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Investigation of soil properties influence on the heavy metals sorption by plants and possibilities for prediction of their bioaccumulation by response surface methodology

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Abstract: The aim of this study was to determine soil properties influence on the heavy metals sorption by vegetables that are used in the diet and possibilities for prediction of their bioaccumulation by response surface methodology (RSM). Lettuce was used as a biosorbent, and cadmium and lead were used as contaminants. Lettuce was grown on compost (previously contaminated with different concentrations of Cd and Pb) which pH was adjusted with different amounts of NPK fertilizers. The content of heavy metals was determined by ICP-MS. Results showed that Cd content in lettuce was below the toxic values, but Pb concentration was above allowable, which indicated that the limit value for Pb is not set in accordance with the food safety regulations. It was found that the heavy metals accumulation in plants depends not only on the content in the soil, but also on the plant affinity for the specific metal, and the individual or the interactive effects of different soil properties. Through the transfer factor it was found that lettuce has a much higher affinity to Cd in relation to Pb. RSM analysis proved to be very good tool for the examination of a large number of variables along with a small number of experiments.

Keywords: lead; cadmium; maximum permissible level; transfer factor.

INTRODUCTION

The composition and properties of the soil depend on the cycles of matter and energy in the environment. Land behaves like a filter, retaining and accumulating toxic substances, which may have negative impact on the environment and the human body by process of circulation of water and other substances.[1,2] The pollutants can enter the body through food, *via* phytosorption of pollutants by vegetable crops during growth on contaminated soil.

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Because of the harmful effects on the body, heavy metals that are common soil pollutants (such as cadmium, lead, mercury, arsenic, chromium, nickel, copper and zinc) are particularly interesting. Health impact and possible genetic effects of the contaminated environment depend on the type of pollutant, its toxicity, exposure time to pollution source and the general health conditions of people. In addition, individual negative effects are amplified when several of them are acting jointly. Increased heavy metal content in the surface layer of the soil may be the result of pollution from human activity, but also natural biogeological processes (plant root systems move elements from deeper soil layers to the upper, arable layers). Heavy metals enter into the food chain *via* plants and may cause adverse consequences in the body of animals and humans.[3,4]

Adoption of elements by plants depends on the physiological characteristics of the plants themselves, growing conditions, soil properties and meteorological conditions. The contaminants mobility depends on their type and soil pH. For example, As and Se are more mobile in alkaline soil, while Hg, Pb, Cd and Zn are more mobile in acidic soil. All plants have the ability to accumulate, from soil and water, those metals that are essential for their growth and development, such as, Fe, Mn, Zn, Cu, Mg, Mo, Ni. Some plants have the ability of the accumulation of heavy metals that have no known biological function such as Cd, Cr, Pb, Co, Ag, Se and Hg. However, a large accumulation of these metals in plants can be toxic to the plant itself and to people and animals who consume it (the plant with an increased accumulation of these metals may exert a harmful effect on their lives and health). The ability to tolerate high concentrations of heavy metals and accumulate them in unusually high concentrations has a number of plants, which is used for phytoremediation of soils (certain plants can adopt (bind) heavy metals from the environment via phytoextraction and than translocate them to the root and/or outgrowth, where the metal is stored until the harvest).[5,6]

Sometimes, vegetable crops can accumulate significant amounts of heavy metals, without the appearance of visible symptoms on the plant itself. Contamination can exist even though the synthesis of plant organic matter and their yield are not reduced. Symptoms suggesting physiological disorders appear only when the concentration of heavy metals becomes multiple times larger that the limit value. However, the negative impact on the quality and endangered biological value of vegetables actually already exist.[3,7]

EXPERIMENTAL

Experimental assumptions

Unfortunately, there is no uniform regulation of the harmful substances content in the soil, food and water, and the maximum permissible level (*MPL*) varies from country to country. In the Republic of Serbia, as well as in the European Union, there are defined maximum allowed levels of Cd and Pb in soil and food, which are very similar (Table I).[8–10]

TABLE I. Maximum permissible level (*MPL* / mg kg^{-1}) in soil and leafy vegetables

No.	Sample	Pb	Cd	Regulative
1.	Soil	150	3	EURS
2.		100	3	Serbia
3.	Leafy vegetables	0.3	0.2	EURS
4.		0.3	0.2	Serbia

For this examination, soils containing different amounts of contaminants – cadmium and lead, different soil pH and different nutrient content in its composition were used. Preparation and contamination of soil by Pb and Cd is carried out according to the new procedure not described previously in the literature (given in Supplementary material to this paper). The initial content of lead and cadmium in the compost is very small (by the manufacturer's declaration) and these starting concentrations were omitted in the calculations. For this examination, we assumed that the maximum permissible level of elements in the soil is safe for crop farming from the standpoint of allowable concentrations of these elements in foods (Table I).

