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ANTAGONISTIC EFFECTS OF BINARY MIXTURE OF TITANIUM DIOXIDE NANOPARTICLES AND LEAD ON BIOMASS AND OXIDATIVE STRESS IN EXPOSED *CHLOROIDIUM ELLIPSOIDEUM* (GERNECK)

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Abstract

Sub-lethal bioassay was performed on *Chloroidium ellipsoideum* to monitor the changes in the algal biomass and antioxidant activities associated with Titanium dioxide Nanoparticles (TiO_2 NPs) and Lead Pb(II). The results showed that there was significant (p < 0.05) decrease in biomass (density, chlorophyll *a* and *b*) of *Chloroidium ellipsoideum* as a result of adsorption of Pb(II) by TiO_2-NPs. However, the binary mixtures of the chemicals significantly increased antioxidant activities (SOD and MDA) of the alga. Furthermore, the study revealed an antagonistic (CI >1) effect of the binary chemicals on the biomass and antioxidant activities of the alga. Based on the study, it was shown that the co-exposure of TiO_2 NPs and Pb(II) decreased the Pb(II) bioavailability causing antagonistic effects on biomass and antioxidant activities in *Chloroidium ellipsoideum*.

Introduction

Increase in anthropogenic activities often leads to a direct or indirect increase in the production of waste effluents in aquatic ecosystems with harmful risks on biodiversity (JAISHANKAR et al. 2014). Mismanagement of the various effluents from industries such as mines, agriculture, phar-

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macy, medicine, and households usually leads to the accumulation of environmental pollutants and nanomaterials in aquatic ecosystems.

The use of nanomaterials is greatly increasing because of their wide application in household products, like sunscreens, cosmetics, paints and surface coating (RAY et al. 2012). In 2010, 50,400 tons of nanomaterials were produced worldwide, which increased to about 201500 tons in 2015 and worth about 2.6 trillion dollars (RAY et al. 2012). This is expected to increase further many years to come.

ZHANG et al. (2015) reported that ZnO_2, SiO_2 and TiO_2 are the most greatly used nanomaterials in the world. TiO_2 nanoparticles are commonly used because of its importance in sunscreens where the particles protect against cell damage and preventing UV light. TiO_2 (NPs) is also used in toothpastes industries, surface coatings, water treatment (CHEN and MAO 2007), and recently TiO_2 NPs bears tremendous hope in helping ease the energy crisis through effective utilization of solar energy based on photovoltaic and water-splitting devices (GRATZEL 2004). Their diversified use has led to a worldwide production of 5000 t/year within 2006–2010, 10 000 t/year within 2011–2014, and an estimated 2.5 million metric tons/year by 2025.

Thus, the large production of TiO_2 NPs with its novel properties is of critical concern because of the likelihood of their fate ending in freshwater ecosystem (ZHANG et al. 2015). However, there is an array of reports that documented the acute and chronic toxicological impacts of TiO_2 NPs in freshwater and marine organisms. Microalga *Pseudokirchneriella subcapitata* exposed to TiO_2 NPs (25–70 nm) was more toxic than bulk TiO_2 with recorded effect of aggregation on algal cells (ARUOJA et al. 2009). Reduced growth and metabolic alterations were observed in three other freshwater species of algae, *Scenedesmus quadricauda*, *Chlamydomonas moewussu* and *Chlorella vulgaris*, exposed to TiO_2 NPs (CARDINALE et al. 2012). Inhibitory effect of titania NPs (EC_{50} of 16.12 mg L^{-1} and 21.2 mg L^{-1}) was reported in both *Scenedesmus* sp. and *Chlorella* sp. (MUKHERJEE et al. 2011). WANG et al. (2012) also reported inhibition of chlorophyll *a* and cell growth as well as increased chrorophyll *b* and carotenoid in *Chlamydomonas reinhardtii* exposed to TiO_2 NPs. The crystal structure of TiO_2 NPs greatly inhibited algal growth after 6 days exposure with recorded EC 30, 30 mg L^{-1} (JI et al. 2010, HUREL et al. 2013).

Only few studies exist on the assessment of ecotoxicological effects and environmental risks of TiO_2 NPs combined with heavy metals on organisms (HARTMAN et al. 2009, MAOET al. 2012, TANG et al. 2013). Lead for instance is one of the most well-known pollutants in the environment and its bioconcentration into biological system is increasing due to anthropogenic activities (TRIPATHI et al. 2006). Lead is also known to be non-biodegradable and have harmful effects on freshwater organisms (GROSELL et al. 2006, MARTINEZ et al. 2004, SEVAKOVA et al. 2011).

However, to the best of our knowledge, studies on the effects of combined chemicals of TiO_2 NPs and Lead on microalgae has not been given due attention, hence the need for its study.

This study investigated the ecotoxicological effects of single and binary mixtures of titanium dioxide nanoparticles and lead on microalgae *Chloroidium ellipsoideum* (chlorophyta) a primary producer in the aquatic ecosystem in order to provide an insight into the impact of the presence of this nanomaterial (TiO_2 NPs) on a conventional pollutant Pb(II).

We aimed to study the effects of binary mixtures of Titanium dioxide Nanoparticles (TiO_2 NPs) and Lead Pb(II) on biomass and antioxidant activities of *Chloroidium ellipsoideum*.

