Effects of Zinc on Some Physiological and Biochemical Parameters of Dunaliella salina

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Abstract

Recently, the application of algae as food and medicinal sources has gained significant attention. Heavy metals are recognized as major environmental pollutants because of their inherent toxicity. Their non-biodegradability and environmental persistence to natural degradation can result in prolonged exposure, posing serious threats to ecosystems and the health of living organisms. Algae have recently attracted considerable interest as valuable sources of food and bioactive compounds. Zinc (Zn) is an essential micronutrient in algal cells, contributing to enzymatic activity, photosynthetic processes, and overall cellular metabolism. This study evaluated the effects of different Zn concentrations (0.03, 0.06, 0.09, 0.25, and 0.5 µg/mL) on the physiological and biochemical characteristics of *Dunaliella salina* using a completely randomized design with three replications. The results showed that 0.03 µg/mL Zn significantly enhanced photochemical efficiency chlorophyll a, chlorophyll b , protein content, soluble sugars, and catalase activity by 50.76%, 154.5%, 88.88%, 13.86%, 89.47%, 71.42%, respectively, compared with the control. In contrast, the highest increase in carotenoid accumulation (92.85% compared to the control) was observed at 0.5 µg/mL Zn. Proline, total amino acids, ascorbate peroxidase, and polyphenol oxidase increased progressively with increasing Zn concentration, reaching their maximum values at 0.5 µg/mL and exceeding the control values by 68.18%, 88.88%, 88.46%, and 90.38%, respectively. Zn also affected carbohydrate metabolism in a biphasic manner, causing an initial increase followed by a decline in soluble sugar levels. Overall, the findings indicate that Zn concentration modulates photosynthetic performance, antioxidant defense, and metabolic activity in *D. salina*, while the species exhibits considerable sensitivity to changes in Zn availability. These characteristics highlight the potential of *D. salina* as a bio indicator for monitoring heavy-metal contamination in aquatic environments. These adaptive responses may also have potential significance for biotechnological applications, including the production of valuable secondary metabolites or the use of algae in heavy metal bioremediation strategies.

Keywords: *Dunaliella salina*, Zn strees, Antioxidant defense, Heavy metal bioremediation

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Introduction

Dunaliella salina is a unicellular alga belonging to the family Dunaliellaceae (Salehipour-Bavarsad, et al., 2022). It was first identified along the Mediterranean coast of France and described by Teodoresco in 1905 (Riahi, 2002). *Dunaliella salina* is a green, halophilic, pear-shaped, biflagellated alga that lacks a cell wall and possesses a simple life cycle, enabling it to grow in a wide range of environmental conditions. This alga is capable of producing valuable pigments, particularly β-carotene (Whittaker, 1978). One of the most remarkable characteristics of *Dunaliella salina* is its ability to adapt to stressful environmental conditions, including extremely high salinity, elevated temperatures, nitrogen deficiency, and intense light exposure. This adaptability contributes to its broad distribution in natural habitats and diverse saline environments such as salt lakes and marshes (Massyuk, 1996). Zn (Zn), an essential micronutrient, plays numerous critical roles in the physiological and biochemical processes of living organisms. It is involved in the structure and function of more than 300 proteins, including key enzymes in metabolic pathways, and acts as a cofactor or structural component in various cellular processes, such as protein synthesis, carbohydrate metabolism, and cell membrane stabilization (Broadley et al., 2007).

In addition, Zn is involved in the regulation of gene expression and the maintenance of DNA stability, and may indirectly influence the photosynthesis process. Photosynthesis, the essential process by which light energy is converted into chemical energy, depends on the activity of several enzymes in which Zn may play either a catalytic or structural role. Zn also contributes to carbon fixation and may enhance photosynthetic efficiency in algae through its role in enzymes such as carbonic anhydrase (Palomares et al., 2004). Under environmental stress conditions, Zn functions as an antioxidant and helps reduce damage caused by reactive oxygen species (ROS). This element can enhance cellular tolerance to environmental stress by promoting the production of non-enzymatic antioxidants and modulating the activity of antioxidant enzymes (Pilon & Várkonyi, 2017). At low concentrations, such as 0.03- 0.09 µg/mL, Zn may improve photosynthetic efficiency, increase the activity of metal-dependent enzymes, and enhance cellular tolerance to environmental stress. These stimulatory effects are generally attributed to meeting cellular physiological Zn requirements and activating metabolic pathways associated with growth and survival (Kabata-Pendias, 2011; Nagajyoti et al., 2010). On the other hand, increasing Zn concentrations beyond the optimal range can exert inhibitory or toxic effects on the algal cell. At higher concentrations, particularly at or above 0.5 µg/mL, Zn may interfere with the uptake and balance of other essential elements, such as iron, magnesium, and copper, thereby disrupting cellular ionic homeostasis (Broadley et al.,2007; Xiong, 2006). Furthermore, excessive accumulation of Zn can promote the generation of reactive oxygen species (ROS) and induce oxidative stress, a condition in which the balance between the ROS production and antioxidant scavenging is disturbed, resulting in increased damage

