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ORIGINAL PAPER

Nitrogen forms and concentration influence the impact of titanium dioxide nanoparticles on the biomass and antioxidant enzyme activities of *Microcystis aeruginosa*

Sadiya Awala Samuel[1] · Mathias Ahii Chia[1] · Waetsi Nya Yusufu[1,2] · Suleiman Dauda[1] · Wisdom Sohunago Japhet[1] · James Dama Habila[3]

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Abstract

Nanoparticles (NPs) are becoming more widely produced, used, and released into the aquatic environment. In aquatic ecosystems, these NPs affect different populations of photosynthesizing organisms, such as cyanobacteria. This study aimed to evaluate the effects of titanium dioxide (TiO_2) NPs (48 mg l^{-1}) combined with low (0.04 mM) and high (9 mM) concentrations of urea and nitrate on *Microcystis aeruginosa*. Microcystins (MCs) production and release were monitored in the cyanobacterium. The results showed that high urea concentration (9 mM) combined with TiO_2 NPs inhibited growth, pigment, and malondialdehyde (MDA) content by 82%, 63%, and 47%, respectively. The treatment also increased the reactive oxygen species (ROS) and glutathione S-transferase (GST) activity by 40.7% and 67.7%, respectively. Similarly, low nitrate (0.04 mM) combined with TiO_2 NPs inhibited growth by 40.3% and GST activity by 36.3% but stimulated pigment production and ROS concentration in *M. aeruginosa.* These responses suggest that high urea combined with TiO_2.NPs and high nitrate combined with TiO_2 NPs induced oxidative stress in cyanobacteria. The peroxidase (POD) activity of *M. aeruginosa* decreased by 17.7% with increasing urea concentrations. Our findings suggest that TiO_2 NPs combined with changing nutrient (urea and nitrate) concentrations may adversely affect cyanobacterial development and antioxidant defense enzymes.

Keywords Titanium dioxide nanoparticles · Nitrogen · Oxidative stress · Microcystin

Introduction

Owing to advances in nanotechnology, the concentration of nanoparticles (NPs) released into water bodies during their manufacture, transport, use, and disposal cycles has been steadily increasing (Ju-Nam & Lead 2008). One of the most popular manufactured nanoparticles is nanosized titanium dioxide (TiO_2 NPs), which is increasingly used in numerous consumer items. The detrimental impact of nanoparticles on aquatic ecosystems has recently garnered much attention (Piotrowska-Niczyporuk et al. 2012). According to estimates, titanium dioxide nanoparticle concentration in effluents from wastewater treatment plants could reach 3 mg/L, which is anticipated to soon rise in the natural ecosystem (Keller et al. 2010; Liang et al. 2019; Xiao et al. 2019; Zhang et al. 2020). To ensure the safety of nanotechnologies and manufacture materials that have minimal adverse effects on aquatic ecosystems, it is essential to carefully analyze the environmental and ecological impacts of extensive nanoparticle use. The worldwide environmental impact of significant NP applications must be considered extensively (Song et al. 2012).

Titanium dioxide nanoparticles have been extensively studied for their inhibitory effects on algae, zooplankton, fish, and higher plants (Chen et al. 2012; Ilona 2008; Wiench et al. 2009). When suspended particles, such as titanium dioxide nanoparticles, come into contact with cyanobacteria, they can alter their photosynthesis and antioxidant defense mechanisms. In some studies, TiO_2 NPs exposure decreased

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✉ Mathias Ahii Chia
chia28us@yahoo.com

[1] Department of Botany, Ahmadu Bello University, Zaria, Nigeria

[2] Department of Biological Sciences, Taraba State University, Jalingo 14, Nigeria

[3] Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

the growth and nitrogen fixation of *Anabaena variabilis*, whereas other studies found no significant association between growth rate and TiO_2 NPs dose in cyanobacterial cultures (Cherchi & Gu 2010; Kulacki & Cardinale 2012). Similarly, *Chlamydomonas reinhardtii* and *Ochromonas danica* exposed to a mixture of Ag nanoparticles (10 nm) and sorbed carboxylated PS nanoparticles (20 nm) suffered an additive decline in their physiological parameters (Huang et al. 2019). However, combining glyphosate and nPS-NH_2 had antagonistic interactive effects on *M. aeruginosa* growth inhibition because they resulted in comparatively lower effects than their effects (Zhang et al. 2018a, b).

