EVALUATING THE UPTAKE OF TEN HEAVY METALS BY KIDNEY BEAN (*PHASEOLUS VULGARIS* L.) GROWN IN A SOIL-SLUDGE MIXTURE USING A REGRESSION MODEL

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(Received 20th Feb 2020; accepted 25th May 2020)

Abstract. Severe human health risks can be caused by consuming vegetables contaminated by heavy metals (HMs); thus, assessing the HM uptake by these plants is important. The current work was performed to construct a regression model for predicting the concentration of ten HMs in four tissues of *Phaseolus vulgaris* (roots, stems, leaves and pods) based on their concentration in a soil-sludge mixture, soil organic matter (OM) and soil pH. For pods, the regression equation with the highest coefficient of determination ($R^2 = 0.99$) and model efficiency ($ME = 1.00$) but the lowest mean normalized bias ($MNB = 0.01$) was that of cobalt. For leaves, the equation with the highest R^2 (0.90) and ME (0.92) but the lowest MNB (0.001) was that of molybdenum. Comparable findings were obtained for molybdenum in the stems and manganese in the roots. All t values that assessed the difference between the actual and predicted values of the ten HMs in the four tissues were nonsignificant. Thus, these models could be used as a risk assessment tool for *P. vulgaris* cultivated in soil-sludge combinations.

Keywords: bioconcentration factor, prediction modelling, sewage sludge, translocation factor, vegetable crops

Introduction

Contamination of cultivated lands by heavy metals (HMs) due to severe human impacts (such as industrial effluents, sewage sludge, chemical fertilizers and pesticides, untreated irrigation water contaminated by wastewater and mining activities) represents a hazardous global problem (Ramadan and Al-Ashkar, 2007). Some HMs are essential to plants at low concentrations (e.g., Zn, Mn, Fe and Cu), but others pose a toxicity risk to living organisms (e.g., Ni, Cd, Pb, As, Hg and Cr). Therefore, many studies have evaluated the risks associated with the accumulation of HMs in soil-crop systems (Belaïd et al., 2012; Galal, 2016; Farahat et al., 2017; Jamali et al., 2007, 2009; Zhao et al., 2010).

Although HMs occur in different forms in the soil, only the dissolved forms are available for uptake by plants (Aydinalp and Marinova, 2003). The adsorption and desorption properties of soil are among the main factors that limit the bioavailability of HMs for plants (Krishnamurti et al., 1999). Some soil factors, such as pH, organic matter (OM), and Fe and Mn oxides, influence the adsorption and desorption of HMs in

soil (Usman et al., 2008). In view of this, soil pH has a significant negative correlation with the mobility and availability of HMs in soil solution, with acidity increasing the availability of HMs and alkalinity having a decreasing effect (Du Laing et al., 2008; Novotná et al., 2015; Zeng et al., 2011).

Consequent to the treatment of drainage water resulting from municipal and industrial activities, sewage sludge (SS) is produced as an organic byproduct that has excess macro- and micro-nutrients (Eid and Shaltout, 2016). Approximately 1.3 billion tons of SS is produced by cities globally per annum, and this is predicted to expand to 2.2 billion tons globally per annum by 2050 worldwide (Hoornweg and Bhada-Tata, 2012). Thus, there is consideration for how such quantities of SS can be disposed of safely to ensure it does not impact the environment (Eid et al., 2017) with the best means indicated as landfills and land application (Li et al., 2018). Some studies have indicated that the mixing of cultivated soil with certain amounts of SS has an enhancing effect on soil fertility and plant nutrients (Aráujo et al., 2007). Many studies (Singh and Agrawal, 2008, 2010; Grotto et al., 2015; Rehman et al., 2018; Asgari Lajayer et al., 2019) reported that SS could be added as a valuable fertilizer that improves the growth and yield of plant crops. However, this practice may lead to a risk to humans and the environment because of the presence of HMs that could contaminate soil and water, cause phytotoxicity, and the entry of such contaminants into the food supply (Mamais et al., 2000; Dolgen et al., 2007; Singh and Agrawal, 2007). The degree of danger is dependent on the quantity of SS applied, its structure, the crop species used and the conventional controls employed (Latare et al., 2014).

