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Effect of titanium dioxide (TIO_2) nanoparticles on photosynthetic pigments, lipids and antioxidant activity of *D. salina*

Prateek Dutt Rawal, GP Singh*
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Abstract

Dunaliella salina, a halophilic green microalga, is recognized for its robust adaptability and commercial potential in producing high-value compounds such as β-carotene and lipids. Its exceptional capacity for β-carotene accumulation, particularly the 9-cis isomer, combined with its adaptability to harsh environmental conditions, solidifies its status as a premier candidate for natural β-carotene production. Its ability to modulate intracellular glycerol levels enables it to thrive under extreme salinity, temperature, and light conditions, making it a resilient model organism in ecotoxicological studies. In this study, the impact of varying concentrations of Titanium dioxide nanoparticles (TiO_2 NPs) ranging from 100 to 5000 ppm was assessed on the photosynthetic pigment profile of *D. salina* over a five-week period. Chlorophyll a, chlorophyll b, and total carotenoids were quantified weekly to evaluate photosynthetic performance. Also, lipid content and antioxidant activity at the end of 5 weeks was also quantified to establish a better correlation between Nanoparticle concentration and Oxidative stress due to ROS generation. The results demonstrated a marked, dose- and time-dependent decline in pigment content, with reductions reaching up to 95% in chlorophyll a, 99.44 % in chlorophyll b, and 94.22% in total carotenoids at the highest concentrations. Lipid content and antioxidant activity at the end of 5 weeks showed enhancement in lipid content of 33.84 % more than control sample and Antioxidant activity spike of 24.28%. These findings highlight the significant phytotoxic effects of Titanium dioxide nanoparticles and emphasize the need for further research into their mechanisms of toxicity and the development of mitigation strategies to safeguard aquatic ecosystems from nanoparticle contamination. Additionally, these results have implications for augmenting lipid contents for biofuel production.

Keywords: *Dunaliella salina*; titanium dioxide nanoparticles, chlorophyll a, chlorophyll b, carotenoids, lipids, DPPH assay, nanotoxicity, aquatic ecotoxicology

Introduction

The manufacture of engineered nanomaterials has amplified exponentially over the past years. This increase has resulted in engineered nanoparticles (NPs) being inevitably released into aquatic systems from the usual sources, including individual care products, urban and industrial sewage, and anti-fouling components of paints which eventually end up in the ocean (Handy *et al.*, 2008 [11]; Klaine *et al.*, 2008 [26]; Matranga and Corsi, 2012) [20]. Consequently, coastal waters are expected to represent the ultimate sink for NPs (Canesi *et al.*, 2012) [6]. Nanoparticles, as emerging contaminants, have become an environmental concern due to their increasing production and application. Once released into the environment, these nanoparticles can enter aquatic systems through various pathways, including atmospheric deposition, wastewater discharge, industrial runoff, and landfill leachate. Their persistence and bioactivity raise growing concerns about potential toxicity and long-term ecological effects, especially in aquatic ecosystems.

Among the various types of NPs, TiO_2 NPs are currently of great interest. TiO_2 NPs are widely used for many applications such as sunscreens, paints, coatings, solar cells, and photocatalytic water purification (Botta *et al.*, 2011) [5]. For Europe and USA, most predicted environmental concentrations in surface water were projected for TiO_2 NPs compared with other NPs such as ZnO NPs, Ag NPs, carbon nanotubes (CNTs), and fullerenes (Gottschalk *et al.*, 2009) [10]. Interestingly, latest eco-toxicological data exhibited that TiO_2 NPs were toxic to marine organisms, such as cyanobacteria (Cherchi and Gu, 2010) [7]. However, the widespread use of TiO_2 NPs has led to their accumulation in natural water bodies, raising concerns about their potential impact on aquatic microorganisms, particularly microalgae (Aruoja *et al.*, 2009; Hund-Rinke & Simon, 2006; Hartmann *et al.*, 2010) [13]. Microalgae are fundamental to aquatic ecosystems, forming the base of the food web and playing key roles in biogeochemical cycles and ecological stability (Field *et al*., 1998) [9]. *Dunaliella salina*, a halotolerant green microalga, is particularly notable for its ability to survive in hypersaline environments and for its industrial value in producing β-carotene, glycerol, and other bioactive compounds (Borowitzka, 2013) [4]. Thanks to its environmental resilience and high sensitivity to stressors, *D. salina* has been widely used as a model organism to assess the ecotoxicological effects of environmental pollutants, including nanoparticles (Khan *et al*., 2015) [15]. It is known that the cell wall is the primary site for NP interaction with algae and acts as a main barrier to the uptake of NPs. The newly synthesized cell wall could be more permeable to entrance of NPs (Ovečka *et al*., 2005) [22]. Additionally, the cells and NPs interact such that new pores that are larger in size compared with general pores are induced, which may increase the likelihood of NP internalization through the cell wall (Navarro *et al.*, 2008) [21]. These interactions often trigger the production of reactive oxygen species (ROS), leading to oxidative stress. As a result, key physiological processes such as photosynthesis, pigment synthesis, and cell division can be disrupted. Studies have shown that TiO_2 NP exposure can damage chloroplast structures, lower chlorophyll content, and impair the function of the photosynthetic machinery in species like *Dunaliella salina*, *Chlorella pyrenoidosa*, and *Scenedesmus obliquus* (Keller *et al.*, 2010; Ma *et al.*, 2019; Liu *et al.*, 2020) [16]. These changes ultimately reduce algal growth and productivity, raising concerns about the broader ecological impact of nanoparticles in aquatic environments.