Vegetable crop, lettuce *(Lactusa sativa)* was used as biosorbent. This plant was selected as a representative sample for the experiment because of its wide usage in the regular diet without prior heat treatment and because of its rate of growth – plant development (good qualitative characteristics to perform the experiment in a short time frame).

In the presented research two experimental investigations were done:

1. Investigation of commercially procured composts that were contaminated with desired amounts of heavy metals, without adjusting the pH value, since it was already at the optimum level of 6.5 to 6.9.
2. Investigation of commercially procured composts that were adjusted to various pH values, with different contents of heavy metals (Pb and Cd) and nutrients (NPK fertilizer), which was done in order to predict the level of adopted heavy metals depending on the soil characteristics.

The experimental plan is defined in accordance to the principles of Response surface methodology (RSM). RSM optimization of heavy metals phytosorption from contaminated soil is done in order to predict bioaccumulation of heavy metals depending on the soil quality. The calculations take into account three factors (content of heavy metals in the soil, the amount of added nutrients in the soil – NPK fertilizers, and pH values) and three levels (–1, 0 and 1). Experimental plan provides that each experiment (except central point) is done in duplicate. The variable values were given in advance and output variable was a level of bioaccumulation of heavy metals in the plant. Data obtained in the experiments were fitted with a second-order polynomial equation; the coefficients of the response function and their statistical significance were evaluated by the least squares method using Design-Expert software, version 9.0.6.2, (Stat-Ease Inc., Minneapolis, MN, USA). The Fisher test was used to determine model adequacy and the Student distribution to evaluate the significance of the coefficients.

The lettuce seedlings were cultivated for 4 weeks (procedure is given in the Supplementary material). During the growth of lettuce, changes were monitored and relevant conclusions were derived. After finished growing, the plant leaves were harvested and prepared for heavy metals concentration measurements by acidic digestion procedure[11] (procedure is given in the Supplementary material to this paper). The concentrations of Cd^{2+} and Pb^{2+} were measured by Agilent Technologies 7500C ICP-MS system (Agilent Technologies, Inc., USA). After obtaining the results of lead and cadmium concentrations in the solution samples, their concentrations in the lettuce leaves were calculated. Subsequently, dependence of the heavy metals concentration in the soil was established.

RESULTS AND DISCUSSION

The influence of the pollutants concentration on the lettuce growth

After two weeks of plant growth there has been an uneven development in the samples grown on contaminated soil with lead concentrations higher than *MPL*. Most likely, discrepancies were influenced by phytotoxicity due to high levels of lead. During the experiment it was observed that the *MPL* for lead, 150 mg kg^{-1} (EURS), does not affect the growth – development of plants. In the sample with the 200 mg Pb kg^{-1} content, minor growth impact was observed; the leave ends began to change the color to yellow and did not reach the same size as the previous sample. In the sample with a concentration of 500 mg Pb kg^{-1}, it was evident that there was no plant development. Concentrations above 500 mg Pb kg^{-1} also led to the plant destruction, *i.e.*, were phytotoxic for lettuce. We assumed that the lead in concentrations higher than *MPL* inhibited lengthening of the root and leaf growth, inhibited photosynthesis and affected the morphological and anatomical structure of plants. Based on the above mentioned results, we concluded that the lead in the soil at concentrations higher than 200 mg kg^{-1} caused complete sterility of the land in respect to the particular plant culture.

Cadmium in all concentrations did not cause changes in the growth of leaves or in the morphological and anatomical structure of the plants. Based on the results, it can be concluded that cadmium in the tested concentration range does not affect the development and growth of lettuce.

The influence of Pb and Cd levels in soil on sorption in lettuce

Based on the results obtained by ICP-MS measuring the concentration of lead and cadmium in solution obtained by acid digestion of the dried leaves of lettuce, concentrations of these metals in fresh lettuce leaves were calculated. Similarly, transfer factor (*TF*) of heavy metals from the earth to a lettuce was calculated.