to lipids, proteins, and nucleic acids. Consequently, photosynthetic activity may decline, cell growth is inhibited, and, in severe cases, cell death may occur (Cakmak, 2000). Determining the concentration at which Zn begins to exert toxic effects on algal growth, photosynthesis, and biochemical parameters is essential for evaluating the health of aquatic ecosystems and establishing permissible pollutant limits (Wang, 2002). Understanding how Zn is absorbed, metabolized, and released by algae can also provide insights into the bioavailability of this element to other organisms within the food chain, as well as its broader biogeochemical cycling in aquatic ecosystems (Sunda & Huntsman, 1995). In some cases, optimal Zn concentrations may enhance the production of valuable secondary metabolites in algae, such as carotenoids, which have significant for biotechnological applications (Rathur et al., 2015). In *Dunaliella salina*, a microalga known for its remarkable adaptability to harsh environmental conditions, responses to Zn may depend on its concentration, exposure time, and culture conditions. Therefore, investigating the effects of different Zn concentrations within the range of 0 -0.5 µg/mL is particularly important for determining the optimal balance between growth stimulation and the onset of Zn toxicity. Such studies can contribute to a better understanding of the mechanisms underlying heavy metal tolerance, photosynthetic regulation, and antioxidant responses in this alga.

Material and methods

Preparation of algae

Dunaliella salina, isolated from Lake Urmia (West Azerbaijan Province, Iran), was used as the experimental microalga in this study. Zn was applied at concentrations ranging from 0.03 to 0.5 µg/mL. The selected concentration range was determined through preliminary range-finding experiments designed to identify both growth-promoting concentrations and the highest non-lethal exposure level. All experimental procedures were conducted in 2023 at the Bioinstrumentation Laboratory, Faculty of Science, University of Mohaghegh Ardabili.

Preparation of Johnson's culture medium

To prepare Johnson's culture medium, 75.87 g of NaCl, 8.9 g of $MgCl_2 \cdot 6H_2O$, 0.53 g of $CaCl_2 \cdot 2H_2O$, 2.3 g of Na_2SO_4, and 0.85 g of K_2SO_4 were dissolved in distilled water and diluted to a final volume of 1 L. The medium was then sterilized by autoclaving at 120°C and 1 atm for 20 minutes. During preparation of the culture medium, 25.3 g of Tris base was added to adjust the pH to 7.5. Because trace elements are heat sensitive, 5 g of each of KNO_3, KH_2PO_4, and trace elements were added to the basal culture medium after it had cooled.

Algal cultivation and treatment

Sterilized 250-mL Erlenmeyer flasks were each filled with 100 mL of the prepared culture medium (1.5 M) and inoculated with algal cells. The remaining volume in the flasks provided sufficient headspace for gas exchange during algal growth. The flasks were then incubated for two weeks at 15°C in a germinator under 16 h light/8 h dark photoperiod. During the incubation period, the cultures were manually agitated every two days to prevent cell sedimentation. The experimental treatments included

a control group and cultures exposed to Zn concentrations of 0.03, 0.06, 0.09, 0.25, and 0.5 μg/mL. For each treatment, the required Zn concentrations were added to the culture medium as Zn sulfate. Subsequently, algal cells obtained from the initial cultivation step were exposed to the respective Zn concentrations and maintained under the same conditions in the germinator for an additional 14 days. The cultures were manually agitated every two days throughout the treatment period.

Measurement of physiological parameters

Optical density(OD)

Before and after the Zn treatments, 3 ml of each algal culture sample was transferred to a cuvette, and its absorbance was measured at 680 nm using a spectrophotometer. The absorbance value indicates the amount of light absorbed by the sample at the specified wavelength, with higher absorbance values generally indicating greater optical density and, under appropriate measurement conditions, a higher cell concentration.