Materials and Methods

Microalgal species

The microalgae *Chloroidium ellipsoideum* was obtained from the National Institute for Fisheries and Freshwater Research (NIFFR), New Bussa, Nigeria. The algae was cultured in a modified BG 11 medium consisting of 31.43 mg L^{-1} organic Nitrate (NO_3), 40.80 mg L^{-1} Phosphorus (PO_4), 170.08 mg L^{-1} Potassium (K_2O), 42.0 mg L^{-1} Magnesium (MgO), 4.66 mg L^{-1} sulfur (SO_4), 0.42 mg L^{-1}, Calcium (Ca) 2.53 mg L^{-1}, Iron (Fe), 39.27 mg L^{-1} Sodium (Na) 20.62 mg L^{-1}, Chlorine (Cl) and Zinc (Zn) 0.29 mg L^{-1}. Prior to the culture, the medium was sterilized by autoclaving at 121°C for 15 minutes. Cultures were maintained under control condition in a culture cabinet at 25 ± 2° C and 12:12 h light: dark cycle.

Nanoparticles of Titanium dioxide (TiO_2) (anatase-rutile) 21 nm and Lead nitrate ($Pb(NO_3)_2$ salt were purchased from Sigma-Aldrich (St Louis, Mo, USA). An Empyrean XRD (Panalytical, The Netherlands) was used to characterize Nano-TiO_2 particles powder by X ray diffraction. The machine was equipped with filtered Cu Kλ radiation (λ = 1.5418 Å) operated at 40 K_v and 40 mA. The XRD patterns were recorded from 10 to 80 degrees with a scanning speed of 0.526° per minute. PDF2 software was used for the analysis of peaks. Images of Nano-TiO_2 particles powder as received from the manufacturer using Scanning electron microscope (Phenom ProX SEM, Phenom-World, The Netherlands) was also obtained.

A stock solution of 100 mg L^{-1} of both chemical was prepared separately by suspension in the ionic water. TiO_2 NPs was rigorously mixed with an ultrasound water bath (100 W, 40 kHz) for 30 mins to prevent agglomeration; samples were diluted to different exposure concentrations. To analyse the interaction of Pb(II) and TiO_2 NPs, batch sorption experiment was performed. In this in-vitro assay, a stock of Pb(II) previously prepared was diluted (0.013, 0.019, 0.025, 0.031, 0.037 and 0.04) with 50 mL of culture medium. The pH of Pb(II) was adjusted to 7.00 using 0.01 M HCl and NaOH. TiO_2NPs of concentration of 0.20 µM was diluted in each flask with distilled water. The mixtures were shaken to achieve sorption equilibrium within 10h (TANG et al. 2013). The binary suspensions were centrifuged at 5000 rpm for 10 mins. The supernatants obtained from centrifugation were then measured.

Chloroidium ellipsoideum cultured at exponential growth phase (6 days) was treated with a nominal concentration of 0.01 and 0.04 µM of Pb; 0.09, 0.20 µM of TiO_2 NP_s and the four (4) couples of binary mixtures (0.01, 0.09); (0.01, 0.20); (0.04, 0.09); (0.04, 0.20) µM respectively for 72 h. In the medium, the effects of the chemicals were observed alone and in combination. Powdered Pb(II) was provided as Pb $(NO_3)_2$ because it is the most common form compared to other Pb(II) sources in aquatic ecosystems (MUTEMBEI et al. 2014).

The chosen TiO_2 NPs and Pb(II) levels were the sub-lethal concentrations. But, for Pb(II), the concentrations were 0.01 µM and 0.04 µM respectively. The concentration (0.01 µM) in this study was considered as allowable permissible concentration and the concentration (0.04 µM) is the maximum allowable limit standard of the United States environmental protection agency (USEPA) (WHO 2013). The concentrations for both chemicals were chosen after a preliminary determination of EC_{50}, where EC_{50} values of 0.190 µM and 0.86 µM were recorded for Pb(II) and TiO_2 NP_s respectively. All the experiments were carried out in triplicates.

Data collection

The suspensions collected from sorption analysis were digested using pure HNO_3 at 120°C for 2 h in a glass beaker. The Pb(II) concentrations in the digested samples were then determined according to the method described by HALTTUNEN et al. (2007). After cooling, the solution was transferred quantitatively to a 10 mL volumetric flask; Pb(II) concentrations were determined using AA-7000 Atomic Absorption spectrophotometer and read at wavelength 283.3 nm.

Sorption of Pb(II) onto TiO_2 NPs

The Langmuir isotherm model was used to fit the adsorption data with $Ce/qe = Ce/q_{max} + 1/q_{max}\, b$, where: q_{max} = maximum adsorption, b = Langmuir coefficient. The interactions of Pb (II) with TiO_2 NPs were computed by examining the sorption equilibrium. In the equilibrium isotherm experiment, a correlation between Pb(II) adsorbed on the TiO_2 NPs (qe, μg/g) and the non adsorbed Pb(II) concentration (Ce, μg L^{-1}) was calculated according to CHEN (2015).

