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The Ability of Ceratophyllum Demersum to Remediate Polluted Water with Cadmium, Nickel and Lead: Optimization Study

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ABSTRACT: Phytoremediation is efficient, eco-friendly and economic selected for the treatment using Ceratophyllum demersum as aquatic plant. The physical characterization of Ceratophyllum demersum shows structural characteristic contributed significantly to the high removal efficiency.

Heavy metal contamination of aquatic environments is a significant environmental concern due to the toxicity and persistence of metals such as cadmium (Cd), nickel (Ni), and lead (Pb). This study investigated the potential of Ceratophyllum demersum biomass for the simultaneous removal of Cd, Ni, and Pb from aqueous solutions.

Response Surface Methodology (RSM) was employed to optimize the treatment conditions and identify the combination of parameters that maximized the predicted removal of the three metals. The initial metal concentrations ranged from 0.5 to 5 mg/L. The optimization results indicated that the maximum predicted simultaneous removal could be achieved at an optimum treatment time of approximately 18.3 days and a biomass dosage of 51.8 g. These findings suggest that Ceratophyllum demersum has potential as a plant-based material for the removal of multiple heavy metals from contaminated water. Furthermore, the application of RSM provided an effective approach for identifying optimal treatment conditions. Further experimental validation is recommended to confirm the predicted optimum and evaluate the feasibility of applying this method to real wastewater treatment systems.

1. INTRODUCTION

Heavy metal pollution of the environment is becoming a bigger issue and a major worry because of the negative consequences it is having globally. Due to inappropriate waste disposal, fertilizers, pesticides, and the constantly expanding agriculture and metal industries, these inorganic pollutants are being dumped into our soils, rivers, and environment (Jessica Briffa, et. al., 2020). The problem of heavy metal pollution in soil and water is becoming more widespread and poses serious threats to both human health and the integrity of the ecosystem. Ecosystems are contaminated by a variety of heavy metals, which are released into the environment by both natural and man-made processes, including coal mining, leather manufacture, metal processing, agriculture, and the disposal of industrial waste. Heavy metals are very poisonous and have a propensity to accumulate in organisms. The removal of heavy metals from contaminated soil and water must be done immediately due to their detrimental consequences [1-7].

Multiple industries produce different types of wastewaters, each of which contributes to the overall wastewater produced (Lin et al., 2012). For example, the textile industry produces wastewater with high levels of organic pollutants, dyes, and heavy metals [8]; food processing produces wastewater rich in suspended solids, fats, and organic matter [9]; pharmaceuticals produce wastewater with active pharmaceutical ingredients (APIs) and other chemical contaminants [10]; and metal processing produces wastewater with heavy metals and other toxic substances [11].

Metals that are frequently detected in industrial effluent might originate from many sources, and each one has various uses and possible adverse effects on health. Below is a summary of several important metals. Cadmium is mostly utilized in nickel-cadmium batteries, ceramic and plastic pigments, and as a stabilizer in some plastics [12]. Concerning the health risks associated with cadmium exposure, it is a carcinogen and can damage kidneys and induce osteoporosis. Respiratory issues and lung damage may result from prolonged exposure [13]. The WHO and EPA set the standard level for Cd in both drinking water and wastewater at 0.003 and 0.005 mg/L, respectively [14]. The production of batteries, electroplating, and stainless steel are the primary sources of nickel. Nickel is utilized in battery components, catalysts, and alloys. According to Genchi et al. (2020), nickel exposure has been related to lung cancer, skin allergies, and respiratory issues. Prolonged exposure can exacerbate pre-existing diseases, cause systemic problems, cardiovascular disease, and have carcinogenic effects. The WHO set the standard level for Ni in drinking water at 0.07 mg/L. The WHO recommends 0.02 mg/L as the safe level for Ni in wastewater. The primary sources are the production of paint, metal plating, batteries, and mining activities. It is extensively utilized in radiation shielding, lead-based solder, lead-acid batteries, and pigments. In terms of health risks, lead is extremely toxic and can have a negative impact on the kidneys, reproductive system, and nervous system. Lead exposure in children can result in cognitive and developmental problems. Adults who are exposed repeatedly may develop hypertension, cardiovascular issues, and kidney impairment. The WHO and EPA set the standard level for Pb in drinking water at 0.01 and 0.015 mg/L, respectively. Meanwhile, it is 0.05 and 0.015 mg/L in wastewater according to the WHO and EPA, respectively [15].

Heavy metals are removed from aqueous solutions by various methods such as adsorption, ion exchange, reverse osmosis, coagulation and flocculation, advanced oxidation, ultra-filtration and photoirradiation [3,4].

