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Effects of wastewater irrigation on accumulation of heavy metals in soil and spinach; optimization by response surface methodology (RSM)

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Abstract

As a result of urbanization, urban wastewater has increased fast, so reusing urban wastewater has become a research topic for a long time. The goals of this study were effects of wastewater irrigation on accumulation of heavy metals in soil and spinach, and optimization by response surface methodology (RSM). The plants were transplanted into pots containing 7 kg of soil, and were irrigated with 400 mL of wastewater to water mixture (in different ratio) every day. The response surface methodology and central composite design have been used to clarify the experimental plan and determine the optimum settings of the independent variables and their responses. The independent factors were ratio of wastewater to water mixture (20, 40 and 60%; v/v) and different lengths of time for taking samples (10, 20 and 30 days). The results showed that the irrigation by wastewater led to accumulation of Mn and Ni in soil and plant. At the optimum time for taking samples (29.99 days) and ratio of wastewater to water mixture (59.85%; v/v) the maximum accumulation of Mn and Ni in soil, and roots and shoots of spinach were 3.25 mg/L (Mn in soil), 4.49 mg/L (Ni in soil), 2.99 mg/L (Mn in root), 1.59 (Mn in shoot), 3.36 mg/L (Ni in root) and 2.20 mg/L (Ni in shoot), respectively.

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Introduction

Water is a vital resource, but a severely limited in most countries. Water supply will be one of the main future challenges in a world of growing population and industrialization (Mojiri *et al.,* 2013a). Water is a scarce commodity in the Middle East and North Africa (MENA) and its availability is declining to a crisis level. As a result of urbanization, municipal wastewater has increased fast, so recycling urban wastewater has become a research topic for a long time (Mojiri and Amirossadat, 2011).

The reuse of wastewaters for purposes such as agricultural irrigation can reduces the amount of water that needs to be extracted from environmental water sources. Land application of treated wastewater (TWW) on cultivated fields may serve as a viable way of disposing of effluents, and sustaining agricultural production in regions experiencing shortage in fresh water (Mojiri and Aziz, 2011).

Effects of wastewater on accumulation of metals in soil and plants were investigated by researchers (Abegunrin *et al.*, 2013; Singh and Agrawal, 2012; Faryal *et al.,* 2007; Mansouri and Ebrahimpour, 2011). Based on the previous studies (Galavi *et al.,* 2010; Xu *et al.,* 2010) the wastewater irrigation led to accumulation of heavy metals in soil and crops. Some researchers (Bahmanyara, 2008; Qishlaqi *et al.*, 2008) investigated effects of wastewater on soil and plants that one of these plants is spinach.

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of *Amaranthaceae*. It is native to central and southwestern Asia. It is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, ovate to triangular-based, very variable in size from about 2–30 cm long and 1–15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm diameter, maturing into a small, hard, dry, lumpy fruit cluster 5–10 mm across containing several seeds. (http://en.wikipedia.org/wiki/Spinach).

The aims of current study were to (1) study on effects of urban wastewater on accumulation of heavy metals in soil and spinach (2) Optimization of these effects by response surface methodology (RSM).

Materials and methods

Sample Preparation

The plants were transplanted into pots containing 7 kg of soil, and were irrigated with 400 mL of wastewater to water mixture (in different ratio) every day. Central composite design and response surface methodology were used in order to clarify the nature of the response surface in the experimental design and explain the optimal conditions of the independent variables. The independent factors were ratio of wastewater to water mixture (20, 40 and 60%; v/v) and different lengths of time for taking samples (10, 20 and 30 days).

Analytical methods

The plant samples were prepared for laboratory analysis by Wet Digestion method (Campbell and Plank, 1998). Extractable manganese (Mn) and nickel (Ni) in soil, and root and shoot of spinach were carried out using a flame atomic absorption spectrometer (Varian Spectra 20 Plus, Mulgrave, Australia) in accordance to the Standard Methods (APHA, 2005). Soil properties before experiments are shown in Table 1. The properties of waste and wastewater are shown in Table 2.

