Effect of lead ions on biochemical behavior of Cladophora glomerata in sterilized and non-sterilized media

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Abstract

Freshwater ecosystems are under peril globally due to anthropogenic influences, most notably metals. The present study aimed to evaluate the morphological and biochemical responses of *Cladophora glomerata* obtained from a freshwater stream to various lead concentrations (0.0, 7.5, 15, 30, and 60 mg/L Pb^{2+}) in sterilized and non-sterilized media. Pigments, proline, malondialdehyde (MDA), total phenolic compounds (TPC), hydrogen peroxide, and protein content of the green alga were determined in response to various growing conditions. Pb^{2+} stress had a detrimental effect not only on biochemical components of *C. glomerata* but also on the algal cell's shape and surface structure. High Pb^{2+} concentrations significantly decreased chlorophyll-a (from 1350 µg/g in non-sterilized and 1340 µg/g in sterilized media for the control group to 1067 µg/g in non-sterilized and 1049 µg/g in sterile media at 60 mg/L Pb^{2+}) and protein content (from 34.47 mg/g for the sterilized and 35.89 mg/g for non-sterilized of the control to 24.82 mg/g for the sterilized and 26.18 mg/g for the non-sterilized at 60 mg/L Pb^{2+}) of algal biomass but increased stress compounds (e.g., MDA, proline, and TPC). Variation in the macroalgal biomass composition was also indicated by FTIR analysis based on interactions between amino, amide, and anionic surface groups on the algal biomass and Pb^{2+} ions. Results indicate that this macroalga shows a broad tolerance to Pb^{2+} levels in non-sterile environments and that the biochemicals generated by *C. glomerata* may be used as biomarkers for oxidative metal stress, but further research is required.

Introduction

Heavy metal pollution particularly in freshwater resources, is a serious environmental issue that poses a threat to living biota (Zhou et al. 2020). These pollutants entering aquatic habitats cause a variety of negative impacts on the aquatic biota that live there, as well as on human health. Among these, human activities such as fossil fuel combustion, mining, and manufacturing contribute to the growth in lead (Pb^{2+}) concentrations in water resources. Lead is a poisonous metal with a well-documented inability to biodegrade in the environment, which is posing a worldwide health threat (Zhou et al. 2020). Lead is a non-essential metal that has harmful effects on organisms, resulting in a variety of human illnesses affecting many organs and systems, including the brain, liver, kidney, and cardiovascular system (WHO 2019).

Changes in the physical and chemical variables of aquatic ecosystems subjected to heavy metals, pesticides, and other contaminants cause stress in aquatic organisms. Metal toxicity results in increase amount of reactive oxygen species (ROS) in organisms, which can cause significant damage to organisms. To prevent detrimental effects of ROS, organisms have enzymatic (e.g., glutathione peroxidase, superoxide dismutase, glutathione S-transferase, and catalase) and non-enzymatic (e.g., glutathione, ascorbic acid, proline, hydrogen peroxide, malondialdehyde) defense systems (Tripathi et al. 2006; Gao and Yan 2012). The metabolic response of organisms to environmental stress can be used as a biomarker.

Cladophora glomerata, a filamentous alga, has a widespread distribution around the world, owing to its high tolerance for changing environmental variables (Zulkifly et al. 2012; Çelekli et al. 2017). *Cladophora glomerata* is prevalent in aquatic habitats such as streams, lakes, and ponds (Zulkifly et al. 2012). Numerous studies have demonstrated that *C. glomerata* is highly resistant to metal stress in laboratory circumstances for Cd^{+2} (Çelekli and Bulut 2020) and Cu^{+2}, and Zn^{+2} (Cao et al. 2015) as well as in complicated natural environments (Çelekli et al. 2016a, 2017).

Cladophora glomerata is frequently used to examine the effect of heavy metals stress, because this filamentous alga is plentiful in nature and easy to collect, To our knowledge, studies on the effects of lead stress on the biochemical composition of *C. glomerata* has not been found in the literature. The purpose of this work was to compare and explain the morphological (biovolume and cell structure) and biochemical (e.g., pigments, malondialdehyde, proline, hydrogen peroxide, and total phenolic compounds) responses of *C. glomerata* to lead stress in sterilized and unsterilized media.

Materials And Methods

Filamentous alga sampling and identification

The biomass of *Cladophora glomerata* was collected from a creek running into Hacarslan Reservoir and placed in 5 L plastic bottles and transported to the laboratory in

a cooler condition. To eliminate undesirable debris and organisms from macroalgal biomass, it was gently washed multiple times with tap water (Çelekli and Bulut 2020). The filamentous alga was identified using a taxonomic key book under an Olympus BX53 type light microscope equipped with a DP73 camera and Cellsens 1.6 imaging system (John et al. 2002).

Lead concentration, environmental variables, cultivation, and biovolume

The lead (Pb^{2+}) solution purchased from Sigma (Sigma-Aldrich GmbH, Germany) was used to prepare 1 g/L stock Pb^{2+} solution with distilled water. The stock Pb^{2+} solution was kept at +4 ^{o}C. Control without Pb^{2+} and required Pb^{2+} (7.5, 15, 30, and 60 mg/L) concentrations were generated using stream water. Two different mediums, sterilized and non-sterilized, were prepared and adjusted to mentioned Pb^{2+} concentrations.

For the sterilized media, the creek's water was autoclaved at 121 °C.

