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Time-Course Bioremediation of Lead, Nickel, and Chromium from Water by the Green Alga *Chlorococcum humicola*

Ibtisam Hussein Muhammad*, Thaer Muhammad Ibrahim

Department of Biology, College of Education for Pure Science (Ibin Al-Haitham), University of Baghdad, Baghdad 10053, Iraq

Corresponding Author Email: Ibtessam.Kadhum2202@ihcoedu.uobaghdad.edu.iq

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ABSTRACT

This study investigates the capability of the green alga *Chlorococcum humicola* to remove heavy metals (Pb, Ni, and Cr) from aqueous solutions through biosorption. The experiments included a blank control (metal solution without algae) to account for abiotic metal loss. The alga, originally isolated from an Iraqi freshwater environment, was cultivated under optimized laboratory conditions at a light intensity of 3000 lux, a photoperiod of 8:16 hours dark: light, a temperature of 25 ± 2℃, and daily shaking. Each experiment was conducted using 30 mL of algal culture mixed with 100 mL of metal solution at an initial concentration of 125 ppm. Samples were collected at intervals of 1, 3, 6, 9, and 12 days. And algal growth was monitored by calculating cell density. The highest metal removal was recorded for chromium, 63.5 ppm, while the lowest removal was observed for lead, 53.6 ppm. Maximum overall removal efficiency for all metals occurred on the ninth day. Differences between days were statistically significant, while differences between metals on the same day were not. In conclusion, the green alga *C. humicola* demonstrated a significant reduction in heavy metal concentrations over the experimental period. These results indicate its potential for bioremediation of heavy metal-contaminated water, although further studies with sterile controls and extended statistical analyses are recommended to confirm its efficiency.

1. INTRODUCTION

Green microalgae are autotrophic organisms belonging to the phylum Chlorophyta, and are often found mostly in fresh and saltwater aquatic ecosystems. Characterized by their ability to photosynthesise and convert solar energy into chemical energy, they contribute significantly to the production of oxygen on Earth [1, 2]. Microalgae are essential to maintaining ecological balance, as they are the first element in the food webs of aquatic systems, as well as playing an important role in carbon fixation and reducing carbon dioxide concentrations [3]. Microalgae have recently gained a lot of attention for their production of high-value bioactive compounds, such as unsaturated fatty acids, carotenoids, and antioxidants, making them an important component of the food, Pharmaceutical, and cosmetic industries [4-6].

Microalgae have the ability to absorb and stabilise heavy metals such as Cd, Pb, Hg, Cr, and As. Due to their cell walls rich in functional groups that easily bind with metal ions, making them effective in purifying contaminated water [7]. Green microalgae play a vital role in aquatic ecosystems, both as primary producers in food webs and as agents of carbon sequestration through photosynthesis. Among these microalgae, *Chlorococcum humicola* has attracted attention due to its potential in the bioremediation of heavy metals and organic pollutants. Studies have shown that this alga can efficiently remove cadmium, nickel, and zinc from aqueous solutions [8], treat wastewater from textile mills while generating biomass [9], and degrade polycyclic aromatic hydrocarbons [10]. Its simple unicellular structure, rapid growth rate, and resilience under environmental stress make it particularly suitable for sustainable and eco-friendly wastewater treatment applications. In the current study, a metal concentration of 125 ppm was selected based on previously reported tolerance ranges for green microalgae and their relevance to typical industrial effluent levels [8]. Investigating the removal efficiency and understanding the tolerance mechanisms of *C. humicola* under these conditions provides new insights into its potential application in bioremediation and highlights the significance of this research.

Chlorococcum humicola consists of single cells or irregular clusters, spherical to oval, with smooth walls, containing polysaccharides, proteins, lipids, fatty substances, soluble yellow phenolic compounds, and volatile acids [11, 12].

Water is considered the foundation of life on planet Earth, as it makes up about 71% of its surface and is involved in all vital processes for humans and other living organisms, in addition to its importance in agricultural and industrial activities [13, 14]. Water resources are an increasing threat due to water pollution, which is the change in their physical, chemical, or biological properties due to the introduction of foreign substances, reducing their suitability for various uses

and negatively impacting aquatic ecosystems and human health [15-17].

Industrial activities are one of the main sources of heavy metal pollution in rivers, as metal industries, mining, and metal smelting, along with chemical and petrochemical industries, contribute to the release of toxic elements such as nickel, lead, and chromium into surface waters. These metals do not decompose biologically and accumulate in food chains, causing serious environmental and health impacts. Studies in Iraq and other countries have shown increased levels of these metals in river waters near industrial areas [18-20].

