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Role of Microalgae in Environmental Biotechnology to Remove Heavy Metals

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A B S T R A C T

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 (5-50 ppm) 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*.

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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].

$$Removal\% = [(Initial\ concentration - Final\ concentration)\ /\ Initial\ concentration] \times 100 \qquad (1)$$

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].

$$Chlorophyll\ a = (9.92 \times A660) – (0.77 \times A643) \qquad (2)$$

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) by using *Tetradesmus nygaardi*.

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) by using *Scenedesmus quadricauda*.

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 (Fig. 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].

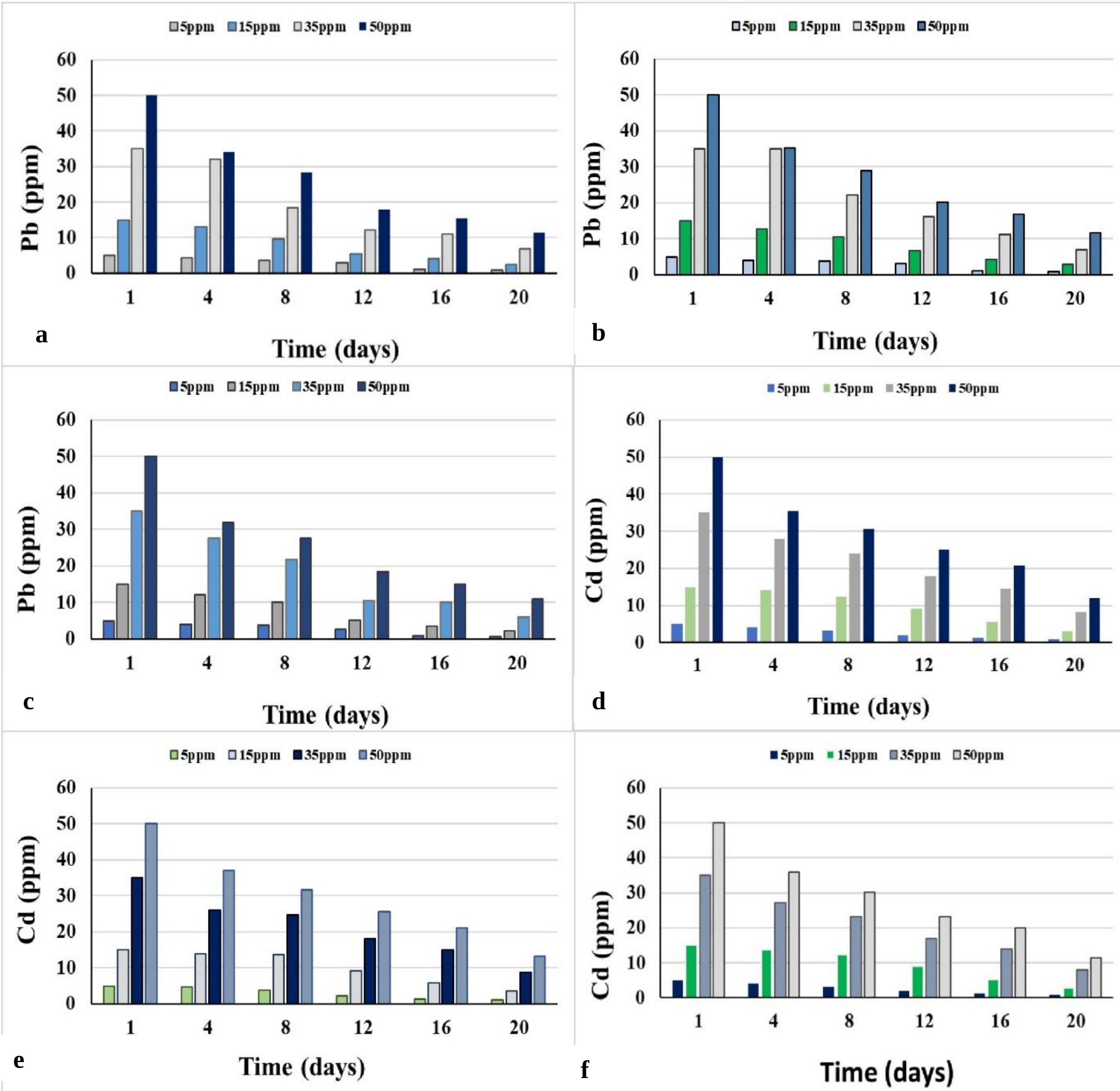

Figure 1: (a)Bioremediation of lead by Tetradesmus nygaardi, (b) Bioremediation of lead by *Scenedesmus quadricauda*, (c) Bioremediation of lead *Coellastrella sp*., (d) Bioremediation of cadmium by *Tetradesmus nygaardi*, (e) Bioremediation of cadmium by *Scenedesmus quadricauda*, (f) Bioremediation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

The highest removal of Pb by *Tetradesmus nygaardi* was observed on day 20^{th} 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 20^{th} (Fig. 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 (Fig. 3) on day 20th of the experiment. According to the results, *Coellastrella sp*. had the highest Cd removal percent at all concentrations.