Environmental variables (e.g., water temperature (°C), dissolved oxygen (mg/l), electrical conductivity (µS/cm), redox potential (mV), pH, total dissolved solids amount (TDS, mg) /l), and salinity (ppt)) of these media were measured using a YSI Professional Plus Model Oxygen-temperature multi-meter before algal inoculation and after harvesting biomass.

About 8 g algal biomass was inoculated into 400 mL of sterilized and unsterilized media in 500 mL flasks as batch cultures (5 groups= control, 7.5, 15, 30, and 60 mg/L Pb^{2+}). These Erlenmayar flasks were shaken at 100 rpm for 7 days under continuous irradiance of 150 µmol m^{-2} s^{-1} and temperature of 24.0±0.5 ^{o}C. Experiments were conducted in duplicate (n= 20 samples as 5 samples sterilized*dublicate and 5 samples non-sterilized media * dublicate).

To asssess cell biovolume, the dimensions of at least 25 randomly selected cells were measured using an Olympus BX53 model light microscope equipped with a DP73 camera attachment and an imaging software system (Olympus CellSens Vers. 1.6).

Analyses of biochemical component

The pigment (total carotenoids, chlorophyll-a, and -b) contents of algal biomass were determined as fresh weight using 80% acetone with a spectrophotometer (UV/VIS Jenway 6305) at 470, 663, and 646 nm wavelength, respectively (Wellburn, 1994).

Harvested algal biomass was dried using freeze-drying procedure to provide dried algal powder for the biochemical analyses. The malondialdehyde (MDA) concentration was evaluated using an UV/VIS spectrophotometer set to 532 nm wavelength according to the method of Zhou (2001) based on a standard curve of MDA (Merck Schuchardt CHG, Germany). The proline level was determined using the Bates et al. (1973) technique and quantified using a standard curve of L-proline (Merck KGaA Darmstadt) at a wavelength of 520 nm. The Folin–Ciocalteu technique (Lowry et al. 1951) was used to quantify the protein content by comparing it to a standard curve of bovine serum albumin using a spectrophotometer set to 750 nm wavelength (Sigma-Aldrich GmbH, Germany). Total phenolic compounds (TPC) were determined using the Folin–Ciocalteu technique using a spectrophotometer set to 765 nm wavelength (Ratkevicius et al. 2003). It was expressed using a calibration curve for gallic acid (purchased from Sigma-Aldrich GmbH, Germany). The hydrogen peroxide level of algal samples was evaluated using Sergiev et al. (1997) spectrophotometric technique at a wavelength of 390 nm. To quantify it, hydrogen peroxide (Sigma-Aldrich GmbH, Germany) was utilized as a reference curve.

Lead analyses

The initial and ultimate Pb^{2+} concentrations of media were determined using a flame atomic absorption spectrometer (FAAS, Perkin Elmer AA 400, USA) operating at 217 nm wavelength before algal inoculation and after algal harvesting.

FTIR analysis

A Fourier transform infrared spectrometer equipped with an attenuated total reflection (Perkin–Elmer Spectrum 100 FTIR–ATR) was used to characterize surface structures of algal biomass exposed to or not exposed to Pb^{2+} in various conditions.

Statistical analyses

The one-way ANOVA and Duncan's multiple range test were used to determine if there are differences in variables between/among groups (SPSS version 15.0, SPSS, USA).

Results And Discussion

Environmental variables for sterilized and non-sterilized media before the inoculation and after the harvesting are listed in Table 1. Environmental factors varied not only prior the inoculation and after the harvesting but also in sterilized and non-sterilized media (Table 1). Similar fluctuation in environmental conditions was seen for *C. glomerata* (Çelekli and Bulut 2020) and *Spirogyra setiformis* (Çelekli et al. 2016b) exposed to the Cd^{+2} stress. The maximal electrical conductivity (EC, 535 µS/cm) value was measured in the non-sterilized medium before the algal inoculation in the control group. *Cladophora glomerata* was often encountered in a variety of streams within the Araban-Yavuzeli basin, having similar EC gradients ranging from 439 to 549 µS/cm (Çelekli and Bozkurt 2021). The sterilized media had relatively low EC values compared to the non-sterilized media. This could be due to the autoclaving process that could affect ionic interactions and microorganisms in the growth media compared to the unsterilized media. There was no significant difference in EC values betwee Pb^{+2} groups using sterilized medium before the inoculation and after the harvesting ($p > 0.05$), however there was a significant difference in non-sterilized media ($p < 0.05$). Only a significant variation in the EC value between sterilized and unsterilized media was seen in the control medium. After the incorporation of Pb^{2+} to growth media, there was no significant difference in EC values between sterilized and non-sterilized media at each Pb^{+2} concentration. This could be due to the interaction between Pb^{2+} ions and anionic groups of media.

Table 1
Physico–chemical variables of media before and after cultivation of *Cladophora glomerata*. TDS-total dissolved solid, DO-dissolved oxygen, and ORP-oxidation reduction potential.

