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Phytoremediation of Cadmium, Nickel and Lead from Wastewater by Limna gibba using Response Surface Methodology: Optimization Study

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ABSTRACT: Water pollution caused by toxic heavy metals remains a critical global issue, driving the search for sustainable and low-cost treatment technologies. This study explores a approach for removing cadmium (Cd), nickel (Ni), and lead (Pb) from industrial wastewater using phytoremediation with aquatic plants. Aquatic species Lemna gibba (Limna G.) was selected for its availability and potential as green remediation agent. In the phytoremediation experiments, synthetic wastewater solutions containing Cd, Ni, and Pb were exposed to the aquatic plant. Metals concentrations were monitored daily, while the percentage removal, was calculated to assess uptake efficiency. Optimization using response surface methodology (RSM) and central composite design (CCD) identified conditions that achieved high removal efficiencies across biomass load, exposure time, and initial concentrations. The plant, Limna G. exhibited the highest and fastest removal rates, particularly for Pb and Cd, classifying it as a hyperaccumulator. Overall, results demonstrate that the combined use of Limna G. for phytoremediation provides an effective, low-cost, and environmentally sustainable strategy for removing heavy metals from industrial wastewater. The dual use of biomass not only maximizes remediation efficiency but also reduces plant waste, offering a circular and eco-friendly treatment solution. The data was analyzed using the response surface methodology (RSM) in order to support activities that optimize the phytoremediation process. Three variables were included in a central composite design (CCD), which included 20 trials with 6 axial points and 6 repetitions at the center point. The most significant component in each experimental design response was determined by the analysis of variance (ANOVA). The prediction error between the experimental and predicted results for the removal of Cd, Ni, and Pb was 2.4, 1.8 and 0.3, respectively. This study recommended to use Limna gibba for the removal of Cd, Ni, and Pb from wastewater.

1. INTRODUCTION

Heavy metals are defined as metallic elements that have a relatively high density compared to water, it has harmful effects on human health, and exposure to these metals has been increased by industrial and anthropogenic activities and modern industrialization. Contamination of water and air by toxic metals is an environmental concern and hundreds of millions of people are being affected around the world. Food contamination with heavy metals is another concern for human and

animal health [1,2]. Heavy metals are released into the environment from various sources including mining, urbanization, chemical industry, sewage plants, pesticide plants, biomedical and unsafe agricultural practices [3].

The order of several specific metals' levels of toxicity to humans is $\text{Co} < \text{Al} < \text{Cr} < \text{Pb} < \text{Ni} < \text{Zn} < \text{Cu} < \text{Cd} < \text{Hg}$. The dosage, emission rate, and exposure duration of heavy metals determine their detrimental effects on humans. Hg, Cd, and Pb are a few heavy metals that have drawn more attention in recent decades [4]. Among its many applications, nickel, a silver-colored metal, is used to make coins, electronics, and stainless steel. An estimated 150 000 to 180 000 metric tons of Ni are released into the environment annually worldwide. Humans are exposed to Ni through food, air, and water. The WHO has recommended acceptable levels of 0.02 and 0.05 parts per million for Ni in agricultural soils and wastewater, respectively [5,6]. The WHO recommended safe limits of Cd in both wastewater and soils for agriculture is 0.003 ppm. The WHO recommended safe limits of Pb in wastewater and soils used for agriculture are 0.01 and 0.1 ppm respectively [7].

There are numerous methods available to remove heavy metals from the environment, including ion exchange, coagulation and precipitation with iron and aluminium salts, adsorption, electrocoagulation, membrane filtration, nanofiltration, electrolysis, reverse osmosis. These techniques, however, are expensive and negatively impact the impacted area. Consequently, novel alternative treatments utilizing inexpensive materials are required, like bioremediation, and phytoremediation [8].

Using real living green plants to remove pollutants from soils, air, surface water, and groundwater is a sophisticated and relatively new process called phytoremediation. Through this process, contaminants are taken up by the roots, stored in the tissues of the body, and broken down into less dangerous forms [9]. Because it is less expensive and takes less expertise to implement, phytoremediation the utilization of plants and allied microorganisms is one of the new technologies that offers developing nations an effective, economical, and sustainable way to accomplish this goal. This technique is an economical plant-based strategy for eliminating heavy metals from water [10]. Different aquatic plants have been used with considerable success in the wastewater phytoremediation process such as blue H Hyacinth (*Eichhornia* sp.), Duckweeds (*Lemna* sp., *Spirodella* sp.), Small water fern (*Azolla* sp.) and blue cabbage (*Pistia* sp.) have been used to remove heavy metals from wastewater [11, 12]. The *Lemna gibba* is a tiny, free-floating aquatic plant that is a member of the Lemnaceae family. Because they can develop quickly, are easier to harvest, and are more tolerant of cold than other species, duckweed species are used in water quality studies to assess heavy metals and are thought to be a superior alternative for wastewater treatment [13].