To reduce the toxicity levels of HMs and their movement through the food chain, it is important to assess the impact of soil variables on the availability and uptake of HMs by plants (Zeng et al., 2011). Regression models are useful mathematical tools for predicting the concentration of HMs in crop plants using certain soil factors such as HMs, pH and OM (Waegeneers et al., 2011). Comparable equations were constructed for barley (Adams et al., 2004; Novotná et al., 2015; Eid et al., 2020a), broad bean (Eid et al., 2019), carrot (Legind and Trapp, 2010; Bešter et al., 2013), cauliflower (Kumar et al., 2019), celery (Wang et al., 2004), chicory (Bešter et al., 2013), Chinese cabbage (Zhang et al., 2016), cucumber (Eid et al., 2018a), endive (Bešter et al., 2013), *Eruca sativa* (Eid et al., 2020b), garden pea (Eid et al., 2020c), hop (Novotná et al., 2015), lettuce (Legind and Trapp, 2010), maize (Tudoreanu and Phillips, 2004; Yang et al., 2013; Novotná et al., 2015), onion (Bešter et al., 2013), potato (Bešter et al., 2013; Novotná et al., 2015), rapeseeds (Novotná et al., 2015), red beet (Bešter et al., 2013), rice (Zeng et al., 2011; Rafiq et al., 2014), rye grass (Tudoreanu and Phillips, 2004), soy bean (Tudoreanu and Phillips, 2004), spinach (Wang et al., 2004; Eid et al., 2018b), tomato (Ramadan and Al-Ashkar, 2007; Bešter et al., 2013), wheat (Adams et al., 2004; Chaudri et al., 2007; Novotná et al., 2015; Eid et al., 2020d) and zucchini (Bešter et al., 2013). However, differences in soil, climate, SS composition and management factors require more specific estimates for different climatic regions or different cropping systems (Binder et al., 2002). Therefore, for safe crop production, it is important to evaluate the toxic levels of the HMs in the soil by establishing regression equations between the HMs in the plants on the one hand and those in the soil supporting the plants on the other hand (Eid et al., 2020d).

The kidney bean (*Phaseolus vulgaris* L.) is a herbaceous annual leguminous plant that has a long history of cultivation (Gentry, 1969). *P. vulgaris* is an annual plant belonging to family *Fabaceae* that originated in Central-South America. It is an erect or

twining plant with compound leaves (each with three leaflets), and its flowers are usually white to purple in colour. Its pods are long, narrow, and straight or slightly curved, with pointed tips. The size and colour of its seeds are variable. The pods are cooked in immature stages, leaves are edible, and dried seeds are cooked and sometimes fried in oils as a salad (Huxley, 1999; Tindall, 1978). *P. vulgaris* is the most important food legume for direct consumption in the world (Jones, 1999). It was therefore selected for assessment in this research study. To the best of the authors' knowledge, no regression models are available for the uptake of HMs by *P. vulgaris* grown in soil amended with SS. Therefore, the present study was carried out to develop a mathematical model for predicting the potential uptake of HMs by *P. vulgaris* after growing it in an agricultural soil with different rates of SS addition under greenhouse conditions. Such models are suitable for assessing the severe impact of cultivating some vegetable crops in soil mixed with different amounts of SS.

Materials and methods

Materials and experimental design

White seeds of *P. vulgaris* (Strike, Seminis Vegetable Seeds, Inc., St. Louis, USA) were obtained from a local market in Abha City, Saudi Arabia. The soil used in the experiment was collected from nearby cultivated fields at a depth of 0-20 cm (Lat.: 18° 14' 36.37" N, Long.: 42° 33' 58.25" E), while the SS was obtained from the municipal sewage treatment station in Abha (Lat.: 18° 13' 59.19" N, Long.: 42° 31' 16.35" E). This station treats some 41,275 m³ day⁻¹ wastewater and produces 90 tons day⁻¹ dry SS (Eid et al., 2017). Soil and SS samples were air dried for 2 weeks, ground and passed through a 2-mm sieve.

The experiment was performed in the greenhouse of the Biology Department, King Khalid University, Abha. Based on a preliminary experiment, the SS was mixed with agricultural soil at concentrations of 0, 10, 20, 30, 40 and 50 g kg⁻¹. Each treatment consisted of six plastic pots (each with a 6-L volume, filled with four kg of the respective treatment, and planted with ten equally sized seeds). The experimental units were arranged following a completely randomized design (CRD). The seeds were sown (directly after mixing the soil with SS) on the 4th of January 2018 for a period of 57 days under a natural day/night regime, irrigated using tap water as required with the soil water holding capacity (SWHC) in each pot varying between 40-50% and weeded manually as needed. The SWHC was determined and maintained on a volume basis using the following formula (Eq. 1):

$$\text{Volumetric SWHC (\%)} = (\text{depth of water in cm} / \text{depth of soil in cm}) \times 100 \quad (\text{Eq.1})$$

Fifteen days after sowing, the emerged plants were manually thinned to one individual in each pot.

Sample analysis

Plant materials were harvested on 1 March 2018, then cleaned under running tap water, separated into four tissues (roots, pods, stems and leaves), dried for one week at 60 °C, powdered in a plastic mill and stored for later use. For physicochemical analyses, post-harvest soil samples were air-dried for two weeks, ground and passed through a 2-

mm sieve. Dried powders (pre- and post-harvest soil and SS samples) were analysed for OM using the loss-on-ignition method at 550 °C for 2 h (Wilke, 2005), while pH was measured in 1:5 soil:water extracts (Allen, 1989).