Transfer factor is the ratio of the concentration of heavy metals in the plant and the concentration of heavy metals in the soil. It is a useful tool to determine the relative differences in the bioavailability of heavy metals in the samples of lettuce or to identify efficiency in the accumulation of heavy metals. Transfer factors for each heavy metal are calculated based on the method described in the previous research,[12,13]:

$$TF = c_{\text{lv}}/c_{\text{total soil}} \quad (1)$$

where: c_{lv} (mg kg^{-1}) – plant metal content; $c_{\text{total soil}}$ (mg kg^{-1}) – total soil metal content.

The results of investigated metals in fresh lettuce leaves and *TF* are presented in Table II. All the obtained values of *TF* in the lead contaminated samples are less than 1. The transfer factor values indicated that the bioavailability of lead from soil is small. It can be seen from the results that with increasing concentrations of lead, transfer factor decreased. Most likely, this is due to a negative impact of increased concentrations of lead, which inhibited growth of root and leaves. The same factor also inhibited photosynthesis thus reducing the intake of lead in the plant.

TABLE II. The content of heavy metals in soil and leaves of investigated samples

Sample No.	Contaminant	C_{Pb} and C_{Cd} in compost, mg kg^{-1}	C_{Pb} and C_{Cd} in leaves samples, mg kg^{-1}	*TF*
1	No contaminant	–	–	–
2	Pb^{2+}	50	0.2900	0.0058
3	Pb^{2+}	100	0.5600	0.0056
4	Pb^{2+}	150	0.8367	0.0055
5	Pb^{2+}	200	1.1001	0.0055
6	Pb^{2+}	250	1.3411	0.0054
7	Pb^{2+}	500	2.1140	0.0042
8	Pb^{2+}	1000	–	–
9	Cd^{2+}	1	0.0255	0.0255
10	Cd^{2+}	3	0.0460	0.0153
11	Cd^{2+}	6	0.0873	0.0145
12	Cd^{2+}	12	0.1620	0.1350
13	Cd^{2+}	25	0.2960	0.1184
14	Pb^{2+} + Cd^{2+}	200 + 6	0.83 + 0.0415	0.00415 + 0.0069

From the *TF* results shown in the Table II, it is evident that the values for cadmium are also less than one, although approximately twenty times higher than the obtained values for lead. These values indicate that the bioavailability of metals from the soil is better for cadmium than for lead. Also, this indicates that lettuce has better ability for cadmium accumulation than for lead. It is also evident that with increased cadmium concentration in the soil, *TF* concentration of Cd in plants decreased, probably due to the decrease of photosynthesis, which is reducing the intake of this element by the plant.

Based on the *TF* value in the sample that had lead and cadmium, it can be concluded that due to the negative effects of both metals on photosynthesis, there was decreased intake of them by plant. Also *TF* value is lower in relation to the same single concentration of these metals.

The values for metal bioaccumulation in plants can be influenced by many factors, such as age of the plants, plant species in question, pH value of the land, the nature of the soil and climate.[3,14]

In order to correlate MPL of Pb^{2+} and Cd^{2+} in the soil (Table II) with *MPL* of these metals in lettuce (Table I), Fig. 1 shows the dependence of the concentration of lead and cadmium in lettuce leaves on the concentration of the soil on which it was grown.

