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Effect of chromium oxide (Cr_2O_3) nanoparticles on photosynthetic pigments in *D. salina*

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Abstract
Dunaliella salina, a halophilic green microalga, has gained significant attention in recent years as a promising biorefinery platform for the sustainable production of high-value carotenoids, particularly β-carotene. Due to its unique physiological and metabolic features, *D. salina* has emerged as a commercially viable organism for industrial biotechnology applications. The studies conducted on *Dunaliella* species have laid the foundation stone of the concept of compatible organic solutes. The mechanism by which *Dunaliella* cells can alter its intracellular glycerol concentration provides it with an ability to thrive in wide range of salt concentrations. *D. salina* can grow under high-light intensity, high temperatures, and a wide pH range. It has a high accumulation of lipids and carotenoids. Chromium pollution, especially in its hexavalent form (Cr (VI)), poses serious environmental and health risks. It contaminates water, inhibits plant and algal growth, disrupts aquatic ecosystems, and is toxic to humans and animals. Prolonged exposure can cause organ damage, cancer, and genetic mutations, making chromium a major pollutant of concern. The current study aims to systematically investigate the effect of different concentration of chromium oxide nanoparticles (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm, 70 ppm, 80 ppm, 90 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500ppm and 600 ppm) on photosynthetic pigments (Chlorophyll and Carotenoids) of *Dunaliella salina* over a period of 5 weeks. Photosynthetic performance was evaluated by measuring chlorophyll a, chlorophyll b, and total carotenoid content over a 5-week exposure period. The results of the study showed significant dose and time dependent decline in algal biomass exposed to nanoparticles. Changes were examined in algal photosynthetic pigments, where exposure to nanoparticles led to percentage decline of 98%, 97% and 94% (in case of chlorophyll a, chlorophyll b and total carotenoids). Therefore, the findings of the current study urge to focus future studies on the investigation of molecular mechanisms of Cr_2O_3 NP toxicity as well as exploration of potential mitigation strategies to evade the malicious nanoparticle toxicity.

Keywords: *Dunaliella salina*, chromium oxide nanoparticles, chlorophyll a, chlorophyll b, total carotenoid content

Introduction

The last few decades witnessed rapid expansion of nanotechnology in various spheres, be it engineering, food industry, cosmetics as well as healthcare. Nanoparticles, a fresher in already known set of pollutants are a pressing issue because of their extensive use in a variety of fields. Particles ranging from 1 to 100 nm in dimension are called nanomaterials. A variety of commercially available products such as paints, semiconductors, sunscreens, and cosmetics contain nanoparticles. Nanoparticles presence has led to alterations in marine and freshwater environments. The nanoparticles find their way into marine and aquatic ecosystems through aerial deposition, effluent discharge, dumping and run-off. However, after being used up, these nanoparticles end up contaminating the environment, leading to exposure of different components of the ecosystem to nanoparticles. To add to the already worrisome scenario, the situation is further worsened by concerns about persistence and potential toxicity in aquatic ecosystems. Among all these nanoparticles, chromium oxide nanoparticles (Cr_2O_3 NPs) are being used in several applications such as industries, ranging from usage in metallurgy, ceramics, catalysis, paints, as well as pigments, owing to their thermal stability, hardness, as well as chemical inertness. However, the discharge of these nanoparticles into water bodies through industrial runoff, has raised several concerns about their ecological impacts on aquatic microorganisms, particularly the microalgae (Wang *et al.*, 2019; Du *et al.*, 2019; Dash *et al.*, 2012) [20, 21, 22]

