TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2020) 44: 222-231

doi:10.3906/bot-2001-12

Biochemical and morphological responses to cadmium-induced oxidative stress in *Cladophora glomerata*

Abuzer ÇELEKLİ*, Hilal BULUT
Department of Biology, Faculty of Arts and Science, University of Gaziantep, Gaziantep, Turkey

Received: 09.01.2020 • **Accepted/Published Online:** 13.03.2020 • **Final Version:** 06.05.2020

Abstract: This study aimed to assess the effect of cadmium (Cd^{2+}) concentrations on biovolume, pigments, malondialdehyde (MDA), hydrogen peroxide, proline, total phenolic compounds, total protein, and total carbohydrate contents of *Cladophora glomerata*. Cultivations of the alga (8.0 ± 0.1 g as fresh weight) were exposed to 7.5, 15, 30, and 60 mg/L of Cd^{2+} ions in 500 mL flasks containing 400 mL of the medium on a shaker at 120 rpm for 7 days. Results of Fourier transform infrared analyses indicated that the amide, anionic, and amino groups had significant rolesin the binding of Cd^{2+} on *C. glomerata*. The detrimental effects of the Cd^{2+} dose not only observed the morphology of the algal cell but also changed the biochemical compounds of *C. glomerata*. Growth gradually decreased when the alga was exposed to Cd^{2+} at 15 and 60 mg/L in comparison with the control. High Cd^{2+} ions concentrations decreased in chlorophyll-a (from 14.27 mg/g in control to 9.97 mg/g at 60 mg/L Cd) and protein content (from 43.60 mg/g in control to 21.66 mg/g at 60 mg/L Cd) in the treated cells compared to the control group, whereas they increased stress molecules (e.g., MDA and proline) as biomarkers in the response mechanisms of algae to Cd^{2+} exposure. Results indicated that this alga had wide tolerance to high cadmium concentrations, and the stress compounds in the alga with exposure of Cd^{2+} seemed to be parameters as a biomarker for metal-induced oxidative stress.

Keywords: Biochemical response, cadmium, *Cladophora glomerata*, pigmentation

1. Introduction

Cadmium (Cd^{2+}) is a toxic metal that influences the physiological activity and growth of biota even at low concentrations. Except for *Thalassiosira weissflogii*, Cd^{2+} is an unessential metal for biota (Lane et al., 2005). The amount of Cd^{2+} in surface waters has been markedly growing with the increase in disposal caused by iron and steel production, melting, manufacturing, electronic (nickel-cadmium batteries) industries, and agricultural activities with phosphate fertilizers (Hayat et al., 2019; Li et al., 2019). Disposal of such huge wastewaters into surface waters (e.g., rivers and lakes) cause major environmental problems for freshwater resources and biota (Wang and Chen, 2009; Zeraatkar et al., 2016). According to the United States Environmental Protection Agency, Cd^{2+} is considered one of the most hazardous contaminant threats to biota and the environment. Cd^{2+}can be easily taken and accumulated by primary producers and transferred to higher trophic levels through the food web (Wang and Chen, 2009; Templeton and Liu, 2010; Andosch et al., 2012). It is known that Cd^{2+}, a mutagenic and carcinogenic metal, affects calcium metabolism in biological systems (Sarwar et al., 2017). Besides, Cd^{2+} binds to organic molecules, and this may cause a wide range of adverse effects on living biota such as cancer, allergies, skin irritation, cell membrane damage, and a change of transporter systems and denaturation of proteins and enzymes (Andosch et al., 2012; Sarwar et al., 2017).

Cd^{2+}exposure promotes the formation of reactive oxygen species in organisms (Pinto et al., 2003), leading to morphological changes in the nucleus (Souza et al., 2011), lipid peroxidation (Pinto et al., 2003; Çelekli et al., 2016), morphological changes in the structure of cells, changes in the electron transport system, and cell death (Verbruggen et al., 2009; Andosch et al., 2012). Organisms under this type of stress demonstrate a few responses via metal detoxification and antioxidant defenses (Branco et al., 2010; Gomes and Asaeda, 2013). Some biomolecules, like ascorbic acid, phenolics, carotenoids, and glutathione, are produced to get rid of these reactive molecules (Branco et al., 2010; Gomes and Asaeda, 2013). Extracellular and/or intracellular metal exclusion through formation with diverse ligands, storage into vacuoles, and the pumping out of metal make up thevarious metal detoxification

* Correspondence: celekli.a@gmail.com

mechanisms (Branco et al., 2010; Gomes and Asaeda, 2013).

Algae as important primary producers (they transform solar energy, inorganic elements, and carbon dioxide into valuable biomass) that support other aquatic trophic levels play a major role in the biogeochemical cycles of the biosphere (Bellinger and Sigee, 2010; Yvon-Durocher et al., 2015) and also play a role in scavenging minerals and molecules from the environment (Li et al., 2019b). The survival of algae in surface waters contaminated with hazardous materials may also depend on their ability to generate signal molecules. Therefore, it is important to search for signal molecules mediating stress tolerance in order to gain a better understanding of how algae respond to hazardous chemicals. The effects of heavy metals on *Cladophora glomerata* have been studied in aquatic ecosystems such as the littoral region of Lake Ashtamudi in India (Murugan and Harish, 2007), the coastal waters of Alexandria City in Egypt (Ismail and Ismail, 2017), freshwater bodies in south-east Anatolia (Çelekli et al., 2017), and in the Farahabad region of the Caspian Sea in Iran (Ebadi and Hisoriev, 2017). However, the biochemical response of *Cladophora glomerata* exposure to Cd^{2+} stress at a lab-scale does not exist in the literature. The present study aimed to assess the effects of Cd^{2+} ions on the morphological and biochemical responses of *C. glomerata* in terms of biovolume, pigmentation, malondialdehyde, hydrogen peroxide, proline, and phenolic compounds. In addition, the algal surface structures under the exposure of Cd^{2+} ions were determined using a Fourier transform infrared spectrometer.

2. Materials and methods

2.1. Macroalga sampling and cultivation

Cladophora glomerata, a green macroalga used in the present study, was obtained from a freshwater creek in Malatya Province (Turkey). The collected filamentous algae was put into 5-L polyethylene bottles at 4 °C and transferred to the laboratory. In the laboratory, the filamentous alga was gently washed with tap water. It was then identified using a microscope (Olympus BX53 Cell Sens, Version 1.6, Olympus Corporation, Tokyo, Japan) and taxonomic books (Prescott, 1982; John, 2002). The morphological properties of *C. glomerata* such as cell dimension and chloroplast type with pyrenoids were examined under the microscope.