A common optimization technique used to investigate the connections between independent variables and input and output parameters as well as to achieve optimal circumstances is response surface methodology (RSM) software [14,15]. All things considered; chemical processes that can be explained by a second order polynomial equation can be optimized using RSM. It has been discovered that response surface methodology (RSM) is a helpful technique for researching how two or more variables interact [16]. RSM is used to estimate an area that satisfies the operating parameters and determine the system's ideal operating conditions [17]. The adsorption of Cd (II) on the root of *Calotropis Procera* was studied by optimized the quadratic model using CCD. The maximum removal percentage was observed at pH 4.48, residence time of 90.58 min, the adsorbent dosage of 2.40 g at an initial Cd (II) concentration of 60 mg/L [18]. It was concluded that RSM-CCD model was best fitted to predict and optimize the Cd and Pb reduction from the aqueous solution. The optimum concentration for Cd and Pb phytoremediation using water hyacinth was found 1.22 mg/L in 54 days experiment in which maximum amount of Cd and Pb was reduced from the aqueous solution [19].

This study explores *Limna gibba* potential as an aquatic plant for phytoremediation because there is a dearth of scholarly research on its application for heavy metal remediation. Synthetic industrial waste water contaminated with varying percentages of Cd, Ni, and Pb at varying exposure durations was treated by this plant at varying weights. Through the use of response surface methodology (RSM), this study seeks to optimize the removal effectiveness of these materials from waste water by optimizing exposure period, *Limna G.* weight, and heavy metals concentration. Because of its highest removal potential, as documented in earlier studies, Ni was chosen as a possible heavy metal to be eliminated utilizing aquatic plant systems out of all the early trials on heavy metals (including Cr, Cd, Ni, Cu, and Zn) [20].

2. MATERIALS AND METHODS

2.1 Aquatic plant

Limna gibba (Limna G.) was selected due to the high metal uptake capacity, rapid growth, environmental adaptability, and their ability to converted to biochar as adsorbent with enhanced physicochemical properties for heavy metal removal.

2.2 Synthesis of Cd, Ni, and Pb Wastewater

A series of combined solutions of Cd, Ni, and Pb (0.5, 1, 3, and 5 mg/L initial concentration) were prepared by diluted certified stock solution of heavy metals (1000 mg/L) Cd, Ni, and Pb, (Sigma-Aldrich, Germany) in distilled water to achieve the required concentrations in order to synthesis wastewater.

2.3 Statistical Analysis

The phytoremediation process parameters were studied with a standard response surface methodology (RSM) design called a central composite design (CCD). This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, and also to analyze the interaction between the parameters. Generally, the CCD consists of a $2n$ factorial runs with $2n$ axial runs and nc center runs (six replicates).

The phytoremediation removal of cadmium, nickel lead ions from synthesis industrial waste water was studied using (RSM). The input variables studied were (i) x_1 , initial concentration of heavy metals (Cd, Ni and Pb); (ii) x_2 , *Limna G.* weight and (iii) x_3 , treatment time. These three variables together with their respective ranges were chosen based on the literature and preliminary studies. The number of experimental runs from the central composite design (CCD) for the three variables consists of eight factorial points, six axial points and six replicates at the centre points indicating that altogether 20 experiments were required, as calculated from the below equation:

$$N = 2n + 2n + nc = 23 + 2 \times 3 + 6 = 20 \quad (1)$$

Where N is the total number of experiments required and n is the number of process variables.