There have also been some documented benefits of TiO_2 NPs in contrast to their negative consequences. For example, Zheng et al. (2005) found that TiO_2 NPs increase the germination, growth, dry weight, pigment production, and metabolism of plants. Nanoparticles affect nutritional dynamics, nutrient (nitrogen and phosphorus) uptake, and metabolism (Chaudhary & Singh 2020; Chen et al. 2012). Nitrogen (N) and phosphorus (P) are the most vital nutrients in waterways, but aquatic environments also deal with environmental contaminants, such as TiO_2 NPs (Liang et al. 2019). In a field investigation, Das et al. (2014) found that the interaction of phosphate fluctuations with silver nanoparticles resulted in a 70–90% reduction in phytoplankton biovolumes. The carbohydrate levels of phytoplankton community were dramatically affected by changing nitrogen levels in the presence of n-TiO_2, as opposed to the overall lipid and protein content (Dauda et al. 2020). Microalgal growth, a significant source of nutrition for several small invertebrates and fish, depends on macronutrients (Chaffin & Bridgeman 2014). Cyanobacterial biomass is controlled by nitrate and urea concentrations and availability (Hampel et al. 2019; Li et al. 2016). While nitrogen forms and concentrations are critical in determining phytoplankton growth, aquatic ecosystems face the reality of the presence and increasing concentrations of emerging environmental contaminants like TiO_2 NPs.

Aquatic nitrogen mainly exists in the form of nitrate (NO_3^-), ammonium (NH_4^+), and urea (Donald et al. 2011). Aquatic ecosystems receive increased quantities of bioavailable nitrogen (Elser et al. 2009) and TiO_2 NPs (Tripathi et al. 2016). In light of the likelihood that nanoparticles interact with nutrients that regulate the development of cyanobacteria, it is critical to study the possible interactive effect of nitrogen forms combined with potential pollutants such as TiO_2 NPs (Mahler et al. 2012; Dauda et al. 2017). Studies on the effect of TiO_2 NPs on *M. aeruginosa* under various nitrogen forms and concentrations are scarce. Cyanobacteria under varying nitrogen forms and concentrations are more likely to interact with suspended materials such as TiO_2 NPs, affecting their photosynthesis and antioxidant protection mechanisms. Therefore, this study aimed to examine the effects of TiO_2 NPs under varying nitrogen forms on *M. aeruginosa* growth, oxidative stress response, and microcystin production. The findings of this research will enhance our knowledge of the impact of TiO_2 NPs on aquatic ecosystems. Our results provide insight into the consequences of nitrogen forms, their concentrations, and TiO_2 NPs exposure on *M. aeruginosa*.

Materials and methods

Sample collection and maintenance

The cyanobacterium *M. aeruginosa* (EAWAG 198) was acquired from the Gobler Laboratory of the State University of New York, USA. *M. aeruginosa* was kept in BG11 medium (pH 7.4) under carefully regulated laboratory settings, including light intensity of 40 µmol m^{-2} s^{-1}, photoperiod of 16:8 h light: dark, and temperature of 23 ± 1 °C. Growth medium was autoclaved (121 °C for 30 min) to ensure sterility. The cyanobacteria were acclimated by a series of culture transfers during the exponential growth phase to acquire accurate physiological results. Pre-cultures were all axenic and samples for the studies were grown under nutrient-sufficient conditions. Cultures were in the exponential growth phase when the experimental vessel was filtered and inoculated to ensure enough biomass and the proper physiological state for effective observation.

Experimental design

Nitrate was provided as sodium nitrate ($NaNO_3$) and urea as carbamide (CH_4N_2O). Sigma-Aldrich (Sigma-Aldrich, USA) provided the titanium dioxide anatase nano-powder (CAS number: 637254), having a particle size of 25 nm. The BG 11 medium was used to suspend the TiO_2 powder to prepare a stock solution. We exposed exponentially growing cultures to low nitrate/urea, high nitrate/urea, low nitrate/urea + TiO_2 NPs, and high nitrate or urea + TiO_2 NPs in 250 mL of modified BG 11. At the same time, experimental control cultures were grown in unmodified BG 11 medium without TiO_2 NPs. Each experimental condition was tested in triplicate. All cultures were started at a cell density of 5.0×10^5 cells mL^{-1}. The study lasted seven days, and samples were taken on the first, third, fifth, and seventh days to measure growth, microcystin, biochemical, and antioxidant responses.

Our limited and high concentrations of both urea and nitrate were 0.04 mM and 9 mM, respectively. These concentrations were based on the levels reported in oligotrophic and eutrophic aquatic environments (Reynolds 2006). The actual urea and nitrate concentrations in the medium were determined using the Magnesium Oxide-Devarda alloy

method, where the first distillation in 2% boric acid was with Mg oxide to estimate the ammonia content, and the second distillation in fresh 2% boric acid was performed with Devarda alloy to estimate the nitrate content (Bremner & Keeney 1965). The TiO_2 NPs level used in this study was 48 mg L^{-1}, which showed substantial activity in phytoplankton, according to our previous studies (Dauda et al. 2017, 2020). The increasing discharge of NPs into aquatic ecosystems may present scenarios where organisms are exposed to concentrations approaching the milligram range (Arabia 2010). The Agilent Technologies Calibration mix 1 for AA and ICP OES was used for the preparation of titanium standards. An Agilent 4200 microwave plasma-atomic emission spectroscopy (Agilent Technologies, CA-USA) was used to measure the amount of TiO_2 NPs in the cultures.