The current study aims to investigate the impact of Titanium dioxide nanoparticles (TiO_2 NPs) on the photosynthetic pigment profile and Lipid Content of *Dunaliella salina*. Photosynthetic performance was assessed by measuring the concentrations of chlorophyll a, chlorophyll b, total carotenoids over a five-week exposure period. Lipid content and Antioxidant activity was measured after completion of 5 weeks. This study evaluates the dose-dependent responses of *D. salina* to varying concentrations of TiO_2 NPs, providing insights into nanoparticle-algae interactions and contributing to ecological risk assessments of nanomaterials in aquatic ecosystems.

Materials and Methods

Isolation and culture of *D. salina*

Alga *Dunaliella salina* were isolated from Sambhar Lake, Rajasthan. Stocks were cultured under laboratory conditions with the temperature of 25±2°C, 2M of salinity and photoperiod of 16:8 light/dark cycle in ASWM medium.

Treatment

Treatment of different concentrations of nanoparticles (Ti-Nps) was given by preparing culture media (ASWM) containing nanoparticles of different concentrations ranging from 100 ppm to 1000 ppm and 1000 ppm to 5000 ppm. The media thus prepared was sonicated for 10 minutes in an ultrasonic bath.

Cultures were initially inoculated with about $5x10^4$ algal cells. *Dunaliella salina* culture were used as control. The experiment was carried out in triplicate for a 5-week period. Details of the used nanoparticles is given in Table 1.

Table 1: Characteristics of the used Titanium dioxide nanoparticles

S.No.	Characteristic	
1.	Chemical Formula/STRUCTURE	TiO_2/ O=Ti=O
2.	Molecular Weight	79.866 g/mol
3.	CAS No.	1317-180-2
4.	Bulk Density	0.15-0.25 g/cm^3
5.	Morphology	Nearly Spherical
6.	Color	White
7.	Density	4.23 g/cm^3
8.	Thickness	30-50 nm
9.	Surface Area	200-230 m^2/g
10.	Purity	99.90%
11.	Physical Form	Powder

Chlorophyll estimation

Chlorophyll content was determined using an extraction method with 90% acetone. The chlorophyll content was measured using a UV-Vis spectrophotometer (Lasany model L1 2704) across a wavelength range of 400–700 nm, using 90% acetone as the blank. The concentrations of chlorophyll a and chlorophyll b were calculated using the SCOR-UNESCO (1966) [24] equations.

Carotenoid estimation

For the estimation of carotenoids, 50 mg of dried *Dunaliella salina* algal powder was homogenized in 50 mL of 80% acetone to extract the pigments. The carotenoid content in the extract was determined spectrophotometrically by measuring absorbance at 480 nm and 510 nm. The pigment concentrations were calculated and expressed in mg/g fresh weight, following the methods described by Arnon (1949) [1] and Mahadevan & Sridhar (1982) [18].

Lipid Estimation

100 mg of fine powdered algal biomass was added to 2 mL n-hexane and sonicated for 30 minutes at 100 W power input, following a modified protocol based on Hui, Meng, and Kassim (2023) [14]. Post sonication, the mixture was filtered, and lipid was weighed after removal of solvent in the rotavapor.

DPPH Radical Scavenging Assay

To evaluate antioxidant activity, the crude methanolic extracts from both control and treated cultures were assessed using the DPPH free radical scavenging method, following the protocol described by Blois (1958) [2]. Extracts were diluted in methanol to a final concentration of 250 µg/mL and mixed with 100 µM DPPH solution. Ascorbic acid (100 µg/mL) served as the positive control. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Lasany model L1 2704).

Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD), along with the corresponding percent change and significance level. Statistical analysis was carried out using Microsoft Excel, with the Student’s t-test applied to determine significant differences between treatment groups. A *p*-value less than 0.05 was considered indicative of statistical significance.

Results

Effect of various concentrations of TiO_2 nanoparticles on chlorophyll a content in *Dunaliella salina*

As per the results in Table 2 and figure 2, the chlorophyll a content of *Dunaliella salina* showed a consistent decline in response to increasing concentrations of TiO_2 nanoparticles, indicating a dose-dependent effect on chlorophyll a across all five weeks.

In Week 1, the chlorophyll a content showed a marked decrease in response to increasing concentrations of TiO_2 nanoparticles. At 5000 ppm, chlorophyll a level decreased by 91.78 %, indicating significant inhibition of photosynthetic activity at higher concentrations. In Week 2, the decrease in chlorophyll a content persisted, with reductions ranging from 27.52 % at 100 ppm to 87.54 % at 5000 ppm. By Week 3, chlorophyll a levels were still significantly reduced at higher nanoparticle concentrations. The decline ranged from 41.91 % at 100 ppm to 93.74 % at 5000 ppm, minimum reduction of 34.54 % was observed at 700 ppm concentration. In Week 4, the decline in chlorophyll a content continued across all concentrations, with the greatest reduction of 89.89 % at 5000 ppm, least reduction (-17.98 %) was observed at 700 ppm concentration. By Week 5, chlorophyll a content showed the highest reduction of 95.09 % at 5000 ppm, indicating nearly complete suppression of chlorophyll a production at the highest concentration. The decline was less severe in the lower concentrations, particularly at 100 ppm (22.87 %) while, least 11.02 % reduction of Chl a was observed at 700 ppm concentration.