Statistical Analysis

Design and analysis of experiments (DOE) have been widely used in planning, analyzing and running experiments in different areas of research, such as wastewater treatment, food analysis, material production and medication intake, helping the researchers to achieve the research objective with less effort, cost and time (Lim *et al.,* 2014). In the current research, the central composite design (CCD) and response surface methodology (RSM) were applied for designing the experiments and data analysis. CCD was recognized through Design Expert Software

Version 6.0.7. RSM was used to control the optimum process parameter levels. RSM collects mathematical and statistical techniques that are appropriate for the modeling and analysis of problems, in which responses of interest are affected by some variables; additionally, the goal was to optimize these responses (Aziz *et al.,* 2011). The appropriate model is the quadratic model Eq. (1).

$$Y = \beta \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_i X_{i2} + \sum_{i<j}^{k} \sum \beta_{ij} X_i X_j + \ldots + e \quad \text{(Eq. 1)}$$

Where Y is the response; Xi and Xj are the variables; βo is a stable coefficient; βj, βjj, and βij illustrate the interaction coefficients of linear, quadratic, and second-order terms, respectively; k is the number of analyzed parameters; and e is the error. The results were investigated by using ANOVA in the Design Expert Software Version 6.0.7. In the current study, each of the operating variables were considered at 3 levels, low (-1), central (0), and high (+1). CCD and RSM were applied to evaluate the relationship between the most significant operating variables i.e. different level of nickel in soil (mg/Kg), time of gathering plants (day), and the total of planted spinach, in addition to optimize the appropriate situations of operating variables in order to expect the best value of responses (Mojiri *et al.,* 2013b,c).

Result and discussion

Table 3 shows the experimental variables and results for the accumulation of heavy metals in soil and spinach. Figures 1 to Figure 3 show the 3D surface plots of accumulation of Mn and Ni in soil, and roots and shoots of spinach.

Table 1. The properties of soil before phytoremediation.

pH	EC (dSm^{-1})	CEC (me $100g^{-1}$)	OM (%)	Clay (%)	Sand (%)	Pb (mg/Kg)
7.0	1.26	10.0	0.72	11.92	59.18	0.0

Accumulation of Mn and Ni in Soil

Table 3 shows that the Mn accumulation in soil varied from 1.39 mg/Kg (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 3.21 mg/Kg (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days). Table 3 shows that the Ni accumulation in soil varied from 1.58 mg/Kg (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 4.31 mg/Kg (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days).

Table 2. The properties of waste and wastewater.

pH	EC (dSm^{-1})	BOD_5 (mg/L)	COD (mg/L)	O.M (%)	Mn (mg/L)	Ni (mg/L)
Water						
7.00	0.32	-	-	-	0.00	0.00
			Wastewater			
6.74	1.33	28.92	817	1.84	1.03	1.89

Accumulation of Mn in Roots and Shoots

Table 3 shows that the Mn accumulation in roots varied from 1.18 mg/L (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 2.99 mg/L (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days). Table 3 shows that the Mn accumulation in shoots varied from 0.79 mg/L (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 1.57 mg/L (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days).

Accumulation of Ni in Roots and Shoots

Table 3 shows that the Ni accumulation in roots varied from 1.26 mg/L (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 3.27 mg/L (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days). Table 3 shows that the Ni accumulation in shoots varied from 0.82 mg/L (ratio of wastewater to water mixture = 20%, and time for taking samples = 10 days) to 2.13 mg/L (ratio of wastewater to water mixture = 60%, and time for taking samples = 30 days).

Table 3. Experimental variables and results for the accumulation of heavy metals in soil and spinach.