The experimental sequence was randomized in order to minimize the effects of the uncontrolled factors. The response (Y_1 , Y_2 and Y_3) were cadmium, nickel and lead removals, respectively. The response was used to develop an empirical model which correlated the response to the three treatment process variables using a second-degree polynomial equation as given by below equation:

$$Y = b_o + \sum_{i=1}^n b_i x_i + \sum b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n b_{ij} x_i x_j \quad (2)$$

Where Y is the predicted ions removal response, the constant coefficient, b_i the linear coefficients, b_{ij} the interaction coefficients, b_{ii} the quadratic coefficients and x_i , x_j are the coded values of the phytoremediation ions removal variables. The parameters involved in the treatment were varied using the response surface methodology (RSM). The three variables studied were, x_1 is the initial concentration of heavy metals (Cd, Ni and Pb) in (mg/L), x_2 is the *Limna gibba* weight in (g), and x_3 is the phytoremediation treatment time in (day).

A total of 20 experiments were conducted based on the CCD design, including factorial points, axial points, and replicates at the central point. The quadratic polynomial model was most suitable for describing the relationship between the independent variables and the percentage removal efficiency. The design matrix consisted of three input variables (X_1 : concentration, X_2 : exposure time, X_3 : biomass) with 20 combinations, including six replicates at the center point to ensure reproducibility and statistical validity.

The responses were analyzed using analysis of variance (ANOVA) to test model adequacy, regression coefficients, and significance of interaction terms. The models were further validated by diagnostic plots such as normal probability plots, three-dimensional surface plots were generated to illustrate the influence of individual and interactive factors on heavy metal removal efficiency. The three dependent responses in this study were: Y_1 : Removal of Cd, Y_2 : Removal of Ni, and Y_3 : Removal of Pb.

The predicted error of experimental and predicted results for Cd, Ni and Pb removal calculated on the following equation:

$$\% \text{Prediction Error} = \frac{(\text{predicted} - \text{experimental})}{\text{predicted}} \times 100 \quad (3)$$

2.4 Phytoremediation experimental

The experiments performed using plastic containers. The Cd, Ni and Pb, phyto remediation efficiency of Lemna G. was assessed using 3L of synthesis wastewater with different initial concentrations over a period of 15 days. 30 g of plant used as active weight for the phyto remediation process. Syringe filter (Membrane: PES, Pore size: 0.45mm, Diameter: 25mm) used to collection synthesis wastewater samples for the purpose of determine Cd, Ni, and Pb concentrations before the experiments and, periodically at days 1, 5, 10, and 15. Atomic absorption spectrometer (Shimadzu, Model 7000, Japan) used for analyze concentrations. All experiments were performed in triplicate, and the results are reported as the mean. Metal removal efficiency calculates using equation (4):

$$\% \text{ (Removal)} = \frac{(C_o - C_e)}{C_o} \times 100 \quad (4)$$

where C_o and C_e (mg/L) are the concentrations of metals at initial and equilibrium, respectively.

3. RESULTS AND DISCUSSION

3.1 Phyto remediation removal matrix of Cd, Ni, and Pb using Lemna G.

Experimental data for the percentage removal of Cd, Ni, and Pb by phyto remediation using Lemna G. are illustrated in Table 1, which shows central composite design and experimental results, indicating the relationship between input parameters (Cd, Ni, and Pb concentration, mg/L), (exposure time, day), and the (weight of Lemna G., gm) and the response to removing (Cd, Ni, and Pb). The three factors were measured at three levels: low (−1), moderate (0), and high (+1). Different initial concentration of Cd, Ni, and Pb (0.5, 1,3, and 5.0 mg/L) were applied in this study.

Table 1. DOE matrix for Cd, Ni, and Pb removal using Lemna G.

Run	Initial Concentration, X1 (mg/L)	Exposure time, X2 (day)	Lemna G weight, X3 (g)	Cd removal, Y1 (%)	Ni removal, Y2 (%)	Pb removal, Y3 (%)
1	2.75	11.00	50.00	100	100	100
2	2.75	11.00	50.00	92	91	94
3	2.75	21.00	50.00	100	100	100
4	2.75	01.00	50.00	90	87	95
5	0.50	21.00	60.00	100	100	100
6	2.75	11.00	50.00	100	100	100
7	2.75	11.00	60.00	95	93	98
8	5.00	21.00	60.00	95	92	98
9	2.75	11.00	50.00	100	100	100
10	5.00	11.00	50.00	94	92	97
11	2.75	11.00	50.00	100	100	100
12	0.50	01.00	60.00	91	88	96
13	2.75	11.00	40.00	99	98	100
14	5.00	01.00	60.00	61	58	70
15	0.50	11.00	50.00	100	100	100
16	0.50	21.00	40.00	100	100	100
17	2.75	11.00	50.00	100	100	100

18	0.50	01.00	40.00	88	86	92
19	5.00	21.00	40.00	94	91	97
20	5.00	01.00	40.00	58	55	68

3.2 Phytoremediation ANOVA results of Cd, Ni, and Pb using Lemna G.

ANOVA revealed significant differences in the removal efficiency of Cd, Ni, and Pb by Lemna gibba ($p < 0.05$). The removal efficiency was influenced by metal concentration and treatment duration, with the magnitude of the effect varying among the three metals. *L. gibba* demonstrated substantial removal capacity for all tested metals, with Ni, Pb, and Cd showing removal efficiencies of approximately 100%, for Cd, Ni, and Pb.