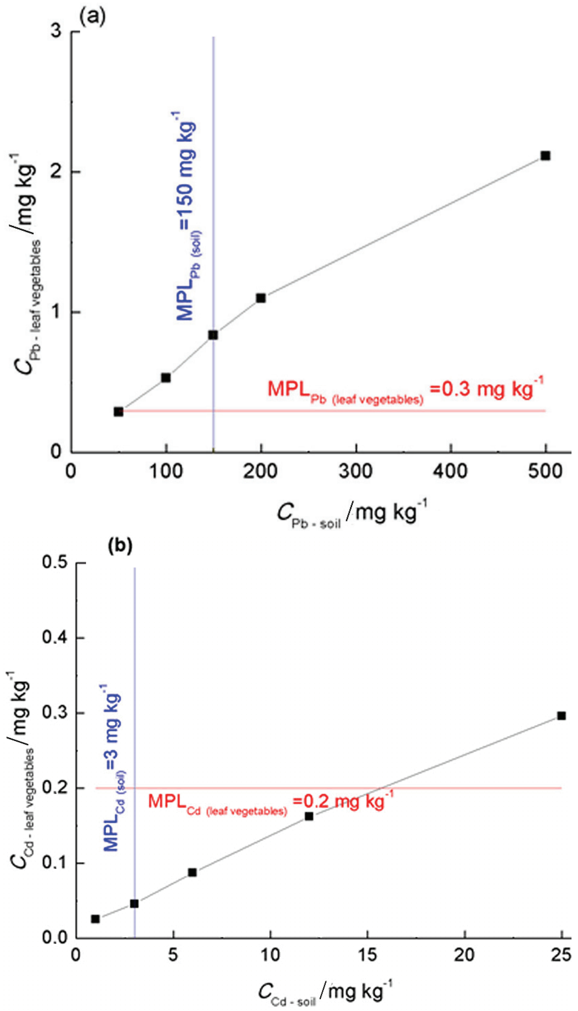

Fig. 1. The dependence of the metal concentration in the lettuce leaves from the concentration in the soil for the lead (a) and cadmium (b).

Figure 1 shows evident linear dependence of heavy metal content in lettuce on their content in the soil. Also, maximum permitted levels of lead in the soil are too high, and to meet the *MPL* in lettuce, limit should be set at 50 mg kg^{-1} soil. As for the cadmium, *MPL* in the soil is properly set and ensures that the content of Cd in lettuce does not exceed the *MPL* according to current standards for food safety.

Prediction of Pb and Cd bioaccumulation in lettuce depending on the soil characteristics

RSM model of investigation of heavy metals phytosorption from soil characteristics has enabled the analysis of all interactions that exist between the analyzed changeable conditions. Application of the method allows assessment of changeable conditions on the result using a clear graphical visualization or by prediction of results depending on the set conditions.

RSM experimental plan for Pb biosorption on lettuce and results of biosorption and TF are presented in Table III.

TABLE III. Experimental plan for RSM (including coded and real values of variables) and experimental results of Pb biosorption on lettuce and transfer factor

No.	X_1, pH	X_2, c_{Pb} / mg kg^{-1}	X_3, m_{NPK} / g kg^{-1}	c / mg g^{-1}	TF
1.	1 (7.5–8)	0 (150)	0 (0.5)	0.801	0.0053
2.	–1 (5.5–6)	1 (250)	1 (1)	1.081	0.0043
3.	–1 (5.5–6)	0 (150)	0 (0.5)	0.852	0.0057
4.	0 (6.5–7)	–1 (50)	0 (0.5)	0.311	0.0062
5.	1 (7.5–8)	–1 (50)	–1 (0)	0.272	0.0054
6.	–1 (5.5–6)	–1 (50)	1 (1)	0.291	0.0058
7.	1 (7.5–8)	1 (250)	–1 (0)	1.095	0.0044
8.	0 (6.5–7)	0 (150)	–1 (0)	0.834	0.0056
9.	0 (6.5–7)	0 (150)	1 (1)	0.831	0.0055
10.	–1 (5.5–6)	–1 (50)	–1 (0)	0.24	0.0048
11.	0 (6.5–7)	1 (250)	0 (0.5)	1.14	0.0046
12.	0 (6.5–7)	0 (150)	0 (0.5)	0.841	0.0056
13.	1 (7.5–8)	–1 (50)	1 (1)	0.231	0.0046
14.	–1 (5.5–6)	1 (250)	–1 (0)	1.111	0.0044
15.	1 (7.5–8)	1 (250)	1 (1)	0.989	0.0039
16.	0 (6.5–7)	0 (150)	0 (0.5)	0.841	0.0056

As it can be seen in Fig. 2, the greatest impact on the accumulation efficiency of lead has a pH value of the soil, which is in accordance with the previous studies.[15] Next important factor is the nutrient content of the soil, which was monitored on the basis of nutrition/non-nutrition of soil samples using NPK fertilizer. For the samples that were not additionally fertilized or had an optimal nutrition level (0.5 g kg^{-1}≈ 50 g m^{-2}), greater sorption of lead was observed since the root system and the whole plant developed normally. As expected, general adoption of nutrients was followed by adoption of lead.