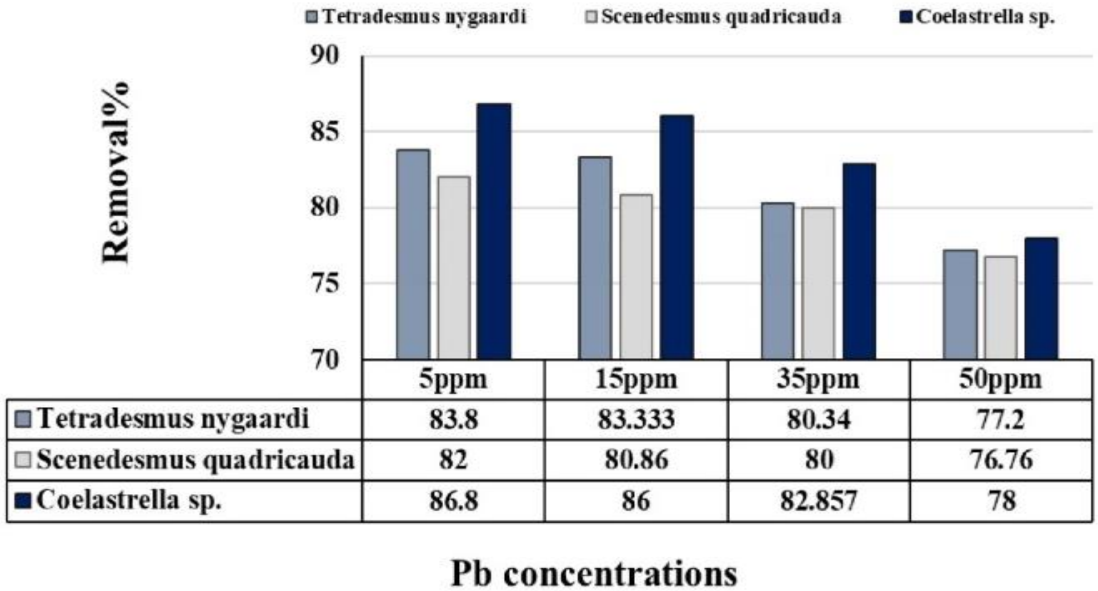

Figure 2: Removal percent of lead by all algal strains (*Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrealla sp*.).

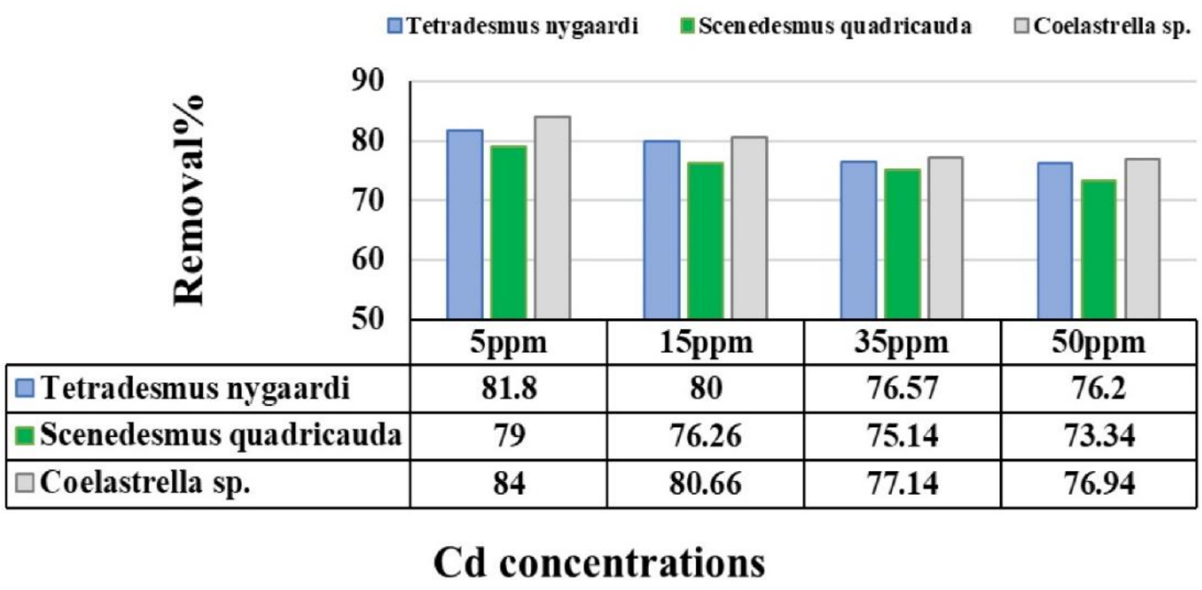

Figure 3: Removal percent of Cd by all algal strains (*Tetradesmus nygaardi*, *Scenedesmus quadricauda* and *Coelastrealla sp*.).

