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Original Article

Assessment of Oxidative Responses in Halophilic Green Microalga *Dunaliella salina* Exposed to Functionalized Carbon Nanotubes: Effects of Functional Group and Particle Type

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Abstract

Background & Aims: The large-scale production and extensive utilization of carbon nanotubes (CNT) in numerous industries have raised significant concerns among environmentalists. The objective of this study was to assess the toxicity and environmental impact of functionalized carbon nanotubes (*f*CNTs) on the halophilic green alga *Dunaliella salina*.

Materials and Methods: The present work evaluated the effects of single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) functionalized with carboxyl and amine groups (as representatives of NTs with negative and positive surface charges, respectively) on photosynthetic pigment (chlorophyll), cell proliferation, and some oxidative stress indicators, including lipid peroxide, protein carbonyl, and hydrogen peroxide content, in a strain of halophilic green algae native to Iran.

Results: The findings indicated that the presence of NTs did not have a notable impact on the levels of chlorophylls a and b, as well as the intracellular hydrogen peroxide levels in algae. However, when examining cell density, it was observed that CNTs exhibited varying degrees of toxicity, particularly at high concentrations and during the logarithmic growth phase of the algae. The results confirmed that the inhibition of alga growth is influenced by the type of functional group. When carboxyl-functionalized SWCNT was used, there was no alteration in the lipid peroxidation (LPX) level at any examined NT concentration. However, when carboxyl-functionalized MWCNT was employed, the level of LPX was consistently lower than the control. On the other hand, amine-functionalized CNTs demonstrated a significantly increasing impact on LPX at high concentrations. Although both functionalized forms of SWCNT can increase the levels of protein carbonyl in algal cells, MWCNTs have no significant effects on cell protein carbonylation.

Conclusion: The results suggest that at concentrations less than 10 mg/L, the anti-stress properties of carboxyl-functionalized CNTs outweigh their cytotoxic properties. Furthermore, SWCNTs have a higher potential for inducing protein carbonylation compared to MWCNTs, regardless of the type of functional group.

Keywords: Oxidative stress, Nanotubes, Carbon, Toxicity, Halophilic green algae

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1. Introduction

Carbon nanotubes (CNTs) are a group of engineered nanoparticles (NPs) that have gained significant attention for their wide range of applications in industries such as polymer composites, electronics, medical equipment, chemical manufacturing, and environmental remediation [1-4]. Production and widespread use of NPs on an industrial scale have caused environmental concerns about the possibility of pollution of the natural environment [5,6]. CNTs are produced in two main types, namely, single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT). Raw CNTs are hydrophobic and do not distribute uniformly in aqueous environments. Therefore, they are often functionalized through chemical processes in order to enhance dispersion in water media. For instance, the acidic oxidation of MWCNTs produces carboxyl-functionalized MWCNT (MWCNT-COOH), which is dispersible in water [7]. Given the anticipated increase in the production and release of CNT derivatives into the environment, it is crucial to study their ecological toxicity and potential long-term impacts on the environment and human health [2,7].

Algae play an important role in monitoring environmental pollution and are commonly utilized as model organisms in nanotoxicology studies. They serve as the foundation of the aquatic food chain and contribute to the nutrient cycle of aquatic ecosystems. Consequently, algae are recognized as valuable indicators of environmental pollution [8]. Based on available findings, exposure of algae to CNTs can result in three different events. Firstly, CNTs can create shadow effects and prevent

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light from reaching the algae. Secondly, the accumulation and physicochemical reactions of algae and CNTs can cause cell membrane damage and the destruction of intracellular structures. Lastly, the production of reactive oxygen species (ROS) can cause oxidative stress (OS) on the cell surface, leading to the peroxidation of membrane lipids, physiological disorders in cell membranes, and DNA damage [9]. It should be pointed out that OS caused by ROS production is considered the primary cause of toxicity in algae [4,10]. Three types of indicators have been introduced with regard to OS. These indicators include direct measurement of ROS, measurement of oxidation products of biomolecules, and measurement of the enzymatic antioxidant defense system [4,10,11].

Measuring the oxidation products of biomolecules, such as lipids and proteins, is commonly used in toxicology studies. Malondialdehyde (MDA) is a significant indicator of OS and the final product of lipid breakdown. Elevated MDA levels signify increased lipid peroxidation (LPX) and damage to cell membranes. MDA is a crucial biomarker for LPX, and its higher levels indicate a disruption in antioxidant defense mechanisms [11,12]. Algal responses to different NPs in the environment are currently unclear, and comprehensive research on the toxic properties of these materials can facilitate the development of safe and effective NP-based technologies for the future [13]. Specifically, it is necessary to research the potential ecological effects and toxicity of CNTs in marine environments.

Different types of CNTs, such as SWCNT and MWCNT, may exhibit varying biological behaviors and cytotoxicity levels in marine algae due to their different geometric structures. Very few studies have been performed on the toxicity of CNTs on algae, and the available information on the potential harmful effects of different forms of these nanomaterials on marine species is extremely limited [9].