Microalgae lie at the centre in food web and act as primary producers in aquatic systems, thereby playing a pivotal role in sustaining food webs as well as maintenance of ecosystem function. Among them, *Dunaliella salina*, a halotolerant green alga, is widely recognized for its ability to thrive in hypersaline environments and its commercial value as a natural source of β-carotene, glycerol, and other high-value compounds. Owing to its high physiological resilience as well as responsiveness to environmental changes, *D. salina* has emerged as a suitable model for evaluation of the toxicological effects of pollutants, including nanoparticles. Furthermore, nanoparticles like Cr_2O_3 NPs, though chemically stable, can interact with algal cells, potentially penetrating algal cell membranes, leading to oxidative stress through the generation of reactive oxygen species (ROS), and disrupting various cellular processes. Exposure of algal cells to nanoparticles has been reported to lead to inhibition of cell division, coupled with impairment of photosynthetic machinery, as well as suppression of pigment biosynthesis, all of which collectively affect algal growth and productivity (Shirazi *et al.*, 2015; Ayatallahzadeh *et al.*, 2016; Johari *et al.*, 2018; Bahador *et al.*, 2019; Luo *et al.*, 2021) [11, 15, 16, 17, 18, 19]

Based on this, the current study has been performed to systematically investigate the effect of Cr_2O_3 NP on photosynthetic pigments of *Dunaliella salina*. Photosynthetic performance was evaluated by measuring chlorophyll a, chlorophyll b, and total carotenoid content over a 5-week exposure period. The current study aims to assess dose-dependent responses of *D. salina* to various Cr_2O_3 NP concentrations, contributing to the enhancement of our understanding of nanoparticle-algae interactions and contribute towards ecological risk assessment of nanopollutants in aquatic environments.

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 Cr_2O_3 nanoparticles (Cr-Nps) was given by preparing culture media (ASWM) containing nanoparticles of different concentrations ranging from 10 ppm to 100 ppm and 100 ppm to 600 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 chromium oxide nanoparticles

S.No.	Characteristic	
1.	Molecular Weight	151.9904 g/mol
2.	CAS No.	1308-38-9
3.	Bulk Density	0.943 g/ cm3
4.	Morphology	Nearly Spherical
5.	Color	Green
6.	Density	5.22 g/cm3
7.	Chemical Formula	Cr_2O_3
8.	Thickness	30-50 nm
9.	Purity	99.90%
10.	Physical Form	Powder
11.	Surface Area	20-40m^2/g

Chlorophyll Estimation

The chlorophyll content was performed by using an extraction with 90% acetone. The obtained algal biomass was added with 90% acetone 1.5ml and homogenized with mortar and pestle. The mixture was incubated in the water bath at 50°C for 30 minutes. The liquid was centrifuged three times in a respective ratio of 1.5 to 8.5ml at 3000 rpm for 10 minutes. The supernatant was then transferred by pipette into a 50 ml centrifuge tube. The solution was allowed to stand for a short period of time prior to an additional 10 minutes of centrifugation. This procedure was completed in subdued lighting. The chlorophyll content of the samples was determined by using the spectrophotometric methods. In order to determine the chlorophyll content of the extract, the sample was measured the absorbance at several wavelengths, between the range of 400 and 700 nm against the solvent (acetone) blank. The concentration of Chlorophyll a and Chlorophyll b were evaluated according to SCOR-UNESCO (1966) [2] equation.

Carotenoid estimation

50 mg dry algal powder of *Dunaliella salina* was taken for the study. For each treatment, algal powder was homogenized with 50 ml of 80 percent acetone. The sample was subjected to centrifugation at a speed of 2000 rpm for a duration of 10 minutes. Subsequently, the resulting volume was adjusted to 10 ml using 80% Acetone. The extract was analysed for carotenoid content using a spectrophotometer at wavelengths of 480 nm and 510 nm. The values of pigments were expressed in terms of mg/g fresh weight as suggested by Arnon (1949) and Mahadevan and Sridhar (1982) [3, 4].

Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean ± standard deviation (SD) followed by Percent change and Significance. Statistical analysis was performed using Microsoft Excel. T-Test was used to assess significant differences among treatment groups. A *p*-value of less than 0.05 was considered statistically significant.

