

# Optimisation of phytoremediation of zinc ion by *Lemnaceae* using response surface methodology

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**Abstract.** Phytoremediation, an eco-friendly approach, offers a green pathway towards cleaner and safer water resources. The efficiency of zinc ion removal through phytoremediation by *Lemnaceae* as a function of *Lemnaceae* species, the mass of duckweed and the initial zinc ion concentration was studied. The central composite design was applied for surface response modelling to analyse the effects of the combination of environmental factors and optimise the phytoremediation process. The model achieved a satisfactory prediction with a coefficient of determination ( $R^2$ ) of 0.985 based on a modified quadratic model for the optimised phytoremediation process. Furthermore, the analysis of variance (ANOVA) was conducted to assess the adequacy and significance of the generated model. The optimal zinc ion removal of 88 % could be achieved by *Spirodela polyrhiza*, *Wolffia arrhiza* and *Lemna minor* at the initial zinc ion concentration of 92.92 mg/L, 39.5 mg/L, and 21.08 mg/L and duckweed mass of 22.56 g, 22.38 g, and 24.81 g under the exposure of ambient conditions with sufficient daylight exposure.

## 1 Introduction

Phytoremediation involves the usage of specific plant species, known as accumulators, to remove contaminants from polluted sources and mitigate environmental damage. The accumulator has evolved specific strategies to cope with environmental threats, as the damage cannot be evaded [1]. During phytoremediation, contaminants are sequestered within the plant biomass or transformed into less harmful forms, ultimately restoring the health and quality of the water sources [2]. As a cost-effective and environmentally friendly method, phytoremediation holds immense potential for wastewater treatment efforts, offering a sustainable pathway towards cleaner and safer water resources.

Duckweed, a floating aquatic plant, is highly adaptable to its living environment, prefers to survive in tropical and temperate regions, and can be found worldwide [3]. Duckweed is well known as a rapidly growing angiosperm with a short doubling time, and it can reproduce

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at 39-105 tons of dry weight per hectare per year [4]. Historical studies have proved the potential of duckweeds in treating types of pollutants, either organic or inorganic pollutants from polluted sources, during phytoremediation with an efficiency of 50% to 80%, depending on the adjustment of the environmental conditions [5–7]. However, optimising phytoremediation through varying environmental conditions still requires further discussion and exploration. The existing study did not provide insight into the combined effects of significant environmental factors during phytoremediation by various duckweed species. The performance of phytoremediation cannot be accurately estimated without adequately developing response surface modelling [8]. Optimization using statistical and mathematical models has been proven to improve the quality and effectiveness of phytoremediation [9]. In this research, the phytoremediation was optimised through response surface methodology to obtain the optimal zinc ion removal efficiency for the short-term phytoremediation by various duckweed species.

## 2 Methodology

### 2.1 Materials and chemicals

Zinc nitrate ( $\text{Zn}(\text{NO}_3)_2$ , 97%) and Hoagland's No. 2 basal salt mixture were purchased from Sigma-Aldrich. Distilled water was used during the experiment. The duckweeds were obtained from the local sellers.

### 2.2 Preparation of cultivation environment

All the duckweed was cultivated in 1/10 diluted Hoagland's solution for one week before being transferred to the synthetic wastewater. The synthetic wastewater was prepared by dissolving the zinc nitrate in the distilled water and performing the dilution based on the desired concentration. After one week of assimilation, the duckweed was washed under the running tap water to remove the dirt attached to the plants. The healthy duckweed with average frond length of 7 mm (*Spirodela polyrhiza*), 3 mm (*Lemna minor*) and 1 mm (*Wolffia arrhiza*) were screened out for phytoremediation purposes.

### 2.3 Phytoremediation of zinc ion by duckweeds

Three different treated duckweed species were transferred to the disposable plastic containers filled with 500 mL of synthetic wastewater. The duckweeds were cultivated in ambient conditions and exposed to sunlight. The removal rate of zinc ions from the synthetic wastewater was analysed after one week of phytoremediation. The zinc ions uptake by the plants was analysed with Optima 7000 DV inductively coupled plasma optical emission spectrometer. The removal efficiency of the zinc ions from the wastewater sources can be determined through Equation (1).

$$\text{Removal efficiency} = \frac{C_0 - C_t}{C_0} \times 100 \% \quad (1)$$

where

$C_0$  = initial concentration of ion, mg/L

$C_t$  = concentration of ion at time  $t$ , mg/L

## 2.4 Optimisation studies

The response surface methodology was conducted through Design Expert (version 11.0.4, state-Ease) to determine the optimal operating conditions of the phytoremediation. The three significant parameters were selected from the preliminary studies as independent variables during the response surface modelling. The independent variables are coded as -1, 0 and +1, representing low to high levels. A central composite design was used to develop the model. Table 1 summarises the ranges and levels of independent variables studied, the range of which was fixed based on a series of preliminary tests.