The water sample taken was passed through Sartorius filtration systems (to 0.80- and 0.45-µm acetate filters) for removal of undesirable compounds and, after this, it was autoclaved. This water was used as a medium for the cultivation of the filamentous alga. Stock Cd^{2+} solution (1 g/L) was prepared using a Cd^{2+}solution (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in distilled water, which passed through a 0.2-µm membrane filtrate used to prepare different Cd^{2+} concentrations (15, 30, 45, and 60 mg/L). Filaments of *C. glomerata* were washed 4 times with distilled water. The fresh weight of the algal biomass (8.0 ± 0.1 g) was inoculated in 500-mL flasks containing 400 mL in the sterilized water. Flasks at 120 rpm were stirred using an orbital shaker for 7 days and at 25 °C with an irradiance of about 150 µmol/m^2/s. Experiments were performed in duplicates.

2.2. Environmental variables

An oxygen-temperature meter (YSI Professional Plus model, YSI Incorporated, Yellow Springs, Ohio, USA) was used to measure the values of water temperature, conductivity, dissolved oxygen concentration, salinity, total dissolved solid (TDS), pH, and the ORP-oxidation-reduction potential of media before and after the experiments in situ.

2.3. Biovolume measurement

Randomly chosen cells of *C. glomerata* (at least 25 cells) were measured using a light microscope (Olympus BX53 model, Olympus CellSens, Version 1.6, Olympus Corporation, Tokyo, Japan) with a DP73 camera attachment to determine cell volumes.The mean cell volume was determined using the following equation: $V = \pi \times r^2 \times h$, where V, r, and $h$are the volume, the radius, and the length of the cell, respectively.

2.4. Analyses of biochemical compounds

Algal pigment (total carotenoids, chlorophyll *a*, and *b*) composition was measured using 80% acetone with a spectrophotometric (UV/VIS Jenway 6305, Jenway Ltd., Staffordeshire, UK) method (Wellburn, 1994) at 470, 663, and 646 nm, respectively. Malondialdehyde (MDA) content as an indication of lipid peroxidation was determined following Zhou's method (2001), using a spectrophotometer at 532 nm. A standard curve of MDA (Merck Schuchardt OHG, Hohenbrunn, Germany) was used to quantify MDA content. Analysis of the proline level in the macroalga was achieved according to the method used in Bates et al. (1973). Proline was spectrophotometrically quantified using a standard curve of L-proline (Merck KGaA, Darmstadt, Germany) at 520 nm. The Folin–Ciocalteu method (Lowry et al., 1951) was used to determine the protein content. The protein was quantified using a spectrophotometer at 750 nm and quantified using a standard of bovine serum albumin (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The Folin–Ciocalteu method (Ratkevicius et al., 2003) was used to specify total phenolic compounds. The absorbance of phenolic compounds was then determined using a spectrophotometer at 765 nm and expressed using a calibration curve of Gallic acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Standard methodswere

applied to determine total carbohydrate (Dubois et al., 1956) and starch (McCready et al., 1950). A standard curve was prepared with known amounts of glucose. The Thiol (-SH) group level in *C. glomerata* was determined following a method proposed by Ellman (1959). Hydrogen peroxide content was estimated according to a standard method presented by Sergiev et al. (1997). The absorbance was spectrophotometrically measured at 390 nm. H_2O_2 solution (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was used as a standard and expressed as μmol H_2O_2/g fw algal mass.

2.5. Cadmium content and residual analyses

The macroalgal powder was suspended in 14 N HNO_3, mineralized, and heated to determine the Cd^{2+} content. The metal content of the alga and residual Cd^{2+} concentration in the aqueous medium was measured using a flame atomic absorption spectrophotometer (Perkin-Elmer AA 400, PerkinElmer Corporation, Waltham, MA USA) at 228.7 nm.

2.6. FTIR–ATR analysis

Changes in the surface structure of *C. glomerata* biomass treated and untreated Cd^{2+} ions were evaluated using a Fourier transform infrared equipped with attenuated total reflection spectrometer (Perkin-Elmer Spectrum 100 FTIR–ATR, PerkinElmer Corporation, Waltham, MA USA).

2.7. Statistical analyses

Analysis of Variance and Duncan's multiple range test (SPSS version 15.0, SPSS Inc., Chicago, IL, USA) were used to compare experimental mean data between/among groups.

3. Results and discussion

The environmental parameters of media before and after algal inoculation are summarized in Table 1. Electrical conductivity (EC) values of media varied from 531 μS/cm to 607 μS/cm for the control to 60 mg/L for the Cd^{2+} group, respectively (Table 1). However, there was no significant difference in EC values among the control, 7.5, 15, and 30 mg/L Cd^{2+} groups ($P > 0.05$). After cultivation, several changes were observed in the physicochemical variables of media (Table 1). The values of salinity, TDS, and EC, except for the 30 and 60 mg/L Cd^{2+} groups, were significantly decreased by *Cladophora glomerata* after cultivation. In particular, the first 3 groups significantly increased the EC, TDS, and salinity levels of the media due

Table 1. Environmental variables (mean ± standard deviation) of medium before and after the cultivation of *Cladophora glomerata*. TDS: total dissolved solid; DO: dissolved oxygen;ORP: oxidation-reduction potential.

