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Bio-remediation capacity for Cd(II) and Pb(II) from the aqueous medium by two novel strains of microalgae and their effect on lipidomics and metabolomics

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ABSTRACT

Microalgae have been the subject of recent research as a sustainable feedstock for the large-scale production of metabolites for commercial purposes. This study presents a green bio-remediation approach towards heavy metal contaminations and biomass production for biofuels in microalgae metabolomics and lipidomics approaches. Two novel microalgae, *Chlorosarcinopsis bastropiensis* and *Polyedriopsis spinulosa*, were isolated during the study and subjected to Pb(II) and Cd(II) pollutants. The isolated microalgae strains have shown a varied behavior towards cell growth, pigment accumulation, and lipids profiles during the impact of short-term (96 h) and long-term (14 d) heavy metal tolerance. Cell viability and IC_{50} value (397.75 mg/L for *C. bastropiensis* and 490.16 mg/L for *P. spinulosa*) have indicated higher tolerance towards Pb(II) in both microalgae. FTIR analysis of microalgal biomass has revealed insignificant differences during long and short-term heavy metal toxicity, clearly indicating the bio-tolerance for Pb(II) and Cd(II) in both microalgae. Principal component analysis has revealed the expression of metabolites (such as glycine, proline, valine, isoleucine, linoleic acid, glucose, sucrose, etc.) under heavy metal stress. ^{1}H NMR analysis has demonstrated the prominent expression of metabolites under heavy metal stress. ICP-MS-based studies do not reflect the correlation between cellular tolerance and bioaccumulation of each heavy metal by both microalgae. Lipidomics based on ^{1}H NMR has revealed an increase in unsaturated fatty acids under the impact of heavy metals. Therefore, this study offers a sustainable bioremediation technique for heavy metal contaminants and biomass production with significant enhancement of metabolites and lipid components for biofuels and/-or other commercial applications.

1. Introduction

Microalgae are an eco-friendly and cost-effective source of metabolites such as lipids, carbohydrates, pigments, sugars, essential/non-essential amino acids, antioxidants, etc., that have commercial importance. Microalgae have been the subject of recent research as a sustainable feedstock for the large-scale production of these metabolites for commercial purposes [1–3]. Another, rapid population growth, urbanization, and industrialization have contributed to serious water contaminations due to the disposal of untreated toxic organic/inorganic

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effluents, including heavy metal pollutants (e.g. Cd, Pb, Hg, Cr, Zn, Cr, etc.) in freshwater bodies. Heavy metal pollutants enter food chains through contaminated water and pose a critical threat to the lives of humans, animals, aquatic bodies, and healthy ecosystems [4,5]. Ignorant and uncontrolled discharge of heavy metals contaminants is gradually increasing their concentrations in wastewater. Lead (Pb) and cadmium (Cd) are among the most toxic and carcinogenic heavy metals that enter healthy ecosystems through industrial effluents. This has drawn the attention of several researchers towards its management and removal from aquatic systems [6,7]. Microalgae are a promising candidate for green bioremediation technologies that several researchers have been focused on around the world [8–10].

Several conventional physicochemical techniques have been employed for the removal of heavy metal contaminants from wastewater such as chemical precipitation, coagulation, membrane filtration, adsorption, chemical oxidation/reduction, solvent extraction, reverse osmosis ion exchange, electro-dialysis, etc. [11–13]. Recently, different bioremediation techniques using various microorganisms (such as bacteria, fungi, and microalgae) have been reported to accumulate heavy metals in their cells [14,15]. Among microorganisms, microalgae are a powerful candidate for the bioremediation of heavy metal contaminants in wastewater through efficient biosorption and bioaccumulation mechanisms. Furthermore, the microalgae biomass produced contains various value-added biomolecules that can be used for different applications, including bioenergy/biofuels products [16–18].

Most microalgae-based approaches for heavy metals bioremediation or biomass to biofuel generation are based on traditional methods of modifying/improving cultivation methods [19]. Developments of advanced and integrated approaches require the comprehensive insight of microalgal metabolomics to understand the biosynthetic pathways responsible for these functions. Research in the field of algomics is limited and imminent researchers are focusing in this direction to make a leap in the research and development of microalgae-based techniques [20–22]. Different researchers have recognized the benefits of microalgae for heavy metals bioremediation, as well as biofuels [21–27]. However, key areas requiring an approach to the development of algal biofuel systems include the development of easy and efficient cultivating methods with high oil yields that can easily be scaled up to large-scale production systems in a sustainable way [21,28–31]. Researchers, therefore, are now focusing on omic approaches in microalgae research [32–34]. The present work suggests a green bioremediation approach towards heavy metal bioremediation and biofuel production using microalgae metabolomics and lipidomics approaches. During the present investigations, ^{1}H NMR-based lipidomics and metabolomics studies were used in conjunction with other methods to reveal lipid profiles in two novel microalgae strains viz. *Chlorosarcinopsis bastropiensis* Ind-Jiht-4 and *Polyedriopsis spinulosa Ind-Jiht-II* in response to Cd(II) and Pb(II) tolerance. ICP-MS investigations were used to determine the bioaccumulation of Cd(II) and Pb(II) by each microalga and to infer any correlation between heavy metal tolerance and simultaneous bioaccumulation by microalgae.

2. Materials and methods

2.1. Isolation and characterization of microalgae

Chlorosarcinopsis bastropiensis Ind-Jiht-4 was isolated using water samples collected from Gangotri, Uttarakhand, India. The isolation of

