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APPLICATION OF D-OPTIMAL DESIGN IN OPTIMIZING MASS RATIO OF BIOMASS TO POLLUTANT FOR NICKEL PHYTOREMEDIATION BY *Ceratophyllum* sp.

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Abstract

Industrial wastewater contaminated with heavy metals when discharged to water surface is a global concern to ecosystem as it can cause harm to environment. Phytoremediation of pollutants using naturally sources like submerged plant of *Ceratophyllum* sp. has significant advantages. In this test, *Ceratophyllum* sp., taken from Tigris River in Iraq, was exposed to different concentrations of Nickel (Ni) (2, 5, and 10 mg/L), and different plant masses (M) (2, 4.2, and 8 g) to fix the ratio of plant mass to Ni mass ($R_{p/Ni}$) (800 to 16000) for 21 days. Twelve beakers of 400 mL volume were used and each was filled up to 250 mL of synthetic Ni-contaminated water. At the same time, the optimum condition for mass ratio of plant to Ni ($R_{p/Ni}$) and time exposure were further determined using D-optimal design based on the maximum removal efficiency of Ni. The D-optimal Design optimisation gave results for $R_{p/Ni}$ of 16000 at day 1 with 84.1% removal efficiency. A validation test at the optimum conditions suggested by the model was conducted in order to validate this model, and it resulted 76% for the maximum Ni removal with error less than 8%. This gives evidence that findings are highly valuable for heavy metal removal with native plant of *Ceratophyllum* sp. from Tigris River.

INTRODUCTION

The rising application of heavy metals in industries becomes the main contributor to environmental problems. Increasing attention regarding soils and water contaminated with heavy metals has emphasized the urgency for treatment technologies that is environmental friendly such as phytoremediation [1]. There is a global need to utilise plants to restore the ecological environment [2] for soil and water contaminated with heavy metals to avoid direct toxicity either to human beings or other living organisms [3].

Heavy metals are elements that exist naturally and have high atomic weight and are denser than water by five times or more [4]. Nickel (Ni), commonly and heavily used in modern technologies, is one among of the major pollutants in water [5]. The least health related effects related with Ni is skin allergies while it can be dragging into more serious health effects such as lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation [6]. Heavy metal pollution in water resources such as rivers and lakes is due to the increased industrial advance along with insufficient available treatment methods [7]. The environmental pollution due to heavy metals is progressively becoming more serious and has attracted large concern owing to the adverse effects it is causing around the world [8]. Traditional techniques has been used in the treatment of metal-polluted water such as chemical precipitation, adsorption, membrane filtration, ion exchange, and electrochemical separation [9]. However, traditional methods are expensive and low effaceable leading to the search for more economy techniques such as biosorption by bacteria [10, 11] and plants in which the utilization of plants through phytoremediation that has emerged as a good substitutional approach [10]. A lot of studies have utilized phytotechnology to uptake heavy metals including iron and aluminium [12-16], lead and chromium [17, 18] and various types of heavy metals [19]. Qadri et. al., tested *Ceratophyllum demersum* with 2, 4, and 6 ppm of Mn, Cu, Co, Cr, Cd, and Zn, and they demonstrated that the plant has reliable metal removal properties and can be used for the removal of the metals from the contaminated water bodies [20].

The optimization for mass ratio of plant to Ni ($R_{p/Ni}$) in phytoremediation studies is infrequent, thus, we have employed a response surface methodology (RSM) using the D-optimal experimental design (DOD) in this study to optimize the required mass of plant in treating wastewater contaminated with different Ni concentrations through phytoremediation. In the current research, two objectives were targeted: (1) to determine the required mass of plant with different Ni concentrations to remove Ni, and (2) to determine the maximum removal of Ni from Ni-contaminated water using RSM through a D-optimal experimental design by optimizing the mass ratio of plant to Ni ($R_{p/Ni}$) and time exposure.

