERJEE et al. 2011). In this study, we attempted to assess the impact of sub-lethal treatments of TiO_2 NPs on microalgae and additionally focus on the response of anti-oxidative stress commonly used as bioindicators of pollution. More knowledge on the impact of TiO_2 NPs on antioxidants is needed to assess the stress factor of TiO_2 NPs on freshwater organisms but also, to enhance awareness, safety and management. *Chloroidium ellipsoideum* was chosen because it is a primary producer in freshwater and it is very important for the survival and equilibrium of aquatic food web.

This study was aimed at evaluating the impact of sublethal TiO_2 NPs on photosynthesis and antioxidant activity in *C. ellipsoideum*.

Materials and Methods

Chemicals

Powder Titanium (IV) oxide nanoparticle (99.50%) of particle size 21 nm, CAS Number 13463-67-7, Pcode: 1002000564 was obtained from Sigma-Aldrich (St. Louis, MO 63103, USA). All other chemicals were of analytical grade. Stock solution was prepared in de-ionized water at a concentration of 1g L^{-1}. TiO_2 NPs was sonicated (600w, 40 KHz, 25ºC) for 30 min to enable full dispersion of NPs. Serial dilution of the stock solution was used to obtain the expected concentrations for chemicals tests.

Characterization of TiO_2 NPs

The characterization of TiO_2 NPs was previously reported in our study (MATOUKE et al. 2018). The determination of phase purity of NPs was carried out with X-ray diffractionometer (XRD) using an Empyrean XRD (Panalytical, The Netherlands) equipped with filtered Cu K λ radiation (λ = 1.5418 Å) that operated at 40 K_v and 40 mA. The XRD patterns were recorded from 10 to 80 2θ degree with a scanning speed of 0.526º per minute. The determined sizes of nanoparticles were confirmed using the Sherrer equation. In addition, image of powder nanoparticles of 0.1 mg L^{-1} was observed using a scanning electron microscope (Zeiss model), Germany. We also determined the zeta-potential in the medium (0.1 mg L^{-1}) using Nanobrook ZetaPlus Brookhaven 220001, USA.

Algal culture

All toxicity tests were conducted using freshwater *C. ellipsoideum* provided by the National Institute for Freshwater and Fisheries Research (NIFFR), Kainji, New Bussa, Nigeria.

The provided axenic cultures of microalgae *C. ellipdoideum* were grown under controlled sterile conditions in 250 mL Erlenmeyer fibre glass flask containing 100 mL of sterilized modified B11 medium (STANIER et al. 1971). In order to determinate the algal inhibition (EC_{50}), alga cultures were maintained at 25±2ºC on a 14: 10-h: light: dark cycle with a light intensity of 100 $\mu Em^{-2}\ s^{-1}$, and continuous shaking (100 rpm) for 72 h. *C. ellipdoideum* cells at exponential growth phase were inoculated in the Elenmeyer flasks containing fresh medium. The exponential growth phase had a density of $2 \cdot 10^3$ cells mL^{-1} and were enriched in triplicate with varying TiO_2 NPs concentrations (10, 20, 40, 60, 80 and 100 mg L^{-1}) according to the Organization for Economic Cooperation and Development (*Proceedings*… 1984) 201 algal growth inhibition test guidelines.

For further study, nominal concentrations of TiO_2 NPs in culture media were: 1.85, 3.88, 6.06, 8.39 and 10.9 mg L^{-1} obtained from the result of algal inhibition (EC_{50}) and represent sub-lethal doses of EC_5, EC_{10}, EC_{15}, EC_{20}, EC_{25}, respectively with the control (without dosage). The experimental culture in Erlenmeyer flask (250 mL) contained initial cell density of $2 \cdot 10^3$ cells mL^{-1} inoculated in media (100 mL of BG 11) at exponentially growing phase. The cultures lasted for 72 h and were kept under 25±2ºC on a 14: 10-h: light: dark cycle with a light intensity of 100 $\mu Em^{-2}\ s^{-1}$, and continuous shaking (100 rpm). Three experimental replicates were performed and growth, chlorophyll and antioxidants were monitored.