In the soil where nutrition was higher than optimal (1 g kg^{-1}≈ 100 g m^{-2}) bioaccumulation of lead was reduced. Applying too much fertilizer causes drying of plant root system and damages that can cause plants dying out;[16] as a side effect there is a reduction of lead bioaccumulation. Based on the data, lead content in soil has the least impact on the level of bioaccumulation – at a lower content of lead in soil transfer factor is higher compared to the soil with a higher content of lead. Lead in higher concentrations inhibited lengthening of root and leaf growth, inhibited photosynthesis, and affected the morphological and anatomical structure of plants and thus the adoption of matter from the soil.

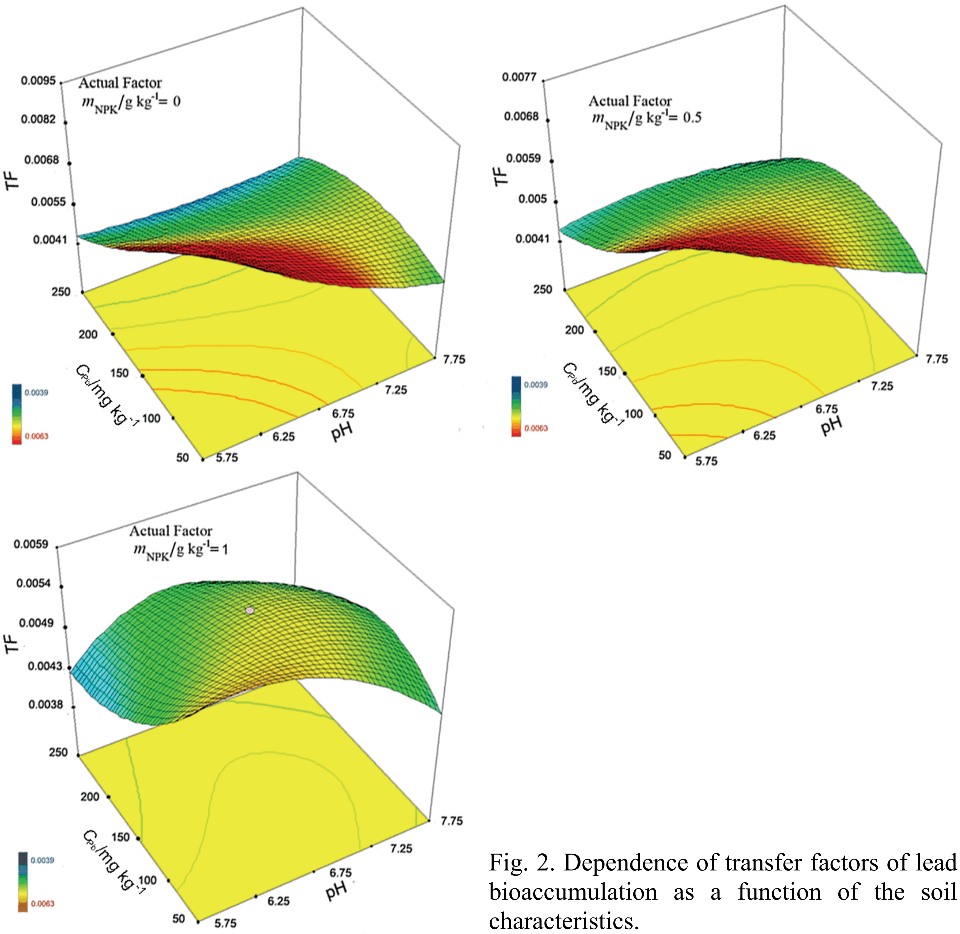

Fig. 2. Dependence of transfer factors of lead bioaccumulation as a function of the soil characteristics.

RSM experimental plan for Cd biosorption on lettuce and results of biosorption and *TF* are presented in Table IV.