				Cd^{2+} (mg L^{-1})			
	Environment	Unit	Control	7.5	15	30	60
Before	Temperature	°C	$23.3 \pm 0.6^{a,A}$	$23.1 \pm 0.1^{a,A}$	$23.3 \pm 0.2^{a,A}$	$23.4 \pm 0.2^{a,A}$	$23.2 \pm 0.2^{a,A}$
	Conductivity	μS/cm	$531 \pm 7^{a,A}$	$540 \pm 2^{a,A}$	$555 \pm 3^{a,A}$	$572 \pm 2^{ab,A}$	$607 \pm 4^{b,A}$
	TDS	mg/L	$0.357 \pm 0.015^{a,A}$	$0.365 \pm 0.004^{a,A}$	$0.368 \pm 0.006^{a,A}$	$0.385 \pm 0.004^{ab,A}$	$0.410 \pm 0.008^{b,A}$
	Salinity	ppt	$0.26 \pm 0.02^{a,A}$	$0.27 \pm 0.01^{ab,A}$	$0.28 \pm 0.01^{ab,A}$	$0.28 \pm 0.01^{ab,A}$	$0.30 \pm 0.02^{b,A}$
	DO	%	$63.8 \pm 4.5^{a,A}$	$77.6 \pm 0.8^{b,A}$	$81.3 \pm 0.8^{b,A}$	$78.1 \pm 0.1^{b,A}$	$78.1 \pm 0.2^{b,A}$
	DO	mg L^{-1}	$5.40 \pm 0.38^{a,A}$	$6.62 \pm 0.01^{b,A}$	$6.88 \pm 0.04^{b,A}$	$6.62 \pm 0.02^{b,A}$	$6.69 \pm 0.10^{b,A}$
	pH		$7.06 \pm 0.50^{a,A}$	$7.44 \pm 0.01^{a,A}$	$7.53 \pm 0.02^{a,A}$	$7.42 \pm 0.03^{a,A}$	$7.26 \pm 0.01^{a,A}$
	ORP	mV	$-113.4 \pm 8.0^{ab,A}$	$-110.8 \pm 1.1^{a,A}$	$-117.9 \pm 0.1^{abc,A}$	$-121.5 \pm 0.7^{bc,A}$	$-125.8 \pm 0.4^{c,A}$
After	Temperature	°C	$25.1 \pm 1.1^{a,A}$	$25.5 \pm 0.1^{a,B}$	$25.1 \pm 0.3^{a,B}$	$25.0 \pm 0.1^{a,B}$	$25.1 \pm 0.1^{a,B}$
	Conductivity	μS/cm	$404 \pm 17^{ab,B}$	$305 \pm 5^{a,B}$	$397 \pm 116^{ab,A}$	$564 \pm 94^{bc,A}$	$746 \pm 115^{c,A}$
	TDS	mg/L	$0.244 \pm 0.010^{ab,B}$	$0.199 \pm 0.002^{a,B}$	$0.258 \pm 0.077^{ab,A}$	$0.367 \pm 0.062^{bc,A}$	$0.484 \pm 0.074^{c,A}$
	Salinity	ppt	$0.18 \pm 0.01^{ab,B}$	$0.15 \pm 0.01^{a,B}$	$0.19 \pm 0.06^{ab,A}$	$0.28 \pm 0.05^{bc,A}$	$0.36 \pm 0.06^{c,A}$
	DO	%	$88.9 \pm 3.8^{b,B}$	$87.4 \pm 8.4^{b,A}$	$83.0 \pm 7.1^{b,A}$	$81.3 \pm 3.9^{b,A}$	$61.0 \pm 8.5^{a,A}$
	DO	mg L^{-1}	$7.35 \pm 0.31^{b,B}$	$7.34 \pm 0.45^{b,A}$	$6.77 \pm 0.47^{b,A}$	$6.68 \pm 0.32^{b,A}$	$5.00 \pm 0.71^{a,A}$
	pH		$7.59 \pm 0.32^{a,A}$	$8.77 \pm 0.26^{b,B}$	$9.24 \pm 0.08^{b,B}$	$9.02 \pm 0.11^{b,B}$	$8.73 \pm 0.24^{b,B}$
	ORP	mV	$-164.2 \pm 7.0^{c,B}$	$-154.6 \pm 3.1^{b,B}$	$-143.8 \pm 2.9^{a,B}$	$-141.8 \pm 0.1^{a,B}$	$-145.6 \pm 0.2^{ab,B}$

Different capital letters A, B, and C indicate a statistical difference between before and after the cultivation at $\alpha = 0.05$ level.
Different lower-case letters a, b, and c indicate a statistical difference amongCd^{2+} concentrations at $\alpha = 0.05$ level.

to the enhancement of their growth. On the other hand, no significant difference was found in these parameters in the 30 and 60 mg/L groups. This could be due to the metal toxicity of high Cd^{2+} concentrations on the growth of *C. glomerata*. Besides, the increment of algal growth, with the exception of 60 mg/L Cd^{2+}, significantly increased the dissolved oxygen of media. Similar behavior was reported for *Spirogyra setiformis* exposed toCd^{2+} stress (Çelekli et al., 2016).

The filamentous alga had large cylindrical cells forming long, regular growths. The cell dimensions were 74.18 ± 0.87 µm in width and 249.27 ± 8.63 µm in length before the experiments. Cells contain many parietal round chloroplasts with pyrenoids, which usually form into a net-like structure and are always multinucleate. The morphological diagnostic properties of filamentous species are related to *Cladophora glomerata* (Linnaeus) Kützing (1843), according to the organization and dimensions of the cells (John, 2002).

Changes in cell morphology of *C. glomerata* in the control without the Cd^{2+}, with 7.5 mg/L, 15 mg/L, 30 mg/L, and 60 mg/L Cd stress are given in Figures 1a–1e, respectively. The visual changes in terms of dimensions and shape of cells and the structure and color of chloroplast were not found in *C. glomerata* cells in the control group after incubation (Figure 1a). The width of cells decreased from 75.90 ± 2.68 µm in the control group to 63.85 ± 1.47 µm in the 60 mg/L Cd^{2+} containing group (Table 2). Consequently, the amount of *C. glomerata*'s biovolume at 60 mg/L Cd^{2+} significantly decreased approximately twice compared to the control group. This decrement in the algal biovolumes was also found in *Spirogyra setiformis* cells under Cd^{2+} stress (Çelekli et al., 2016). The morphological response of algal species exposed to ecological stressors can be important to assess the dynamic of surface waters (Reynolds et al., 2002; Çelekli et al., 2017). Furthermore, the shape of chloroplast and the color of pigments were deformed, especially at high Cd^{2+} concentrations (Figures 1c–1e). The structure of the chloroplast of *Micrasterias* was probably severely damaged because ofCd^{2+} exposure (Andosch et al., 2012). Regularly coiled spiral chloroplasts of *S. setiformis* were deformed at high cadmium concentrations (Çelekli et al., 2016), and the structure of the chloroplast of *Chlamydomonas reinhardtii* was broken down under the exposure of copper oxide (Perreault et al., 2012).

The biovolume of the algal cells gradually decreased when the Cd^{2+} concentration increased from 7.5 to 60 mg/L (Table 2). Cd^{2+} of 7.5 mg/L could not significantly affect the cell volume level but was significantly reduced at higher metal exposure levels ($P > 0.05$), according to Duncan's test. The highest cellvolume was measured in the control group ($P < 0.05$), but the difference found among high Cd^{2+} containing groups was not substantial. Previous studies also showed that Cd^{2+} can affect the cell biovolumes of the species (Peña-Castro et al., 2004; Paquet et al., 2015).

