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Evaluation of the Heavy–Metal Bioremediation and Removal Efficiency of Premna tomentosa Leaf–Derived Metabolites Against Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} Under Controlled Laboratory Conditions

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ABSTRACT

The present study evaluated the heavy-metal bioremediation/removal potential of methanolic leaf-derived metabolites of Premna tomentosa against Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) under controlled laboratory conditions. A metabolite-rich methanolic extract was applied at a fixed dose of 100 mg per 100 mL of metal solution, and removal efficiency was assessed at initial concentrations of 10, 25, 50, and 100 mg L^{-1}. The influence of contact time was examined over 0–72 h, and residual metal concentrations were quantified using Atomic Absorption Spectrophotometry. The extract showed clear concentration- and time-dependent removal of all four contaminants. At 10 mg L^{-1}, the highest removal was recorded for Pb^{2+} at 82.40 ± 1.24%, followed by Cd^{2+} at 76.80 ± 1.18%, Cr(VI) at 69.50 ± 1.31%, and As(III) at 61.20 ± 1.26%. At 100 mg L^{-1}, removal efficiencies decreased to 66.70 ± 1.46%, 60.90 ± 1.39%, 52.60 ± 1.51%, and 44.30 ± 1.42%, respectively. In contrast, maximum uptake capacities increased to 66.70 ± 1.46 mg g^{-1} for Pb^{2+}, 60.90 ± 1.39 mg g^{-1} for Cd^{2+}, 52.60 ± 1.51 mg g^{-1} for Cr(VI), and 44.30 ± 1.42 mg g^{-1} for As(III). Contact-time analysis at 50 mg L^{-1} showed progressive removal up to 72 h, reaching 73.20 ± 1.22% for Pb^{2+}, 67.40 ± 1.34% for Cd^{2+}, 59.10 ± 1.48% for Cr (VI), and 50.80 ± 1.42% for As(III). Two-way ANOVA confirmed highly significant effects of metal type, initial concentration, and contact time ($p < 0.001$). Overall, the removal pattern followed $Pb^{2+} > Cd^{2+} > Cr(VI) > As (III)$, demonstrating the potential of P. tomentosa leaf metabolites as a plant-derived system for heavy-metal remediation.

Keywords: *Premna tomentosa; heavy-metal removal; bioremediation; Pb^{2+}; Cd^{2+}; Cr(VI); As(III); methanolic extract; biosorption; plant metabolites.*

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Introduction

Heavy-metal contamination of aquatic and terrestrial environments is a major environmental concern because metallic and metalloid contaminants persist for long periods and may enter biological food chains. Industrialization, mining, electroplating, agriculture, and untreated effluent discharge are important sources of potentially toxic metals. Among them, lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As) are particularly important because of their persistence, toxicity, bioaccumulation potential, and adverse ecological and human-health effects [11,23,28].Conventional methods for heavy-metal removal include chemical precipitation, ion exchange, membrane filtration, electrochemical treatment, and adsorption. However, these methods may involve high operating costs, energy requirements, and secondary-waste generation. Therefore, plant-based biosorbents and natural metabolites have gained considerable attention as sustainable alternatives because they contain functional groups capable of interacting with metal ions [12,13,19].

Heavy metals cannot be biologically degraded; instead, their concentration, mobility, oxidation state, and bioavailability can be reduced through adsorption, complexation, chelation, ion exchange, precipitation, and sequestration. Plant-derived biosorbents contain hydroxyl, carboxyl, carbonyl, amino, and phenolic groups that can bind metal species. Their removal efficiency is influenced by pH, contact time, initial metal concentration, biosorbent dose, and temperature [12,13,21]. Plant secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, terpenoids, and organic acids possess electron-donating functional groups that may participate in metal binding. These metabolites can act as antioxidants, metal chelators, and metal-precipitating agents, thereby contributing to metal detoxification and removal [19,2]. Polyphenolic compounds are particularly important because their hydroxyl groups can form complexes with metal ions. Plant phenolics have also been associated with Pb-binding and metal-stress tolerance [9,16].

Cadmium exposure induces oxidative stress and affects several biochemical processes in plants.

Phenolic and flavonoid metabolites may contribute to Cd detoxification through antioxidant activity and metal coordination. Similar relationships between secondary metabolites and detoxification of Cd, Pb, and As have also been reported [2,9]. Chromium remediation depends strongly on its oxidation state. Cr(VI) is generally more toxic and mobile than Cr(III) and may occur as chromate, hydrogen chromate, or dichromate species in aqueous environments. Its remediation can involve adsorption, electrostatic interaction, complexation, and reduction of Cr(VI) to the less mobile Cr(III) form [11,29]. Arsenic also exhibits pH- and oxidation-state-dependent speciation. Plant-derived metabolites have been reported to contribute to arsenic detoxification through binding and sequestration mechanisms [1,2]. Medicinal plants represent valuable sources of chemically diverse metabolites and are increasingly investigated for environmental remediation. Phenolics, flavonoids, alkaloids, tannins, terpenoids, and glycosides present in these plants may provide functional sites for metal interaction. Recent studies have highlighted the potential of ethnomedicinal plants and plant-derived biosorbents for removal and stabilization of heavy metals from contaminated environments [12,21,23].

Premna tomentosa is a promising source of bioactive metabolites. [14] reported that the methanolic leaf extract of *P. tomentosa* contained high levels of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and other phytochemicals. FTIR characterization revealed hydroxyl, amino, carbonyl, C–N, C–O, and ether functional groups, while GC–MS analysis identified several bioactive compounds including phytol, squalene, campesterol, stigmasterol, β-sitosterol, lupeol, farnesol, cycloartenol, α-tocopherol, fatty acids, and their derivatives [14]. These functional groups and metabolites may interact differently with Pb^{2+}, Cd^{2+}, Cr(VI), and As(III). Pb^{2+} and Cd^{2+} may be removed mainly through adsorption, coordination, and chelation, whereas Cr(VI) and As(III) may also involve speciation-dependent electrostatic and redox interactions. Metal speciation, solution pH, contact time, initial concentration, and availability of binding sites are therefore important determinants of removal efficiency [12,13,21]. Plant secondary metabolites can be extracted, characterized, standardized, and evaluated independently, providing a useful approach for understanding direct metabolite–metal interactions [2,6,19]. However, information on the direct interaction of *P. tomentosa* leaf-derived metabolites with Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) remains limited.

Material and Methods

Preparation of *Premna tomentosa* Metabolite Working Solution

The dried methanolic leaf extract of *Premna tomentosa* was used for preparation of the metabolite stock solution. Exactly 1.0 g of dried crude methanolic extract was weighed and initially dissolved in 10 mL of analytical-grade methanol. The solution was then transferred into a 100 mL volumetric flask, and the final volume was made up to 100 mL with deionized water to obtain a 10 mg mL^{-1} stock solution. The solution was mixed thoroughly and filtered to remove insoluble particles. Fresh metabolite working solutions of 0.25, 0.50, 0.75, and 1.00 mg mL^{-1} were prepared from the stock solution using deionized water. For the comparative metal-removal experiment, a final extract concentration of 1.00 mg mL^{-1} was used, corresponding to 100 mg of crude extract per 100 mL of metal solution. The methanol concentration in the final treatment system was maintained at a minimal level to avoid interference with metal removal.