MATERIALS AND METHODS

Experiment Work Set-Up

This research was done in an open environment at University of Baghdad. First, the *Ceratophllyum* sp. was collected from Tigris River in Baghdad. Nickel sulfate salt was used ($NiSO_4.6H_2O$) for its ability to quickly dissolved in distilled water with 800 rpm and 20 °C. A total of 12 400 mL-beakers were filled with 250 mL of synthetic wastewater contaminated with different nickel concentration of 2, 5, and 10 mg/L as shown in **Figure 1**. After that, three different masses of *Ceratophyllum* sp. (2, 4.2, and 8 g) were poured in nine beakers for each concentration and another three beakers acted as control contaminant (without plants) for each concentration 2, 5, and 10 mg/L.

Selection of Plant and Physical Water Quality Parameters

The plant species of *Ceratophyllum* sp. was selected because it is a native perennial plant that has the ability to grow under different conditions throughout the years and also has the ability to endure contaminants. *Ceratophyllum* plant species is available in large quantities in Tigris River. In this research, we look forward to using it in a beneficial way and withdrawing pollution from rivers and lakes because of its great abundance and require less cost. The plant was freshly collected from the river and directly exposed to the synthetic contaminated water without any acclimatization. Three different masses were used to get different mass ratio of plant to Ni ($R_{p/Ni}$) (800 to 16000) for 21 days (**Table 1**). A physical observation of *Ceratophyllum* sp. growth was performed for 21 days at different plant masses and Ni concentrations to determine plant mass that can tolerate with different Ni concentrations.

A multi-probe of IQ 150 (IQ Scientific Instruments, UK) was used throughout experimental work to monitor the physicochemical changes of treated water in which temperature (T, °C), conductivity (COND, µS), total dissolved solid (TDS mg/L), and pH were recorded. All parameters were measured directly in beakers throughout the 21 days of exposure.

Ni Analysis in Water

20 mL of water samples were taken from each beaker at each sampling day (0, 6, 11, 16, and 21) and poured in clean tubes. No nutrient was added during the test. The Ni content in the medium of water was analyzed using Atomic Absorption Spectrophotometer (Model AA-7000, Shimadzu, U.S.A.). The following Equation (1) was used to determine the removal of Ni from the contaminated water at each sampling day:

$$\%\text{Nickel removal} = \left(\frac{Ni_0 - Ni_d}{Ni_0}\right) \times 100 \quad (1)$$

with, Ni_0 is the concentration at day 0, Ni_d is the concentration at each sampling day.