Collection of data

Measurements of cell density, growth, and pigment content

Microcystis aeruginosa cells were counted using an improved bright-lined Neubauer hemocytometer under a light microscope. Daily growth observations were also performed using a UV–VIS spectrophotometer to determine optical density at 750 nm. Specific growth rate (μ) was determined according to Liang et al. (2013).

For chlorophyll-*a* quantification, 10 mL of *M. aeruginosa* culture was centrifuged at 4000 rpm for 10 min. The resulting pellets were extracted with 3 mL acetone at − 20 °C for 24 h in the dark. The absorbance of the extracts was measured using a UV–VIS spectrophotometer, and the total chlorophyll-*a* concentrations were calculated using the equation of Ritchie (2006).

Quantification of total proteins and oxidative stress parameters

Cultures were extracted to quantify total proteins and oxidative stress parameters. A 40 mL culture aliquot collected from the cultures was centrifuged at 4000 rpm for 10 min, and the resulting pellets were stored at − 20 °C. The pellets were homogenized in a vortex for 10 s before being mixed with 2.5 mL of 0.1 M phosphate buffer [pH 6.5, containing 1% (*w*/*v*) polyvinylpyrrolidone (PVP)]. The resulting homogenate was centrifuged at 4000 rpm for 10 min, and the supernatant was stored at − 20 °C until analysis.

Bovine serum albumin (BSA) was used as a standard to quantify the total intracellular protein from the extracted supernatant, according to the Bradford (1976) technique. The supernatant (500 µL) was mixed with Bradford reagent (2.5 mL) containing 0.01% Coomassie blue, 4.7% methanol, and 8.5% phosphoric acid. After incubation at room temperature for 5 min, the absorbance was measured at 595 nm. The concentration of BSA used as a standard ranged from 10 to 400 µg mL^{-1} in phosphate buffer solution (pH 7.2).

The Jana & Choudhuri (1982) approach was used to extract and quantify the intracellular hydrogen peroxide (H_2O_2). 400 µL H_2O_2 extract was combined with 133 µL 0.1% titanium chloride in 20% H_2SO_4. After one minute, the absorbance of the red–orange mixture at 410 nm was measured spectrophotometrically. Using the extinction coefficient of 0.28 L $mmol^{-1}$ cm^{-1}, the amount of H_2O_2 in µmol/mg was calculated.

Malondialdehyde (MDA) levels in the cells of *M. aeruginosa* were measured to determine lipid peroxidation according to the method of Heath & Packer (1968). One milliliter of the extract was added to 2 mL 10% trichloroacetic acid and 0.5% thiobarbituric acid. The mixture was maintained at 95 °C in a water bath for 15 min before being cooled quickly in an ice bath. A spectrophotometer was used to read the absorbance of the mixture at 532 and 600 nm, and an extinction coefficient of 155 mM^{-1} cm^{-1} was used to compute the MDA content.

Glutathione S-transferase (GST) activity was assessed following the method of Habig et al. (1974). One hundred microliters of enzyme extract was combined with 2 mL of the reaction mixture (3.6 mM reduced glutathione and 1 mM 1-chloro-2,4-dinitrobenzene in 0.1 M potassium phosphate buffer, pH 6.5) to start the reaction. The GST activity showed a linear relationship with the change in absorbance at 340 nm. The method of Reddy et al. (1996) for measuring peroxidase activity was used. After combining 3 mL of pyrogallol solution (0.05 M in 0.1 M phosphate buffer, pH 6.5) and 0.5 mL of 1% H_2O_2, 0.1 mL of enzyme extract was introduced into the mixture in a cuvette. The activity of the enzyme, measured in nkat per mg protein, was directly related to the change in spectrophotometric absorbance at 430 nm per minute.

Quantification of microcystins

Microcystins were extracted from the cell pellet obtained after centrifuging 20 mL culture aliquots of *M. aeruginosa* for 10 min at 4000 rpm. The pellet was vortexed for 10 s in 3 mL of 80% methanol before storing at − 20 °C. (Turner et al. 2018). Microcystin levels were quantified with the Abraxis Microcystins-ADDA ELISA 96 well plate kit (Eurofins Abraxis Inc., Warminster, PA, USA), with absorbance read at 450 nm.

Statistical analyses

A two-way analysis of variance (ANOVA) was used to measure significant differences among the treatments. Where significant differences occurred, Tukey's HSD test separated the means at a 5% significance level. The homogeneity of variance and normality of the experimental data were determined using Levene's homogeneity of variance and Shapiro–Wilk tests, respectively. Principal component analysis (PCA) was used to ascertain whether the response parameters and treatments were related.