Preparation of Heavy-Metal Solutions and Experimental Treatments

Individual stock solutions of Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) were prepared separately at a nominal metal concentration of 1000 mg L^{-1} using analytical-grade reagents. For Pb^{2+}, 1.599 g of $Pb(NO_3)_2$ was dissolved in deionized water and the volume was made up to 1 L. For Cd^{2+}, 1.631 g of anhydrous $CdCl_2$ was dissolved and adjusted to 1 L. For Cr(VI), 2.829 g of $K_2Cr_2O_7$ was dissolved and made up to 1 L, while for As(III), 1.320 g of $NaAsO_2$ was dissolved and the final volume was adjusted to 1 L. From these stock solutions, working concentrations of 10, 25, 50, and 100 mg L^{-1} were prepared separately. For preparation of 100 mL of each working solution, 1.0, 2.5, 5.0, and 10.0 mL, respectively, of the 1000 mg L^{-1} stock solution was transferred into separate 100 mL volumetric flasks and diluted to the mark with deionized water. For the treatment experiment, 100 mL of each metal working solution was transferred into a 250 mL Erlenmeyer flask, and *P. tomentosa* metabolite extract was added to obtain a final concentration of 1.00 mg mL^{-1}, equivalent to 100 mg of crude extract per 100 mL of metal solution. Each metal was evaluated independently at initial concentrations of 10, 25, 50, and 100 mg L^{-1}. Corresponding control flasks containing the same metal concentrations without plant extract were maintained under identical conditions. An extract blank containing 100 mg of *P. tomentosa* extract in 100 mL of deionized water without added metal was also included. All experimental treatments were performed in triplicate (n = 3).

Preparation of Heavy-Metal Stock and Working Solutions

Individual stock solutions of Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) were prepared separately at a concentration equivalent to 1000 mg L^{-1} of the respective element using analytical-grade metal salts and deionized water. For Pb^{2+}, 1.599 g of lead nitrate [$Pb(NO_3)_2$] was dissolved in deionized water and the final volume was adjusted to 1 L. For Cd^{2+}, 1.631 g of anhydrous cadmium chloride ($CdCl_2$) was dissolved and made up to 1 L. The Cr(VI) stock solution was prepared by dissolving 2.829 g of potassium dichromate ($K_2Cr_2O_7$) and adjusting the final volume to 1 L, whereas the As(III) stock solution was prepared using 1.320 g of sodium arsenite ($NaAsO_2$) in a final volume of 1 L. From each 1000 mg L^{-1} stock solution, working concentrations of 10, 25, 50, and 100 mg L^{-1} were prepared separately using deionized water. For preparation of 100 mL working solutions, 1.0, 2.5, 5.0, and 10.0 mL of the respective stock solution were transferred into separate 100 mL volumetric flasks and diluted to the mark with deionized water. All solutions were prepared separately to prevent cross-contamination and were stored in pre-cleaned, appropriately labelled glass containers until use.

Experimental Design for Heavy-Metal Removal

The metal-removal experiment was conducted separately for Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) under controlled laboratory conditions. For each metal, 100 mL of the respective working solution at 10, 25, 50, or 100 mg L^{-1} was transferred into a 250 mL Erlenmeyer flask. The methanolic leaf-derived metabolite extract of *Premna tomentosa* was added to each treatment flask at a fixed dose of 100 mg crude extract per 100 mL of metal solution, corresponding to a final extract concentration of 1.0 mg mL^{-1}.

The contents of each flask were mixed thoroughly to ensure uniform contact between the plant-derived metabolites and dissolved metal species. For every metal concentration, an untreated control containing the corresponding metal solution without *P. tomentosa* extract was maintained under identical conditions. A separate extract blank containing 100 mg of *P. tomentosa* extract in 100 mL of deionized water without added metal was also included to determine any background contribution from the plant extract. All control, treatment, and blank experiments were performed in triplicate (n = 3).

Control of Experimental Conditions

The heavy-metal treatment systems were maintained under standardized laboratory conditions throughout the experiment. The initial pH of each treatment solution was adjusted to 6.0 ± 0.2 using dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH), with only minimal adjustment where necessary to maintain the stability of the respective metal species. Each treatment flask was incubated at 25 ± 2 °C on an orbital shaker operated at 120 rpm. Continuous agitation was maintained to provide uniform contact between the dissolved metal species and the bioactive constituents present in the *Premna tomentosa* methanolic leaf extract. Identical experimental conditions, including temperature, agitation speed, solution volume, extract dose, pH, and treatment period, were maintained for all metal treatments and corresponding controls to ensure reliable comparison of metal-removal efficiency.

Contact-Time Study

The effect of contact time on the interaction between *Premna tomentosa* leaf-derived metabolites and the selected metals was evaluated over a total experimental period of 72 h. Samples were collected at 0, 6, 12, 24, 48, and 72 h. The 0 h sample represented the initial metal concentration before effective interaction with the plant-derived metabolites and was used as the baseline value for subsequent removal calculations. At each subsequent sampling interval, a measured aliquot was withdrawn from the respective flask and processed for determination of the residual concentration of Pb^{2+}, Cd^{2+}, Cr(VI), or As(III). The decrease in metal concentration at each contact period, relative to the corresponding initial concentration, was used to determine the time-dependent metal-removal efficiency of the *P. tomentosa* metabolite treatment. All contact-time measurements were carried out in triplicate (n = 3) under the same experimental conditions.

Separation of Treated Samples and Determination of Residual Heavy-Metal Concentration

At each sampling interval, an aliquot of the treatment mixture was withdrawn and centrifuged at 8,000–10,000 rpm for 10 min to separate precipitated, complexed, and insoluble plant–metal fractions. The resulting supernatant was carefully collected and passed through a 0.45 µm membrane filter to obtain a clear soluble fraction. Where required, the filtrate was acidified with trace-metal-grade nitric acid to stabilize the dissolved metal species prior to instrumental analysis. Residual concentrations of Pb, Cd, Cr, and As in both treated and control samples were quantified using Atomic Absorption Spectrophotometry (AAS) under appropriate element-specific operating conditions. Separate calibration curves were prepared using certified standard solutions of Pb, Cd, Cr, and As, and reagent blanks were analysed before sample measurement. Calibration performance was periodically verified using reference standards, and samples exceeding the established calibration range were appropriately diluted prior to analysis. Metal concentrations were expressed as mg L^{-1}. The decrease in metal concentration relative to the corresponding initial concentration was considered as the amount of metal removed by the *Premna tomentosa* metabolite fraction.

Calculation of Removal Efficiency, and Uptake Capacity

The heavy-metal removal efficiency of *Premna tomentosa* metabolites was calculated from the initial and residual metal concentrations using the equation:

Removal efficiency (%) = $[(C_0 - C_t) / C_0] \times 100$

where C_0 represents the initial metal concentration before treatment (mg L^{-1}) and C_t represents the residual metal concentration at the respective sampling time (mg L^{-1}). The metal removal capacity of the extract was calculated using:

$q_t = [(C_0 - C_t) \times V] / m$

where q_t is the amount of metal removed at time *t* (mg g^{-1}), V is the volume of the metal solution (L), and m is the mass of *P. tomentosa* crude extract used (g). The final equilibrium removal capacity was calculated using the residual metal concentration obtained at the end of the experimental period. Removal efficiencies and uptake capacities of Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) were compared under identical experimental conditions to determine the relative affinity of the plant-derived metabolites toward the selected contaminants. The influence of initial metal concentration was evaluated using 10, 25, 50, and 100 mg L^{-1} treatment levels, and percentage removal and uptake capacity were calculated separately for each concentration. This comparison was used to determine whether increasing metal loading affected the availability or saturation of metabolite-binding sites.