Results

Effect of various concentrations of Cr_2O_3 nanoparticles on chlorophyll a content in *Dunaliella salina*

As per the results in Table 2, the chlorophyll a content of *Dunaliella salina* showed a consistent decline in response to increasing concentrations of Cr_2O_3 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 Cr_2O_3 nanoparticles. At 600 ppm, chlorophyll a level decreased by 97.3 %, indicating significant inhibition of photosynthetic activity at higher concentrations. In Week 2, the decrease in chlorophyll a content persisted, with reductions ranging from 9.49 % at 10 ppm to 95.9 % at 600 ppm. By Week 3, chlorophyll a levels were still significantly reduced at higher nanoparticle concentrations. The decline ranged from 7.89 % at 10 ppm to 97.41 % at 600 ppm. In Week 4, the decline in chlorophyll a content continued across all concentrations, with the greatest reduction of 97.42 % at 600 ppm. By Week 5, chlorophyll a content showed the highest reduction of 98.43 % at 600 ppm, indicating nearly complete suppression of chlorophyll a production at the highest concentration. The decline was less severe in the lower concentrations, particularly at 10 ppm (32.73%).

Table 2: Effect of various concentrations of Cr_2O_3 nanoparticles on chlorophyll a content in *Dunaliella salina* (µg/ml)

S.No.	Concentration of Cr_2O_3 (ppm)	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
1	control	3.36 ± 0.01	5.33 ± 0.01	11.43 ± 0.01	16.68 ± 0.03	26.09 ± 0.18
2	10	2.7 ± 0.02 (-19.77 %) *	4.82 ± 0.06 (-9.49 %)*	7.89 ± 0.06 (-30.95 %) **	11.16 ± 0.13 (-33.08 %) **	17.55 ± 0.15 (-32.73 %) **
3	20	2.63 ± 0.13 (-21.82 %) *	4.5 ± 0.12 (-15.51 %) *	7.57 ± 0.08 (-33.78 %) **	9.77 ± 0.3 (-41.43 %) **	13.16 ± 0.02 (-49.57 %) **
4	30	2.75 ± 0.14 (-18.32 %) *	4.56 ± 0.02 (-14.29 %) **	7.56 ± 0.13 (-33.86 %) **	10.4 ± 0.22 (-37.64 %) **	15.26 ± 0.26 (-41.5 %) **
5	40	2.86 ± 0.06 (-14.77 %) *	4.85 ± 0.07 (-8.86 %) *	7.51 ± 0.05 (-34.25 %) **	10.32 ± 0.06 (-38.17 %) **	17.69 ± 0.25 (-32.2 %) **
6	50	2.93 ± 0.09 (-12.86 %) *	4.89 ± 0.11 (-8.17 %) ns	7.8 ± 0.23 (-31.76 %) *	10.09 ± 0.01 (-39.5 %) ***	17.77 ± 0.08 (-31.89 %) **

