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PHYTOREMEDIATION OF SOIL CONTAMINATED WITH NICKEL BY *LEPIDIUM SATIVUM;* OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Phytoremediation is an alternative to traditional chemical and ways of treating polluted soils. The current study was carried out to investigate the phytoremediation of soil contaminated with nickel (Ni) by *Lepidium sativum*. Soil samples from 0 to 10 cm depth were collected. *Lepidium sativum* was transplanted in pots containing 5 kg of the collected soils. Central composite design and response surface methodology were employed in order to illustrate the nature of the response surface in the experimental design and explain the optimal conditions of the independent variables. Different concentrations for Ni (1 to 20 mg kg^{-1}) and times for collecting samples (10 to 40 days) were used. The results showed the amount of Ni removed was ranged from 8.62 mg kg^{-1} (Ni concentration of 20 mg kg^{-1} and time for taking samples of 10 days) to 7.066 mg kg^{-1} (Ni concentration of 10.50 mg kg^{-1} and time for taking samples of 40days). Additionally, the findings explained that the *Lepidium sativum* is an effective accumulator plant for phytoremediation of Ni polluted soils. Optimum conditions for nickel concentration and time for taking samples were 19.66 mg kg^{-1} and 39.28 days, respectively. For the optimum condition, the amount of Ni removed was 10.8095 mg kg^{-1}.

KEYWORDS: *Lepidium sativum*, Nickel, Phytoremediation, RSM, Soil contaminated.

1. INTRODUCTION

Environmental contamination with heavy metals is a global disaster that is related to human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and melting operations. All the heavy metals with high concentrations have strong toxic effects and are regarded as environmental pollutants (Chehregani *et al.*, 2009). Metals deposited to soil may transform (solubilise) into more mobile forms capable of migrating into the soil water where they may pose an elevated risk to plants, other soil biota and to groundwater (Ermakov *et al.,* 2007). Restoration of soils polluted with potentially toxic metals and metalloids is of major global concern (Shelmerdine *et al.*, 2009). Without doubt, environmental pollution is a major threat to our life. Industrialization, urbanization, and phenomenal growth in population are the factors for increasing pollution. In the last decade, much information has been obtained on the effect of heavy metal ions like nickel (Ni) on the soil. Ni is a naturally occurring element that exists mostly in the form of sulphide ores found underground, and in silicate minerals, found on the surface. In the environment, Ni is found primarily combined with oxygen (oxides) or sulphur (sulfides) (Ministry of the Environment, 2001). Elevated levels of Ni (Ni^{++}) can pose a major threat to both human health and the environment (Hussain *et al.*, 2010).

The researches aims in recent years, obtain to methods to be appropriate environmental and cost-effective for the cleanup of contaminated soils. Phytoremediation is a new and novel strategy to remove toxic heavy metals from soils through hyperaccumulator plant species. This is a low cost and eco-friendly means of reclaiming heavy metal contaminated soils, resulting from developmental activities, e.g. discharge of industrial effluents, city wastes, etc. (Panwar *et al.*, 2002).

Phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments (Greipsson, 2011). Phytoremediation is an alternative to traditional chemical and ways of treating polluted soils (Mathur *et al.*, 2007). However, detoxification of organic pollutants by plants is slow, leading to the accumulation of toxic compounds that could be later released into the environment (Aken, 2008).

Phytoremediators have been studied for using in cleaning up heavy metals such as aluminium, cadmium, chromium, copper, mercury, Ni, lead and zinc (Ndimele, 2010). Plants that accumulate high concentrations of metals are sometimes referred to as ‘‘hyperaccumulators’’ (Visoottiviseth *et al.*, 2002). Hyperaccumulator plants show a resource for remediation of metal contaminated sites, as they are able to extract wide range of metals and to concentrate them in their upper parts with the character of metal tolerance (Sarma, 2011). In literature, many plants were used for phytoremediation of heavy metals from soil (Mojiri, 2011; Cruz-Landero *et al.*, 2010; Jadia and Fulekar, 2009; Chehregani *et al.*, 2009; Zhang *et al.,* 2009; Xiao *et al.*, 2008; Subroto *et al.*, 2007; Al-Farraj and Al-Wabel, 2007; Ok and Kim, 2007; Cho-Ruk *et al.*, 2006; Lombi *et al.*, 2001).