Run	ratio of wastewater to water mixture (%; v/v)	time for taking samples (day)	Accumulation of Mn in Soil (mg/L)	Accumulation of Ni in Soil (mg/L)	Accumulation of Mn in Roots (mg/L)	Accumulation of Mn in Shoots (mg/L)	Accumulation of Ni in Roots (mg/L)	Accumulation of Ni in Shoots (mg/L)
1	60.00	30.00	3.21	4.31	2.99	1.57	3.27	2.13
2	40.00	30.00	2.69	3.78	2.33	1.23	2.57	1.49
3	60.00	20.00	2.81	4.13	2.57	1.34	2.99	1.84
4	60.00	10.00	2.43	3.54	2.27	1.10	2.27	1.28
5	40.00	20.00	2.11	3.25	1.92	1.04	1.97	1.02
6	40.00	20.00	2.28	3.29	1.94	1.00	2.03	1.04
7	40.00	20.00	2.11	3.25	1.92	1.02	1.98	1.02
8	40.00	20.00	2.21	3.25	1.96	1.00	1.94	0.97
9	20.00	20.00	1.57	1.71	1.39	0.82	1.48	0.88
10	40.00	10.00	1.89	2.03	1.79	0.85	1.81	0.94
11	20.00	30.00	1.74	1.93	1.52	0.87	1.64	0.94
12	20.00	10.00	1.39	1.58	1.18	0.79	1.26	0.82
13	40.00	20.00	2.23	3.15	1.91	1.00	1.94	1.01

Kibria *et al.* (2012) investigated influence of wastewater irrigation on heavy metal accumulation in soil and plant. The results showed the Cd, Pb, Zn, Cu, Mn and Fe contents of soils were significantly higher in wastewater irrigated location than those in the control location.

Table 4. ANOVA results for response parameter.

Response	Prob.	R^2	Adj. R^2	Adec. P.	SD	CV	PRESS	Prob. LOF
Accumulation of Mn in Soil	0.0001	0.9843	0.9731	33.588	0.083	3.76	0.27	0.3483
Accumulation of Ni in Soil	0.0001	0.9449	0.9056	16.889	0.280	9.28	5.33	0.0070
Accumulation of Mn in Roots	0.0001	0.9947	0.9910	56.79	0.046	2.33	0.10	0.0208
Accumulation of Mn in Shoots	0.0001	0.9907	0.9841	43.14	0.028	2.67	0.043	0.0940
Accumulation of Ni in Roots	0.0001	0.9742	0.9557	25.78	0.12	5.73	0.70	0.0550
Accumulation of Ni in Shoots	0.0001	0.9685	0.9459	21.799	0.093	7.87	0.42	0.0036
Equation for Accumulation of Mn in Soil	$0.7701 + 0.0297A – 0.0109B – 0.0004A^2 + 0.0005B^2 + 0.005AB$							
Equation for Accumulation of Ni in Soil	$-1.213 + 0.850A + 0.1111B – 0.0004A^2 – 0.002B^2 + 0.0005AB$							
Equation for Accumulation of Mn in Roots	$0.8031 + 0.0232A – 0.0211B – 0.0001A^2 + 0.0007B^2 + 0.0004AB$							
Equation for Accumulation of Mn in Shoots	$0.8710 – 0.008A – 0.0102B + 0.0001A^1 + 0.0001B^2 + 0.0004AB$							
Equation for Accumulation of Ni in Roots	$1.1419 – 0.0003A – 0.0162B + 0.0002A^2 + 0.0005B^2 + 0.0007AB$							
Equation for Accumulation of Ni in Shoots	$1.5619 – 0.0399A – 0.0401B + 0.0005A^2 + 0.0007B^2 + 0.0009AB$							

* Prob.: Probability of error; R^2: Coefficient of determination; Adj. 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.

*A is ratio of wastewater to water mixture and B is time for taking samples.

Table 5. The value of factors and responses at optimum conditions.

Factors		Responses					
ratio of wastewater to water mixture (%)	Time for taking samples (day)	Mn in Soil (mg/L)	Ni in Soil (mg/L)	Mn in Roots (mg/L)	Mn in Shoots (mg/L)	Ni in Roots (mg/L)	Ni in Shoots (mg/L)
59.85	29.99	3.25	4.49	2.99	1.59	3.36	2.20

Karatas *et al.* (2006) investigated heavy metal accumulation in wheat plants irrigated by wastewater. This result showed that sewage channel water application for field watering increased the heavy metals (Cu, Cr, Mn, Ni, Pb and Zn) content in the root, body and seed parts of wheat plant, the increases recorded being higher for Mn and Zn.