Growth determination

Growth of algal cells was monitored by direct count of viable cells under microscope using a Neubauer haemacytometer. Percentage inhibition of growth was calculated as (ADELEYE and KELLER 2016) [6]:
where:

$$GI = \frac{N_c - N_t}{N_c} \cdot 100$$

GI – the percent inhibition in average cell density

N_c – the average cell density in the control group,

N_t – the average cell density for the treatment group.

The EC_{50} values, which represent the concentrations of the test substances leading to 50% reduction in the algal growth compared to the control, were calculated from the dose-response curve Weibull model (MONTEIRO et al. 2011) analysis on Regression toxicology software for Excel. For further analysis, sub-lethal concentrations (EC_5, EC_{10}, EC_{15}, EC_{20}, EC_{25}) derived from the acute concentrations of TiO_2 NPs were used.

Chlorophyll *a* and *b* determination

The extraction and analysis of chlorophyll a were done according to the procedure described by (AMARAL 2012). Chlorophyll *a* and *b* extracted using absolute methanol. The chlorophyll *a* (Chl*a*) concentration was calculated using the equation:

$$\text{Chl}a\ [\text{mg L}^{-1}] = (11.47 \cdot \text{OD}_{664}) - (0.4 \cdot \text{OD}_{630})\ x/y$$

Where *x* is the total volume of extraction solvent used and *y* represents the volume of culture filtered.

Chlorophyll *b* (Chl*b*) was calculated according to the formula (LICHTENTHALER and WELLBURN 1985):

$$(\text{Chl}b)\ [\text{mg L}^{-1}] = 27.05\ \text{OD}_{653} - 11.21\ \text{OD}_{666.}$$

OD_{630}, OD_{653}, OD_{664}, and OD_{666}, is optical density at a wavelength of 630, 653, 664 nm and 666 nm, respectively. OD was determined using a UV-2600 spectrophotometer (Shimadzu Scientific Instrument, China).

Antioxidant bioassays

Microalgal cells (50 mL) of each culture were centrifuged at 100 rpm for 10 min. Centrifuged algal cells were ground in 1 mL of 20 mM phosphate buffer (pH 7.4) , 0.1 g of white quartz sand in a chilled tissue grinder was added to the mixture. The mixture was centrifuged at 12000 g for 10 min at 4ºC to obtain the supernatant for further analysis. The supernatant was stored as aliquot for antioxidant estimations. Protein measurements were performed according to LOWRY et al. (1951).

Glutathione peroxidase (GPX, EC 1.11.1.7)

Peroxidase activity was measured using guaiacol and H_2O as the donor as substrate. The substrate mixture contained 10 mL 1% guaiacol, 10 mL 0.3% hydrogen peroxide and 100 mL 0.05 M sodium phosphate buffer (pH 6.5). The mixture in the cuvette was made up of 2.87 mL substrate, 0.1 mL of crude extract, and 0.03 mL antioxidant solution in a total vol-

ume of 3 mL (HEMEDA and KLEIN 1990). The control contained 0.03 mL of ethanol. Peroxidase activity was determined spectrophotometrically at 25ºC and 470 nm. The result was expressed as μg mg^{-1} FW min^{-1}.

Superoxide dismutase (SOD, EC. 1.15.1.1)

Superoxide dismutase (SOD) activity followed the method described by (GAO 2005) which consisted of the reduction of tetrazolium. One unit is the amount required to inhibit 50% of NBT photoreduction. The control tube, the light tube and the measuring tube were divided for each sample. Each tube contained 550 mmol L^{-1} potassium phosphate buffer (pH 7.8), 130 mmol L^{-1} methionine solution, 750 μmol L^{-1} NBT solution, 20 μmol L^{-1} riboflavin solution,100 μmol L^{-1} EDTA-Na_2, distilled water, and the enzyme solution was added to the measuring tube, the same amount of distilled water was added to the other tubes. The experiment was conducted under 1000 Lx Fluorescent color reaction for 15 min, the dark control tube was used as a blank. The absorbance was read at 560 nm and the result expressed as U mg^{-1}FW h^{-1}.

Glutathione reductase (GRx, EC 1.6. 4.2)

Glutathione reductase (GRx, EC 1.6. 4.2) activity was determined according to (SCHAEDLE and BASSHAM 1977). GR catalyzed following reaction:

$$GSSH + NADPH \rightarrow GSH + NADP+.$$

GR activity was evaluated by measuring the change of NADPH. 1 mL reaction mixture containing 50 mmol L^{-1} potassium phosphate buffer (pH 7.8), 20 mmol L^{-1} EDTA, 1.5 mM NADPH, 5 mM GSSG, 200 μL enzyme solution, and measured the change of absorbance at 340 in 1 min under 20°C immediately (extinction coefficient is 6.2 mmol L^{-1} cm^{-1}). The result was expressed in U mg^{-1} protein.