Table 2: Effect of various concentrations of TiO_2 nanoparticles on chlorophyll a content in *Dunaliella salina* (µg/ml)

S.No.	Conc. of TiO_2 (ppm)	Week 1	Week 2	Week 3	Week 4	Week 5
1	control	2.75 ± 0.07	4.83 ± 0.06	7.69 ± 0.08	15.73 ± 0.14	27.06 ± 0.09
2	100	1.76 ± 0.28 (-36.22 %) *	3.5 ± 0.29 (-27.52 %) ns	4.47 ± 0.44 (-41.91 %) *	9.29 ± 0.37 (-40.96 %) *	20.87 ± 0.22 (-22.87 %) *
3	200	1.37 ± 0.02 (-50.11 %) *	3.23 ± 0.17 (-33.16 %) *	3.39 ± 0.69 (-55.96 %) *	9.56 ± 0.1 (-39.23 %) **	18.3 ± 0.5 (-32.38 %) *
4	300	1.48 ± 0.02 (-46.35 %) **	2.85 ± 0.34 (-40.94 %) *	2.5 ± 0.04 (-67.48 %) **	8.61 ± 0.53 (-45.24 %) *	16.02 ± 0.04 (-40.8 %) **
5	400	1.46 ± 0.28 (-46.83 %) ns	2.71 ± 0.43 (-43.98 %) *	3.75 ± 0.11 (-51.29 %) *	9.47 ± 0.63 (-39.82 %) *	20.86 ± 0.3 (-22.92 %) *
6	500	1.67 ± 0.1 (-39.33 %) *	3.11 ± 0.57 (-35.68 %) ns	4.64 ± 0.36 (-39.67 %) *	10.7 ± 0.38 (-31.95 %) *	21.21 ± 0.3 (-21.63 %) **
7	600	1.83 ± 0.09 (-33.66 %) *	3.19 ± 0.17 (-33.95 %) *	4.63 ± 0.44 (-39.84 %) *	12.48 ± 0.51 (-20.66 %) *	21.66 ± 0.13 (-19.96 %) **
8	700	1.91 ± 0.06 (-30.7 %) *	3.33 ± 0.06 (-31.02 %) *	5.04 ± 0.08 (-34.54 %) ***	12.9 ± 0.03 (-17.98 %) **	24.08 ± 0.45 (-11.02 %) *
9	800	1.48 ± 0.03 (-46.41 %) *	2.86 ± 0.44 (-40.8 %) *	4.02 ± 0.77 (-47.76 %) ns	10.89 ± 0.49 (-30.75 %) *	23.65 ± 0.17 (-12.61 %) **
10	900	1.16 ± 0.09 (-57.81 %) *	2.2 ± 0.87 (-54.39 %) ns	2.24 ± 0.05 (-70.89 %) **	9.68 ± 0.27 (-38.47 %) *	19.91 ± 0.39 (-26.4 %) **
11	1000	0.82 ± 0.06 (-70.12 %) **	1.62 ± 0.29 (-66.44 %) *	2.18 ± 0.05 (-71.67 %) **	8.09 ± 0.83 (-48.54 %) *	16.09 ± 0.12 (-40.55%) **
12	2000	0.6 ± 0.01 (-78.06 %) **	1.53 ± 0.21 (-68.24 %) *	2.16 ± 0.19 (-71.87 %) *	7.69 ± 0.6 (-51.12 %) *	12.74 ± 0.53 (-52.94 %) **
13	3000	0.31 ± 0.02 (-88.65 %) **	0.94 ± 0.05 (-80.45 %) **	1.4 ± 0.55 (-81.84 %) *	6.26 ± 0.34 (-60.18 %) **	10.21 ± 0.35 (-62.25 %) **
14	4000	0.24 ± 0.02 (-91.33 %) **	0.83 ± 0.02 (-82.74 %) **	0.77 ± 0.03 (-90.03 %) **	2.29 ± 0.3 (-85.45 %) **	4.06 ± 0.13 (-84.98 %) ***
15	5000	0.23 ± 0.03 (-91.78 %) **	0.6 ± 0.05 (-87.54 %) **	0.48 ± 0.01 (-93.74 %) **	1.59 ± 0.45 (-89.89 %) **	1.33 ± 0.52 (-95.09 %) **

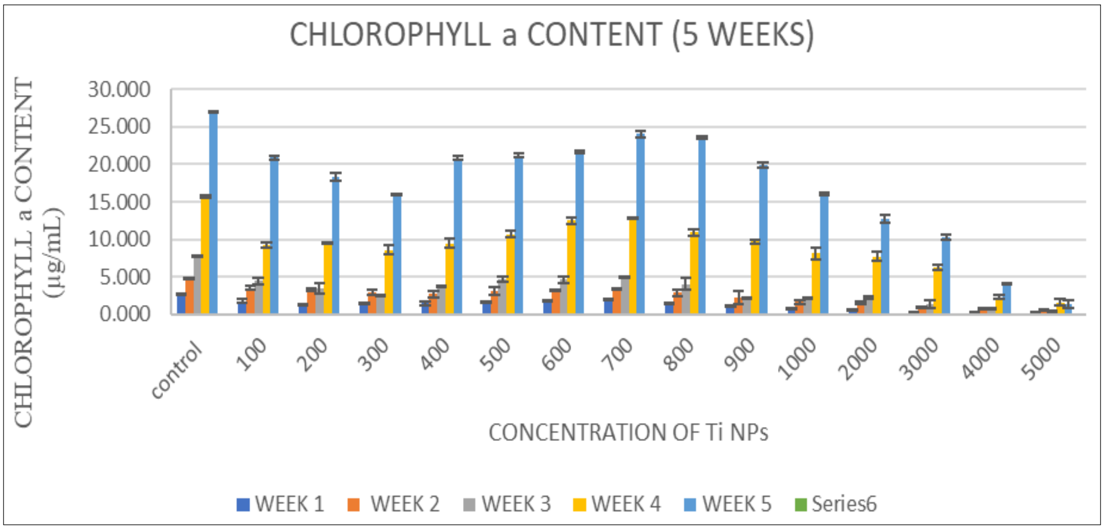

Fig 2: Effect of various concentrations of TiO_2 nanoparticles on chlorophyll a content in *Dunaliella salina* (µg/ml)