	Sterilized media Pb^{2+} (mg/L)						
	Variable	Unit	Control	7.5	30	60	
Before	Temperature	˚C	24.2	23.9	24.2	23.8	24.1
	pH		8.27	7.94	7.91	7.88	7.87
	DO	mg/L	8.1	7.82	7.29	7.10	6.97
	Conductivity	µs/cm	372	312	344	362	370
	TDS	mg/L	237	213	226	238	236
	Salinity	ppt	0.18	0.16	0.17	0.18	0.18
	ORP	mV	-71.0	-51.5	-49.7	-48.1	-47.4
After	Temperature	˚C	24.4	24	23.8	23.9	23.9
	pH		8.09	7.96	8.01	8.02	8.04
	DO	mg/L	7.06	5.5	5.27	5.12	
	Conductivity	µs/cm	336	304	301	300	294
	TDS	mg/L	227	207	207	209	201
	Salinity	ppt	0.17	0.16	0.16	0.16	0.16
	ORP	mV	-60.2	-52.6	-55.5	-56.1	-57.3
	Non-sterilized media Pb^{2+} (mg/L)						
	Variable	Unit	Control	7.5 mg/L	15 mg/L	30 mg/L	60 mg/L
Before	Temperature	˚C	24.2	23.9	24.2	23.8	24.1
	pH		8.21	8.11	8.07	7.98	7.92
	DO	mg/L	9.32	8.33	8.23	8.05	7.82
	Conductivity	µS/cm	535	390	380	344	322
	TDS	mg/L	370.5	268.7	260	235.95	219.7
	Salinity	ppt	0.27	0.20	0.19	0.17	0.16
	ORP	mV	-65.9	-61.4	-57.7	-53.4	-49.8
After	Temperature	˚C	24.1	24.2	24.1	24.1	24.2
	pH		8.10	8.04	8.02	7.97	7.89

	Sterilized media Pb^{2+} (mg/L)					
DO	mg/L	8.96	5.97	5.96	5.88	5.95
Conductivity	µS/cm	473	343	301	294	289
TDS	mg/L	323.7	234	205.4	200.8	197.6
Salinity	ppt	0.24	0.17	0.15	0.15	0.14
ORP	mV	-60.7	-57.0	-56.0	-53.0	-49.0

The filamentous macroalga had large cylindrical cells with net-like chloroplasts. The cell dimensions were determined to be 107 µm in width and 264 µm in length before the tests. The morphological and cell dimensions of the filamentous alga addressed to *Cladophora glomerata* (Linnaeus) (John et al. 2002). Çelekli and Bulut (2020) also observed similar findings. Table 2 summarizes the cell diameters and biovolumes of *C. glomerata* grown in sterilized and unsterilized medium following the experiment. *Cladophora glomerata* cells varied in width, length, and biovolume when exposed to various lead concentrations in sterilized and non-sterile conditions. Cell sizes were significantly different between sterilized and unsterilized medium ($p < 0.05$). Unlike the Pb^{2+} concentrations in this work, the biovolume level of *C. glomerata* cells decreased with increasing cadmium concentrations in media (Çelekli and Bulut 2020).

Table 2

Effect of Pb^{2+} concentrations on the morphological properties of *C. glomerata*

	Sterilized environment		
Pb^{2+} (mg/L)	Wide (µm)	Lenght (µm)	Biovolume x 10^{-3} ($µm^3$)
0	$109.5 \pm 6.3^{b,A}$	$300.3 \pm 5.9^{c,A}$	$2.84 \pm 0.37^{b,A}$
7.5	$108.6 \pm 3.2^{a,b,A}$	$263.4 \pm 20.4^{b,A}$	$2.44 \pm 0.26^{a,A}$
15	$105.5 \pm 3.4^{a,b,A}$	$274.7 \pm 8.4^{b,A}$	$2.40 \pm 0.17^{a,A}$
30	$103.1 \pm 3.6^{a,A}$	$340.3 \pm 7.5^{d,A}$	$2.84 \pm 0.25^{b,A}$
60	$125.5 \pm 5.4^{c,A}$	$246.2 \pm 12.9^{a,A}$	$3.04 \pm 0.20^{b,A}$
	non-sterilized environment		
Pb^{2+} (mg/L)	Wide (µm)	Lenght (µm)	Biovolume ($µm^3$)
0	$106.1 \pm 2.7^{a,b,A}$	$217.1 \pm 5.6^{a,B}$	$1.91 \pm 0.57^{a,B}$
7.5	$109.5 \pm 6.1^{c,A}$	$284.5 \pm 21.9^{b,B}$	$2.69 \pm 0.46^{b,B}$
15	$102.7 \pm 3.4^{a,B}$	$222.4 \pm 28.6^{a,B}$	$1.76 \pm 0.16^{a,B}$
30	$120.7 \pm 2.2^{d,B}$	$256.1 \pm 19.7^{a,b,B}$	$2.93 \pm 0.28^{b,A}$
60	$105.5 \pm 3.6^{a,b,B}$	$313.8 \pm 47.5^{b,B}$	$2.64 \pm 0.36^{b,B}$

The shape of *C. glomerata* cells altered in the sterilized and non-sterilized conditions for the control and 60 mg/L Pb^{2+} concentrations (Figs. 1a-d). The chloroplast structure and pigment concentration of green filamentous alga in sterilized (Fig. 1a) and non-sterilized (Fig. 1c) conditions were alive and similar to those seen in nature. Visual alterations in the structure and color of chloroplast and the morphology of cells of *C. glomerata* were observed at 60 mg/L Pb^{2+} concentrations for both mentioned media compared to the control group. However, elevated Pb^{2+} concentrations cause degradation of the macroalgal chloroplast structure, resulting in the formation of voids. The degradation of pigment structure and void size were greater in sterilized media (Fig. 1b) than in non-sterilized media (Fig. 1c). Along with the increase in lead concentrations in the environment, there was an increase in the deterioration of the algal chloroplast structure. Similar findings were found in a research on the biochemical reaction of *C. glomerata* and *S. setiformis* to Cd^{2+} concentrations (Çelekli and Bulut 2020) and *S. setiformis* (Çelekli et al. 2016b).