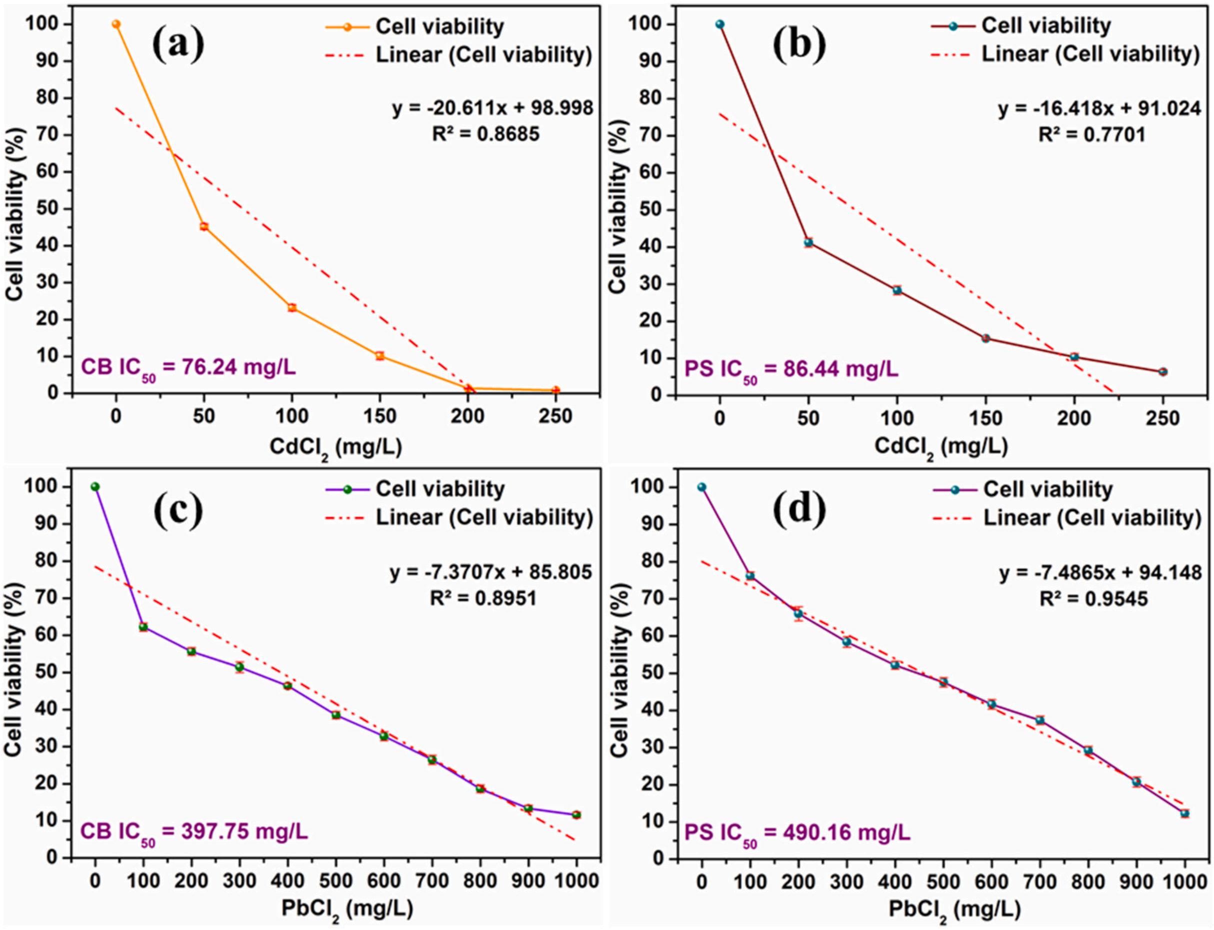

Fig. 1. Cell viability (%) and IC$_{50}$ of *C. bastropiensis* (CB) and *P. spinulosa* (PS); (a) CB in CdCl$_2$, (b) PS in CdCl$_2$, (c) CB in PbCl$_2$, and (d) PS in PbCl$_2$.

Polyedriopsis spinulosa Ind-Jiht-II was done from water samples collected from Naraingarh, Haryana, India. Pure cultures were isolated in Bold's basal medium (BBM) by streaking of 1% water samples [35,36]. Furthermore, molecular characterization and identification were performed by constructing phylogenetic trees based on 26S rRNA sequences using MEGA 6 software [37].

2.2. Microalgae cultivation and short term assay of heavy metal toxicity to microalgal cells

C. bastropiensis and *P. spinulosa* were cultured in BBM for stock culture. The logarithmic phase of the stock cultures was used as inoculum and the initial optical density (OD) of microalgae culture was measured ~0.2 for heavy metal toxicity studies. During the growth of the microalgae, we have observed the stationary phase around 14th days. For this purpose, both isolated microalgae were cultured in BBM supplemented with varying concentrations of $PbCl_2$ (100–1000 mg/L) and $CdCl_2$ (50–250 mg/L) along with the control for 96 h to determine the half-maximal inhibitory concentration (IC_{50}) values. IC_{50} values were calculated by plotting separate graphs between the growth rates of the microalgae against the different concentrations of heavy metals after 96 h of culture using linear interpolation analysis in Microsoft Excel 2010. The estimated concentration of the IC_{50} values of $PbCl_2$ and $CdCl_2$ was used to cultivate the microalgae *C. bastropiensis* and *P. spinulosa* for 14 days.

Heavy metal toxicity is known to greatly influence pigment accumulation, growth rate, and biomass productivity in microalgal cells. In order to establish a correlation between them, the above-mentioned parameters were also recorded. Pigments were determined on the 10th day of culture by centrifuging 2 mL of culture at 5500 rpm. The collected cell pellets were incubated at 45 °C for 30 min with 2 mL of methanol. The supernatant was finally separated by centrifuging the contents again at 5500 rpm and recording the absorbance at 665.2 nm, 652.4 nm, 470 nm, and 750 nm. Finally, the following equations (Eqs. (1)–(3)) were used to calculate the amounts (μg/mL) of various pigments [38]. For short-term metal toxicity studies, 100 mg of the microalgae biomass was collected on the 4th day of culture and subjected to FTIR analysis.

$$\text{Chlorophyll‘a’}\,(\text{Chl‘a’};\,\mu g/mL) = 16.72\ A_{665.2} - 9.16\ A_{652.4} \tag{1}$$

$$\text{Chlorophyll‘b’}\,(\text{Chl‘b’};\,\mu g/mL) = 34.09\ A_{652.4} - 15.28\ A_{665.2} \tag{2}$$

$$\text{Carotenoids}\ (\mu g/mL) = (1000\,A_{470} - 1.63\ \text{Chl‘a’} - 104.9\ \text{Chl‘b’})/221 \tag{3}$$

where ‘$A_{665.2}$’ is the absorbance at 665.2 nm, ‘$A_{652.4}$’ is the absorbance at 652.4 nm, and ‘A_{470}’ is the absorbance at 470 nm.