7	60	2.87 ± 0.07 (-14.63 %) *	4.98 ± 0.12 (-6.54 %) ns	7.91 ± 0.08 (-30.75 %) **	11.65 ± 0.17 (-30.19 %) **	18.29 ± 0.15 (-29.92 %) **
8	70	2.95 ± 0.05 (-12.35 %) *	5.39 ± 0.2 (1.18 %) ns	9.62 ± 0.02 (-15.87 %) **	13.78 ± 0.04 (-17.43 %) ***	19.4 ± 0.29 (-25.66 %) **
9	80	2.54 ± 0.02 (-24.35 %) **	4.37 ± 0.12 (-17.93 %) *	8.3 ± 0.18 (-27.34 %) *	10.71 ± 0.31 (-35.78 %) *	16.72 ± 0.04 (-35.92 %) **
10	90	2.41 ± 0.05 (-28.16 %) **	3.94 ± 0.08 (-25.98 %) *	8.24 ± 0.09 (-27.94 %) **	8.93 ± 0.08 (-46.49 %) **	14.17 ± 0.14 (-45.7 %) **
11	100	2.36 ± 0.02 (-29.84 %) **	3.68 ± 0.24 (-30.99 %) *	7.7 ± 0.32 (-32.65 %) *	7.5 ± 0.11 (-55.02 %) **	11.6 ± 0.42 (-55.55 %) **
12	200	1.54 ± 0.06 (-54.25 %) **	2.53 ± 0.51 (-52.43 %) *	5.75 ± 0.05 (-49.71 %) **	6.16 ± 0.11 (-63.1 %) **	10.26 ± 0.4 (-60.68 %) **
13	300	1.06 ± 0.04 (-68.58 %) **	2.44 ± 0.36 (-54.27 %) *	4.35 ± 0.05 (-61.91 %) **	5.41 ± 0.34 (-67.57 %) **	7.79 ± 0.01 (-70.13 %) **
14	400	0.17 ± 0.02 (-94.81 %) **	0.26 ± 0.01 (-95.03%) **	2.29 ± 0.04 (-79.99 %) ***	3.55 ± 0.07 (-78.71 %) ***	5.07 ± 0.11 (-80.55 %) ***
15	500	0.12 ± 0.01 (-96.34 %) **	0.22 ± 0.01 (-95.89 %) **	0.38 ± 0.03 (-96.72 %) ***	0.67 ± 0.02 (-95.98 %) ***	0.77 ± 0.07 (-97.06 %) ***
16	600	0.09 ± 0 (-97.3 %) **	0.22 ± 0 (-95.9 %) ***	0.3 ± 0.01 (-97.41 %) ***	0.43 ± 0.04 (-97.42 %) ***	0.41 ± 0.11 (-98.43 %) ***

Effect of various concentrations of Cr_2O_3 nanoparticles on chlorophyll b content in *Dunaliella salina*

As per the results in Table 3, the chlorophyll b content of *Dunaliella salina* showed a consistent decline in response to increasing concentrations of Cr_2O_3 nanoparticles, indicating a dose-dependent effect on chlorophyll b across all five weeks.

In Week 1, chlorophyll b levels showed a decline similar to that of chlorophyll a. The reduction ranged from 33.24 % at 10 ppm to 97.97% at 600 ppm. In Week 2, chlorophyll b content remained low at higher concentrations, showing a decrease from 24.7 % at 10 ppm to 97.51 % at 600 ppm. By Week 3, chlorophyll b continued to decline, with the highest reduction of 97.62 % at 600 ppm. The concentrations between 20 ppm and 100 ppm also exhibited substantial declines. In Week 4, chlorophyll b content was again reduced at all nanoparticle concentrations, with the greatest reduction (97.21%) at 600 ppm. At the lower concentrations, there was a decrease of around 35-40%, indicating some resilience at these levels. By Week 5, chlorophyll b levels had almost completely diminished at higher nanoparticle concentrations, particularly at 600 ppm (97.41%), while lower concentrations still displayed marked decreases in pigment content.

Table 3: Effect of various concentrations of Cr_2O_3 nanoparticles on chlorophyll b content in *Dunaliella salina* (µg/ml)