Lepidium sativum commonly called ‘‘Garden Cress’’ is a polymorphic species (Karazhiyan *et al.*, 2009). It is a native plant of South West Asia, which spread many centuries ago to western European. It was used by ancient Egyptians as a food source and became well known in various parts of Europe, including Britain, France, Italy and Germany in due course, where it is still used as a minor crop. Persian used to eat this plant even before bread was known (Sharma and Agarwal, 2011). In literature, *Lepidium sativum* is a plant that is used for phytoremediation of soil heavy metals contaminated (Gunduz *et al.*, 2012; Kiayee *et al.*, 2012; Kathi and Khan, 2011).

The goals of this study were: 1) the phytoremediation of soil contaminated with Ni by *Lepidium sativum* and 2) Optimization by response surface methodology (RSM).

2. MATERIALS AND METHODS

2.1. Site description, Sample preparation

The experiment was carried out at the greenhouse. Soil samplings from 0 to 10 cm depth were collected in 2011. *Lepidium sativum* was transplanted in pots containing 5 kg of the collected soils. Central composite design (CCD) and response surface methodology (RSM) were employed to illustrate the nature of the response surface in the experimental design and to show the optimal conditions of the variables. Different Ni concentrations (1 to 20 mg kg^{-1}) and times for taking samples (10 to 40 days) were applied.

2.2. Laboratory determinations

Soil texture was determined by the Bouyoucos hydrometer method (Gee and Bauder, 1986). Soil pH and electrical conductivity (EC) were measured on 1:1 extract (Soil:Water). Total Ni in soil samples was carried out in accordance the Standard Methods (APHA, 2005). Soil organic matter (OM) was determined as in Walkley and Black and cation exchange capacity (CEC) was determined (ASA, 1982).

Soil samples were allowed to air dry in a greenhouse at a temperature between 25ºC and 30ºC and were then ground to pass a 2-mm mesh sieve for prepared of soil samples (Makoi and Verplancke, 2010; Mojiri and Amirossadat, 2011). The plant tissues were prepared by Wet Digestion Method (Campbell and Plank, 1998).

2.3. Experimental design and data analysis

CCD and RSM were employed in order to illustrate the nature of the response surface in the experimental design and elucidate the optimal conditions of the independent variables. CCD was established through Design Expert Software (6.0.7). The behavior of the system is described through Eq. (1) an empirical second-order polynomial model:

$$Y = \beta \sum_{i=1}^{k} \beta i X i + \sum_{i=1}^{k} \beta i X i 2 + \sum_{i<j}^{k} \quad \sum_{j}^{k} \beta i j X i X j + \cdots + e \qquad \text{(Eq. 1)}$$

where Y is the response; Xi and Xj are the variables; β0 is a constant coefficient; βj, βjj, and βij are the interaction coefficients of linear, quadratic and second-order terms, respectively; k is the number of studied factors; and e is the error.

The results were completely analyzed by analysis of variance (ANOVA) in the Design Expert Software. Nickel concentrations (1, 10.50, and 20 mg kg^{-1}) and times for taking samples (10, 25, and 40 days) were used. To carry out an adequate analysis, one dependent parameter (reducing nickel concentration in soil) was measured as responses (Table 2).

Descriptive statistical analysis including mean comparison of Ni accumulation in roots and shoots of plant using Duncan's Multiple Range Test (DMRT) was conducted using SPSS software.

3. RESULTS AND DISCUSSIONS

Soil properties before experiment, results of the experiments, ANOVA results for response parameter, comparing the means of Ni accumulation in *Lepidium sativum* roots, and shoots are shown in Tables 1, 2, 3 and 4, respectively. Design expert plot; response surface plot for Ni removal and design-expert plot; predicted vs. actual values plot for nickel removal are shown in Figures 1 and 2, respectively.

In this work, RSM was used for analyzing the correlation between the variables (Ni concentrations and times for taking samples) and the important process response (the amount of Ni removed). Considerable model terms were preferred to achieve the best fit in a particular model. CCD permitted the development of mathematical equations where predicted results (Y) were evaluated as a function of Ni concentration (A) and times for taking samples (B). The results were computed as the sum of a constant, two first order effects (terms in A and B), one interaction effect (AB), and two second-order effects (A^2 and B^2), as shown in the equation (Table 3). The results were analyzed by ANOVA to determine the accuracy of fit. Table 3 shows the quadratic models in terms of actual factors, it means that the arrangement of variables such as A, B, A^2, B^2 and A*B are exist in the equation. 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 was 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 significant model relationship between variables and process responses. The R^2 gave the proportion of total variation in the response predicted by the model, indicating the ratio of sum of squares due to regression to total sum of squares. R^2 value close to 1 was desirable, and a high R^2 coefficient ensured acceptable modification of the quadratic model to the experimental data. Adequate precision compared the range of the predicted values at the design points to the mean prediction error. The suitability of the model could be judged by diagnostic plots i.e. predicted vs. actual values. Figure 2 shows the predicted vs. actual value plots of the response parameters. This plot signified a sufficient agreement between the real data and the values achieved from the model. The coefficient of variance (CV) is the ratio of the standard error of estimate to the average value of the observed response defined by the reproducibility of the model.