**Table 1.** Actual values of the dependent variables and their respective coded levels.

Variables	Factors	Unit	Actual values for the coded levels				
			-α	-1	0	+1	+α
<b>Numerical</b>							
Mass of macrophyte	$x_1$	g	0.8579	5	15	25	29.1421
Initial Zn ion concentration	$x_2$	mg/L	3.4316	20	60	100	116.568
Coded level							
			L1	L2	L3		
<b>Categoric</b>							
Duckweed species	$x_3$		<i>Spirodela polyrhiza</i>	<i>Lemna minor</i>	<i>Wolffia arrhiza</i>		

The model was analysed with a second-order polynomial modelling equation, as shown in Equation (2), where  $R$  is the response,  $\beta_0$  is the intercept,  $\beta_i$ ,  $\beta_{ii}$ , and  $\beta_{ij}$  are interaction coefficients of linear, quadratic and second-order terms,  $x_i$  and  $x_j$  are independent variables, and  $\varepsilon$  is the error. The ANOVA was conducted to ensure the model generated was statistically significant at a 95 % confidence level. The F-test was employed to assess the adequacy of the developed model in predicting variations within experimental data without surpassing the confidence limit. Furthermore, the three-dimensional response was created to evaluate the influence of each factor and the interactions between parameters.

$$R = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^k \beta_{ij} x_i x_j + \varepsilon \tag{2}$$

## 3 Results and discussion

### 3.1 Regression analysis

The zinc ion removal efficiency during phytoremediation by duckweed species ranged from 0 to 87.3 % in response to the variation in the environmental conditions during phytoremediation. The final equation of the response in terms of coded factors obtained with a coefficient of determination of 0.985 is shown in Equation (3).

$$\begin{aligned} \text{Response}_{\text{pred}} = & 25.22 + 6.91x_1 - 9.01x_2 + 20.14x_3[1] - 31.01x_3[2] + 1.16x_1x_2 - 1.78x_1x_3[1] + \\ & 1.06x_1x_3[2] + 2.24x_2x_3[1] - 1.52x_2x_3[2] - 10.73x_1^2 + 0.8419x_2^2 + 6.49x_1x_2x_3[1] - 4.56x_1x_2x_3[2] \\ & - 8.29x_1^2x_3[1] + 14.48x_1^2x_3[2] - 4.29x_2^2x_3[1] + 12.26x_2^2x_3[2] \end{aligned} \tag{3}$$

Table 2 demonstrates the data of the ANOVA for the developed model through response surface methodology. The high  $R^2$  obtained indicates a good fit between the model and the data. The zinc ion removal efficiency in the model was highly effective in explaining the variation observed in the mass of macrophytes, initial zinc ion concentration and the

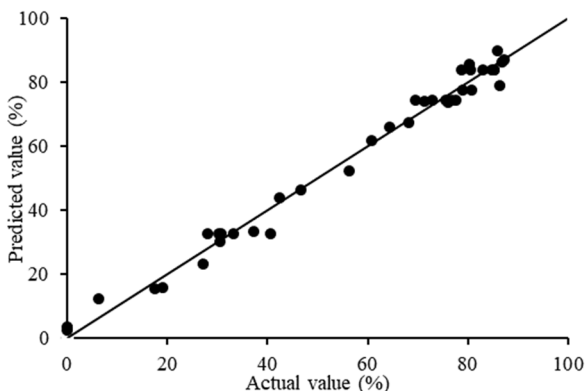
duckweed species. This implied that about 98 % of the variations in the zinc ion removal efficiency during phytoremediation could be explained by the independent variables and only 2 % of the total variability in the response could not be clarified by the model. The model F-value of 81.47 implied the model was significant, with just a 0.01% probability that such a large F-value could occur by chance alone. In this model, the dependent variable  $x_1$ ,  $x_2$ ,  $x_3$ ,  $x_1^2$ ,  $x_1x_2x_3$ ,  $x_1^2x_3$ , and  $x_2^2x_3$  were significant model term with p-value <0.05. However, the insignificant model terms have remained in this model to support the model hierarchy. The lack of fit test proved that the developed model was insignificant to the lack of fit, which implied that there was a 7.22 % possibility that such a large F-value could occur by chance due to noise. The non-significant lack of fit suggested that the model has good predictability. Furthermore, the adequate precision of 28.25, which was greater than 4 is desirable to claim the adequate model discrimination.