The predicted responses obtained from RSM were compared to the actual responses for confirmation of the predicted data. According to the sequential model sum of squares, the models were selected based on highest order polynomials where the additional terms were significant and the models were not aliased. For the removal of metals, the quadratic model was selected as suggested by the software.

Positive sign in front of the terms indicates synergistic effect, whereas negative sign indicates antagonistic effect. The quality of the model developed was evaluated based on the correlation coefficient value. The closer the R^2 values to unity for metals yield equations, the better the model will be as this will give predicted values which are closer to the actual values for the response. The R^2 from these equations were considered high, indicating that there was agreement between the experimental and the predicted in the removal of cadmium, nickel and lead from this model.

The ANOVA for response surface quadratic model for Cd removal (Y1) onto Lemna G. is given in Table 2. The model (F-value) of 14.18 and (Prob > F) of less than 0.0001 implied that this model was also significant. The ANOVA for response surface quadratic model for Ni removal (Y2) onto Lemna G. listed in Table 3. The model (F-value) of 166.61 and (Prob > F) of less than 0.0001 implied that this model was also significant. The ANOVA for response surface quadratic model for Pb removal (Y3) onto Lemna G. is also given in Table 4. The model (F-value) of 58.82 and (Prob > F) of less than 0.0001 implied that this model was also significant. In the above case x_2 was significant model term whereas x_1 , x_3 , x_{12} , x_{22} , x_{32} , $x_1 x_2$, $x_1 x_3$ and $x_2 x_3$ were insignificant to the response of removal Cd. In the case of Ni, x_1 , x_2 and x_{22} were significant model term whereas x_3 , x_{12} , x_{32} , $x_1 x_2$, $x_1 x_3$ and $x_2 x_3$ were insignificant to the response of removal Ni. In the case of Pb phytoremediation removal by Lemna G., x_2 and x_{22} were significant model terms whereas x_1 , x_3 , x_{12} , x_{32} , $x_1 x_2$, $x_1 x_3$ and $x_2 x_3$ were insignificant to the response of removal Pb.

Table 2: ANOVA results for Cd phytoremediation removal by Lemna G.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	Prob > F
Model	2572.98	9	285.89	14.18	0.0001
X1	592.90	1	592.90	29.41	0.0003
X2	1020.10	1	1020.10	50.61	< 0.0001
X3	0.90	1	0.90	0.045	0.8369
X12	39.14	1	39.14	1.94	0.1937
X22	91.64	1	91.64	4.55	0.0588
X32	39.14	1	39.14	1.94	0.1937
X1X2	300.12	1	300.12	14.89	0.0032
X1X3	0.13	1	0.13	6.E-003	0.9388
X2X3	3.13	1	3.13	0.16	0.7020

Residual	201.57	10	20.16		
Lack of Fit	148.24	5	29.65	2.78	0.1431
R ²	92.7		86.2		

Table 3. ANOVA results for phytoremediation removal of Ni by Lemna G.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	Prob > F
Model	13970.63	9	1552.29	166.61	< 0.0001
X1	409.60	1	409.60	43.96	< 0.0001
X2	10176.10	1	10176.10	1092.20	< 0.0001
X3	36.10	1	36.10	3.87	0.0773
X12	1.45	1	1.45	0.16	0.7011
X22	1687.64	1	1687.64	181.13	< 0.0001
X32	1.64	1	1.64	0.18	0.6835
X1X2	253.13	1	253.13	27.17	0.0004
X1X3	3.13	1	3.13	0.34	0.5753
X2X3	15.13	1	15.13	1.62	0.2314
Residual	93.17	10	9.32		
Lack of Fit	93.17	5	18.63		
R ²	99.3		98.7		

Table 4. ANOVA results for phytoremediation removal of Pb by Lemna G.