The deterioration of chloroplast structure reflected pigment content of filamentous alga and findings of the sterilized and non-sterilized media are shown in Figs. 2 and 3, respectively. The control group had high chlorophyll-a levels of 1350 g/g fw in non-sterilized medium (Fig. 3) and 1340 g/g fw in sterilized

media (Fig. 2). The lowest results were 1049 g/g fw in sterilized medium and 1067 g/g fw in unsterilized media with 60 mg/L Pb^{2+}. At each metal concentration, the chlorophyll-a content of non-sterilized medium is greater than that of sterilized media. but the difference is not significant ($p > 0.05$). Chlorophyll-b and carotenoid levels exhibited a similar pattern (Figs. 2 and 3). On the other hand, increasing the lead content resulted in a significant decrease in the pigment values of both medium tested ($p < 0.05$). Metals also have advers effects on the pigment content of *Scenedesmus quadricauda* (Çelekli et al. 2013), *Chlorella vulgaris* (Rai et al. 2013), *Spirogyra setiformis* (Çelekli et al. 2017), *Chladophora* (Cao et al. 2015), and *C. glomerata* (Çelekli and Bulut 2020). High metal concentrations can induce the generation of reactive oxygen species, which has a substantial negative effect on the pigment components (Calatayud et al. 1999). Additionally, metal ions, such as Mg^{2+} ions substituted for chlorophyll pigment, have a limiting influence on algal pigment synthesis (Küpper et al. 2003; Rai et al. 2013; Çelekli and Bulut 2020).

Table 3 summarizes the biochemical results of *C. glomerata* to changes in Pb^{2+} concentrations. Total protein content showed a decrement trend with increasing Pb^{2+} concentrations in the media. The greatest protein concentration, 35.88 mg/g, was detected in the non-sterilized medium without lead ions, whereas the lowest concentration, 24.82 mg/g at 60 mg/L Pb^{2+}, was determined in the sterilized medium. Similar results were obtained previously for the protein content of *C. vulgaris* exposed to chromate (Rai et al. 2013), *C. glomerata* subjected to Cd^{2+} (Çelekli and Bulut 2020), and *S. setiformis* exposed to Cd^{2+} (Çelekli et al. 2016b). Heavy metals have also been shown to have a detrimental influence on the protein content of filamentous algae in a variety of complex environments (Çelekli et al. 2016a, 2017). Protein and pigment production in *C. glomerata* is highly sensitive to high metal ions.

Table 3

Effect of Pb^{2+} concentrations on the biochemical components of *Cladophora glomerata*. MDA and TPC are malondialdehyte and total phenolic compounds, respectively.

	Pb^{2+}	MDA	Proline	H_2O_2	TPC	Protein
	(mg/L)	(µg/g)	(µg/g)	(µg/g)	(mg/g)	(mg/g)
Sterilized	0	57.48 ± 4.07[a]	45.91 ± 3.25[a]	34.92 ± 2.47[a]	2.82 ± 0.21[a]	34.47 ± 2.43[a]
	7.5	69.52 ± 4.92[a,b]	51.60 ± 3.65[a,b]	42.00 ± 2.97[a]	3.35 ± 0.24[b,c]	32.11 ± 2.27[a,b]
	15	75.75 ± 5.36[b,c]	64.00 ± 4.53[b,c]	54.21 ± 3.83[b]	3.62 ± 0.25[b]	29.08 ± 2.05[a - c]
	30	88.62 ± 6.26[c,d]	76.61 ± 5.42[c]	65.43 ± 4.62[c]	3.89 ± 0.28[b,c]	27.21 ± 1.92[b,c]
	60	99.63 ± 7.04[d]	100.28 ± 7.09[d]	83.39 ± 5.90[d]	4.38 ± 0.31[c]	24.82 ± 1.75[c]
Non-sterilized	0	56.07 ± 3.96[a]	41.43 ± 2.93[a]	33.95 ± 2.40[a]	2.76 ± 0.20[a]	35.89 ± 2.54[a]
	7.5	64.69 ± 4.57[a,b]	47.60 ± 3.37[b]	38.52 ± 2.72[a]	3.16 ± 0.23[a,b]	33.24 ± 2.35[a,b]
	15	72.58 ± 5.13[b,c]	60.80 ± 4.30[b]	49.55 ± 3.50[b]	3.47 ± 0.25[b,c]	31.51 ± 2.23[a - c]
	30	81.15 ± 5.74[c]	74.00 ± 5.23[c]	60.86 ± 4.31[c]	3.78 ± 0.27[b,c]	28.78 ± 2.04[b,c]
	60	84.81 ± 6.00[c]	96.80 ± 6.84[d]	76.35 ± 5.40[d]	3.96 ± 0.28[c]	26.18 ± 1.85[c]

Different letters indicate a statistical difference at a 0.05 level in each column. Values with the same letters in the same column indicate that the values did not differ by the Duncan test at 0.95 confidence interval.

Bioaccumulation of MDA, proline, H_2O_2 and TPC in C. *glomerata* was enhanced when Pb^{2+} concentrations increased (Table 3). The concentration of MDA in the sterilized medium changed significantly between 57.48 g/g for control and 99.63 g/g for 60 mg/L ($p < 0.05$). MDA levels were somewhat lower in the non-sterilized media than in the sterilized medium, but this difference was not significant. High metal ions promote the enheancement of MDA content of algae (Rai et al. 2013; Çelekli and Bozkurt 2021).