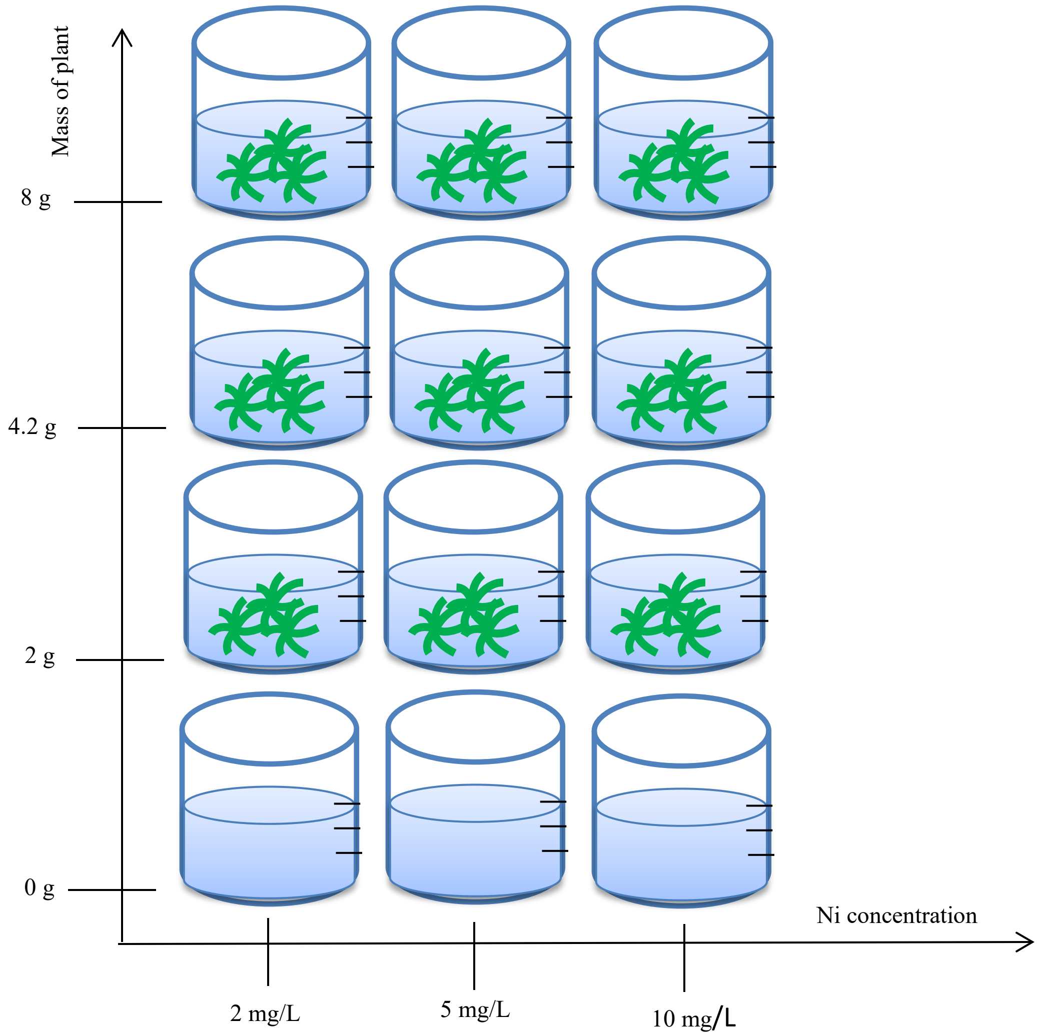

Table 1. Selection Ni concentrations and plant mass to get ratio ($R_{p/Ni}$).

Ni Concentration (mg/L)	Mass of Ni (g)	Mass of *Ceratophyllum* sp. (g)	Mass Ratio = Mass of *Ceratophyllum* sp. (g) / Mass of Ni (g)
2	0.0005	2	4000
2	0.0005	4.2	8400
2	0.0005	8	16000
5	0.00125	2	1600
5	0.00125	4.2	3360
5	0.00125	8	6400
10	0.0025	2	800
10	0.0025	4.2	1680
10	0.0025	8	3200

Statistical Analysis for Removal Efficiency

All data obtained from studies were subjected to statistical analysis using SPSS version 16 (SPSS Inc., USA). The results were presented as mean ± standard deviation (SD) for all experiments conducted in triplicate. Two-way analysis of variance (ANOVA) [21] and Pearson linear correlation were practical to investigate the significance of Ni removal as dependent variable with time, Ni concentration and plant mass as independent variables. Duncan's test for post hoc multiple comparisons with the *p* value of significant at 0.05 [22] for all analyses was considered for any differences between the values obtained in the result of analyses.

Independent Variables and Response for Optimization Test

In the optimization study, the interaction between the main factors and the response was studied. The independent variables selected were the mass ratio of plant to Ni ($R_{p/Ni}$) and exposure time. While, the response was the removal of Ni from the contaminated water. **Table 2** includes the variables in this design as time (1, 11 and 21 days) and the mass ratio of plant to Ni ($R_{p/Ni}$) (800, 8400, and 16000). An appropriate model for these factors can be applied for future study and for larger scale application.

Table 2. The levels of variables in the D-Optimal design.