This study aims to evaluate the ability of the green algae *C. humicola* to remove heavy metals lead, nickel, and chromium from aqueous solutions at a concentration of 125 ppm. It also seeks to compare the differences in removal efficiency among these metals and to understand how the alga tolerates their presence. The results are expected to provide useful information about the potential use of *C. humicola* in treating industrial wastewater containing heavy metals.

2. MATERIALS AND METHODS

2.1 Cultivation and growth medium of algae

Chlorococcum humicola was grown in BG11 culture medium (Figure 1) in optimized laboratory conditions at a light intensity of 3000 lux, a photosystem of 8:16 of lighting: dark, and a temperature of 25±2℃, with daily shaking. The PH of both the culture media and stored solutions was measured daily using a Cond 3320 device to ensure stable chemical conditions. The shaking was performed manually, and the PH was regularly monitored and controlled during the experiment. Growth was monitored by calculating the number of cells and reading absorbance daily, in duplicate to ensure the accuracy [21, 22].

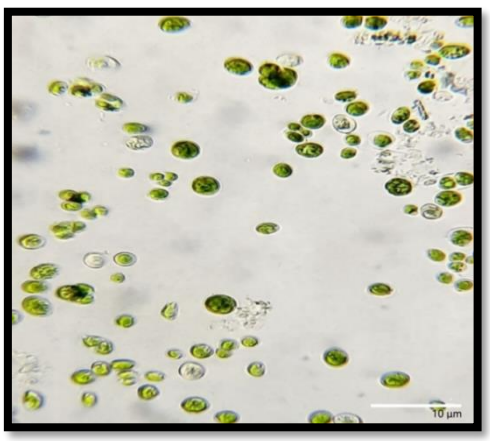


Figure 1. Characteristics of *Chlorococcum humicola* under the microscope (40x)

2.2 Preparation of solutions of lead, chromium, and nickel elements

The stock solutions for heavy elements were prepared by dissolving 1 gram each of lead acetate $Pb(CH_3COO)_2$, potassium dichromate $K_2Cr_2O_7$, and nickel sulfate $NiSO_4$ in 100 mL of distilled water separately, while continuously stirring with a glass rod for 30 minutes to obtain solutions with a concentration of 10.000 mg/L for lead, chromium, and nickel, respectively [23, 24].

2.3 Biomass measurement and adsorption capacity calculation

The initial biomass for each algal species was monitored over a 45-day period using cell density measurements to establish a baseline before the biosorption experiments. During the treatment, metal concentration and cell densities were recorded every three days. Adsorption capacity was calculated at each time point using the equation:

$$q = \frac{(C_0 - C_t) \cdot V}{m}$$

where, C_0 and C_t represent the initial and residual metal concentrations (mg/L). V is the volume of the solution (L), and m corresponds to the biomass in terms of cell density. This procedure allows the performance of each species in metal uptake to be directly related to the available biomass over time.

2.4 Experiment design for elements determination

The experiment was designed to evaluate the efficiency of *C. humicola* in removing heavy metals: lead (Pb), chromium (Cr), and nickel (Ni), each prepared at the same initial concentration. Metal stock solutions were added to achieve a final concentration of 125 ppm foreach metal in the culture flasks. Algal cultures were grown in 500 mL flasks containing 100 mL of GB-11 medium, supplemented with 10 mL of actively growing algae inoculum, giving a total working volume of 110 mL. The flasks were incubated under the same conditions described above, and the remaining metal concentrations in the medium were measured using atomic absorption spectrophotometry every three days over a 12-day period to determine the removal efficiency.

2.5 Blank control

Each heavy metal had a blank control prepared by using 100 mL of the metal solution (125 ppm) added to a sterile 500 mL Erlenmeyer flask containing no algal biomass. All blanks followed identical incubation conditions as the treatment groups (3000 lux, 8:16 light/dark photoperiod, 25 ± 2℃), which included daily manual movement. The samples for every blank were taken on days 1, 3, 6, 9, and 12. Following the collection of the samples, the samples were chemically treated with nitric acid, and they underwent analysis for metal concentration using AAS, just as was done for the flasks containing algal biomass. The blank controls confirmed that any detected changes in metal concentration were not due to environmental factors or contaminants.

2.6 Estimation of the residual concentration of heavy metals in polluted aqueous media

The concentrations of the elements under study were estimated as stated in study [25], where the method included the following:

A volume of 50 mL was taken from the samples, placed inside a clean 100 mL glass flask, and 5 mL of concentrated nitric acid was added to digest the samples. The flask was heated on a hot plate, and heating continued on the hot plate until just before drying. An additional 5 mL of concentrated nitric acid was added while continuing to heat, in order to

obtain a precipitate. The solution was left to cool, and then the volume was completed to 25 mL with distilled water. The solution was filtered using a filtration membrane (0.45 µm), making the samples ready for estimating heavy metal concentrations. Filtration through this membrane completely removed the algal cells, ensuring that only the unadsorbed dissolved metals remaining in the supernatant were determined by atomic absorption. The absorbance of these digested samples was measured using an atomic absorption device (SHEMADZU AA 7000).