MDA results at high metal ion concentrations indicated membrane lipid peroxidation. Due to their inability to attach to Pb^{2+} ions, high metal concentrations accelerate the degradation of the cell membrane. Thus, increased metal ions entrance into the cell boosts ROS formation, protein breakdown, and some biochemical synthesis (Salama et al. 2019). Metal-stressed organisms exhibit several mechanisms: metal detoxification (extracellular and/or intracellular metal exclusion via ligand synthesis, storage in vacuoles, and metal pumping out) and antioxidant defenses (enzymatic and non-enzymatic) (Gomes and Asaeda 2013). These processes have a significant effect on algal tolerances to metal ion gradients.

Pb^{2+} ions increased the quantity of proline in *C. glomerata*. The maximum proline concentration (100.28 g/g) was determined in the sterilized medium containing 60 mg/L lead ions. Additionally, earlier research has established that exposure to heavy metals increases the proline concentration of algae (Rai et al. 2013; Çelekli and Bozkurt 2021). A similar trend was found in hydrogen peroxide (H_2O_2) and total phenolic compound (TPC) of *C. glomerata*. The highest values of H_2O_2 (83.39 µg/g) and TPC (4.38 mg/g) were found in the sterilized media containing 60 mg/L Pb^{2+} ions ($p < 0.05$). Increased lead concentrations in sterilized and unsterilized medium could stimulate the H_2O_2 and TPC production in algal biomass. Previously, metal stress led to stimulate the increase in proline levels in *Scenedesmus* sp. (Tripathi et al. 2006), *C. glomerata* (Çelekli and Bulut 2020), and *S. quadricauda* (Kováčik et al. 2010) in both laboratory and complex natural environments (Çelekli and Bozkurt 2021). The total phenolic content is considered to be one of the primary non-antioxidant components, perhaps counteracting the detrimental effect of reactive oxygen species (ROS) generated by metals (Ismaiel and Said 2018). Total phenolics may have the capacity to donate electrons, effectively stabilizing free radicals. Additionally, phenolics can bind active metals involved in the start and generation of reactive oxygen species (Pinto et al. 2003). The present study's findings demonstrated that the alga exhibits metabolic reactions to metal exposure, reiterating the relevance of signaling molecules such as MDA and proline (Murugan and Harish 2007; Çelekli and Bulut 2020). Thus, these biochemical compounds could be used to assess the detection of free amino acids and membrane lipid peroxidation, respectively. These bioindicator molecules colud be used to monitor environmental conditions and organism health (Çelekli and Bozkurt 2021).

MDA, proline, H O , and TFB concentrations in *C. glomerata* were higher in the sterilized environment than in the non-sterilized environment. On the contrary, the total protein content of non-sterilized media was greater than that of sterilized media. The biochemical composition of *C. glomerata* did not differ statistically across research groups in sterile and non-sterile conditions ($p > 0.05$). The autoclaving process not only affects interactions among ions in media but also leads to the death of available microorganisms in media. Additionally, both microorganisms and the ion composition of non-sterilized media may mitigate the effects of Pb^{2+} on the biochemical and morphological responses of *C. glomerata.* Microorganisms growing on unsterilized medium may absorb some lead ions. Additionally, microbes may contribute to this process by converting organic components to inorganic molecules in non-sterile conditions. The results suggested that when algae cells are exposed to oxidative metal stress, they create certain compounds (as defensive mechanisms) such as MDA, glutathione, proline, and TPC to counteract the detrimental effects of ROS (Volland et al. 2014; Çelekli and Bozkurt 2021).

The residual Pb^{2+} concentrations in the media were found to differ little between sterilized and unsterilized media. The non-sterilized media had 0.69, 0.84, 0.97, and 1.28 mg/L of Pb^{2+} in the 15, 30, 45, and 60 mg/L Pb^{2+} groups, respectively, whereas the sterilized media contained 0.63, 0.80, 0.89, and 1.16 mg/L of Pb^{+2}. The results suggested that *C. glomerata* could tolerate a wide range of Pb^{2+} gradients. *Cladophora glomerata* had an important potential for lead ion bioaccumulation. *Chlorella vulgaris* (Rai et al. 2013) and *Chlorella glomerata* (Çelekli and Bulut 2020) were also shown to be metal hyperaccumulators. All of them corroborate *C. glomerata*'s widespread distribution on the planet (Higgins et al. 2008; Çelekli et al. 2017).

The results of FTIR-ATR studies indicated that the surface structure of *C. glomerata* changed under different Pb^{2+} levels in sterilized (Fig. 4) and non-sterilized (Fig. 5) medium. *Cladophora glomerata* displayed comparable main bands in both media without Pb^{2+} ions as a control group: 3336–3340 assigned to –OH and $-NH_2$ groups, 2924–2926 related to –CH stretching, 1643–1644 associated with amide (-C = O) or N–H bending, 1375–1384 related to C–H bending and antisym stretch, 1234–1235 associated with NO2 and antisym stretch, 1056–1057 related to –OH stretching, 1033–1034 assigned to the C-O groups, and 871–874 1/cm assigned to CH–CH2 in vinyl compounds (Arief et al. 2008). Similar peaks were also found for the surface structure of *C. glomerata* (Çelekli and Bulut 2020). The spectrum of algal biomass showed new values at 60 mg/L Pb^{2+} compared to control. The results suggested that amino, amide, and anionic surface groups all had a substantial impact in Pb*2+-C. glomerata* interaction.