Figure 1 shows the predicted zinc ion removal efficiency versus the experimental values. It was observed that the predicted value calculated from Eq. (1) was well-fixed with the experimental values and provided an accurate response estimate. Hence, this model could be used to estimate the zinc ion removal efficiency in the variation of environmental conditions.

**Table 2.** ANOVA analysis for reduced cubic model.

Factor	Sum of squares	Degrees of freedom	Mean square	F-value	p-value	
Model	28463.88	17	1674.35	81.47	<0.0001	Significant
$x_1$	15409.33	1	15409.33	749.76	<0.0001	
$x_2$	1946.41	1	1946.41	94.70	<0.0001	
$x_3$	4816.41	2	2408.46	117.19	<0.0001	
$x_1x_2$	16.17	1	16.17	0.79	0.3851	
$x_1x_3$	38.64	2	19.32	0.94	0.4065	
$x_2x_3$	62.58	2	31.29	1.52	0.2413	
$x_1^2$	2402.39	1	2402.39	116.89	<0.0001	
$x_2^2$	14.79	1	14.79	0.72	0.4058	
$x_1x_2x_3$	266.31	2	133.15	6.48	0.0064	
$x_1^2x_3$	2201.84	2	1100.92	53.57	<0.0001	
$x_2^2x_3$	1614.78	2	807.39	39.28	<0.0001	
Residual	431.60	21	20.55			
Lack of fit	280.72	9	31.19	2.48	0.0722	insignificant
Pure error	150.87	12	12.57			
Corrected total	28895.48	38				