TABLE IV. Experimental plan for RSM (including coded and real values of variables) and experimental results of Cd biosorption on lettuce and transfer factor

No.	X_1, pH	X_2, c_{Cd} / mg kg^{-1}	X_3, m_{NPK} / g kg^{-1}	c / mg g^{-1}	*TF*
1.	1 (7.5–8)	0 (6)	0 (0.5)	0.0804	0.0134
2.	–1 (5.5–6)	1 (12)	1 (1)	0.1908	0.0159
3.	–1 (5.5–6)	0 (6)	0 (0.5)	0.1038	0.0173
4.	0 (6.5–7)	–1 (3)	0 (0.5)	0.1377	0.0153
5.	1 (7.5–8)	–1 (3)	–1 (0)	0.0435	0.0145

TABLE IV. Continued

No.	X_1, pH	X_2, c_{Cd} / mg kg^{-1}	X_3, m_{NPK} / g kg^{-1}	c / mg g^{-1}	*TF*
6.	–1 (5.5–6)	–1 (3)	1 (1)	0.0541	0.018
7.	1 (7.5–8)	1 (12)	–1 (0)	0.1548	0.0129
8.	0 (6.5–7)	0 (6)	–1 (0)	0.1014	0.0169
9.	0 (6.5–7)	0 (6)	1 (1)	0.0948	0.0158
10.	–1 (5.5–6)	–1 (3)	–1 (0)	0.0525	0.0175
11.	0 (6.5–7)	1 (12)	0 (0.5)	0.1824	0.0152
12.	0 (6.5–7)	0 (6)	0 (0.5)	0.0936	0.0156
13.	1 (7.5–8)	–1 (3)	1 (1)	0.0402	0.0134
14.	–1 (5.5–6)	1 (12)	–1 (0)	0.1932	0.0161
15.	1 (7.5–8)	1 (12)	1 (1)	0.1488	0.0124
16.	0 (6.5–7)	0 (6)	0 (0.5)	0.0936	0.0156

The impact of variable conditions on phytosorption of cadmium from the soil (Fig. 3) is the same as for phytosorption of lead.

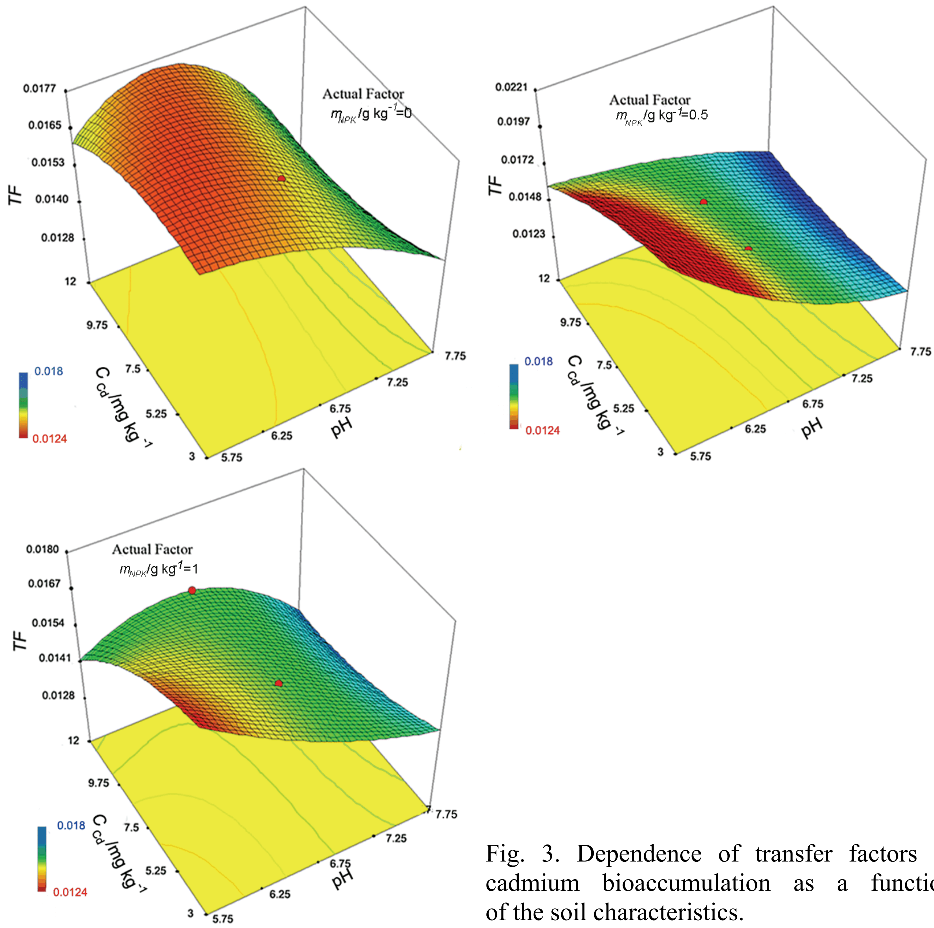

Fig. 3. Dependence of transfer factors of cadmium bioaccumulation as a function of the soil characteristics.