Statistical Analysis

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). Differences in removal efficiency among metals, initial concentrations, and contact times were evaluated statistically using analysis of variance (ANOVA) followed by Tukey's post hoc multiple-comparison test where significant differences were observed. Statistical significance was considered at $p < 0.05$, while $p < 0.001$ was considered highly significant. The final comparative efficiency of the four metals was ranked from the highest to the lowest removal based on percentage removal and metal uptake capacity.

Results

Effect of *Premna tomentosa* Leaf-Derived Metabolites on Heavy-Metal Removal

The methanolic leaf-derived metabolites of *Premna tomentosa* showed concentration-dependent removal of Pb^{2+}, Cd^{2+}, Cr(VI), and As(III). At an initial concentration of 10 mg L^{-1}, Pb^{2+} exhibited the highest removal efficiency (82.40 ± 1.24%), with a residual concentration of 1.76 ± 0.12 mg L^{-1} and an uptake capacity of 8.24 ± 0.12 mg g^{-1}. This was followed by Cd^{2+} with 76.80 ± 1.18% removal, a residual concentration of 2.32 ± 0.12 mg L^{-1}, and an uptake capacity of 7.68 ± 0.12 mg g^{-1}. Cr (VI) showed 69.50 ± 1.31% removal with 3.05 ± 0.13 mg L^{-1} remaining in solution and an uptake capacity of 6.95 ± 0.13 mg g^{-1}, whereas As (III) showed the lowest efficiency at 61.20 ± 1.26%, with a residual concentration of 3.88 ± 0.13 mg L^{-1} and an uptake capacity of 6.12 ± 0.13 mg g^{-1}. At 25 and 50 mg L^{-1}, the same order of removal was maintained.

Pb^{2+} removal was 78.60 ± 1.16% and 73.20 ± 1.22%, Cd^{2+} removal was 72.30 ± 1.27% and 67.40 ± 1.34%, Cr(VI) removal was 64.80 ± 1.36% and 59.10 ± 1.48%, and As(III) removal was 56.50 ± 1.48% and 50.80 ± 1.42%, respectively.

At the highest initial concentration of 100 mg L^{-1}, the percentage removal decreased further to 66.70 ± 1.46% for Pb^{2+}, 60.90 ± 1.39% for Cd^{2+}, 52.60 ± 1.51% for Cr(VI), and 44.30 ± 1.42% for As(III). Despite this decline in percentage removal, the absolute uptake capacity increased with increasing metal loading, reaching 66.70 ± 1.46 mg g^{-1} for Pb^{2+}, 60.90 ± 1.39 mg g^{-1} for Cd^{2+}, 52.60 ± 1.51 mg g^{-1} for Cr(VI), and 44.30 ± 1.42 mg g^{-1} for As(III) at 100 mg L^{-1}. These results indicate that higher initial metal concentrations increased the total amount of metal associated with the fixed dose of *P. tomentosa* extract, while the proportion of metal removed decreased. Tukey's post hoc grouping consistently separated Pb^{2+} (a), Cd^{2+} (b), Cr(VI) (c), and As(III) (d), confirming significant differences among the metals at the same initial concentrations ($p < 0.05$). Overall, the metal-removal performance of the *P. tomentosa* metabolite fraction followed the order $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$ (Table. 1).

Table 1: Residual metal concentration, removal efficiency, and uptake capacity after 72 h treatment with P. tomentosa metabolites

Metal	Initial concentration (mg L^{-1})	Residual concentration (mg L^{-1})	Metal removed (mg L^{-1})	Removal efficiency (%)	Uptake capacity, q_e (mg g^{-1})	Tukey group
Pb^{2+}	10	1.76 ± 0.12	8.24 ± 0.12	82.40 ± 1.24	8.24 ± 0.12	a
Pb^{2+}	25	5.35 ± 0.29	19.65 ± 0.29	78.60 ± 1.16	19.65 ± 0.29	a
Pb^{2+}	50	13.40 ± 0.61	36.60 ± 0.61	73.20 ± 1.22	36.60 ± 0.61	a
Pb^{2+}	100	33.30 ± 1.46	66.70 ± 1.46	66.70 ± 1.46	66.70 ± 1.46	a
Cd^{2+}	10	2.32 ± 0.12	7.68 ± 0.12	76.80 ± 1.18	7.68 ± 0.12	b
Cd^{2+}	25	6.93 ± 0.32	18.08 ± 0.32	72.30 ± 1.27	18.08 ± 0.32	b
Cd^{2+}	50	16.30 ± 0.67	33.70 ± 0.67	67.40 ± 1.34	33.70 ± 0.67	b
Cd^{2+}	100	39.10 ± 1.39	60.90 ± 1.39	60.90 ± 1.39	60.90 ± 1.39	b
Cr(VI)	10	3.05 ± 0.13	6.95 ± 0.13	69.50 ± 1.31	6.95 ± 0.13	c
Cr(VI)	25	8.80 ± 0.34	16.20 ± 0.34	64.80 ± 1.36	16.20 ± 0.34	c
Cr(VI)	50	20.45 ± 0.74	29.55 ± 0.74	59.10 ± 1.48	29.55 ± 0.74	c
Cr(VI)	100	47.40 ± 1.51	52.60 ± 1.51	52.60 ± 1.51	52.60 ± 1.51	c
As(III)	10	3.88 ± 0.13	6.12 ± 0.13	61.20 ± 1.26	6.12 ± 0.13	d
As(III)	25	10.88 ± 0.37	14.13 ± 0.37	56.50 ± 1.48	14.13 ± 0.37	d
As(III)	50	24.60 ± 0.71	25.40 ± 0.71	50.80 ± 1.42	25.40 ± 0.71	d
As(III)	100	55.70 ± 1.42	44.30 ± 1.42	44.30 ± 1.42	44.30 ± 1.42	d

Values represent mean ± SD (n = 3). Different lowercase letters indicate significant differences among metals at the same initial concentration according to Tukey's test ($p < 0.05$).

Effect of Initial Metal Concentration

The initial concentration of the metal solution had a clear influence on the percentage removal achieved by the methanolic leaf-derived metabolites of *Premna tomentosa*. For all four contaminants, removal efficiency progressively declined as the starting concentration increased from 10 to 100 mg L^{-1}. Pb^{2+} showed the highest removal at every concentration, decreasing from 82.40 ± 1.24% at 10 mg L^{-1} to 78.60 ± 1.16% at 25 mg L^{-1}, 73.20 ± 1.22% at 50 mg L^{-1}, and 66.70 ± 1.46% at 100 mg L^{-1}, with an overall mean removal of 75.23%. Cd^{2+} removal declined from 76.80 ± 1.18% to 72.30 ± 1.27%, 67.40 ± 1.34%, and 60.90 ± 1.39%, giving an overall mean of 69.35%. A similar concentration-dependent decline was observed for Cr(VI), from 69.50 ± 1.31% at 10 mg L^{-1} to 52.60 ± 1.51% at 100 mg L^{-1}, with a mean removal of 61.50%. As(III) consistently showed the lowest efficiency, decreasing from 61.20 ± 1.26% to 56.50 ± 1.48%, 50.80 ± 1.42%, and 44.30 ± 1.42%, resulting in an overall mean removal of 53.20%.