Catalase (CAT, EC 1. 11.1.6)

Catalase activity was determined using UV absorption method (GAO 2005). Sample was divided into two tubes added with live enzymes in one and another with dead enzymes, then Tris-HCl buffer (pH 7.0), distilled water were added in each tube and the mixture in each tube was pre-heated with 200 mmol L^{-1} H_2O_2 for 3min using a water bath at 25°C. The absorbance was measured immediately at 240 nm. The result was expressed in U mg^{-1} FW min^{-1}.

Malondialdehyde (MDA)

Malondialdehyde (MDA) measurements, used to estimate the level of lipid peroxidation in algal cells, were carried out according to (HEATH and PACKER 1968). 2 mL of 10% trichloroacetic acid (TCA), containing 0.5% thiobarbituric acid (TBA) was added to 1 ml of the microalgal suspension. The mixture was then heated in a water bath for 15 min and allowed to cool in an ice bath then, centrifuged at 3000 rpm for 5 min and the absorbance of the supernatant was read at 532 nm and 600 nm. Lipid peroxidation was expressed as the MDA content in nanomoles per 10^5 cells (nmol mg^{-1} FW) based on the difference between absorbance at 535 and 600 nm, using a molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Data analysis

For the algal growth inhibition tests, the EC_{50} values (metal concentration required to cause a 50% reduction in growth) were computed using curve Weibull model analysis on Regression toxicology software for Excel. The data obtained from the study was normalized and subjected to Levene's test for homogeneity of variance and one way analysis of variance (ANOVA) was used to determine the differences in means parameters (chlorophyll and antioxidant activities) using GraphPad Prism 8.3 for windows. Where significant differences were observed, separation of means was done using Tukey's HSD post hoc test. Values were considered significantly different when the probability was less than 0.05 or 0.01.

Results

The results of the x-ray Diffractogram (XRD) for TiO_2 NPs indicated eleven (11) diffracted peaks that reckon their tetragonal structure. From the diffractogram we derived the two main textures Anatase (82%) and rutile (31%) phase. The pictogram TiO_2 NPs powder with the Scanning electron microscope (SEM) showed an agglomeration of particles of 21 nm according to the manufacturer. However, 1 mg L^{-1} of prepared TiO_2 NPs revealed an average zeta potential of 0.19 mV informing us of an unstable mixture (Figure 1).

The 72 h EC_{50} values obtained from inhibition of cells of *C. ellipsoideum* exposure to TiO_2 NPs was 69.90 mg L^{-1}. The assay revealed sub-lethal concentrations with EC_5, EC_{10} EC_{15}, EC_{20} and EC_{25}: 1.85, 3.88, 6.06, 8.37 and 10.90 respectively showed TiO_2 threshold concentrations on microalgae cells (Table 1).