Cladophora glomerata produced the largest biomass values at 8.4 ± 0.1 g in the control group without Cd^{2+} ions, whereas it gradually decreased to 7.7 ± 0.1 g at 60 mg/L Cd^{2+}. The detrimental effects of heavy metals have been observed on the growth of *Scenedesmus quadricauda* (Çelekli et al., 2013; Štork et al., 2013), *Chladophora* (Cao et al., 2015), and *Ulva lactuca*(Saleh, 2015).

The effects of Cd^{2+} ions on the pigment content of *C. glomerata*are given in Figure 2. When the initial Cd^{2+} concentration increased from the control to 60 mg/L Cd^{2+},chlorophyll-*a* content significantly decreased ($P < 0.05$) from 14.27 mg/g to 9.97 mg/g. A similar tendency was found in the variation of chlorophyll-*b* and total carotenoids exhibited by the filamentous alga under Cd^{2+}stress (Figure 2). High metal concentrations decreased in the chlorophyll content compared to the control group. This could be related to the fact that the cell wall at low concentrations can provide some protection against metal ions (Macfie and Welbourn, 2000). On the other hand, the cell walls at higher Cd^{2+} concentration are not able to bind well enough to the metal ions on the surface and, thus, some ions can enter the cell and cause damage to the chloroplast and pigments.

Exposure of heavy metals induced the chlorosis of various algae. This phenomenon was also reported for *Ulva lactuca* exposed to *Scenedesmus obliquus* under carbamazepine stress (Zhang et al., 2012), *Chlorella vulgaris* with Cr exposure (Rai et al., 2013), *Scenedesmus quadricauda* exposed to Cu (Kováčik et al., 2010), *Chlorella pyrenoidosa* with perfluorooctanoic acid exposure (Xu et al., 2013), *S. obliquus* exposed to Cu (Chen et al., 2012), and *Ulva australis* and *Pyropia yezoensis* under hypo- and hypersalinity (Samanta et al., 2019). As a result, chlorosis phenomena could be used as a biomarker for metal stress. Küpper et al. (2003)reported that Cd^{2+} could damage the biosynthesis of chlorophyll in algae due to the combination of the substitution of Mg^{2+} ions with the tetrapyrole ring of chlorophyll. Moreover, chlorophyll reduction was also found for *Chlorella vulgaris* under chromate exposure (Rai et al., 2013). Cd^{2+} ions showed an inhibitory effect on the production of pigments in *C. glomerata*. Biosynthesis of pigments in this filamentous macroalga could be limited by the toxicity of Cd^{2+}. Previously, detrimental effects were also observed for the carotenoids content of *Ulva prolifera* and *Ulva linza* under Cd^{2+} exposure (Jiang et al., 2013) and *S. incrassatulus* (Perales-Vela et al., 2007) and *S. obliquus* (Li et al., 2005) under toxicity of Cu^{2+}. The biochemical responses of *C. glomerata* exposure to Cd^{2+} concentrations are given in Table 3. The amounts of MDA, H_2O_2, proline,

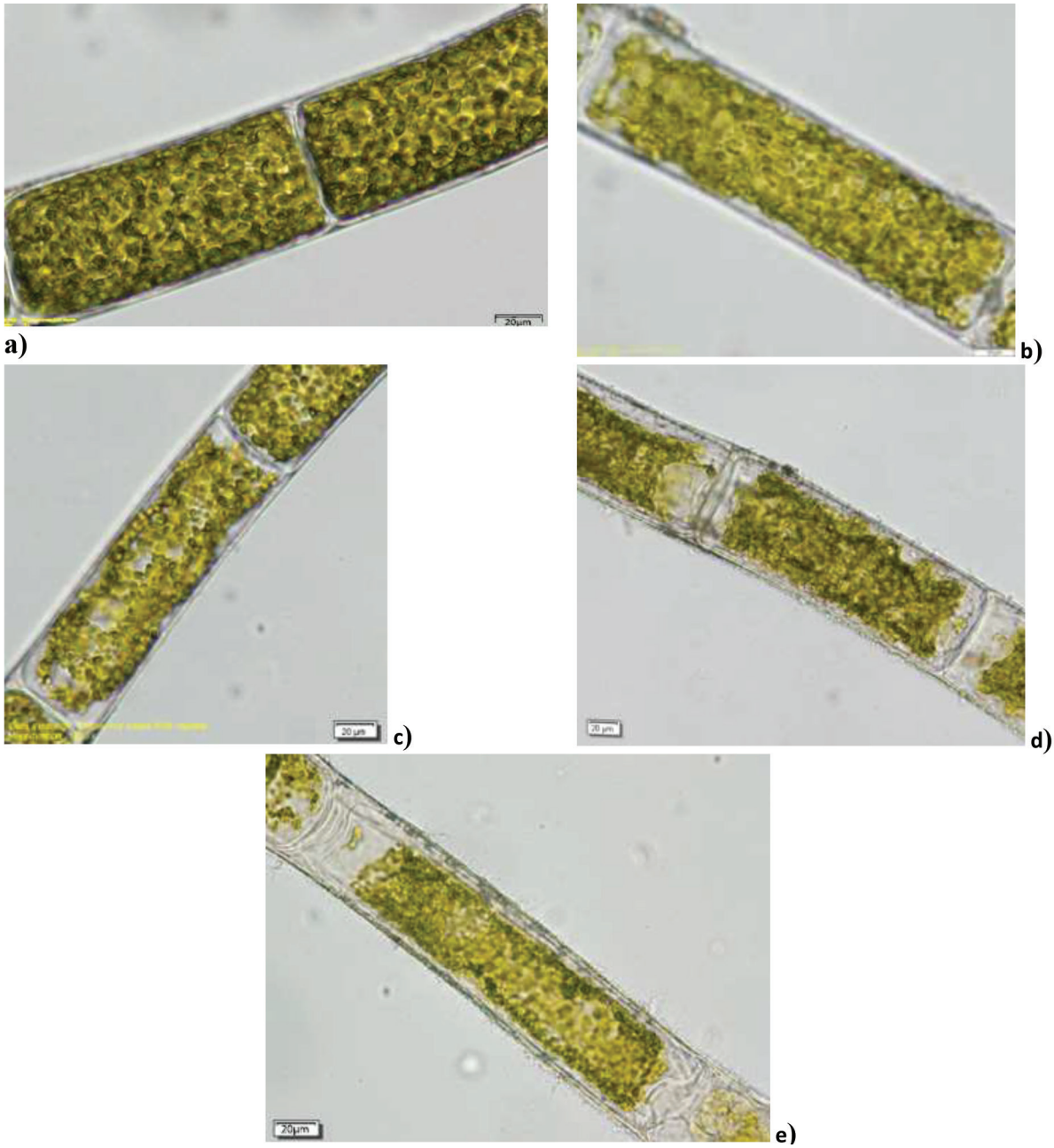

Figure 1. Morphology of *Cladophora glomerata* at (a) the control;(b) 7.5 mg/L; (c) 15 mg/L; (d) 30 mg/L;and (e) 60 mg/L Cd^{2+}.