Although percentage removal decreased at higher initial concentrations, the absolute uptake capacity increased because a greater quantity of metal was available for interaction with the fixed dose of *P. tomentosa* metabolites. At 100 mg L^{-1}, the maximum uptake capacity was observed for Pb^{2+} at 66.70 ± 1.46 mg g^{-1}, followed by Cd^{2+} at 60.90 ± 1.39 mg g^{-1}, Cr(VI) at 52.60 ± 1.51 mg g^{-1}, and As(III) at 44.30 ± 1.42 mg g^{-1}. The declining percentage efficiency at increasing metal loading suggests progressive occupation and eventual limitation of available metabolite-binding sites, whereas the increase in q_e indicates enhanced absolute metal loading on the extract. Overall, the mean removal pattern followed the order $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$, confirming a stronger apparent affinity of the *P. tomentosa* metabolite fraction toward Pb^{2+} and Cd^{2+} than toward Cr(VI) and As (III) (Table. 2).

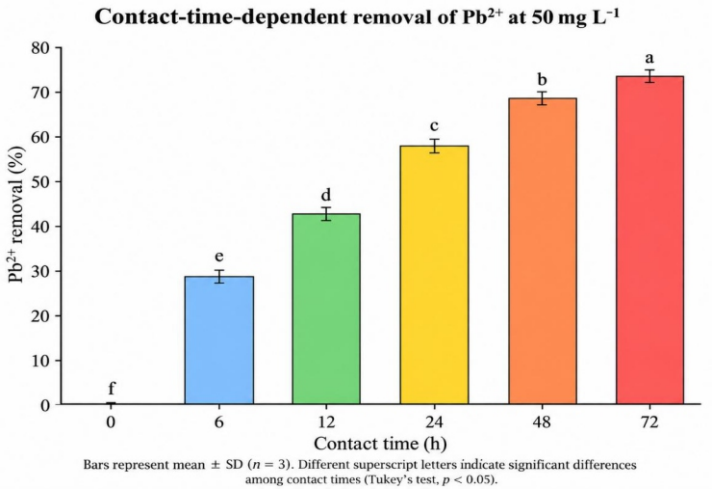
Table 2: Percentage decrease in removal efficiency with increasing initial metal concentration

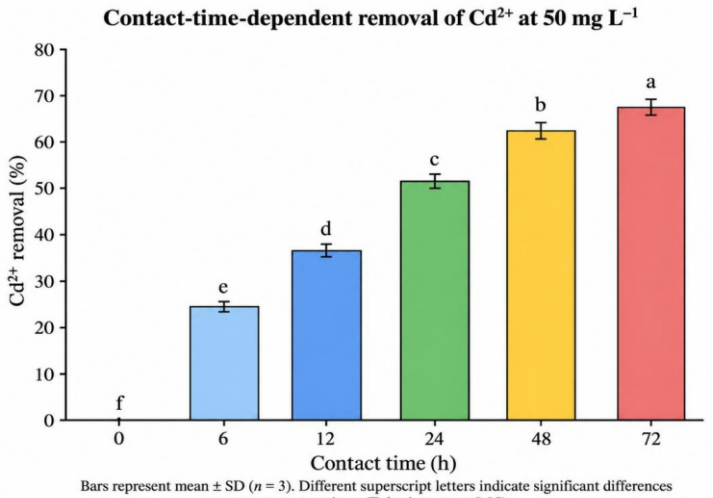
Metal	Removal at 10 mg L^{-1} (%)	Removal at 25 mg L^{-1} (%)	Removal at 50 mg L^{-1} (%)	Removal at 100 mg L^{-1} (%)	Overall mean removal (%)
Pb^{2+}	82.40 ± 1.24	78.60 ± 1.16	73.20 ± 1.22	66.70 ± 1.46	75.23
Cd^{2+}	76.80 ± 1.18	72.30 ± 1.27	67.40 ± 1.34	60.90 ± 1.39	69.35
Cr(VI)	69.50 ± 1.31	64.80 ± 1.36	59.10 ± 1.48	52.60 ± 1.51	61.50
As(III)	61.20 ± 1.26	56.50 ± 1.48	50.80 ± 1.42	44.30 ± 1.42	53.20

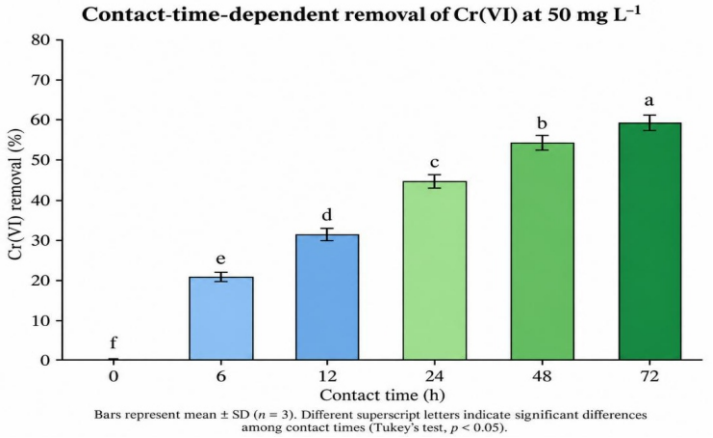
Effect of Contact Time on Heavy-Metal Removal

Contact time markedly influenced the removal of all four tested contaminants at the fixed initial concentration of 50 mg L^{-1}. No removal was recorded at 0 h, while a rapid increase was observed during the early phase of exposure. After 6 h, removal efficiencies reached 28.60 ± 1.08% for Pb^{2+}, 24.40 ± 0.96% for Cd^{2+}, 20.80 ± 0.88% for Cr(VI), and 17.20 ± 0.81% for As(III). These values increased further at 12 h to 42.80 ± 1.17%, 36.90 ± 1.12%, 31.50 ± 1.04%, and 25.90 ± 0.96%, respectively. By 24 h, removal had risen to 57.90 ± 1.31% for Pb^{2+}, 51.60 ± 1.24% for Cd^{2+}, 44.70 ± 1.17% for Cr(VI), and 37.40 ± 1.12% for As(III), demonstrating that the most pronounced increase occurred during the first 24 h of contact.

A further increase in removal was observed between 24 and 72 h, although the rate of improvement became comparatively slower. At 48 h, the removal efficiencies were 68.50 ± 1.28% for Pb^{2+}, 62.30 ± 1.31% for Cd^{2+}, 54.10 ± 1.39% for Cr(VI), and 46.30 ± 1.28% for As(III). Maximum removal was recorded at 72 h, reaching 73.20 ± 1.22%, 67.40 ± 1.34%, 59.10 ± 1.48%, and 50.80 ± 1.42%, respectively. The comparatively smaller increase between 48 and 72 h indicated that the system was approaching equilibrium as the available binding sites became progressively occupied. Tukey's post hoc grouping showed statistically distinct contact-time responses, with 72 h assigned to group a, followed by 48 h (b), 24 h (c), 12 h (d), 6 h (e), and 0 h (f), confirming significant differences among exposure periods ($p < 0.05$). Overall, the contact-time response followed the order $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$ throughout the experiment (Fig. 1).