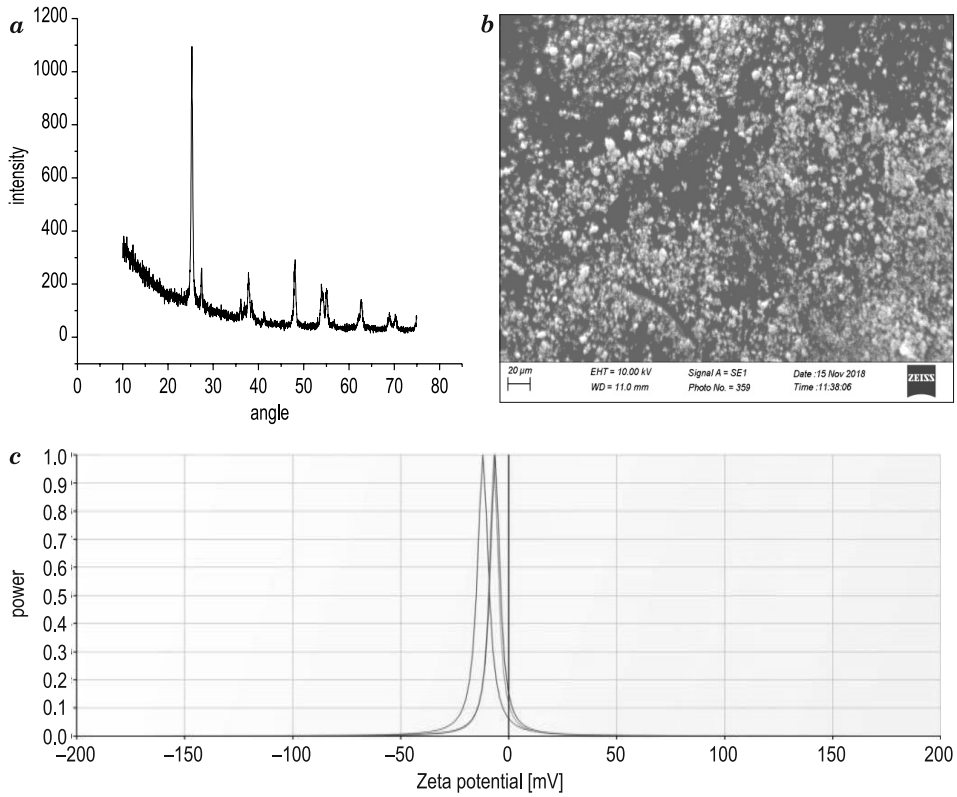

Fig. 1. Characterization of titanium dioxide nanoparticles (TiO2 NPs): a – X-ray diffraction pattern; *b* – Scanning electron micrograph; *c* – Zetapotential of 1 mg L^{-1} TiO_2 NPs. Scale bar represents 30 µm. Images at a magnification of 2000× in culture medium. Scale bar 10 µm. Images at a magnification of 2000× in culture medium. Scale bar 10 µm. Images at a magnification of 2000×

Table 1

Effective concentration (EC_{50}) after 72 h of exposure of TiO_2 NPs to *C. ellipsoideum*, Estimation of parameters of Weibull model

Effective concentration [mg L^{-1}]	TiO_2 NPs
EC_{50}	69.9
EC_{25}	10.9
EC_{20}	8.37
EC_{15}	6.06
EC_{10}	3.88
EC_5	1.85

Chlorophyll *a* and *b* exposed to sub-lethal concentration of TiO_2 NPs decreased significantly ($p < 0.01$) with increasing concentrations of TiO_2 NPs compared to the controls (Figure 2), and the highest inhibition of chlorophyll *a* and *b* were observed after 72 h in cells exposed to 10.90 mg L^{-1} of TiO_2 NPs. Chlorophyll *a* and *b* are inversely proportional to the level of concentrations. The decreases in chlorophyll *a* were 38.76, 53.37, 67.98, 70.78 and 76.68% compared to control; while chlorophyll *b* decreased as follows: 87.64, 88.20, 91.57, 92.97 and 93.25% compared to the control. This indicates that chlorophyll b was highly decreased compared to chlorophyll *a*.

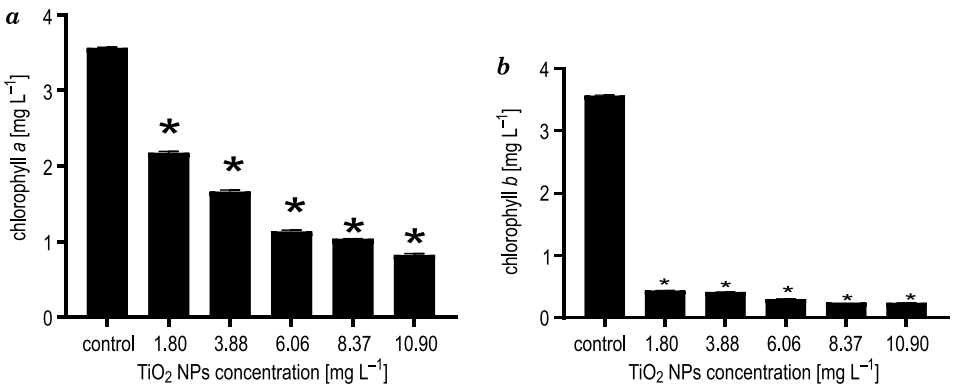

Fig. 2. Comparison of Chlorophyll *a* and *b* levels in *C. ellipsoideum*: *a* – comparison of Chlorophyll *a*; *b* – chlorophyll *b* levels in *C. ellipsoideum* with exposed concentrations (control 0.0, 1.80, 3.88, 6.06, 8.37 and 10.90 mg L^{-1}) of TiO_2 NPs after 72 h. Data were expressed as mean ± SD of three replicate samples. $^*p < 0.01$ indicate significant differences between exposure group and the corresponding control group (ANOVA followed by Tukey's test)