total phenolic compounds, total protein, and total carbohydrate by *C. glomerata* significantly enhanced with increasing Cd^{2+} ion values, whereastotal protein values significantly decreased ($P < 0.05$). The highest MDA value (14.16 µg/g) was measured at 60 mg/L Cd^{2+} ions. Increasing the MDA content of algae due to exposure of heavy metal has been previously proven by many researchers (Piotrowska-Niczyporuk et al., 2012; Rai et al., 2013; Çelekli et al., 2016). In addition, salinity gradients increased MDA content in *Polygonum equisetiforme* (Boughalleb et al., 2020) and *Zea mays* (Rıffat and Ahmad, 2020). The consequent formation of MDA is a breakdown product of membrane lipid peroxidation when insufficient detoxification of ROS by biota occurs (Pinto et al., 2003).

Duncan's test revealed that significant differences in the H_2O_2, proline and phenolic compounds among the groups were observed ($P < 0.05$). Previous studies confirmed that metal stress enhanced the proline levels in algal assemblages in *Scenedesmus* sp. (Tripathi et al., 2006), *Spirulina platensis* (Choudhary et al., 2007), and

Table 2. Effect of Cd^{2+} concentrations on the morphological properties of *Cladophora glomerata*.

Cd^{2+}(mgL)	Width (µm)	Length (µm)	Biovolume x 10-3 (mm^3)
0	75.90 ± 2.68^{b}	251.51 ± 16.11^{b}	1.11 ± 0.15^{b}
7.5	75.15 ± 2.75^{b}	235.75 ± 14.69^{ab}	1.05 ± 0.14^{b}
15	69.01 ± 2.68^{ab}	250.33 ± 15.03^{b}	0.94 ± 0.13^{ab}
30	65.70 ± 1.69^{a}	205.51 ± 17.81^{a}	0.70 ± 0.01^{a}
60	63.85 ± 1.47^{a}	215.35 ± 16.11^{ab}	0.69 ± 0.08^{a}

Different lower-case letters a, b, and c indicate a statistical difference between the concentrations at α= 0.05 level among Cd^{2+} groups.

S. quadricauda (Kováčik et al., 2010). The results of our research and previous studies (Choudhary et al., 2007; Murugan and Harish, 2007; Ismail and Ismail, 2017) seem to reinforce the importance of signal molecules like MDA and proline in the response mechanisms of algae to Cd^{2+} exposure. From this vantage point, proline and MDA could be used as a biomarker for the detection of free amino acids and membrane lipid peroxidation, respectively. Biomarkers could also be used as an indicator tool to monitor ecological quality conditions and organism health (Çelekli et al., 2017).

Cladophora glomerata with exposure of Cd^{2+} ions showed a decreasing trend in the protein content (from 43.60 mg/g in the control to 21.66 mg/g at 60 mg/L Cd^{2+}) with increasing metal concentrations (Table 3). There was no significant difference in protein values between the control and 7.5 mg/L Cd^{2+} ions. This could be due to the binding of Cd^{2+} ions on the cell wall at low metal concentration, which then prevented the damage of its organic molecules (Salama et al., 2019). A possible adverse effect was observed in the protein content of *S. setiformis* exposure to Cd^{2+} levels (Çelekli et al., 2016) and *Chlorella vulgaris* under chrome stress (Rai et al., 2013). The result of our study indicated that membrane lipid peroxidation by measuring MDA was the highest at 60 mg/L. The cell wall damage at higher concentrations was not able to bind Cd^{2+} ions. Thus, increasing the entry of metal into the cell caused damage to protein (Salama et al., 2019). Extracellular and/or intracellular metal exclusion processes through formation with diverse ligands, storage into vacuoles, and metal pumping out are several metal detoxification mechanisms (Branco et al., 2010; Gomes and Asaeda, 2013). Besides, organisms under stress demonstrate a few responses via metal detoxification and antioxidant defenses (enzymatic and nonenzymatic) (Branco et al., 2010; Gomes and Asaeda, 2013). These and other mechanisms closely affect algal tolerances to the gradient of metal ions.

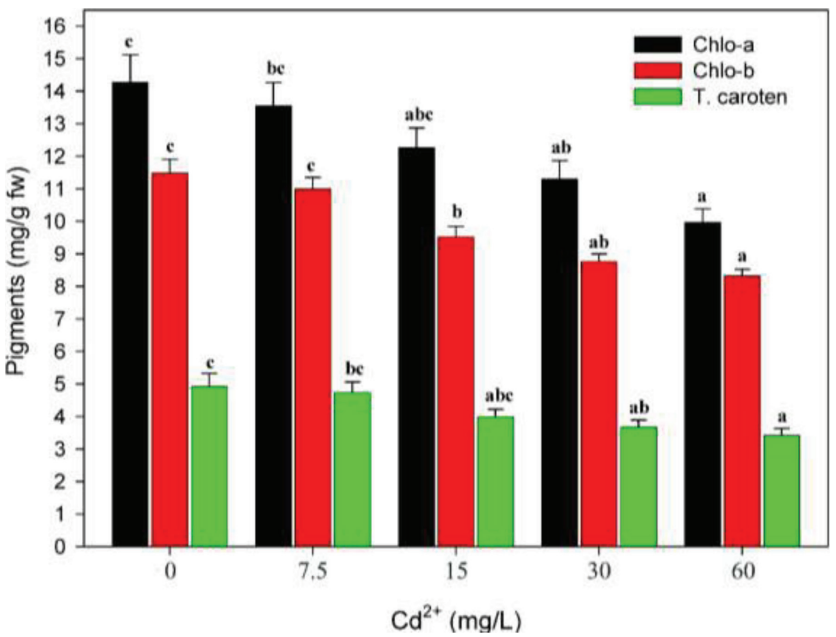

Figure 2. Effect of Cd^{2+} concentrations on chlorophyll-a (Chlo-a), chlorophyll-b (Chlo-b), and total carotenoids (T. caroten) contents of *Cladophora glomerata*. Different lower-case letters show statistical differences among groups at α = 0.05 level; fw: fresh weight.