The current study investigated the impact of SWCNTs and MWCNTs with amine (i.e., $-NH_2$) and carboxyl (i.e., –COOH) functional groups on chlorophyll, cell proliferation, and OS indicators in *Dunaliella salina*, a green algal strain native to the Persian Gulf. The aim was to understand the toxicity and environmental effects of different CNTs in saline waters. Due to the diversity in the synthesis methods of CNTs as well as ecological conditions and biodiversity, the present study was conducted on CNTs made at Iran's Research Institute of Petroleum Industry and a green algal strain native to the Persian Gulf to accurately document their biological effects in the saltwater ecosystems of the country.

2. Materials and Methods

2.1. Preparation of functionalized carbon nanotubes and structure evaluation

To prepare carboxyl-functionalized CNTs (i.e., MW-COOH or SW-COOH), 10 mg of raw CNTs were mixed with a 40 mL solution of H_2SO_4 and HNO_3 in a volume ratio of 3:1. The mixture was then placed in an ultrasonic bath at a temperature of 40 ˚C for 4 hours. The resulting solution was then slowly added to two liters of deionized water and passed through a 0.45-μm Whatman filter. Next, the filter cake (CNT-COOH) was washed with deionized water until neutral pH was obtained and finally placed in an oven at 70 ˚C until complete drying [14]. The prepared CNT-COOH was then utilized to produce amine-functionalized CNTs (i.e., MW-NH_2 or SW-NH_2). In this regard, CNT-COOH was dispersed in dimethylformamide and thionyl chloride ($SOCl_2$) and subjected to reflux conditions at 120 ˚C with magnetic stirring at 240 rpm for 24 hours. Subsequently, ethylenediamine (EDA) was introduced to the refluxed solution, and incubation was prolonged at 90 ˚C for 48 hours. The obtained product was centrifuged at 10 000 rpm for 30 minutes. Finally, the product containing CNT-NH2 was washed with tetrahydrofuran, followed by ethanol [14].

2.2. Characterization of functionalized carbon nanotubes

Fourier-transform infrared spectroscopy was utilized to ensure the functionalization of CNTs. Additionally, transmission electron microscope imaging was employed to examine the structure and size of NTs.

2.3. Preparation of algal strain and preparation of culture medium

The algal strain examined in this study is *D. salina*, a species native to the Persian Gulf, acquired from the Iranian Biological Resources Center. This unicellular halophilic green alga is predominantly present in high-salinity water environments and possesses chloroplasts and two flagella of equal length [15]. Its advantageous compounds, including carotenoid pigment, glycerol, and unsaturated fatty acids, have been shown to possess antioxidant, anticancer, and antimicrobial properties, making it a viable option for use in the food, pharmaceutical, and cosmetic industries [16]. It was cultured in a modified Johnson culture medium by suspending a loop of purchased algae in 50 mL of sterile culture medium in an Erlenmeyer flask and incubating at a temperature of 25–30 ˚C under light.

2.4. Alga exposure tests

Four different types of the prepared CNTs, including SWCNT-COOH, SWCNT-NH2, MWCNT-COOH, and MWCNT-NH2, at five different concentrations between 2.5 and 40 mg/L, were examined to investigate the toxic effects of the functionalized CNTs on *D. salina* [17]. The CNT concentrations were chosen based on our unpublished preliminary studies demonstrating that exposing algae to low concentrations of NTs (less than 2 mg/L) did not result in any significant cellular responses. There is a significant effect on the alga growth rate at CNT concentrations of more than 50 mg/L; however, due to the aggregation

of CNTs and the strong changes in the color of the environment and shadowing effects, concentrations higher than 40 mg/L were excluded from our investigation. It should be mentioned that the experiments were conducted under controlled conditions in an incubator at 25 ˚C with a light intensity of 2000 lux. Culture media sampling and cell counting were also performed during the growth period of this algae on days 4, 8, 14, and 21.

2.5. Determination of the cell density of green algae

To determine cell density for the assessment of the median effective concentration (EC_{50}), direct counting was performed using a Neubauer chamber [18]. The cells were treated with formaldehyde before counting at different time points (days 4, 8, 14, and 21).

2.6. Chlorophyll measurement

To measure the amount of chlorophyll a and b at different times of alga culture, one milliliter of alga culture was removed from the culture medium under sterile conditions and centrifuged at 3000 rpm for 15 minutes. Then, 90% acetone (1 mL) was added to the cell pellet. The samples were refrigerated for 24 hours at 4 ˚C and subsequently centrifuged. The ultraviolet-visible (UV-Vis) spectrophotometer was then used to measure the optical absorption of the supernatant, which contained chlorophylls, within the wavelength range of 370–700 nm. The amount of chlorophyll a and b was calculated using the following formulas:

$$\text{Chl-a} = 15.65\ A_{666} - 7.340\ A_{653} \quad (1)$$

$$\text{Chl-b} = 27.05\ A_{653} - 11.21\ A_{666} \quad (2)$$

2.7. Measurement of protein content

The protein content of the alga cells was measured using the Bradford method [19]. The working solution of Bradford's reagent was prepared by mixing 5 mg of Coomassie Brilliant Blue dye G-250 with 5 mL of ethanol and 10 mL of phosphoric acid (w/v% 85). The final volume of the reagent was adjusted to 100 mL with deionized water [19]. To measure the protein content, the alga cells were disrupted using a probe sonicator in an ice bath. Subsequently, the resulting samples were centrifuged at 15000 rpm for 15 minutes, and then the supernatant solution was utilized to quantify the concentration of cellular protein. In this regard, one milliliter of Bradford's reagent was added to a specific amount of algal protein extract, and the optical density (OD) of the samples was measured after 5 minutes at the wavelength of 595 nm using a UV-Vis spectrophotometer. Bovine serum albumin was utilized to prepare the protein standard curve. The protein concentration of each sample was calculated using the graph line equation.

2.8. Alga cellular responses

2.8.1. Measurement of intracellular content of hydrogen peroxide

The method suggested by Alexieva ⁋et al [20] was employed to measure intracellular hydrogen peroxide (H_2O_2) content. Briefly, alga samples were treated with 10% trichloroacetic acid (TCA) in an ice bath and centrifuged at 7000 g for 10 minutes. The resulting supernatant was mixed with 50 mM phosphate buffer and 1 M potassium iodide. Optical absorption was measured at 390 nm using a UV-Vis spectrophotometer. The commercial solution of H_2O_2 was used as a standard, and the amount of cellular H_2O_2 was expressed as nanomoles of H_2O_2 per mg of cell protein [20].

2.8.2. Lipid Peroxidation Assay: Measuring the Amount of Cellular Malondialdehyde

The estimation of lipid LPX in cells involves the reaction of MDA with thiobarbituric acid [21, 22]. In this method, cells were treated with a mixture of 20% TCA and 0.67% thiobarbituric acid (prepared in hydrochloric acid 1 M), followed by incubation in a water bath at 95 ˚C for 45 minutes and subsequent cooling to room temperature. The samples were then centrifuged for 15 minutes at 10,000 g. The absorbance of the supernatant solution was measured at a wavelength of 532 nm using a UV-Vis spectrophotometer. The concentration of MDA in each sample was determined using 1,1,3,3-tetramethoxypropane as the standard. To prepare the standard chart, a 100-µM stock solution of 1,1,3,3-tetramethoxypropane was prepared in water. Dilutions ranging from 2 to 100 µM of this compound were used to construct the standard graph. The optical absorption of each sample at a wavelength of 532 nm was measured, and the concentration of MDA in alga samples was determined as MDA µM per mg of cell protein.

2.8.3. Measurement of protein carbonyl content of the cell

The content of protein carbonyl in algal cells was measured by breaking the cells in an ice bath using the probe sonicator and centrifuging the resulting mixture at 15000 rpm for 15 minutes. The supernatant solution was then mixed with streptomycin sulfate 10% in an ice bath and centrifuged at 14000 g for 10 minutes. After separating the supernatant solution, it was mixed with 10 mM dinitrophenyl hydrazine (prepared in hydrochloric acid 2 M) and left at room temperature for one hour. Subsequently, a 20% TCA solution was added and allowed to react for an additional 15 minutes to facilitate protein precipitation. Following centrifugation at 4500 g for 5 minutes, the resulting supernatant was discarded, and the remaining precipitate was washed twice with an ethanol:ethyl acetate (1:1) mixture to remove any excess dinitrophenyl hydrazine. The final precipitate was treated with guanidine hydrochloride and dithiothreitol solution, and optical absorption was then measured at a wavelength

of 370 nm using a UV-Vis spectrophotometer [23].

2.9. Statistical calculations

In this research, the physiological responses of alga samples exposed to different CNTs were compared to the control unexposed group. The experiments were conducted with a single variable (CNT concentration), and the environmental factors such as temperature, light intensity, and pH were kept constant. The experiments were conducted in triplicate at each selected concentration. The data were presented as means ± standard deviations (SD) and analyzed using the Student's T-test statistical method, with a significance level of $P < 0.05$.

3. Results

3.1. Evaluation of functionalized carbon nanotube morphology

Figure 1 displays the FTIR spectra of raw, carboxyl-functionalized, and amine-functionalized CNTs. The presence of hydroxyl groups is indicated by a broad peak at 3400 cm^{-1}. Additionally, the spectrum reveals peaks at 1101 cm^{-1} and 1720 cm^{-1}, representing the C-O and C = O bonds, respectively, in the carboxylic acid (COOH) group (Figure 1B). In the case of CNTs derivatized with EDA, the stretching vibrations of the methylene group are observed at 2957 cm^{-1}, 2922 cm^{-1}, and 2871 cm^{-1} (Figure 1C). Furthermore, peaks at 1666 cm^{-1} and 1233 cm^{-1} confirm the presence of the amine functional group (Figure 1C). The transmission electron microscope images of CNTs are depicted in Figure 2. SWCNTs have a diameter of 10–15 nm (Figure 2A), while MWCNTs have a diameter of approximately 20–25 nm (Figure 2B). The length of the prepared CNTs is about 10–20 µm.

