Optimization of Lead Removal via Napier Grass in Synthetic Brackish Water using Response Surface Model

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Abstract The efficiency of the lead (Pb) phytoremediation by Napier grass was studied on the plant's growth and plant's tolerance on the Pb toxicity in synthetic brackish water. It was found that the plant was high tolerance to high level of Pb concentration (10 mg/l) in synthetic brackish water. Which revealed on the possibilities of plant's growth under the presence of Pb contaminated condition. According to the Pb removal efficiency, the highest one (88.63±4.9%) was found at 10 ppm Pb concentration, 0.3 g/l NaCl concentration during the period 45 day. However, this study investigated the optimum condition for lead (Pb) removal from synthetic brackish water using phytoremediation treatment with Napier grass through a Box-Behnken Design. Three operational variables, i.e. Pb concentration (1, 5.5, 10 mg/l), NaCl (0.1, 0.3, 0.5 g/l) and period time (7, 26, 45 day), were determined. The results were provided evidence that the highest Pb removal efficiency (93.56%) from synthetic brackish water via Napier grass was Pb and NaCl concentration at 10 mg/l and 0.5 g/l during 45 day.

1. Introduction
High concentration of lead (Pb) is one of the most a detectable heavy metal in aquatic environment. Their contamination in marine and coastal environment (sediment, sea water, and brackish water) has been recognized as the major problem worldwide that affect to human health through both water and food consumption. Source of the various heavy metal releasing to the aquatic environment are both of anthropogenic source; leaching of municipal wastes, agricultural pesticides and effluent and wastewater from industrial manufacturing and mining, and naturally source such as leaching during soil erosion and flooding [1], [2].

According to the heavy metal remediate approach, phytoremediation has considered as a green alternative solution to reduce high concentrations of heavy metal due to the public acceptance, the restoration of contaminated environment with low costs and applicable in many ecosystems. However, the limitation of the phytoremediation is long time required for the clean up contaminated site and toxic plant left over after the remediation approach. Therefore, utilization and effective remediation of contaminated land synergistic bonding by using energy crop for bioenergy production has been considered as sustainable approach of phytoremediation [3], [4].

In recent year, cellulosic biomass including Napier grass has been identified as good candidates for bioenergy production due to their high growth rate and high biomass production. However, few studies have investigated their heavy metal removal efficiency [5].
Response surface methodology (RSM), a collection of mathematical and statistical approach for experimental design useful for analyzing and evaluating the effects of variable as well as searching optimum conditions of variable to predict targeted responses, has been proven to be successfully implemented in optimization of phytoremediation conditions. The effectiveness of southern cattail (Typha domingensis) for phytoremediation of heavy metals from municipal waste leachate was investigated using Central composite design in order to clarify the optimal conditions of the independent variables[6]. Therefore, this study objected to investigate the Pb phytoremediation efficiency and optimization of remediation conditions via Napier grass in synthetic brackish water using response surface methodology.

2. Materials and methods

2.1. Experimental design

Respond surface methodology (RSM) is a statistical method that uses quantitative data from appropriate experiments to determine regression model equations and operating conditions. RSM is a collection statistical techniques for modeling and analysis of problems that a response result influenced by several variables Box-Behnken Design (BBD); the standard RSM design, was applied in this work to investigate the variables for removal of lead by Napier grass. BBD for three variables (initial Pb concentration, initial NaCl concentration, and time), each with two level (minimum and maximum), was used as experimental design model. In experimental design model, initial Pb concentration (1-10 mg/l), initial NaCl concentration (0.1-0.5 g/l), and time (7-45 day) were taken as input variables. Percentage removal of Pb was taken as the response of the system. Three independent variables were studied at three different levels, such as low (-1), medium (0) and high (+1) as shown in Table 1.

Table 1. Actual values of variables of the experimental design

| Factor                     | Levels of variables | -100  | 0 0  | 100  |
|----------------------------|---------------------|-------|------|------|
| Initial Pb concentration (mg/l) | X₁                  | 1     | 5.5  | 10   |
| Initial NaCl concentration (g/l) | X₂                  | 0.1   | 0.3  | 0.5  |
| Period Time (day)           | X₃                  | 7     | 26   | 45   |

2.2. Plant cultivation

Firstly, Napier grass was pre-cultivated in enrich nutrient soil for 30 days until the roots grew. Prior to the study the removal of lead in brackish water, stem’s plant was conducted with approximately length of 20 cm. According to plant cultivation, it was cultivated in a hydroponic system using a half-strength Hoagland’s nutrient solution supplemented with each sodium chloride (NaCl) and Lead (Pb) as the experimental design by BDD as expressed in Table 2.