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	Prob > F
Model	8830.39	9	981.15	58.82	< 0.0001
X1	547.60	1	547.60	32.83	0.0002
X2	5017.60	1	5017.60	300.80	< 0.0001
X3	25.60	1	25.60	1.53	0.2437
X12	3.84	1	3.84	0.23	0.6417
X22	1560.09	1	1560.09	93.53	< 0.0001
X32	3.84	1	3.84	0.23	0.6417
X1X2	684.50	1	684.50	41.03	< 0.0001
X1X3	2.00	1	2.00	0.12	0.7363
X2X3	32.00	1	32.00	1.92	0.1962
Residual	166.81	10	16.68		
Lack of Fit	166.81	5	33.36		
R ²	98.2		96.5		

3.3 Predicted three-dimensional plots of heavy metals Phytoremediation using Lemna G.

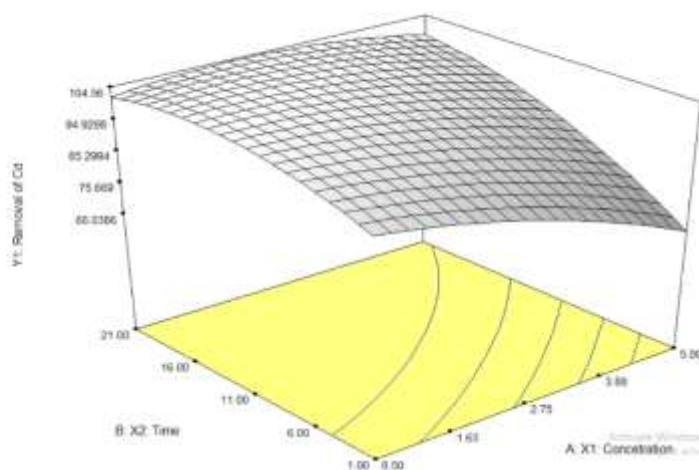
The three-dimensional plots were designed based on the experimental results. The results of the interaction between the input factors (initial concentration of metals used, exposure time and the weight of Lemna G.) on response (percentage removal of Cd, Ni, and Pb) was then analyzed by using polynomial regression.

The experimental observation for both the exposer time and initial concentration revealed that they have significant effects on the response of Cd, Ni, and Pb removal onto Lemna G., whereas Lemna G. weight showed the least effect on this response. The interaction effect between x1, x2 and x3 on the response of Cd, Ni, and Pb removals were found to moderate. Figures 1,2, and 3 shows the three-dimensional response and the interaction effects between the variable's initial concentration, exposer time and Lemna G weight on the Cd, Ni, and Pb removals.

DESIGN-EXPERT Plot

Y1: Removal of Cd
X = A: X1: Concentration
Y = B: X2: Time

Actual Factor
C: X3: Weight = 50.00

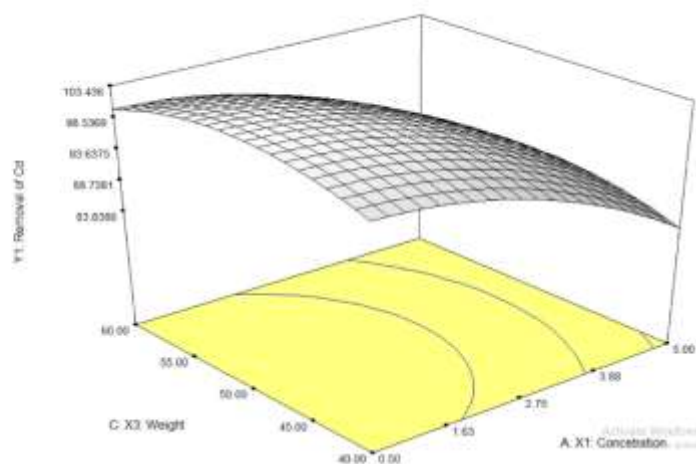


(a)

DESIGN-EXPERT Plot

Y1: Removal of Cd
X = A: X1: Concentration
Y = C: X3: Weight

Actual Factor
B: X2: Time = 11.00



(b)