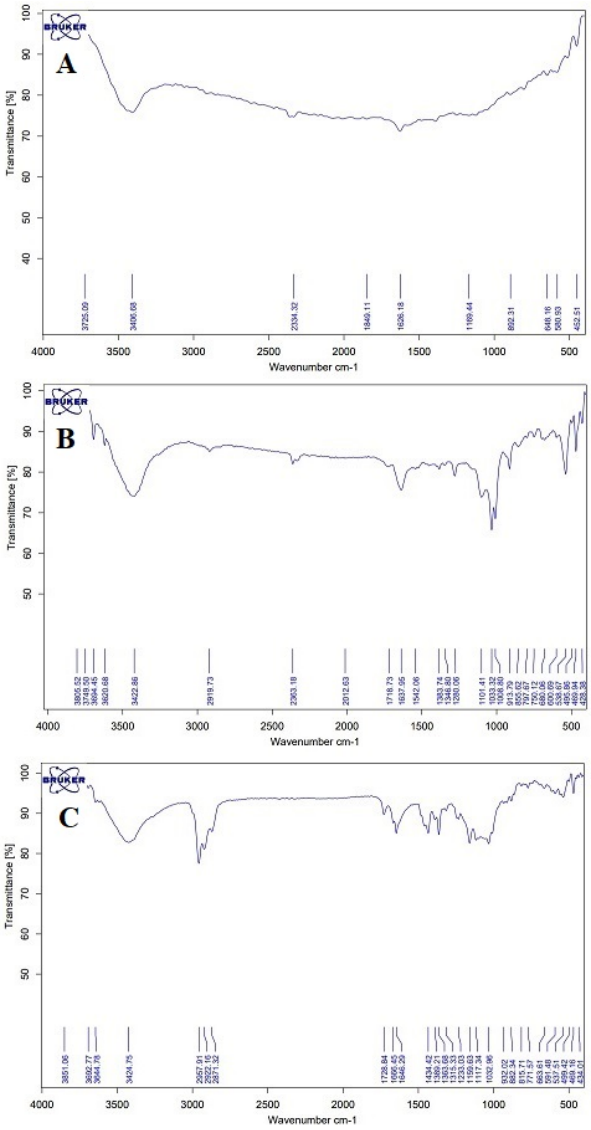

Figure 1. FTIR Spectra of (A) raw CNT, (B) carboxyl-functionalized CNT, and (C) amine-functionalized CNT. *Note*. FTIR: Fourier-transform infrared spectroscopy; CNT: Carbon nanotube

3.2. Effect of functionalized carbon nanotubes on alga cell density

The changes in the number of algal cells in the culture

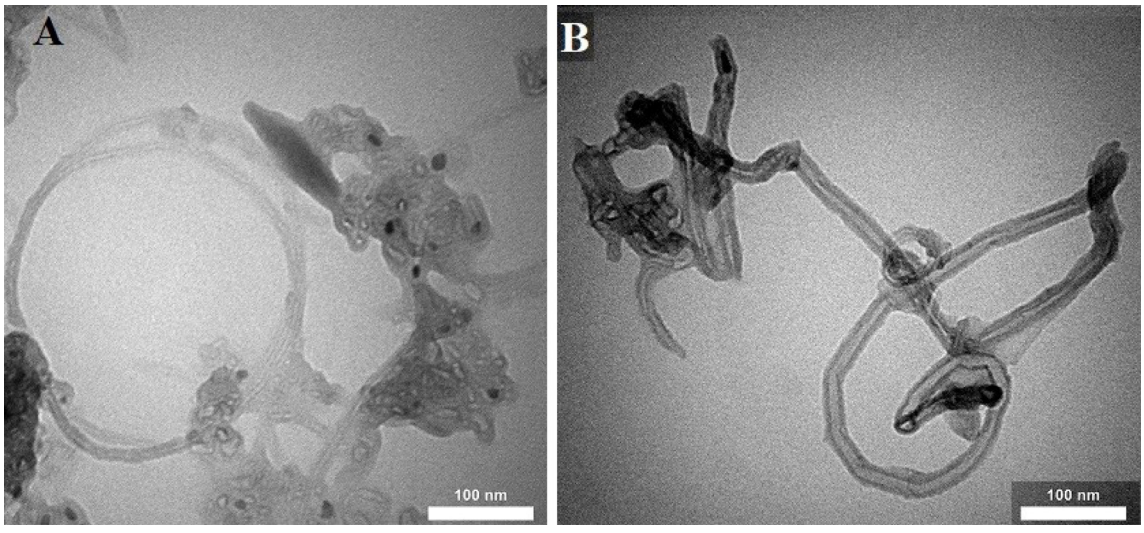

Figure 2. TEM Images of (A) Single-walled and (B) Multi-walled CNT. *Note*. TEM: Transmission electron microscope; CNT: Carbon nanotube