For HM estimation, 0.5-1.0 g of each dried powders and plant sample was digested following the mixed-acid digestion method (HNO₃ and HClO₄; 3:1, v/v). A microwave sample preparation system was used for digestion (PerkinElmer Titan MPS, PerkinElmer Inc., USA). Blank samples were used to verify the accuracy of the digestion procedure and the subsequent analyses. Ten HMs (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) were estimated by inductively coupled plasma optical emission spectrometry (Thermo Scientific iCAP 7000 Plus Series; Thermo Fisher Scientific, USA) as outlined by Allen (1989). The detection limits of the HMs (in µg l⁻¹) were as follows: 6.0 for Ni; 2.0 for Co, Cr and Cu; 1.0 for Fe, Pb and Zn; 0.3 for Mn; and 0.1 for Cd and Mo. The settings of the instrument and the conditions of operation were as specified by the manufacturer. Standard solutions with known concentrations of the ten HMs were prepared for system standardization.

Quality assurance and control

Certified reference material (tomato leaves, SRM 1573a) was used to verify the accuracy of HM estimates. The reference material was digested and analysed using the methods applied to the *P. vulgaris* samples. Digestion and measurement of the ten HMs were performed in triplicate. Accuracy was assessed by comparing the actual concentration with the certified value and then expressed as a percentage. Recovery rates had a range of 95-104% for SRM 1573a.

Data analysis

Before performing the one-way analysis of variance (ANOVA-1), the distribution of the HM, bioconcentration factor (BCF) and translocation factor (TF) data was tested for normality using the Shapiro-Wilk W test and for homogeneity of variance using Levene's test; when necessary, the data were log-transformed. The HM data for *P. vulgaris* tissues were subjected to an ANOVA-1 to evaluate the differences among *P. vulgaris* tissues. Significant difference between means among the four tissues were identified using the Tukey's HSD test at $p < 0.05$. The BCF determines the capacity of a plant, such as *P. vulgaris*, to accumulate an HM in its roots (Galal et al., 2017) and is calculated using the following formula (Eq. 2):

$$\text{BCF} = C_{\text{Root}} / C_{\text{Soil}} \quad (\text{Eq.2})$$

where C_{Root} is the concentration of an HM in the root (mg kg⁻¹) and C_{Soil} is its concentration in the soil (mg kg⁻¹). In addition, the TF determines the ability of *P. vulgaris* to translocate an HM from the root to each of the stem, leaf and pod (Eid and Shaltout, 2016) and is calculated using the following formulas (Eqs. 3, 4 and 5):

$$\text{TF}_{\text{Stem}} = C_{\text{Stem}} / C_{\text{Root}} \quad (\text{Eq.3})$$

$$\text{TF}_{\text{Leaf}} = C_{\text{Leaf}} / C_{\text{Root}} \quad (\text{Eq.4})$$

$$\text{TF}_{\text{Pod}} = C_{\text{Pod}} / C_{\text{Root}} \quad (\text{Eq.5})$$

where C_{Root} , C_{Stem} , C_{Leaf} and C_{Pod} are the concentrations of an HM in these tissues (mg kg⁻¹). The BCFs and TFs data were subjected to an ANOVA-1 to evaluate the differences among the ten HMs. Significant difference between means among the ten HMs were identified using the Tukey's HSD test at $p < 0.05$. Pearson's simple linear correlation coefficient (r) was estimated for the BCF of a certain HM in the plant tissues with soil pH and soil OM. The same coefficient was determined to assess the relationship between the plant HMs and soil HMs, pH and OM.

Eighteen observations from the dataset for each of the root, stem and leaf were selected randomly as a validation dataset. The other eighteen observations were used to establish the regression models for predicting the HM contents in *P. vulgaris* tissues, based on some soil variables used as independent variables (pH, OM, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn). The number of individuals that produced pods was twelve (in treatments with concentrations of 10 and 20 g kg⁻¹ only); thus, six observations were used as a validation dataset, while the other six observations were used in the regression procedure. Following Novotná et al. (2015) and Yang et al. (2013), the OM content, pH value and HM content of the soil are considered the most important factors with which to explain plant HM concentrations. The general equation of the model was established using the following formula (Eq. 6):

$$C_{\text{Plant}} = a + b \times C_{\text{Soil}} + c \times \text{pH} + d \times \text{OM} \quad (\text{Eq.6})$$

where C_{Plant} is the concentration of a given HM in any *P. vulgaris* tissue; C_{Soil} is its concentration in the soil; OM is the soil organic matter content (%); and a , b , c and d are the regression coefficients.

Model quality was evaluated using the coefficient of determination (R^2), model efficiency (ME), model strength ($MNAE$) and model bias (MNB) using the formulas by Novotná et al. (2015) (Eqs. 7, 8 and 9):

$$ME = 1 - (\sum (C_{\text{Model}} - C_{\text{Measured}})^2 / \sum (C_{\text{Measured}} - C_{\text{Mean}})^2) \quad (\text{Eq.7})$$

$$MNAE = (\sum (|C_{\text{Model}} - C_{\text{Measured}}| / C_{\text{Measured}})) / n \quad (\text{Eq.8})$$

$$MNB = \sum (C_{\text{Model}} - C_{\text{Measured}}) / \sum C_{\text{Measured}} \quad (\text{Eq.9})$$

where C_{Model} is the predicted concentration of an HM given by the model, C_{Measured} is the actual HM concentration, C_{Mean} is the mean of the actual HM concentrations, and n is the number of observations. Student's t -test was applied to assess the significance of the difference between the predicted concentration of an HM in a tissue and its actual value in the same tissue. All these analyses were carried out in SPSS 15.0 (SPSS, 2006).

