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Removal of Cr, Ni, and Pb from Sterilized Wastewater Using Dried *Cladophora*: Influence of pH and Contact Time

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Abstract

This study investigated the effect of pH and contact time on the removal efficiency of heavy metals, including chromium (Cr), nickel (Ni), and lead (Pb), from sterilized wastewater using dried *Cladophora* biomass. The experiments were conducted at three pH levels (5.5, 7, and 10) and different contact times (2, 6, and 24 hours), using wastewater matrices supplemented with selected heavy metals under controlled conditions. The results demonstrated that both pH and contact time significantly influenced the removal efficiency of all tested metals. Increasing the pH from acidic (5.5) to alkaline conditions (10) led to a marked improvement in removal efficiency. The highest removal rates were consistently observed at pH 10, reaching up to 97.14% for Cr, 88.92% for Ni, and 94.21% for Pb after 24 hours of contact time. Similarly, extending the contact time enhanced the removal performance at all pH levels, indicating a time-dependent biosorption process. Chromium exhibited the highest removal efficiency across all conditions, followed by lead and nickel. Overall, the findings confirm that alkaline conditions and prolonged contact time play a crucial role in optimizing heavy metal removal. The use of sterilized wastewater provides a controlled yet realistic system, demonstrating the effectiveness of dried *Cladophora* biomass for potential application in wastewater treatment.

Keywords: Heavy metals, Biosorption, *Cladophora* biomass, Wastewater, pH

Introduction

Heavy metals are considered among the most hazardous environmental pollutants present in industrial wastewater, particularly chromium (Cr), nickel (Ni), and lead (Pb), due to their toxicity, persistence, and bioaccumulative nature. These metals are non-biodegradable and tend to accumulate in living organisms, leading to severe ecological and human health risks, including neurological, renal, and carcinogenic effects [1]. Consequently, the removal of such contaminants from aqueous environments has become a major global concern. Conventional treatment methods, including chemical precipitation, ion exchange, membrane filtration, and electrochemical processes, often suffer from several limitations such as high operational cost, sludge generation, and low efficiency at low metal concentrations [2]. Therefore, alternative eco-friendly and cost-effective approaches have been increasingly explored, among which biosorption using algal biomass has emerged as a promising technique.

Algae, particularly green macroalgae such as *Cladophora* spp., have demonstrated significant potential as biosorbents due to their abundance, low cost, high surface area, and the presence of various functional groups such as hydroxyl, carboxyl, amino, and phosphate groups that facilitate metal binding [3]. These functional groups play a critical role in metal uptake through mechanisms including ion exchange, complexation, adsorption, and micro-precipitation. Several studies have reported the efficiency of dried *Cladophora* biomass in removing heavy metals from aqueous solutions under controlled laboratory conditions. For instance, Al-Homaidan *et al.* demonstrated the capability of *Cladophora* glomerata to remove Cr (VI) with considerable efficiency [4]. Similarly, Ross *et al.* highlighted that the biochemical composition of *Cladophora* significantly influences its biosorption capacity [5]. Furthermore, Rangabhashiyam and Balasubramanian reported high adsorption capacities for Cr (VI), confirming the suitability of *Cladophora* as an effective biosorbent [6].

In addition, Metwally observed that *Cladophora* glomerata could achieve up to 100% removal efficiency for lead under optimal conditions, emphasizing its applicability in wastewater treatment systems [7]. Other studies also confirmed the high removal efficiency of *Cladophora* and related freshwater algae for multiple heavy metals, including Pb, Cd, and Ni, indicating their versatility and effectiveness in environmental remediation [8]. Notably, several regional studies, including those conducted in Iraq, have demonstrated the effectiveness of dried *Cladophora* and similar algae in removing heavy metals from contaminated water. Al-Qaisi *et al.* reported removal efficiencies reaching up to 100% for lead and cadmium using dried algal biomass under controlled conditions [9]. Similarly, Hassan and Nisa highlighted the potential of macroalgae

collected from Iraqi marshlands for removing chromium and other heavy metals, confirming their suitability as low-cost biosorbents [10].

