Journal of Applied Sciences and Nanotechnology, Vol. 3, No. 1 (2023)

Journal of Applied Sciences and Nanotechnology

Journal homepage: https://jasn.uotechnology.edu.iq/

Role of Microalgae in Environmental Biotechnology to Remove Heavy Metals

Muzhda Q. Qader*, Yahya A. Shekha

Environmental Science and Health Department, College of Science, Salahaddin University, Erbil – Iraq

Article information

Article history:

Received: July, 30, 2022

Accepted: August, 16, 2022

Available online: March, 10, 2023

Keywords:

Bioremediation,

Bioaccumulation,

Microalgae

Corresponding Author:

Muzhda Q. Qader

mzhda.qadir@su.edu.krd

Abstract

The objective of the study is to evaluate the role of microalgae in heavy metal remediation (Pb and Cd). Three microalgae species were used (Tetradesmus nygaardi, Scenedesmus quadricauda, and Coelastrella sp.) with four concentrations of both tested heavy metals (lead and cadmium) were, (5, 15, 35, and 50 ppm). Samples were analyzed every 4th day during the experimental study for 20 days. The result showed that during experimental days the lower concentration (5ppm) has the highest reduction percent for Pb and Cd by all microalgal species (Tetradesmus nygaardi, Scenedesemus quadricauda, and Coellastrella sp.). Whereas Coellastrella sp. had the highest efficiency for Pb and Cd uptake in all concentrations (5, 15, 35, and 50 ppm) for Pb removal percent were (86.8%, 86%, 82.85%, 78%) respectively, while for Cd were (84%, 80.66%, 77.14%, 76.94%) respectively. The lead had a higher reduction percent for all concentrations (5-50ppm) in comparison with cadmium by all microalgal strains during 20 days of the experiment. The removal of Cd and Pb by microalgal strains by order, Coellastrella sp. > Tetradesmus nygaardi > Scenedesemus quadricauda.

DOI: 10.53293/jasn.2022.5346.1183, Department of Applied Sciences, University of Technology

1. Introduction

Biotechnology is a methodology that makes use of living organisms to create new products, improve their quality, and reduce human suffering [1]. Heavy metals exist in form of organic compounds, oxides, hydroxides, sulfides, sulfates, phosphates, and silicates. They exist in their metallic, elemental form as well, but they are mobilized by humans through anthropogenic activities or natural phenomena action[2]. When toxic heavy metal concentrations are high, they cannot be biodegraded, and their accumulation has been linked to significant diseases and disorders [3]. The most common heavy metal contaminants include Cu, Hg, Pb, Zn, Cd, and Cr. Water contamination by heavy metal (HM) ions is one of the main environmental problems in the world today [4]. Natural geological weathering and industrialization, including the production of plastics, paint, gasoline, and fossil fuels as well as the mining, smelting, and metallurgical sectors, as well as the chemical industry, are two factors that contribute to the enrichment of HMs in aquatic environments, agrochemical, and animal feed industries [5]. Water is an essential element of life and a valuable resource for human society. A significant global issue in the twenty-first century is clean water. Thus, the distribution of freshwater is already unequal, and global climate change highlights this [6]. Clean water is water that is free of pathogens, chemicals, pollutants, and toxic substances [7]. Releasing of heavy metals into the environment has been rising steadily, due to human activity and technological development, due to their toxicity, accumulation in the food chain, and persistence in nature, pose a serious threat to the environment and public health. The main causes of the rise of metallic species released into the environment include mining operations, agricultural runoff, industrial, and domestic effluents [8]. In general, unlike many other contaminants, removing HMs from the environment is extremely difficult because they cannot be destroyed chemically or biologically and are hence indestructible. HMs released into water bodies through Waste have a permanent negative damage to the aquatic system and inhibit self-purification of the water body [9]. Microalgae have been used in a variety of environmental biotechnology applications, including bioremediation. Bioremediation is a branch of environmental biotechnology that treats pollutants through a biological process [10, 11]. Microalgae are currently being used to treat wastewater and toxicants due to their efficient photosynthetic uptake of high concentrations of minerals, inorganics, and organics [12, 13]. Algae in the natural environment have a significant effect on controlling the concentration of heavy metals in oceans and lakes [14]. As a result of tolerance mechanisms, some algae have a high potential for heavy metal accumulation, and many algae generate Metallothionein and phytochelatins that can combine with heavy metals to form complexes and transfer those complexes into vacuoles [15]. According to Bulgariu and Gavrilescu [2015], when designing bioremediation processes, one should consider, process efficiency, which depends on the microalgal characteristics, the retention process, and the process of environmental impact (pH, time, temperature, concentration of heavy metals, bio sorbent dosage, solution, and aqueous solution flow rate) [16]. Because heavy metals cannot be decomposed or destroyed, they are stable and persistent environmental contaminants. As a result, their toxicity poses significant environmental and health risks, requiring a continual search for efficient, cost-effective technology for detoxification of metal-contaminated sites, so by using microorganisms such as microalgae this can be solved. The purpose of this study is to demonstrate the role of different micro-algal species to reduce or remove toxic heavy metals (Pb and Cd) from an aqueous solution.