2.3. Determination of Pb removal efficiency

According to the remaining Pb concentration, brackish water samples were collected and digested in nitric acid (HNO₃ 65%) and hydrogen peroxide (H₂O₂ 30%) solution using microwave digestion-model speedwave four, berghof Then Pb concentration was determined using Microwave Plasma Atomic Emission Spectroscopy (MP-AES 4100, Agilent Technologies). Removal efficiency of Pb removal (%) was calculated as equation (1);

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\text{Pb Removal Efficiency (\%) = \left(\frac{C_i - C_f}{C_i}\right) \times 100}
\] (1)

Where \(C_i\) was the initial Pb concentration (mg/l) and \(C_f\) was Pb concentration at the period time indicated (mg/l).
2.4. Phytotoxicity of Pb
Plant's growth such as amount of leaves, amount of root and stem length was directly measurement subject to phytotoxicity of Pb via Napier grass in synthetic brackish water. The results were investigated using SPSS Version 11.5 software (SPSS Inc., USA). The data were subjected to ANOVA, and differences between means were determined using the least squares deviation (LSD) test.

3. Results and discussions

3.1. Phytotoxicity of Pb
To evaluate phytotoxicity of Pb via Napier grass in synthetic brackish water (NaCl concentration range between 0.1-0.5 g/l), the plant growth was direct measured as indicator such as the amount of root, shoot length and amount of leaf. In comparing between the beginning of cultivation and after treatment, a significantly increase in amount of root significant was found in Pb and NaCl concentration increased (p < 0.05) (Figure 1(a)). Beside the amount of leaf, the increased NaCl seem to increase their leaf that might because of the increasing osmotic pressure. That bring more nutrients to it's leaf and increase the plant growth. However, there were no significant differences in amount of leaf (p < 0.05) (Figure 1(c)). On the other hand, the shoot length was significant decreased after 45 day (p < 0.05) (Figure 1(b)). These results indicated that shoot has more sensitive to heavy metal than root [7]. Moreover, it was interesting that plant were high tolerated to high level of Pb concentration (10 mg/l) in synthetic brackish water.

![Figure 1](image)
*Figure 1. Pb phytotoxicity indicators; a) amount of root, b) shoot length and c) amount of leaf, via Napier grass in synthetic brackish water (NaCl concentration range between 0.1-0.5 g/l)*

3.2. Phytoremediation of Pb
The Pb removal efficiency via Napier grass in synthetic brackish water has shown in Figure 2. The highest Pb removal efficiency (88.63±4.9%) found at 10 ppm Pb concentration, 0.3 g/l NaCl concentration during the period of 45 day. Moreover, it revealed that most of Pb content (80%) could
be removed using phytoremediation within 7 day. According to the lower concentration of Pb and NaCl, plant removed Pb lower than the higher one. It is suggesting that the increased removal efficiencies were revealing in the increased Pb concentration. That might cause by the higher osmotic pressure can be enhance more mobilization of Pb from solution into the different part of plant in this present study. However, the accumulation of Pb in different part of plant should investigate in further study. Beside the heavy metal phytoremediation via Napier grass, it was the good representing plant for the removal of Cu from contaminated soil. In comparing to the other plant, S. monticola has found to be suited for Pb phytoremediation from contaminated watershed at low concentration (15 - 145 µg/l) [8].

![Figure 2](image)

**Figure 2.** Removal efficiency of Pb via Napier grass in synthetic brackish water according to Box-Behnken design experimental condition

### 3.3. Optimization of the removal of Pb via Napier grass using response surface method

The experimental design matrix derived from BBD which involved 15 runs was shown in Table 2. Analysis of variance has been calculated to analyze the accessibility of the model. The model was significant at the 5% confidence level because probability values were less than 0.05. The lack of fit (LOF) F-test explains variation of the data around the modified model. LOF would be significant, if the model did not fit the data well. Generally, large probability values for LOF (>0.05) explained that the F-statistic was insignificant, implying a significant model relationship between variables and process responses.

To investigate the relationships between the response and the independent variables, BBD showed relationships as the polynomial equation as equation (2);

$$ Y = 43.80 + 32.71X_1 + 0.94X_2 + 10.95X_3 + 0.70X_1^2 + 3.47X_2^2 + 3.09X_3^2 + 6.77X_1X_2 + 4.33X_1X_3 + 1.35X_2X_3 $$

(2)

Where Y is the percentage removal of Pb, $X_1$ is initial Pb concentration (mg/l), $X_2$ is initial NaCl concentration (g/l) and $X_3$ is period time (day). The regression coefficients of the full polynomial model using BBD are given in Table 3.