Results

Cell density, chlorophyll content, total protein, and specific growth rate of M. aeruginosa

The cell density of *M. aeruginosa* on day 7 (Fig. 1a) was significantly ($p < 0.05$) reduced by all treatments (low and high nitrogen and urea and their combinations with TiO_2 NPs), with high urea combined with TiO_2 NPs producing the lowest cell density. Chlorophyll *a* content of *M. aeruginosa* (Fig. 1b) declined to a lesser degree than cell density to the treatments, with only high urea and high urea combined with TiO_2 NPs producing significantly ($p < 0.05$) reduced chlorophyll *a*. The growth rate of *M. aeruginosa* (Fig. 1c) was significantly ($p < 0.05$) reduced by urea (both low and

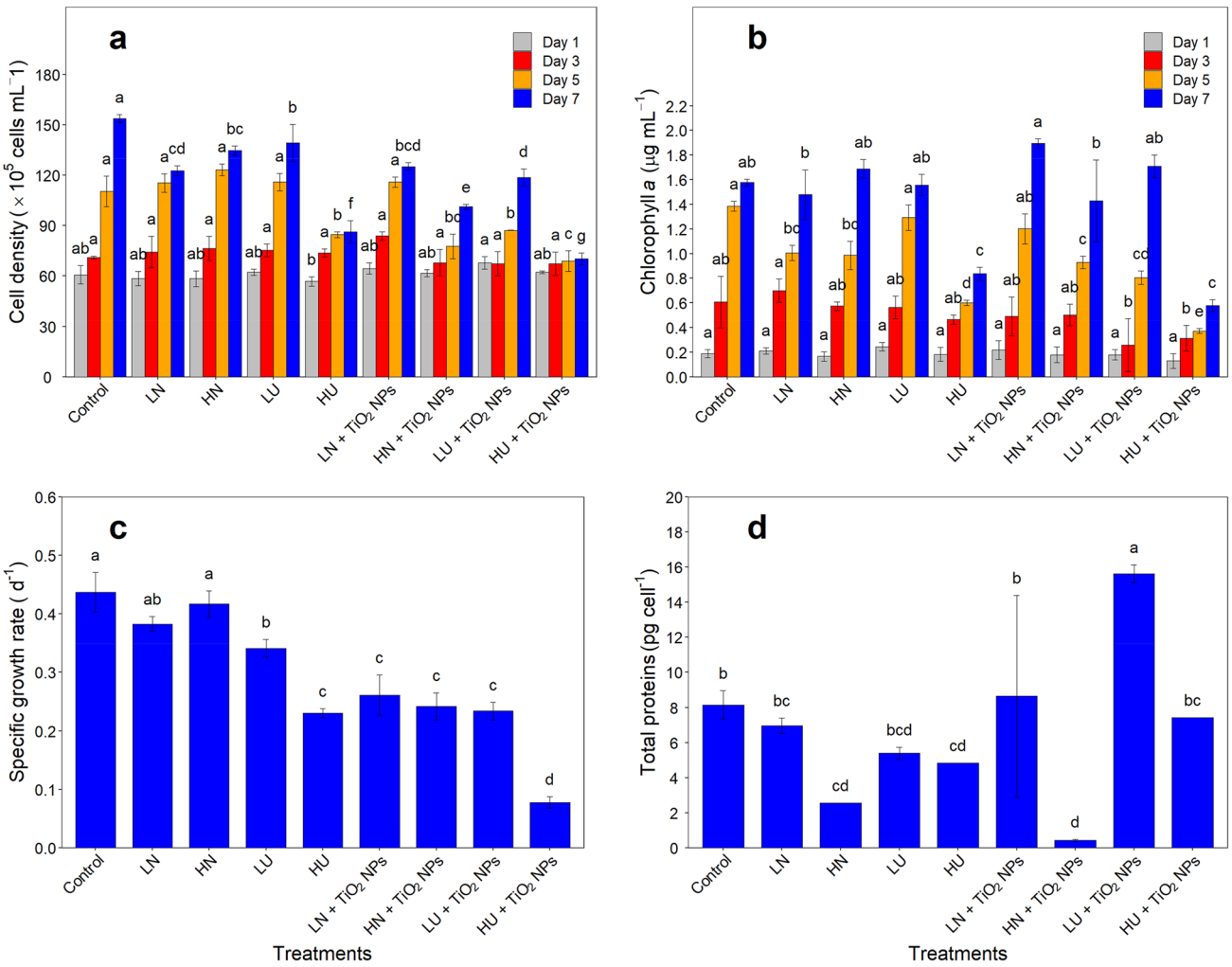

Fig. 1 Cell density (cells mL^{-1}) (**a**), chlorophyll-*a* (μg mL^{-1}) (**b**), total protein (pg $cell^{-1}$) at day 7 (**c**), and specific growth rate (day^{-1}) at day 7 (**d**) of *Microcystis aeruginosa* EAWAG 198 exposed to low and high urea and nitrate concentrations, and their combinations with titanium dioxide nanoparticles. Error bars indicate the standard deviation for a sample size of 3. Bars with different letters are significantly different ($p < 0.05$)

high) and all urea and nitrate combined with TiO_2 NPs treatments, and the total protein content of *M. aeruginosa* was significantly ($P < 0.05$) increased by the low urea combined with TiO_2 exposure, whereas high nitrate and its combination with TiO_2 NPs resulted in the lowest total protein content (Fig. 1d).