2. Materials and Methods

2.1. Experimental Setup

A stock solution (1000 mg/l) of Cd (II) (EMPARTA) purity (99.9%) and Pb (II) (BDH) purity (99%) ion was prepared by dissolving analytical grade substance of $PbCl_2$, and $CdCl_2$ in deionized water. This comparative study was using different metal concentrations ranging from 5, 15, 35 to 50 ppm. Added 50 ml of pure culture of *Tetradesmus nygaardi* (MZ801740), *Scenedesemus quadricauda* (MZ801741), and *Coellastrella* sp. (MZ801742) separately to the 500 ml of the BG11 broth media which contain different concentrations of each tested heavy metals [17, 18]. Daily and at the same time, samples were withdrawn from flasks, and centrifuged to separate algae. Atomic Absorption Spectrometry (AAS Perkins Elmer USA 1100D) was used to measure the concentrations of heavy metals (HMs) [19]. Data were analyzed by calculating the removal efficiency by comparing the metal concentration before and after treatment [20].

$$\text{Removal\%} = [(\text{Initial concentration} - \text{Final concentration}) / \text{Initial concentration}] \times 100 \quad (1)$$

2.2. Chlorophyll Estimation

For estimation of chlorophyll-*a* 10 ml of culture was taken from each flask of sample and centrifuged at 3000 rpm for 5 min and the supernatant was discarded, and the cell was suspended with 5 ml of diethyl ether. The absorbance value of supernatant was measured using UV-spectrometer at 660 nm and 643 nm [21].

$$\text{Chlorophyll } a = (9.92 \times A_{660}) - (0.77 \times A_{643}) \quad (2)$$

3. Results and Discussion

The mean value of heavy metals Pb, and Cd by using microalgae (*Tetradesmus nygaardi*, *Scenedesemus quadricauda*, and *Coellastrella* sp.) (Tables 1-3).

Table 1: Bioremediation of different concentrations of heavy metals (Pb and Cd).

Tetradesmus nygaardi							
Heavy metals	**Concentrations, ppm**	**1day**	**4day**	**8day**	**12day**	**16day**	**20day**
Pb	5	5	4.2	3.7	2.9	1.012	0.81
	15	15	13	9.6	5.5	4.1	2.5
	35	35	32	18.5	12.2	10.9	6.88
	50	50	34	28.4	18	15.5	11.4
Cd	5	5	4	3.16	2.02	1.27	0.91
	15	15	14	12.5	9.14	5.5	3
	35	35	28	24	17.9	14.5	8.2
	50	50	35.5	30.6	25	20.8	11.9

Table 2: Bioremediation of different concentrations of heavy metals (Pb and Cd).

Scenedesmus quadricauda							
Heavy metals	**Concentrations, ppm**	**1day**	**4day**	**8day**	**12day**	**16day**	**20day**
Pb	5	5	4	3.8	3.2	1.16	0.9
	15	15	12.8	10.5	6.8	4.18	2.87
	35	35	35	22.3	16.1	11.1	7
	50	50	35.2	29	20.1	16.8	11.62
Cd	5	5	4.69	3.85	2.1	1.35	1.05
	15	15	13.92	13.65	9.2	5.87	3.56
	35	35	26	24.6	18.11	15	8.7
	50	50	37	31.6	25.6	21.1	13.33

Table 3: Bioremediation of different concentrations of heavy metals (Pb and Cd) by using *Coelastrella* sp.

***Coelastrella* sp.**							
Heavy metals	**Concentrations, ppm**	**1day**	**4day**	**8day**	**12day**	**16day**	**20day**
Pb	5	5	4	3.8	2.7	0.82	0.66
	15	15	12.1	10.1	5.2	3.6	2.1
	35	35	27.7	21.8	10.6	10	6
	50	50	31.9	27.7	18.3	15	11
Cd	5	5	4	3	2	1.18	0.8
	15	15	13.7	12.3	9.1	5.24	2.9
	35	35	27.2	23.13	17	13.9	8
	50	50	36	30.14	23.2	20	11.53

The initial value (control) of heavy metals Pb and Cd were (5, 15, 35, and 50 ppm), then reduced by using *Tetradesmus nygaardi*, *Scenedesemus quadricauda*, and *Coellastrella* sp. during 20 days of the experiment (Figure 1). The capacity of algae to absorb metals is affected by temperature, initial metal ion concentration, pH, initial biomass concentration, and contact time. Several researchers have conducted studies to see how these variables affect the algae's capability to remove Pb and Cd from wastewater, they revealed that the ability of algae to uptake and remove heavy metals is largely determined by the initial metal concentration in the solution [22-25].