After harvesting biomass of *C. glomerata*, remaining Cd^{2+} values in the media were found as 0.64, 0.77, 0.98, and 1.21 mg/L in the 15, 30, 45, and 60 mg/L Cd^{2+} groups, respectively. Results indicated that *C. glomerata* had wide tolerance to the gradient of Cd^{2+} ions. *C. glomerata* is widespread on earth due to its broad tolerance to changing environmental conditions (Higgins et al., 2008; Bellinger and Sigee, 2010; Çelekli et al., 2017). The present study indicated that this green macroalga has great potential for the bioaccumulation of Cd^{2+} ions. Hyperaccumulation of metal was also observed for the accumulation of chrome by *Chlorella vulgaris*(Rai et al., 2013), CuO by *Chlamydomonas reinhardtii* (Perreault et al., 2012), and Ni, Pb, and Cd by *Ulva* (Rybak et al., 2012).

The surface composition of *C. glomerata* was evaluated using a FTIR-ATR spectrum of algal biomass to understand the metal binding mechanism. Several major peaks, at 3340, 2925, 1644, 1518, 1398, 1236, 1160, 1110, 1056, and 1034 1/cm, were observed on the spectrum of *C. glomerata* biomass in the control (Figure 3a). Peaks at 3340 1/cm and 2925 1/cm could be assigned to the –OH and $-NH_2$ groups and –CH stretching vibrations, respectively (Çelekli et al., 2016). Other peaks of spectra could be attributed as follows: 1644 1/cm ($-NH_2$ group or –C-N (amide)), 1518 1/cm (–N–H bending), 1398 1/cm($-CH_3$ stretching), 1236 1/cm (NO_2 antisym stretch), 1160 1/cm (–C-O stretching of COOH), 1056 1/cm (–C-N and –C-C stretching vibrations), and 1034 1/cm (–C-N and –C-C stretching vibrations) (Arief et al., 2008).

Similar major peaks were observed on the surface structure of *C. glomerata* under the different Cd^{2+} values. From this, to prevent the overlapping of curves, the plots

Table 3. Effect of Cd^{2+} on malondialdehyde (MDA), proline, H_2O_2, totalphenolic compounds (TPC), total protein, and total carbohydrate (charbo) contents of *Cladophora glomerata.* Data are fresh weight.

Cd^{2+}	MDA	Proline	H_2O_2	TPC	Protein	Charbo
(mg/L)	(µg/g fw)	(µg/g fw)	(µg/g fw)	(mg/g fw)	(mg/g fw)	(mg/g fw)
0	7.56 ± 0.38^{a}	5.7 ± 0.29^{a}	2.31 ± 0.11^{a}	0.66 ± 0.04^{a}	43.60 ± 1.74^{a}	78.51 ± 3.92^{a}
7.5	8.88 ± 0.17^{b}	6.05 ± 0.06^{a}	2.60 ± 0.04^{ab}	0.96 ± 0.33^{a}	37.05 ± 0.49^{a}	115.26 ± 5.10^{b}
15	11.25 ± 0.18^{c}	7.33 ± 0.15^{b}	2.89 ± 0.1^{bc}	2.45 ± 0.14^{b}	31.16 ± 0.41^{b}	147.49 ± 3.16^{c}
30	12.43 ± 0.33^{d}	8.05 ± 0.12^{c}	3.16 ± 0.06^{c}	2.88 ± 0.28^{bc}	24.88 ± 0.78^{c}	169.08 ± 2.11^{d}
60	14.16 ± 0.3^{e}	8.74 ± 0.18^{d}	3.6 ± 0.13^{d}	3.49 ± 0.27^{c}	21.66 ± 0.21^{d}	194.6 ± 13.60^{e}

Different lower-case letters a, b, and c indicate a statistical difference between the concentrations at $\alpha = 0.05$ level among Cd^{2+} groups.

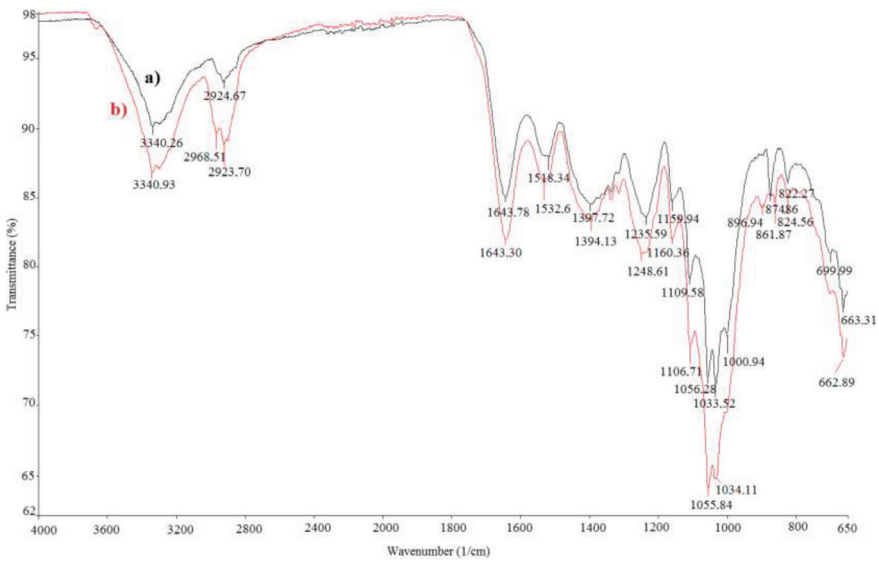

Figure 3. FTIR-ATR spectra of *Cladophora glomerata* for (a) the control and (b) after Cd^{2+} exposure.

of the control (Figure 3a) and 60 mg/L Cd^{2+} ions (Figure 3b) were selected in the FTIR spectra. Many peaks of 3340, 2925, 1644, 1518, 1398, 1236, 1110, and 1034 1/cm on the surface structure of alga without Cd^{2+} stress were shifted to 3341, 2924, 1643, 1533, 1394, 1249, 1107, and 1056 1/cm on the algal biomass under Cd^{2+} exposure. These changes may explain the Cd^{2+} binding on the surface of macroalga. Results based on FTIR–ATR studies indicated that theamino (at 3341, 2924, and 1533 1/cm), amide (at 1643 1/cm), and anionic (at 1056 1/cm) groups played a significant role in the binding of Cd^{2+} on *C. glomerata*. Similar results were also found in previous studies on the effect of Cd^{2+} on *Synechocystis sp*. (Ozturk et al., 2010) and *Scenedesmus quadricauda* var. *longispina* (Çelekli et al., 2013).