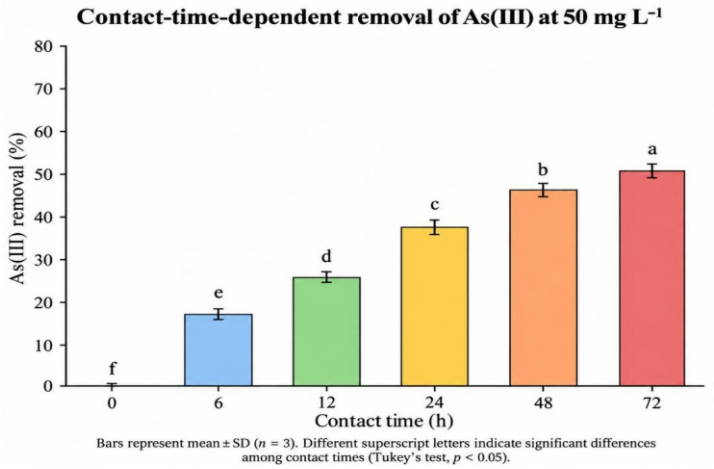
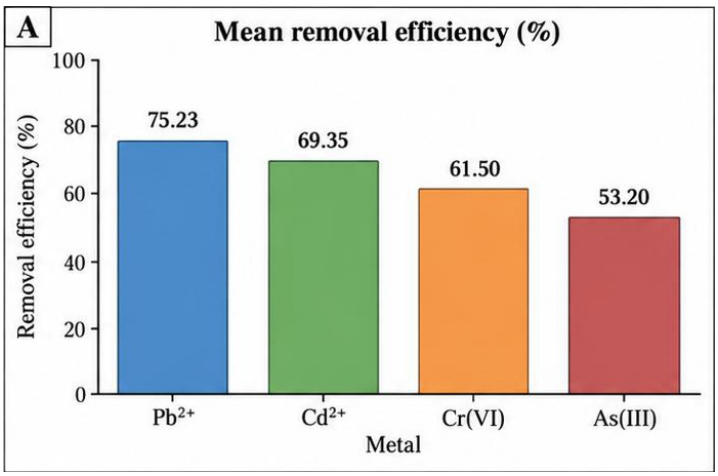


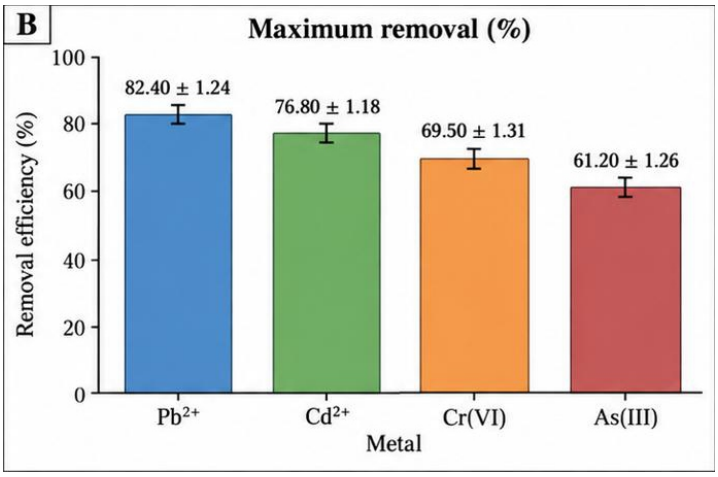
Figure. 1: Contact-time-dependent removal of selected metals at 50 mg L^{-1}

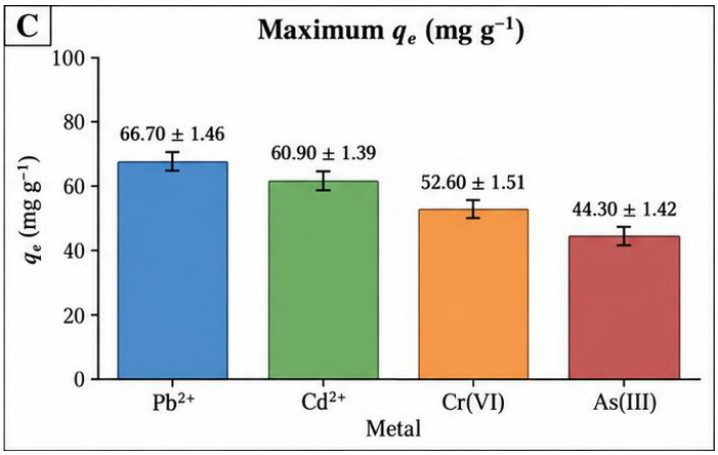
Comparative Bioremediation Performance

The comparative assessment of the four selected contaminants showed clear differences in the removal performance of the *Premna tomentosa* metabolite fraction. Pb^{2+} exhibited the highest overall mean removal efficiency at 75.23%, together with a maximum removal of 82.40 ± 1.24% and a maximum uptake capacity of 66.70 ± 1.46 mg g^{-1}. Cd^{2+} ranked second, with an overall mean removal of 69.35%, a maximum removal efficiency of 76.80 ± 1.18%, and a maximum q_e value of 60.90 ± 1.39 mg g^{-1}. Cr(VI) showed intermediate performance, recording a mean removal efficiency of 61.50%, a maximum removal of 69.50 ± 1.31%, and a maximum uptake capacity of 52.60 ± 1.51 mg g^{-1}. Among the tested contaminants, As(III) showed the lowest overall response, with a mean removal efficiency of 53.20%, a maximum removal of 61.20 ± 1.26%, and a maximum q_e of 44.30 ± 1.42 mg g^{-1}.

Based on the combined removal efficiency and uptake-capacity data, the relative bioremediation performance followed the order $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$. Pb^{2+} was therefore ranked first and classified as showing very high removal efficiency, followed by Cd^{2+} in second position with high efficiency. Cr (VI) occupied the third rank with moderate removal, while As (III) was ranked fourth with comparatively lower efficiency. The consistent ranking across mean removal, maximum percentage removal, and maximum q_e values indicates that the *P. tomentosa* metabolite fraction displayed the greatest apparent affinity toward Pb^{2+} and Cd^{2+}, whereas Cr (VI) and particularly As (III) were removed less efficiently under the tested conditions (Fig. 2).







D Relative efficiency and rank

Metal	Relative efficiency	Rank
Pb^{2+}	Very high	1
Cd^{2+}	High	2
Cr(VI)	Moderate	3
As(III)	Comparatively lower	4

Figure. 2: Overall comparative efficiency of P. tomentosa metabolites toward selected metals

ANOVA for Effect of Metal Type and Initial Concentration

Two-way analysis of variance demonstrated that both the type of metal and the initial metal concentration exerted strong effects on the removal efficiency of the *Premna tomentosa* metabolite fraction. The main effect of metal type was highly significant ($F_{3,32}$ = 604.79, p < 0.001), with a sum of squares of 3297.98 and a mean square of 1099.33, confirming that Pb^{2+}, Cd^{2+}, Cr(VI), and As (III) differed markedly in their response to treatment. Initial concentration also produced a highly significant effect ($F_{3,32}$ = 328.88, p < 0.001), with a sum of squares of 1793.44 and a mean square of 597.81. This statistical outcome supports the observed decline in percentage removal as the starting metal concentration increased from 10 to 100 mg L^{-1}.