In the current study, the irrigation of wastewater caused accumulation of Mn and Ni in soil and plants. The accumulation of Mn and Ni in roots was more important than shoots. This is in line with findings of Vakili and Aboutorab (2013), Kiayee (2013) and Mojiri *et al.* (2013a).

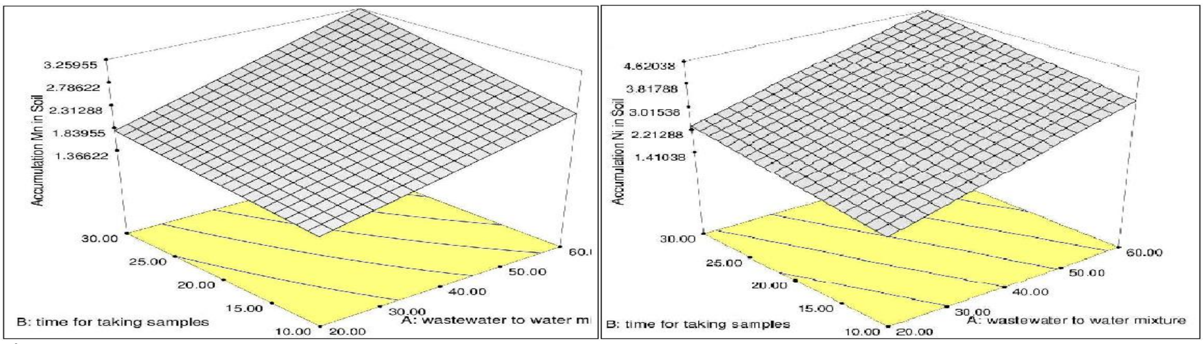

Fig. 1. The 3D surface plots of accumulation of Mn and Ni in soil.

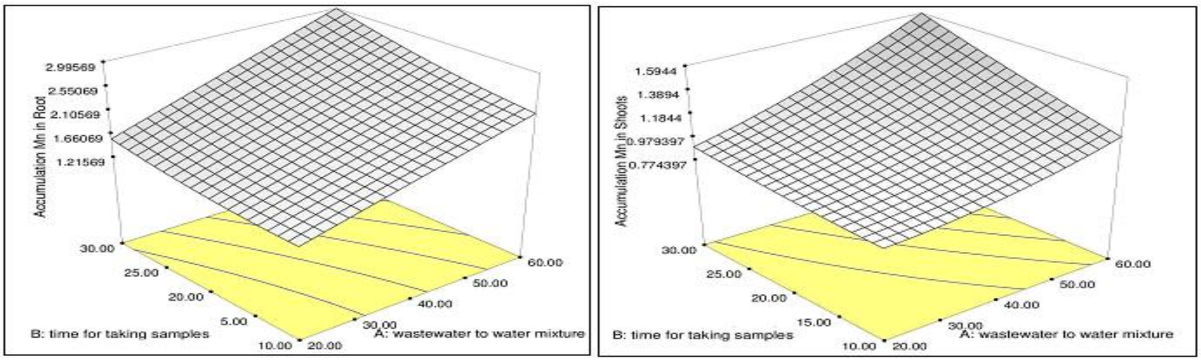

Fig. 2. The 3D surface plots of accumulation of Mn in roots and shoots.