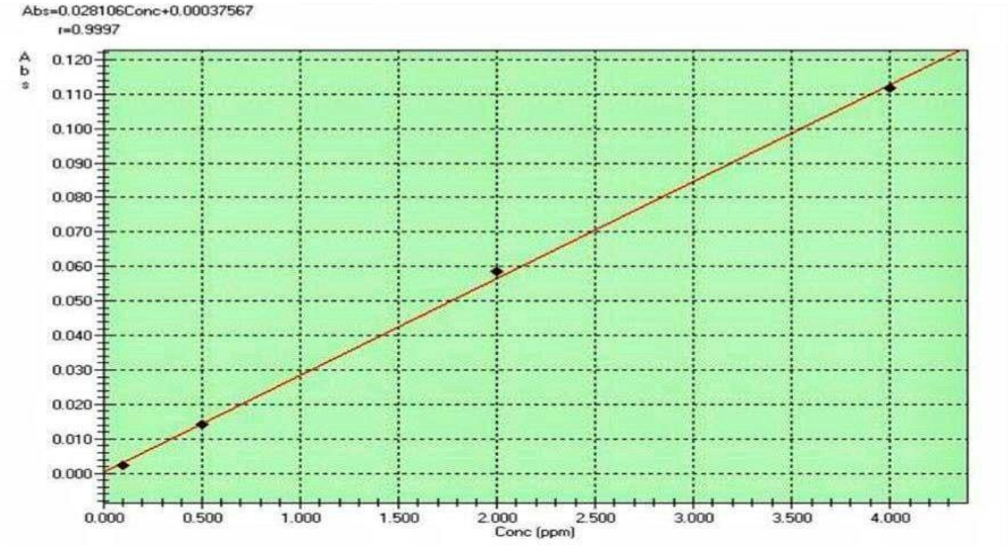


Figure 2. The standard calibration curve for lead (Pb) using Atomic Absorption Spectroscopy (AAS)

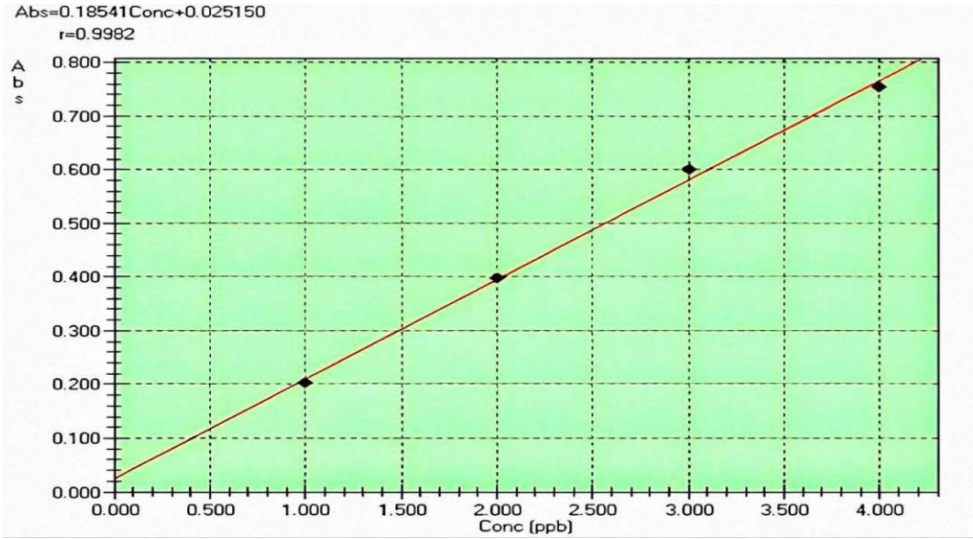


Figure 3. The standard calibration curve for nickel (Ni) using Atomic Absorption Spectroscopy (AAS)