DESIGN-EXPERT Plot

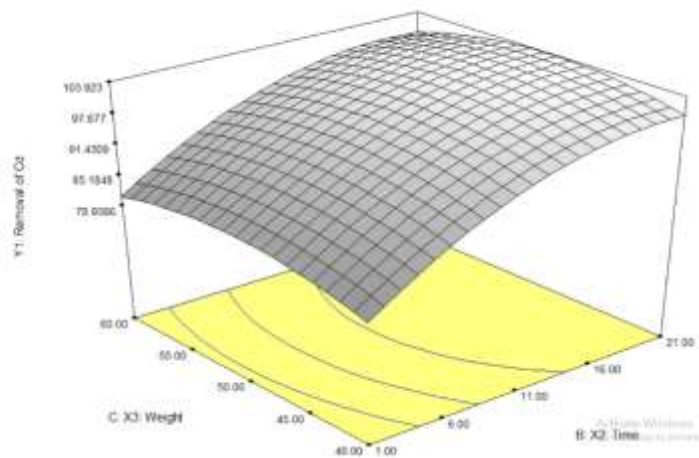
Y1: Removal of Cd

X = B: X2: Time

Y = C: X3: Weight

Actual Factor

A: X1: Concentration = 2.75



(c)

Figure 1: Combined effect of (a) initial concentration and exposure time, (b) initial concentration and Limna G. weight, (c) Limna G. weight and exposure time on phytoremediation removal of Cd

DESIGN-EXPERT Plot

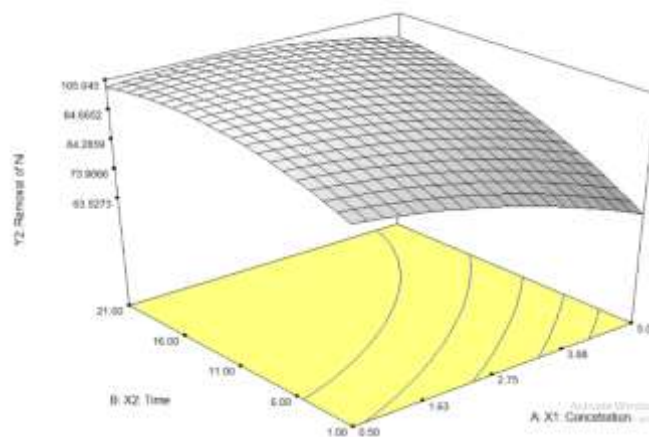
Y2: Removal of Ni

X = A: X1: Concentration

Y = B: X2: Time

Actual Factor

C: X3: Weight = 50.00



(a)