S.No.	Concentration of Cr_2O_3 (ppm)	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
1	control	0.95 ± 0.04	1.62 ± 0.03	2.57 ± 0	3.87 ± 0.07	6.93 ± 0.05
2	10	0.64 ± 0.06 (-33.24 %) **	1.22 ± 0.02 (-24.7 %) **	1.89 ± 0.12 (-26.37 %) *	2.49 ± 0.16 (-35.73 %) *	5.58 ± 0.15 (-19.41 %) *
3	20	0.61 ± 0.02 (-36.07 %) *	1.07 ± 0.01 (-34.03 %) *	1.81 ± 0.12 (-29.64 %) *	2.47 ± 0.22 (-36.17 %) *	3.55 ± 0.1 (-48.71 %) **
4	30	0.65 ± 0.01 (-31.62 %) *	1.03 ± 0.05 (-36.52 %) *	1.71 ± 0.09 (-33.29 %) *	2.27 ± 0.41 (-41.37 %) ns	4.44 ± 0.25 (-35.89 %) *
5	40	0.65 ± 0.01 (-31.7 %) *	1.15 ± 0.01 (-28.81 %) *	1.91 ± 0.1 (-25.76 %) *	2.42 ± 0.06 (-37.4 %) **	4.8 ± 0.12 (-30.7 %) *
6	50	0.68 ± 0 (-28.74 %) *	1.16 ± 0.03 (-28.59 %) *	1.91 ± 0.1 (-25.58 %) *	2.64 ± 0.24 (-31.97 %) *	4.69 ± 0.21 (-32.26 %) *
7	60	0.69 ± 0.01 (-27.86 %) *	1.13 ± 0 (-30.18 %) *	1.95 ± 0.07 (-24.27 %) *	2.63 ± 0.1 (-32.12 %) *	4.75 ± 0.34 (-31.45 %) *
8	70	0.74 ± 0.02 (-22.29 %) ns	1.34 ± 0.02 (-17.57 %) ns	2.07 ± 0.03 (-19.5 %) *	3.33 ± 0.13 (-14.13 %) *	5.63 ± 0.01 (-18.79 %) *
9	80	0.55 ± 0.04 (-42.1 %) *	1.01 ± 0.1 (-37.96 %) *	1.95 ± 0.09 (-24.2 %) *	2.4 ± 0.13 (-38.08 %) *	4.4 ± 0.25 (-36.56 %) *
10	90	0.54 ± 0.01 (-43.62 %) *	1.02 ± 0.02 (-36.76 %) *	1.36 ± 0.05 (-47.09 %) **	2.85 ± 0.09 (-26.5 %) *	4.35 ± 0.45 (-37.27 %) *
11	100	0.54 ± 0.01 (-43.5 %) *	1.01 ± 0.14 (-37.61 %) *	1.15 ± 0.05 (-55.44 %) **	2.68 ± 0.07 (-30.92 %) *	3.96 ± 0.1 (-42.8 %) **
12	200	0.37 ± 0.01 (-61.08 %) *	0.65 ± 0.1 (-60.04 %) *	1.33 ± 0.12 (-48.22 %) *	1.78 ± 0.05 (-54.01 %) **	2.78 ± 0.21 (-59.86 %) **
13	300	0.21 ± 0.01 (-77.93 %) **	0.34 ± 0.18 (-79.31 %) **	0.49 ± 0.25 (-81.11 %) *	1.32 ± 0.03 (-65.88 %) **	2.86 ± 0.08 (-58.66 %) **
14	400	0.06 ± 0.01 (-93.67 %) **	0.08 ± 0.01 (-94.82 %) **	0.16 ± 0.02 (-93.88 %) **	0.19 ± 0.02 (-94.98 %) **	0.31 ± 0.07 (-95.56 %) ***
15	500	0.03 ± 0 (-96.94 %) **	0.05 ± 0.02 (-96.61 %) **	0.07 ± 0.02 (-97.42 %) **	0.1 ± 0.01 (-97.49 %) **	0.18 ± 0.04 (-97.42 %) ***
16	600	0.02 ± 0 (-97.97 %) **	0.04 ± 0.01 (-97.51 %) **	0.06 ± 0.01 (-97.62 %) **	0.11 ± 0.01 (-97.21 %) **	0.18 ± 0 (-97.41 %) **

Effect of various concentrations of Cr_2O_3 nanoparticles on total carotenoid content in *Dunaliella salina*

As per the results in Table 4, the total carotenoid content of *Dunaliella salina* showed a consistent decline in response to increasing concentrations of Cr_2O_3 nanoparticles, indicating a dose-dependent effect on total carotenoid content across all five weeks.