Results

For prior testing of the quality of the used agricultural soil and SS, the OM, pH value, and concentrations of ten common HMs were analyzed. The data presented in *Appendix 1* show that the agricultural soil from the study region is poor in fertility because it contains less than 1% OM. The pH of this soil is 8.7 (basic), which reduces the availability of most nutrients (Sharma et al., 2017). The HM concentrations in the agricultural soil ranged between a minimum of 1.1 mg kg⁻¹ for Mo and a maximum of

42,400 mg kg⁻¹ for Fe; while in the SS they have a range of 0.9 mg kg⁻¹ for Mo and 24,100 mg kg⁻¹ for Fe (*Appendix 1*). In addition, the concentration of 5 HMs (Cd, Co, Fe, Mn and Mo) are higher in the agricultural soil than in the SS, while the reverse is true regarding the other 5 HMs.

After plant harvesting, the pH values of the soil-sludge mixture were alkaline, with a range of 6.8 - 8.5, a mean of 7.5 and a coefficient of variance (CV) of 7%; the OM ranged between 1 and 9%, with a mean of 5% and a CV of 47% (*Table 1*). The concentrations of the ten HMs in the soil descended as follows: Fe > Mn > Cr > Zn > Ni > Co > Cu > Pb > Cd > Mo. Iron had the highest mean concentration of 41,878 mg kg⁻¹, while Mo had the lowest mean concentration of 1 mg kg⁻¹. Regarding the plant tissues, most of the HM concentrations were lower in the pods than in the other tissues, except Co, Mo and Ni (*Table 2*). In contrast, the HMs were more abundant in the roots than in all the areal tissues, except Mo. In descending order, the ten HMs in the three areal tissues were Fe > Mn > Zn > Mo > Ni > Cu > Cr > Co > Pb > Cd, while those in the roots were Fe > Mn > Zn > Cr > Cu > Ni > Co > Mo > Pb > Cd.

Table 1. Chemical characteristics of the soil (*n* = 36) amended with sewage sludge after harvesting *Phaseolus vulgaris* plants grown for 57 days

Value	pH	OM (%)	Heavy metal concentration (mg kg ⁻¹)									
			Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Minimum	6.8	1	2	24	123	15	23,488	557	1	29	3	64
Maximum	8.5	9	4	49	159	39	67,273	796	2	44	5	120
Mean	7.5	5	3	29	141	28	41,878	623	1	34	4	95
CV (%)	7	47	18	17	7	28	16	8	14	10	20	21

CV: coefficient of variance, OM: organic matter content

Table 2. Heavy metal concentrations in pods, leaves, stems and roots of *Phaseolus vulgaris* plants harvested after 57 days

Tissue	Value	Heavy metal concentration (mg kg ⁻¹)									
		Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Pod	Minimum	0.1	1	1	7	118	25	12	7	0.1	21
	Maximum	0.3	2	2	11	165	38	17	14	1	29
	Mean (<i>n</i> = 12)	0.2a	2a	2a	9a	147a	32a	14b	11b	0.3a	26a
	CV (%)	30	9	21	17	12	15	13	29	83	13
Leaf	Minimum	0.1	1	2	7	183	138	7	3	0.2	20
	Maximum	0.4	2	5	13	1,230	228	20	9	2	33
	Mean (<i>n</i> = 36)	0.2a	1a	3a	10a	389a	187b	15b	5a	1a	27a
	CV (%)	30	38	35	17	78	16	26	30	53	15
Stem	Minimum	0.2	1	1	8	85	24	10	3	1	19
	Maximum	1	2	6	18	734	191	48	8	4	60
	Mean (<i>n</i> = 36)	0.4b	1a	3a	12a	272a	76a	29c	5a	1a	34a
	CV (%)	40	40	55	19	78	73	41	24	57	34
Root	Minimum	0.2	6	19	2	4,078	258	2	11	2	53
	Maximum	2	16	67	46	17,760	1190	15	27	9	222
	Mean (<i>n</i> = 36)	1c	11b	39b	27b	10,283b	673c	7a	18c	4b	126b
	CV (%)	52	24	38	47	41	42	50	26	53	50
F-value		47.4***	346.9***	167.9***	44.7***	155.5***	105.1***	55.9***	146.7***	56.5***	64.3***

F-values represent one-way ANOVA, degrees of freedom = 3. Means in the same column followed by different letters are significantly different at *p* < 0.05 according to Tukey's HSD test. ***: *p* < 0.001, CV: coefficient of variance

The BCFs and TFs were < 1 for most HMs, except the BCFs of Mn (1.07), Mo (6.12), Pb (1.03) and Zn (1.25) and the TFs of Mo (2.53-4.57) in the three areal tissues (Table 3). In addition, the BCFs and TFs varied among the studied HMs (Table 3). Correlations were significant and negative between the BCFs of all ten HMs and soil pH, with the highest r -value of 0.89 for Mn and the lowest of 0.37 for Pb (Fig. 1), but significant and positive between the BCFs and soil OM, with a maximum r -value of 0.84 for Mn and a minimum of 0.36 for Pb (Fig. 2).