In contrast, the interaction between metal type and initial concentration was not statistically significant ($F_{9,32}$ = 0.18, p = 0.995). The interaction accounted for a sum of squares of only 2.90, with a mean square of 0.32, compared with the residual error mean square of 1.82. The absence of a significant interaction indicates that although the absolute removal efficiencies differed among the four contaminants, the effect of increasing concentration followed a broadly comparable pattern across all metals. Thus, metal identity and initial concentration independently influenced removal performance, while their combined interaction contributed negligibly to the overall variation.

Table 3: Two-way ANOVA for effects of metal type and initial concentration on removal efficiency

Source of variation	df	Sum of squares	Mean square	F-value	p-value	Significance
Metal type	3	3297.98	1099.33	604.79	<0.001	Highly significant
Initial concentration	3	1793.44	597.81	328.88	<0.001	Highly significant
Metal × concentration	9	2.90	0.32	0.18	0.995	NS
Error	32	58.17	1.82	–	–	–

NS = not significant.

One-Way ANOVA Comparing Metals at Individual Concentrations

One-way ANOVA performed separately at each initial metal concentration confirmed that the removal efficiencies of Pb^{2+}, Cd^{2+}, Cr (VI), and As (III) differed significantly across the entire concentration range. At 10 mg L^{-1}, the differences among the four metals were highly significant ($F_{3,8}$ = 162.46, p < 0.001). A similarly strong difference was observed at 25 mg L^{-1} ($F_{3,8}$ = 156.22, p < 0.001) and 50 mg L^{-1} ($F_{3,8}$ = 153.19, p < 0.001). Even at the highest tested concentration of 100 mg L^{-1}, the removal responses remained clearly separated among the metals, with $F_{3,8}$ = 137.26, p < 0.001.

These results demonstrate that metal identity significantly influenced the removal performance of the *Premna tomentosa* metabolite fraction at every initial concentration tested.

Tukey's post hoc multiple-comparison test further separated the four metals into distinct significance groups throughout the experiment. Pb^{2+} consistently showed the highest removal and was assigned to group a, followed by Cd^{2+} in group b, Cr (VI) in group c, and As (III) in group d. The general statistical ranking therefore followed $Pb^{2+a} > Cd^{2+b} > Cr(VI)^{c} > As(III)^{d}$, confirming that the apparent affinity of the *P. tomentosa* metabolites differed significantly among the selected metal species at all tested concentrations (Table. 4).

Table 4: One-way ANOVA comparison of metal-removal efficiencies at each initial concentration

Initial concentration (mg L^{-1})	df between	df within	F-value	p-value	Interpretation
10	3	8	162.46	<0.001	Highly significant
25	3	8	156.22	<0.001	Highly significant
50	3	8	153.19	<0.001	Highly significant
100	3	8	137.26	<0.001	Highly significant

Tukey's multiple-comparison analysis demonstrated the general grouping $Pb^{2+a} > Cd^{2+b} > Cr(VI)^{c} > As(III)^{d}$ across the tested concentration range.

ANOVA for Effect of Contact Time

Two-way ANOVA demonstrated that both metal type and contact time had highly significant effects on the removal efficiency of the *Premna tomentosa* metabolite fraction at an initial concentration of 50 mg L^{-1}. The effect of metal type was highly significant ($F_{3,48}$ = 687.97, $p < 0.001$), with a sum of squares of 2445.54 and a mean square of 815.18, confirming that Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) differed substantially in their overall removal response. Contact time produced an even greater statistical effect ($F_{5,48}$ = 5642.38, $p < 0.001$), accounting for a sum of squares of 33428.50 and a mean square of 6685.70. This result confirmed that extending the exposure period from 0 to 72 h markedly increased the removal of all four contaminants. A significant interaction between metal type and contact time was also observed ($F_{15,48}$ = 36.38, $p < 0.001$), with a sum of squares of 646.58 and a mean square of 43.11, indicating that the rate and magnitude of removal over time were not identical for all metals, the statistical findings were consistent with the observed experimental pattern. Maximum removal at the lowest tested initial concentration was recorded for Pb^{2+} at 82.40 ± 1.24%, followed by Cd^{2+} at 76.80 ± 1.18%, Cr(VI) at 69.50 ± 1.31%, and As(III) at 61.20 ± 1.26%. Percentage removal declined as the initial metal concentration increased, whereas the absolute uptake capacity increased. In the contact-time experiment, removal rose progressively and began to approach a comparatively stable level between 48 and 72 h, indicating movement toward equilibrium. Taken together, the ANOVA results confirmed highly significant effects of metal identity, initial concentration, and contact time ($p < 0.001$) on the performance of the *P. tomentosa* metabolite fraction. The overall removal efficiency followed the order $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$, demonstrating a clear difference in the apparent affinity of the plant-derived metabolites toward the selected metal species.

Table 5: Two-way ANOVA for effects of metal type and contact time on removal efficiency

Source of variation	df	Sum of squares	Mean square	F-value	p-value	Significance
Metal type	3	2445.54	815.18	687.97	<0.001	Highly significant
Contact time	5	33428.50	6685.70	5642.38	<0.001	Highly significant
Metal × contact time	15	646.58	43.11	36.38	<0.001	Highly significant
Error	48	56.88	1.18	–	–	–

Discussion

The present investigation demonstrated that the methanolic leaf-derived metabolite fraction of *Premna tomentosa* was capable of removing Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) from aqueous solutions under controlled laboratory conditions. The magnitude of removal varied considerably among the four contaminants and was strongly influenced by initial metal concentration and contact time. At an initial concentration of 10 mg L^{-1}, the highest removal was observed for Pb^{2+} (82.40 ± 1.24%), followed by Cd^{2+} (76.80 ± 1.18%), Cr(VI) (69.50 ± 1.31%), and As(III) (61.20 ± 1.26%). At 100 mg L^{-1}, removal declined to 66.70 ± 1.46%, 60.90 ± 1.39%, 52.60 ± 1.51%, and 44.30 ± 1.42%, respectively. The overall mean removal efficiencies of 75.23% for Pb^{2+}, 69.35% for Cd^{2+}, 61.50% for Cr(VI), and 53.20% for As(III) clearly established the affinity sequence $Pb^{2+} > Cd^{2+} > Cr(VI) > As(III)$. Such differences are consistent with the general behaviour of plant-based biosorbents, where the affinity toward individual metal species depends on ionic properties, metal speciation, surface chemistry, accessibility of functional groups, and competition for chemically favourable binding sites [4,7]. The comparatively high Pb^{2+} removal observed in the present study agrees with a substantial body of work describing strong interactions between Pb^{2+} and lignocellulosic or plant-derived biosorbents. [7] reported that low-cost natural and biological adsorbents can achieve a broad range of Pb^{2+} removal efficiencies and adsorption capacities, depending on surface composition and experimental conditions. [4] similarly emphasized that Pb^{2+} biosorption is strongly controlled by solution pH, initial metal concentration, adsorbent dosage, contact time, and the chemical nature of available binding sites. Plant materials containing oxygen-rich functional groups are particularly effective because hydroxyl, carboxyl, carbonyl, and related groups can participate in complexation, electrostatic interaction, ion exchange, and surface coordination. This provides a plausible explanation for the relatively strong Pb^{2+} response of the *P. tomentosa* metabolite fraction, where Pb^{2+} removal remained the highest at every tested concentration.