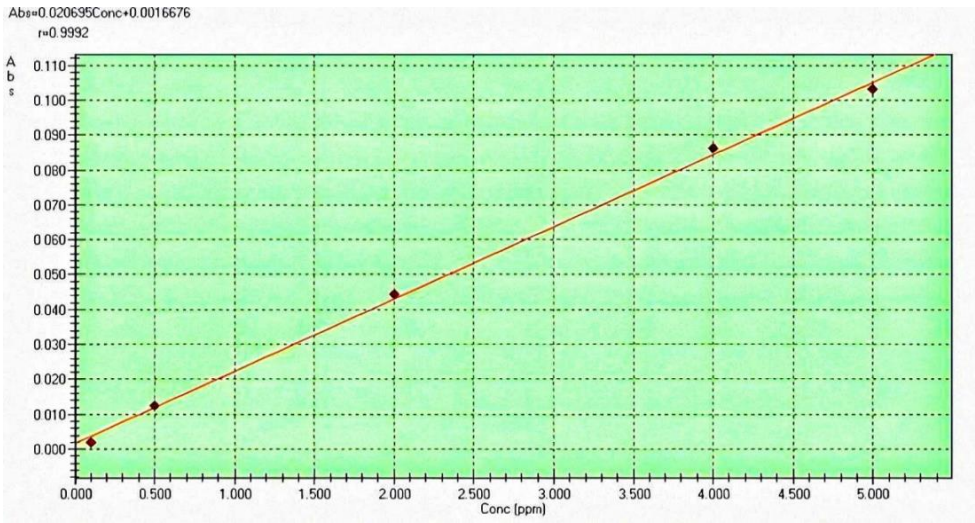


Figure 4. The standard calibration curve for chromium (Cr) using Atomic Absorption Spectroscopy (AAS)

The standard calibration curves for lead, nickel, and chromium are shown in Figures 2, 3, and 4, respectively.

2.7 Statistical analysis

The statistical analysis was conducted using GraphPad Prism by means of t-test, ANOVA, and the results were presented as mean ± standard error of the mean at a significance level of $p \leq 0.05$.

3. RESULTS

The algae removed Pb, Ni, and Cr efficiently, with the highest removal on day 9. The highest removal values reached 53.6 ppm for Pb, 56.1 ppm for Ni, and 63.5 ppm for Cr on day 9, indicating the peak biosorption efficiency of *C. humicola*. Metal concentrations continued to decrease until they reached undetectable or zero levels by day 12 (Tables 1-3). Day 12 values are reported with UDL to indicate that all residual concentrations were below the detection limit, reflecting complete metal removal.

Table 1. Amount of lead (Pb), nickel (Ni), and chromium (Cr) removed (ppm) from the aqueous medium by the green alga *Chlorococcum humicola* during the bioremediation experiment

Type of Pollutant / Days	Pb Blank	Ni Blank	Cr Blank	Pb Removed (ppm)	Ni Removed (ppm)	Cr Removed (ppm)
The first day	0.00	0.00	0.00	1	1.2	1.1
The third day	0.00	0.00	0.00	16.2	13	23.4
The sixth day	0.02	0.01	0.02	19.2	30.1	16.4
The ninth day	0.1	0.02	0.03	53.6	56.1	63.5
The twelfth day	0.2	0.03	0.04	35 (UDL)	24.6 (UDL)	20.6 (UDL)

UDL = Under Detection Limit.
The detection limit of the AAS instrument used in this study was 0.01 ppm (for Pb, Ni, and Cr).
UDL values were treated as zero for descriptive presentation but were excluded from statistical analysis to avoid artificial bias.

Blank controls were included to verify that no significant metal removal occurred in the absence of algal biomass. The residual metal concentrations in the blanks remained close to zero throughout the experiment, confirming that the observed reduction in metal levels was primarily due to the biosorption and uptake by *C. humicola* cells.

The blank control (metal solution without algae) revealed concentrations that were zero or very close to zero (0.00-0.20 ppm), which is below the detection limit for AAS. The low indications for concentration show that there has not been any significant abiotic metal loss; therefore, any metal removal that occurred in the experimental flasks can be attributed to algal biosorption and not through chemical precipitation or adsorption to the glass surfaces.

The trends observed in Figure 5 are consistent with the residual metal concentrations presented in Table 2, confirming the reliability of the graphical and tabulated data.