2.3. Long term heavy metal toxicity assay by metabolites analysis and lipidomics studies

Lipids were estimated after 14 days of culture in the control, as well as in cultures stressed by supplemented heavy metals. Microalgae biomass was harvested by centrifugation for 10 min at 8000 rpm. The harvested biomass was dried and stored for further analysis. Lipids were extracted from microalgal biomass using the modified method of Bligh and Dyer [39]. The lipid contents and productivity were estimated using the following equations (Eqs. (4) and (5)). For lipidomics studies, 10 mg of the extracted microalgae lipids were added to 550 μL of deuterated chloroform ($CDCl_3$) and applied for ^1H NMR (500 MHz NMR) to record the spectra and identify any chemical shifts in lipid profiles [39]. For metabolite analysis, we have followed the sample preparation and NMR method according to Azizan et al., (2018) [40]. Briefly, 100 mg of the lyophilized microalgae biomass was mixed in 50 mL of methanol and vortexes for 5 min. Cell disruption was achieved by sonication for 30 min using an ultrasonic water bath at room temperature. The extract was separated from cell debris by filtration through Whatman filter paper No. 1. The residue was dried and used for re-extraction with methanol as above twice. The filtered extracts were collected together and dried using a rotary evaporator, stored at −20 °C and used for the ^1H NMR analysis of the metabolites.

$$Lipid\ content\ (\%) = Total\ lipids\ (g)/Dry\ biomass\ (g) \times 100 \tag{4}$$

$$Lipid\ productivity\ (mg/L/d) = Lipid\ content \times Biomass\ productivity/100 \tag{5}$$

2.4. Metabolomics studies for Pb(II) and Cd(II) tolerance using ICP-MS

Biomass productivity and dry cell weight (dcw) were determined after 14 days of microalgae cultivation by recording the OD at 686 nm. The microalgae biomass harvested by centrifugation was washed 2–3 times with distilled water to remove unbound heavy metals. Another, 100 mg of biomass was subjected to acid digestion by incubating with hydrochloric acid: nitric acid (3:1 *v*/v) at 120 °C until a clear solution was obtained. Finally, the solution was diluted with H_2O and filtered. Intracellular concentrations of Pb(II) and Cd(II) were then analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, ELAN DRC-e). The concentration of Pb(II) and Cd(II) remaining in the BBM medium was also analyzed by ICP-MS. The following equations (Eqs. (6)–(8)) were used for the calculations:

$$DCW\ (g/L) = 0.274\ OD_{686} + 0.002 \tag{6}$$

$$Biomass\ productivity\ (mg/L/d) = \frac{Final_{dcw} - Initial_{dcw}}{Cultivation\ time} \tag{7}$$

$$Bioaccumulation\ (mg/Kg\ dcw) = \frac{Heavy\ metal\ concentration\ in\ microalgae\ biomass\ (dcw)}{Initial\ heavy\ metal\ concentration\ in\ the\ medium} \tag{8}$$

3. Results and discussion

3.1. Isolation and characterization of microalgae

The isolated microalgae were identified as *Chlorosarcinopsis bastropiensis* Ind-Jiht-4 (GenBank accession number MW485748) and *Polyedriopsis spinulosa* Ind-Jiht-II (GenBank accession number MW485748). The 23S rRNA phylogenetic trees based on neighbor-joining analysis are shown in Figs. S1 and S2. Microalgae are also being explored for their tolerance to heavy metals and are used by various researchers for bioremediation and biofuel production in order to develop integrated systems [24,26,41]. Efficient absorption of Cr, Cd, Zn, Pb, Ni, and Cu has been reported in *Phormidium* sp. and *Spirullina* sp. [42].

3.2. Values and short-term toxicity assay for heavy metals on cell viability and biomass

Chlorosarcinopsis bastropiensis Ind-Jiht-4 (CB) and *Polyedriopsis spinulosa* Ind-Jiht-II (PS) were able to grow in the presence of $PbCl_2$ and $CdCl_2$, and have shown considerable tolerance to Pb(II) and Cd(II) during cultivation. However, the differences in the tolerances to Pb(II) and Cd(II) in both microalgae have been indicated by the respective IC_{50} values for the supplemented heavy metals. The IC_{50} value for Cd(II) in the case of *C. bastropiensis* and *P. spinulosa* was observed to be 76.24 mg/L and 86.44 mg/L, respectively. Whereas, the IC_{50} value for Pb(II) in the case of *C. bastropiensis* and *P. spinulosa* was 397.75 mg/L and 490.16 mg/L, respectively. The respective IC_{50} values indicate the concentrations of Pb(II) and Cd(II) that do not induce cell toxicity, as evidenced by cell viability. Cell viability and IC_{50} values of heavy metal stressed microalgae cultures are presented in Fig. 1 (a, b, c, and d). The observed IC_{50} values and cell viabilities of both microalgae suggest higher tolerances towards Pb(II) and comparatively lower towards Cd(II). Microalgae have demonstrated different intracellular and extracellular mechanisms to resist heavy metal toxicity and also to differentiate between essential and non-essential heavy metals [9]. The stress-induced by heavy metals and their exertions on cell growth as well as cell viability has also been reported by other researchers. The researchers have highlighted the differential responses and behavior of microalgae cultures towards different heavy metals [43,44].