The preferential removal of Pb^{2+} over Cd^{2+} is also supported by experimental studies using intact plant biomass. [22] investigated broadleaf cattail and water hyacinth biomass and observed that both materials displayed a greater affinity for Pb^{2+} than Cd^{2+}. Their study also demonstrated that removal efficiency decreased when the initial metal concentration increased, while adsorption increased with contact time until equilibrium was attained. This behaviour closely parallels the pattern obtained for *P. tomentosa*. In the present data, Pb^{2+} removal declined from 82.40 ± 1.24% at 10 mg L^{-1} to 66.70 ± 1.46% at 100 mg L^{-1}, while Cd^{2+} decreased from 76.80 ± 1.18% to 60.90 ± 1.39% over the same concentration range. The consistent superiority of Pb^{2+} may therefore reflect a stronger thermodynamic or coordination affinity of Pb^{2+} for the electron-donating sites present in the plant-derived metabolite mixture. The observed Cd^{2+} removal was nevertheless substantial, with a maximum efficiency of 76.80 ± 1.18% and a maximum calculated uptake capacity of 60.90 ± 1.39 mg g^{-1}. Plant-derived materials have repeatedly been shown to possess substantial affinity for Cd^{2+}. [15] reviewed agricultural-waste-based adsorbents and identified carboxyl, hydroxyl, sulphydryl, and amide groups as important contributors to Cd^{2+} binding. [3], using shea fruit shell biomass, similarly demonstrated that Cd^{2+} removal was dependent on pH, contact time, temperature, and initial concentration and that the kinetics were well represented by a pseudo-second-order model, suggesting a strong contribution from surface chemical interactions. The present Cd^{2+} removal therefore agrees well with the established ability of plant-derived matrices to coordinate Cd^{2+} through oxygen- and nitrogen-containing functional sites.

Further support is provided by [5], who developed biosorbents from *Capparis decidua* branches and *Ziziphus mauritiana* leaves for Cd^{2+} removal. They reported maximum adsorption capacities of 248.62 and 235.65 mg g^{-1}, respectively, and showed that sorption depended on pH, contact time, initial concentration, and adsorbent dose. Although these adsorption capacities are higher than the values calculated for the crude *P. tomentosa* metabolite fraction, direct numerical comparison should be made cautiously because the adsorbent type, dose,

metal concentration, preparation method, and physicochemical properties differed substantially. Nevertheless, the study confirms that leaf-derived materials can provide abundant chemical sites for Cd^{2+} interaction and supports the present observation of appreciable Cd^{2+} removal. [24] also demonstrated that low-cost agri-food residues can remove multiple divalent metals, including Cd^{2+} and Pb^{2+}, from aqueous systems. Their findings are particularly relevant to the present comparative experiment because they show that a single biological matrix may exhibit markedly different adsorption efficiencies toward different cations. Such selectivity reflects differences in hydrated ionic radius, electronegativity, hydration energy, charge density, ligand affinity, and accessibility of surface-binding groups. The separation of Pb^{2+} and Cd^{2+} into statistically distinct Tukey groups in the present study therefore suggests that *P. tomentosa* metabolites did not act as a nonspecific precipitating material but displayed differential interaction with the selected metals.

The concentration-dependent decline in percentage removal represented one of the clearest trends in the present investigation. Pb^{2+} removal declined from 82.40 to 66.70%, Cd^{2+} from 76.80 to 60.90%, Cr(VI) from 69.50 to 52.60%, and As(III) from 61.20 to 44.30% as the initial concentration increased from 10 to 100 mg L^{-1}. At the same time, the calculated uptake capacity increased, reaching 66.70 ± 1.46 mg g^{-1} for Pb^{2+}, 60.90 ± 1.39 mg g^{-1} for Cd^{2+}, 52.60 ± 1.51 mg g^{-1} for Cr(VI), and 44.30 ± 1.42 mg g^{-1} for As(III) at the highest concentration. This apparently contrasting response is typical of adsorption systems. At low concentrations, the number of available binding sites is large relative to the number of dissolved metal species, enabling a high proportion of the contaminant to be removed. As concentration increases, the available sites become progressively occupied and the percentage removed declines, although the higher concentration gradient increases the total mass transferred to the sorbent. [22] reported a comparable decrease in Pb^{2+} and Cd^{2+} removal efficiency with increasing initial concentration, while [8] identified initial concentration as an important determinant of Cd^{2+} biosorption by *Averrhoa carambola* leaf waste. The behaviour of Cr(VI) differed from that of Pb^{2+} and Cd^{2+}, which is expected because Cr(VI) is present primarily as oxyanion species rather than as a simple divalent cation. The present maximum Cr(VI) removal of 69.50 ± 1.31% was lower than that of Pb^{2+} and Cd^{2+}, while its overall mean efficiency was 61.50%. Plant-derived materials can nevertheless remove Cr(VI) efficiently through a combination of electrostatic attraction, adsorption, complexation, and in some systems reduction to Cr(III). [20] reviewed agricultural waste materials for Cr(VI) and Cd^{2+} removal and concluded that adsorption efficiency is strongly governed by adsorbent surface chemistry and operating conditions. More recently, [10] reported that natural adsorbents can remove Cr(VI) through protonated functional groups followed in some cases by electron transfer and reduction to Cr(III), with leaf-derived materials showing particularly promising performance.

Direct experimental comparisons with plant leaves further support the present Cr(VI) findings. [26] studied *Prosopis cineraria* leaf powder and reported monolayer capacities of approximately 10.046 mg g^{-1} for Cr(VI) and 10.238 mg g^{-1} for Pb^{2+}, demonstrating that the same leaf-derived biosorbent can interact with both cationic Pb^{2+} and oxyanionic Cr(VI) through different mechanisms. Their kinetic analysis supported significant roles for hydrogen bonding and electrostatic interactions.

[27] subsequently reported a maximum chromium removal of 89.65% using *P. cineraria* leaf powder under optimized acidic conditions and an adsorption capacity of 55.55 mg g^{-1}. The higher optimum removal reported in that study compared with the 69.50% maximum Cr(VI) removal observed here can reasonably be attributed to differences in pH and material form: Soleimani et al. optimized Cr removal near pH 2, whereas the present *P. tomentosa* experiment was designed as a comparative multi-metal assay rather than a Cr-specific optimization study. The comparatively lower removal of As(III) in the current experiment is also chemically reasonable. The maximum As(III) removal was 61.20 ± 1.26%, the mean removal efficiency was 53.20%, and the maximum calculated uptake capacity was 44.30 ± 1.42 mg g^{-1}. Unlike Pb^{2+} and Cd^{2+}, As(III) commonly occurs under near-neutral aqueous conditions largely as uncharged arsenous acid, which reduces the contribution of simple electrostatic attraction to unmodified organic binding sites. [25] emphasized that arsenic removal by biomass-derived adsorbents is strongly dependent on arsenic speciation, solution pH, sorbent modification, surface functional groups, and mineral components. This helps explain why As(III) removal by an unmodified metabolite-rich extract may be lower than cation removal under a single standardized pH condition.