The $R^2$ value of model had 0.859. The optimum values of initial concentration of Pb, initial concentration of NaCl and period time from Box-Behnken design were found to be 10 mg/l, 0.5 g/l and 45 day, respectively. The maximum predicted Pb Removal Efficiency was found to be 93.56%.
### Table 2. Experimental design and results for the Pb removal by Napier grass.

| Condition | Initial Pb concentration (mg/l), $X_1$ | Initial NaCl concentration (g/l), $X_2$ | Time (day), $X_3$ | % Pb Removal Efficiency |
|-----------|---------------------------------|---------------------------------|----------------|-----------------|
| 1         | 1.0                             | 0.1                             | 26             | 10.29           |
| 2         | 10.0                            | 0.1                             | 26             | 47.63           |
| 3         | 1.0                             | 0.5                             | 26             | 18.04           |
| 4         | 10.0                            | 0.5                             | 7              | 82.44           |
| 5         | 1.0                             | 0.3                             | 7              | 0.00            |
| 6         | 10.0                            | 0.3                             | 7              | 71.32           |
| 7         | 1.0                             | 0.3                             | 45             | 0.00            |
| 8         | 10.0                            | 0.3                             | 45             | 88.63           |
| 9         | 5.5                             | 0.1                             | 7              | 29.76           |
| 10        | 5.5                             | 0.5                             | 7              | 9.53            |
| 11        | 5.5                             | 0.1                             | 45             | 62.20           |
| 12        | 5.5                             | 0.5                             | 45             | 47.38           |
| 13        | 5.5                             | 0.3                             | 26             | 51.09           |
| 14        | 5.5                             | 0.3                             | 26             | 42.18           |
| 15        | 5.5                             | 0.3                             | 26             | 38.06           |

### Table 3. Regression coefficient of full polynomial model.

| Coefficient | Parameter estimate | p-Value |
|-------------|--------------------|---------|
| $\beta_0$   | 43.80              | 0.008*  |
| $\beta_1$   | 32.71              | 0.004*  |
| $\beta_2$   | 0.94               | 0.888   |
| $\beta_3$   | 10.95              | 0.146   |
| $\beta_{11}$| -0.70              | 0.943   |
| $\beta_{22}$| -3.47              | 0.726   |
| $\beta_{33}$| -3.09              | 0.755   |
| $\beta_{12}$| 6.77               | 0.486   |
| $\beta_{13}$| 4.33               | 0.651   |
| $\beta_{23}$| 1.35               | 0.886   |

*Significant, if $P < 0.05$

#### 3.3 Influence of variables interaction on the Pb removal via Napier grass

The results of interactional between three independent variables and dependent variable are shown in three-dimention surface plots. The combined effect of initial of Pb and NaCl on removal efficiency of Pb is depicted in Figure 3(a). The percentage removal of Pb increased with increasing both of initial of Pb and NaCl. The effect of initial Pb and period time on Pb removal efficiency is shown in Figure 3(b). It was observed that percentage of Pb removal increased with increase in initial Pb and period time. The interaction between initial of NaCl and period time on Pb removal is presented in Figure 3(c). It was observed that an increase in the Pb removal occurred when the NaCl in the solutions changed from 0.3 to 0.5 g/l and the period time increase.
Figure 3. 3D-Respond surface plots showing the effect of variable parameter on the removal of Pb via Napier grass a) initial concentration of Pb and NaCl, b) initial Pb concentration and time and c) initial NaCl concentration and time

4. Conclusions

Napier grass; energy crop, was suggesting to be a good candidate for Pb phytoremediation under the brackish water that revealed in the high phytotoxicity (at 10 mg/l Pb concentration) and removal efficiency (88.63±4.9%). On the other hand, Napier grass was as high tolerated to high level of Pb concentration in synthetic brackish water. Which revealed on the possibilities of plant growth under the presence of Pb contaminated condition (10 mg/l). According to the experimental condition, the highest Pb removal efficiency via Napier grass in synthetic brackish water revealed at Pb and NaCl concentration at 10 mg/l and 0.5 g/l, respectively. BBD and RSM were used in the design of experiments, statistical analysis and optimization of the parameters. The optimum conditions for initial of Pb, initial of NaCl and period time 10 mg/l, 0.5 g/l and 45 day, respectively. At the optimized factors, the amount of removed pollutants Pb was 93.56%. Since this study was performed the optimum condition for Pb phytoremediation using Napier grass under brackish water, a further work will look on the continuous operation constructed system according to these prediction results.

5. References

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