medium were monitored over 3 weeks. Based on probit analysis, the EC_{50}-96 hour values of SWCNT-COOH, MWCNT-COOH, SWCNT-NH2, and MWCNT-NH2 against *D. salina* were calculated as 37 mg/L, 40 mg/L, 18 mg/L, and 11 mg/L, respectively. Based on Figure 3, in both control and treated samples, the cell count increased until the eighth day of incubation. The algal cells entered the death phase, and cell destruction occurred by culture aging. Notably, on the fourth day of incubation, algae exposed to a concentration of 40 mg/L of either SWCNT-COOH or MWCNT-COOH exhibited a remarkable decrease in cell density compared to the control, unexposed cells. This decreasing effect was maintained after 8 days of incubation only in the cells exposed to SWCNT-COOH, while in the cells treated with 40 mg/L of MWCNT-COOH, a growth-stimulating effect was observed, and cell density reached the control level. In other words, the inhibitory effect of MWCNT-COOH at this concentration is temporary, and by continuing the incubation, MWCNT-COOH promotes growth, probably by removing harmful substances from the culture medium, creating conditions similar to the control level.

The behavior of *D. salina* in dealing with amine-functionalized CNTs is comparable to that of species exposed to carboxyl-functionalized CNTs. As shown, SWCNT-NH2 at 20 mg/L and 40 mg/L (Figure 3C) and MWCNT-NH2 at 10 mg/L, 20 mg/L, and 40 mg/L (Figure 3D) caused a significant reduction in cell density on the fourth day of incubation compared to the control group. This reduction in cell density persisted until the eighth day of incubation and became less pronounced by increasing the incubation period to more than eight days. It was found that functionalized CNTs can prolong the growth phase of cells, despite a decrease in cell density. This behavior can be explained by the scavenging of harmful substances of metabolism by CNTs or by the lower depletion rate of essential salts required for growth from the culture medium due to a decrease in cell density. Compared to the carboxyl-functionalized CNTs, the amine-functionalized CNTs exhibited stronger inhibitory effects on the growth of algae, particularly at concentrations of 40 mg/L. This enhanced inhibitory effect can be attributed to the higher affinity of the amine group to bind and penetrate the cells of algae.

3.3. Effects of functionalized carbon nanotubes on the chlorophyll content of algae

The photochemically active pigment in photosynthetic organisms is chlorophyll a. In many phylogenetic groups, the presence of other photochemically active pigments, such as chlorophyll b, enhances the light-trapping efficiency. Chlorophyll b, an important auxiliary pigment, is found in higher plants and green algae, and the ratio of chlorophyll a to b (Chl a/b) is in the range of 2–3. The toxicity of NPs on photosynthetic organisms can be demonstrated by the inhibition of photosynthetic activity, which serves as a primary biomarker [24]. This inhibition is attributed to the accumulation of ROS in the chloroplast and changes in the level of proteins and lipids within this intracellular organelle [25–28]. In the present study, it was