Independent variables	Symbols	Code levels		
		-1	0	+1
Time (day)	A	1	11	21
Mass ratio of plant to Ni (g/g)	B	800	8400	16000

Optimization of Ni Removal with D-Optimal Design (DOD)

The optimization of Ni removal from water by *Ceratophyllum* sp. was conducted by applying Design Expert version 10 (State Ease Inc., USA) with a DOD. All experiments for Ni removal from water by *Ceratophyllum* sp. as a response (dependent variable), *Y*, were designed according to DOD with two independent variables of time (A) and mass ratio of plant to Ni (B). With two independent variables and one response, the DOD suggested 12 total experiments, including two replicates, to minimize random errors (**Table 3**). In this optimization, the mathematical relations between the responses and the variables can be represented by a quadratic model.

Table 3. Experimental runs of DOD and response for Ni removal from water.

Run no.	Time	Ratio	Response (%)
1	21	800	82.5
2	21	16000	100
3	11	8400	53.6
4	11	16000	100
5	1	16000	83
6	1	800	20.4
7	11	800	82.5
8	1	8400	64.6
9	21	8400	100
10	21	16000	100
11	21	800	82
12	1	16000	83.2

RESULTS AND DISCUSSION

Plant Tolerance to Ni and Physical Parameter Monitoring

Throughout 21 days of the experiment, *the Ceratophyllum* sp. was seen in good condition after the passing this period with respect to the three concentrations of 2, 5, and 10 mg/L (**Figure 2**).

The variations in parameters of pH, Temperature (°C), Electrical Conductivity (EC), Total dissolved solid (TDS) were recorded in the chemical lab as shown in **Table 4** for all beakers with and without plants. The results illustrate that the temperature mean values ranged between 27 °C and 29 °C throughout the 21 day-exposure which is normal weather during November in Iraq. The water medium pH of the beakers were ranged from 7 to 9 which do not differ significantly between the treatments (p<0.05). Next, EC, and TDS values were ranged between 39.3-363.0 μS, and 21.3-394.0 mg/L respectively. All results of EC, and TDS parameters were due to dissolved Ni contamination and impurities from plant parts throughout the 21-day treatment.

Beaker set up

Day 0

Day 21

Figure 2: physical observation of *Ceratophyllum* sp.

Table 4. Variation of physical water quality parameter throughout 21-day exposure.

Parameter	With plants	Without plants
T (℃)	25 -28	25 - 29
pH	7 - 9	6 - 9
EC (μs)	125.2 - 363	39.3 - 207
TDS (mg/L)	85.8 - 394	21.3 - 132

Ni Removal Efficiency by *Ceratophyllum* sp.

Figure 3 displays the Ni removal percentage for three different Ni concentrations (2, 5 and 10 mg/L) and plant masses (2, 4.2, and 8 g). It was crystal clear that there were significant differences in Ni removal by *Ceratophllyum* sp. between three different concentrations and plant masses (p<0.05). For Ni concentrations of 5 and 10 mg/L, the removal efficiency increased when plant mass increased, while for 2 mg/L (the low concentration), the best removal efficiency achieved after 1 day was 86%.

The correlation coefficients for Ni removal with time, Ni concentrations and plant masses are presented in **Table 5**. Ni removal showed a significantly correlation (r=+0.606, p<0.01) with time, while it was negatively correlation (r=-0.367, p<0.01; r=-0.351, p<0.01) related to Ni concentrations and plant masses. It is concluded that the Ni removal decreased with increasing Ni concentration and plant mass throughout the 21-day experimental period. High Ni concentration has affected the ability of plant mass to accumulate contaminant in *Ceratophyllum* sp. It can be concluded that high Ni concentrations has greatly impacted *Ceratophyllum* sp.; however, the growth of *Ceratophyllum* sp. with Ni phytoremediation is in parallel to the findings of Parnian et. al., [23]. Parnian et. al., demonstrated that the *Ceratophyllum demersum* was very effective in removing Ni (2 mg/L) with 52.5% efficiency.

