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Phytoremediation of Aqueous Lead Ions by Using Leaves of *Clematis terniflora*

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ABSTRACT
Heavy metals persist in the environment abundantly. They are excessively released into the environment through various anthropogenic activities. Lead is a principal pollutant that entails a serious nature. Remediation of land pollutants is consequently of utmost significance. Phytoremediation has surged in popularity, due to its lower cost and fewer detrimental outcomes in contrast to chemical and physical approaches. Clematis terniflora (Ranunculaceae) is an invasive plant which is investigated as an operational alternative for the removal of heavy metal contaminants. The study examines the potentiality of C. terniflora against aqueous lead ions by varying time intervals and plant powder concentrates. The results were concluded based on atomic absorption spectroscopy and atomic emission spectroscopy. The findings were analysed statistically using Pearson correlation coefficient. Further preliminary phytochemical analysis was carried out to detect the presence of various phyto-constituents in the leaf extract of C. terniflora.
Keywords*: Clematis terniflora, Phytoremediation, lead, Pearson's Coefficient.*

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INTRODUCTION

Heavy metal accumulation in aquatic ecosystems is known to have a negative effect on aquatic life, in addition to the fact that they kill microorganisms during the biological processing of contaminated water [1]. The majority of heavy metal salts are soluble in water and form aqueous solutions, making it inaccessible to separate them by using conventional physical and chemical approaches [2]. The problems of lead (Pb) pollution in the soil arise along with the rapid urbanization and industrialization [3, 4]. As a pollutant, Pb possess a direct threat to human health, lingering in the environment for a long time due to its non-biodegradable nature, or accumulating in the food chain [5].

Toxic pollutants from polluted soils can be eliminated through an assortment of remedial approaches, including physical and chemical procedures. These techniques are often exorbitant and laborious [6]. A substantial environmental and health concern prompted by contaminated soils and waterways may be mitigated by phytoremediation approach [7]. It is an innovative method that can be utilised for treating both organic and inorganic contaminants that are prevalent in soil, water, or the natural environment. Phytoremediation is an efficient technique in which plants are utilized to remove the heavy metals present in the soil or aquatic ecosystem [9]. Phytoremediation is ecologically sustainable and economically affordable approach towards removal of heavy metals [8].

Clematis terniflora is a perennial woody vine belonging to the family Ranunculaceae. It is native to China, the Russian Far East, and Temp. E. Asia [10]. It has opposite and pinnately compound leaves. The flowers are white in colour. *C. terniflora* has therapeutic as well as anti-inflammatory properties [11]. The present paper deals with evaluating the potency of the leaf extract of *C. terniflora* against aqueous lead ions. The use of this natural material for water treatment can provide a cost-effective and eco-friendly solution to water pollution.

MATERIAL AND METHODS

Fresh leaves of *Clematis terniflora* DC. were collected from the campus of M.M. College of Arts, N.M. Institute of Science, and H.R.J. College of Commerce, Bhavans College (Autonomous), Andheri (West), Mumbai 400 058, Maharashtra, India (72°50'07.6"E and 19°07'28.6"N) district Mumbai of Maharashtra. The collected leaves were washed and dried in an oven at 60°C for 24 hours. The dried leaves were then powdered into a fine powder. The powder was used for phytochemical screening and lead treatment analysis.

Preliminary qualitative phytochemical analysis was carried out on crude extracts of the leaves of *C. terniflora* using standard procedures to identify the presence or absence of various chemical constituents such as phenolic compounds, terpenoids, flavonoids, tannins, quinones, and coumarins [12]. The presence of phenolic compounds was determined using the procedures described by Rohit [12].

Test for phytochemicals

Detection of Phenols

Ferric chloride test: Dissolve 500mg of extract in 5ml of distilled water and add a few drops of neutral 5% ferric chloride solution. Observe the development of a dark green colour, indicating the presence of phenolic compounds.

Detection of Terpenoids:

Salkowski test: Dissolved 5ml of leaf extract in 2ml of chloroform and 3ml of conc. Sulfuric acid was precisely applied to create a coating. Reddish brown colouration confirmed the presence of terpenoids [13].

Detection of Flavonoids

Lead acetate test: Add 1 ml of 10% lead acetate solution to 1 ml of extract and observe the formation of a yellow precipitate, indicating the presence of flavonoids.

Detection of Tannins

Braymer's test: Mix 2ml of extract with 2ml of distilled water and add a few drops of $FeCl_3$ solution. The occurrence of a green precipitate shows the presence of tannin.

Detection of Quinones

Borntrager's test: 3 ml of leaf extract was treated with 3 ml of chloroform and the chloroform layer was separated. To this 5% potassium hydroxide dissolution was added. The occurrence of red colour in the alkaline phase confirms the presence of Quinones [14].

Detection of Coumarins

NaOH test: 2ml of the extract was treated with a few drops of chloroform and later 10% Sodium hydroxide was added. The formation of yellow colour confirms the test [15].