Using aquatic plants to absorb, collect, and cleanse contaminants from wastewater which called phytoremediation. This has the advantages of being inexpensive, long-lasting, and suitable for contaminated or expansive areas. Additionally, it enhances the landscape's attractiveness [3]. It could take place in several ways: Phytoextraction: After taking up pollutants from the soil or water, plants concentrate them in their tissues. Phytostabilization: plants stabilize pollutants in the soil or water, hence lowering their mobility and bioavailability. Phytodegradation: Pollutants are converted by plants or the microbes that coexist with them into less dangerous compounds. Limmer and Burken (2016) state that phytovolatilization is the process by which contaminants from the soil or water are taken up by plants and released as volatile molecules into the atmosphere.

Aquatic plants are essential to phytoremediation because they clean up wastewater. *Ceratophyllum demersum* one of these of aquatic plants that are employed in phytoremediation. It is floats and growth well in a range of water situations growth in rivers, ponds or artificial wetlands, use for wastewater treatment can be utilized with *Ceratophyllum demersum*, it aids in clearing the water of impurities and pollutants with great potential for water phytoremediation.

Response Surface Methodology (RSM) was selected in this study due to its proven effectiveness in modelling and optimizing processes influenced by multiple interacting variables. RSM allows simultaneous evaluation of several independent variables and their interaction effects while significantly reducing the number of experimental runs. RSM offers a balanced combination of experimental efficiency, interpretability of results, and statistical rigor. Furthermore, RSM has been widely applied in similar studies, enabling meaningful comparison and validation of findings with previously published work [13-18].

In this research study, 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 of heavy metals selected.

2. MATERIALS AND METHODS

2.1 Aquatic plant

Ceratophyllum demersum (*Ceratophyllum* D.) was selected due to the rapid growth, environmental adaptability, and for heavy metal removal. The *Ceratophyllum* D. was collected from the Tigris River near Baghdad city, and transferred to the laboratory.

2.2 Heavy metals

The chemicals, cadmium (Cd), nickel (Ni) and lead (Pb) used in this research study and their properties are listed in Table 1

Table 1: List of chemicals used in this work

Chemical	Supplier	Concentration	Appearance
Cadmium (Cd)	Sigma–Aldrich, Iraq	1000 ppm	Colourless liquid
Nickel (Ni))	Sigma–Aldrich, Iraq	1000 ppm	Clear blue-green solution
Lead (Pb)	Sigma–Aldrich, Iraq	1000 ppm	Colourless liquid

2.3 Response surface methodology (RSM)

Response Surface Methodology (RSM) was selected in this study due to its proven effectiveness in modelling and optimizing processes influenced by multiple interacting variables. RSM allows simultaneous evaluation of several independent variables and their interaction effects while significantly reducing the number of experimental runs. The literature consistently reports that RSM provides reliable second-order polynomial models capable of describing both linear and non-linear system behaviour, which is essential for identifying true optimal conditions. Compared with other statistical and computational optimization techniques, RSM offers a balanced combination of experimental efficiency, interpretability of results, and statistical rigor. Furthermore, RSM has been widely applied in similar studies, enabling meaningful comparison and validation of findings with previously published work [19-25].

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.

The phytoremediation removal of cadmium, nickel lead ions from synthesis wastewater was studied using (RSM). The input variables studied were (i) x1, initial concentration of heavy metals (Cd, Ni and Pb) with range of (0.5-5.0 mg/L); (ii) x2, *Ceratophyllum D.* weight with range of (50-60 g), and (iii) x3, treatment time with range of (1-21 day). These three variables interact together with their respective ranges were chosen based on the literature and preliminary studies for the maximum removal of metals. The number of experimental runs from the central composite design (CCD) for the three variables consists of 20 experiments were required [3,4,6]. The responses were analyzed using analysis of variance (ANOVA) to test model adequacy, regression coefficients, and significance of interaction terms. The three dependent responses in this study were: Y1: Removal of Cd, Y2: Removal of Ni, and Y3: Removal of Pb.

Based on the RSM software, Table 2, illustrate the generated matrix, which outlined 20 trials involving varying conditions for initial metals concentrations, *Ceratophyllum D.* biomass, and treatment durations. According to this matrix, twenty phytoremediation experiments were conducted in the laboratory.