Effect of various concentrations of TiO_2 nanoparticles on chlorophyll b content in *Dunaliella salina*

As per the results in Table3 and Figure 3, the chlorophyll b content of *Dunaliella salina* showed a consistent decline in response to increasing concentrations of TiO_2 nanoparticles, indicating a dose-dependent effect on chlorophyll b across all five weeks.In Week 1, chlorophyll b levels showed a decline like that of chlorophyll a. The reduction ranged from 32.40 % at 100 ppm to 99.44 % at 5000 ppm. In Week 2, chlorophyll b content remained low at higher concentrations, showing a decrease from 19.58 % at 100 ppm to 87.35 % at 5000 ppm, minimum decline in Chl b amount was observed at 700 ppm (1.97%). By Week 3, chlorophyll b continued to decline, with the reduction of 29.76 % at 100 ppm to 93.18 % at 5000 ppm. Minimum reduction of 14.16 % was found at 700 ppm. In Week 4, chlorophyll b content was again reduced at all nanoparticle concentrations, with the greatest reduction (92.21 %) at 5000 ppm. At the lower concentrations, there was a decrease of around 25-40%, indicating some resilience at these levels. By Week 5, chlorophyll b levels had almost completely diminished at higher nanoparticle concentrations, particularly at 5000 ppm (89.36 %), while lower concentrations still displayed marked decreases in pigment content while 700 ppm concentration exhibited minimum decline (1.17%).

Table 3: Effect of various concentrations of TiO_2 nanoparticles on chlorophyll b content in *Dunaliella salina* (μg/ml)

S.No.	Conc. of TiO_2 (ppm)	Week 1	Week 2	Week 3	Week 4	Week 5
1	control	0.45 ± 0	0.95 ± 0.07	2.05 ± 0.17	3.49 ± 0.31	5.74 ± 0.05
2	100	0.3 ± 0.01 (-32.4 %) **	0.76 ± 0.01 (-19.58 %) ns	1.44 ± 0.05 (-29.76 %) *	2.61 ± 0.18 (-25.29 %) ns	5.18 ± 0.02 (-9.8 %) *
3	200	0.29 ± 0.01 (-34.64 %) *	0.7 ± 0.07 (-26.1 %) ns	0.99 ± 0 (-51.55 %) *	2.71 ± 0.33 (-22.32 %) **	4.49 ± 0.01 (-21.85 %) **
4	300	0.37 ± 0.02 (-17.65 %) ns	0.7 ± 0 (-25.66 %) ns	1.21 ± 0.49 (-40.89 %) *	2.51 ± 0.49 (-28.24 %) *	4.47 ± 0.62 (-22.18 %) ns
5	400	0.37 ± 0.01 (-18.62 %) *	0.73 ± 0.1 (-22.36 %) *	1.39 ± 0.29 (-32.4 %) *	2.66 ± 0.19 (-23.76 %) *	5.19 ± 0.05 (-9.61 %) **
6	500	0.43 ± 0.01 (-3.42 %) ns	0.75 ± 0.06 (-20.24 %) ns	1.58 ± 0.18 (-22.98 %) *	2.95 ± 0.29 (-15.4 %) **	5.32 ± 0.04 (-7.28 %) *
7	600	0.44 ± 0.01 (-3.14 %) ns	0.79 ± 0.14 (-16.45 %) ns	1.51 ± 0.34 (-26.26 %) *	3.18 ± 0.21 (-8.86 %) ns	5.37 ± 0.07 (-6.51 %) ns
8	700	0.44 ± 0.01 (-1.46 %) ns	0.93 ± 0.05 (-1.97 %) ns	1.76 ± 0.17 (-14.16 %) **	3.1 ± 0.03 (-11.32 %) ns	5.67 ± 0.07 (-1.17 %) ns
9	800	0.44 ± 0.02 (-2.95 %) ns	0.7 ± 0 (-26.36 %) ns	1.22 ± 0.01 (-40.34 %) *	2.78 ± 0.58 (-20.5 %) ns	5.11 ± 0.08 (-10.94 %) *
10	900	0.21 ± 0.06 (-52.47 %) ns	0.51 ± 0.14 (-46.29 %) ns	0.95 ± 0.41 (-53.77 %) *	2.52 ± 0.18 (-27.69 %) ns	4.97 ± 0.08 (-13.41 %) **
11	1000	0.15 ± 0.01 (-67.37 %) **	0.41 ± 0.04 (-56.48 %) *	0.82 ± 0.04 (-59.81 %) *	2.14 ± 0.39 (-38.71 %) *	4.03 ± 0.02 (-29.73 %) **
12	2000	0.11 ± 0.01 (-74.94 %) **	0.26 ± 0.09 (-72.34 %) ns	0.7 ± 0.1 (-65.94 %) *	1.44 ± 0.17 (-58.69 %) ns	2.23 ± 0.09 (-61.15 %) **
13	3000	0.06 ± 0 (-86.18 %) ***	0.28 ± 0.07 (-70.22 %) *	0.58 ± 0.08 (-71.72 %) *	0.49 ± 0 (-85.96 %) *	1.25 ± 0.16 (-78.2 %) **
14	4000	0.04 ± 0 (-90.87 %) ***	0.21 ± 0.06 (-78.28 %) **	0.23 ± 0.03 (-88.82 %) *	0.38 ± 0.02 (-89.06 %) *	0.96 ± 0.03 (-83.35 %) **
15	5000	0.003 ± 0 (-99.44 %) **	0.12 ± 0.06 (-87.35 %) **	0.14 ± 0.05 (-93.18 %) *	0.27 ± 0.03 (-92.21 %) *	0.61 ± 0.04 (-89.36 %) ***