The standard lead solution was prepared by dissolving 2g of lead acetate in 1L of distilled water. In two sets of experiments, we conducted one keeping the leaf concentration constant and the other with time as a constant. In the first set of experiments, three test tubes bearing 1g of pre-weighed dry leaf powder were mixed with a standard lead solution. The samples kept standing and were filtered after 24, 48, and 72 hours. The filtrate was then subjected to AAS analysis. In the second set of experiments, a varied amount of leaf powder (i.e., 2g, 3g, 4g, and 5g) was mixed with a standard lead solution while keeping the treatment period restricted to 12 hours. Each sample was filtered and subjected to AES analysis.

RESULTS AND DISCUSSIONS

The analysis shows that the crude extract of leaves of *Clematis terniflora* contains various constituents, including polyphenols, terpenoids, flavonoids, and tannins (Table I.)

Table. I. Preliminary qualitative phytochemical screening

Phyto-constituent	Test	Observation	Inference
Polyphenols	Ferric chloride test	Dark green colour	Present
Terpenoids	Salkowski test	Reddish brown colour	Present
Flavonoids	Lead acetate test	Yellow precipitate	Present
tannins	Braymer's test	Green precipitate	Present
Quinones	Borntrager's test	No red colour	Absent
Coumarins	NaOH test	No yellow fluorescence	Absent

AES analysis was carried out through Inductive- Coupled Plasma- Atomic Emission Spectroscopy. The absorbance of lead in the control group (1323.84 ppm) was significantly higher than all other concentrations of leaf powder, indicating that the use of *C. terniflora* is effective in reducing lead contamination in water. The highest concentration of leaf powder (5g) resulted in the absorbance of lead (12.707 ppm), which was significantly lower than the control (1323.84 ppm). With the increase in concentration of leaf powder, the absorption rate of lead decreases gradually. The AES result showed that there was effective absorption of lead; however, this absorption was seen to be forming a linear graph.

These results align with the findings of Swain *et al*. [18], who reported that *Eichhornia crassipes* showed an effective phytoremediation rate against copper and cadmium. The reduction in lead absorbance may be attributed to the presence of phytochemicals such as flavonoids, alkaloids, and terpenoids in *C. terniflora,* which have been reported to exhibit metal chelating and antioxidant activities [15]. However, it should be noted that this study only assessed the absorbance of lead in the water, and additional analysis, such as the measurement of plant biomass and metal accumulation in the plant tissue, would provide a more comprehensive understanding of the phytoremediation potential of *C. terniflora*. Furthermore, the effectiveness of *C. terniflora* as a phytoremediation agent for lead-contaminated water may vary depending on factors such as the initial concentration of lead, pH, and temperature of the water. The results support the potential use of *C. terniflora* leaf powder as a phytoremediation agent for lead-contaminated water. Further research is needed to fully evaluate its efficacy under varying conditions and to explore its potential use in field applications. The Pearson correlation coefficient between the different concentrations of leaf powder *of C. terniflora* and the corresponding absorbance of lead was calculated to be -0.977. This indicates a strong negative correlation between the two variables, meaning that as the concentration of leaf powder increases, the absorbance of lead decreases (Fig. I; Table II, III.).

The second experiment was conducted to evaluate the effect of varying time intervals on the phytoremediation potential of *C. terniflora* on lead-contaminated water. Different time intervals, including 0 hours (control), 24 hours, 48 hours, and 72 hours, were used to observe the phytoremediation potential of the plant. The absorbance of lead was evaluated using inductive-coupled plasma-atomic absorption spectroscopy (ICP-AAS). The results showed that the absorbance of lead was highest in the control (563 ppm); later, it gradually decreased with an increase in time intervals. The lowest absorbance of lead was observed after 72 hours (323 ppm).

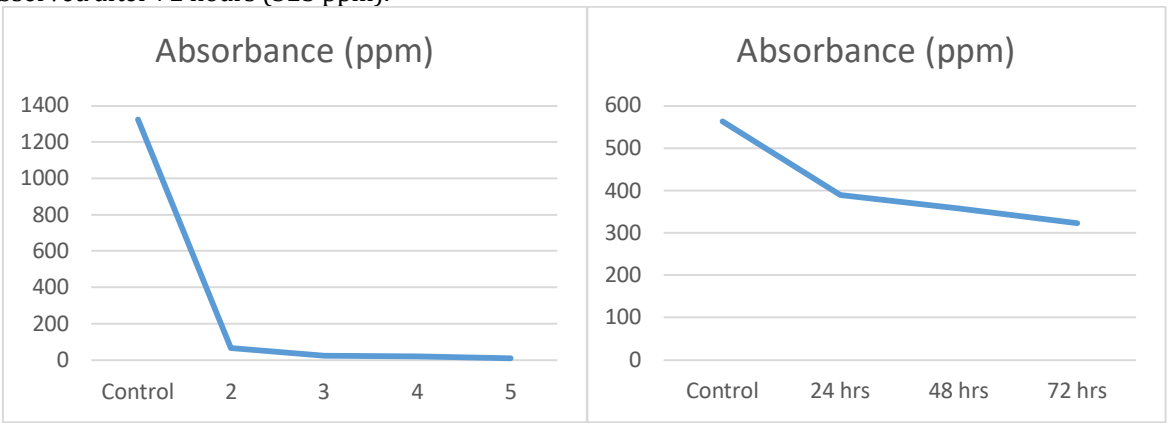

Figure I. Absorption of lead ions by varying leaf powder amount (gm) and varying time (hrs).