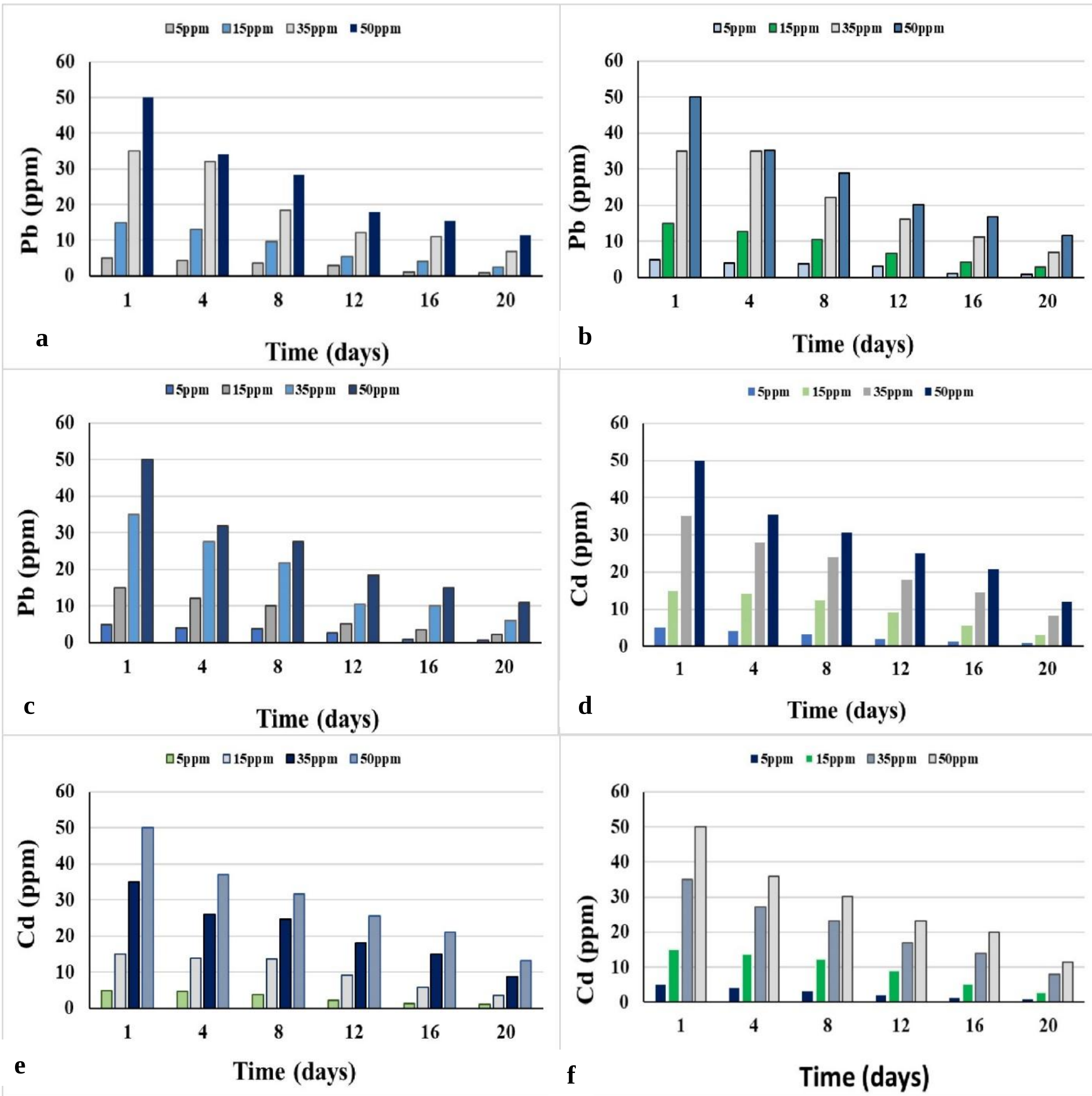

Figure 1: (a)Bioremediation of lead by *Tetradesmus nygaardi*, (b) Bioremediation of lead by *Scenedesmus quadricauda*, (c) Bioremediation of lead *Coellastrella* sp., (d) Bioremediation of cadmium by *Tetradesmus nygaardi*, (e) Bioremediation of cadmium by *Scenedesmus quadricauda*, (f) Bioremediation of Cd by *Coellastrella sp.*

The highest removal of Pb by *Tetradesmus nygaardi* was observed on day 20th of an experiment for all concentrations (5, 15, 35, and 50 ppm) were (83.8, 83.33, 80.34, and 77.2%) respectively, while percent reduction of Pb by *Scenedesemus quadricauda* were (82, 80.86, 80, 76.6%) and the highest removal of Pb by *Coellastrella* sp. were (86.8, 86, 82.85, and 78%) respectively on day 20th (Figure 2). According to the present study the lower concentration (5ppm) has the highest reduction percent of all microalgal species (*Tetradesmus nygaardi*, *Scenedesemus quadricauda*, and *Coellastrella* sp.). The result showed that *Coellastrella* sp. has a higher removal percent of Pb for all concentrations. The removal of Pb showed by order, *Coellastrella* sp. > *Tetradesmus nygaardi* > *Scenedesemus quadricauda*. Remediation percent of cadmium (Cd) by *Tetradesmus nygaardi*, for all concentrations (5, 15, 35, and 50 ppm) were (81.8, 80, 76.57, and76.2%) respectively, whereas by *Scenedesemus quadricauda* were (79, 76.26, 75.14, and 73.34%) respectively, and *Coellastrella* sp. removed (84, 80.66, 77.14, and 76.94%) of Cd (Figure 3) on day 20th of the experiment. According to the results, *Coellastrella* sp. had the highest Cd removal percent at all concentrations.

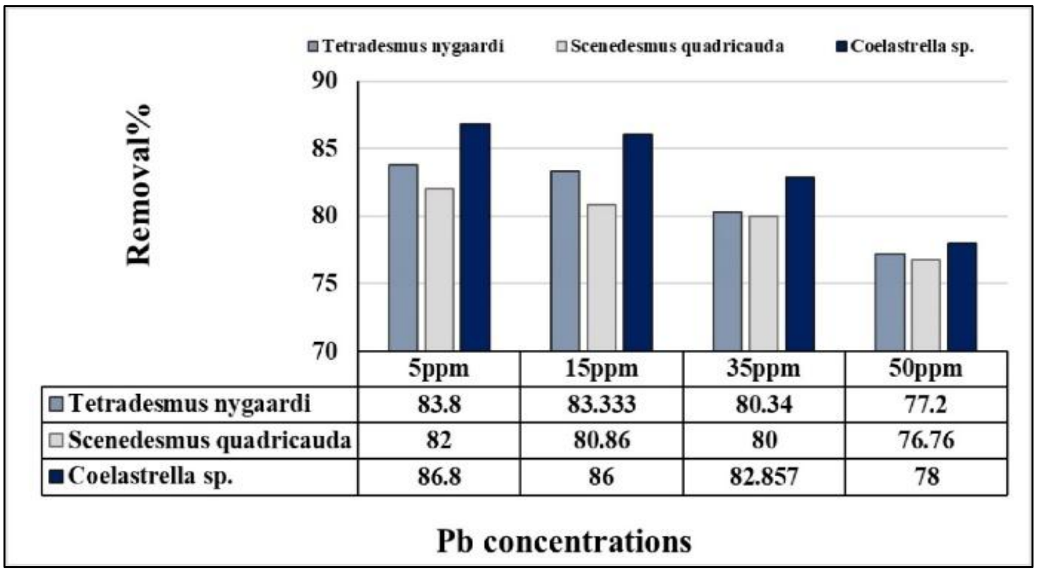

Figure 2: Removal percent of lead by all algal strains (*Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrealla* sp.).