3.3. Effect of short and long term exposure to Pb(II) and Cd(II) on pigments

A decrease in the photosynthetic pigments was recorded on the 4th day of culture in both microalgae. However, *P. spinulosa* was more affected by Cd(II) and Pb(II), which might be due to the rapid drop in photosynthetic pigments under heavy metals stress (Fig. 2a). A slight reduction in Chl. 'a' and Chl. 'b' has been observed under heavy metals stress in comparison to the control on the 14th day of culture, however, it has been observed that the carotenoid levels were slightly elevated compared to the other pigments (Fig. 2b). An essential and fundamental metabolic process of microalgae is photosynthesis, which drives nutrient cycling and energy flow. In photosynthetic metabolism, Chl. 'a' is represented as the key component and Chl. 'b' as alternative assessor components. Thereafter, the improved total content of Chl. 'a' and Chl. 'b' could be due to the increase in Chl. 'b'. Furthermore, the decreasing Chl. 'a'/Chl. 'b' ratio further indicated that photosynthesis could be altered by heavy metal stress within the 4th day of exposure. Interestingly, the increase in Chl. 'a' content and the similar trend in Chl. 'b' content appeared to indicate that the pigments had achieved the alteration to resist heavy metal stress in exponential stages. The total content of Chl. 'a' and Chl. 'b' have not shown any obvious distinction under different heavy metals on the 14th day of exposure. However, compared to the 4th day of exposure to the 14th day of heavy metal stress, the Chl. 'a'/Chl. 'b' ratio showed an inverse trend along with the increasing duration of stress exposure. The concentration of IC_{50} of heavy metals stimulated a higher Chl. 'a'/Chl. 'b' ratio after the 4th day of exposure, which implies that the photosynthetic process could be restarted and promoted under stress conditions [45]. We have also analyzed the activity of photosystem II (PS II) for *C. bastropiensis* and *P. spinulosa* (control and heavy metal stress) at 4th and 14th days using the ratio of carotenoids to Chl. 'a' + Chl. 'b' as previously reported methods [46]. Similar values of control PS activity were observed for both microalgae (CB and PS). However, microalgae stressed by heavy metals have shown higher PS II activity, indicating the viability and versatility of photosystem II under stress conditions (Fig. 3). The photosynthetic process is influenced by various abiotic factors such as radiation, temperature, heavy metals stress, etc. [47]. Photosystem II activity is regulated by photosynthetic pigments and may have an adverse impact on prolonged exposure to heavy metals [48]. The drop in the level of carotenoids is less compared to the other pigments due to their protective role on the photosynthetic apparatus against the lethal

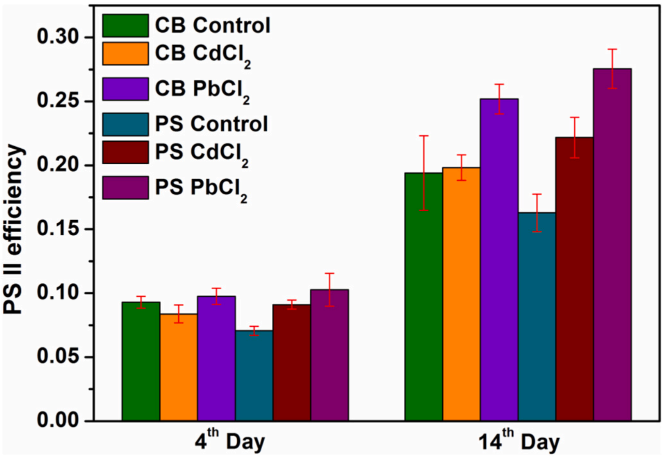

Fig. 3. Photosystem II activity for C. bastropiensis and *P. spinulosa* on 4th and 14th day.

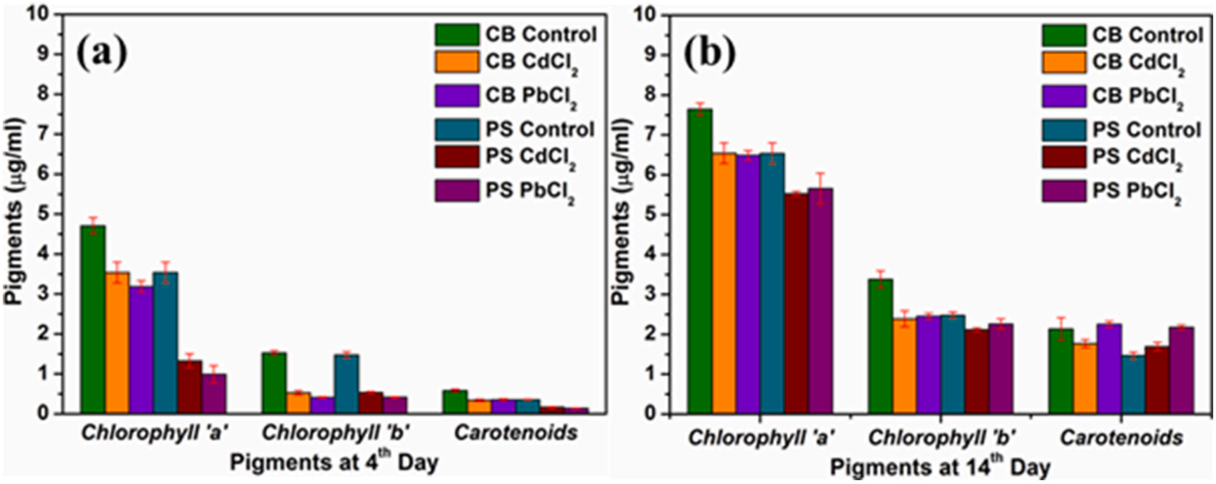

Fig. 2. Comparative estimation of photosynthetic pigments in *C. bastropiensis* (CB) and *P. spinulosa* (PS) in exposure to Pb(II) and Cd(II); (a) short term (4 d) and (b) long term (14 d).