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].

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 (Fig. 4), for *Scenedesemus quadricauda* (3.85, 4.42, 4.70, and 4.88 mg/L) (Fig. 4), and for *Coellastrella sp*. were (3.94,4.56, 4.9, and 4.99 mg/L) respectively (Fig. 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 (Fig. 4)., at the same time *Scenedesemus quadricauda* reached (3.63,3.49, 3.13 and 3.05 mg/L) (Fig. 4), and *Coellastrella sp*. (3.74, 3.48, 3.38 and 3.17 mg/L) respectively (Fig. 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.

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].

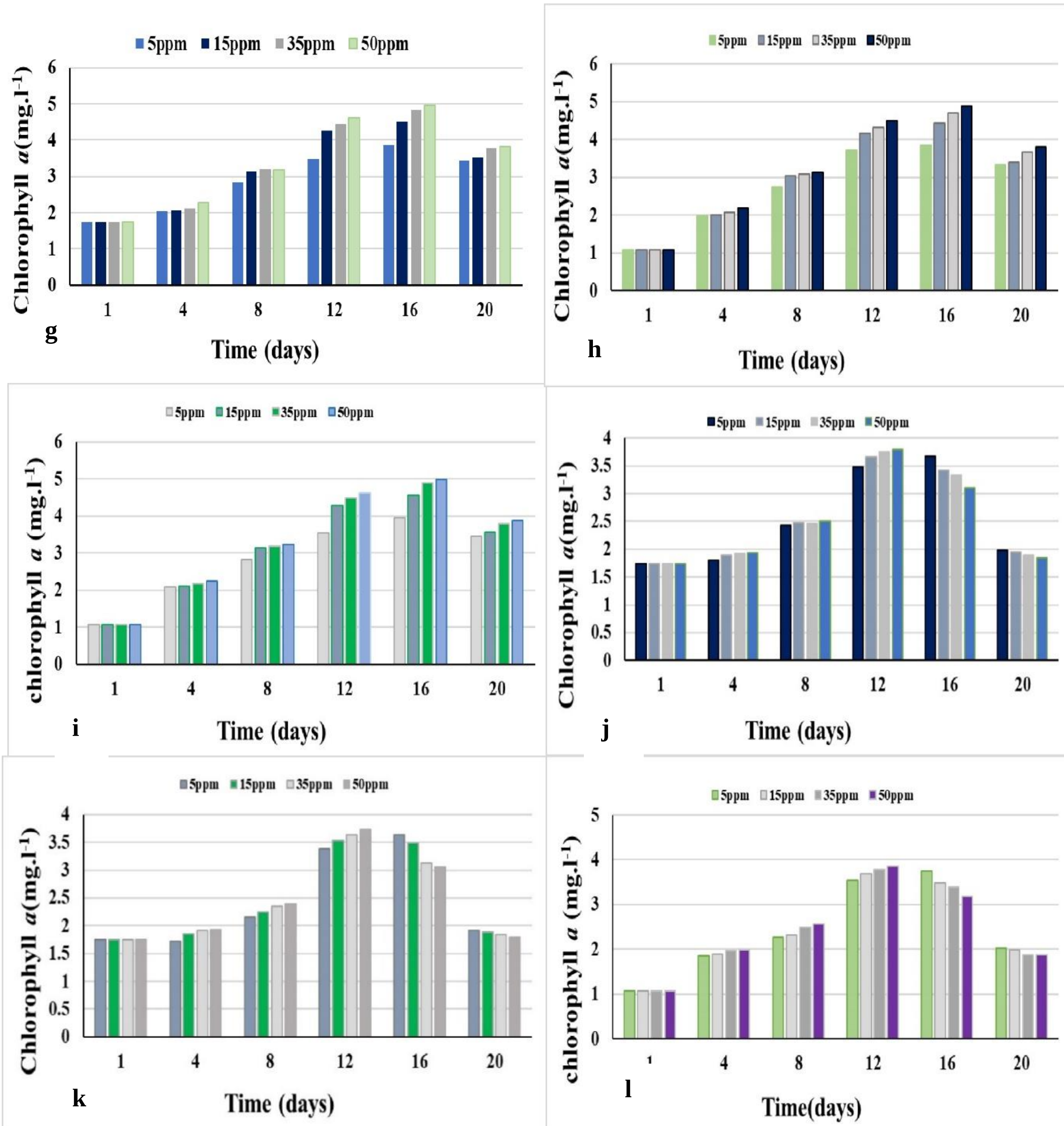

Figure 4: (g) Effects of different lead concentrations on *Tetradesmus nygaardi* chlorophyll content, (h) Effects of different lead concentrations on *Scenedesmus quadricauda*. chlorophyll content, (i) Effects of different lead concentrations on *Scenedesmus quadricauda*. chlorophyll content, (j) Effect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

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.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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