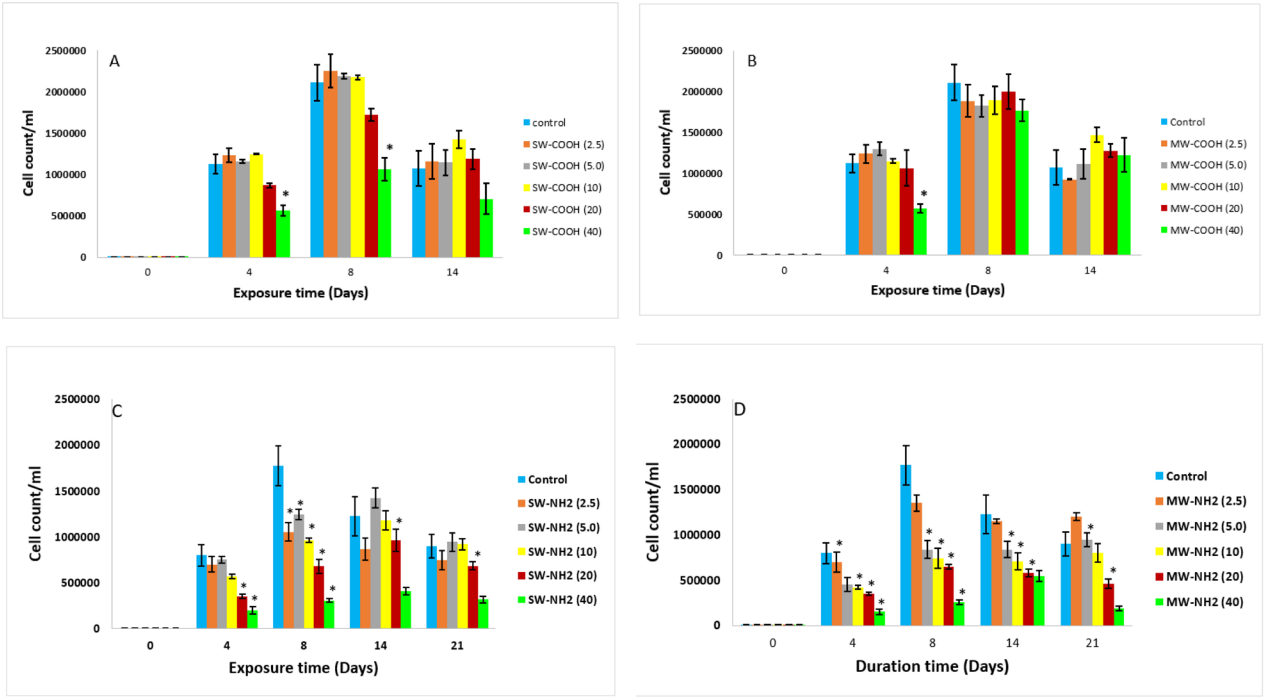

Figure 3. Effect of functionalized CNTs on alga cell density: (A) Carboxyl-functionalized SWCNT, (B) carboxyl-functionalized MWCNT, (C) amine-functionalized SWCNT, and (D) amine-functionalized MWCNT. *Note*. CNT: Carbon nanotube; SWCNT: Single-walled carbon nanotube; MWCNT: Multi-walled carbon nanotubes. The results were significant at $P<0.05$

demonstrated that the presence of CNTs in the media had no notable impact on the levels of chlorophylls a and b in green alga *D. salina*. Additionally, the analysis of the chlorophyll a: chlorophyll b ratio, ranging from 2.5 to 3.1 in all the control and treated groups, revealed that there was no significant difference between different treated groups and the control group based on this parameter.

3.4. Cellular responses of algae exposed to functionalized carbon nanotubes

3.4.1. Changes in lipid peroxidation index

NP toxicity in algae is primarily caused by ROS production and subsequent LPX. In this regard, active substances such as superoxide, hydroxyl radicals, and hydrogen peroxide react with the methylene groups of polyunsaturated fatty acids. During this reaction, the oxidation of polyunsaturated fatty acids leads to the formation of various byproducts, such as lipid peroxyl radicals, hydroperoxides, conjugated dienes, and MDA. Among these byproducts, MDA, as the primary end-product of LPX in cells, is commonly used as a marker for OS.

In this study, the MDA content of *D. salina* was measured after exposure to CNTs. Based on the data in Figures 4A and 4B, the MDA level in the alga cells treated with carboxyl-functionalized CNTs within the concentration range of 2.5–40 mg/L represents no significant increase. As shown, exposure to MWCNT-COOH (2.5 mg/L and 5 mg/L) or SWCNT-COOH (5 mg/L and 10 mg/L) could result in a significant reduction in the MDA level compared to the control. This decrease in the MDA level may be attributed to the potential of carboxyl-functionalized CNTs to scavenge ROS. This reduction in MDA production has also been observed in animal models and human cultured cells [29,30]. This behavior indicates that at low concentrations of carboxyl-functionalized CNTs, anti-stress properties are superior to cytotoxic properties.

In the case of amine-functionalized CNTs at low concentrations, there was no significant effect on intracellular MDA production in *D. salina*. However, at higher concentrations, both SWCNT-NH2 and MWCNT-NH2 induced a significant increase in the MDA level compared to the control.

3.4.2. Changes in protein carbonylation index

Protein carbonyls are known as reliable indicators of protein oxidation in cells. They are primarily formed as a result of covalent and irreversible modifications of the side chains of cysteine, histidine, and lysine residues within proteins, catalyzed by ROS. During environmental stress conditions, primary (e.g., alpha-amino adipic acid and gamma-glutamic semialdehyde) and secondary