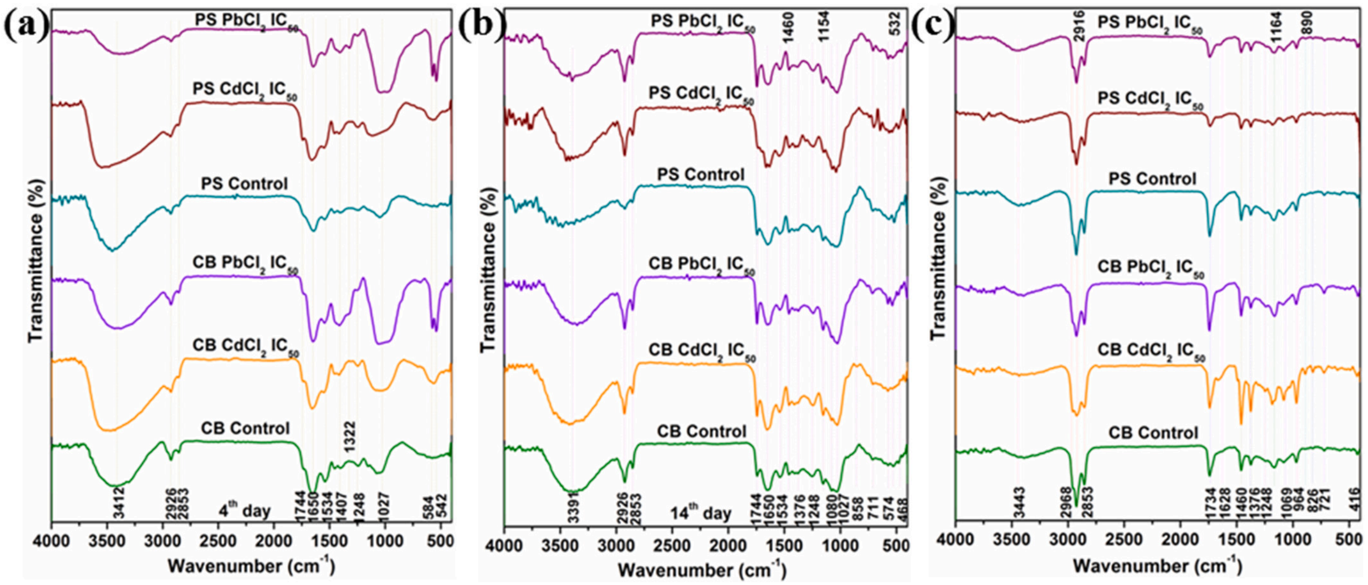

Fig. 4. FTIR spectra of microalgae (CB and PS); (a) biomass on 4th day, (b) biomass on 14th day, and (c) lipids on 14th day.

effects of heavy metals [49]. Dao and Breadall, (2016) have also reported the negative effects of heavy metal on photosystem II in microalgae [50].

3.4. Effect of short and long-term exposure of Pb(II) and Cd(II) on biomass and lipids

The chemical structures of the biomass of microalgae grown in the control medium and under heavy metal stress have shown insignificant variances on the 4th and 14th day of culture as analyzed by FTIR spectroscopy (Fig. 4a and b). However, the intensity of the absorption peaks corresponding to the hydrocarbons and other functional groups has been increased on the 14th day (Fig. 4b). In the spectra of the FTIR absorption band, significant positive upsurge has been observed at 1027 cm^{-1}, 584 cm^{-1}, 542 cm^{-1}, indicating C—O—C stretching vibrations due to carbohydrates molecules, 1248 cm^{-1} (P═O asymmetric stretching or bending of the C—H ring), 1407 cm^{-1} (bending of C—H), 1534 cm^{-1}, 1650 cm^{-1} (protein amide bands), 1744 cm^{-1} (methyl and methylene groups) in control as well as the biomass under heavy metals stress [51]. Thus, it could be inferred that there were no significant alterations in the chemical structure of the microalgae biomass under the influence of heavy metals stress. This clearly indicates the bio-tolerance for both Pb(II) and Cd(II) among these microalgal species (CB and PS).

FTIR-based lipid analysis of control and metal-stressed microalgae reflects the chemical construction of cellular lipids (Fig. 4c). The extracted lipids from the biomass of *C. bastropiensi* and *P. spinulosa* on the 14th day have demonstrated intense absorption peaks at 2968 cm^{-1}, 1734 cm^{-1}, and 1460 cm^{-1} corresponding to the hydrocarbons of lipid molecules. Other chemical fingerprints of the extracted lipids have been observed in the absorption peaks at 2853 cm^{-1}, 1376 cm^{-1}, and 964 cm^{-1}. Similar patterns of absorption bands have also been investigated in microalgal lipids under stress conditions [52].

3.5. ^{1}H NMR based metabolomics and lipidomics analysis

The ^{1}H NMR-based metabolite profiles of microalgae (CB and PS) in control and under stress conditions are illustrated in Fig. 5. The interpretation and comparison of the signals were analyzed according to previous reports, databases in Biological Magnetic Resonance (BMR), and PubChem [53,54]. The spectra have indicated different classes of carbohydrates, amino acids, and fatty acids (such as alanine, valine, isoleucine, proline, glutamic acid, glycine, leucine, methionine, glutamine, palmitic acid, linoleic acid, α-linolenic acid, glucose, and sucrose). Some insignificant signals for alanine, leucine, and methionine have been observed in the spectra of *C. bastropiensis* metabolites. Likewise, an insignificant signal for glutamine, linoleic acid, and α-linolenic acid was observed for *P. spinulosa*. The composition of the microalgal metabolites from the control and heavy metal stressed cultures appeared similar; however, stronger peaks were recorded under the influence of heavy metals compared to the control indicating overexpression of the metabolites. This indicates the toxic behavior of Pb(II) and Cd(II) generates stress to microalgae cells which respond in terms of elevated expression of metabolites.

The ^{1}H NMR-based spectra of the lipids isolated from the control medium and from the microalgae cells cultured under heavy metals stress are shown in Fig. 6. The chemical structure of the lipids isolated from the control culture and the culture stressed under Pb(II) is relatively analogous to each other (such as methyl, methylene, methine, α to carbonyl, alkynyl, nitromethylene, alcohols, α to oxygen, esters, pH-H aromatics, vinyl, and Ar—H), while distinct peaks were recorded in lipids of microalgae stressed under Cd(II). This can be attributed to characteristic lipid accumulation behaviors under different heavy metal stresses. A strong singlet signal at 5.5 ppm representing fatty acid residues (proton of Ar—H) was reported in both microalgae grown in control and under the stress of Pb(II). A similar signal has been reported in *Scenedesmus* sp. IITRIND2 [55]. The insignificant signals were recorded at 0.5–1.0 ppm (—CH_3) and 1.0–1.5 ppm (—$(CH_2)_n$) in the control as well as in the heavy metal stressed microalgal lipids. A similar signal has been described in *Chlorella sorokiniana* lipids grown under stress conditions [52]. A proton signal has been detected in microalgae growing in wastewater at 0.5–1.0 ppm [39]. A strong signal was noticed at 3.5–4.0 ppm (RO—CH_3) in the lipids of *P. spinulosa* grown in the control medium. A weak signal at this position has been observed in lipids obtained from microalgae grown in Pb(II) and Cd(II) medium during the present study.