Biomass (density, dry weight and chlorophyll content) and the biomolecular composition (protein, lipid and carbohydrate) of the alga which are the key parameters of growth and productivity assessment were determined (MATOUKE et al. 2018). Density was monitored by direct count of viable cells under the microscope (Motic Digital DMB 1 series) using a Neubauer haemacytometer (Optik labor, Qiujing, QJ1102). Dry weight was determined gravimetrically using previously oven dried (2 h, 60°C) Whatman's GF/C filters (0.45 μm pore size) with a sensitive weighing balance (Sartorius; AG Germany). Chlorophyll extraction was carried out using 80% (v/v) acetone according to the method of SHOAF and LIUM (1976) and chlorophyll *a* and *b* were determined using the equation of RITCHIE (2008).

Antioxidant activities were measured based on standard methods, Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and malondialdehyde activities were evaluated as a measure of oxidative stress in the exposed *C. ellipsoides*.

After 72 h of exposure to the chemicals, the microalgal cells cultures were collected, centrifuged and then the pellets homogenized in 2 mL of 100 mM potassium phosphate buffer (pH 7.5), 1 mM ethylene diamine tetra acetic acid (EDTA) and 1%w/w polyvinyl polypyrrolidone (PVP) using a vortex. The homogenate was centrifuged at 12,000 rpm for 15 min at 4ºC. For enzymes activities the supernatant was separated and stored as aliquot at 80ºC.

The enzymatic activities of Glutathion peroxidase (GPx), Superoxide dismutase (SOD), Glutathione reductase (GR) and Malondialdehyde (MDA) were evaluated and described in supplementary document by the methods of ENSIBI and MOHAMMED (2017), BEAUCHAMP and FRIDOVISH (1971), SCHAEDLE and BASSHAM (1977) and HEATH and PACKER (1968) respectively.

Data Analysis

The obtained data were subjected to Levene's test for homogeneity of variance and one way ANOVA was used to determine the differences in means of the parameters (Biomass and antioxidant responses using Origin-Pro 8.5 (OriginalLab Corp., Northampton, MA, USA). 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. The determination of interaction between chemicals or combination index used the method of Chou-Talalay (1976).

Results

Characterization of TiO_2 nanoparticles

X Ray Diffraction analysis demonstrated the crystal phases and the crystallite size of TiO_2 NPs. Figure 1*a* revealed eleven (11) peaks on the the X-ray diffractograms. SEM revealed the whitish colour of TiO_2 NPs.

The study showed the powder form of TiO_2 NPs before and after dispersion into the medium (Figures 1*a*, 1*b* and 1*c*).

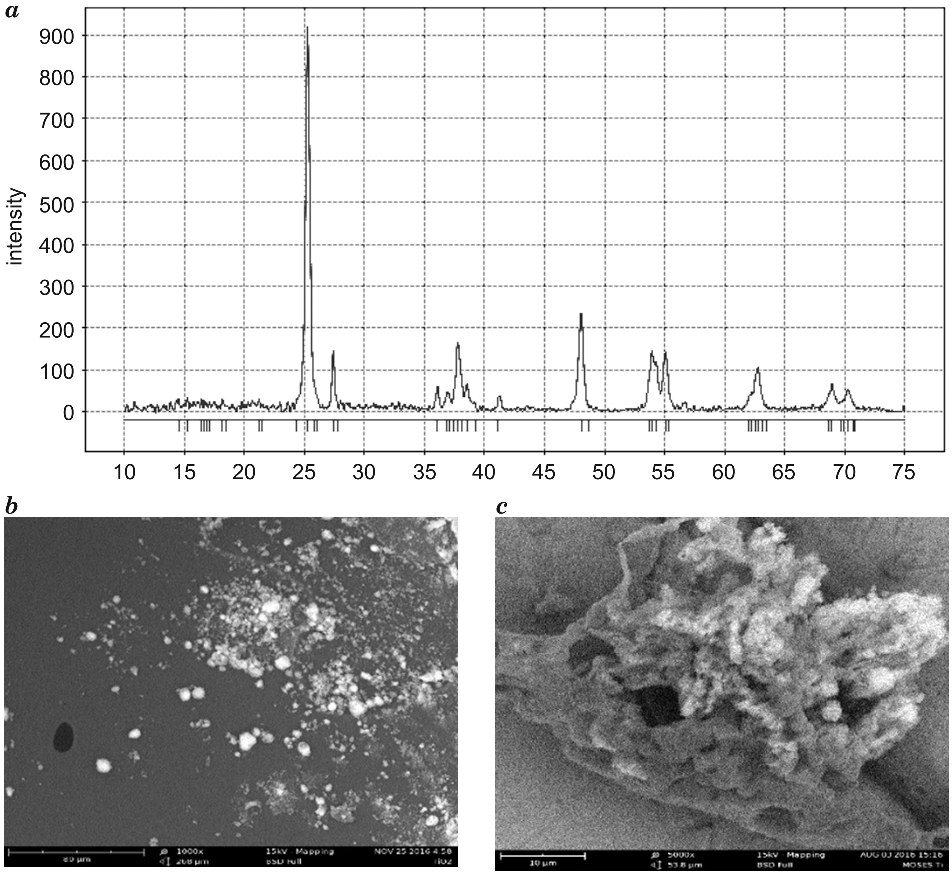


Fig. 1. Characterization of TiO_2 NPs: *a* – X-ray diffraction pattern of TiO_2 NPs; *b* – SEM image of TiO_2 NPs as received from the manufacturer; *c* – SEM image of TiO_2 NPs after dispersion in the medium

Sorption of Pb(II) onto TiO_2 NPs

The computed adsorption capacity q_{max} was 30.296 μg g^{-1}, the parameter b corresponding to the affinity of the TiO_2 NPs to Pb(II) or Langmuir constant was 33.57 μg g^{-1} and the correlation ($R^2 = 0.999$) is shown in Figure 2. The result indicated a positive and good correlation suggesting a monolayer adsorption of Pb(II) on TiO_2 NPs.