DESIGN-EXPERT Plot

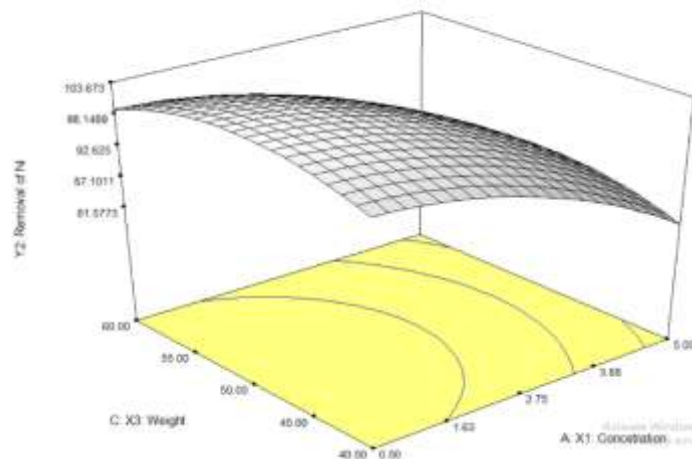
Y2: Removal of Ni

X = A: X1: Concentration

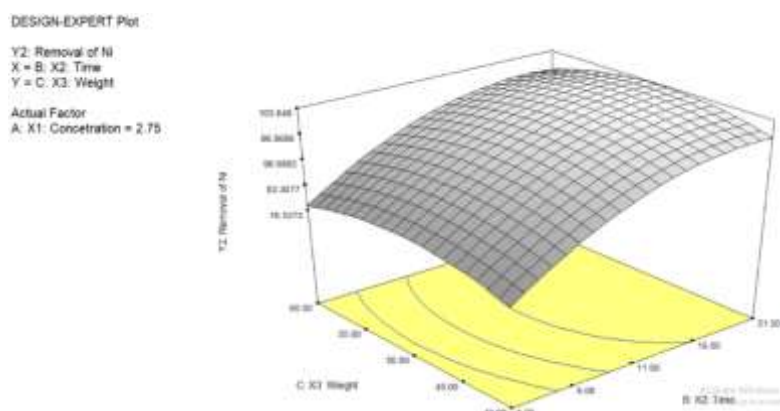
Y = C: X3: Weight

Actual Factor

B: X2: Time = 11.00

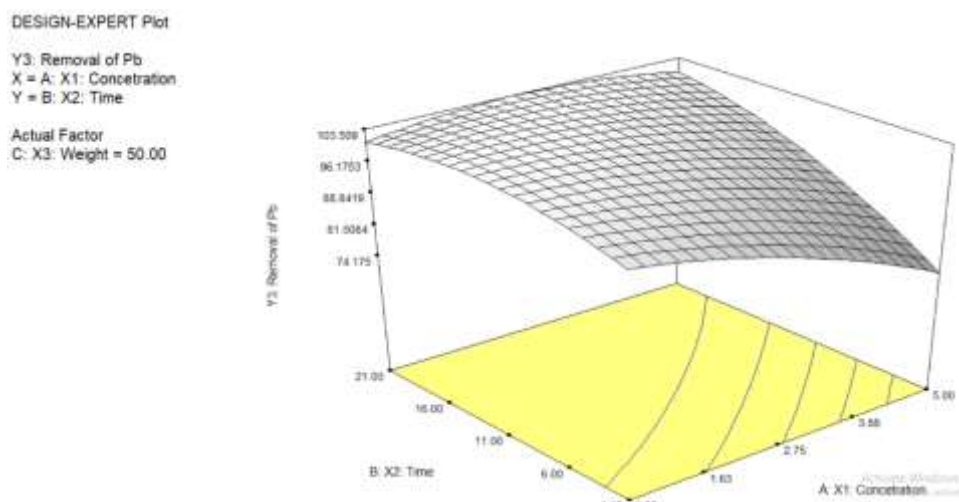


(b)

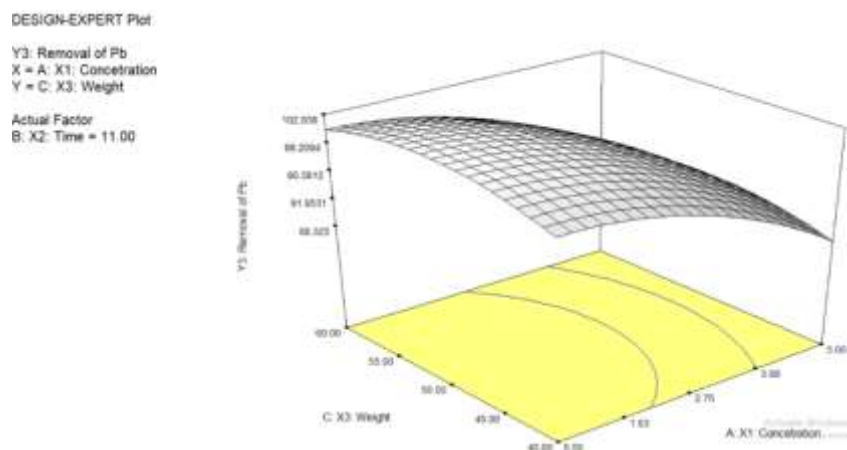


(c)

Figure 2: Combined effect of (a) initial concentration and exposure time, (b) initial concentration and Limna G. weight, (c) Limna G. weight and exposure time on phytoremediation removal of Ni



(a)



(b)