3.6. Principal component analysis (PCA)

The effect of heavy metal stress by Pb(II) and Cd(II) on microalgae was determined by principal component analysis (PCA). PCA is a powerful statistical tool that helps to classify samples based on their chemical composition [56]. Fig. 7 illustrates the PCA score plot for control and metal-stressed microalgae (CB and PS). The stress was separated by PC1, the control microalgae extract separated from Cd(II)

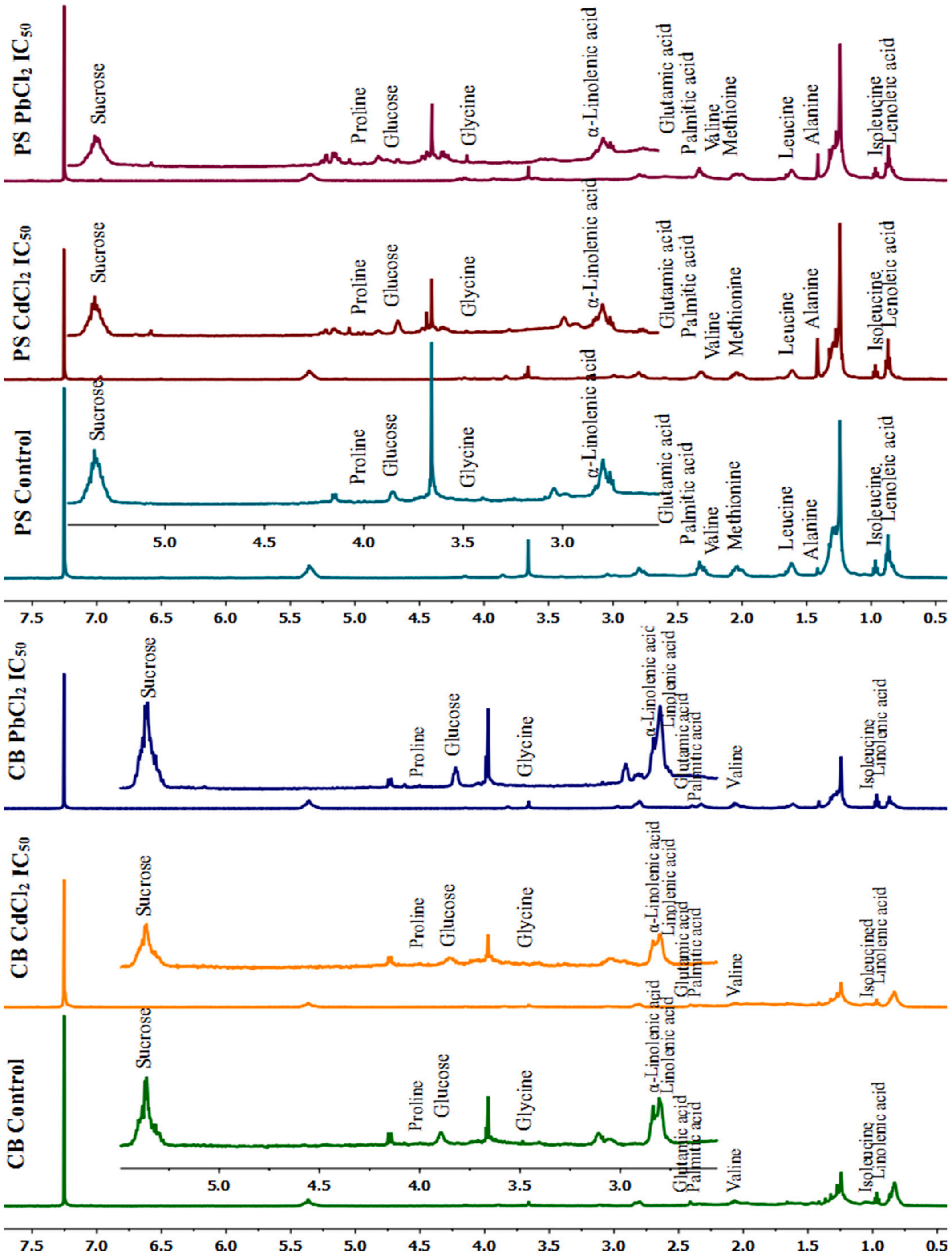

Fig. 5. ^{1}H NMR-based metabolite profiles of microalgae (CB and PS) on 14th day of cultivation.

and Pb(II) by PC1 at a variance of 71.3% and PC2 at a variance of 21%. Fig. 8 elucidates the heat map for control and metal-stressed microalgae (CB and PS) showing variations in metabolites under heavy metals stress as well as under control conditions. The stress was separated by PC1, the control microalgae extract separated from Cd(II) and Pb(II) by PC1 at a variance of 59.5% and PC2 at a variance of 23.2%.

3.7. Metabolomics analysis for Pb(II) and Cd(II) tolerance using ICP-MS

The biomass productivity (in dcw) of *C. bastropiensis* in the control, Pb(II), and Cd(II) were observed to be 38.74 ± 1.3 mg/L/d, 39.29 ± 0.3 mg/L/d, and 38.48 ± 0.5 mg/L/d, respectively. Similarly, it was observed that the biomass productivity of *P. spinulosa* in the control, Pb (II), and Cd(II) was 33.13 ± 0.6 mg/L/d, 33.22 ± 0.4 mg/L/d, and 33.56 ± 0.5 mg/L/d, respectively (Fig. 9). The results revealed that the biomass productivity of *C. bastropiensis* was higher than that of *P. spinulosa* under control and heavy metal stress conditions. Furthermore, the biomass productivity (dcw) under heavy metal stress was slightly high with insignificant differences in the stationary stages. A slight reduction in cell growth and biomass productivity was noted in microalgae (*C. bastropiensis*) under the influence of Cd(II). On the contrary, an upsurge in cell growth and biomass productivity was observed both in microalgae in the presence of Pb(II), which indicates a greater tolerance and, therefore, a greater affinity for Pb(II) than for Cd(II) in