Additional studies using various algal species have also supported the efficiency of biosorption processes. Abioye *et al.* demonstrated significant removal of lead and cadmium using *Cladophora* biomass [11], while Lee and Chang confirmed that biosorption by filamentous algae follows well-established adsorption isotherms such as Langmuir [12]. Moreover, Elmaci *et al.* showed that *Cladophora* exhibits strong adsorption capacity for multiple heavy metals under different pH conditions [13]. The efficiency of biosorption processes is strongly influenced by several operational parameters, among which pH and contact time are considered the most critical. The pH of the solution affects both the speciation of metal ions and the surface charge of the biosorbent, thereby directly influencing the adsorption capacity. Generally, acidic conditions reduce biosorption efficiency due to proton competition, whereas alkaline conditions enhance metal uptake by increasing the availability of binding sites [3].

Similarly, contact time plays a crucial role in determining the kinetics of metal uptake. Most biosorption processes exhibit rapid initial adsorption followed by a slower phase until equilibrium is reached, as reported in several kinetic studies [14]. Prolonged contact time allows for greater interaction between metal ions and active sites on the biosorbent surface, resulting in improved removal efficiency. Despite the extensive research on algal biosorption, there remains a need for further investigation into the combined effects of pH and contact time on the removal efficiency of specific heavy metals using dried *Cladophora* biomass under controlled experimental conditions. Therefore, the present study aims to evaluate the removal efficiency of chromium, nickel, and lead from sterilized wastewater using dried *Cladophora* biomass, with particular emphasis on the influence of pH and contact time in optimizing the biosorption process.

Keywords: Heavy metals, Biosorption, *Cladophora* biomass, Wastewater, pH

Materials and Methods

Collect study samples

The wastewater collected from the wastewater treatment plant in Al-Rumaitha city, Al-Muthanna Governorate, Iraq. The sampling location was selected from the final sedimentation basin, where treated water is discharged into the drainage channel.

Collecting and Algae Preparation

Samples of *Cladophora* algae were collected from the local environment. The samples were thoroughly washed several times with distilled water to remove impurities and debris. The cleaned biomass was then dried in an oven at 60°C until a constant weight was achieved. After drying, the algae were ground into a fine powder to obtain a homogeneous biosorbent material.

Preparation of Heavy Metal Solutions

Stock solutions of heavy metals (Pb, Ni, and Cr) were prepared at a concentration of 1000 mg/L by dissolving accurately weighed amounts of their respective nitrate salts ($\text{Pb}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2$, and $\text{Cr}(\text{NO}_3)_3$) in distilled water. The required weights were calculated based on the molecular weights of each compound to obtain the desired metal ion concentration. The stock solutions were then diluted to obtain a final concentration of 5 ppm for each metal ion using standard dilution procedures [15].

Experimental Design

1. Wastewater samples were collected from the final sedimentation basin of the wastewater treatment plant.
2. The collected samples were sterilized using an autoclave to eliminate microbial activity.
3. Initial concentrations of heavy metals (Pb, Cr, Ni) were measured and found to be low; therefore, the samples were spiked with 5 ppm of each metal.
4. Initial metal concentrations were recorded before treatment.
5. The pH of the samples was adjusted to 5.5, 7, and 10.

Treatments

For each treatment, 1 g of dried *Cladophora* biomass was added to 250 mL of sterilized wastewater under the following conditions:

- Temperature: 25°C
- pH levels: 5.5, 7, and 10
- Contact time: 2, 6, and 24 hours
- Continuous shaking was carried out using an incubator shaker at 150 rpm

After treatment, samples were analyzed to determine heavy metal concentrations. All experiments were conducted in triplicate.

The removal percentage (R.P) was calculated using the following equation:

$$R.P = (A - B) / A \times 100$$

where A is the initial concentration and B is the final concentration after treatment.

Statistical Analysis

The obtained data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS). The Least Significant Difference (LSD) test was used to determine significant differences between treatments.

Results

Effect of pH on Heavy Metal Removal Efficiency

After 2 Hours of Contact Time

As shown in table (1) and Figure (1), increasing the contact time to 2 hours led to a noticeable improvement in removal efficiency at all pH levels. At pH 5.5, removal efficiencies increased to 68.40% for Cr, 38.15% for Ni, and 55.03% for Pb. At pH 7, intermediate values were recorded (82.14 %, 48.50 %, and 66.08 %, respectively). The highest efficiencies were observed at pH 10, reaching 90.15 % for Cr, 72.80 % for Ni, and 85.46 % for Pb. The differences among treatments remained statistically significant ($p \leq 0.05$).