Response of Microcystis aeruginosa EAWAG 198 cells to oxidative stress

The intracellular H_2O_2 content of *M. aeruginosa* (Fig. 2a) increased significantly ($p < 0.05$) in the urea treatments (both low and high urea) and in all nitrate and urea combinations with TiO_2 NPs. However, a significant ($p < 0.05$) decrease in H_2O_2 was recorded in nitrate-only (low and high) treatments. The peroxidase activity of *M. aeruginosa* increased significantly ($p < 0.05$) after high urea treatment (Fig. 2b). To a greater degree, the combination of TiO_2 NPs with high and low nutrient (urea and nitrate) concentrations stimulated POD activity ($p < 0.05$). Because of lipid peroxidation, the increase in malondialdehyde (MDA) content can be used to evaluate the extent of membrane lipid damage. High urea significantly ($p < 0.05$) increased the MDA levels of *M. aeruginosa* (Fig. 2c), whereas the combination of high nitrate, and low and high urea with TiO_2 NPs produced the lowest MDA levels. High urea combined with TiO_2 NPs caused the greatest increase in GST activity in *M. aeruginosa*, whereas nitrate (low and high) combined with TiO_2 NPs produced the lowest GST activity (Fig. 2d).

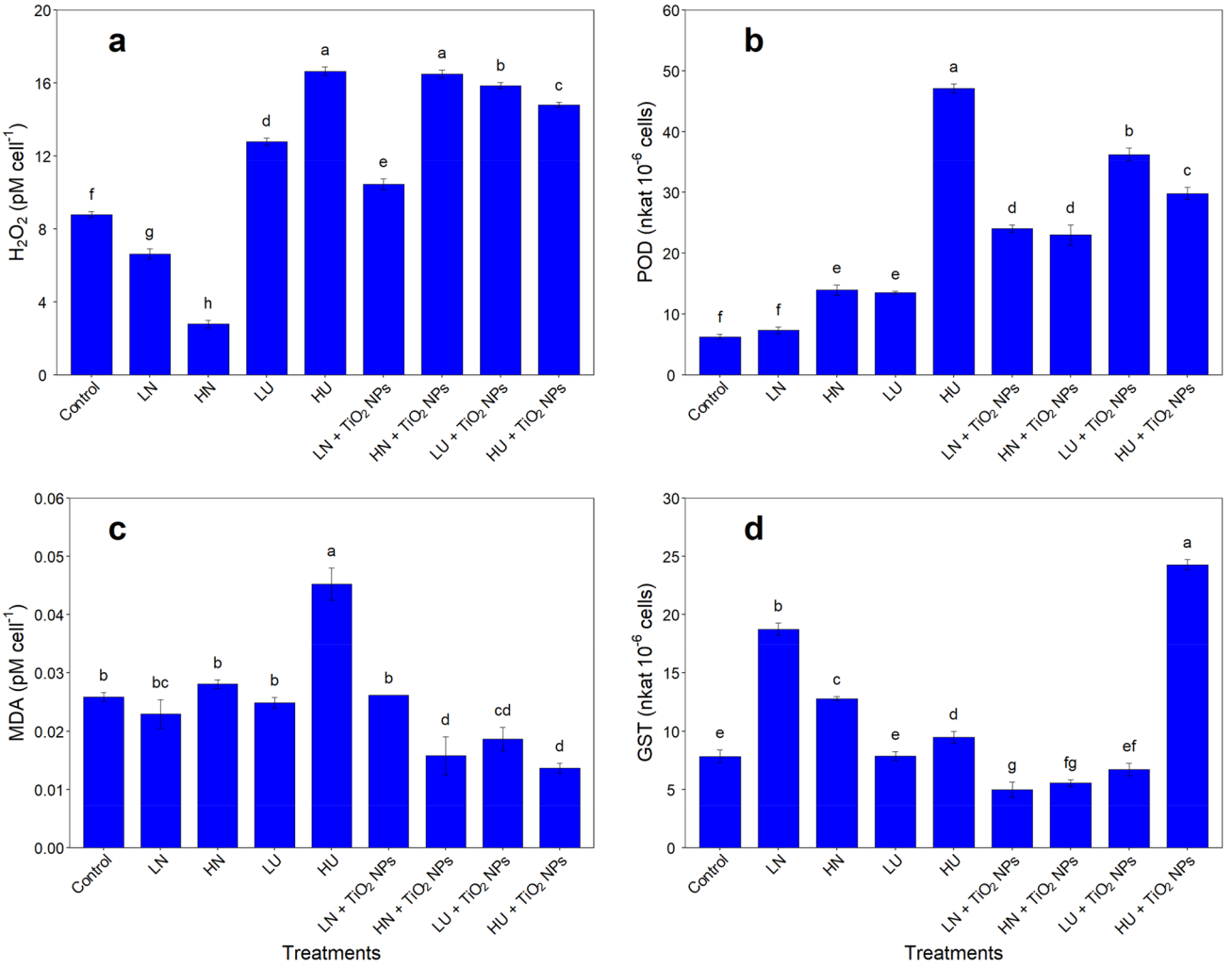

Fig. 2 Changes in intracellular H_2O_2 (pM cell^{-1}) (**a**), malondialdehyde content (pM cell^{-1}) (**b**), peroxidase activity (nKat 10^{-6} cells) (**c**), and glutathione-S-transferase activity (nKat 10^{-6} cells) (**d**) of *Microcystis aeruginosa* EAWAG 198 after 7 days of exposure to low and high urea and nitrate concentrations, and their combinations with titanium dioxide nanoparticles. Error bars show the standard deviation for a sample size of 3. Bars with different letters are significantly different ($p < 0.05$)

Microcystin production by Microcystis aeruginosa