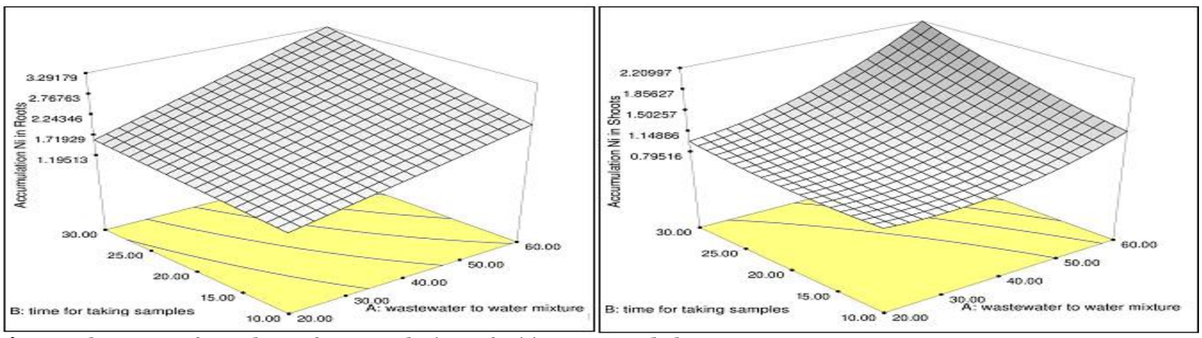

Fig. 3. The 3D surface plots of accumulation of Ni in roots and shoots.

Statistical Analysis and Experimental Condition Optimization

In this work, RSM was used for analyzing the correlation between the variables (ratio of wastewater to water mixture, lengths of time for taking samples) and the important process response (accumulation Mn and Ni in soil, and shoots and root of spinach). 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 ratio of wastewater to water mixture (A), and lengths of time for taking samples (B). The results were computed as the sum of a constant, three first order effects (terms in A and B), one interaction effects (AB), and two second-order effects (A^2 and B^2), as shown in the equation (Table 4). The results were analyzed by ANOVA to determine the accuracy of fit. Table 4 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. Figures 4 to Figure 6 show the design expert statistical plots - predicted versus actual plot: accumulation of Mn and Ni in soil, and roots and shoots of spinach. This plot signifies sufficient agreement between real data and the ones achieved from the models. The coefficient of variance (CV) is the ratio of the standard error of estimate to the average of observed response defined by reproducibility of the model. If the CV is more than 10%, then it is considered reproducible (Bashir *et al.*, 2010). Table 5 shows the value of factors and responses at optimum conditions.

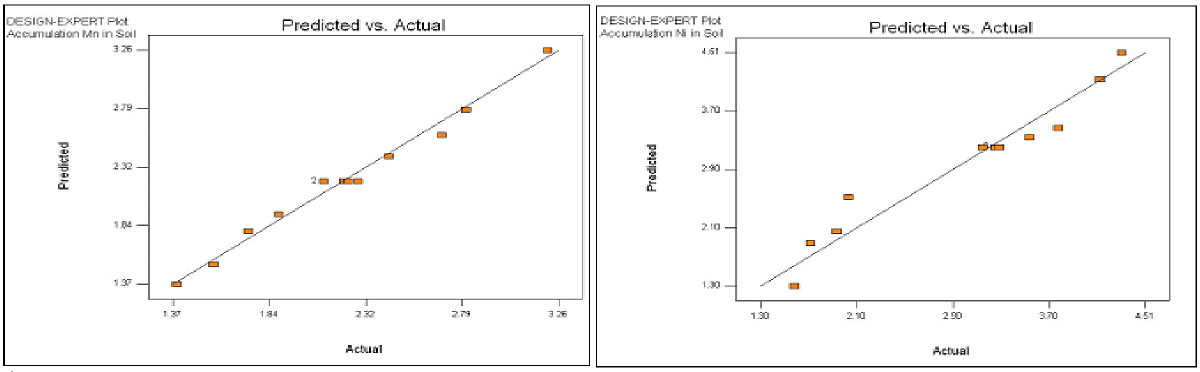

Fig. 4. The design expert statistical plots - predicted versus actual plot: accumulation of Mn and Ni in soil.