Table (1): Effect of pH on the removal efficiency of heavy metals (Cr, Ni, Pb) after 2 hours of contact time by *Cladophora spp.*

pH	Cr (%)	Ni (%)	Pb (%)
5.5	68.40 ± 1.65 ^a	38.15 ± 1.20 ^a	55.03 ± 1.44 ^a
7	82.14 ± 1.35 ^b	48.50 ± 1.40 ^b	66.08 ± 1.15 ^b
10	90.15 ± 0.95 ^c	72.80 ± 1.85 ^c	85.46 ± 2.30 ^c
LSD	2.45	2.88	3.24

Note: Values are expressed as mean ± standard deviation. Different superscript letters (a, b, c) within the same column indicate significant differences at ($p \leq 0.05$).

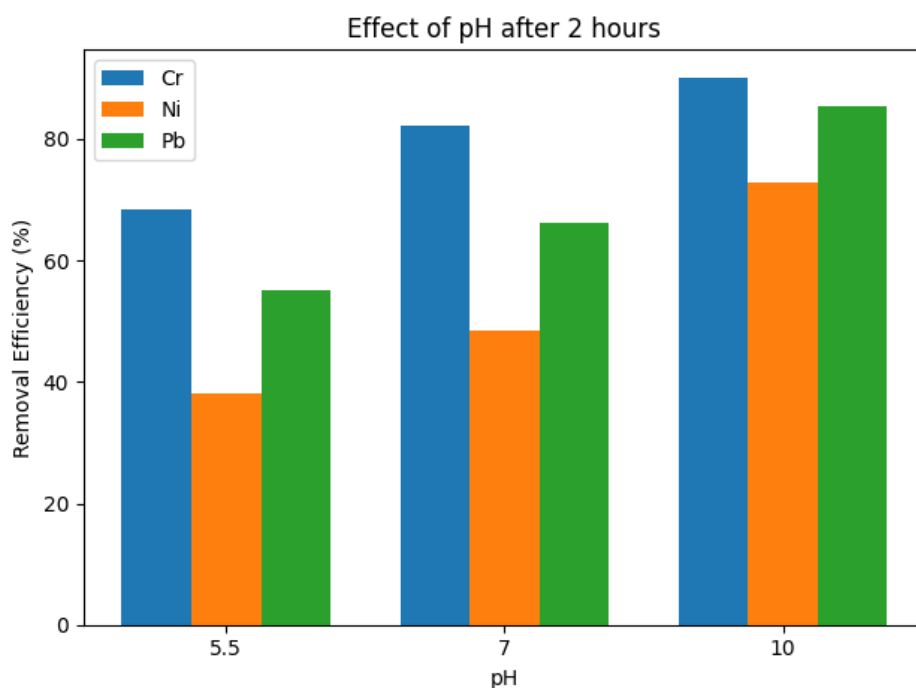


Figure (1): Effect of pH on the removal efficiency of heavy metals after 2 hours by *Cladophora spp.*

After 6 Hours of Contact Time

As shown in Table (2) and Figure (2), increasing the contact time to 6 hours led to a noticeable improvement in removal efficiency at all pH levels. At pH 5.5, removal efficiencies increased to 75.12% for Cr, 44.20% for Ni, and 58.31% for Pb. At pH 7, intermediate values were recorded (88.60%, 55.40%, and 71.97%, respectively). The highest efficiencies were observed at pH 10, reaching 94.30% for Cr, 82.15% for Ni, and 92.81% for Pb. The differences among treatments remained statistically significant ($p \leq 0.05$).

Table (2): Effect of pH on the removal efficiency of heavy metals (Cr, Ni, Pb) after 6 hours of contact time by *Cladophora spp.*

pH	Cr (%)	Ni (%)	Pb (%)
5.5	75.12 $\pm$ 1.45 ^a	44.20 $\pm$ 1.10 ^a	58.31 $\pm$ 1.65 ^a
7	88.60 $\pm$ 1.10 ^b	55.40 $\pm$ 1.60 ^b	71.97 $\pm$ 1.30 ^b
10	94.30 $\pm$ 0.85 ^c	82.15 $\pm$ 1.45 ^c	92.81 $\pm$ 1.10 ^c
LSD	2.10	2.65	2.98

Note: Values are expressed as mean $\pm$ standard deviation. Different superscript letters (a, b, c) within the same column indicate significant differences at ($p \leq 0.05$).