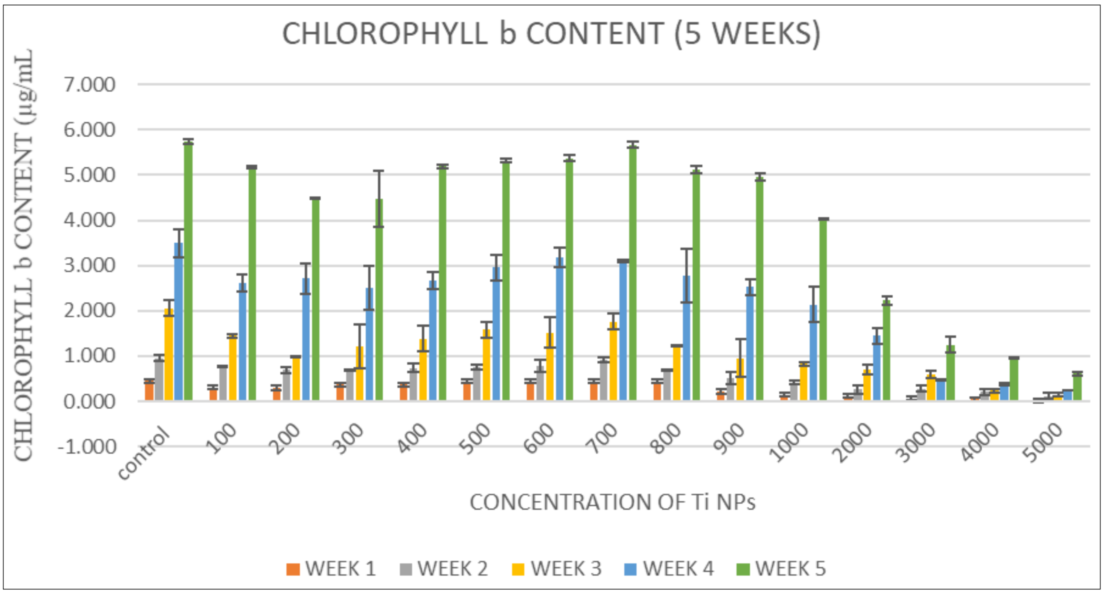

Fig 3: Effect of various concentrations of TiO_2 nanoparticles on chlorophyll b content in *Dunaliella salina* (μg/ml)

Effect of various concentrations of TiO_2 nanoparticles on total carotenoid content in *Dunaliella salina* (mg/gm)

Table 4 and Figure 4 demonstrates a dose-dependent response of total carotenoid content in *Dunaliella salina* to TiO_2 nanoparticle (Ti-NP) exposure. At lower Ti-NP concentrations, carotenoid levels increased, likely as an adaptive antioxidant response to mitigate oxidative stress. However, at higher concentrations, a marked reduction in carotenoid content was observed, indicating potential nanoparticle-induced toxicity. This pattern persisted consistently throughout the five-week treatment period.

In Week 1, total carotenoid content was significantly reduced in the nanoparticle-treated groups, with a 16.85 % decrease at 100 ppm and an 86.43 % reduction at 5000 ppm. Extremely higher concentrations led to a marked decrease, suggesting a significant impairment in carotenoid biosynthesis but at around 600-700 ppm carotenoid amount was 0.83% and 4.79% more than the control sample. In Week 2, there was a continued decline in total carotenoids, with the most substantial reduction of 86.96 % at 5000 ppm. By Week 3, total carotenoid content decreased by 94.22 % at 5000 ppm, indicating a strong negative impact on carotenoid production at concentrations more than 1000 ppm, however 13.90% increase was observed at 700 ppm. The decrease at lower concentrations (100-1000 ppm) was less severe but still noticeable. In Week 4, carotenoid content remained substantially reduced in the higher nanoparticle concentrations, with 87.61 % reduction at 5000 ppm whereas a 6.55 % increase was seen at 700 ppm. The declines were still noticeable at concentrations around 100 ppm to 1000 ppm, though they were less severe. By Week 5, carotenoid content showed a substantial decline at the highest nanoparticle concentration, with an 88.32% reduction indicating near-complete inhibition. An exception was observed at 700 ppm, which resulted in a modest enhancement of 6.24%.

Table 4: Effect of various concentrations of TiO_2 nanoparticles on total carotenoid contents in *Dunaliella salina* (mg/g)