Table 3. Descriptive statistical results (mean $\pm$ standard error) of the bioconcentration factors (BCFs) of heavy metals from soil to *Phaseolus vulgaris* roots and the translocation factors of heavy metals from *Phaseolus vulgaris* roots to stems (TF_{stem}), leaves (TF_{leaf}) and pods (TF_{pod})

Heavy metal	BCF (n = 36)	TF_{stem} (n = 36)	TF_{leaf} (n = 36)	TF_{pod} (n = 12)
Cd	0.29 $\pm$ 0.02ab	0.49 $\pm$ 0.03ab	0.27 $\pm$ 0.02a	0.27 $\pm$ 0.03a
Co	0.38 $\pm$ 0.01ab	0.11 $\pm$ 0.01a	0.12 $\pm$ 0.01a	0.17 $\pm$ 0.01a
Cr	0.27 $\pm$ 0.02ab	0.07 $\pm$ 0.00a	0.07 $\pm$ 0.00a	0.05 $\pm$ 0.00a
Cu	0.92 $\pm$ 0.05bcd	0.83 $\pm$ 0.21b	0.63 $\pm$ 0.14b	0.42 $\pm$ 0.02a
Fe	0.25 $\pm$ 0.02a	0.03 $\pm$ 0.00a	0.04 $\pm$ 0.00a	0.02 $\pm$ 0.00a
Mn	1.07 $\pm$ 0.07cd	0.10 $\pm$ 0.01a	0.32 $\pm$ 0.02a	0.06 $\pm$ 0.00a
Mo	6.12 $\pm$ 0.47e	4.57 $\pm$ 0.37c	2.53 $\pm$ 0.21c	3.84 $\pm$ 0.73b
Ni	0.53 $\pm$ 0.02abc	0.31 $\pm$ 0.01ab	0.29 $\pm$ 0.01a	0.77 $\pm$ 0.05a
Pb	1.03 $\pm$ 0.08cd	0.32 $\pm$ 0.03ab	0.21 $\pm$ 0.02a	0.11 $\pm$ 0.02a
Zn	1.25 $\pm$ 0.07d	0.30 $\pm$ 0.01ab	0.26 $\pm$ 0.02a	0.35 $\pm$ 0.02a
F-value	128.7***	101.8***	83.4***	24.7***

F-values represent one-way ANOVA, degrees of freedom = 9. Means in the same column followed by different letters are significantly different at $p < 0.05$ according to Tukey's HSD test. ***: $p < 0.001$

With a few exceptions, the correlation between an HM in the four tissues and the same HM in the soil was significant and positive (Table 4). In addition, significant negative correlations were detected between soil pH and only some HMs in the pods ($r = -0.76$ with Co, -0.67 with Mo, -0.89 with Ni and -0.81 with Zn, $p < 0.001$) but between soil pH and all HMs in the other three tissues. In contrast, soil OM showed the reverse trend with some HMs in the pods and all HMs in the other three tissues.

Correlations between the measured and predicted HM values, with high R^2 , high MEs and low mean MNAEs, reflected the goodness of fit of the model. Additionally, the presence of a non-significant difference (p -value) between the measured and predicted concentrations of the HMs indicated good performance of the developed model. For the pods, the regression equation that had the highest R^2 (0.99) and highest ME (1.00) but the lowest MNB (0.01) was that of Co. For the leaves, the equation with the highest R^2 (0.90) and highest ME (0.92) but the lowest MNB (0.001) was that of Mo. Similar results were obtained for Mo in the stems and Mn in the roots (Table 5). Applying a t test that assessed the significance of the differences between the actual and predicted values of the ten HMs in the four tissues revealed that the differences were nonsignificant ($p > 0.05$).

Discussion

The soil-sludge combination supporting *P. vulgaris* was slightly alkaline (pH = 7.5). High soil pH is known to enhance the adsorption of many HMs, thus decreasing their solubility in soil solutions (Chaudri et al., 2007; Zeng et al., 2011). The adsorption and

desorption properties of soil are among the factors affecting the mobility and availability of HMs (Krishnamurti et al., 1999). Both properties are associated with soil pH, OM, clay minerals and oxidation-reduction status (Antoniadis et al., 2008; Usman et al., 2008). Significant negative correlations were detected between soil pH and most of the estimated HM concentrations in the four tissues of *P. vulgaris*. Zhao et al. (2010) reported that this soil factor plays a vital role in determining the solubility and availability of soil HMs.

Figure 1. Linear relationships (r -values, $n = 36$) between bioconcentration factors (BCFs) of heavy metals in *Phaseolus vulgaris* roots and soil pH. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Figure 2. Linear relationships (r -values, $n = 36$) between bioconcentration factors (BCFs) of heavy metals in *Phaseolus vulgaris* roots and soil organic matter content. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

In contrast, soil OM had significant positive correlations with most of the estimated HM concentrations in the tissues of the studied species. OM content plays an important role in determining the availability and mobility of HMs in soils, and it helps provide organic chemicals to the soil solution that act as chelates to increase HM availability to plants (Zeng et al., 2011). Decomposition of soil OM forms soluble organic-HM complexes and consequently more bioavailable HMs (Antoniadis and

Alloway, 2002). Therefore, SS must be applied carefully under appropriate conditions to reduce the accumulation of HMs in soil and consequently their transfer to plant tissues.