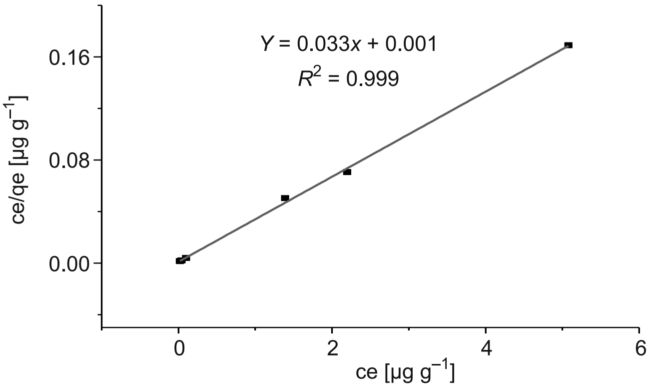


Fig. 2. Adsorption isotherms of Pb(II) on TiO_2 NPs in the culture medium; pH = 7.00; temperature = 298 K

Biomass production of *C. ellipsoideum*

The inhibitory effect of single and combined chemicals on density, chlorophyll *a* and *b* after 72 h exposure are shown in Figure 3 and Figure 4. The chemical concentrations density of Pb (0.01) and Pb (0.04) μM were significantly ($p < 0.05$) inhibited to 79%, 76%; chlorophyll *a* content to 61%, 83% and chlorophyll *b* content to 68%, 57% of the control respectively. However, TiO_{2-} NPs (0.09) and TiO_{2-} NPs (0.20) μM concentrations density was significantly ($p < 0.05$) inhibited to 58%, 63%; chlorophyll *a* content to 87%, 69% and chlorophyll *b* content to 62%, 85% of the control respectively. The combined treatment of both chemicals indicated an inhibitory effect on the density, chlorophyll *a* and *b* contents to 79%, 83%, 78% and 77%; 69%, 66%, 81%, 88%, 51,%, 44%, 70%, 66% of the control after exposure to Pb (0.01) + TiO_2 NPs (0.09), Pb (0.01)+ TiO_2 NPs (0.20), Pb (0.04) + TiO_2 NPs (0.09), Pb (0.04) + TiO_2 NPs (0.20) μM respectively. However, the decrease observed in the biomass content was not concentration dependent. The comparison between the single and the combined chemicals showed no significant ($P > 0.05$) decrease. Furthermore, the interaction between both chemicals on the biomass (density, chlorophyll *a* and *b*) showed a combination index (CI >1) which exhibited a strong antagonistic effect on the biomass.