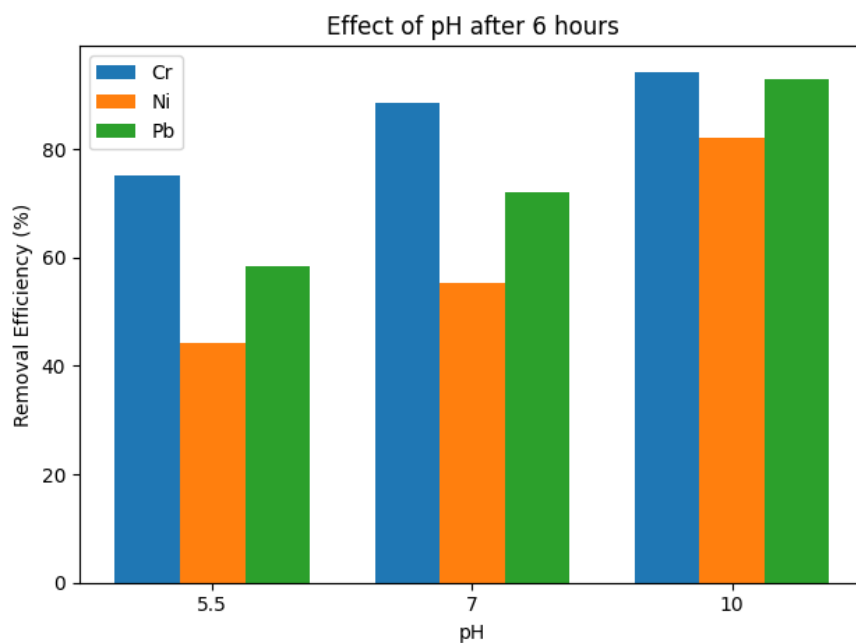


Figure (2): Effect of pH on the removal efficiency of heavy metals after 6 hours by *Cladophora spp.*

After 24 Hours of Contact Time

The results in Table (3) and Figure (3) indicate that extending the contact time to 24 hours significantly enhanced the removal efficiency of all metals. At pH 5.5, removal efficiencies reached 83.88% for Cr, 50.25% for Ni, and 75.45% for Pb. At pH 7, higher values were recorded (96.43% for Cr, 62.74% for Ni, and 85.05% for Pb). The maximum removal efficiencies were observed at pH 10, reaching 97.14% for Cr, 88.92% for Ni, and 94.21% for Pb. Statistical analysis confirmed significant differences among treatments ($p \leq 0.05$).

Table (3): Effect of pH on the removal efficiency of heavy metals (Cr, Ni, Pb) after 24 hours of contact time by *Cladophora spp.*

pH	Pb (%)	Ni (%)	Cr (%)
5.5	75.45 ± 14.2 ^a	50.25 ± 3.54 ^a	83.88 ± 4.09 ^a
7	85.05 ± 6.36 ^b	62.74 ± 14.6 ^b	96.43 ± 3.32 ^b
10	94.21 ± 1.46 ^c	88.92 ± 3.68 ^c	97.14 ± 2.19 ^b
LSD	5.55	1.11	3.60

Note: Values are expressed as mean ± standard deviation. Different superscript letters (a, b, c) within the same column indicate significant differences at ($p \leq 0.05$).