Table 4. Pearson correlation coefficient (*r*-value) between heavy metals in *Phaseolus vulgaris* tissues and their concentrations in the soil

<i>P. vulgaris</i> heavy metals	Soil heavy metals										Soil pH	Soil OM
	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn		
<i>Pod (n = 12)</i>												
Cd	<u>-0.33</u>	<u>-0.36</u>	<u>0.09</u>	<u>0.10</u>	<u>-0.36</u>	<u>-0.29</u>	<u>-0.10</u>	<u>-0.11</u>	<u>-0.09</u>	<u>-0.16</u>	<u>-0.19</u>	<u>0.43</u>
Co	<u>0.28</u>	0.83	<u>0.08</u>	0.83	0.72	<u>0.25</u>	0.89	<u>0.02</u>	0.96	0.94	-0.76	0.77
Cr	<u>-0.48</u>	<u>0.50</u>	<u>-0.53</u>	<u>0.29</u>	0.67	<u>-0.51</u>	<u>0.53</u>	<u>-0.66</u>	<u>0.47</u>	<u>0.40</u>	<u>-0.51</u>	<u>0.54</u>
Cu	<u>0.15</u>	<u>-0.52</u>	0.74	<u>0.48</u>	-0.68	<u>0.19</u>	<u>-0.09</u>	<u>0.51</u>	<u>0.03</u>	<u>0.01</u>	<u>-0.54</u>	<u>0.55</u>
Fe	<u>-0.52</u>	<u>0.52</u>	-0.59	<u>0.26</u>	0.70	<u>-0.55</u>	<u>0.56</u>	-0.71	<u>0.49</u>	<u>0.38</u>	<u>-0.51</u>	<u>0.52</u>
Mn	0.83	<u>-0.20</u>	0.96	<u>0.43</u>	<u>-0.53</u>	0.86	<u>-0.07</u>	0.99	<u>0.10</u>	<u>0.21</u>	<u>-0.11</u>	<u>0.11</u>
Mo	<u>0.28</u>	<u>-0.31</u>	0.80	0.67	<u>-0.52</u>	<u>0.32</u>	<u>0.11</u>	0.58	<u>0.25</u>	<u>0.24</u>	-0.67	0.69
Ni	<u>-0.23</u>	<u>0.32</u>	<u>0.00</u>	0.68	<u>0.33</u>	<u>-0.24</u>	<u>0.60</u>	<u>-0.23</u>	0.64	<u>0.54</u>	-0.89	0.90
Pb	<u>-0.20</u>	<u>-0.32</u>	<u>0.25</u>	<u>0.29</u>	<u>-0.32</u>	<u>-0.16</u>	<u>0.11</u>	<u>0.03</u>	<u>-0.05</u>	<u>-0.03</u>	<u>-0.33</u>	<u>0.38</u>
Zn	0.58	<u>0.28</u>	0.76	0.97	<u>-0.01</u>	0.59	<u>0.56</u>	0.63	0.73	0.75	-0.81	0.84
<i>Leaf (n = 36)</i>												
Cd	0.54	0.70	<u>0.22</u>	0.48	<u>0.24</u>	0.53	0.54	0.57	0.45	0.45	-0.67	0.63
Co	0.66	0.78	0.44	0.75	0.36	0.64	0.59	0.77	0.81	0.83	-0.85	0.81
Cr	0.56	0.71	0.35	0.56	<u>0.16</u>	0.61	0.69	0.80	0.64	0.62	-0.76	0.74
Cu	0.66	<u>0.31</u>	0.73	0.82	0.46	0.33	0.47	0.54	0.74	0.61	-0.89	0.86