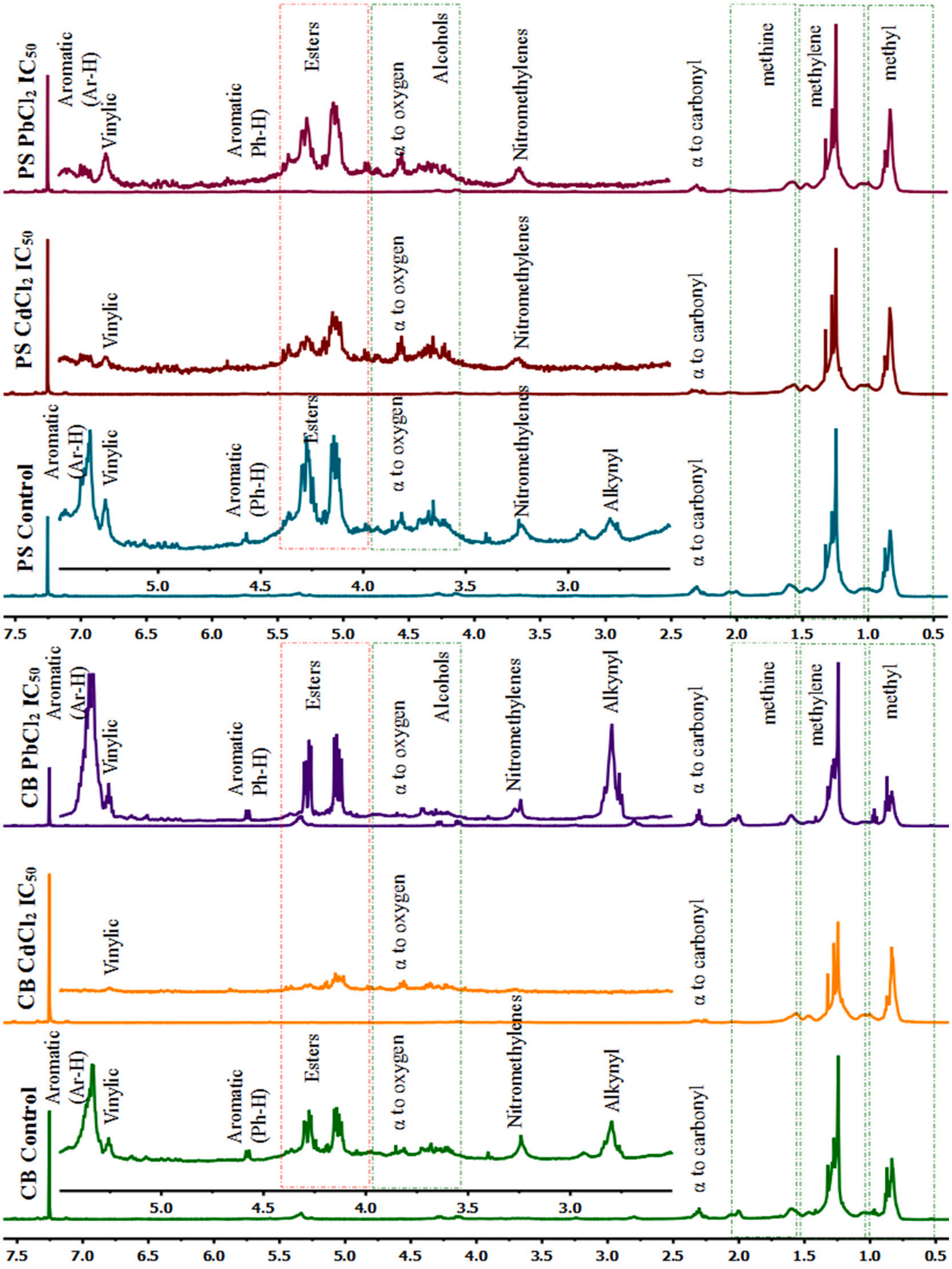

Fig. 6. ^{1}H NMR-based lipid profiles of microalgae (CB and PS) on 14th day of cultivation.

both microalgae.

The concentration of heavy metals in the biomass of *C. bastropiensis* was analyzed as 329.32 mg/L and 26.81 mg/L for Pb(II) and Cd(II), respectively. Similarly, the concentration of heavy metals in *P. spinulosa* biomass was analyzed as 406.72 mg/L and 401.6 mg/L for Pb(II) and Cd (II), respectively. The concentration of heavy metals remaining in the medium (BBM) was found to be 10.81 mg/L and 11.17 mg/L for Pb(II) and Cd(II), respectively, in *C. bastropiensis*. However, in *P. spinulosa*, it was observed that the concentration of heavy metals remaining in the medium (BBM) was 13.73 mg/L and 7.7 mg/L for Pb(II) and Cd(II), respectively. Furthermore, the bioaccumulation concentration of Pb(II) and Cd(II) was found to be 0.073 mg/Kg dcw and 0.430 mg/Kg dcw, in *C. bastropiensis*. Similarly, the bioaccumulation concentration of Pb(II) and Cd(II) was found to be 0.622 mg/Kg dcw and 0.182 mg/Kg dcw, in *P. spinulosa*. The bioaccumulation studies were also synchronized with the above-mentioned findings, where a greater accumulation of Pb(II) was recorded in the biomass obtained from both microalgae compared to Cd(II). *P. spinulosa* have shown the highest bioaccumulation of Pb(II) as to be 0.622 mg/Kg dcw. The maximum bioaccumulation of Cd(II) in *C. bastropiensis* was observed to be 0.430 mg/Kg dcw. Bioaccumulation assays suggest that there is no correlation between cellular tolerance and bioaccumulation of heavy metals by microalgae, supporting the findings of Debelius et al., (2009), where they have reported similar bioaccumulation behavior in/on cells by different microalgae irrespective