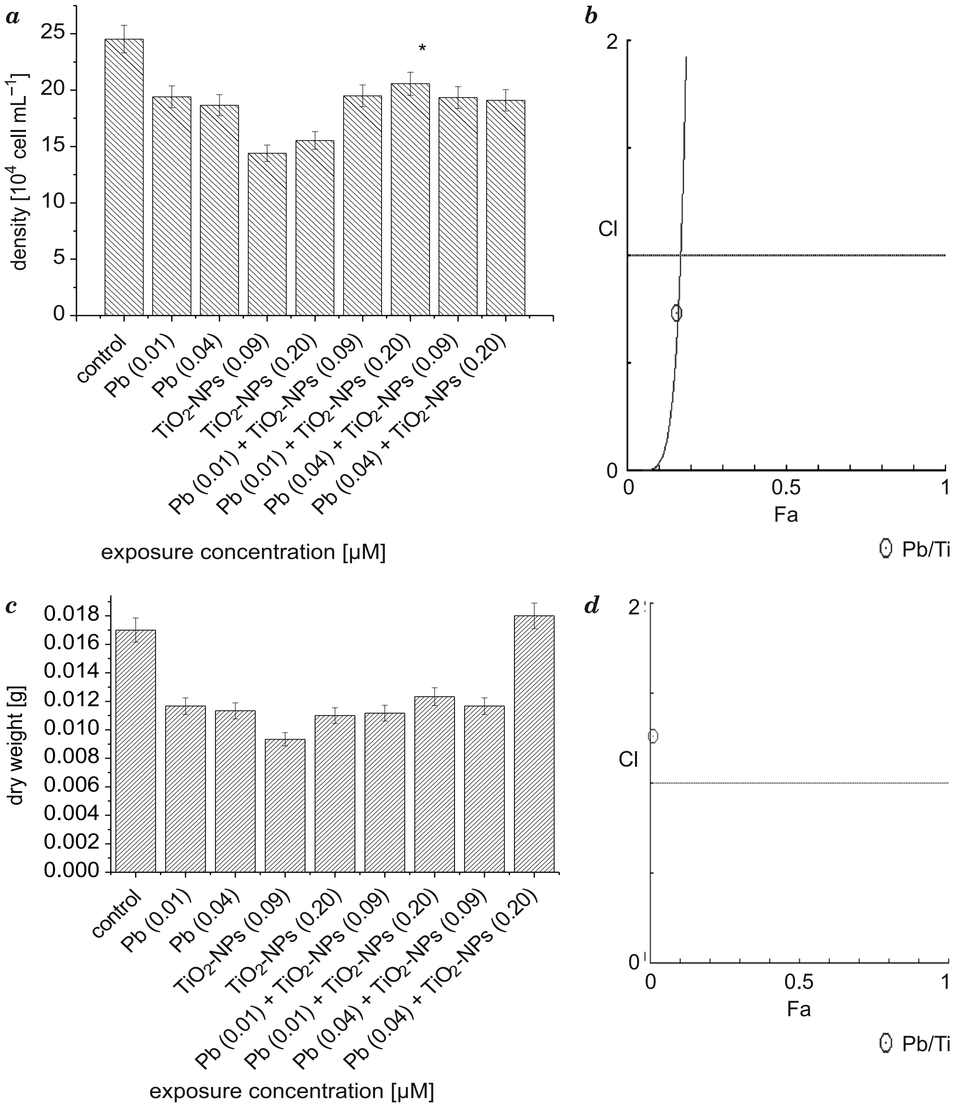


Fig. 3. *C. ellipsoideum* denisity and dry weight responses: *a* – cells density of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 2.409, p = 0.0029; threshold replicate were included (n = 3); errors bars indicate; standard deviation (SE), *significant difference. Chi-square test (p > 0.05)]; *b* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on cells density of *C. ellipsoideum* [combination index (CI); CI > 1]; c – dry weight of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 0.476, p = 0.866; threshold replicate were included (n = 3); errors bars indicate Standard deviation (SE), *significant difference. Chi-square test (p > 0.05)]; *d* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on cells dry weight of *C. ellipsoideum* [combination index (CI); CI > 1]