In Week 1, total carotenoid content was significantly reduced in the nanoparticle-treated groups, with a 36.61% decrease at 10 ppm and a 93.64 % reduction at 600 ppm. Higher concentrations led to a marked decrease, suggesting a significant impairment in carotenoid biosynthesis. In Week 2, there was a continued decline in total carotenoids, with the most substantial reduction of 91.29 % at 600 ppm. By Week 3, total carotenoid content decreased by 94.52 % at 600 ppm, indicating a strong negative impact on carotenoid production. The decrease at lower concentrations (10-100 ppm) was less severe but still noticeable. In Week 4, carotenoid content remained substantially reduced in the higher nanoparticle concentrations, with 92.64 % reduction at 600 ppm. The declines were still noticeable at concentrations around 10 ppm and 20 ppm, though they were less severe. By Week 5, the decrease in carotenoid content at the highest concentration (94.83%) was the most drastic, indicating near-total inhibition.

Table 4: Effect of various concentrations of Cr_2O_3 nanoparticles on total carotenoid contents in *Dunaliella salina* (mg/g)

S.No.	Concentration of Cr_2O_3 (ppm)	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
1	control	20.99 ± 0.64	27.26 ± 0.42	76.63 ± 0.09	113.81 ± 0.52	236.04 ± 0.36
2	10	13.31 ± 0.45 (-36.61 %) *	29.9 ± 0.52 (9.69 %) **	52.86 ± 0.45 (-31.02 %) **	71.67 ± 1.54 (-37.03 %) **	126.33 ± 1.82 (-46.48 %) **
3	20	14.16 ± 0.22 (-32.52 %) *	27.42 ± 0.18 (0.59 %) ns	50.13 ± 0.36 (-34.59 %) **	70.88 ± 0.26 (-37.73 %) **	100.48 ± 1.11 (-57.43 %) **
4	30	15.13 ± 0.32 (-27.9 %) *	28.24 ± 0.05 (3.59 %) ns	53.28 ± 1.18 (-30.47 %) *	75.06 ± 0.56 (-34.05 %) **	115.45 ± 1.18 (-51.09 %) **
5	40	15.31 ± 0.11 (-27.09 %) *	28.57 ± 0.35 (4.8 %) ns	54.38 ± 1.57 (-29.03 %) *	74.26 ± 0.02 (-34.75 %) **	132.02 ± 0.73 (-44.07 %) **
6	50	15.41 ± 0.03 (-26.61 %) *	28.5 ± 0.16 (4.55 %) *	54.72 ± 2.31 (-28.59 %) *	78.49 ± 0.5 (-31.04 %) **	131.39 ± 1.62 (-44.34 %) **
7	60	15.71 ± 0.12 (-25.18 %) *	29.35 ± 0.28 (7.68 %) *	62.39 ± 1.67 (-18.58 %) *	79.55 ± 0.03 (-30.11 %) **	130.41 ± 1.13 (-44.75 %) **
8	70	16.17 ± 0.35 (-22.97 %) *	32.8 ± 0.05 (20.32 %) *	70.05 ± 0.73 (-8.59 %) *	87.15 ± 2.07 (-23.42 %) *	145.28 ± 1.82 (-38.45 %) **
9	80	15.33 ± 0.48 (-26.98 %) *	30.46 ± 0.71 (11.75 %) *	57.64 ± 0.86 (-24.78 %) *	75.84 ± 0.75 (-33.36 %) **	124.3 ± 1.66 (-47.34 %) **
10	90	14.31 ± 0.09 (-31.84 %) *	28.08 ± 0.3 (3.02 %) *	57.16 ± 1.14 (-25.4 %) *	72.28 ± 0.45 (-36.49 %) **	120.56 ± 1.5 (-48.92 %) **
11	100	14.01 ± 0.02 (-33.26 %) *	25.41 ± 0.21 (-6.79 %) ns	52.98 ± 0.44 (-30.86 %) **	70.65 ± 1.37 (-37.93 %) **	107.74 ± 2.1 (-54.36 %) **
12	200	8.79 ± 0.08 (-58.13 %) *	15.18 ± 0.37 (-44.29 %) *	38.28 ± 0.74 (-50.04 %) **	45.53 ± 2.33 (-60 %) **	66.22 ± 2.04 (-71.95 %) **
13	300	5.07 ± 0.11 (-75.84 %) *	9.54 ± 0.34 (-64.98 %) **	27.12 ± 0.99 (-64.61 %) **	34.3 ± 3.07 (-69.86 %) **	52.94 ± 1.32 (-77.57 %) **
14	400	4.32 ± 0.45 (-79.43 %) *	7.31 ± 0.07 (-73.17 %) **	10.13 ± 0.45 (-86.78 %) **	17.14 ± 1.63 (-84.94 %) **	23.81 ± 1.26 (-89.91 %) ***
15	500	3.19 ± 0.12 (-84.82 %) **	6.09 ± 0.65 (-77.67 %) **	7.15 ± 0.85 (-90.67 %) **	13.8 ± 1.64 (-87.87 %) **	18.51 ± 0.21 (-92.16 %) ***
16	600	1.34 ± 0.16 (-93.64 %) **	2.37 ± 0.07 (-91.29 %) **	4.2 ± 0.24 (-94.52 %) ***	8.38 ± 1.47 (-92.64 %) **	12.19 ± 0.8 (-94.83 %) ***