Chlorophyll fluorescence

Chlorophyll fluorescence of the algal samples was measured using a PAM (Pulse-Amplitude Modulation) fluorometer. Prior to measurement, the samples were dark-adapted for 30 min to ensure that all photosynthetic reaction centers were in the open state. Basal fluorescence (F_0) was then measured under dim light conditions, followed by the application of a saturating light pulse to record maximum fluorescence (F_m). The maximum quantum efficiency of photosystem II (F_v/F_m) was calculated using the following equation: $F_v/F_m = (F_m - F_0) / F_m$ All measurements were performed at room temperature using three biological replicates. For data normalization, when necessary, cell concentration was determined based on light absorbance at a specific wavelength or dry biomass weight (Maxwell & Johnson, 200 0).

Chlorophyll a, b and Carotenoid levels

First, 0.5 g of a fresh algal sample was ground in a porcelain mortar. The resulting extract was then brought to a final volume of 20 mL using 90% acetone and centrifuged at 6000 rpm for 20 min. The supernatant obtained after centrifugation was transferred to a cuvette, and the absorbance values of chlorophyll a, chlorophyll b, and carotenoids were measured at wavelengths of 663, 645, and 470 nm, respectively, using a spectrophotometer. chlorophyll content was expressed as mg g^{-1} fresh weight, whereas carotenoid content was expressed as μg g^{-1} fresh weight using the following equations. In these equations, V represents the final volume of the extract, and W represents the fresh weight of the sample (Lichtenthaler, 1987)

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V / (1000 \times W)$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V / (1000 \times W)$$

$$\text{Carotenoids} = [1000 \times A_{470} - 3.27 \times (\text{Chl a}) - 104 \times (\text{Chl b})] / 227$$

Proline content determination

To determine proline content, 0.5 g of frozen algal sample was ground, and 5 mL of 3% sulfosalicylic acid was added. The mixture was immediately transferred to microtubes and kept on ice. Samples were then centri-

fuged using a refrigerated centrifuge for 15 min at 15,000 rpm at 4°C. Next, 1 mL of the supernatant was transferred to a new tube, and 1 mL of acetic acid and 1 mL of ninhydrin were added. The mixture was incubated in a 100°C water bath for 1 h, followed by cooling in an ice bath. Then, 2 mL of toluene was added to each sample and mixed for 20 s using a vortex mixer. After mixing, two phases were formed, and the absorbance of the upper toluene phase was measured using a spectrophotometer (PG Instruments, Model T80 +, UK) at 520 nm (Bates et al.,1973).

Measuring biochemical parameters

Protein content measurement

For protein extraction, 0.1 g of fresh algal biomass was thoroughly ground in liquid nitrogen, and 6 mL of 0.01 M phosphate buffer was added. The resulting protein extract was transferred to microtubes. Samples were then centrifuged at 4 °C for 15 min at 13,000 rpm using a refrigerated centrifuge. After centrifugation, the supernatant (upper phase) was transferred to new microtubes, stored at −80 °C, and used to determine protein concentration and the activities of various enzymes. For protein quantification, 0.5 mL of the prepared protein extract was mixed with 2 mL of Bradford reagent. The absorbance of the resulting mixtures was measured at 595 nm using a spectrophotometer (Bradford, 1976).

soluble sugar determination

For soluble sugar determination, 0.1 g of fresh algal tissue was mixed with 2.5 mL of 80% ethanol. The mixture was then placed in a water bath at 90°C for 60 minutes to extract soluble carbohydrates. Following extraction, the mixture was filtered through filter paper, and the residual alcohol was allowed to evaporate. The resulting precipitate was dissolved in 2.5 mL of distilled water, and 0.2 mL of the extract was transferred to a tube. Next, 5 mL of anthrone reagent was added to each tube, and the contents were mixed thoroughly. The tubes were then incubated in a water bath at 90°C for 17 minutes. After cooling, the absorbance of the samples was measured at 625 nm using a spectrophotometer (Dubois et al., 1956).