These shifts in the spectrum peaks may account for the presence of Pb^{2+} on the surface of macroalga. Similar results were also found in the effect of Cd^{2+} on *S. setiformis* (Çelekli et al. 2016b), *Synechocystis* sp. (Ozturk et al. 2010), *C. glomerata* (Çelekli and Bulut 2020), *S. quadricauda* (Çelekli et al. 2013). Besides, these interactions between metal ions and surface structures of filamentous algae were also indicated in nature-complex ecosystems (Çelekli et al. 2016a, 2017).

Conclusion

The results of this investigation demonstrated that *C. glomerata* exhibited biochemical and morphological responses to the Pb^{2+} gradients in sterilized and non-sterile medium. Pigment and protein content decreases in response to elevated Pb^{2+} concentrations. On the other hand, stress molecules (e.g., MDA and proline) were raised as biomarkers in *C. glomerata*'s response mechanisms to Pb^{2+} exposure. These signaling molecules may be utilized to identify the detrimental effects of hazardous substances on biota in advance, which may be useful for environmental monitoring purposes such as analyzing ecological quality conditions. These diverse biological reactions of *C. glomerata* provide critical information about how it survives in a variety of natural situations.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Author Contribution

AÇ: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing-review & editing, Software, Supervision, Visualization, Formal analysis, Project administration. EA: Methodology, Data curation, Investigation, and Visualization.

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Figures

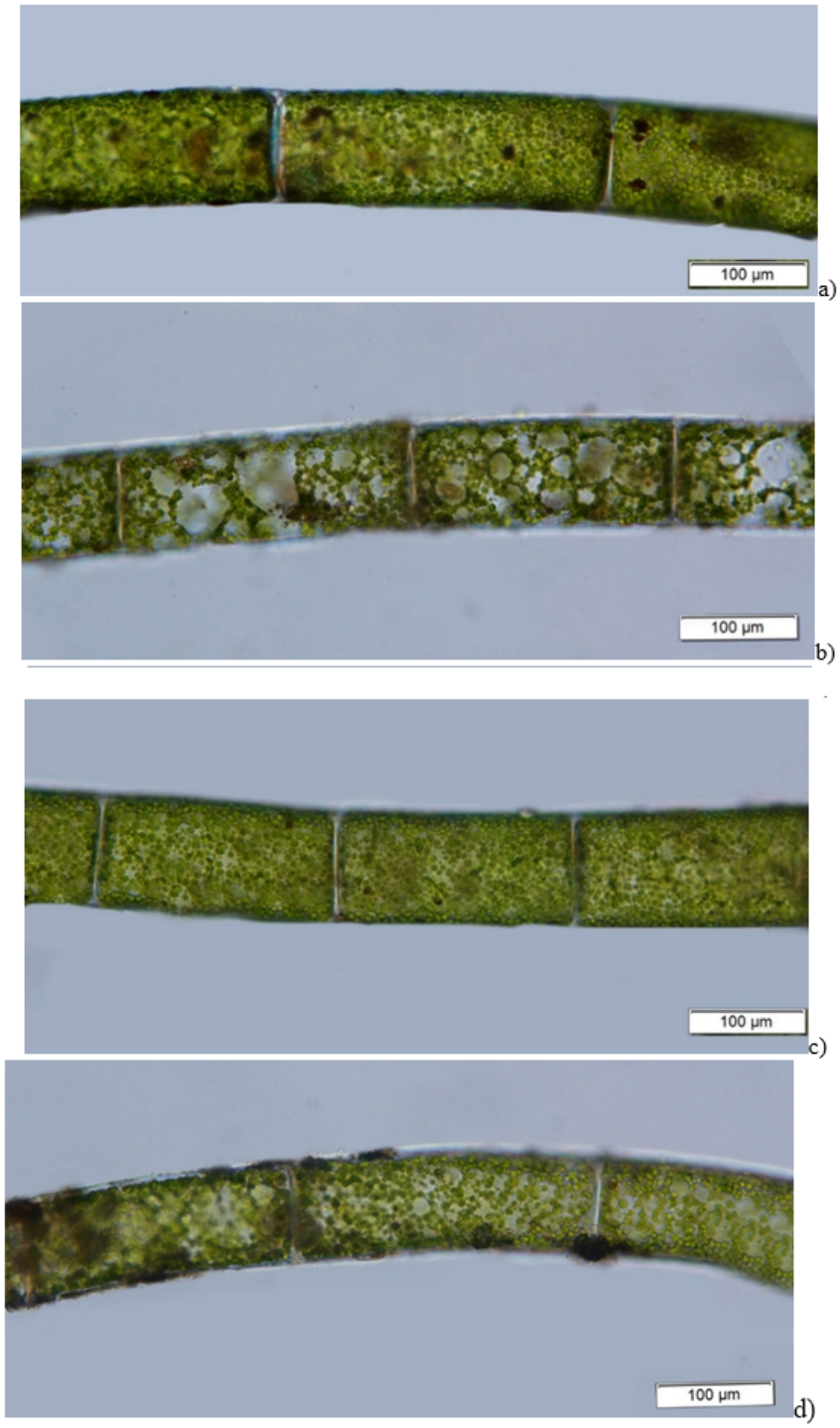

Figure 1

Changes in cell morphology of *C. glomerata* at (a) control and (b) 60 mg/L Pb^{2+} for sterilized and (c) control and (b) 60 mg/L Pb^{2+} for non-sterilized media after the experiment.