Discussion

The current study highlights a clear and consistent dose-dependent inhibitory effect of Cr_2O_3 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 Cr_2O_3 nanoparticles (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm, 70 ppm, 80 ppm, 90 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm and 600 ppm) was examined on the microalga *Dunaliella salina* over a period of 5 weeks.

We assessed the impact of Cr_2O_3 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 maybe linked with nanoparticle induced impairment of photosystem II as well as impaired electron transport, both of which are critical for photosynthesis. A decrease in chlorophyll a content may have occurred owing to ROS-induced oxidative degradation of pigment molecules. In addition, chlorophyll a content may have decreased owing to the disruption of chloroplast membranes by Cr_2O_3 NPs, leading to pigment leakage or breakdown. Besides this, chlorophyll biosynthetic enzymes (e.g., protochlorophyllide reductase) may have led to decreased chlorophyll a content.

Furthermore, the results also showed decline in chlorophyll b content of the algae, which is indicative of generalized pigment destabilization or reduced chloroplast function. Interestingly, while low concentrations showed relatively moderate impacts on content of photosynthetic pigments, concentrations ≥100 ppm resulted in drastic pigment losses,

owing to immense amount of oxidative stress induced by Cr_2O_3 NPs. Thereafter, the researchers assessed the impact of nanoparticle exposure on total carotenoids. Total carotenoids, which play a very important 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 2), carotenoid levels showed slight 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 in the body. Therefore, this decline in carotenoid amount maybe attributed to excessive ROS generation by Cr_2O_3 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, which are the sites of carotenoid synthesis, may be adversely affected owing to nanoparticle accumulation, leading to decreased carotenoid biosynthesis (Makhi *et al.*, 2022; Hanifi *et al.*, 2022 [5]; Constantinescu-Aruxandei *et al.*, 2019; Romero *et al.*, 2020; Shi *et al.*, 2025) [1, 6, 7, 8].

Concluding the results of the study in a nutshell, the findings of the current study provide strong evidence that Cr_2O_3 nanoparticles exert a sustained and concentration-dependent toxic effect on *Dunaliella salina*, impairing its growth, pigment content, as well as overall biomass. These findings emphasize the need for careful regulation and environmental monitoring of nanoparticle contaminants, particularly in aquatic and hypersaline habitats. The study showed that toxic effects of Cr_2O_3 NPs were both dose-dependent as well as time-dependent. With increasing duration, even lower concentrations began to exhibit stronger suppressive effects, suggesting a cumulative or persistent impact of Cr_2O_3 nanoparticles. Nanoparticles may exert toxic effects in the algae owing to direct physical interactions with cell membranes causing mechanical disruption. Apart from this, exposure to nanoparticles may cause internalization and generation of ROS by nanoparticles coupled with disruption of enzymatic pathways involved in photosynthesis and cell division. Considering this, future studies should be focussed on the investigation of molecular mechanisms of Cr_2O_3 NP toxicity and explore potential mitigation strategies using antioxidants, biosorption agents, or resistant algal strains.

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