Table 1. Soil properties before experiment

Ph	**EC (dSm^{-1})**	**CEC (me 100g^{-1})**	**OM (%)**	**Clay (%)**	**Sand (%)**	**Silt (%)**	**Ni (ppm)**
			Main Soil (T1)				
6.97	1.14	9.6	0.70	10.00	60.90	29.10	0.0

Table 2. Experimental variables and results for the removal nickel concentration in soil

Run	**Variables**		**Response**
	A: Nickel concentration (mg kg^{-1})	B: Time for taking samples (days)	Amount of Ni removed. (mg kg^{-1})
1	10.50	25.00	5.838
2	10.50	25.00	5.922
3	20.00	25.00	10.0
4	10.50	10.00	5.575
5	10.50	25.00	5.890
6	20.00	40.00	10.78
7	20.00	10.00	8.62
8	10.50	25.00	6.079
9	10.50	40.00	7.066
10	1.00	40.00	0.574
11	1.00	25.00	0.561
12	1.00	10.00	0.540
13	10.50	25.00	5.575

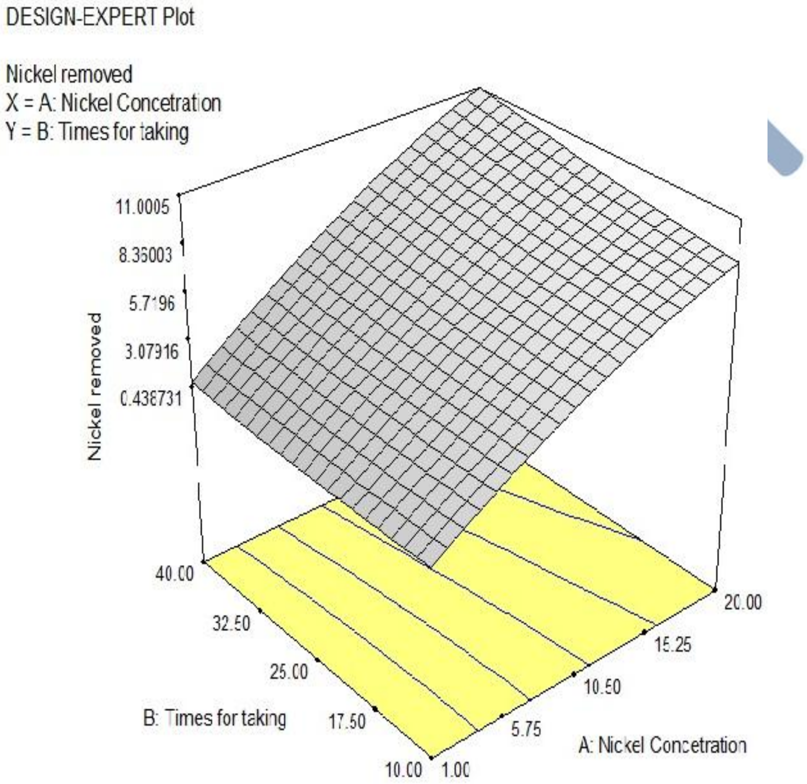

Figure 1. Design expert plot; response surface plot for nickel removal

Table 3. ANOVA results for response parameter

Response	**Prob.**	**R^2**	**Adj. R^2**	**Adec. P.**	**SD**	**CV**	**PRESS**	**Prob. LOF**
Nickel Removal	0.0001	0.9965	0.9945	59.724	0.26	4.63	2.78	0.136

Prob.: Probability of error; R^2: Coefficient of determination; Ad. R^2: Adjusted R^2; Adec. P.: Adequate precision; SD: Standard deviation; CV: Coefficient of variance; PRESS: Predicted residual error sum of square; Prob. LOF: Probability of lack of fit

Final equation in terms of actual factor= $0.18042+ 0.59690A-0.034751B-0.003A^2-0.004B^2-0.003AB$ where A is Ni concentration) and B is time for taking samples