CONCLUSION

Increased concentration of pollutants in the soil adversely affects the development of plants, *e.g.*, lettuce, destroying their morphological and anatomical structure. The lead concentration greater than 200 mg kg^{-1} has shown phytotoxicity and caused infertility of soil for lettuce growth.

The concentration of heavy metals in the soil affects the concentration of heavy metals in the vegetable crops grown on contaminated soil. Increasing concentrations of heavy metals in the soil increased their concentrations in crops. Limit of lead maximum permitted level in the soil of 150 mg kg^{-1} per EURS and 100 mg kg^{-1} per Serbian standard does not ensure that the level of lead is in the permitted range, according to the regulations on food safety (for leafy vegetables $MPL_{Pb} < 0.3$). Limit is set correctly for cadmium, ensuring that the concentration of the metal in grown plants is within permissible limits according to the regulations on food safety ($MPL_{Cd} < 0.2$ mg kg^{-1}). Calculated transfer factors for lead and cadmium showed that the bioavailability of cadmium is much higher in relation to lead, which is in accordance with previous studies.[12] It also showed that increase of heavy metals concentration in the soil reduced the transfer factors. Consequently, increasing concentrations of heavy metals in the soil increased the negative impact on the crop plants, because they are less developing and less adopting elements from the soil.

RSM has proved to be very good for the examination of a large number of variables with a small number of experiments. Also, it gives a clear graphical visualization and noticeable interaction of changing conditions, which allows prediction of results in the desired condition range that were not examined. This method is particularly useful in order to reduce the number of necessary experiments for examination, which saves time and reduces the amount of hazardous and/or expensive reagents thus reducing material costs and contributing to environmental protection.

SUPPLEMENTARY MATERIAL

Experimental details and additional results are available electronically from http:// //www.sbd.org.rs/JSCS/ or from the corresponding author on request.

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ИЗВОД

ИСТРАЖИВАЊЕ УТИЦАЈА КАРАКТЕРИСТИКА ЗЕМЉИШТА НА СОРПЦИЈУ ТЕШКИХ МЕТАЛА БИЉКАМА И МОГУЋНОСТИ ПРЕДИКЦИЈЕ ЊИХОВЕ БИОАКУМУЛАЦИЈЕ ПОМОЋУ МЕТОДОЛОГИЈЕ ОДЗИВНИХ ПОВРШИНА

ЗЛАТЕ ВЕЛИЧКОВИЋ, НЕГОВАН ИВАНКОВИЋ, ВАЊА СТРИКОВИЋ, РАДОВАН КАРКАЛИЋ, ДАЛИБОР ЈОВАНОВИЋ, ЗОРАН БАЈИЋ и ЈОВИЦА БОГДАНОВ

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Циљ рада је био да се утврди утицај карактеристика земљишта на сорпцију тешких метала поврћем које се користи у исхрани и могућности предвиђања њихове био-акумулације методологијом одзивних површина (RSM). Као биосорбент је коришћена зелена салата, а као контаминанти су коришћени кадмијум (Cd) и олово (Pb). Зелена салата је гајена на компосту (раније контаминиран различитим концентрацијама Pb и Cd) подешене pH вредности различитим количинама NPK ђубрива. Садржај тешких метала је одређиван са ICP-MS. Резултати су показали да је садржај кадмијума у зеленој салати био испод токсичних вредности, али концентрације олова су биле изнад дозвољених што указује да гранична вредност за олово није постављена у складу са правилницима о здравственој исправности хране. Утврђено је да акумулација тешких метала у биљкама не зависи само од укупног садржаја у земљишту, него и од афинитета биљке према одређеном металу, те индивидуалног или интерактивног дејства разних земљишних својстава. Преко трансфер фактора утврђено је да зелена салата има много већи афинитет према Cd у односу на Pb. RSM се показала као веома добра за испитивање великог броја променљивих са малим бројем експеримената.

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