Amino acids analysis

To determine amino acid content, 1 g of fresh algal tissue was homogenized in 10 mL of 0.05 M phosphate buffer. The mixture was then centrifuged at 3000 rpm for 20 mn. After centrifugation, the supernatant was collected, and ninhydrin reagent was added at a supernatant-to-reagent ratio of 1:5. The solution was incubated in a water bath at 100°C for 7 minutes. Following incubation and cooling, the absorbance of the samples was measured at 570 nm using a spectrophotometer, following the method described by Moore and Stein (1963).

Catalase enzyme activity measurement

For the determination of catalase activity, the reaction mixture was prepared by combining 2.8 mL of 0.05 M phosphate buffer, 0.1 mL of 3% hydrogen peroxide, and 0.1 mL of enzyme extract. The absorbance of this mixture was measured every 10 seconds using a spectrophotometer at a wavelength of 290 nm (Aebi, 1984).

Ascorbate peroxidase enzyme activity analysis

Ascorbate peroxidase activity was determined by preparing the reaction mixture in an ice bath. Specifically, 2.0 mL of 0.05 M phosphate

buffer was mixed with 0.2 mL of 3% hydrogen peroxide and 0.2 mL of 5 mM ascorbic acid. Immediately after mixing, 0.1 mL of enzyme extract was added, and the initial absorbance was measured spectrophotometrically at 290 nm (Nakano & Asada, 1981).

polyphenol oxidase activity determination

To measure polyphenol oxidase activity, 0.2 mL of pyrogallol was added to 2.5 mL of 0.2 M phosphate buffer in a test tube. The mixture was placed in a water bath until the temperature of the tubes reached 40°C. Subsequently, 0.2 mL of the previously prepared enzyme extract was added, and the change in absorbance was monitored spectrophotometrically at 430 nm (Mayer & Harel, 1979).

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three replications. All statistical analyses were performed using SPSS software. Differences among treatments were evaluated using one-way analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test at a significance level of $p \leq 0.05$.

Results

Physiological parameters

The results of the statistical analysis showed that Zn treatment significantly affected physiological parameters ($p \leq 0.05$). Exposure to 0.03 µg/mL Zn resulted in a 35.51% increase in light absorbance relative to the control group. Under Zn treatment, F_0 and F_m increased in a concentration-dependent manner, reaching 40.82% and 91.84% above the control values, respectively, at 0.5 µg/mL of Zn. m. The highest F_v/Fm value was also recorded at 0.03 µg/mL Zn, showing a 50.76% increase compared with the control. At a Zn concentration of 0.03 µg/mL, chlorophyll a and chlorophyll b contents increased by 154.5% and 88.88%, respectively, relative to the control group. In contrast, carotenoid content showed the greatest increase, reaching 92.85% above the control at 0.5 µg/mL Zn. Proline content also increased in a concentration-dependent manner under Zn treatment, with the highest value observed at 0.5 µg/mL Zn, representing a 68.18% increase relative to the control (Table 1).

Table 1. Mean values regarding to effects of different Zn content of medium on physiological parameters of *Dunaliella salina*

Zn content (µg ml^{-1})	Control	0.03	0.06	0.09	0.25	0.5
Physiological parameters						
OD (680)	0.01^{f}± 1.07	0.06^{a}±1.45	0.03^{b}±1.40	0.04^{c} ±1.36	0.02^{d}±1.28	0.01^{e}±1.15
F_0	0.33^{f}±25.33	0.97^{e}±29.72	1.1^{d} ±30.52	1.3^{c}±31.12	1.47^{b}± 33.67	1.45^{a} ±35.67
F_m	0.57^{f}20.12	0.35^{e}±24.64	0.29^{d} ±26.53	0.31^{c}±28.71	0.45^{b}±32.67	0.33^{a} ±38.60
F_V/F_m	0.01^{f}±0.065	0.07^{a}±0.098	0.03^{b}±0.093	0.02^{c} ±0.082	0.06^{d}±0.074	0.05^{e}±0.070
Chlorophyll a (mg g^{-1} FW)	0.006^{f}±0.11	0.011^{a}±0.28	0.005^{b}±0.26	0.003^{c}±0.24	0.002^{d}±0.19	0.004^{e}±0.13
Chlorophyll b (mg g^{-1} FW)	0.003^{f}±0.09	0.005^{a}±0.17	0.002^{b}±0.15	0.001^{c}±0.14	0.003^{d}±0.12	0.002^{e}±0.10
Carotenoids (µg g^{-1} FW)	0.002^{f}±0.14	0.004^{e}±0.16	0.001^{d}±0.19	0.003^{c}±0.21	0.002^{b}±0.25	0.001^{a}±0.27
Proline (µm g^{-1} FW)	0.98^{f}±14.52	1.1^{e}±15.82	1.3^{d} ±16.64	1.5^{c} ±17.72	1.7^{b} ± 20.84	1.9^{a} ±24.42