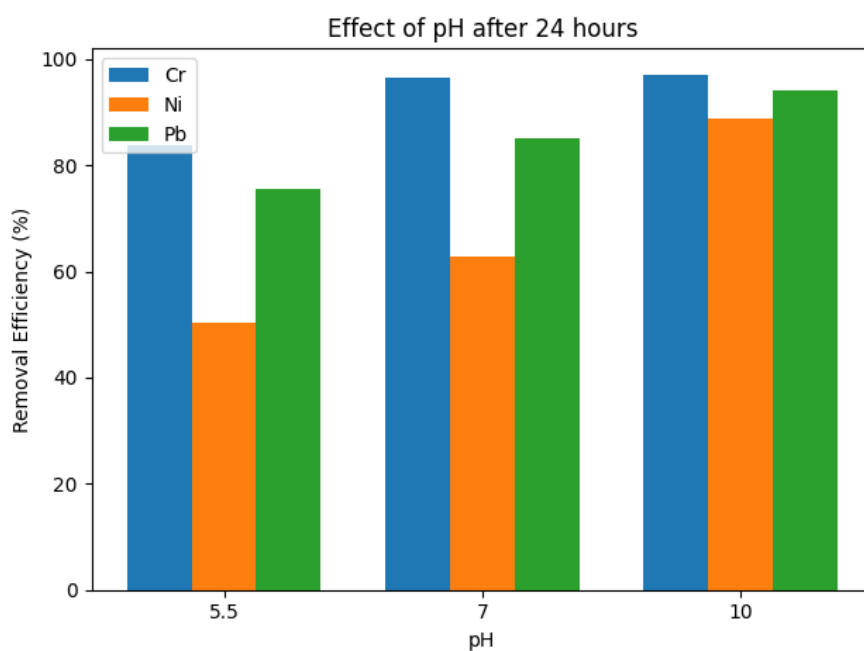


Figure (3): Effect of pH on the removal efficiency of heavy metals after 24 hours by *Cladophora spp.*

Effect of Contact Time on Heavy Metal Removal Efficiency at pH 10

Figure (4) illustrates the effect of contact time on the removal efficiency of heavy metals under optimal conditions (pH 10). The results show a continuous increase in removal efficiency with increasing contact

time from 2 to 24 hours. Chromium removal increased from 90.15% at 2 hours to 97.14% at 24 hours. Similarly, lead removal improved from 85.46% to 94.21%, while nickel increased from 72.80% to 88.92%. These findings indicate that prolonged contact time enhances the biosorption process, resulting in higher removal efficiency.

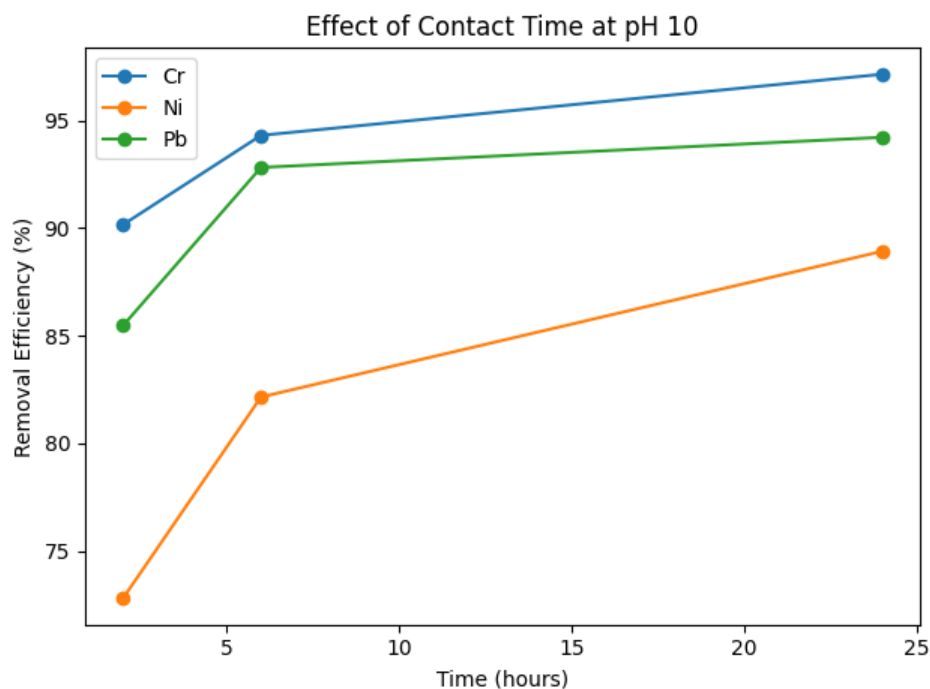


Figure (4): Effect of contact time on the removal efficiency of heavy metals at pH 10 by *Cladophora spp.*

Discussion

Effect of pH on Heavy Metal Removal Efficiency by *Cladophora spp.*

The obtained results indicate that pH is a key factor influencing the removal efficiency of chromium (Cr), nickel (Ni), and lead (Pb) in sterilized wastewater by *Cladophora spp.*, as shown in Tables (1–3) and Figures (1–3). The increase in removal efficiency with increasing pH can be attributed to the reduction in competition

between hydrogen ions and metal ions for active binding sites on the biosorbent surface [13]. At low pH levels, the high concentration of H^+ ions lead to protonation of functional groups, thereby reducing their ability to bind metal ions. As the pH increases, deprotonation of functional groups such as carboxyl and hydroxyl groups enhance the availability of negatively charged sites, which promotes electrostatic attraction with positively charged metal ions [16]. Similar findings have been reported in studies using *Cladophora glomerata*, where adsorption efficiency improved significantly under alkaline conditions due to increased surface reactivity [17].