$R^2 = 0.985$ ; adequate precision = 28.25

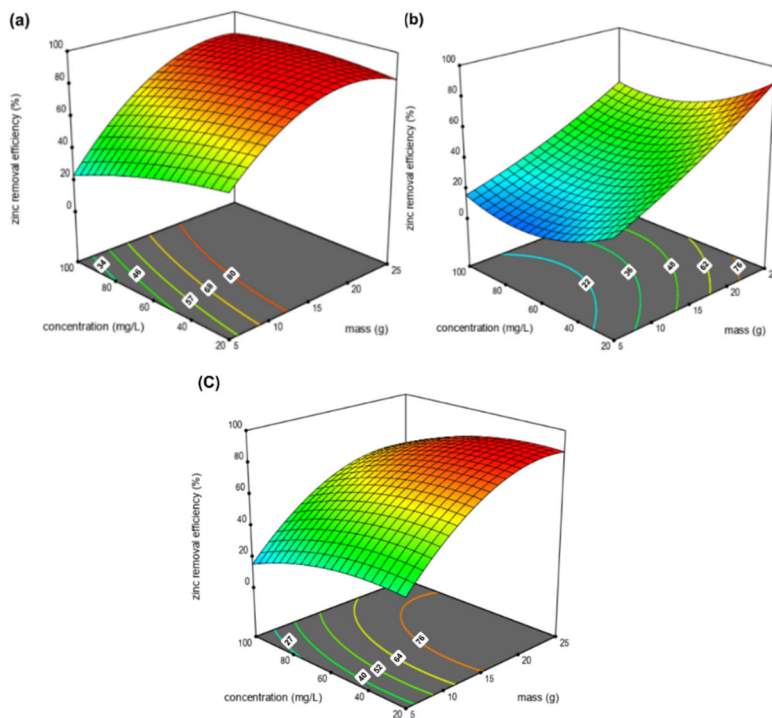


**Fig. 1.** Actual and predicted values of zinc ion removal efficiency.

### 3.2 Optimisation conditions and response surface analysis

Figure 2(a) shows the three-dimensional response surface plot of zinc ion removal efficiency during phytoremediation by *Spirodela polyrhiza*. It can be observed that zinc ion removal efficiency as high as 80 % could be achieved by treating the wastewater with 12 g of *Spirodela polyrhiza*. When the mass of macrophytes increased, the zinc ion removal efficiency increased. In contrast, the increasing initial zinc ion concentration contributed to a lower removal efficiency. By comparing run 3 and run 4, it can be observed that a high initial zinc ion concentration (100 mg/L) might require a higher mass of *Spirodela polyrhiza* to achieve the optimum zinc ion removal efficiency. This might be due to the saturation of the sorption sites, which subsequently inhibited the ion's sorption process when the pollutant concentration was high [10]. As a result, the sorption process was restricted, and the phytoremediation rate was slowed down.

Figure 2(b) and Figure 2(c) show the three-dimensional response surface plot of zinc ion removal efficiency during phytoremediation by *Lemna minor* and *Wolffia arrhiza*, respectively. For *Lemna minor*, increasing the macrophyte's mass was required to improve the zinc ion removal efficiency even at a low initial zinc ion concentration. It can be observed that the trend of zinc ion removal efficiency by *Wolffia arrhiza* followed a similar trend as *Spirodela polyrhiza*. Still, its zinc ion removal efficiency was lower than that of *Spirodela polyrhiza* under the same environmental conditions. By comparing run 4, run 17 and run 21, it can be observed under similar environmental conditions, the zinc ion removal efficiency was higher by *Spirodela polyrhiza* (80.21 %) compared to *Lemna minor* (60.73 %) and *Wolffia arrhiza* (64.39 %). This might be attributed to the number of roots owned by the duckweeds, as the larger surface area for contaminant uptake was available with the increasing root length and density [11]. The genus *Spirodela polyrhiza* has 7 to 21 roots per frond with round or point root cap, while the genus *Lemna minor* has only one root per frond. In contrast, the species of *Wolffia* lacks root [12]. Furthermore, the fibrous roots with a longer length increased the surface area exposed for contaminant uptake, which is preferable during phytoremediation [13]. Hence, *Spirodela polyrhiza* performed excellent zinc ion removal efficiency during phytoremediation among the studied *Lemnaceae* species.



**Fig. 2.** Three-dimensional response surface plot of zinc removal efficiency (a) *Spirodela polyrhiza*, (b) *Lemna minor*, and (c) *Wolffia arrhiza*.

Overall, it can be observed that the zinc ion removal efficiency during phytoremediation was highly dependent on the duckweed species. A similar opinion was supported by [8], who claimed that the sorption mechanism of the plant during phytoremediation was influenced by the type of pollutant and its concentration. Table 3 summarises the optimal zinc ion removal efficiency by different duckweed species with the desirability of 1. Based on the developed model, 100 solutions can be found during the optimisation process, but only the solutions with the highest initial zinc ion concentration were summarised in Table 3. From Table 3, *Spirodela polyrhiza* can achieve optimal zinc ion removal efficiency at a higher initial zinc ion concentration than the remaining duckweed species.

**Table 3.** Optimised environment conditions through response surface methodology.

Mass of macrophyte, $x_1$ (g)	Initial zinc ion concentration, $x_2$ (mg/L)	Duckweed species, $x_3$	Removal efficiency (%)	Desirability
22.56	92.92	<i>Spirodela polyrhiza</i>	87.53	1
22.38	39.5	<i>Wolffia arrhiza</i>	87.89	1
24.81	21.08	<i>Lemna minor</i>	87.96	1

## 4 Conclusion

Phytoremediation of zinc ion by duckweed species appeared to be an effective alternative wastewater treatment with low operating costs. The second-order polynomial model showed a high coefficient of determination which proved the suitability of the proposed model in describing the phytoremediation of zinc ion by duckweed species and its optimisation

process. The optimised environmental conditions for *Spirodela polyrhiza*, *Wolffia arrhiza* and *Lemna minor* in this study were found at the initial zinc ion concentration of 92.92 mg/L, 39.5 mg/L, and 21.08 mg/L and duckweed mass of 22.56 g, 22.38 g, and 24.81 g, respectively. Under the optimised environmental conditions, zinc ion removal efficiency was around 88 %.

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