Fe	0.46	0.74	<u>0.22</u>	0.53	<u>0.23</u>	0.64	0.65	0.80	0.67	0.55	-0.64	0.60
Mn	0.76	0.55	0.40	0.75	<u>0.21</u>	0.46	0.62	0.63	0.72	0.80	-0.85	0.86
Mo	0.72	0.47	0.72	0.80	0.36	0.42	0.75	0.62	0.83	0.82	-0.87	0.88
Ni	0.57	0.69	0.45	0.78	0.47	0.48	0.59	0.62	0.82	0.76	-0.85	0.80
Pb	0.42	<u>0.11</u>	0.68	0.66	0.44	<u>0.16</u>	0.35	0.38	0.69	0.47	-0.72	0.66
Zn	0.75	0.53	0.53	0.80	<u>0.32</u>	0.48	0.57	0.63	0.88	0.78	-0.94	0.88
<i>Stem (n = 36)</i>												
Cd	0.51	<u>0.24</u>	0.54	0.50	<u>0.21</u>	<u>0.28</u>	<u>0.29</u>	0.40	0.44	0.59	-0.70	0.69
Co	0.60	0.46	0.35	0.65	<u>0.15</u>	0.42	0.52	0.60	0.78	0.71	-0.81	0.78
Cr	0.55	0.58	0.52	0.76	0.44	0.48	0.61	0.69	0.77	0.64	-0.80	0.78
Cu	0.61	0.80	0.41	0.70	0.34	0.70	0.42	0.81	0.55	0.68	-0.81	0.81
Fe	0.45	0.48	0.46	0.68	0.42	0.36	0.55	0.59	0.74	0.58	-0.74	0.70
Mn	0.49	0.54	0.56	0.76	0.47	0.43	0.55	0.65	0.75	0.65	-0.79	0.78
Mo	0.81	0.67	0.47	0.83	<u>0.28</u>	0.61	0.63	0.75	0.81	0.83	-0.90	0.91
Ni	0.37	0.45	0.53	0.62	0.39	0.38	<u>0.19</u>	0.59	0.50	0.50	-0.75	0.75
Pb	0.48	0.76	<u>0.28</u>	0.56	<u>0.25</u>	0.71	0.71	0.82	0.65	0.53	-0.64	0.63
Zn	0.68	0.82	0.37	0.71	<u>0.27</u>	0.74	0.60	0.84	0.72	0.72	-0.82	0.81
<i>Root (n = 36)</i>												
Cd	0.54	<u>0.31</u>	0.64	0.69	0.42	<u>0.16</u>	0.51	0.40	0.79	0.67	-0.77	0.70
Co	0.46	0.64	<u>0.26</u>	0.56	<u>0.29</u>	0.35	0.49	0.51	0.56	0.64	-0.78	0.78
Cr	0.58	0.49	0.48	0.54	<u>0.21</u>	0.46	0.52	0.68	0.49	0.59	-0.78	0.76
Cu	0.66	0.42	0.72	0.86	0.43	0.43	0.72	0.62	0.84	0.66	-0.83	0.81
Fe	0.56	0.44	0.70	0.69	0.40	0.48	0.38	0.63	0.45	0.50	-0.76	0.76
Mn	0.71	0.57	0.54	0.79	0.42	0.47	0.59	0.65	0.82	0.77	-0.92	0.87
Mo	0.64	<u>0.28</u>	0.67	0.76	0.42	<u>0.18</u>	0.48	0.35	0.80	0.83	-0.79	0.80
Ni	0.55	0.64	0.48	0.73	0.39	0.48	0.47	0.68	0.70	0.70	-0.89	0.89
Pb	0.33	0.56	0.39	0.44	0.35	0.39	0.50	0.56	0.43	0.50	-0.59	0.56
Zn	0.66	0.56	0.44	0.73	<u>0.27</u>	0.42	0.58	0.61	0.80	0.87	-0.86	0.87