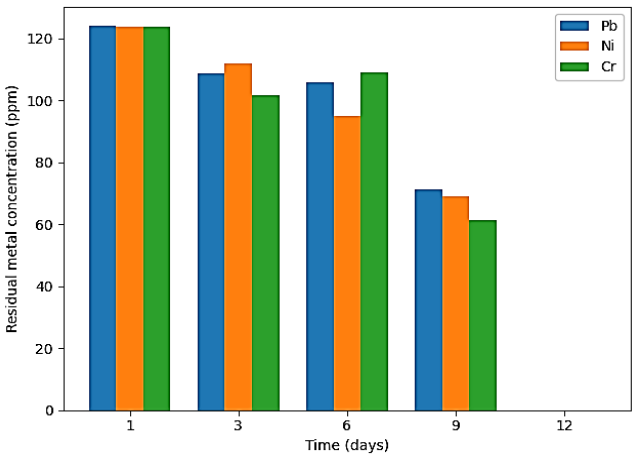


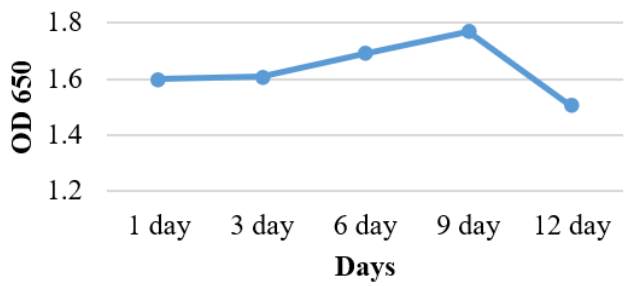
Figure 5. Residual concentrations of Pb, Ni, and Cr during the bioremediation process by *Chlorococcum humicola* at an initial concentration of 125 ppm

The removal of heavy metals by *Chlorococcum humicola* was monitored throughout the experimental period. According to Table 2, lead, nickel, and chromium concentrations decreased from 125 ppm to low residual levels by the end of the experiment. Values for day 12 correspond to (UDL), indicating complete removal of metals. Algal growth was measured by optical density (OD) using a spectrophotometer at a wavelength of 650 nm, which is suitable for detecting chlorophyll a as an indicator of algal growth. A significant reduction in heavy metal concentrations was observed on day 9 of the experiment. Lead and Chromium levels showed a marked decrease during this period, while chromium exhibited the highest removal efficiency among the studied metals. This indicates that day 9 represents the peak biosorption activity of *Chlorococcum humicola* under the experimental conditions (Figure 6).

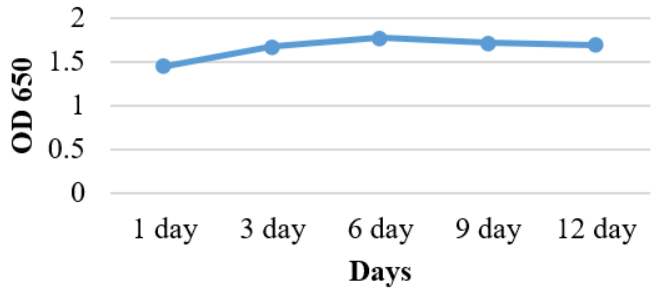
Table 2. Residual concentration of lead (Pb), nickel (Ni), and chromium (Cr) in the bioremediation experiment by the green alga *Chlorococcum humicola*

Type of Pollutant / Days	Pb Remaining (ppm)	Ni Remaining (ppm)	Cr Remaining (ppm)
The first day	124.0	123.8	123.9
The third day	108.8	112	101.6
The sixth day	105.8	94.9	109.1
The ninth day	71.4	68.9	61.5
The twelfth day	UDL	UDL	UDL

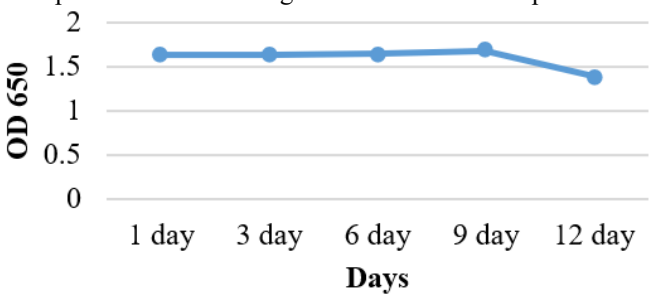
UDL = Under Detection Limit. The detection limit of the AAS instrument used in this study was 0.01 ppm.



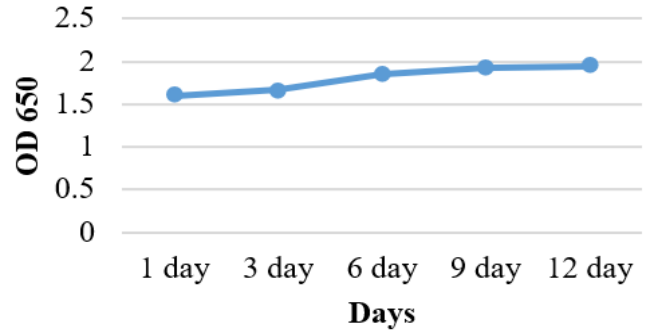
A. Growth curve of the green alga *Chlorococcum humicola* exposed to lead during the bioremediation experiment