This study confirmed that *C. glomerata* has a tolerance to the gradient of Cd^{2+} ions. Detrimental effects of a Cd^{2+} dose on both the morphology and biochemical compounds of *C. glomerata* were observed. Results based on FTIR–ATR studies revealed that the amide, amino, and anionic groups have a significant role in the binding of Cd^{2+} on *C. glomerata*. A Cd^{2+} ions gradient-dependentdecrease in pigment and protein content was observed in the exposed cells compared to the control ones and an increase in stress molecules (e.g., MDA and proline) as biomarkers in the response mechanisms of *C. glomerata* to Cd^{2+} exposure. Biomarkers could be used as an indicator tool to monitor ecological quality condition and organism health. These signal molecules can be used to earlier detect the adverse effects of hazardous compounds on biota, which may be of use in environmental monitoring while assessing ecological quality conditions.

Acknowledgments

This research was supported by TÜBITAK (The Scientific and Technical Research Council of Turkey), project No: 112Y054. The authors would also like to thank the Scientific Research Projects Executive Council of the University of Gaziantep.

References

Andosch A, Affenzeller MJ, Lütz C, Lütz-Meindl U (2012). A freshwater green alga under cadmium stress: ameliorating calcium effects on ultrastructure and photosynthesis in the unicellular model *Micrasterias*. Journal of Plant Physiology 169 (15): 1489-1500.

Arief VO, Trilestari K, Sunarso J, Indraswati N, Ismadji S (2008). Recent progress on biosorption of heavy metals from liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies. Clean 36 (12): 937-962.

Bates L, Waldren R, Teare I (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39 (1): 205-207.

Bellinger E, Sigee D (2010). Introduction to Freshwater Algae. Freshwater Algae: Identification and Use As Bioindicators. Oxford, UK: John Wiley & Sons.

Boughalleb F, Abdellaouı R, Mahmoudi M, Bakhshandeh E (2020). Changes in phenolic profile, soluble sugar, proline, and antioxidant enzyme activities of *Polygonum equisetiforme* in response to salinity. Turkish Journal of Botany 44: 25-35.

Branco D, Lima A, Almeida SF, Figueira E (2010). Sensitivity of biochemical markers to evaluate cadmium stress in the freshwater diatom *Nitzschia palea* (Kützing) W. Smith. AquaticToxicology 99 (2):109-117.

Cao DJ, Xie PP, Deng JW, Zhang HM, Ma RX et al. (2015). Effects of Cu^{2+} and Zn^{2+} on growth and physiological characteristics of green algae, *Cladophora*. Environmental Science and Pollution Research 22 (21): 16535-16541.

Chen H, Chen J, Guo Y, Wen Y, Liu J et al. (2012). Evaluation of the role of the glutathione redox cycle in Cu (II) toxicity to green algae by a chiral perturbation approach. AquaticToxicology 120:19-26.

Choudhary M, Jetley UK, Khan MA, Zutshi S, Fatma T (2007). Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicology and Environmental Safety 66 (2): 204-209.

Çelekli A, Gültekin E, Bozkurt H (2016). Morphological and biochemical responses of *Spirogyra setiformis* exposed to cadmium. Clean 44 (3): 256-262.

Çelekli A, Kapı E, Soysal Ç, Arslanargun H, Bozkurt H (2017). Evaluating biochemical response of filamentous algae integrated with different water bodies. Ecotoxicology and Environmental Safety 142: 171-180.

Çelekli A, Kapı M, Bozkurt H (2013). Effect of cadmium on biomass, pigmentation, malondialdehyde, and proline of *Scenedesmus quadricauda* var. *longispina*. Bulletin of Environmental Contamination and Toxicology 91 (5): 571-576.

Dubois M, Gilles KA, Hamilton JK, Rebers Pt, Smith F (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28 (3): 350-356.

Ellman GL (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82 (1): 70-77.

Gomes PI, Asaeda T (2013). Phytoremediation of heavy metals by calcifying macro-algae (*Nitella pseudoflabellata*): implications of redox insensitive end products. Chemosphere 92 (10):1328-1334.

Hayat MT, Nauman M, Nazir N, Ali S, Bangash N (2019). Environmental hazards of cadmium: past, present, and future. In:Hasanuzzaman, M, Prasad, M, Fujita, M. (editors). Cadmium Toxicity and Tolerance in Plants. Amsterdam, Netherlands: Elsevier, pp 163-183.

Higgins SN, Malkin SY, Todd Howell E, Guildford SJ, Campbell L et al. (2008). An ecological review of *Cladophora glomerata* (Chlorophyta) in the Laurentian Great Lakes 1. Journal of Phycology 44 (4): 839-854.

Jiang HP, Gao BB, Li WH, Zhu M, Zheng CF et al. (2013). Physiological and biochemical responses of *Ulva prolifera* and *Ulva linza* to cadmium stress. Scientific World Journal 2013: 1-11.

John DM, BA Whitton, JA Brook (2002). The Freshwater Algal Flora of the British Isles. 1st edition. Cambridge, UK: Cambridge University Press.

Kováčik J, Klejdus B, Hedbavny J, Bačkor M (2010). Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chlorophyceae). Plant Science 178 (3):307-311.

Küpper H, Šetlík I, Šetliková E, Ferimazova N, Spiller M et al. (2003). Copper-induced inhibition of photosynthesis: limiting steps of in vivo copper chlorophyll formation in *Scenedesmus quadricauda*. Functional Plant Biology 30 (12): 1187-1196.

Lane TW, Saito MA, George GN, Pickering IJ, Prince R et al. (2005). Biochemistry: a cadmium enzyme from a marine diatom. Nature 435 (7038): 42.

Li H, Yang Z, Dai M, Diao X, Dai S et al. (2019). Input of Cd from agriculture phosphate fertilizer application in China during 2006–2016. The Science of the Total Environment 698: 134-149.