S.No.	Conc. of TiO_2 (ppm)	Week 1	Week 2	Week 3	Week 4	Week 5
1	control	9.76 ± 0.21	20.64 ± 0.48	42.72 ± 0.6	73.48 ± 0.81	164.76 ± 0.74
2	100	8.12 ± 0.13 (-16.85 %) *	19.43 ± 0.72 (-5.89 %) *	34 ± 1.35 (-20.42 %) *	61.73 ± 0.71 (-16 %) *	134.66 ± 0.61 (-18.27 %) ***
3	200	8.25 ± 0.07 (-15.47 %) *	17.37 ± 0.58 (-15.87 %) ns	32.04 ± 0.2 (-25.02 %) *	56.5 ± 0.41 (-23.11 %) *	112.19 ± 0.7 (-31.91 %) ***
4	300	8.62 ± 0.08 (-11.66 %) ns	16.98 ± 0.73 (-17.77 %) ns	29.31 ± 0 (-31.39 %) *	57.94 ± 0.51 (-21.15 %) *	109.69 ± 0.91 (-33.43 %) **
5	400	9.12 ± 0.04 (-6.55 %) ns	16.85 ± 0.51 (-18.37 %) ns	30.42 ± 0.42 (-28.8 %) **	59.07 ± 0.65 (-19.62 %) *	134.88 ± 0.27 (-18.14 %) **
6	500	9.57 ± 0.12 (-1.96 %) ns	18.78 ± 0.94 (-9.01 %) ns	34.83 ± 0.03 (-18.48 %) *	61.6 ± 0.35 (-16.17 %) **	140.95 ± 0.82 (-14.45 %) ***
7	600	9.84 ± 0.17 (0.83 %) ns	18.77 ± 0.18 (-9.07 %) ns	40.36 ± 0.19 (-5.53 %) ns	62.93 ± 0.93 (-14.37 %) *	151.98 ± 0.52 (-7.76 %) *
8	700	10.23 ± 0.05 (4.79 %) ns	21.27 ± 0.09 (3.05 %) ns	48.66 ± 0.12 (13.9 %) *	78.29 ± 0.17 (6.55 %) *	175.03 ± 0.47 (6.24 %) *
9	800	8.91 ± 0.07 (-8.77 %) *	17.36 ± 0.25 (-15.9 %) *	26.4 ± 0.39 (-38.2 %) *	71.83 ± 0.29 (-2.25 %) ns	169.55 ± 3.27 (2.91 %) ns
10	900	5.43 ± 0.62 (-44.34 %) *	15.33 ± 0.92 (-25.72 %) ns	18.63 ± 0.51 (-56.4 %) ***	46.35 ± 0.07 (-36.92 %) **	130 ± 0.45 (-21.09 %) **
11	1000	4.38 ± 0.5 (-55.17 %) *	10.08 ± 0.6 (-51.17 %) *	12.8 ± 0.44 (-70.03 %) **	44.93 ± 0.51 (-38.86 %) *	102.74 ± 0.22 (-37.64 %) **
12	2000	3.42 ± 0.58 (-64.99 %) *	9.91 ± 0.4 (-52.02 %) *	10.31 ± 0.72 (-75.86 %) ***	31.72 ± 0.26 (-56.83 %) **	65.93 ± 0.63 (-59.98 %) ***
13	3000	1.87 ± 0.12 (-80.82 %) **	5.33 ± 0.16 (-74.18 %) **	7.75 ± 0.11 (-81.87 %) **	23.35 ± 0.72 (-68.22 %) ***	58.9 ± 0.38 (-64.25 %) ***
14	4000	1.66 ± 0.03 (-82.99 %) **	4.75 ± 0.4 (-76.98 %) *	5.11 ± 0.15 (-88.04 %) **	15.76 ± 0.45 (-78.55 %) **	27.81 ± 1.1 (-83.12 %) ***
15	5000	1.32 ± 0.13 (-86.43 %) **	2.69 ± 0.93 (-86.96 %) **	2.47 ± 0.55 (-94.22 %) ***	9.1 ± 0.78 (-87.61 %) **	19.25 ± 0.2 (-88.32 %) ***

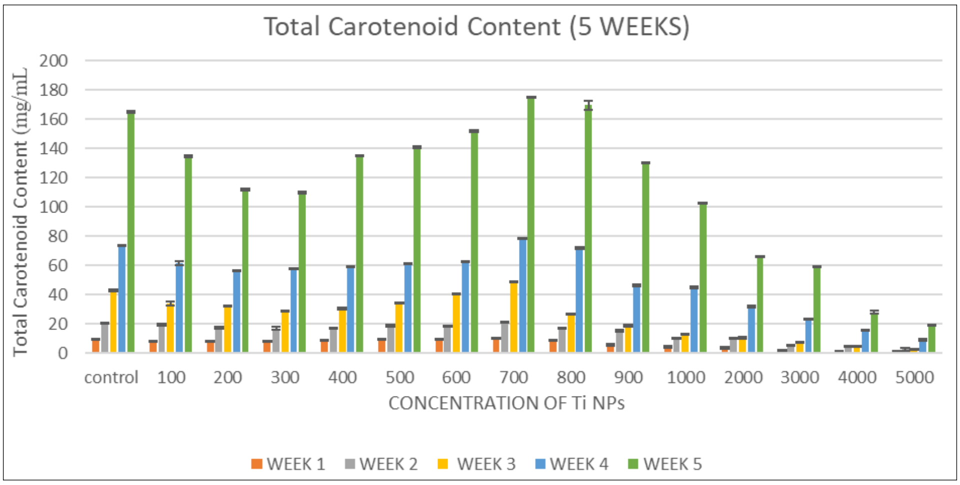

Fig 4: Effect of various concentrations of TiO_2 nanoparticles on total carotenoid contents in *Dunaliella salina* (mg/g)

Effect of various concentrations of TiO_2 nanoparticles on lipid contents in *Dunaliella salina* (mg/g)

Table 5 and Figure 5 reveals that lipid content in *Dunaliella salina* increased in response to TiO_2 nanoparticle (Ti-NP)-induced oxidative stress in the culture medium. However, lipid quantification was challenging across all concentrations due to low biomass yield over the five-week cultivation period.

Despite this limitation, a clear trend was observed: at lower Ti-NP concentrations (100 ppm), lipid content increased by approximately 4.57%, indicating a stress-induced lipid accumulation. This response became more pronounced at 500 ppm, where lipid yield showed a significant enhancement of 33.84%. Conversely, at very high Ti-NP concentrations, lipid content declined sharply, likely due to nanoparticle-induced cytotoxicity. This is supported by a substantial reduction up to (83.23%) in lipid synthesis, suggesting near-complete inhibition under excessive nanoparticle exposure.