The removal of Cd showed by order, *Coellastrella* sp. > *Tetradesmus nygaardi* > *Scenedesemus quadricauda*. The result showed that *Coellastrella* sp. was the most resistant microalga against all concentrations of Pb and Cd, while *Scenedesemus quadricauda* was the most sensitive one. According to the studies *C. vulgaris* has great efficiency in metal removal, according to the [26], removed Hg, Cd and Pb 94, 89 and 88% respectively, Cd and Ni were removed by 57.3 and 38.9% [27], Pb 99.4% [28], Pb and Cd were 94-86% [29]. The metal removal efficiency, in contrast to metal sorption, decreases as the concentration of heavy metals in the solution increases [30, 31]. During growing, algae release metabolites into the environment which decrease the concentration of toxic heavy metal ions when they are chelated [32]. The decline in algal number, lowers the ability of algae cultures to resist the presence of heavy metals, heavy metals have been reported to have inhibited *Chlorella vulgaris* growth [33]. However, different organisms have different sensitivities to the same metal and the same organisms may be more or less damaged by different metals. The uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the medium [34]. Eidizadeh *et al*. [35], in their study, compared *C. vulgaris* and *Nanocloropsis oculata* to remove lead and nickel from industrial wastewater. In the case of lead metal, C. vulgaris has a higher capacity, so the maximum removal of the lead after 5 days was 94% for *C. vulgaris* and 88.3% for *N. oculata*. However, in the case of nickel, the two microalgae function similarly, the maximum efficiency of *C. vulgaris* to remove nickel was

94.9% and in compression to *N. oculata* was 93.4 %. A present study revealed that microalga tolerated high lead and cadmium concentrations up to 50 ppm. The lead solution was less toxic than cadmium for *Tetradesmus nygaardi*, *Scenedesemus quadricauda*, and *Coellastrella* sp. growth. The lowest concentration (5ppm) of heavy metals (Pb and Cd) had the highest removal percent in comparison with other concentrations. The same pattern of variations they found that at low doses of Cd (0.05 mg/L), algal growth was observed to be slightly inhibited, however at higher concentrations (>1.0 mg/L), it was reported to be significantly inhibited [36, 37]. Based on the results, removal efficiency is significantly decreased as HM concentrations are increased [38]. Researchers detected the effects of Cd, Cu, Zn, Pb, and Fe on *Scenedesmus quadricauda*, a green alga, and also discovered that the toxicity for all of the measured parameters increased with the concentration of these metals in the growth medium [36, 39-41].

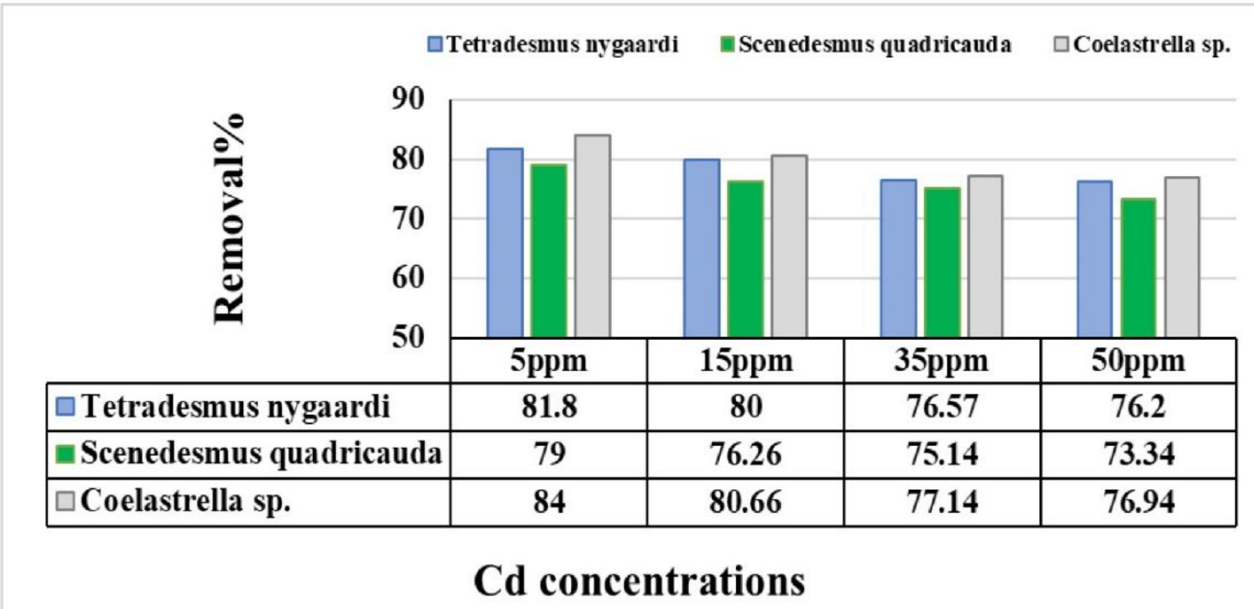

Figure 3: Removal percent of Cd by all algal strains (*Tetradesmus nygaardi, Scenedesmus quadricauda* and *Coelastrealla* sp.).