B. Growth curve of the green alga *Chlorococcum humicola* exposed to nickel during the bioremediation experiment



C. Growth curve of the green alga *Chlorococcum humicola* exposed to chromium during the bioremediation experiment



D. Growth curve of the green alga *Chlorococcum humicola* exposed to control during the bioremediation experiment

Figure 6. Growth curves of the green alga *Chlorococcum humicola* in terms of optical density (OD) during the bioremediation experiment for (A) lead, (B) nickel, (C) chromium (Cr), and (D) control

3.1 Experimental conditions and statistical analysis

The algal growth experiments were conducted under strictly controlled conditions, with a constant temperature of 25±2°C and a photoperiod of 16 hours light/8 hours dark in a specialized incubator designed for algal cultivation Figure 7. The culture media were specifically prepared by the researcher to ensure uniformity and reproducibility. Blank controls and initial algal biomass were included to verify the effect of algae on metal removal (Figure 8).

Statistical analyses were performed using GraphPad Prism, version 2025. The reported statistical values for Pb, Ni, and Cr reflect the uniform experimental setup and the reproducible conditions under which the data were obtained. The analyses were based on representative experimental means, and all procedures were repeated under identical conditions to verify the reliability and consistency of the results. Minor variations existed in the raw data, but after averaging and rounding for presentation in Table 3, similar mean differences and P-values were observed across the three metals. Minor variations existed in the yaw data, but the reported means represent averages of repeated experiments, ensuring consistency across metals.

Table 3. Mean (±SE) concentrations of Pb, Ni, and Cr (ppm) remaining over time (125 ppm initial), based on triplicate measurements (n = 3)

Metal	Time (day)	Mean (ppm)	SE	Significance
Pb	1	124.000	2.082	a
	3	107.800	0.666	b
	6	88.600	0.321	c
	9	35.000	0.577	d
	12	0.010	0.000	e
ANOVA (Pb)		**F = 2591.563**	**P ≤ 0.0001**	
Ni	1	123.800	0.400	a
	3	110.800	0.153	b
	6	80.700	0.115	c
	9	24.600	0.058	d
	12	0.010	0.000	e
ANOVA (Ni)		**F = 72,581.053**	**P ≤ 0.0001**	
Cr	1	123.900	0.115	a
	3	100.500	0.115	b
	6	84.100	0.100	c
	9	20.600	0.173	d
	12	0.010	0.000	e
ANOVA (Cr)		**F = 211,611.594**	**P ≤ 0.0001**	

Different letters indicate significant differences among time points for each metal (Fisher's LSD, P ≤ 0.05). SE = standard error.

Figure 7. Growth of *Chlorococcum humicola* cultures inside the incubation chamber under controlled conditions

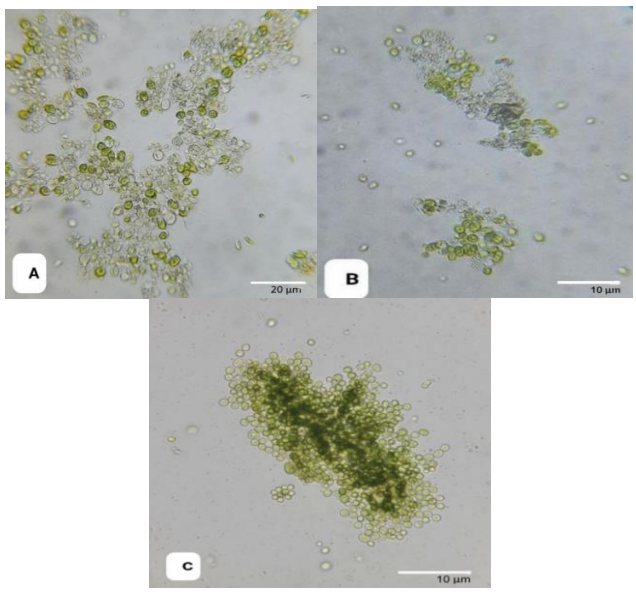


Figure 8. Alga *C.humicola* under the influence of heavy metals (A) lead, (B) nickel, (C) chromium at a concentration of 125 parts per million on the twelfth day

4. DISCUSSION

The results of the statistical analysis showed no significant differences between the studied minerals on the same day, while significant differences were recorded between the days for the same mineral at a significance level of $P \leq 0.05$. The alga *C. humicola* showed significant differences between the first day and each of the third, sixth, ninth, and twelfth days. Significant differences were also found between the third and ninth days, as well as between the sixth and ninth days, and between the ninth and twelfth days, at a significance level of $P \leq 0.05$ (Table 3).