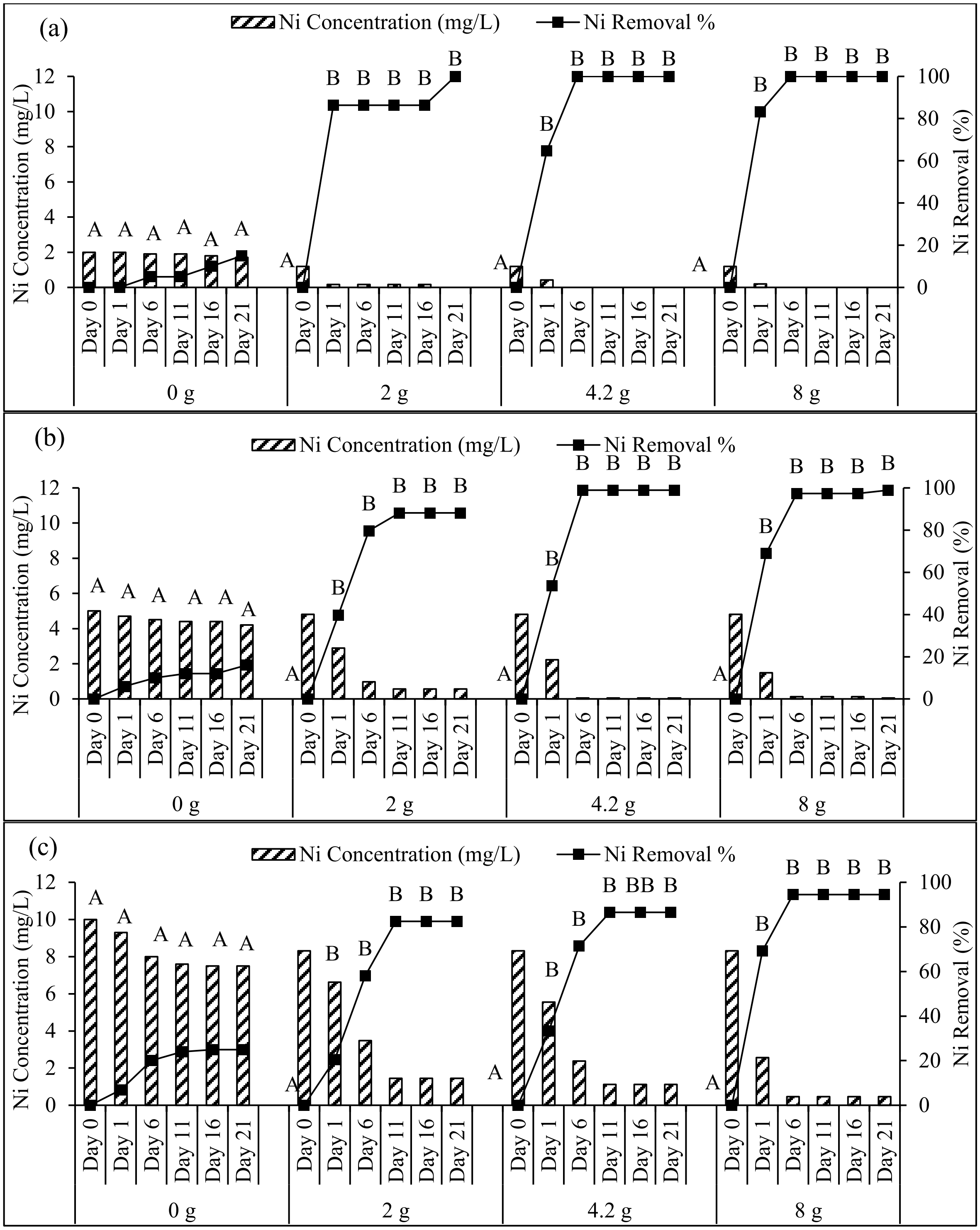

Figure 3: Variation in Ni concentration and its removal for different Ni concentrations, (a) 2 mg/L, (b) 5 mg/L and (c) 10 mg/L, according to plant mass (2, 4.2 and 8 g) and control (without plants). Different letters of A and B denote statistically significant differences of Ni removal from water between treatment with plant and control (treatment without plant) for the different mass of plant within same day ($p < 0.05$).