Table 5: Effect of various concentrations of TiO_2 nanoparticles on lipid contents in *Dunaliella salina* (mg/g)

S.no	Conc.(ppm)	Lipid Content After 5 Weeks (mg/gm)
1	control	164 ± 1.41 (0 %)
2	100	171.5 ± 2.12 (4.57 %) ns
3	500	219.5 ± 0.71 (33.84 %) **
4	1000	169.5 ± 0.71 (3.35 %) *
5	5000	27.5 ± 3.54 (-83.23 %) **

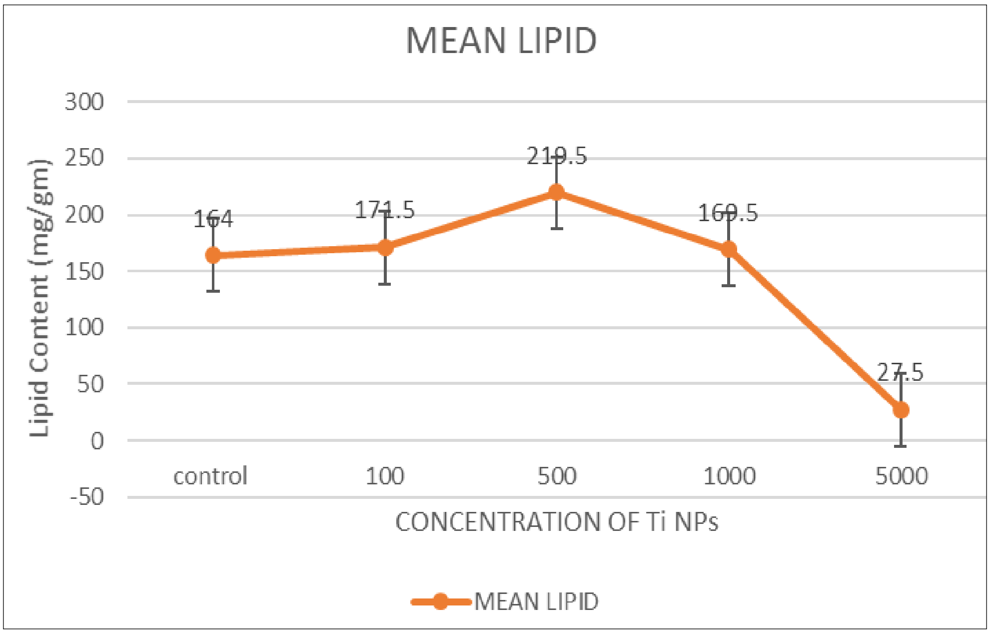

Fig 5: Effect of various concentrations of TiO_2 nanoparticles on lipid contents in *Dunaliella salina* (mg/g)

Effect of various concentrations of TiO_2 nanoparticles on percentage DPPH radical scavenging activity in *Dunaliella salina.*

Table 6 and Figure 6 shows that the antioxidant activity of *Dunaliella salina* increased in response to TiO_2 nanoparticle exposure, with moderate concentrations improving its free radical scavenging ability due to oxidative stress. However, due to low biomass production during the five-week growth period, it was difficult to measure antioxidant activity accurately at all concentrations.

By Week 5, a concentration-dependent trend in DPPH radical scavenging activity was evident. Treatment with 100 ppm and 500 ppm resulted in significant enhancements of 18.86% and 24.28%) respectively, relative to control sample. A marginal increase of 2.15% was observed at 1000 ppm. In contrast, exposure to 5000 ppm led to a pronounced decrease in activity, with an 88.14% reduction suggesting potential oxidative stress at higher concentrations. The positive control exhibited no radical scavenging activity.

Table 6: Effect of various concentrations of TiO_2 nanoparticles on percentage DPPH radical scavenging activity in *Dunaliella salina.*

S.no	Conc.(ppm)	% DPPH *radical* scavenging activity week 5
1	**Positive Control**	**0 ± 0**
2	control	21.17 ± 0.08
3	100	25.17 ± 0.2 (18.86 %) **
4	500	28.27 ± 0.14 (24.28 %) **
5	1000	21.63 ± 0.06 (2.15 %) *
6	5000	2.51 ± 0.14 (-88.14 %) **