It can be observed that the *C. humicola* algae have a low effectiveness in treating the three metals, with a slight difference in chromium removal compared to the other metals, where the removal percentages (Pb, Ni, Cr) were 16.2, 13, and 23.4 ppm, respectively, on the third day of the experiment. This decrease is attributed to the Chlorophyta demonstrating high physiological flexibility that enables them to survive and adapt under environmental stress conditions, including exposure to high concentrations of pollutants (Table 1) [7, 26].

During the early stages of exposure to pollutants, algae may show a decrease in metabolic activity or growth, which is interpreted as a necessary dormant phase for adaptation. Over time, algae activate detoxification systems, modify membrane permeability, and reorganize their metabolic pathways to cope with chemical stresses. This flexibility is a key factor in their use in bioremediation and environmental pollution monitoring (Tables 1 and 2) [26].

The experiment included a blank control, and the blank control maintained a value of zero or close to zero (in almost all cases), therefore proving that the decrease in metal concentration was primarily a result of algal activity. This was confirmed in the abstract because the blank control showed that there was no considerable abiotic loss throughout the entire experiment.

The limitation is that the initial algal biomass was not measured; therefore, metal removal could only be expressed as concentration changes (ppm) and not as adsorption capacity (mg/g biomass). This limits the ability to compare the results with other studies. This limitation restricts the ability to make quantitative comparisons with previously published work. Future studies should include precise biomass measurements (e.g., dry weight or ash-free dry weight) to allow accurate estimation of adsorption capacity and to strengthen cross-study comparability.

The high concentration of metals is considered a toxic factor that can inhibit enzyme activity and cause damage to cellular membranes, negatively affecting the absorption capacity of algae initially [27, 28]. In addition, the active binding sites on the cell wall, such as carboxyl and phosphate groups, require time to become fully active under new environmental conditions [29].

On the sixth day, a slight improvement in metal removal efficiency was observed compared to the early days; however, the extraction levels remained relatively low, indicating that the *C. humicola* algae are still at the end of the cellular adaptation phase [29]. At this stage, some functional groups on the cell surface, such as carboxyl and phosphate groups, begin to gradually release and open, enabling the cells to gradually restore their metabolic activity, but this activity is not yet at a full level. The slow metabolic response is due to the continued toxic effect of the heavy metals and the slow activation of the enzymes and transporters responsible for the uptake of these metals. Also, some binding sites may lack sufficient charge due to changes in pH value or reduced efficiency of photosynthesis. This period represents a transitional phase in which cells are biologically prepared and reconfigure their internal structure to maximize uptake efficiency in subsequent days, which is consistent with what has been suggested in previous studies [30].

On the sixth day, a clear clustering of algal cells was observed, while retaining the green color indicative of chlorophyll. This behavior indicates a collective physiological response of the cells to heavy metal stress, as their aggregation helps to reduce the area directly exposed to the metal's toxicity. The cells at this stage also showed remarkable resistance, with no visible signs of degradation or pigment loss, reflecting their continued biological activity and photosynthetic capacity (Tables 1 and 2) [31]. This is attributed to the secretion of extracellular polymeric substances (EPS) that trap metals outside the cells, as well as the role of the cell wall in the initial biosorption, followed by slower accumulation inside the cell. These findings are consistent with recent studies showing that algae can activate antioxidant enzymes and produce proteins such as phytochelatins and metallothioneins that bind to toxic metals and reduce their impact [31, 32]. When analyzing the metal removal efficiency on the sixth day, it was observed that the algae removed nickel more efficiently compared to lead, followed by chromium. This difference is attributed to the chemical properties and toxicity of each metal. Nickel is characterized by low toxicity and a small ionic size that facilitates its binding to adsorption sites, while lead shows a gradual and moderate effect on cells. Chromium, especially in its Cr^{+6} isoform, is highly toxic and rapidly affects cellular processes, reducing the ability of algae to absorb it. Algae also show a differential response depending on the type of metal [33], supported by biological mechanisms such as the secretion of EPS and the activation of internal defenses, making the absorption of nickel and lead more effective compared to chromium (Tables 1 and 2).

During the ninth day, a clear increase was observed in the algae's ability to remove heavy metals from the medium, with a removal rate of 53.6 ppm for lead, 56.1 ppm for nickel, and 63.5 ppm for chromium. The ninth day started with a significant decrease in metal concentration, which completely disappeared by the twelfth day, indicating a peak in the algae's activity.

This is partly attributed to a deficiency in essential nutrients such as nitrogen (N) and phosphorus (P), which has led to the stimulation of an environmental stress response in algal cells. When algae are exposed to nutrient deficiency, they increase the exposure or activation of functional groups (carboxyl and phosphate) on the cell walls and enhance the production of extracellular polymers (EPS) that have a high capacity to bind metal ions. Therefore, an increase in bioremediation efficiency has been observed as a defensive mechanism to obtain alternative sources for the deficient element or to eliminate toxins [34].