Table 2: RSM matrix for Cd, Ni, and Pb removal using *Ceratophyllum D*

Run	Type	Initial Concetration, X1 (mg/L)	Exposer time, X2 (day)	Ceratophyllum weight, X3 (g)	Cd removal Y1 (%)	Ni removal, Y2 (%)	Pb removal, Y3 (%)
1	Center	2.75	11.00	55.00			
2	Center	2.75	11.00	55.00			
3	Axial	0.50	11.00	55.00			
4	Axial	2.75	21.00	55.00			
5	Center	2.75	11.00	55.00			
6	Fact	0.50	1.00	60.00			
7	Axial	2.75	11.00	60.00			
8	Fact	5.00	21.00	50.00			
9	Fact	5.00	1.00	60.00			
10	Fact	0.50	21.00	50.00			
11	Center	2.75	11.00	55.00			
12	Center	2.75	11.00	55.00			

13	Fact	0.50	21.00	60.00			
14	Fact	5.00	01.00	50.00			
15	Axial	2.75	11.00	50.00			
16	Center	2.75	11.00	55.00			
17	Fact	0.50	1.00	50.00			
18	Axial	2.75	1.00	55.00			
19	Axial	5.00	11.00	55.00			
20	Fact	5.00	21.00	60.00			

2.4 Phytoremediation experimental

According to the above matrix, 20 phytoremediation experiments done for different initial metals concentrations, *Ceratophyllum D.* biomass, and treatment durations. Each experiment using four plastic containers (4L for each) that had been previously cleaned made comprised the experimental setup. Three litres of synthesis industrial wastewater of combined Cd, Ni, and Pb. Amount of fresh aquatic plant biomass of *Ceratophyllum D.* as mentioned for each experiment add to the containers and left for the duration mentioned.

The whole 20 experiments achieved; wastewater samples were collected in order to measure metals concentrations. The water samples were then analyzed for elements by means of Atomic Absorption Spectroscopy (AAS) (AA-7000, Shimadzu, Japan).. The results were expressed as a mean of outcomes of the three experimental runs. The removal efficiency of heavy metals calculates using equation (1):

$$\% \text{ (Removal)} = \frac{(C_o - C_e)}{C_o} \times 100 \quad (1)$$

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

3. RESULTS AND DISCUSSION

3.1 Phytoremediation removal of Cd, Ni, and Pb using *Ceratophyllum D.*

Experimental data for the percentage removal of Cd, Ni, and Pb by phytoremediation using *Ceratophyllum D.* are illustrated in Table 3, which shows central composite design and experimental results matrix, indicating the relationship between input parameters (Cd, Ni, and Pb concentration, mg/L), (exposure time, day), and the (weight of *Ceratophyllum D.*, g) and the response to removing (Cd, Ni, and Pb). Different initial concentration of Cd, Ni, and Pb (0.5, 1,3, and 5.0 mg/L) were applied in this study.

Table 3: Predicted results matrix for Cd, Ni, and Pb removal using *Ceratophyllum D.*

Run	Initial Concentration, X1 (mg/L)	Exposer time, X2 (day)	<i>Ceratophyllum</i> weight, X3 (g)	Cd removal Y1 (%)	Ni removal, Y2 (%)	Pb removal, Y3 (%)
1	2.75	11.00	55.00	95	90	100
2	2.75	11.00	55.00	95	90	100
3	0.50	11.00	55.00	100	95	100
4	2.75	21.00	55.00	97	93	100
5	2.75	11.00	55.00	95	90	100
6	0.50	1.00	60.00	60	50	80
7	2.75	11.00	60.00	97	92	100
8	5.00	21.00	50.00	98	96	100
9	5.00	1.00	60.00	25	21	41
10	0.50	21.00	50.00	100	100	100
11	2.75	11.00	55.00	95	90	100
12	2.75	11.00	55.00	95	90	100
13	0.50	21.00	60.00	100	100	100
14	5.00	01.00	50.00	21	18	35
15	2.75	11.00	50.00	92	88	100
16	2.75	11.00	55.00	95	90	100

17	0.50	1.00	50.00	51	40	70
18	2.75	1.00	55.00	41	39	50
19	5.00	11.00	55.00	92	88	100
20	5.00	21.00	60.00	100	98	100

3.2 ANOVA Predicted phytoremediation removal results of metals using *Ceratophyllum D*

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 R^2 values for metals yield equations which corresponding to unity, the better the model will be as this will give predicted values which are closer to the actual values for the response, 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 *Ceratophyllum D.* is given in Table 4., the model (F-value) of 330.5 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 *Ceratophyllum demersum* is as listed in Table 5, 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 *Ceratophyllum demersum* is also given in Table 6. The model (F-value) of 58.82 and (Prob > F) of less than 0.0001 implied that this model was also significant.

In all these three cases x1, x2, and x22 were significant model terms whereas x3, x12, x32, x1 x2, x1x3 and x2x3 were insignificant to the response of removal Cd, Ni, and Pb.

The experimental observation for both the exposurer time and initial concentration revealed that they have significant effects on the response of metals removal onto *Ceratophyllum D.* whereas *Ceratophyllum D.* weight showed the least effect on this response. The quadratic effect of exposurer time was also higher compared to the quadratic effects of initial concentration and plant weight on the same response.