In each row, values followed by the same letters are not significantly different according to Duncan's multiple range test at a significance level of $p \leq 0.05$ (n = 3)

Biochemical parameters

Data analysis showed that protein content increased under Zn treatment; however, the changes were not concentration-dependent. Protein content increased by 13.86% at 0.03 µg/ml compared with the control group, followed by a slight decrease at higher Zn concentrations, although the values remained higher than the control group. Soluble sugar content initially increased and subsequently decreased under Zn treatment, indicating a non-concentration-dependent response. The highest soluble sugar content was recorded at 0.03 µg/mL Zn, showing an 89.47% increase compared with the control. Total amino acid content increased in a concentration-dependent manner under Zn treatment, reaching its maximum value at 0.5 µg/mL Zn, with an 88.88% increase relative to the control. The results further demonstrated that catalase activity was significantly affected by Zn treatment ($p \leq 0.05$), although the changes were not concentration-dependent. The highest catalase activity, representing a 71.42% increase compared with the control, was observed at 0.03 µg/mL Zn. In contrast, the activities of ascorbate peroxidase and polyphenol oxidase increased in a concentration-dependent manner, reaching maximum increases of 88.46% and 90.38%, respectively, at 0.5 µg/mL Zn relative to the control (Table 2).

Discussion

Zinc (Zn) is an essential micronutrient for the growth and metabolism of microalgae and plays important roles enzyme structure and activity, photosynthetic processes, and antioxidant defense systems (Elleuch et al., 2021). Therefore, the physiological and biochemical responses observed in *Dunaliella salina* under different Zn concentrations should be interpreted from two distinct perspectives: the nutritional and stimulatory effects at low concentrations and the stress-inducing or toxic effects at higher concentrations. The significant increase in F_v/F_m observed in Zn-treated cultures, particularly at 0.03 µg/mL, suggests that this concentration provided favorable conditions

Table 2. Mean values regarding to effects of different Zn content of medium on biochemical parameters of *Dunaliella salina* . In each row, values followed by the same letters are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (n = 3)

Treatments (µg ml^{-1})	Control	0.03	0.06	0.09	0.25	0.5
Biochemical parameters						
Total protein (ng ml^{-1})	0.06^{e}± 1.01	0.06^{a} ±1.15	0.05^{b} ±1.14	0.05^{b} ± 1.14	0.07^{c} ±1.06	0.06^{d} ±1.04
Soluble sugar (mg g^{-1} FW)	0.007^{f}±0.19	0.004^{a}±0.36	0.004^{b}±0.32	0.002^{c}±0.30	0.003^{d} ± 0.28	0.005^{e} ±0.21
Free amino acid (mg g^{-1} FW)	0.001^{e}±0.009	0.001^{d}±0.011	0.002^{c}±0.012	0.004^{c}±0.012	0.003^{b}± 0.015	0.005^{a} ±0.017
Catalase activity (µmol. min−1 mg−1)	0.003e±0.007	0.005a ±0.012	0.006b ±0.011	0.002b±0.011	0.002c ±0.010	0.001d ±0.008
Ascorbate peroxidase activity (µmol. min^{-1} mg^{-1}	0.002^{f}±0.052	0.001^{e}±0.059	0.001^{d}±0.061	0.002^{c}±0.063	0.003^{b} ± 0.072	0.006^{a} ±0.098
Polyphenol oxidase activity (µmol. min^{-1} mg^{-1})	0.001f±0.52	0.002e ± 0.60	0.003d±0.61	0.005c±0.62	0.004b ± 0.78	0.007a ±0.99