The present As(III) values are nevertheless consistent with the documented ability of leaf-derived materials to adsorb arsenic. [17] used okra leaves for As(III) and As(V) sorption and reported that adsorption was both pH- and contact-time-dependent, with equilibrium occurring after approximately 180 min and substantial differences between the two arsenic oxidation states. Their results demonstrated that heterogeneous plant surfaces can retain As through both surface sorption and intraparticle-related processes. [18], using agricultural-waste-derived biochar, reported more than 60% arsenic immobilization under optimized conditions around pH 6, with adsorption described by pseudo-second-order kinetics and Langmuir-type behaviour. The 61.20% maximum As(III) removal observed for *P. tomentosa* therefore falls within a biologically plausible range for biomass-associated As retention, even though the chemical form of the present material differs from biochar and intact leaf biomass. Contact time had a pronounced effect on all four contaminants. At 50 mg L^{-1}, removal after 6 h was 28.60 ± 1.08% for Pb^{2+}, 24.40 ± 0.96% for Cd^{2+}, 20.80 ± 0.88% for Cr(VI), and 17.20 ± 0.81% for As(III). By 24 h, these values had increased to 57.90 ± 1.31%, 51.60 ± 1.24%, 44.70 ± 1.17%, and 37.40 ± 1.12%, respectively, while maximum values at 72 h reached 73.20 ± 1.22%, 67.40 ± 1.34%, 59.10 ± 1.48%, and 50.80 ± 1.42%. The faster initial phase can be attributed to a relatively large number of readily accessible binding sites and a strong concentration gradient between the aqueous phase and metabolite-associated sites. As contact continued, progressive occupation of these sites reduced the rate of additional uptake, resulting in the smaller differences observed between 48 and 72 h. This general pattern has been widely reported for biomass adsorption systems and is consistent with the contact-time behaviour described for Cd^{2+}, Pb^{2+}, Cr(VI), and arsenic in plant-derived sorbents [3,17,26].

The significant contact-time response also indicates that the interaction between the metals and *P. tomentosa* metabolites was not instantaneous. The statistical analysis showed a strong effect of contact time ($F_{5,48}$ = 5642.38, $p < 0.001$) and a significant metal × contact-time interaction ($F_{15,48}$ = 36.38, $p < 0.001$), demonstrating that the rate of removal differed among metal species.

This is expected because Pb^{2+} and Cd^{2+} can interact directly with negatively charged or electron-rich ligands, whereas Cr(VI) and As(III) may require different adsorption, protonation, diffusion, or redox pathways. [26] demonstrated strong kinetic dependence during Cr(VI) adsorption by plant leaves, while [5] reported pseudo-second-order Cd^{2+} adsorption by plant-derived biosorbents. These comparisons support the interpretation that multiple, metal-specific surface interactions contributed to the observed time-dependent behaviour rather than a single identical mechanism for all contaminants. The statistical separation of the four metals further strengthens the comparative interpretation. Two-way ANOVA showed a highly significant effect of metal type ($F_{3,32}$ = 604.79, $p < 0.001$) and initial concentration ($F_{3,32}$ = 328.88, $p < 0.001$), whereas the metal × concentration interaction was not significant ($F_{9,32}$ = 0.18, $p = 0.995$). Thus, although each metal displayed a different absolute removal efficiency, increasing initial concentration produced a broadly similar directional effect across the four contaminants. The one-way ANOVA performed at each concentration likewise showed highly significant differences among metals, and Tukey analysis maintained the grouping Pb^{2+a} > Cd^{2+b} > $Cr(VI)^{c}$ > $As(III)^{d}$. This strong statistical differentiation is consistent with literature showing that biosorption selectivity depends on both sorbent chemistry and contaminant-specific properties [7,24].

The use of a metabolite-rich extract rather than intact dried biomass is an important feature of the present study. Many published biosorption studies rely on lignocellulosic plant powders, shells, peels, biochars, or chemically modified biomass. By contrast, the *P. tomentosa* system contains soluble and semi-soluble phytochemical constituents capable of participating in complexation, chelation, association, precipitation, and redox reactions. Consequently, the term "removal" is more appropriate than assuming that all decreases in dissolved metal concentration resulted from classical surface adsorption. Some fraction of the Pb^{2+} and Cd^{2+} may have been retained through complex formation with metabolite ligands, whereas Cr(VI) removal may have included adsorption together with partial reduction, and As(III) removal may have involved weaker coordination or precipitation pathways. Current reviews of biosorbent systems emphasize that apparent pollutant removal can result from several simultaneous mechanisms and that direct mechanistic confirmation requires additional spectroscopic and surface characterization [10,20,25]. The present results also suggest practical advantages and limitations. Maximum removal efficiencies of 82.40% for Pb^{2+} and 76.80% for Cd^{2+} indicate promising activity of the *P. tomentosa* metabolite fraction without chemical surface modification. However, performance declined at higher metal loading, particularly for As(III), whose efficiency decreased to 44.30 ± 1.42% at 100 mg L^{-1}. This indicates that the metabolite dose used in the experiment may become limiting at elevated contaminant concentrations. Published biosorbent studies similarly demonstrate that optimization of adsorbent dose, pH, initial concentration, and contact time is necessary before laboratory observations can be translated into practical treatment systems [4,7,15].

Conclusion

The present study demonstrated that methanolic leaf-derived metabolites of *Premna tomentosa* possess appreciable potential for the removal of Pb^{2+}, Cd^{2+}, Cr(VI), and As(III) from aqueous systems under controlled laboratory conditions.

Among the tested contaminants, Pb^{2+} showed the highest overall removal efficiency, followed by Cd^{2+}, Cr(VI), and As(III), establishing the order Pb^{2+} > Cd^{2+} > Cr(VI) > As(III). The maximum removal efficiencies recorded at 10 mg L^{-1} were 82.40 ± 1.24% for Pb^{2+}, 76.80 ± 1.18% for Cd^{2+}, 69.50 ± 1.31% for Cr(VI), and 61.20 ± 1.26% for As(III). Although percentage removal declined with increasing initial metal concentration, uptake capacity increased, indicating greater absolute metal loading on the metabolite fraction at higher concentrations. Contact time also played a major role in the remediation process, with removal increasing progressively up to 72 h and approaching equilibrium between 48 and 72 h. At 50 mg L^{-1}, the 72 h removal efficiencies reached 73.20 ± 1.22% for Pb^{2+}, 67.40 ± 1.34% for Cd^{2+}, 59.10 ± 1.48% for Cr(VI), and 50.80 ± 1.42% for As(III). Statistical analysis confirmed highly significant effects of metal type, initial concentration, and contact time on removal efficiency ($p < 0.001$). Overall, the findings indicate that *P. tomentosa* leaf-derived metabolites can serve as a promising plant-based material for heavy-metal remediation. Further studies involving optimization of pH and extract dose, kinetic and isotherm modelling, spectroscopic confirmation of metal-binding mechanisms, regeneration studies, and validation using real contaminated water are required to establish its practical applicability in environmental treatment systems.

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