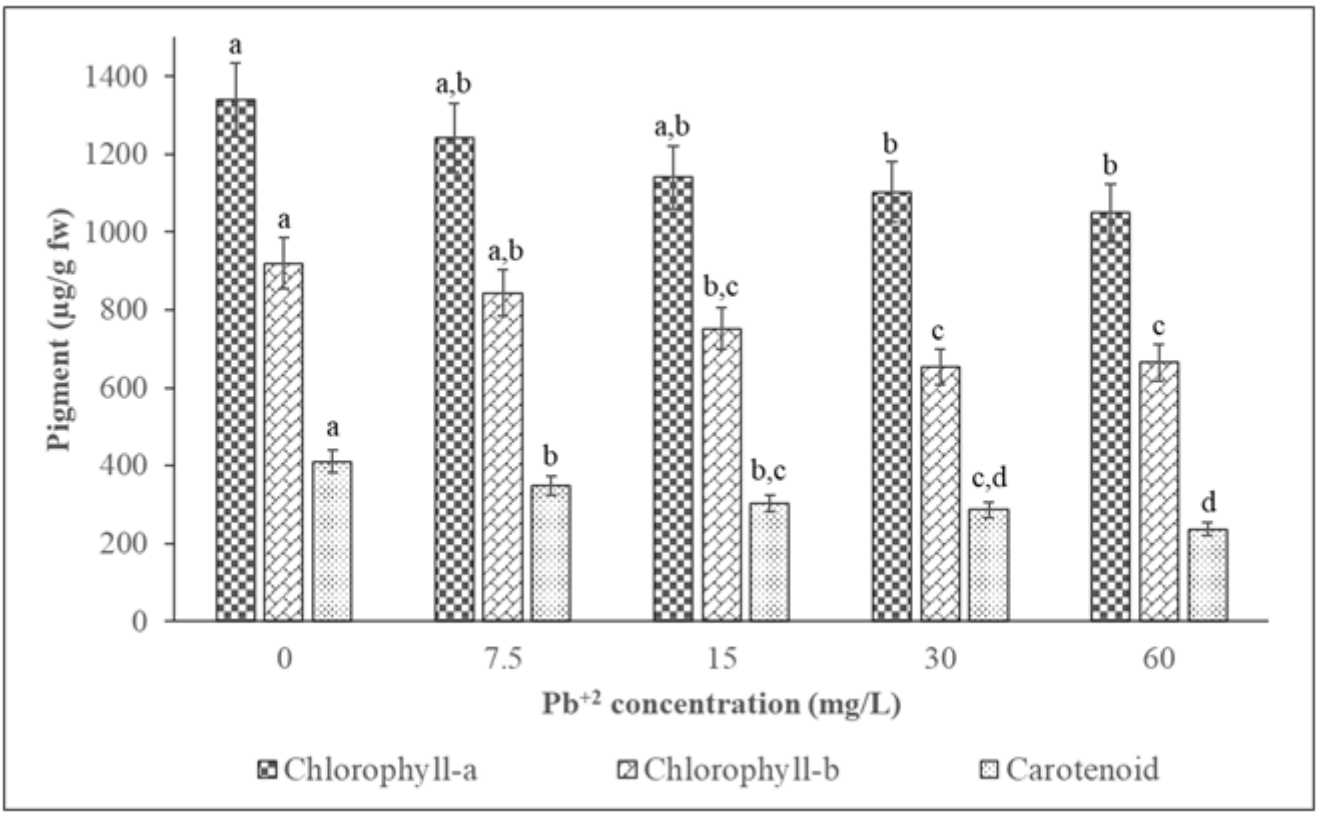

Figure 2

Cladophora glomerata pigment levels at different lead concentrations in sterilized media.