Li S, Ji X, Zhu J, Li C, Jian Y et al. (2019). Utilizing algae for agricultural non-point source pollution control: a review. Journal of Agro-Environment Science 38 (5): 970-979.

Li X, Ping X, Xiumei S, Zhenbin W, Liqiang X (2005). Toxicity of cypermethrin on growth, pigments, and superoxide dismutase of *Scenedesmus obliquus*. Ecotoxicology and Environmental Safety 60 (2): 188-192.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry193: 265-275.

Macfie S, Welbourn P (2000). The cell wall as a barrier to uptake of metal ions in the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophyceae). Archives of Environmental Contamination and Toxicology 39 (4): 413-419.

McCready R, Guggolz J, Silviera V, Owens H (1950). Determination of starch and amylose in vegetables. Analytical Chemistry 22 (9): 1156-1158.

Ozturk S, Aslim B, Suludere Z (2010). Cadmium (II) sequestration characteristics by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition. BioresourceTechnology 101 (24): 9742-9748.

Paquet N, Lavoie M, Maloney F, Duval JF, Campbell PG et al. (2015). Cadmium accumulation and toxicity in the unicellular alga *Pseudokirchneriella subcapitata*: Influence of metal-binding exudates and exposure time. EnvironmentalToxicologyand Chemistry 34 (7):1524-1532.

Peña-Castro JM, Martínez-Jerónimo F, Esparza-García F, Cañizares-Villanueva RO (2004). Phenotypic plasticity in *Scenedesmus incrassatulus* (Chlorophyceae) in response to heavy metals stress. Chemosphere 57 (11): 1629-1636.

Perales-Vela HV, González-Moreno S, Montes-Horcasitas C, Cañizares-Villanueva RO (2007). Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae). Chemosphere 67 (11): 2274-2281.

Perreault F, Oukarroum A, Melegari SP, Matias WG, Popovic R (2012). Polymer coating of copper oxide nanoparticles increases nanoparticles uptake and toxicity in the green alga *Chlamydomonas reinhardtii*. Chemosphere 87 (11): 1388-1394.

Pinto E, Sigaud-Kutner TC, Leitao MA, Okamoto OK, Morse D et al. (2003). Heavy metal–induced oxidative stress in algae 1. Journal of Phycology 39 (6): 1008-1018.

Piotrowska-Niczyporuk A, Bajguz A, Zambrzycka E, Godlewska-Żyłkiewicz B (2012). Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae). Plant Physiology and Biochemistry 52: 52-65.

Rai U, Singh N, Upadhyay A, Verma S (2013). Chromate tolerance and accumulation in *Chlorella vulgaris* L.: role of antioxidant enzymes and biochemical changes in detoxification of metals. Bioresource Technology 136: 604-609.

Ratkevicius N, Correa J, Moenne A (2003). Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev.(Chlorophyta) from heavy metal-enriched environments in northern Chile. Plant Cell& Environment 26 (10): 1599-1608.

Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S (2002). Towards a functional classification of the freshwater phytoplankton. Journal of Plankton Research 24 (5): 417-428.

Rıffat A, Ahmad MSA (2020). Regulation of antioxidant activity in maize (*Zea mays* L.) by exogenous application of sulfur under saline conditions. Turkish Journal of Botany 44: 62-75.

Rybak A, Messyasz B, Łęska B (2012). Freshwater *Ulva* (Chlorophyta) as a bioaccumulator of selected heavy metals (Cd, Ni and Pb) and alkaline earth metals (Ca and Mg). Chemosphere 89 (9):1066-1076.

Salama E, Roh H, Dev S, Khan MA, Abou-Shanab RAI et al. (2019). Algae as a green technology for heavy metals removal from various wastewater. World Journal of Microbiology and Biotechnology 35: 75: 1-19.

Saleh B (2015). Physiological response of the green algae *Ulva lactuca* (Chlorophyta) to heavy metals stress. Journal of Stress Physiology & Biochemistry 11 (3): 38-51.

Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA et al. (2017). Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171: 710-721.

Sergiev I, Alexieva V, Karanov E (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Comptes Rendus del'Academie Bulgare des Sciences 51 (3): 121-124.

Souza VL, de Almeida AAF, Lima SG, Cascardo JCDM, Silva DDC et al. (2011). Morphophysiological responses and programmed cell death induced by cadmium in *Genipa americana* L.(Rubiaceae). Biometals 24 (1): 59-71.

Štork F, Bačkor M, Klejdus B, Hedbavny J, Kováčik J (2013). Changes of metal-induced toxicity by H 2 O 2/NO modulators in *Scenedesmus quadricauda* (Chlorophyceae). Environmental Science and Pollution Research 20 (8): 5502-5511.

Templeton DM, Liu Y (2010). Multiple roles of cadmium in cell death and survival. Chemico-BiologicalInteractions 188 (2): 267-275.

Tripathi B, Mehta S, Amar A, Gaur J (2006). Oxidative stress in *Scenedesmus* sp. during short-and long-term exposure to Cu^{2+} and Zn^{2+}. Chemosphere 62 (4):538-544.

Verbruggen N, Hermans C, Schat H (2009). Mechanisms to cope with arsenic or cadmium excess in plants. Current Opinion in Plant Biology 12 (3): 364-372.

Wang J, Chen C (2009). Biosorbents for heavy metals removal and their future. Biotechnology Advances 27 (2): 195-226.

Wellburn AR (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometersof different resolution. Journal of Plant Physiology 144 (3): 307-313.

Xu D, Li C, Chen H, Shao B (2013). Cellular response of freshwater green algae to perfluorooctanoic acid toxicity. Ecotoxicology and Environmental Safety 88: 103-107.

Yvon-Durocher G, Dossena M, Trimmer M, Woodward G, Allen AP (2015). Temperature and the biogeography of algal stoichiometry. Global Ecolology and Biogeography 24 (5): 562-570.

Zeraatkar AK, Ahmadzadeh H, Talebi AF, Moheimani NR, McHenry MP (2016). Potential use of algae for heavy metal bioremediation, a critical review. Journal of Environmental Management 181: 817-831.

Zhang W, Zhang M, Lin K, Sun W, Xiong B et al. (2012). Eco-toxicological effect of Carbamazepine on *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Environmental Toxicology and Pharmacology 33 (2): 344-352.

Zhou Q (2001). The measurement of malondialdehyde in plants. In: Zhou Q (editor): Methods in Plant Physiology.Beijing, China: China Agricultural Press.