Table II: Absorbance of lead (in ppm) by varying amount of leaf powder of *C. terniflora* and by varying the time period

	Experiment 1 (leaf powder amount variation)			Experiment 2 (time variation)		
Test No.	**Amt of leaf powder (gm)**	**Time (hrs)**	**Absorbance (ppm)**	**Amt of leaf powder (gm)**	**Time (hrs)**	**Absorbance (ppm)**
	Control	12	1323.84	Control		563
1	2	12	65.891	1	24	389
2	3	12	23.707	1	48	357
3	4	12	19.208	1	72	323
4	5	12	12.707	-	-	-

This reduction in lead concentration is attributed to the phytoremediation process, where it showcases 563 ppm, 389 ppm, 357 ppm, and 323 ppm at the time intervals of 0 hours (control), 24 hours, 48 hours, and 72 hours effectively. These findings are consistent with previous studies that have reported a decrease in lead concentration in soil after the application of phytoremediation techniques [16, 17]. The Pearson correlation coefficient for the given data is -0.886, indicating a strong negative correlation between the time interval and the absorbance of lead concentration. This means that as the time interval increases, the absorbance of lead concentration by *C. terniflora* decreases (Fig. 1; Table 2, 3.).

The results suggest that *C. terniflora* has effective potential to remove lead from contaminated water through phytoremediation. However, the efficacy of the process may depend on the duration of exposure to the plant material. Further studies are required to investigate the optimal duration of exposure and other factors that may affect the phytoremediation potential of *C. terniflora* in lead-contaminated water.

1. $\boldsymbol{Pearson\ co-efficient\ (r) = \Sigma\ ((Xi - \bar{X}) \times (Yi - \bar{Y}))/((n-1) \times dX \times dY)}$

- Xi and Yi are the individual values of X and Y,
- n is the number of data points,
- dX and dY are the standard deviations of X and Y, respectively.

2. $\boldsymbol{Co-variance = \dfrac{(n \times \Sigma XY) - (\Sigma X \times \Sigma Y)}{n \times (n-1)}}$

- Where n is the number of data points (in this case, n = 4),
- ΣXY is the sum of the product of the deviations of X and Y from their respective means
- ΣX and ΣY are the sums of the deviations of X and Y from their respective means.

3. $\boldsymbol{pearson\ co-relation\ co-efficient\ (r) = \dfrac{Co-variance}{dX \times dY}}$

- dX and dY are the standard deviations of X and Y, respectively.

Table III. Pearson coefficient for experiment 1 and 2

	Amount of leaf powder (gm)	**Time (hrs)**	**Absorbance of Lead (ppm)**
EXPERIMENT 1 (PLANT AMOUNT VARIATON)			
	X		**Y**
1	2	12	1323.84
2	3	12	65.891
3	4	12	19.207
4	5	12	12.707
Mean	3.5	-	289.0678
Std. dev	1.118	-	624.245
Pearson coefficient (r)			**-0.977**
EXPERIMENT 2 (TIME VARIATION)			
		X	**Y**
1	Control	**-**	563
2	1	24	389
3	1	48	357
4	1	72	323
Mean	-	36	408
Std. dev	-	25.7	110.4
Co-variance			-2610.5
Pearson coefficient (r)			**-0.886**

CONCLUSION

In this study, *Clematis terniflora* leaf extract was used for phytoremediation, which contains polyphenols, terpenoids, flavonoids, and tannins with various biological activities, including antioxidant, antibacterial, antifungal, and anticancer properties. The results of the study demonstrated that phytoremediation using plant powder can be successful in removing contaminants such as heavy metals, organic pollutants, and radionuclides from water. The concentration of the leaf powder of *C. terniflora* affected the absorption of lead ions by the plant material, as the rate of ion absorption decreased with increasing the concentration of leaf powder. In the second set of experiments, the time duration was varied, and the concentration of leaf powder was kept constant in all the samples. The results showed that the absorption rate decreased at 72 hours, indicating a decrease in the rate of absorption. The Pearson correlation coefficient for varying the time period data was -0.886, indicating a strong negative correlation between the time interval and the absorbance of lead concentration. Similarly, the Pearson correlation coefficient between the different concentrations of leaf powder of *C. terniflora* and the corresponding absorbance of lead was -0.977, indicating a strong negative correlation between the two variables. The study demonstrated that phytoremediation using the plant powder of *C. terniflora* is a promising technology for water remediation, showing success in removing a considerable amount of lead ions from the sample. Further research is

needed to determine the most optimal absorption of ions by *C. terniflora*. The findings of this study will contribute to the understanding of the phytoremediation potential of *C. terniflora* for lead removal from water and may lead to the development of more efficient and sustainable methods for the removal of contaminants from water.

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