Table 5. Correlation coefficients for Ni removals with time, concentration, and mass of plant.

		Time	Ni concentration	Mass of plants
Ni Removal	Pearson Correlation	.606**	-.367**	.351**
	Sig. (2-tailed)	0	0	0.001
	N	90	90	90

** Correlation is significant at the 0.01 level

Optimization of Operating Variables on Ni Removal Using D-Optimal Design Analysis of variance

The interaction of the independent variable parameters including time (A) and mass ratio of plant to Ni (B) with the Ni removal by *Ceratophyllum* sp. was further optimized using DOD. Equation (2) is the quadratic regression model given by the RSM model to correlate the two independent variables with the predicted response (Ni removal) as follows:

$$\text{Ni Removal} = +75.81+18.33 \times \text{A}+16.82 \times \text{B} - 4.61 \times A^2+4.34 \times \text{B}^2-10.09 \times A \times B \quad (2)$$

The ANOVA statistics of the Ni removal responses are summarized in **Table 6**. The associated *p*-value of model (0.0446) was lower than 0.05, implying that the quadratic model of Ni removal was statistically significant. In addition, the model is also within the range of factors. The DOD model fits well for Ni removal with adequate precision of 6.859 (F-value of 4.626351) [24]. Furthermore, the two factors have significantly affected the Ni removal ($p<0.05$). With the given R^2 correlation coefficient of 0.794 for the model, it reveals a good fit between the experimental values and the regression model. And the model is proven to be significant by a relatively medium value of the adjusted R^2 coefficients (R^2adj= 0.622). The signal to noise ratio is determined by the "Adeq Precision" with value greater than 4 indicates an adequate signal. For this model, the signal to noise ratio was found to be 6.658 (for Ni removal from water), hence, the quadratic model can be used to pilot the design space [25].

Table 6. ANOVA results for RSM Model.

Source	Sum of Squares	DF	Mean Square	*F*-value	Prob > F	Significance
Model	4860.173	5	972.0346	4.626351	0.0446	significant
A	2848.724	1	2848.724	13.55836	0.0103	
B	2398.705	1	2398.705	11.41652	0.0149	
A^2	46.66683	1	46.66683	0.222109	0.6541	
B^2	41.40838	1	41.40838	0.197081	0.6726	
AB	664.9119	1	664.9119	3.164616	0.1256	
Residual	1260.65	6	210.1083			
Pure Error	0	3	0			
Cor Total	6120.823	11				

R^2=0.794, R^2adj=0. 622, adequate precision=6.859, *Significant at $p<0.05$

RSM for the Optimization of Mass Ratio of Plant to Ni

Figure 4 shows the combined effect of time (A) and ratio of plant mass to Ni mass (B) on the Ni removal. Based on this result, the removal was determined to be affected by time (A) and mass ratio of plant to Ni (B). The highest achievable removal was 100% for plant masses (4.2 and 8.0 g) at 6 days as depicted in **Figure 3**. However, at a high ratio of 16000, the removal percentage was 83.2% at day 1 for plant mass (2.0 and 4.2 g) with the low Ni concentration of 2 mg/L. Based on these results, it could be concluded that high plant mass accelerated Ni removal within low Ni concentration. The finding was in good agreement with [26], that reported *Azolla filiculoides* removal efficiency of Ni increased from 40% to 70% at 10 days along with the increasing mass from 2.0 to 8.0 g. The optimum conditions given by the model with a desirability of 0.895 for maximum Ni removal (84.1%) by setting the exposure time as minimum and mass ratio as in the range, were at 1 day with mass ratio of 16,000 (**Table 7**).