Microcystin concentrations significantly ($p < 0.05$) increased when *M. aeruginosa* was subjected to both low and high amounts of nitrate in the culture medium (Fig. 3). The highest significant ($p < 0.05$) stimulation of microcystins was recorded for low nitrate combined with TiO2 NPs and high urea combined with TiO_2 NPs exposure (Sadiq et al. 2011).

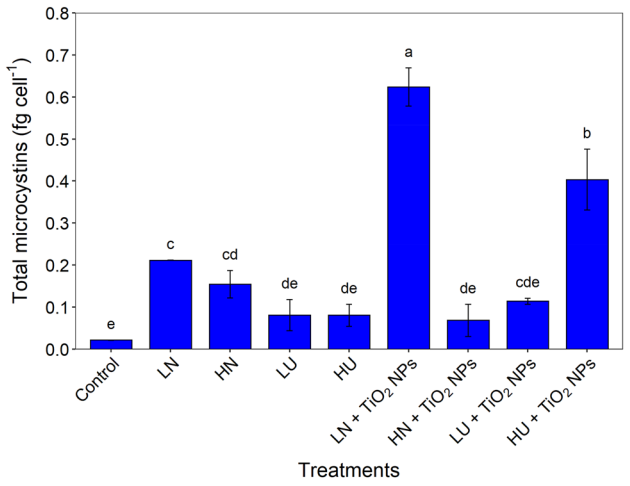

Fig. 3 Microcystins concentration (fg cell^{-1}) of *Microcystis aeruginosa* EAWAG 198 after 7 days of exposure to low and high urea and nitrate and their combinations with titanium dioxide nanoparticles. Error bars show the standard deviation for a sample size of 3. Bars with different letters are significantly different ($p < 0.05$) different

Relationship between response parameters of the investigated organisms and treatment conditions: PCA

The first two principal components represented 61.6% of the total variation in the data generated from experiments involving *M. aeruginosa* (Fig. 4). The highest growth and biomass (cell density and chlorophyll-*a* content) of *M. aeruginosa* were recorded in the control treatment. However, *M. aeruginosa* growth and biomass were negatively correlated with peroxidase activity and H_2O_2 content.

Discussion

Growth and pigment content of Microcystis aeruginosa

In this study, the cell density, chlorophyll *a*, and growth rate of *M. aeruginosa* responded differently to the two concentrations (low and high) of both nitrate and urea and their combinations with TiO_2 NPs. Cell density was most sensitive to the treatments, indicating that cyanobacterial cell division was the first to be impaired. The difference in the growth response of *M. aeruginosa* to nitrate and urea shows that this cyanobacterium preferentially uses nitrate and not urea as a nitrogen source. In this study, we found that high urea (9 mM) inhibited the chlorophyll-*a* content and growth of *M. aeruginosa,* whereas high nitrate (9 mM) level maintained these response variables within the control range. Thus, depending on the nutrient and TiO_2 NPs scenario, cyanobacteria are likely to change their intracellular carbon