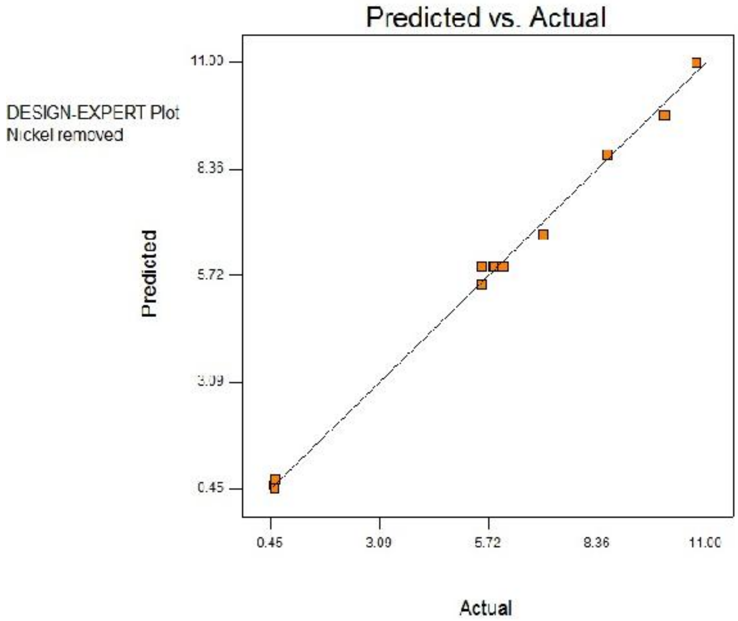

Figure 2. Design-expert plot; predicted vs. actual values plot for nickel removal

The amount of Ni removed ranged from 8.62 mg kg^{-1} (Ni concentration of 20 mg kg^{-1} and time for taking samples of 10 days) to 7.066 mg kg^{-1} (Ni concentration of 10.50 mg kg^{-1} and time for taking samples of 40 days). The phytoremediation of Ni increases, when the Ni concentration was increased till 10.50 and after that pheytoremedion of Ni was decreased. It can be noticed from Figure 1 that the increase in the times for taking samples was resulted in amount of removed Ni.

Optimum conditions for nickel concentration and time for taking samples were 19.66 mg kg^{-1} and 39.28 days, respectively. For the optimum condition, the amount of Ni removed was 10.8095 mg kg^{-1}.

Table 4. Comparing the means of treatments in *Lepidium sativum*

Nickel (ppm)	**Time for taking (days)**	***Lepidium sativum***		**TF***	**Time for taking (days)**	**Lepidium sativum**		**TF**	**Time for taking (days)**	**Lepidium sativum**		**TF**
		Root	**Shoot**	**-**		**Root**	**Shoot**	**-**		**Root**	**Shoot**	**-**
1.00	10	0.04a+	0.04f	1.00	25	0.07a+	0.08f	1.14	40	0.10a+	0.11f	1.1
10.50		3.02b	3.81g	1.26		4.39b	5.17g	1.17		5.92b	7.49g	1.26
20.00		5.99c	6.93h	1.15		7.89c	8.69h	1.10		11.00c	12.91h	1.17

+ Row means followed by the same letter are not significantly different at 0.05 probability level

*TF: translocation factor

The efficiency of phytoremediation can be quantified by calculating translocation factor. The translocation factor indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots. It is calculated as follows (Padmavathiamma and Li, 2007).

$$\text{Translocation Factor (TF)} = \frac{C\text{shoot}}{C\text{root}}$$

where C_{shoot} is concentration of the metal in plant shoots and C_{root} is concentration of the metal in plant roots.

Based on Table 4, translocation factors (TF) were more than 1 in all treatments. Translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part (Jamil *et al.*, 2009). According to Yoon *et al.* (2006), only plant species with TF greater than 1 have the potential to be used for phytoextraction.

Accumulation of Ni in roots was 0.40 (Ni concentration of1and time for taking samples of 10) to 11.00 (Ni concentration of 1and time for taking samples of 40 days). Heavy metals when present at an elevated level in soil are absorbed by the root system, accumulate in different parts of plants, reduce their growth and impair metabolism (Kiyaee *et al.*, 2012).

4. CONCLUSIONS

Environmental pollution with heavy metals is a global disaster that is related to human activities. Phytoremediation is a new and novel strategy to remove toxic heavy metals from soils through hyperaccumulator plant species. At the optimum conditions of Ni concentration (19.66 mg kg^{-1}) and time for taking samples (39.28 days), the amount of Ni removed was 10.8095 mg kg^{-1}. The evidence provided by this experiment indicated that *Lepidium sativum* is an effective accumulator plant for phytoremediation of Ni polluted soils because the translocation factor was greater than 1.

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