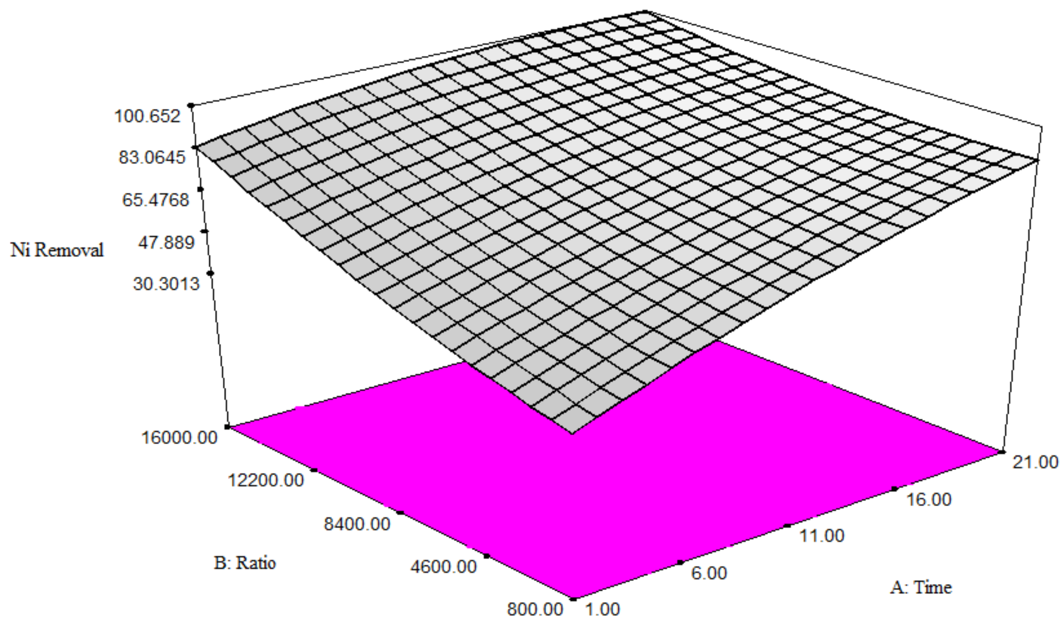

Figure 4: 3D response surface plot of time (A) and ratio of plant mass to Ni mass (B) on the Ni removal.

Table 7. Optimum conditions for Ni removal by *Ceratophyllum* sp.

Time (day)	Mass ratio $R_{p/Ni}$, (g/g)	Ni Removal (%)	Desirability
1.0	16,000	84.1	0.895

Validation Experiment of Ni Removal by *Ceratophyllum* sp.

A validation tests were conducted under the optimum conditions given by the model to confirm the predicted optimum response. The phytoremediation by *Ceratophyllum* sp. experiments was repeated with hourly observation at optimum conditions of 1 day and 16,000 $R_{p/Ni}$ (**Table 7**), with 2 mg/L of Ni concentration and of 8.0 g of plant mass (**Figure 5**).

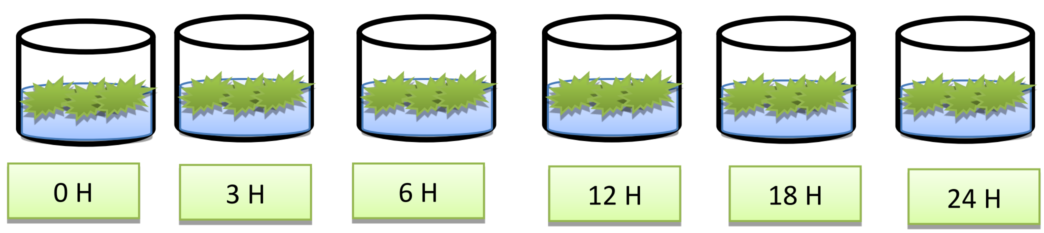

Figure 5: The hourly observation with optimum conditions of *Ceratophyllum*.