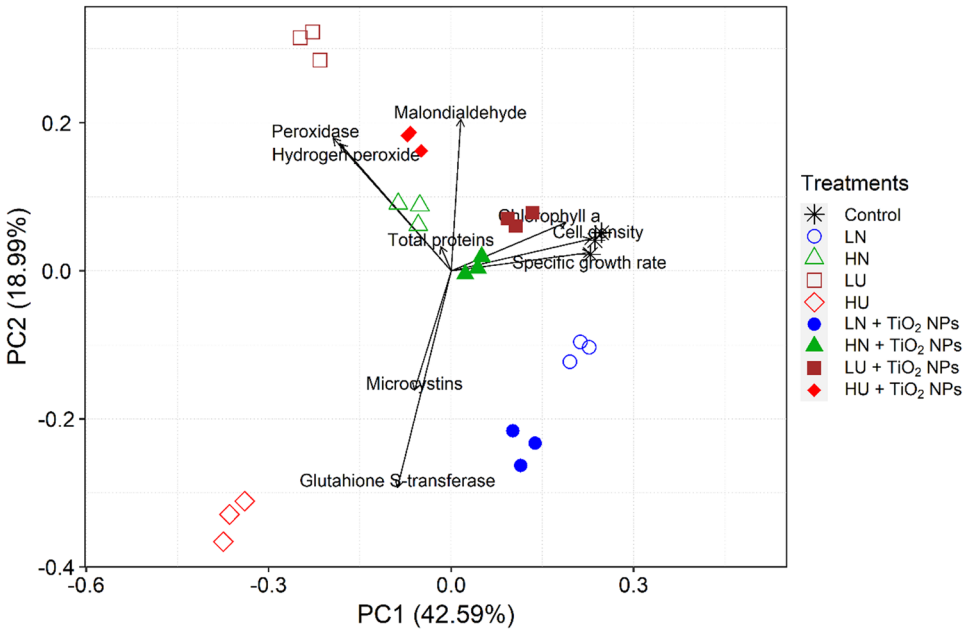

Fig. 4 Principal component analysis biplot of biomass, growth, antioxidant response, and microcystin content of *Microcystis aeruginosa* as a function of low and high nitrate and urea levels and their combination with TiO_2 NPs. The variables on opposing axes are negatively related, while those on the same orthogonal axis are positively correlated

and nitrogen equilibria (Cherchi et al. 2021). Phytoplankton species, such as *Microcystis,* have preferences for the form of nitrogen and the concentration thresholds needed for optimum growth (Glibert et al. 2016).

Our findings extend the understanding of the impact of urea on *M. aeruginosa* growth, showing that while low concentrations maintain high growth rates, high concentrations of urea do not. Thus, the extra nitrogen building blocks and low energy investment (Finlay et al. 2010), when urea is used as a nitrogen source, become compromised at substantially high concentrations, resulting in *M. aeruginosa* growth reduction. Another explanation for the influence of high urea on cyanobacteria may be related to the cost decision by the cell to either produce microcystins or invest in growth. Therefore, the carbon and nitrogen produced by urea hydrolysis are probably used for physiological processes other than growth and photosynthesis, such as toxin synthesis (Erratt et al. 2018; Harke et al. 2016). However, when uptake and cellular incorporation are impaired, high concentrations of ammonium cation in the medium further create unfavorable growth conditions for cyanobacteria and microalgae (Collos and Harrison 2014; Dai et al. 2012; Glibert et al. 2016). Previous studies by Wu et al. (2015) and Dai et al. (2016) revealed that high urea levels hindered the growth and development of *M. aeruginosa,* whereas lower concentrations stimulated growth. On the other hand, our findings support the existing knowledge that *Microcystis* spp. can withstand high nitrogen levels, explaining the non-significant differences between the control and high nitrate conditions. It is also essential to note that the control also contained nitrate as the nitrogen source, albeit at a lower concentration than the high nitrate treatment. These concentrations fall within the levels reported for natural waters that experience *Microcystis* blooms (Brêda-Alves et al. 2021).

A considerable decrease in cell density, growth, chlorophyll *a*, and total protein was recorded under high urea concentration and in combination with TiO_2 NPs. The reduction in the growth of *M. aeruginosa* as a result of the addition of TiO_2 NPs could have been a result of the TiO_2 NPs coating phytoplankton cells and self-shading, which inhibited cell division (Aruoja et al. 2009; Hartmann et al. 2010; Clément et al. 2013). Similarly, Dauda et al. (2017) found that TiO_2 NPs suppressed the growth of *Chlorella vulgaris* in a nutrient-rich environment. Treatment with TiO_2 NPs decreased the growth rate and photosystem II activity, while boosting the LPO of *Scenedesmus obliquus, Anabaena sp.,* and *Chattonella subsalsa* (Li et al. 2020; Tang et al. 2013). These changes indicate the induction of oxidative stress. The high urea concentration in the culture medium could also have aggregated with TiO_2 NPs, causing damage to the cell membrane by increasing its permeability and disrupting its structural integrity (Ma et al. 2014; Perreault et al. 2012). Such changes alter the structural integrity of organelles, consequently affecting their function. Our findings also demonstrated a decline in the chlorophyll-*a* levels of *M. aeruginosa* when exposed to high urea and TiO_2 NPs, which correlated with the additive inhibitory effect recorded on the growth of the cyanobacterium. Following our findings, (Deng et al. 2017) also discovered that 40 mg L^{-1} TiO_2 NPs inhibited photosynthetic activity in *Phaedactylum tricornutum* in a nutrient-replete culture. Exposure to TiO_2 NPs has been associated with reduced chlorophyll content, which leads to a reduction in electron transport through PSII, and an overall decrease in photosynthetic activity in phytoplankton (Wu et al. 2019). High nitrogen generally interacts with different pollutants to cause antagonistic effects, as our findings showed that high nitrate and urea combined with TiO_2 NPs had an interactive effect in reducing the cell density of *M. aeruginosa*.