The mechanisms behind this efficiency include: cellular adaptation and chemical stress response, which confirm that microalgae increase the production of metal-associated compounds and interact to enhance bioavailability [35]. Additionally, the secretion of extracellular polymeric substances (EPS) increases with continuous exposure to metals, which enhances surface adsorption (biosorption) [36]. On the ninth day, it was observed that the highest removal rate was for chromium and the lowest for nickel. This shift is

attributed to the completion of environmental adaptation of the algae, which led to an expansion in its vital activity and an increase in the efficiency of heavy metal binding, especially chromium, known for its strong interaction with surface groups, after the cell stabilized physiologically. In contrast, the efficiency of nickel began to decline due to the saturation of its binding sites, or the cells transitioning to internal pathways for isolating or precipitating the element (Tables 1 and 2) [37, 38].

On the twelfth day, the removal of metals was sequentially Pb, Ni, Cr (35, 24.6, 20.6), where the algae *C. humicola* effectively removed the metals as its concentration decreased from 125 ppm to a value below the laboratory detection limit. Recent studies have shown that the use of algae in the bioremediation of water contaminated with heavy metals leads to a reduction in the concentrations of these metals to levels below the detection limit due to the large amounts of metals that algae can absorb and remove efficiently and effectively. Additionally, algae produce low waste and possess good biodegradation capabilities (Tables 1 and 2) [33].

It should be emphasized that the values presented in Tables 1 and 2 refer to the amount of metal removed rather than the residual concentrations in the medium. The decline in removal efficiency observed on the twelfth day does not indicate an increase in residual metals but reflects the onset of physiological stress and possible site saturation after prolonged exposure. Such late-stage decreases in biosorption performance have been previously reported in green microalgae under extended heavy metal stress, where loss of cellular integrity and metabolic inhibition limit further uptake [39-41]. The algal cells showed significant morphological changes under the light microscope (Figure 8), with a clear aggregation observed in cells exposed to lead, nickel, and chromium elements, indicating a potential physiological response to metal stress. This aggregation may be related to the initiation of the secretion of extracellular polysaccharides (EPS) to reduce metal absorption or to the activation of defensive mechanisms such as the production of antioxidant enzymes (like glutathione) and metal-binding proteins (metallothioneins), which are known for resisting oxidative stress and safely storing toxic metals inside the cell [38-41].

In addition, the continued presence of the green pigment within the cells indicates that the damage has not reached the point of complete breakdown of the three metals, suggesting the possibility of partial adaptation or the activation of cellular repair pathways. These responses may be delayed as a result of reaching an internal metal balance after a period of exposure, which is consistent with what recent studies have shown on this type of algae when exposed to lead, nickel, and chromium, where it was observed that the cells begin to adapt after several days of exposure, especially through the activation of cellular defense systems [38, 39, 41, 42].

Although this study did not include direct biochemical or molecular analyses to confirm the proposed mechanisms (EPS secretion, antioxidant enzymes, functional groups, metabolic pathway reorganization), these interpretations are based on previously published findings on similar algal species and are consistent with the observed removal patterns and morphological responses in the current work.

The results indicate that the alga *Chlorococcum humicola* exhibited a gradual increase in growth during the initial days of exposure to pollutants, as shown in the optical density (OD) curves. This increased growth was correlated with higher pollutant removal efficiency, as removal percentages rose alongside the algal metabolic activity. A slight decline in growth and removal efficiency was observed after the ninth day, which can be attributed to the saturation of adsorption sites on the cell surface or the physiological adaptation of the algae under metal stress. The algae demonstrated a clear ability to continue uptake even after prolonged exposure, confirming their effectiveness in the bioremediation of aquatic pollutants [8].

5. CONCLUSIONS

Chlorococcum humicola was found to be the most effective for the removal of lead, nickel, and chromium from aqueous solution at its greatest rate of removal after 9 days, and all metal concentrations were reduced to undetectable levels at the end of 12 days. The result of the alkaline solution without the algae (the blank control) provides additional evidence that the reduction in metal concentration in the algae was not due to any loss from the solution; therefore, it indicates that metals were removed from solution primarily by the process of biosorption by algae and uptake by cellular components of the algae. Methods and procedures in this study contain some limitations; however, due to these limitations, it is clear that *C.humicola* demonstrates a high potential for bioremediation of lead, nickel, and chromium. Future studies will incorporate additional information on biomass and also investigate the mechanisms that support heavy metal removal processes.

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