Chlorophyll-*a* concentration in all batches cultured with *Tetradesmus nygaardi*, *Scenedesemus quadricauda*, and *Coellastrella* sp. was increased during the experimental period. The initial value of chlorophyll-*a* for all concentrations of Pb and Cd was (1.749 mg/l). Then on day 16th chlorophyll-*a* reached the maximum values for all concentrations of Pb (5, 15, 35, and 50 ppm) for *Tetradesmus nygaardi* were (3.87, 4.51, 4.83, and 4.94 mg/l) respectively (Figure 4), for *Scenedesemus quadricauda* (3.85, 4.42, 4.70, and 4.88 mg/l) (Figure 4), and for *Coellastrella* sp. were (3.94,4.56, 4.9, and 4.99 mg/l) respectively (Figure 4). The maximum values of chlorophyll-*a* were recorded on day 16th for all concentrations of Cd (5, 15, 35, and 50 ppm), for *Tetradesmus nygaardi* were (3.67, 3.42, 3.32 and 3.1 mg/l) respectively (Figure 4)., at the same time *Scenedesemus quadricauda* reached (3.63,3.49, 3.13 and 3.05 mg/l) (Figure 4), and *Coellastrella* sp. (3.74, 3.48, 3.38 and 3.17 mg/l) respectively (Figure 4). The microorganism obtains energy by catalyzing energy producing chemical reactions and this energy is used in the production of new cells [42]. On day 20 of the experiment, there was a significant reduction in biomass and chlorophyll-a in all treatments, which demonstrated the algae were entering the death stage and the number of algae cells decrease and chlorophyll a content drops to (1.98, 1.94, 1.89 and 1.84 mg/l) for *Tetradesmus nygaardi*, *Scenedesemus quadricauda* (1.90, 1.88, 1.84 and 1.79 mg/l), for *Coellastrella* sp. were (2.01, 1.98, 1.87 and 1.86 mg/l) for all concentrations (5, 15, 35, and 50) respectively.

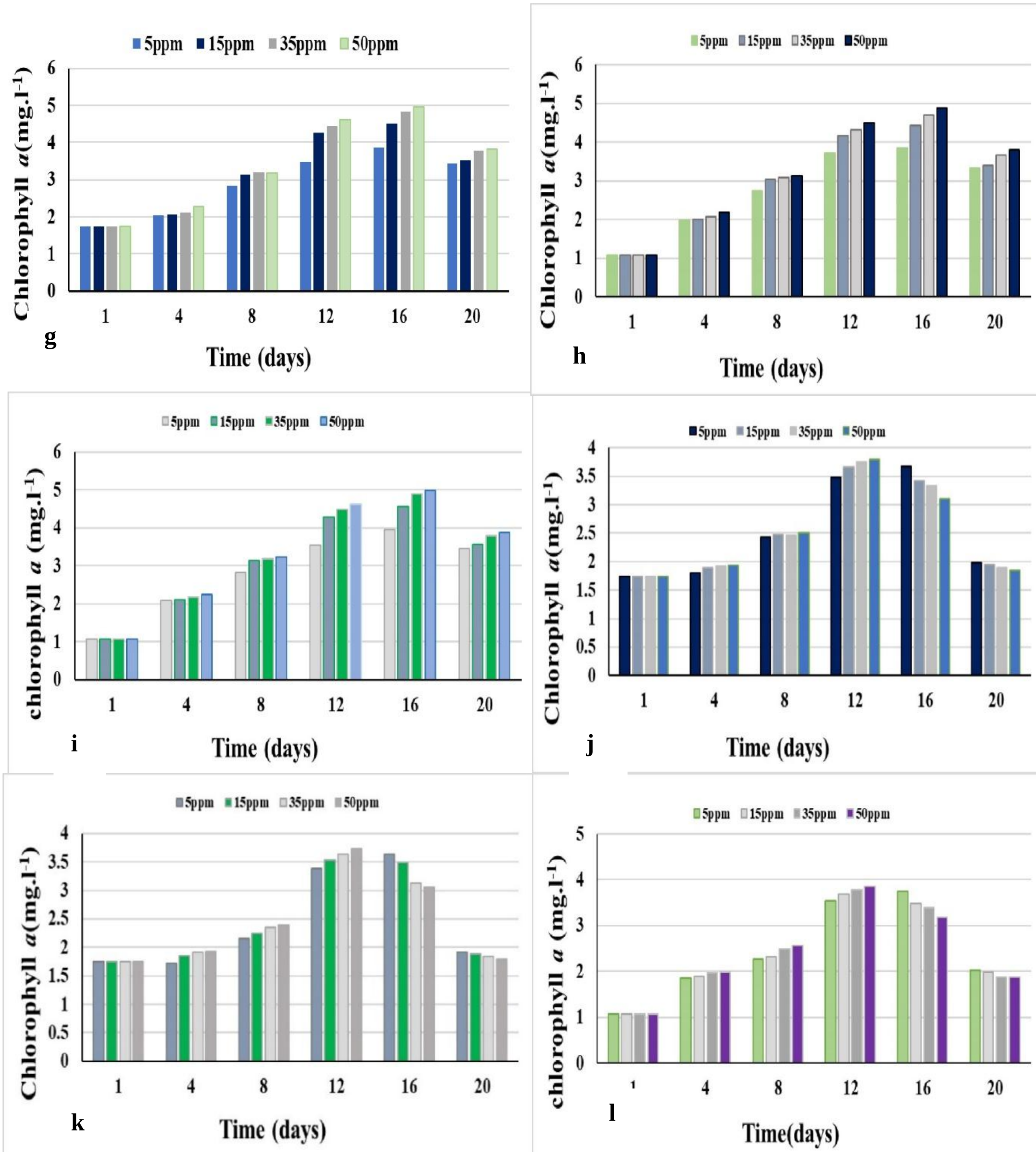

Figure 4: (g) Effects of different lead concentrations on *Tetradesmus nygaardi* chlorophyll content, (h) Effects of different lead concentrations on *Scenedesmus quadricauda.* chlorophyll content, (i) Effects of different lead concentrations on *Scenedesmus quadricauda.* chlorophyll content, (j) Effect of different cadmium concentrations on *Tetradesmus nygaardi* chlorophyll content, (k) Effect of different cadmium concentrations on *Scenedesmus quadricauda* chlorophyll content, (l) Effect of different cadmium concentrations on *Coelastrella sp.* chlorophyll content.