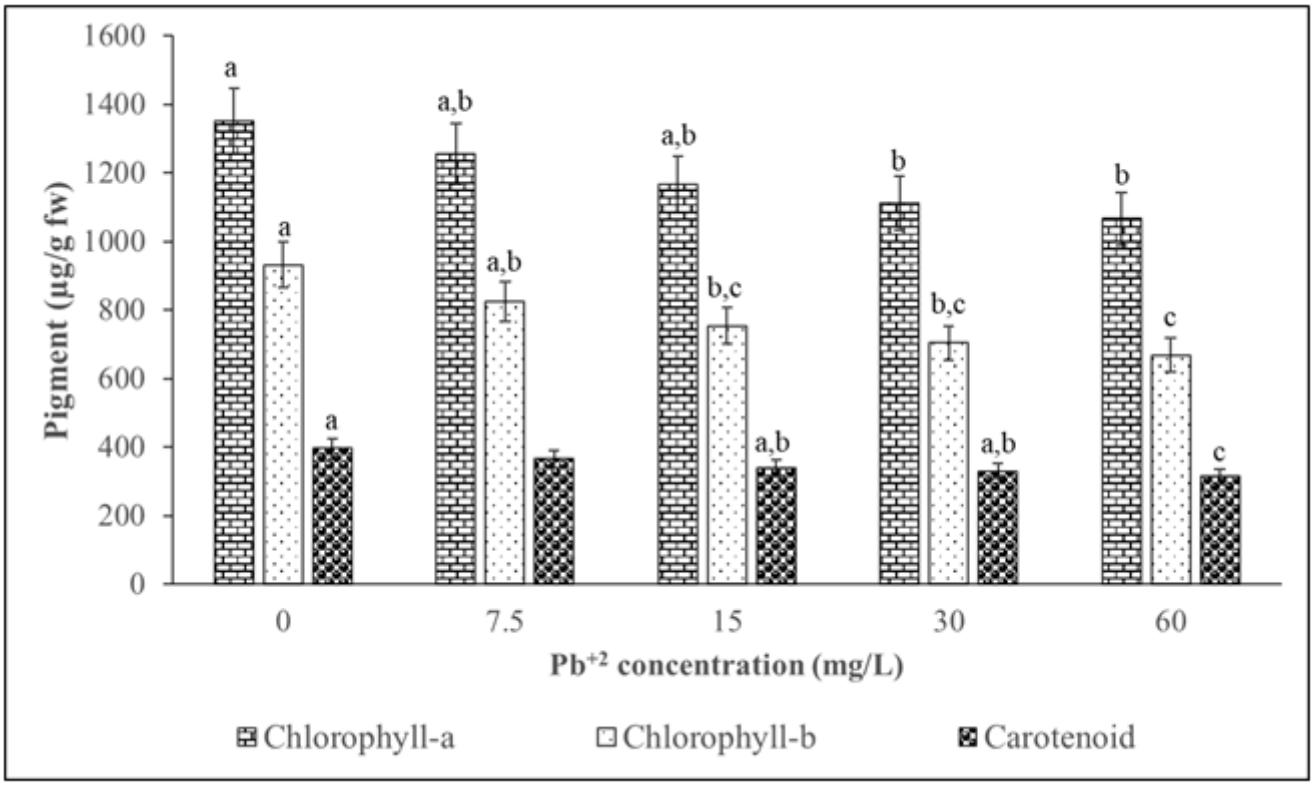

Figure 3

Cladophora glomerata pigment levels at different lead concentrations in non-sterile environment

Figure 4

FTIR analysis results of *C. glomerata* biomass in sterile environment (A) control group and (B) 60 mg/L Pb^{2+} group.

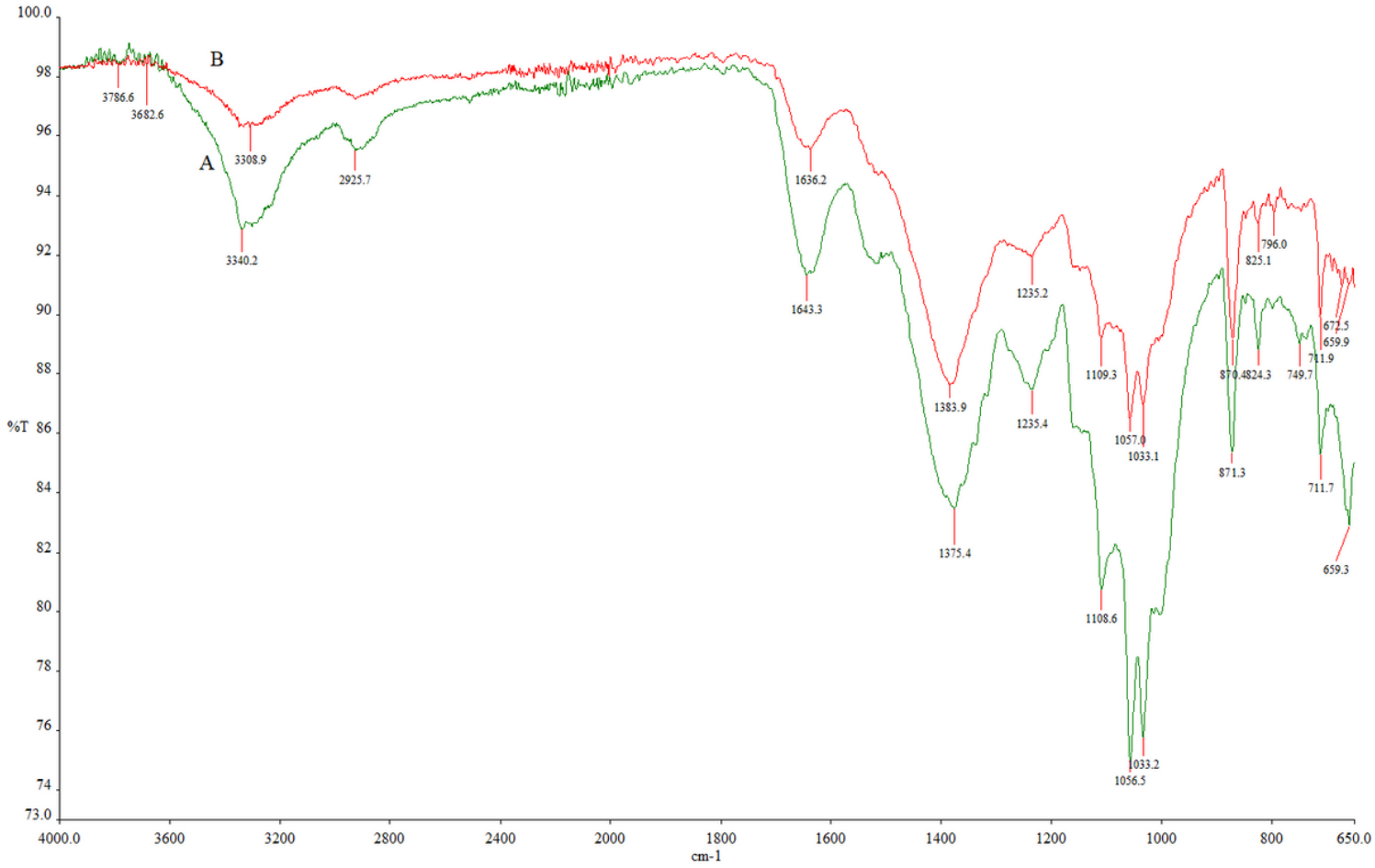

Figure 5

FTIR analysis results of *C. glomerata* biomass in non-sterile media (A) control group and (B) 60 mg/L Pb^{2+} group.