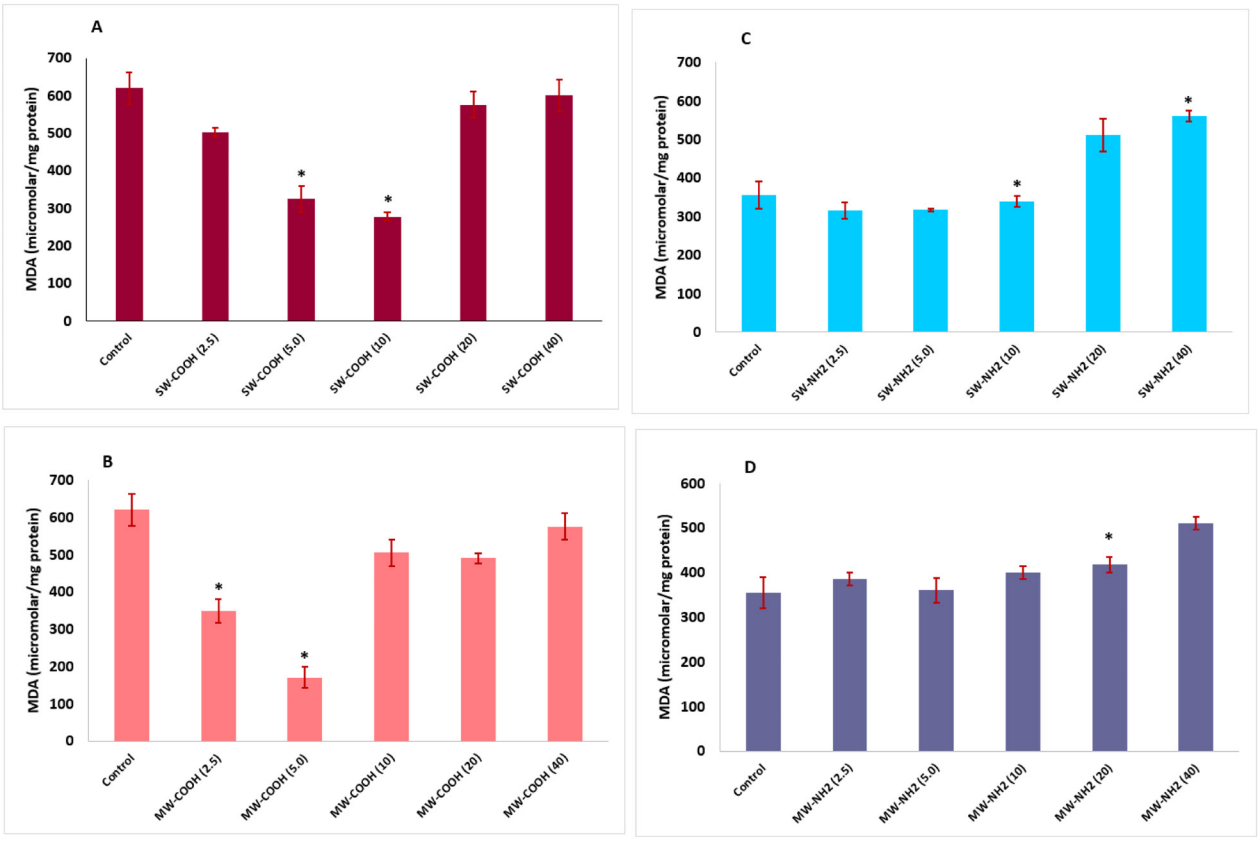

Figure 4. MDA levels in alga cells treated with functionalized CNTs: (A) Carboxyl-functionalized SWCNT, (B) carboxyl-functionalized MWCNT, (C) amine-functionalized SWCNT, and (D) amine-functionalized MWCNT. *Note*. CNT: Carbon nanotube; SWCNT: Single-walled carbon nanotube; MWCNT: Multi-walled carbon nanotube. The data were significant at $P<0.05$

(formed through covalent binding of lipid carbonyls such as MDA with proteins) protein carbonyls are generated within the cell. These compounds may collect up to 70% of intracellular ROS [31,32]. ROS production and activation of molecular signaling pathways associated with OS have been reported by researchers using raw or functionalized CNTs in animal models [33]. Studies have demonstrated that carboxyl-functionalized CNTs, compared to their original raw materials, exhibit enhanced interactions with cellular proteins [34]. The impact of NPs on ROS production and cell death has also been found to be influenced by their size and type of functional groups [35]. Furthermore, the chemical structure and type of nanomaterials may determine the type of produced ROS [36]. As a result, the presence and type of the functional group are crucial factors in determining the toxicity potential of CNTs in living organisms, including algae.

Our findings showed that when *D. salina* was treated with SWCNT-COOH (in the range of 2.5–40 mg/L), protein carbonyl levels increased significantly (Figure 5A), while in cells treated with MWCNT-COOH, only a non-significant decrease was observed in protein carbonyl levels at concentrations of 2.5 mg/L and 5 mg/L (Figure 5B). The impact of MWCNT-NH2 on the protein carbonyl level in *D. salina* cells was found to be negligible (Figure 5D). However, a noticeable rise in protein carbonyl level was observed in cells treated with SWCNT-NH2 (≥5 mg/L, Figure 5C). In general, regardless of the type of functional group, SWCNTs have a higher potential to induce protein carbonyl in algal cells when compared to MWCNTs.

3.4.3. Effects of functionalized carbon nanotubes on the hydrogen peroxide content of algae

Environmental stress can disrupt cellular balance and result in the generation of ROS. However, a coordinated network of defense mechanisms can safeguard the cell against OS and enable it to adapt to new conditions. Superoxide within cells can be converted into hydrogen peroxide either spontaneously or with the help of superoxide dismutase. Hydrogen peroxide is a relatively stable molecule that readily passes through cell membranes and damages various cellular components. In addition, hydrogen peroxide operates as a signaling molecule and activates the defense system. Small intracellular antioxidant molecules, such as glutathione and ascorbate, along with antioxidant enzymes, can effectively capture hydrogen peroxide and mitigate its detrimental impact.