According to Bajguz [2011], Heavy metals affect essential cell components like photosynthetic pigments, enzymes and protein change, and fat or sugar content of algae cells. Also they have an impact on vital metabolic processes like the movement of vital cells and ions from cellular sites and the photosynthetic process [43]. After heavy metal treatments, an electron-dense layer was observed on the algal cell surfaces. This layer may be made up of bios orbed (adsorbed) metal ions that bind to various functional groups on the surfaces of the algae, which is thought to be a protective mechanism for limiting most of the toxic ions[44]. Studies noted that Cd reduced the number of photosynthetic pigments, the rate of cell growth, and cellular volume [45-48]. The researcher found that with increasing metal concentration, the percentage of insoluble and adsorbed metal ions increased [49]. Results of Wong et al.[50], in *chlorella fusca* illustrated that the accumulation of starch grains in the heavy metal treated *P. typicum* cells may function as an energy reserve for the cell after the deterioration of organelles, particularly chloroplast, pyrenoid, and mitochondria. Some studies examined the impact of Pb on the specific growth rate of *Lemna gibba*. They discovered that the specific growth rate of *L. gibba* was greatly decreased by high Pb concentrations in the media (200–500 mg/L) [34, 51-54].This may be related to Pb enhancing the activity of the enzyme peroxidase, which is responsible for breaking down indole acetic acid (IAA), the hormone that promotes growth and multiplication. Several attempts have examined the relationship between Cd and algal growth [34, 55-57].

4. Conclusions

The accumulation of heavy metals by microalgae provides an advantage for phytoremediation over other methods which are costlier and not environmentally friendly. The obtained results showed that concentrations of Pb^{2+} and Cd^{2+} (5–50 ppm) enhanced the algal growth (chlorophyll *a*) during 16^{th} day of the experiment, while on 20^{th} days the elevation of the growth inhabited by all concentrations (5-50 ppm). *Scenedesmus quadricauda* was the most sensitive alga to the two metal ions while *Coelastrella* sp. was most tolerant to high metal concentrations that had a higher removal percent. Microalgae are more efficient in Pb removal than Cd.

Acknowledgement

Special thanks to Dr. Sewgil S. Anwer from Department of Clinical biochemistry, Hawler medical University for the help and valuable scientific advices.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] B. O. Olatunji, J. M. Cisler, and D. F. Tolin, "Quality of life in the anxiety disorders: a meta-analytic review," (in eng), *Clin Psychol Rev,* vol. 27, no. 5, pp. 572-81, Jun 2007.
[2] S. Mitra *et al.*, "Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity," *Journal of King Saud University-Science,* p. 101865, 2022.
[3] C. Cassardo and J. A. A. Jones, "Managing water in a changing world," vol. 3, ed. Water: MDPI, 2011, pp. 618-628.
[4] K.-S. Hong *et al.*, "Removal of heavy metal ions by using calcium carbonate extracted from starfish treated by protease and amylase," *Anal. Sci. Technol,* vol. 2, no. 2, pp. 75-82, 2011.
[5] K. Li, J. Wang, and Y. Zhang, "Heavy metal pollution risk of cultivated land from industrial production in China: Spatial pattern and its enlightenment," *Science of The Total Environment,* vol. 828, p. 154382, 2022.
[6] X. Qu, P. J. Alvarez, and Q. Li, "Applications of nanotechnology in water and wastewater treatment," *Water research,* vol. 47, no. 12, pp. 3931-3946, 2013.
[7] Z. A. Hassan and R. T. Rasheed, "Preparation of V2O5 and SnO2 Nanoparticles and Their Application as Pollutant Removal," *Journal of Applied Sciences Nanotechnology,* vol. 1, no. 4, pp. 69-80, 2021.