Table 4: phytoremediation results for (Cd) removal by *Ceratophyllum D.*

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	Prob > F
Model	13266.59	9	1474.07	330.46	< 0.0001
X1	562.50	1	562.50	126.10	< 0.0001
X2	8820.90	1	8820.90	1977.48	< 0.0001
X3	40.00	1	40.00	8.97	0.0135
X12	2.51	1	2.51	0.56	0.4708
X22	1865.51	1	1865.51	418.21	< 0.0001
X32	0.82	1	0.82	0.18	0.6775
X1X2	496.12	1	496.12	111.22	< 0.0001
X1X3	1.13	1	1.13	0.25	0.6264
X2X3	15.13	1	15.13	3.39	0.0954
Residual	44.61	10	4.46		
Lack of Fit	44.61	5	8.92		
R^2	99.7		99.4		

Table 5: phytoremediation results for (Ni) removal by *Ceratophyllum D.*

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 6: phytoremediation results for (Pb) removal by *Ceratophyllum D.*

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 Phytoremediation removal of Cd, Ni, and Pb using *Ceratophyllum D.*

The effect of exposure time and initial concentration on the response surface (removal of Cd, Ni, and Pb) with weight of *ceratophyllum D.* (Figures not shown) being fixed at zero level (W=55.0 g), the effect of *ceratophyllum D.* weight and initial concentration on the response surface (removal of Cd, Ni, and Pb) with exposure time (Figures not shown) being fixed at zero level of (11 day), and effect of exposure time and *ceratophyllum D.* weight on the response surface (removal of Cd, Ni, and Pb) with initial concentration of elements being fixed at zero level (Conc.= 2.75 mg/L). It was observed that the removal of Cd, Ni, and Pb by phytoremediation using *ceratophyllum* 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 Cd, Ni and Pb concentration had a significant impact on the reaction and percentage elimination of metals. The media and *ceratophyllum* surface adsorption active sites were still depleted at an early exposure time. The quantity of metal ions rose along with the metal's concentration, which had an impact on Cd, Ni, and Pb elimination.

3.4 Optimization of phytoremediation parameters

The optimum phytoremediation conditions for Cd, Ni, and Pb removal by *Ceratophyllum D.* aquatic plant were determined using RSM. ANOVA results provided the best combinations of initial concentration, exposure time, and plant biomass that maximize metal removal summarize in Table 7.

Table 7: Predicted optimum solutions for phytoremediation of (Cd, Ni, and Pb) by *Ceratophyllum D.*

No.	X1: Concentration	X2: Time	X3: Weight	Y1: Cd Removal	Y2: Ni Removal	Y3: pb Removal	Desirability
1	1.90	18.13	51.85	100.00	100.00	100.00	1.000
2	1.28	18.09	50.68	100.00	100.00	100.00	1.000
3	2.77	18.19	59.83	100.00	100.00	100.00	1.000
4	1.72	14.55	59.13	100.00	100.00	100.00	1.000
5	2.25	17.00	55.30	100.00	100.00	100.00	1.000
6	3.13	17.52	55.87	100.00	100.00	100.00	1.000
7	2.58	18.62	56.30	100.00	100.00	100.00	1.000

8	2.13	17.27	52.29	100.00	100.00	100.00	1.000
9	3.08	18.40	58.82	100.00	100.00	100.00	1.000
10	5.00	18.65	53.19	100.00	98.66	100.00	0.995

4. CONCLUSION

The Response Surface Methodology (RSM) results demonstrated that the optimum conditions for maximizing the simultaneous removal of cadmium (Cd), nickel (Ni), and lead (Pb) from aqueous solutions were approximately 18.3 days of treatment time and 51.8 g of *Ceratophyllum demersum* biomass, at initial metal concentrations ranging from 0.5 to 5 mg/L. These findings highlight the potential of *Ceratophyllum demersum* as a promising plant-based material for the simultaneous removal of multiple heavy metals from contaminated water. The optimized conditions obtained through RSM provide a useful basis for further experimental validation and the development of efficient and sustainable wastewater treatment strategies. According to the results of this study, complete removal of cadmium, nickel, and lead was achieved under optimal conditions: initial metal concentrations of 1–5 mg/L, a treatment period of 14–18 days, and aquatic plant (*Ceratophyllum D.*). biomass of 50–60 g.

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AUTHOR CONTRIBUTIONS

Alaa Jassem Mohammed: Investigation, Laboratory Analysis. Data Curation, Formal Analysis

Zahraa Najim Abd: Methodology, Conceptualization

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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