All antioxidant enzymes activities (Catalase, SOD and GRx) on the algal cells exposed to TiO_2 NPs (Figure 3) significantly increased ($p < 0.01$). The range of percentages increment compared to the control was between: 22–58.66, 2.68–16.85, 26.50–86.67 and 33.33–100 for Catalase, SOD, GRx and MDA respectively. However, lipid peroxidation (MDA) increase in this study, demonstrated a high fluctuation with the concentrations 6.06 and 8.37 mg L^{-1}.

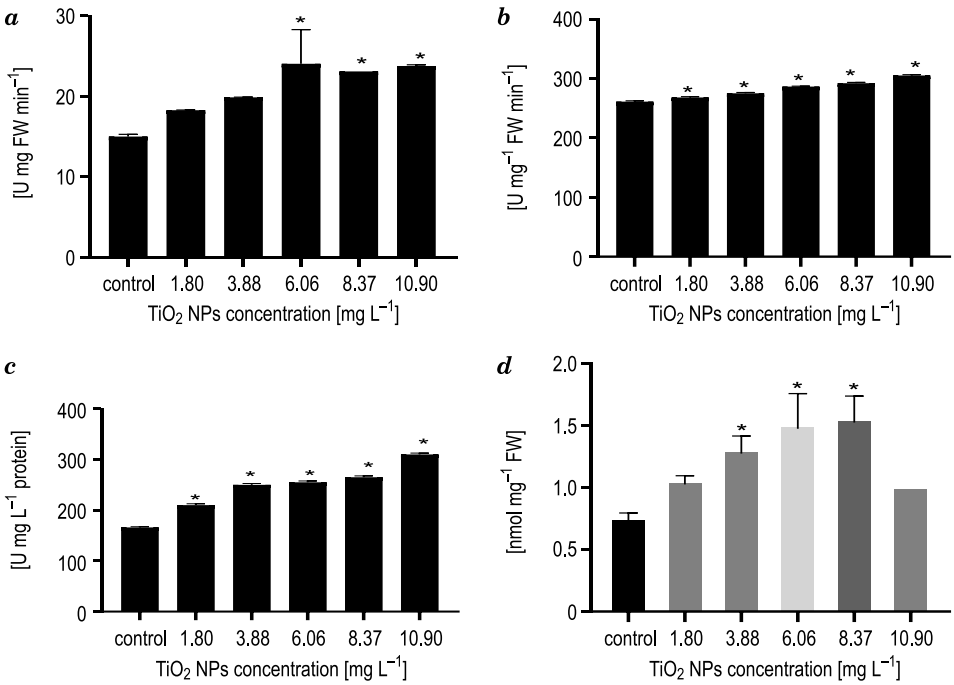

Fig. 3. Antioxidant enzymes concentrations in *C. ellipsoideum*: *a* – catalase activity; *b* – SOD activity; *c* – GRx activity; *d* – MDA activity with exposed concentrations (control 0.0, 1.80, 3.88, 6.06, 8.37 and 10.90 mg L^{-1}) of TiO_2 NPs after 72 h in *C. ellipsoideum*. Data are expressed as mean ± SD of three replicate samples. $^*p < 0.01$ indicate significant differences between exposure group and the corresponding control group (ANOVA followed by Tukey's test)

Discussion

In this study, the acute concentration of TiO_2 NPs was sufficient to induce toxicity to *C. ellipsoideum* with harmful effects on its physiology. This pollutant in aqueous solution probably released free radicals capable of scavenging for microalgal cells and forms ligands that chelate and alter the survival of the exposed microalgae. The effective concentration (EC_{50}) in response to stress caused by TiO_2 NPs is probably due to their toxicity. However, based on the EC_{50} value recorded (69.90 mg L^{-1}), exposures of microalgae showed that *C. ellipsoideum* is intolerant to TiO_2 NPs compared with the recorded values of 9.10 mg L^{-1} to *C vulgaris*, 4.90 mg L^{-1} to *C. pyrenoisa* and 10.91 mg L^{-1} to *Phaeodactylum tricornutum* (DAOHUI et al. 2018, HUREL et al. 2012, ZHU et al. 2010).