for Photosystem II (PSII) performance and enhanced photosynthetic efficiency. Since Zn is involved in the activity of several enzymes associated with photosynthesis and electron transport, this response may reflect the significant nutritional role of Zn rather than a direct stress response (Wongsnansilp et al., 2018). In contrast, the increase in F_0 observed in some treatments may indicate alterations in PSII function and, at higher Zn concentrations, may be associated with the onset of photoinhibition and photosynthetic disturbances. The increase in chlorophyll content under Zn supplementation, particularly at 0.03 μg/mL, may be attributed increased pigment biosynthesis, improved activity of chlorophyll-synthesizing enzymes, and enhanced pigment stability (Alirahimi et al., 2022). Therefore, the elevated chlorophyll levels at this concentration may reflect the essential nutritional role of Zn. Conversely, the relative decline in chlorophyll content at 0.5 μg/mL may indicate the onset of inhibitory or toxic effects associated with excessive Zn accumulation and potential impairment to photosynthetic structures (Elleuch et al., 2021). The increase in soluble sugar content, particularly at 0.03 μg/mL Zn, may be associated with improved photosynthetic performance and enhanced carbon fixation. Although soluble sugars are known to function as osmoprotectants under stress conditions, their accumulation at low Zn concentrations may instead reflect enhanced carbon metabolism and energy storage rather than a direct stress response (Elleuch et al., 2021).

Similarly, the increase in total protein content at 0.03 μg/mL supports the potential role of Zn in promoting protein synthesis and metabolic activity. As a cofactor for numerous enzymes and a regulator of gene expression, Zn may promote protein production under optimal nutritional conditions. In contrast, the reduction in total protein content at 0.5 μg/mL may be associated with impaired protein synthesis or increased protein degradation under Zn-induced stress (Folgar et al., 2009). Regarding free amino acids, their accumulation at higher Zn concentrations, particularly at 0.5 μg/mL, may be associated with the synthesis of protective metabolites and the regulation of osmotic balance. This interpretation is consistent with the marked increase in proline observed at the same Zn concentration. Proline is widely recognized as a compatible osmolyte and antioxidant-related metabolite that accumulates under oxidative and metal-induced stress conditions (Hamed et al., 2017). Therefore, the substantial increase in proline and total amino acids at higher concentrations may indicate the activation of cellular defense mechanisms in response to Zn-induced stress, whereas the responses observed at lower Zn concentrations may primarily reflect the nutritional effects of Zn, (Sui et al., 2021). The pattern of antioxidant enzyme activities further supports this distinction. The increase in catalase (CAT) activity at 0.03 μg/mL may reflects enhanced cellular antioxidant capacity associated with adequate Zn availability. This response does not necessarily indicate severe stress but may instead suggests at enhanced capacity of the cells to detoxify reactive oxygen species (ROS) (Gao et al., 2024).

In contrast, the elevated ascorbate peroxi-

dase (APX) activity observed at 0.5 μg/mL may indicate enhanced antioxidant responses associated with increased ROS generation and a greater requirement for hydrogen peroxide detoxification under Zn-induced stress conditions (Roy, 2021). Likewise, the increase in polyphenol oxidase (PPO) activity at 0.5 μg/mL may be attributed to the activation of defense pathways involved in phenolic metabolism and oxidative stress mitigation. The higher PPO activity at elevated Zn concentrations is consistent with its proposed role in cellular defense against environmental stress and oxidative damage (Mishra & Jha, 2011). Overall, the results demonstrate that the response of *Dunaliella salina* to Zn varies with concentration and depends on the physiological and biochemical parameter examined. The low Zn concentration of 0.03 μg/mL enhanced photosynthetic performance, chlorophyll accumulation, soluble sugar content, total protein content, and selected antioxidant responses, suggesting a predominantly nutritional and stimulatory role of Zn in cellular metabolism. In contrast, the higher concentration of 0.5 μg/mL was associated with increased levels of proline, free amino acids, APX, and PPO, together with relative reductions in some metabolic parameters, suggesting the activation of stress-related defense responses and the possible onset of Zn toxicity. Therefore, not all observed responses should be interpreted as stress-related effects; rather, a distinction should be made between the beneficial effects of adequate Zn nutrition and the potentially detrimental effects associated with excessive Zn accumulation.