In the present work, it was found that exposure to varying concentrations of functionalized CNTs did not

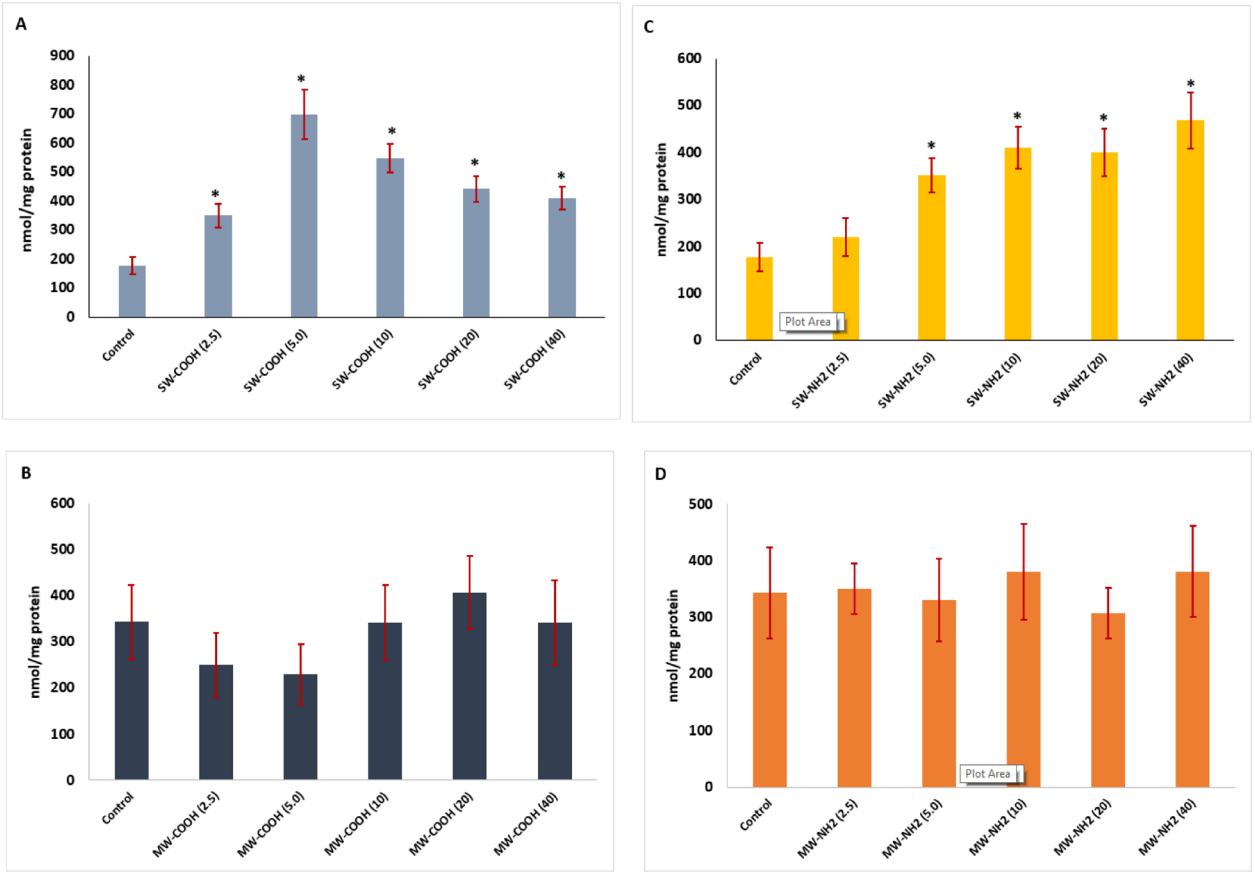

Figure 5. Protein carbonyl levels in alga cells treated with functionalized CNTs: (A) Carboxyl-functionalized SWCNT, (B) Carboxyl-functionalized MWCNT, (C) Amine-functionalized SWCNT, and (D) Amine-functionalized MWCNT. *Note*. CNT: Carbon nanotube; SWCNT: Single-walled carbon nanotube; MWCNT: Multi-walled carbon nanotubes. The significance level is $P<0.05$

result in any significant alteration in the intracellular hydrogen peroxide levels. Considering that remarkable changes were observed in the levels of LPX and protein carbonylation of algal cells exposed to CNTs (Figures 4 and 5), firstly, the level of the generated ROS was low, and secondly, the produced superoxide was inactivated during the reaction with cellular lipids and proteins before being converted into hydrogen peroxide and diffusing inside the cells.

Conclusion

The current investigation aimed to explore the impact of carboxyl- and amine-functionalized CNTs on OS in halophilic green microalga *D. salina*. The findings revealed that the MDA level significantly decreased in cells exposed to low concentrations of MWCNT-COOH or SWCNT-COOH compared to the control group. SWCNT had the potential to induce protein carbonylation in algal cells, regardless of the functional group type. However, the intracellular level of hydrogen peroxide remained unchanged when algal cells were exposed to different concentrations of functionalized CNTs. Based on the changes in the levels of LPX and protein carbonylation in algal cells, the levels of ROS produced during exposure to CNTs were low. Furthermore the produced superoxide was inactivated due to reactions with cellular lipids and proteins before conversion into hydrogen peroxide.

Authors' Contribution

Conceptualization: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
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Formal analysis: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Funding acquisition: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Investigation: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Methodology: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Project administration: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Resources: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Software: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Supervision: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Validation: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Visualization: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Writing–original draft: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.
Writing–review & editing: Akram Hosseinnia, Samar Mortazavi, Majid Zeinali, Ebrahim Alaei.

Competing Interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This article was written based on the thesis approved by the 109th session of Malayer University's Graduate Education Council on 2020.8.16.

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