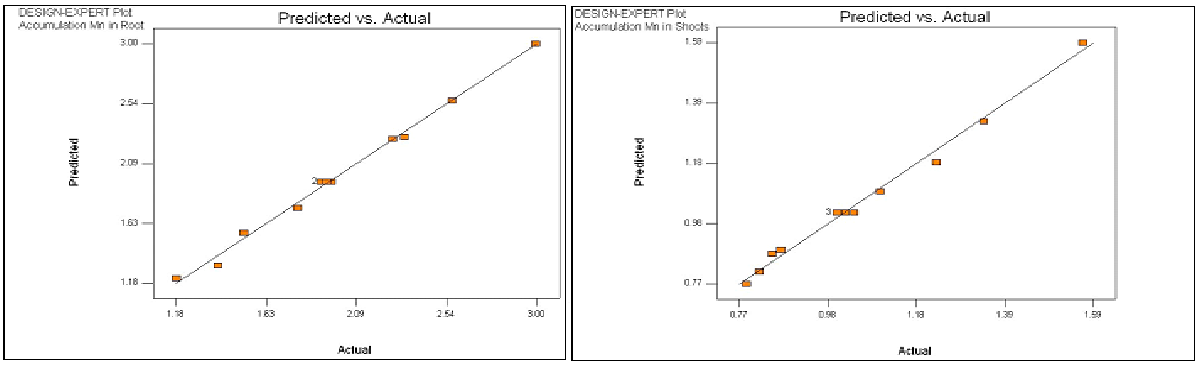

Fig. 5. The design expert statistical plots - predicted versus actual plot: accumulation of Mn in roots and shoots.

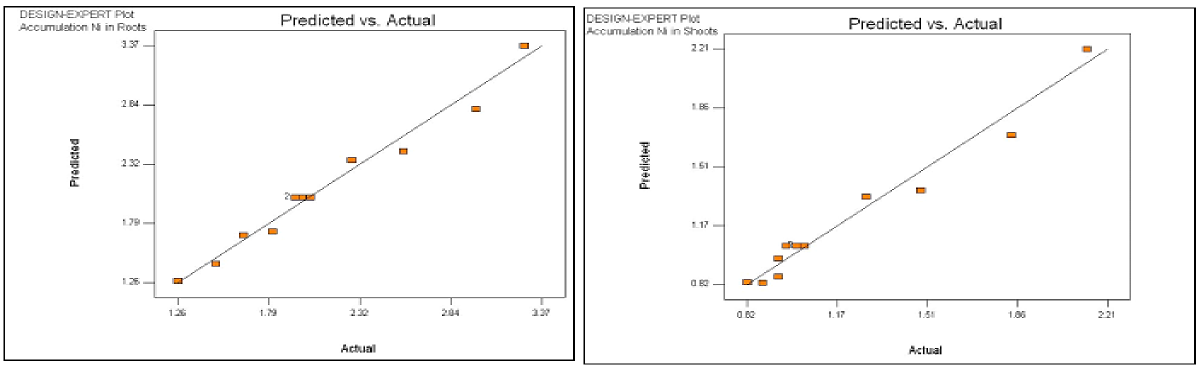

Fig. 6. The design expert statistical plots - predicted versus actual plot: accumulation of Ni in roots and shoots.

Conclusion

Water is a vital resource but a severely limited in most countries. The reuse of wastewaters for purposes such as agricultural irrigation can reduces the amount of water that needs to be extracted from environmental water sources. The goal of the current study was to investigate the effects of wastewater irrigation on accumulation of heavy metals in soil and spinach; optimization by response surface methodology (RSM). The evidences provided by this experiment indicated that urban wastewater caused increase of heavy metals in soil and spinach. The accumulation of Mn and Ni in roots was important than shoots. At the optimum time for taking samples (29.99 days) and ratio of wastewater to water mixture (59.85%; v/v) the maximum accumulation of Mn and Ni in soil, and roots and shoots of spinach were 3.25 mg/L (Mn in soil), 4.49 mg/L (Ni in soil), 2.99 mg/L (Mn in root), 1.59 (Mn in shoot), 3.36 mg/L (Ni in root) and 2.20 mg/L (Ni in shoot), respectively.

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