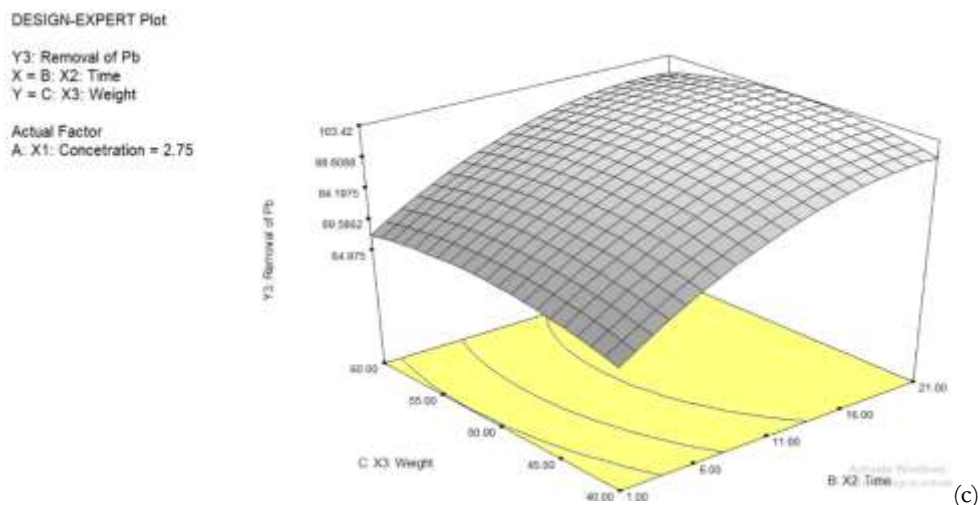


Figure 3: Combined effect of (a) initial concentration and exposure time, (b) initial concentration and Limna G. weight, (c) Limna G. weight and exposure time on phytoremediation removal of Pb

Figures 1,2 and 3 depict the effect of exposure time, the weight of aquatic plant selected and initial concentration on the response surface (removal of Cd, Ni, and Pb). The Figures shows the effect of Limna G. weight and initial concentration with weight of Limna G. being fixed at zero level ($W=50.0$ g), also the effect of exposure time and Lemna gibba weight exposure time with fixed at zero level of (11 day), and the effect of exposure time and Limna G. weight with initial concentration of elements being fixed at zero level ($\text{Conc.} = 2.75$ mg/L) on the response surface (removal of Cd, Ni, and Pb). It was observed from these Figures that the removal of Cd, Ni, and Pb by phytoremediation using Limna G. generally increased with increasing exposure time. More of Cd, Ni, and Pb were removed as a result of longer exposure times and lower starting Cd, Ni and Pb concentrations. The results demonstrated that the exposure duration and starting metals concentration had a significant impact on the reaction and percentage elimination of metals.

It was reported that the increase in exposure time and decrease in initial concentration of Ni resulted in an increased removal of Ni. Furthermore, increasing the time beyond 10 days did not significantly affect the percentage removal. Treatment with a longer exposure time and lower concentration resulted in a higher percentage removal of Ni. The findings showed that the response and percentage removal of Ni were highly influenced by the exposure time and initial concentration of Ni. At an early exposure time, the active sites for the media and plant surface adsorption were still emptied. With the increasing concentration of Ni, the number of metal ions also increased, which affected Ni removal.

3.4 Optimization results

According to the analysis of variance (ANOVA) the optimum predicted results of phytoremediation parameters (concentration, time and weight) and Cd, Ni and Pb removal obtained at optimum condition are listed in Table 5.

Table 5: Phytoremediation predicted optimum conditions of Cd, Ni, and Pb by Limna G.

No.	X1: Concentration, mg/L	X2: Time day	X3: Weight g	Y1: Cd Removal	Y2: Ni Removal	Y3: Pb Removal	Desirability
1	0.50	8.00	53.74	100.0	100.0	100.0	1.000
2	2.37	19.26	40.20	100.0	100.0	100.0	1.000
3	2.75	12.07	48.34	100.0	100.0	100.0	1.000
4	3.15	20.19	55.70	100.0	100.0	100.0	1.000
5	3.13	14.24	51.62	100.0	100.0	100.0	1.000

6	1.94	15.21	56.84	100.0	100.0	100.0	1.000
7	0.65	10.81	41.23	100.0	100.0	100.0	1.000
8	3.19	16.16	46.18	100.0	100.0	100.0	1.000
9	2.45	16.66	54.82	100.0	100.0	100.0	1.000
10	1.99	14.12	48.60	100.0	100.0	100.0	1.000

4. CONCLUSION

A starting concentration of 0.5 mg/L, an exposure period of 8 days, and a *Limna G.* weight of 53.7 g were the optimum parameters for the highest removal of Cd, Ni, and Pb from wastewater. The findings showed that, in ideal circumstances, a removal rate of 100.0% could be accomplished for these three metals. *Limna G.* is a suitable plant for the phytoremediation of Cd, Ni, and Pb, according to the current study. *Limna gibba* is therefore advised for the removal of these metals from wastewater, and the data gathered from this study can be applied to large-scale pilot projects.

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AUTHOR CONTRIBUTIONS

Alaa Jassem Mohammed: Conceptualization, Investigation.

Alaa Jassem Mohammed: Methodology, Laboratory Analysis.

Raad Abdulhadi Nayyef: Data Curation, Formal Analysis.

Jasim Mohammed Salman: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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