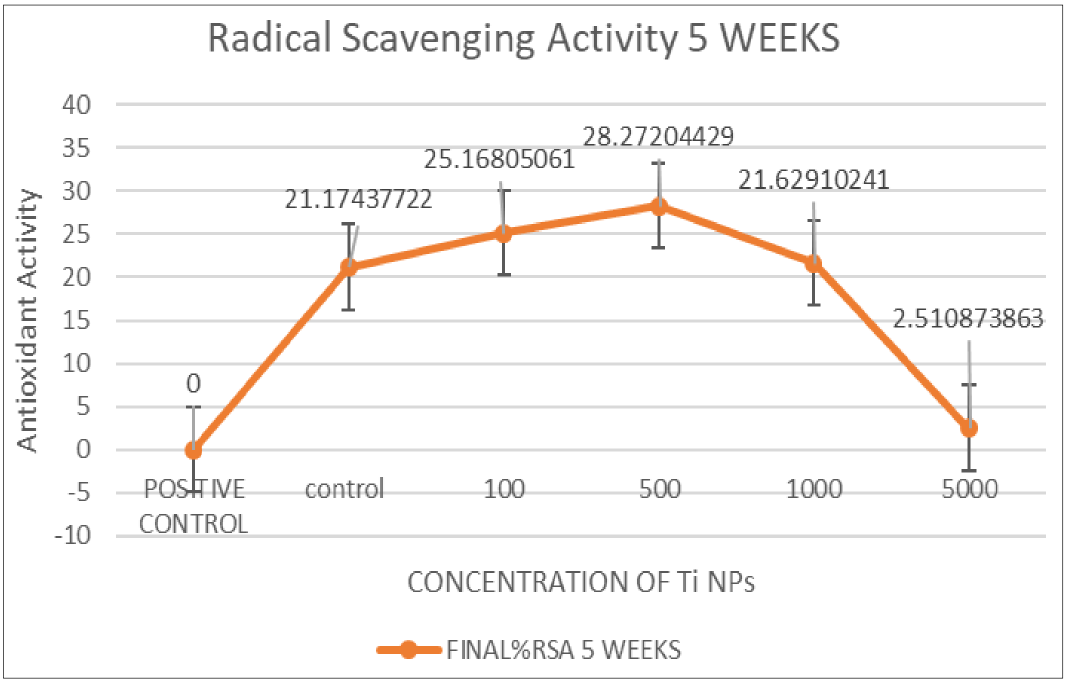

Fig 6: Effect of various concentrations of TiO_2 nanoparticles on percentage DPPH radical scavenging activity in *Dunaliella salina.*

Discussion

The current study highlights a clear and consistent dose-dependent inhibitory effect of TiO_2 nanoparticles on the green microalga *Dunaliella salina*, as evident from a significant decrease in chlorophyll a, chlorophyll b, as well as total carotenoids. The effect of different doses of TiO_2 nanoparticles (100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm, 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm) was examined on the microalga *Dunaliella salina* over a period of 5 weeks.

We assessed the impact of TiO_2 nanoparticles on the photosynthetic content of the green microalga *Dunaliella salina.* The results showed a drastic decline in chlorophyll a, chlorophyll b and total chlorophyll content of *Dunaliella salina* on exposure to nanoparticles. Chlorophyll a declined substantially with increasing NP doses, which may be linked with nanoparticle induced impairment of photosystem II as well as impaired electron transport or ROS-induced oxidative degradation of pigment molecules. In addition, chlorophyll a content may have decreased owing to the disruption of chloroplast membranes. Besides this, chlorophyll biosynthetic enzymes (e.g., protochlorophyllide reductase) may have led to decreased chlorophyll a content.

Similar results were obtained by Barreto & Lombardi while treating *Scenedesmus bijugus* with different concentrations of Nano-TiO_2 (Barreto & Lombardi, 2016) [3] and in *Pseudokirchneriella subcapitata* where altered nanoparticle size caused reductio n in biopigments. (Hartmann *et al.*, (2010) [13].

The results also showed that chlorophyll b levels dropped in the algae, suggesting pigment destabalization. Severe pigment loss was observed, beyond concentration 1000 ppm or higher, most likely because of intense oxidative stress. Total carotenoids, which play a crucial role in photoprotection as well as reactive oxygen species (ROS) scavenging, followed the same inhibitory trend on exposure to nanoparticles. However, at certain time points week 1 (600 and 700 ppm), week 2 (700 ppm), week 3 (700 ppm), week 4 (700 ppm) and week 5 (700 and 800 ppm), carotenoid levels showed considerable increases at lower NP concentrations, possibly reflecting a temporary compensatory antioxidant response. Nonetheless, this protective mechanism appears to be overwhelmed at higher concentrations and prolonged exposures, leading to significant carotenoid degradation by week 5. Carotenoids play a very crucial role in light harvesting as well as ROS detoxification. Therefore, this decline in carotenoid amount maybe attributed to excessive ROS generation by TiO_2 NPs, which overwhelms and depletes carotenoid reserves. Furthermore, nanoparticle exposure may lead to inhibition of carotenoid biosynthesis enzymes such as phytoene synthase. Also, possible structural damage to plastids (sites of carotenoid synthesis) may be due to nanoparticle accumulation, leading to decreased carotenoid biosynthesis (Makhi *et al.*, 2022 [19]; Hanifi *et al.*, 2022 [12]; Constantinescu-Aruxandei *et al.*, 2019 [8]; Romero *et al.*, 2020; [23] Shi *et al.*, 2025) [25].

To sum up, our findings clearly show that TiO_2 nanoparticles have a lasting toxic effect on *Dunaliella salina*, which is both the concentration and duration dependent. These nanoparticles not only retarded the algae's growth but also reduced pigment levels and overall biomass. This provides a hint to possible effects of nanoparticle accumulation in the living organisms and the environment.

Conclusion

To sum up, our findings clearly show that TiO_2 nanoparticles have a lasting toxic effect on *Dunaliella salina*, which is both the concentration and duration dependent. These nanoparticles not only retarded the algae's growth but also reduced pigment levels and overall biomass. Interestingly, even low concentrations became more harmful over time, suggesting that the impact of TiO_2 accumulation with prolonged exposure. This provides a hint to possible effects of nanoparticle accumulation in the living organisms and the environment.

These findings emphasize the need for careful regulation and environmental monitoring of nanoparticle contaminants, particularly in aquatic and hypersaline habitats. Exposure to nanoparticles may cause internalization and generation of ROS by nanoparticles coupled with disruption of enzymatic pathways involved in photosynthesis and cell division. This study highlights why it is so important to monitor and regulate nanoparticle pollution, especially in sensitive aquatic and saline environments. Moving forward, it will be valuable to dig deeper into exactly how these nanoparticles cause damage at the molecular level and to explore ways to protect algae, such as using antioxidants, biosorption agents, or developing more resistant strains.

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