To understand the action of TiO_2 NPs on *C. ellipsoideum*, chlorophyll content was evaluated and significant decrease was recorded in this study. Similar changes have been identified in other findings on microalgae

Scenedesmus obliquus (YUAN et al. 2010), implying that this decrease in chlorophyll content commonly occur in microalgae exposed to TiO_2 NPs. The changes observed due to the selected sub-lethal concentrations used in the present study are probably linked to the adsorption of TiO_2 NPs on algal cells. The bioaccumulation of this chemical in *C. ellipsoideum* cells could be responsible for the disruption and impairment of photosynthesis electron transfer system (HUREL et al. 2012) involved in the mechanisms pathways of photosynthesis. Moreover, the decrease of chlorophyll levels in this study may be ascribed to the presence of TiO_2 NPs into algal cells that are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient during photosynthesis (QIAN et al. 2009). Furthermore, *C. ellipsoideum* exposure to TiO_2 NPs could block light penetrating the cells and induce disruptive effects in the cells thereby causing decreasing chlorophyll content. Recently, the presence of TiO_2 NPs on *C. vulgaris* was characterized by cell wall/membrane damage, plasmolysis and internalization of TiO_2 NPs with critical effects on photosynthesis (HUREL et al. 2012).

The mechanism involved for the prevention and inhibition of cells damage due to environmental stressors is the synthesis of antioxidant activities. These stressors enhanced the ability of the organism to accumulate hydrogen peroxide and superoxide anion commonly known as reactive oxygen species (ROS). Previous studies have reported the increase of ROS production on algae following the exposure of nanomaterial (CHENG et al. 2016, ZHONG et al. 2014, RAI et al. 2013). In this study, *C. ellipsoideum* after exposed to TiO_2 NPs demonstrated an alteration of antioxidants. This probably suggests that antioxidants could act synergistically to mitigate the effect of toxicity. Similar results have been reported in the effect of cadmium on the growth and antioxidant response of freshwater *C. vulgaris* (CHENG et al. 2016, ZHU et al. 2007).

Catalase, SOD and MDA activities were significantly increased in this study. This shows the tendency of TiO_2 NPs to alter the functioning ability of H_2O_2 as reported by (SHARMA et al. 2012) [29]. Catalase promotes the dismutation of H_2O_2 into H_2O and O_2. H_2O_2 which is a product of SOD is detoxified by CAT, which is one of the main ROS-scavenging enzymes. SOD plays protective roles against oxidative damage and its increase might be due to the direct effect of the metal-oxide on the SOD genes. The great induction of SOD by TiO_2 NPs in this study could be due to the enhancement of ROS.

The alteration of MDA activity in this study was observed to vary with the change in concentrations; however exposure of *C. ellipsoideum* to TiO_2

NPs significantly increased MDA activity. The increase of secondary end-product of oxidation which is eventually considered as biomarker of lipid peroxidation in this study could be attributed to the effect of TiO_2 NPs which could have stressed the algae thereby oxidizing polyunsaturated fatty acid with their free ions (Hemeda 1990).

The relatively significant decrease of GRx activity of *C. ellipsoideum* exposed to TiO_2 NPs was recorded. This decrease could be assigned to the sudden change on the specific gene expressed on the treated *C. ellipsoideum*. Similar depletion of GRx was reported on *C. ellipsoides* exposed to TiO_2 NPs and phosphorous (MATOUKE et al. 2018)

Conclusions

In this study, exposure of *C. ellipsoideum* to TiO_2 NPs indicated an alteration of the biomass (chlorophyll *a*, *b*) and antioxidant activities (catalase, superoxide dismutase, glutathione reductase and malondialdehyde). The study also demonstrated that *C. ellipsoideum* is sensitive to TiO_2 NPs therefore, monitoring of this microalga in natural ecosystem for this metallic compound is necessary for its conservation. Thus, research studies on the impacts of TiO_2 NPs on other freshwater plankton's organisms are relevant in order to have an insight on their challenges in aquatic ecosystems.

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