[8] N. R. Bishnoi, R. Kumar, S. Kumar, and S. J. J. o. H. M. Rani, "Biosorption of Cr (III) from aqueous solution using algal biomass spirogyra spp," vol. 145, no. 1-2, pp. 142-147, 2007.
[9] M. A. Khan, R. A. K. Rao, and M. Ajmal, "Heavy metal pollution and its control through nonconventional adsorbents (1998-2007): a review," *Journal of International Environmental Application Science Journal of University of Zakho*, vol. 3, no. 2, pp. 101-141, 2008.
[10] K. S. Kumar, K. Singh, S. Kumari, P. Kushwaha, and P. S. Rao, "Phycoremediation–A Potential Approach for Heavy Metal Removal," *Marine Microbial Bioremediation*, pp. 76-121, 2021.
[11] I. Sharma, "Bioremediation techniques for polluted environment: concept, advantages, limitations, and prospects," in *Trace Metals in the Environment-New Approaches and Recent Advances*: IntechOpen, 2020.
[12] B. K. Körbahti and A. Tanyolaç, "Electrochemical treatment of simulated textile wastewater with industrial components and Levafix Blue CA reactive dye: Optimization through response surface methodology," *ournal of hazardous Materials*, vol. 151, no. 2-3, pp. 422-431, 2008.
[13] X. Zeng, M. K. Danquah, C. Zheng, R. Potumarthi, X. D. Chen, and Y. Lu, "NaCS–PDMDAAC immobilized autotrophic cultivation of Chlorella sp. for wastewater nitrogen and phosphate removal," *Chemical Engineering Journal*, vol. 187, pp. 185-192, 2012.
[14] S. Naeher, H. Suga, N. O. Ogawa, C. J. Schubert, K. Grice, and N. Ohkouchi, "Compound-specific carbon and nitrogen isotopic compositions of chlorophyll a and its derivatives reveal the eutrophication history of Lake Zurich (Switzerland)," *Chemical Geology*, vol. 443, pp. 210-219, 2016.
[15] K. B. Chekroun and M. Baghour, "The role of algae in phytoremediation of heavy metals: a review," *J Mater Environ Sci*, vol. 4, no. 6, pp. 873-880, 2013.
[16] L. Bulgariu and M. Gavrilescu, "Bioremediation of heavy metals by microalgae," in *Handbook of marine microalgae*, S. Kim, Ed., pp. 457-469, Elsevier, 2015
[17] S. Sadettin and G. Dönmez, "Simultaneous bioaccumulation of reactive dye and chromium (VI) by using thermophil Phormidium sp," *Enzyme Microbial Technology*, vol. 41, no. 1-2, pp. 175-180, 2007.
[18] S. Sadettin and G. Dönmez, "Bioaccumulation of reactive dyes by thermophilic cyanobacteria," *Process Biochemistry*, vol. 41, no. 4, pp. 836-841, 2006 DOI:10.1016/j.procbio.2005.10.031.
[19] A. A. P. H. Association, *Standard methods for the examination of water and wastewater* American Public Health Association, American Water Works Association, Water Environment Federation, 2012.
[20] S. G. K. Al Ahmed, "Dairy wastewater treatment using microalgae in Karbala city–Iraq," *Int J Env Ecol Family Urban Stud.*, vol. 22., 2014 http://www.tjprc.org/publishpapers/--1395914676-Dairy%20wastewater%20treatment%20-full.Pdf.
[21] E. W. Becker, *Microalgae: biotechnology and microbiology*. Cambridge University Press, 1994.
[22] K. Jahan, P. Mosto, C. Mattson, E. Frey, and L. Derchak, "Metal uptake by algae," *WIT Transactions on Ecology the Environment*, vol. 78, 2004.
[23] Y. Taamneh and S. Sharadqah, "The removal of heavy metals from aqueous solution using natural Jordanian zeolite," *Applied Water Science*, vol. 7, no. 4, pp. 2021-2028, 2021.
[24] K. G. Akpomie, F. A. Dawodu, and K. O. Adebowale, "Mechanism on the sorption of heavy metals from binary-solution by a low cost montmorillonite and its desorption potential," *Alexandria Engineering Journal*, vol. 54, no. 3, pp. 757-767, 2015.
[25] E. N. Mahmoud, F. Y. Fayed, K. M. Ibrahim, and S. Jaafreh, "Removal of Cadmium, Copper, and Lead From Water Using Bio-Sorbent From Treated Olive Mill Solid Residue," *Environmental Health Insights*, vol. 15, p. 11786302211053176, 2021.
[26] D. Inthorn, N. Sidtitoon, S. Silapanuntakul, and A. Incharoensakdi, "Sorption of mercury, cadmium and lead by microalgae," *Sci Asia*, vol. 28, no. 3, pp. 253-261, 2002.
[27] Z. Aksu and G. J. P. B. Dönmez, "Binary biosorption of cadmium (II) and nickel (II) onto dried Chlorella vulgaris: Co-ion effect on mono-component isotherm parameters," vol. 41, no. 4, pp. 860-868, 2006.
[28] W. L. Aung, N. N. Hlaing, and N. Aye, "Biosorption of lead (Pb 2+) by using Chlorella vulgaris," *International Journal of Chemical, Environmental amd Biological Sciences*, vol. 1, no. 2, pp. 2320-4087, 2013.
[29] L. Wang, Y. Wang, P. Chen, and R. Ruan, "Semi-continuous cultivation of Chlorella vulgaris for treating undigested and digested dairy manures," *Applied biochemistry biotechnology*, vol. 162, no. 8, pp. 2324-2332, 2010.