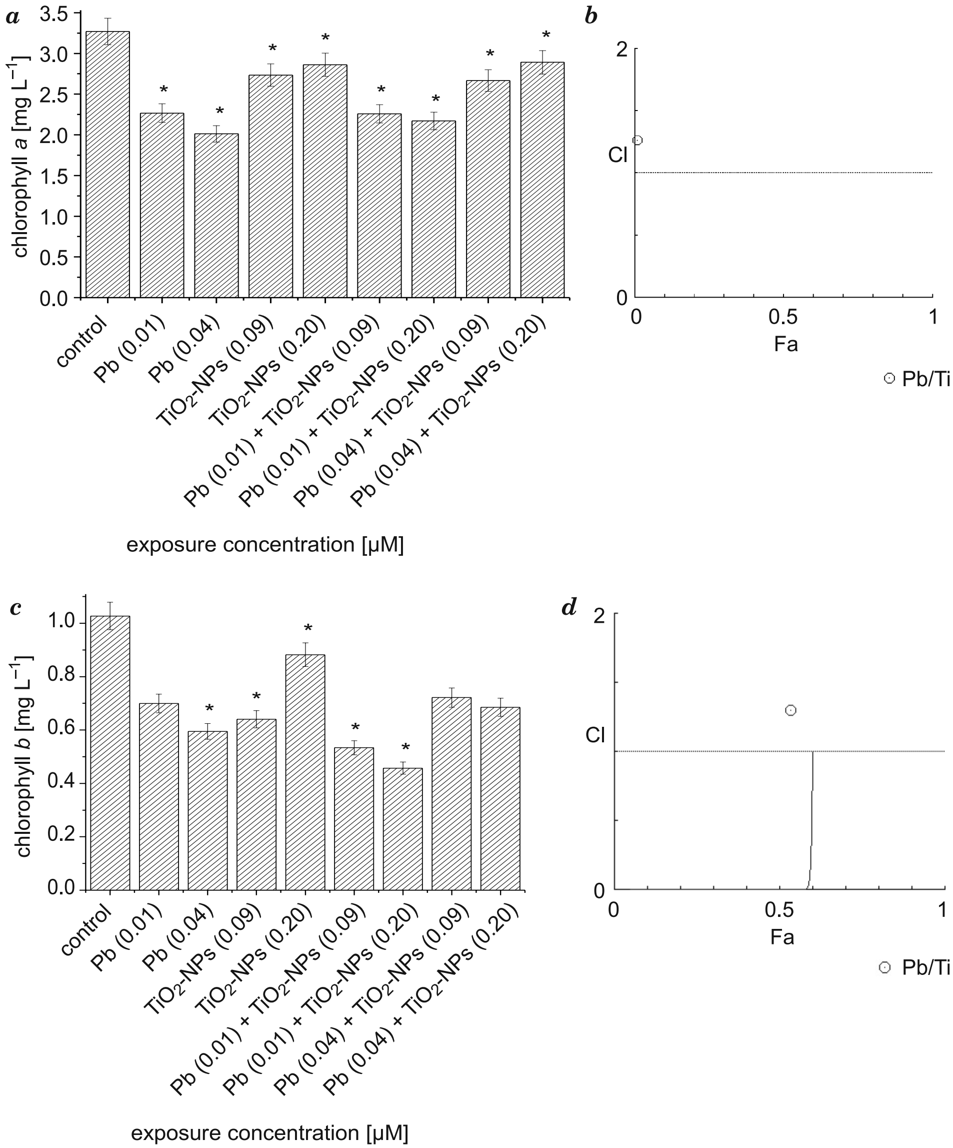
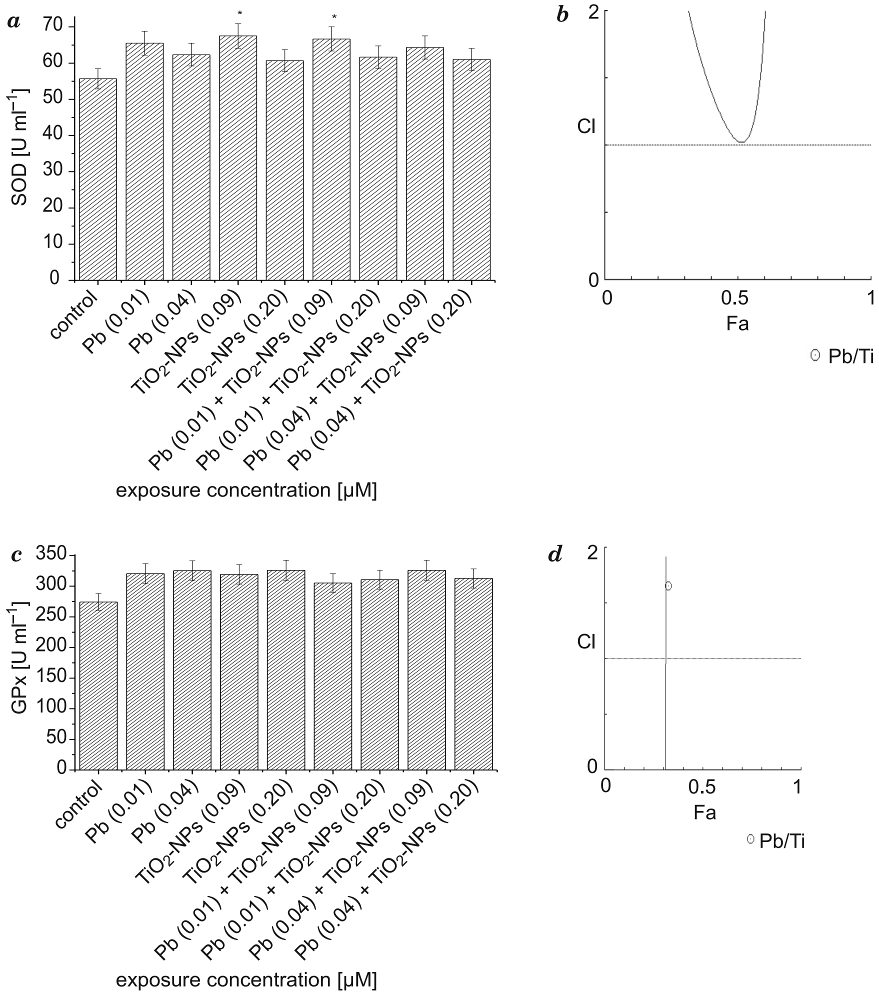


Fig. 4. Chlorophyll responses of *C. ellipsoideum*: *a* – chlorophyll a of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs; [one-way ANOVA, F = 6.505, p = 0.0001.73; threshold replicate were included (n = 3); errors bars indicate Standard deviation (SE), *significant difference; chi-square test (p > 0.05)]; *b* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on cells chlorophyll a of *C. ellipsoideum* [combination index (CI); CI > 1]; *c* – chlorophyll b of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 4.786, $p < 2.6610^{-4}$; threshold replicate were included (n = 3); errors bars indicate; standard deviation (SE), *significant difference; chi-square test (p > 0.05)]; *d* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on cells chlorophyll *b* of *C. ellipsoideum* [combination index (CI); CI > 1]

Oxidative stress and antioxidant enzymes responses of *C. ellipsoideum*

The single and combined chemicals used in this study increased the antioxidant enzymes activities (SOD, GPx, GR and MDA) as shown in Figures 5*a*, 5*c*, 5*e*, and 5*g*. However, one way ANOVA showed that only SOD and MDA were increased significantly ($P < 0.05$) as compared to the control. The antioxidant enzyme activities were observed to increase irrespective of the concentration employed, indicating that responses to the activities were not concentration dependent. Chi-square showed that single and combined chemicals had no significant ($P > 0.05$) increase on antioxidant enzyme activities. Furthermore, the interaction between the