In addition, pH influences the speciation of metal ions in solution, which can affect their interaction with the biosorbent. Under alkaline conditions, the formation of metal hydroxide complexes may contribute to enhanced removal efficiency through combined adsorption and precipitation mechanisms [14]. Therefore, the high efficiency observed at pH 10 in the present study confirms that alkaline conditions are optimal for heavy metal removal using algal biomass in sterilized wastewater systems.

Effect of Contact Time on Heavy Metal Removal Efficiency by *Cladophora spp*

The results also demonstrated that increasing contact time from 2 to 24 hours significantly improved the removal efficiency of heavy metals in sterilized wastewater by *Cladophora spp* (Figure 4), indicating that biosorption is a time-dependent process. The initial rapid adsorption observed at shorter contact times is attributed to the availability of abundant active sites on the biosorbent surface [18]. As contact time increases, the adsorption rate decreases due to saturation of available binding sites and increased resistance to mass transfer [19]. This two-stage kinetic behavior is characteristic of biosorption processes and has been widely reported in studies involving algal biomass [20]. Furthermore, extended contact time allows for diffusion of metal ions into internal pores of the biomass, resulting in enhanced adsorption capacity [21]. The gradual increase in removal efficiency up to 24 hours suggests that equilibrium conditions were not fully achieved at earlier time points, emphasizing the importance of sufficient contact time for maximum removal efficiency in complex wastewater matrices.

Comparison Among Metals (Cr, Pb, Ni) Removed by *Cladophora spp*

The results showed that chromium exhibited the highest removal percentage, followed by lead and then nickel under all experimental conditions. This variation can be explained by differences in physicochemical properties of the metal ions, such as ionic radius, hydration energy, and affinity toward functional groups on the biosorbent surface [22]. Chromium demonstrated higher removal percentage due to its stronger interaction with functional groups such as hydroxyl and carboxyl groups, which enhances its adsorption

capacity [23]. Lead also showed high removal percentage, which can be attributed to its lower hydration energy and higher tendency to form stable complexes with binding sites on the biomass surface [24]. In contrast, nickel exhibited lower removal percentage, likely due to its higher hydration energy and weaker interaction with functional groups, which limits its adsorption potential [25]. Similar trends have been reported in previous studies where nickel showed lower affinity toward algal biosorbents compared to other heavy metals [26]. Additionally, competitive adsorption among multiple metal ions may influence removal percentage, as metals with higher affinity occupy available binding sites preferentially. This effect may be further influenced by the presence of other dissolved constituents in sterilized wastewater, which can compete with target metal ions for available binding sites [27].

Combined Effect of pH and Contact Time

The combined effect of pH and contact time highlights the importance of optimizing these parameters to achieve maximum biosorption efficiency. The highest removal efficiency observed at pH 10 and 24 hours indicates that both alkaline conditions and prolonged contact time are essential for effective heavy metal removal in sterilized wastewater systems [1]. Under these conditions, increased availability of active binding sites and sufficient interaction time enhance the adsorption process, resulting in higher removal efficiency [2]. This synergistic effect has been reported in several studies, confirming that optimization of operational parameters significantly improves biosorption performance.

Environmental Implications

The results of the present study demonstrate the potential of dried *Cladophora* biomass as an efficient and eco-friendly biosorbent for heavy metal removal from sterilized wastewater. The high removal efficiency observed for multiple metals indicates its suitability for treating wastewater containing mixed contaminants under controlled conditions [21]. Moreover, the low cost, availability, and environmental compatibility of algal biomass make it a promising alternative to conventional treatment methods. Similar applications have been reported in studies focusing on phycoremediation using algal biomass, where significant improvements in water quality were achieved even in complex wastewater systems [22].

Conclusion

The present study demonstrated that pH and contact time significantly influence the removal efficiency of heavy metals from sterilized wastewater. Increasing pH enhanced the removal process, with the highest

efficiencies recorded under alkaline conditions (pH 10), while longer contact time (24 hours) further improved performance. Among the tested metals, chromium showed the highest removal efficiency, followed by lead and nickel. These findings indicate that optimizing pH and contact time plays a crucial role in improving biosorption efficiency. Overall, dried *Cladophora* biomass proved to be an effective and promising biosorbent for heavy metal removal under controlled wastewater conditions.

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