[30] E. M. Torres, *Microalgae sorption of ten individual heavy metals and their effects on growth and lipid accumulation*. Utah State University, 2016.
[31] F. Manzoor, A. Karbassi, and A. Golzary, "A theoretical and experimental study on removal of nickel, lead, and zinc metals from wastewater using Chlorella vulgaris microalgae," *International Journal of Environmental Engineering*, vol. 10, no. 4, pp. 350-373, 2020.
[32] A. Bajguz, "Metabolism of brassinosteroids in plants," *Plant Physiology Biochemistry,* vol. 45, no. 2, pp. 95-107, 2007.
[33] A. Bajguz, "An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in Chlorella vulgaris cultures under heavy metals stress," *Environmental Experimental Botany,* vol. 68, no. 2, pp. 175-179, 2010.
[34] R. M. Alharbi, "Toxicity and bioaccumulation of lead, cadmium and zinc in Chroococcus minutus and Chlorococcum aegyptiacum," *Int J Pharm Res Allied Sci,* vol. 6, pp. 290-300, 2017.
[35] A. Eidizadeh, M. Khajehalichalehshtari, D. Freyer, and G. Trendelenburg, "Assessment of the therapeutic potential of metallothionein-II application in focal cerebral ischemia in vitro and in vivo," *PloS one,* vol. 10, no. 12, p. e0144035, 2015.
[36] A. Fargasova, "The green alga Scenedesmus quadricauda-a subject for the study of inhibitory effects of Cd, Cu, Zn and Fe," *Section Cellular Molecular Biology,* 1999.
[37] A. Khosmanesh, F. Lawson, and I. Prince, "Cadmium uptake by unicellular green microalgae," *The Chemical Engineering Journal the Biochemical Engineering Journal*, vol. 62, no. 1, pp. 81-88, 1996.
[38] F. Manzoor, A. Karbassi, and A. Golzary, "Removal of heavy metal contaminants from wastewater by using Chlorella vulgaris beijerinck: a review," *Current Environmental Management,* vol. 6, no. 3, pp. 174-187, 2019.
[39] G. Marinova, J. Ivanova, P. Pilarski, G. Chernev, and G. Chaneva, "Effect of heavy metals on the green alga Scenedesmus incrassatulus," *Oxidation Communications,* vol. 41, no. 2, pp. 318-328, 2022.
[40] J. F. Lukavský and V. Cepák, "Toxicity of metals, Al, Cd, Co, Cr, Cu, Fe, Ni, Pb and Zn on microalgae, using microplate bioassay 1: Chlorella kessleri, Scenedesmus quadricauda, sc. subspicatus and Raphidocelis subcapitata (Selenastrum capricornutum)," *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes,* pp. 127-141, 2003.
[41] M. Mohammed and B. Markert, "Toxicity of heavy metals on Scenedesmus quadricauda (Turp.) de Brébisson in Batch Cultures (7 pp)," *Environmental Science Pollution Research,* vol. 13, no. 2, pp. 98-104, 2006.
[42] C. Goudar and P. J. I. Subramanian, "Bioremediation for hazardous waste management," vol. 16, no. 2, pp. 124-128, 1996.
[43] A. Bajguz, "Suppression of Chlorella vulgaris growth by cadmium, lead, and copper stress and its restoration by endogenous brassinolide," *Archives of environmental contamination toxicology,* vol. 60, no. 3, pp. 406-416, 2011.
[44] I. Tüzün, G. Bayramoğlu, E. Yalçın, G. Başaran, G. Celik, and M. Y. Arıca, "Equilibrium and kinetic studies on biosorption of Hg (II), Cd (II) and Pb (II) ions onto microalgae Chlamydomonas reinhardtii," *Journal of Environmental Management,* vol. 77, no. 2, pp. 85-92, 2005.
[45] I. Azizi, B. Esmaielpour, and H. Fatemi, "Effect of foliar application of selenium on morphological and physiological indices of savory (Satureja hortensis) under cadmium stress," *Food Science Nutrition,* vol. 8, no. 12, pp. 6539-6549, 2020.
[46] J. A. Pérez-Romero, S. Redondo-Gómez, and E. Mateos-Naranjo, "Growth and photosynthetic limitation analysis of the Cd-accumulator Salicornia ramosissima under excessive cadmium concentrations and optimum salinity conditions," *Plant Physiology Biochemistry,* vol. 109, pp. 103-113, 2016.
[47] F. Pietrini, M. Zacchini, V. Iori, L. Pietrosanti, D. Bianconi, and A. Massacci, "Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses," *International Journal of Phytoremediation,* vol. 12, no. 1, pp. 105-120, 2009.
[48] H. Zhao, J. Guan, Q. Liang, X. Zhang, H. Hu, and J. Zhang, "Effects of cadmium stress on growth and physiological characteristics of sassafras seedlings," *Scientific Reports,* vol. 11, no. 1, pp. 1-11, 2021.
[49] T. Volke-Sepúlveda *et al.*, "Remediación de sitios contaminados por metales provenientes de jales mineros en los distritos de El Triunfo-San Antonio y Santa Rosalía, Baja California Sur," *Centro Nacional de Investigación y Capacitación Ambiental Instituto Nacional de Ecología*, 2003.

[50] S. Wong, L. Nakamoto, and J. Wainwright, "Identification of toxic metals in affected algal cells in assays of wastewaters," *Journal of Applied phycology*, vol. 6, no. 4, pp. 405-414, 1994.
[51] M. Mkandawire, B. Taubert, and E. G. Dudel, "Capacity of Lemna gibba L.(Duckweed) for uranium and arsenic phytoremediation in mine tailing waters," *International Journal of Phytoremediation,* vol. 6, no. 4, pp. 347-362, 2004.
[52] M. Mkandawire and E. G. J. B. Dudel, Biodiversity Bioavailability, "Are Lemna spp. effective phytoremediation agents," *Dudel, E Gert %J Bioremediation, Biodiversity Bioavailability,* vol. 1, no. 1, pp. 56-71, 2007.
[53] M. Sasmaz, E. I. A. Topal, E. Obek, and A. Sasmaz, "The potential of Lemna gibba L. and Lemna minor L. to remove Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey," *Journal of environmental management*, vol. 163, pp. 246-253, 2015.
[54] N. Khellaf and M. Zerdaoui, "Growth response of the duckweed Lemna gibba L. to copper and nickel phytoaccumulation," *Ecotoxicology* vol. 19, no. 8, pp. 1363-1368, 2010.
[55] M. R. Lasheen, S. A. Shehata, and G. H. Ali, "Effect of cadmium, copper and chromium (VI) on the growth of Nile water algae," *Water, Air, Soil Pollution*, vol. 50, no. 1, pp. 19-30, 1990.
[56] G. F. Leborans and A. Novillo, "Toxicity and bioaccumulation of cadmium in Olisthodiscus luteus (Raphidophyceae)," *Water Research*, vol. 30, no. 1, pp. 57-62, 1996.
[57] A. J. S. C. Fargasova and M. Biology, "The green alga Scenedesmus quadricauda-a subject for the study of inhibitory effects of Cd, Cu, Zn and Fe," 1999.