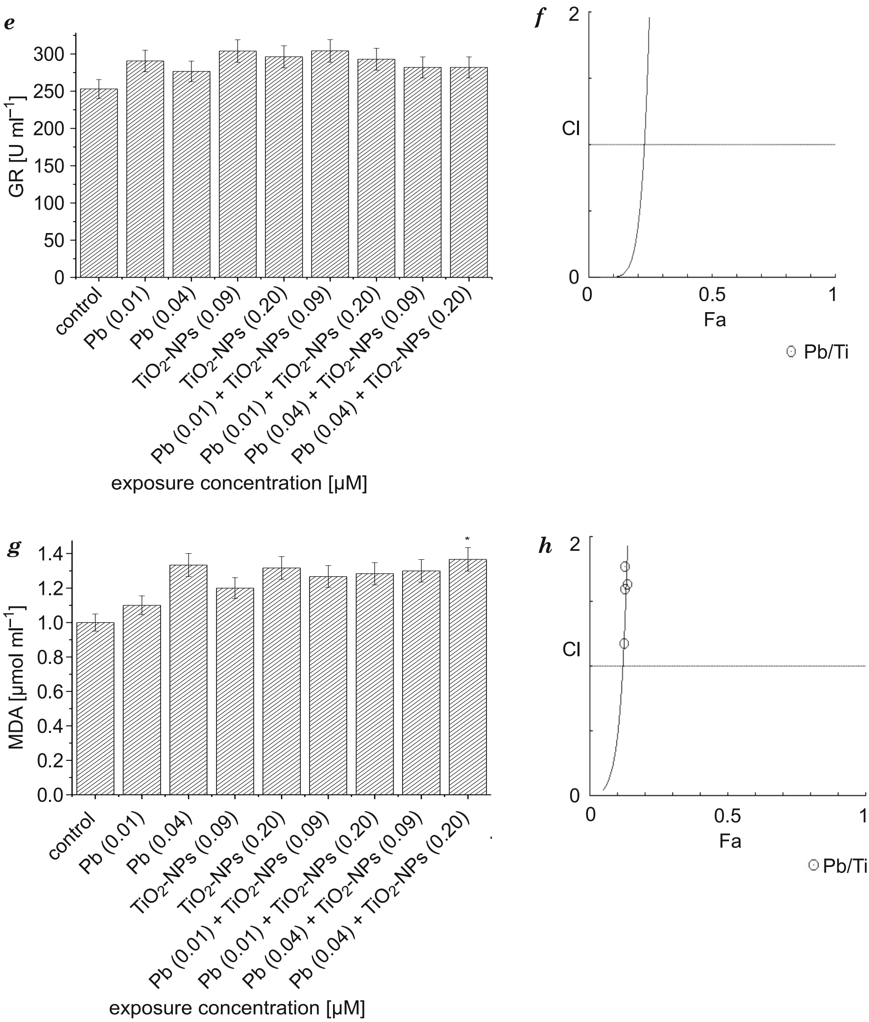


Fig. 5. Oxidative stress and antioxidant enzymes responses of *C. ellipsoideum*: *a* – SOD of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 2.409, p = 0.029; threshold replicate were included (n = 3); errors bars indicate; standard deviation (SE), *significant difference; Chi-square test (p > 0.05)]; *b* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on SOD of *C. ellipsoideum* [combination index (CI)]; *c* – GPx of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 0.573, p = 0.793; threshold replicate were included (n = 3); errors bars indicate standard deviation (SE), *significant difference; chi-square test (p > 0.05); CI > 1]; *d* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on GPx of *C. ellipsoideum* [combination index (CI); CI > 1]; *e* – GR of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 0.816, p = 0.591; threshold replicate were included (n = 3); errors bars indicate standard deviation (SE), *significant difference; chi-square test (p > 0.05)]; *f* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on GR of *C. ellipsoideum* [Combination index (CI); CI > 1]; *g* – MDA of *C. ellipsoideum* exposed to single and combined Pb and TiO_2 NPs [one-way ANOVA, F = 2.299, p = 0.0036; threshold replicate were included (n = 3); errors bars indicate Standard deviation (SE); *significant difference; chi-square test (p > 0.05)]; *h* – combined effect of Pb and TiO_2 NPs (Pb/Ti) on MDA of *C. ellipsoideum* [combination index (CI); CI > 1]

combined chemicals on antioxidant enzymes exhibited strong antagonistic activities with the combination index greater than one (CI > 1) shown in Figure 5*b*, 5*d*, 5*f* and 5*h*.

Discussion

The Langmuir model is a nonlinear sorption which suggests that uptake occurs on a homogeneous surface by monolayer adsorption without interaction between adsorbed molecules (DĄBROWSKI 2001). Based on the q_{max} and the Langmuir constant (b), it can be concluded that TiO_2 NPs highly adsorbed Pb(II) with higher affinity. This result also indicates the highest applicability of the model with the best coefficient $R^2 = 0.99$. The study indicated that the large surface area and pore volume generally known of nanoparticles resulted in higher and favourable adsorption capacity of titanium dioxide nanoparticles probably with consequences on the bioavailability of Pb(II). ZHANG et al. (2010) reported that titanium nanoparticles adsorption of Pb II through electrostactic interaction reduced the bioavailability of Pb II during the acute toxicity interaction of TiO_2 NPs and lead acetate exposed to mice.