As shown in **Figure 6**, 68.0% Ni removal was achieved after 3 h of exposure (under the optimum conditions) while 76.0% for Ni removal from water after 1 day. This result was close to the predicted value (84.1%), which indicates the viability of the model to optimize Ni removal from contaminated water. The deviations between the measured and predicted values were within 8%. According to Li et al. (2021), 10% of the relative error between model and validation experiment was acceptable [27]. Therefore, it can be concluded that the regression models were appropriate in their reduced forms. The results show that *Ceratophyllum* sp. has ability to biosorb Ni contaminant. Aquatic plants are able to absorb heavy metals like Ni and stores in their biomass [28].

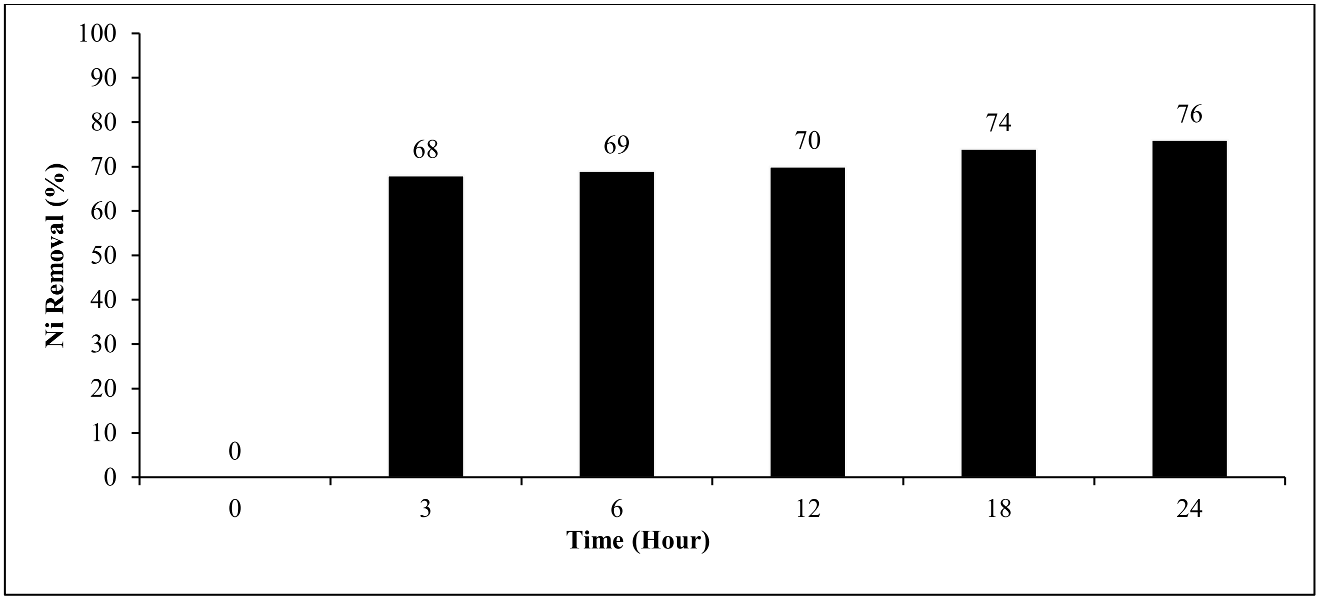

Figure 6: Ni removal within 24 h of exposure using *Ceratophyllum* sp. with 16,000 ratio of plant mass to 2 mg/L Ni mass during validation test.

CONCLUSIONS

A perennial plant species, *Ceratophyllum* sp. was selected to remove nickel (Ni) from contaminated water with respect to mass ratio of plant to Ni, and the optimum conditions of nickel (Ni) removal were determined. The optimum conditions given by the RSM model were at 1 day with a mass ratio of 16,000 that will give 84.1% for the Ni removal. According to a validation run with a mass ratio of plant to Ni of 16,000 and 1 day, 76.0% of Ni removal was achieved with only 8% error between the model and experimental values. Thus, *Ceratophyllum* sp. is a good candidate to remove nickel in the future because of its abundant availability in large amount and will contribute to low cost for the water treatment.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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