Oxidative stress, POD, and GST activity of Microcystis aeruginosa

During photosynthesis, chloroplasts are more likely to produce more reactive oxygen species ROS, which leads to high cellular ROS levels. Phytoplankton stress leads to ROS overproduction, which can disrupt normal metabolism by oxidizing lipids, nucleic acids, and proteins. Our study showed that high urea and nitrate induced high intracellular H_2O_2 content, lipid peroxidation, and antioxidant enzyme activity (POD and GST) in *M. aeruginosa*. Fluctuations in POD and GST activities defend cells against the harmful effects of ROS and lipid peroxidation (Pejić et al. 2009). The combination of TiO_2 NPs with different nitrate and urea concentrations increased the levels of H_2O_2, but to varying degrees depending on the nutrient concentration. A smaller increase in H_2O_2 was produced in the TiO_2 NPs treatment combined with high nitrate than in that combined with high urea, which means that high nitrate provided a better growth condition. This hypothesis is further supported by the change in POD activity, which is not surprising as H_2O_2 is among the major substrates of the enzyme. Despite the differences in POD and GST activities to suppress the toxic effects of ROS (Pejić et al. 2009; Xia et al. 2014) induced by the investigated conditions, membrane damage via peroxidation was significantly increased by high urea treatment.

Despite being dependent on the form and concentration of nitrogen, the changes in oxidative stress recorded in *M. aeruginosa* when subjected to TiO_2 NPs under different nutrient conditions confirmed the TiO_2 NPs' stress-inducing mechanism in cyanobacteria (Melegari et al. 2013; Planchon et al. 2013). The augmented GST activity observed under high urea plus TiO_2 NPs revealed that the enzyme detoxified the TiO_2 NPs (Li et al. 2015; Xu et al. 2021). GST repairs oxidized macromolecules in damaged cellular organelles. Hence, the highest GST activity was observed

under the treatment (high urea combined with nanoparticles) that caused the most elevated stress and growth inhibition.

Microcystin production

Microcystis spp. toxin concentrations can be regulated in part by nutrients (Chia et al. 2018). Microcystins biosynthesis is associated with nitrogen availability (Harke et al. 2016; Harke & Gobler 2013). In the current study, the stimulation of microcystin production by *M. aeruginosa* when treated with low and high nitrate concentrations was most likely caused by toxin production in these circumstances. It is generally hypothesized that microcystins are produced in proportion to their growth. Nevertheless, *M. aeruginosa* cultured under low/high nitrate and high urea conditions in this study had a decreased growth rate and increased microcystin concentration. Accordingly, our results showed that nitrogen forms and concentrations affected microcystin production, but did not show that microcystin production was controlled by growth. This outcome is in agreement with previous findings (Chen et al. 2019; Peng et al. 2018; Wagner et al. 2021). We discovered that a combination of low nitrate and high urea with titanium dioxide increased microcystin formation. This stimulation could be because microcystin is synthesized as a defense mechanism to protect/adapt the cells to stress (Chen et al. 2010; Wang et al. 2017). In several studies, increased microcystin production has also been linked to the suppression of protein membrane formation and cell permeability (Zhang et al. 2018a, b). Microcystin synthesis is promoted by high inorganic nitrate (Hu 2006; Pimentel et al. 2014) and low urea concentrations, but it is decreased by high urea concentration (Hu 2006; Pimentel et al. 2014; X. Wu et al. 2015). However, some studies show that amino acids and urea can make *M. aeruginosa* produce more microcystins.

Conclusion

The results of this study reveal variable responses of *M. aeruginosa* to urea and nitrate levels, together with their combinations with TiO_2 NPs, depending on the treatment conditions. Cyanobacteria were more sensitive to changes in urea conditions with and without TiO_2 NPs. Specifically, growth and chlorophyll a synthesis inhibition were recorded, and oxidative stress was generally induced when *M. aeruginosa* was exposed to high urea and high urea combined with TiO_2 NPs conditions. Variations in pigment content lead to changes in light absorption and photosynthesis. These findings indicate that the increasing presence of urea and nanometals in the environment could influence aquatic organisms and decrease the population structure and primary productivity of *M. aeruginosa* in the aquatic environment. Therefore, the effect of TiO_2 NPs on aquatic organisms in water bodies may depend on the form and concentration of nitrogen. Adding TiO_2 NPs to high urea disrupted normal metabolism by oxidizing lipids, resulting in lipid peroxidation by 43% compared to the control. Microcystin production was also stimulated by high urea and low nitrate combined with TiO_2 NPs by 81% and 97%, respectively, to combat the adverse effects of the induced stress.

Author contributions SSA collected data and wrote the main manuscript text, and MAC and SD performed data analysis and prepared all the Figs. 1, 2, 3, 4. WNY, MAC, WSJ, and JH supervised the project and revised the manuscript. All authors reviewed and approved the final manuscript.

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Data availability The corresponding author will provide the datasets produced and analyzed during the current work upon request.

Declarations

Conflict of interest The authors state that they have no known competing financial interests or personal ties that could be perceived as having influenced the work described in this study.

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