Lead (Pb) and TiO_2 NPs either as individuals or in binary mixture decreased the cells density of *Chloroidium ellipsoideum*, indicating the inhibitory effect of these chemicals on the microalgae. The decrease of cells density due to Pb(II) indicated the ability of the chemicals to alter cells division. These results corroborated with the inhibitory effect of some other heavy metal (Cd, Pb) after two days exposure to *Chlorella vulgaris* as reported by BAJGUZ (2011). The decrease of cells density after treatment with TiO_2 NPs could be due to the coating ability of the metal to bind on algal cells and therefore, altering cells reproduction and their growth. A similar report by HARTMAN et al. (2009) which is in agreement with this study, demonstrated the inhibitory effect of alga exposed to TiO_2 NPs with the tendency of the nanomaterial to shade the incidence of light by coating on algal cells. The result revealed that the addition of TiO_2NPs to Pb(II) inhibit the growth of microalgae cells resulting in the reduction of cell density; however the reduction was antagonistic. This could be attributed to the adsorptive effect of TiO_2NPs in the mixture suggesting its ability to prevent the synergy of the combined compounds by hindering the Pb(II) bioavailability and thereby reducing its toxicity. Our result agreed with the findings of MIAO et al. (2012) who reported that combined $nTiO_2$ NPs and Cd (a heavy metal) had alleviating effect on alga growth.

Our result revealed a general decreased of dry weight when exposed to single and combined chemicals ($P > 0.05$). This implied that both compounds either single or mixed could lead to death and reduction of cells in the tested medium. This was in agreement with the findings obtained by SILVERBERG (1977) who reported a decrease of dry weight by 85% and 32% respectively compared to the control on freshwater green algae *Scenedesmus quadricauda* exposed to Tetramethyl lead.

The decrease in chlorophyll *a* and *b* observed in this study exposed to Pb and TiO_2 NPs demonstrated that these chemicals had prevented the photosynthetic activity indicating an absence of modulation of photosynthetic mechanism (PSII) as a result of damage of the thylakoid membranes of the chloroplast (KIRCHOFF 2014, NEELAM and RAI 2003). The interaction between Pb(II) and TiO_2 NPs concentration had an antagonistic effect on chlorophyll production. Titanium dioxide nanoparticles have the capacity to act as a carrier and entrapped nutrient that increased the growth and production of chlorophyll. The binary mixtures antagonistically decreased the chlorophyll content due to the alleviation of the inhibitory effect of Pb(II) by TiO_2 NPs. This study contradicts the result of TANG et al. (2013) who reported a synergistic response of nano-sized titanium dioxide and zinc on *Anabaena* sp.

Superoxide dismutase (SOD), GPx, GR and MDA are indicators of oxidative stress in many organisms including microalgae. Superoxide dismutase increased under the effects of individual Pb II and TiO_2 NPs respectively. This could be attributed to the absorption of these chemicals inside the cells of microalgae thereby causing the formation of reactive oxygen species (ROS). The increase of SOD activity was to quench the reactive oxygen species (ROS). Our results disagreed with that of Fan et al. (2016) who reported a decreased of SOD activity in *Scenedesmus obliquus* exposed to nanomaterials aluminium oxide ($AlO_{3)}$ and copper. The interaction of both compounds led to increase in SOD activity suggestive of the ability to protect against ROS. However, there was antagonistic response of combined compounds in binary mixtures. This result is also not in agreement with the findings of Fan et al. (2016) who reported decreased SOD activity in *Scenedesmus obliquus* exposed to nano-Al_2O_3 and copper.

Glutathione peroxidase and glutathione reductase increased in microalgae treated with single chemicals Pb (II) and TiO_2 NPs respectively, which could be due to the scavenging and inactivation of hydrogen and lipids peroxides thereby protecting the microalga against oxidative stress. Our results agreed with STOIBER et al. (2009) who reported increased GR level following exposure of *Chlamidomonas reinhardhtii* to cadmium. The combined compounds also increased GPx and GR activities in the exposed microalga. The implication is that Pb II and TiO_2 NPs in a mixture inhibit the release of GPx in the cells thereby reducing the protection of the cells against ROS. This is in disagreement with the result of FAN et al. (2016) who reported reduced GPx activity in *Scenedesmus obliquus* exposed to nano-Al_2O_3 and copper. However, there was increased GR response in the presence of Pb/TiO_2NPs indicating an increase in protection against oxidative stress similar to this study.

The increased MDA in all treatments compared to the control was an indication of lipid membrane damage and cell lysis. Our results is consistent with XIONG et al. (2013) who reported increased MDA level in *Chlorella vulgaris* exposed to a xenobiotic metals.

Conclusion

Based on the specific properties of TiO_2 NPs, it is more likely it adsorbs other non-essential metals in water. The study suggests that co-exposure of TiO_2 NPs and Pb(II) decreased the bioavailability of Pb(II). This study demonstrated an adsorptive interaction between TiO_2 NPs and Pb(II). Data from this study revealed that combined exposure of the two chemicals decreased the biomass of *Chloroidium ellipsoideum* and increased antioxidant activities with dosage. We also observed that the mode action of Pb(II) and TiO_2 NPs has an antagonistic effect on biomass and antioxidant activities. Further studies are required to understand the mechanical pathway of the interactions of this binary mixture.

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