Conclusion

Zn supplementation induced concentration-dependent changes in several physiological and biochemical parameters in *Dunaliella salina*, reflecting both its essential nutritional role and its potential adverse effects at elevated concentrations. The lowest Zn concentration (0.03 μg/mL) enhanced photosynthetic efficiency, chlorophyll content, soluble sugar content, protein accumulation, and catalase activity, suggesting improved metabolic performance and antioxidant responses. These responses suggest a predominantly beneficial and stimulatory effect of Zn rather than a pronounced stress response. In contrast, the highest Zn concentration (0.5 μg/mL) increased proline, total free amino acid content, APX activity, and PPO activity while reducing protein content, suggesting the activation of stress-related protective responses. The control treatment generally exhibited lower levels of several metabolic and antioxidant parameters, highlighting the importance of Zn for optimal cellular function. Overall, *Dunaliella salina* exhibited considerable metabolic flexibility and modulation of antioxidants in response to Zn availability. These findings emphasize the importance of optimizing Zn concentrations to support algal physiological performance and potentially enhance applications in metabolite production and bioremediation.

Author contribution statement

S. M. R. conceived and designed the experiments. M. Kh. prepared the initial draft of the manuscript. Data analysis was performed by S. M. R., L. B., S. M. M., A. Gh., and M. Kh. All authors read and approved the final

version of the manuscript.

References

Aebi, H. (1984) 'Catalase in vitro', Methods in Enzymology, 105, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Alirahimi, N., Kordenaeej, A., Rezaei, A. and Talei, D. (2022) 'Changes in growth, beta-carotene production, traits, and protein banding patterns in *Dunaliella salina* microalgae in the presence of colchicine', *Journal of Marine Science and Technology*, 20(4), pp. 86–94. https://doi.org/10.22113/jmst.2016.38962.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) 'Rapid determination of free proline for water-stress studies', Plant and Soil, 39(1), pp. 205–207. https://doi.org/10.1007/BF00018060.

Bradford, M.M. (1976) 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding', *Analytical Biochemistry*, 72(1–2), pp. 248–254. https://doi.org/10.1006/abio.1976.9999.

Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and Lux, A. (2007) 'Zn in plants', *New Phytologist*, 173(4), pp. 677–702. https://doi.org/10.1111/j.1469-8137.2007.01996.x.

Cakmak, I. (2000) 'Possible roles of Zn in protecting plant cells from damage by reactive oxygen species', *New Phytologist*, 146(2), pp. 185–205. https://doi.org/10.1046/j.1469-8137.2000.00630.x.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) 'Colorimetric method for determination of sugars and related substances', *Analytical Chemistry*, 28(3), pp. 350–356. https://doi.org/10.1021/ac60111a017.

Elleuch, J., Hmani, R., Drira, M., Michaud, P., Fendri, I. and Abdelkafi, S. (2021) 'Zinc biosorption by *Dunaliella* sp. AL-1: Mechanism and effects on cell metabolism', *Science of the Total Environment*, 773, 145024. https://doi.org/10.1016/j.scitotenv.2021.145024.

Folgar, S., Torres, E., Pérez-Rama, M., Cid, A., Herrero, C. and Abalde, J. (2009) '*Dunaliella salina* as a marine microalga highly tolerant to, but a poor remover of, cadmium', *Journal of Hazardous Materials*, 165(1–3), pp. 486–493. https://doi.org/10.1016/j.jhazmat.2008.10.004.

Gao, M., Ling, N., Tian, H., Guo, C. and Wang, Q. (2024) 'Toxicity, physiological response, and biosorption mechanism of *Dunaliella salina* to copper, lead, and cadmium', *Frontiers in Microbiology*, 15, 1374275. https://doi.org/10.3389/fmicb.2024.1374275.

Hamed, S.M., Zinta, G., Klöck, G., Asard, H., Selim, S. and AbdElgawad, H. (2017) 'Zn-induced differential oxidative stress and antioxidant responses in *Chlorella sorokiniana* and *Scenedesmus acuminatus*', *Ecotoxicology and Environmental Safety*, 140, pp. 256–263. https://doi.org/10.1016/j.ecoenv.2017.02.039.

Kabata-Pendias, A. (2011) *Trace Elements in Soils and Plants*. 4th edn. Boca Raton, FL: CRC Press.

Lichtenthaler, H.K. (1987) 'Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes', *Methods in Enzymology*, 148, pp. 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Massyuk, N.P. (1996) 'Mass culture of the carotene-bearing alga *Dunaliella salina* Teod.', *Ukrainian Botanical Journal*, 23, pp. 12–19.

Maxwell, K. and Johnson, G.N. (2000) 'Chlorophyll fluorescence—A practical guide', *Journal of Experimental Botany*, 51(345), pp. 659–668. https://doi.org/10.1093/jex-

bot/51.345.659.