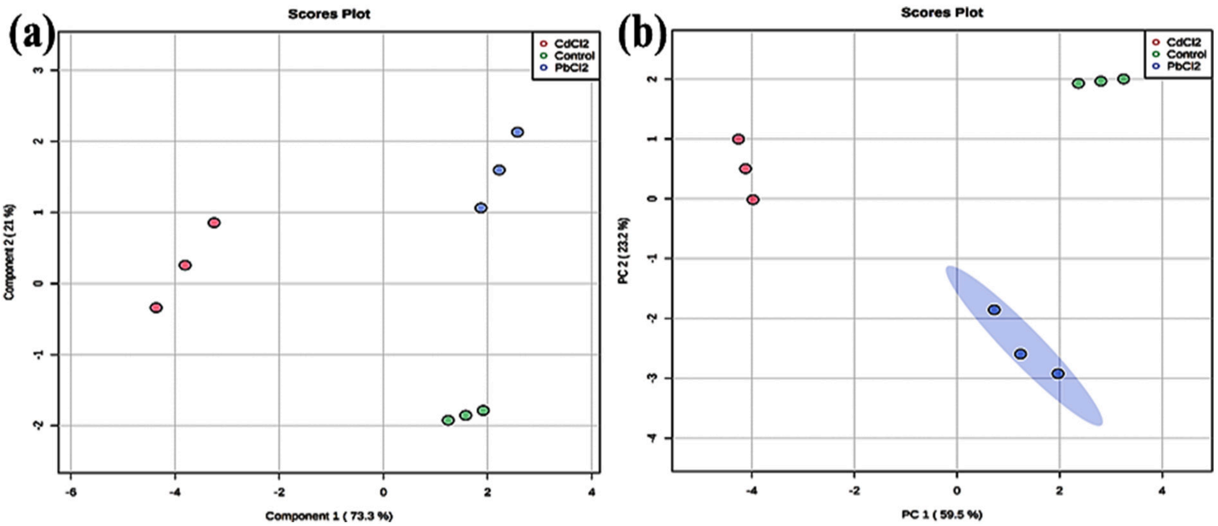

Fig. 7. Score plot for metal-stressed microalgae; (a) *C. bastropiensis* (CB) and (b) *P. spinulosa* (PS).

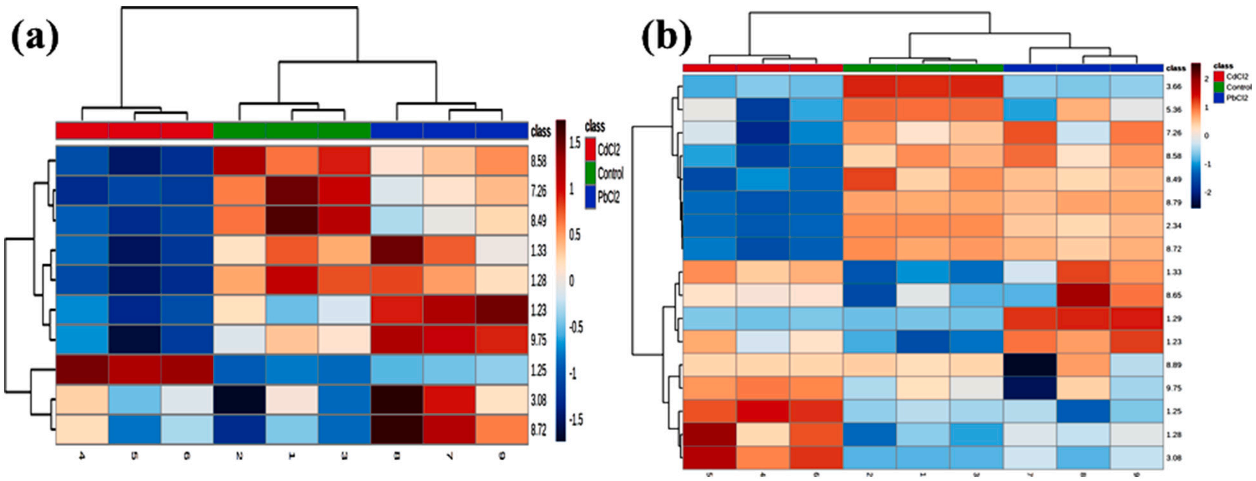

Fig. 8. Heat map for metal-stressed microalgae; (a) *C. bastropiensis* (CB) and (b) *P. spinulosa* (PS).

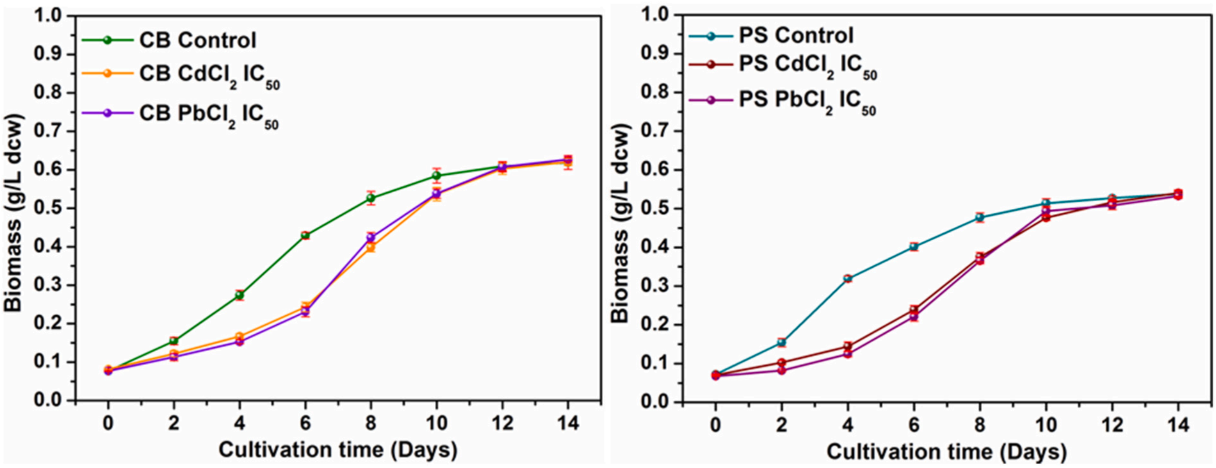

Fig. 9. Comparative biomass production (dcw) of microalgae; (a) *C. bastropiensis* (CB) and (b) *P. spinulosa* (PS).

of their heavy metal tolerance [57]. The results also support the findings of Abirhire and Kadiri, (2011), where they have reported that each microalga has different metal concentration factors for specific heavy metals [58]. The bioaccumulation of Cd(II) and Pb(II) by both species of microalgae during this study was also specific for the distinctive heavy metals.

4. Conclusions

The findings of the metabolomics and lipidomics studies during the present study suggest the following conclusions. There is no correlation between cellular tolerance and the bioaccumulation of each heavy metal by both microalgae. There are significant alterations in the biochemical composition of the microalgae metabolites under heavy metal stress; however, its levels upsurge in response to stress conditions. As a defense strategy, microalgae accumulate higher amounts of unsaturated fatty acids under the influence of heavy metals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2021.102404.

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