Mayer, A.M. and Harel, E. (1979) 'Polyphenol oxidases in plants', *Phytochemistry*, 18(2), pp. 193–215. https://doi.org/10.1016/0031-9422(79)80057-6.

Mishra, A. and Jha, B. (2011) 'Antioxidant response of the microalga *Dunaliella salina* under salt stress', *Botanica Marina*, 54(2), pp. 195–199. https://doi.org/10.1515/BOT.2011.027.

Moore, S. and Stein, W.H. (1963) 'Methods for amino acid analysis', *Analytical Chemistry*, 35(13), pp. 1965–1970. https://doi.org/10.1021/ac60205a045.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M. (2010) 'Heavy metals, occurrence and toxicity for plants: A review', *Environmental Chemistry Letters*, 8, pp. 199–216. https://doi.org/10.1007/s10311-010-0297-8.

Nakano, Y. and Asada, K. (1981) 'Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts', *Plant and Cell Physiology*, 22(5), pp. 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Palomares, A.J., Rubio, F. and Florencio, F.J. (2004) 'Role of carbonic anhydrase in inorganic carbon uptake by phytoplankton', *Biochemical Journal*, 382(2), pp. 353–361. https://doi.org/10.1042/BJ20040484.

Pilon, M. and Várkonyi, A. (2017) 'Plant metal homeostasis—Challenges and opportunities', *Current Opinion in Plant Biology*, 38, pp. 151–159. https://doi.org/10.1016/j.pbi.2017.05.004.

Rathore, D., Singh, A.K. and Pandey, A. (2015) 'Effect of Zn on growth and β-carotene production in *Dunaliella salina*', *Journal of Environmental Biology*, 36(1), pp. 141–147.

Riahi, H. (2002) *Algology*. 2nd edn. Tehran: Alzahra University Press.

Roy, U.K., Nielsen, B.V. and Milledge, J.J. (2021) 'Tuning *Dunaliella tertiolecta* for enhanced antioxidant production by modification of culture conditions', *Marine Biotechnology*, 23(3), pp. 482–500. https://doi.org/10.1007/s10126-021-10039-7.

Salehipour-Bavarsad, F., Riahi, H., Hejazi, M.A. and Shariatmadari, Z. (2022) 'Screening terrestrial and aquatic strains of *Dunaliella* from the eco-taxonomical perspective: A comparative study on fatty acid compositions as habitat indicators', *Journal of Applied Phycology*, 34(1), pp. 461–474. https://doi.org/10.1007/s10811-021-02645-3.

Sui, Y., Mazzucchi, L., Acharya, P., Xu, Y., Morgan, G. and Harvey, P.J. (2021) 'A comparison of β-carotene, phytoene, and amino acid production in *Dunaliella salina* DF15 (CCAP 19/41) and *Dunaliella salina* CCAP 19/30 under different light wavelengths', *Foods*, 10(11), 2824. https://doi.org/10.3390/foods10112824.

Sunda, W.G. and Huntsman, S.A. (1995) 'Effect of free metal ion concentration on the survival of the marine diatom *Thalassiosira weissflogii*', *Limnology and Oceanography*, 40(3), pp. 512–517. https://doi.org/10.4319/lo.1995.40.3.0512.

Wang, W.-X. (2002) 'Interactions of trace metals and different marine food chains', *Marine Ecology Progress Series*, 243, pp. 295–309. https://doi.org/10.3354/meps243295.

Whittaker, R.H. and Margulis, L. (1978) 'Protist classification and the kingdoms of organisms', *BioSystems*, 10(1–2), pp. 3–18. https://doi.org/10.1016/0303-2647(78)90006-3.

Wongsnansilp, T., Juntawong, N. and Wu, Z. (2018) 'Effects of phosphorus on the growth and chlorophyll fluorescence of a *Dunaliella salina* strain isolated from saline soil under nitrate limitation', *Journal of Biological Research-Bollettino della Società Italiana di Biologia Sperimentale*, 91(1). https://doi.

org/10.4081/jbr.2018.7541.

Xiong, Z.T. (2006) ‘Bioaccumulation and physiological effects of excess Zn in plants’, *Reviews of Environmental Contamination and Toxicology*, 186, pp. 67–113. https://doi.org/10.1007/0-387-32883-2-3.