OM: organic matter content. $n = 12$: $p < 0.05$ ($r \geq 0.58$), $p < 0.01$ ($r \geq 0.72$), $p < 0.001$ ($r \geq 0.86$). $n = 36$: $p < 0.05$ ($r \geq 0.33$), $p < 0.01$ ($r \geq 0.43$), $p < 0.001$ ($r \geq 0.55$). Nonsignificant r values ($p > 0.05$) are underlined

Table 5. Regression models between heavy metal concentrations in *Phaseolus vulgaris* tissues (mg kg⁻¹) and soil heavy metals (mg kg⁻¹), pH and organic matter (OM) content (%)

Equation	R^2	ME	$MNAE$	MNB	Student's t -test	
					t -value	p
<i>Pod</i>						
$Cd_{pod} = -3.576 - 0.054 \times Cd_{soil} + 0.460 \times pH + 0.099 \times OM$	0.54	0.98	0.21	0.00	0.02	0.98
$Co_{pod} = 2.317 + 0.025 \times Co_{soil} - 0.217 \times pH + 0.052 \times OM$	0.99	1.00	0.01	0.01	1.56	0.18
$Cr_{pod} = 8.114 - 0.036 \times Cr_{soil} - 0.347 \times pH + 0.274 \times OM$	0.89	0.99	0.07	0.07	2.59	0.12
$Cu_{pod} = 23.505 - 0.010 \times Cu_{soil} - 2.686 \times pH + 0.611 \times OM$	0.32	0.89	0.18	0.12	1.67	0.16
$Fe_{pod} = -191.906 + 0.008 \times Fe_{soil} - 0.254 \times pH + 8.584 \times OM$	0.66	0.99	0.13	0.12	1.69	0.23
$Mn_{pod} = -21.811 + 0.107 \times Mn_{soil} - 1.444 \times pH - 0.176 \times OM$	0.74	0.99	0.07	0.01	0.30	0.78
$Mo_{pod} = 99.371 - 34.628 \times Mo_{soil} - 6.639 \times pH - 1.001 \times OM$	0.62	0.99	0.07	0.00	0.08	0.94
$Ni_{pod} = 92.323 - 0.558 \times Ni_{soil} - 9.183 \times pH + 2.105 \times OM$	0.97	0.99	0.05	0.01	0.21	0.85
$Pb_{pod} = 1.808 - 0.629 \times Pb_{soil} - 0.023 \times pH + 0.261 \times OM$	0.39	0.87	0.54	0.10	0.40	0.71
$Zn_{pod} = 55.093 + 0.050 \times Zn_{soil} - 5.102 \times pH + 1.648 \times OM$	0.77	0.99	0.06	0.01	0.22	0.84
<i>Leaf</i>						
$Cd_{leaf} = 0.930 + 0.004 \times Cd_{soil} - 0.093 \times pH - 0.002 \times OM$	0.44	0.39	0.17	0.03	0.69	0.50
$Co_{leaf} = 3.561 + 0.048 \times Co_{soil} - 0.500 \times pH + 0.024 \times OM$	0.87	0.88	0.10	0.00	0.10	0.93
$Cr_{leaf} = 16.501 - 0.029 \times Cr_{soil} - 1.356 \times pH + 0.095 \times OM$	0.63	0.64	0.15	0.01	0.20	0.85
$Cu_{leaf} = 26.640 - 0.045 \times Cu_{soil} - 2.391 \times pH - 0.009 \times OM$	0.80	0.77	0.07	0.01	0.34	0.74
$Fe_{leaf} = 4480.959 - 0.002 \times Fe_{soil} - 514.335 \times pH - 24.985 \times OM$	0.42	0.52	0.45	0.04	0.29	0.77
$Mn_{leaf} = 276.061 + 0.050 \times Mn_{soil} - 19.950 \times pH + 5.949 \times OM$	0.75	0.75	0.06	0.00	0.12	0.91
$Mo_{leaf} = 17.856 + 8.829 \times Mo_{soil} - 2.170 \times pH + 0.632 \times OM$	0.90	0.92	0.07	0.00	0.00	1.00
$Ni_{leaf} = 24.194 + 0.070 \times Ni_{soil} - 2.771 \times pH - 0.078 \times OM$	0.73	0.73	0.14	0.00	0.10	0.92
$Pb_{leaf} = 5.113 + 0.170 \times Pb_{soil} - 0.633 \times pH - 0.051 \times OM$	0.56	0.50	0.58	0.04	0.41	0.68
$Zn_{leaf} = 97.118 + 0.009 \times Zn_{soil} - 9.185 \times pH - 0.423 \times OM$	0.89	0.90	0.04	0.00	0.29	0.78
<i>Stem</i>						
$Cd_{stem} = 1.770 - 0.037 \times Cd_{soil} - 0.174 \times pH + 0.019 \times OM$	0.51	0.32	0.31	0.08	1.03	0.32
$Co_{stem} = 6.229 + 0.005 \times Co_{soil} - 0.694 \times pH + 0.014 \times OM$	0.66	0.67	0.19	0.02	0.33	0.75
$Cr_{stem} = 16.967 - 0.005 \times Cr_{soil} - 1.877 \times pH + 0.110 \times OM$	0.65	0.68	0.26	0.03	0.37	0.71
$Cu_{stem} = 28.016 - 0.029 \times Cu_{soil} - 2.288 \times pH + 0.385 \times OM$	0.67	0.66	0.07	0.02	0.79	0.44
$Fe_{stem} = 2386.891 - 0.004 \times Fe_{soil} - 302.592 \times pH - 2.840 \times OM$	0.56	0.62	0.46	0.06	0.50	0.62
$Mn_{stem} = 466.786 + 0.084 \times Mn_{soil} - 61.969 \times pH + 4.757 \times OM$	0.64	0.62	0.48	0.02	0.19	0.86
$Mo_{stem} = 53.364 + 14.133 \times Mo_{soil} - 7.149 \times pH + 2.519 \times OM$	0.87	0.85	0.16	0.00	0.02	0.99
$Ni_{stem} = 9.916 + 0.066 \times Ni_{soil} - 0.986 \times pH + 0.143 \times OM$	0.60	0.59	0.14	0.04	0.95	0.35
$Pb_{stem} = 1.533 + 0.340 \times Pb_{soil} - 0.255 \times pH + 0.041 \times OM$	0.46	0.48	0.29	0.03	0.41	0.69
$Zn_{stem} = 122.663 + 0.056 \times Zn_{soil} - 13.081 \times pH + 0.847 \times OM$	0.69	0.69	0.13	0.01	0.33	0.75
<i>Root</i>						
$Cd_{root} = 11.361 - 0.138 \times Cd_{soil} - 1.263 \times pH - 0.073 \times OM$	0.61	0.63	0.20	0.08	1.06	0.30
$Co_{root} = 5.812 + 0.191 \times Co_{soil} - 0.434 \times pH + 0.616 \times OM$	0.70	0.68	0.12	0.03	0.96	0.35
$Cr_{root} = 170.335 - 0.131 \times Cr_{soil} - 16.199 \times pH + 1.772 \times OM$	0.61	0.55	0.20	0.04	0.69	0.50
$Cu_{root} = 87.997 + 0.915 \times Cu_{soil} - 11.069 \times pH - 0.629 \times OM$	0.77	0.74	0.38	0.01	0.15	0.88
$Fe_{root} = 28452.324 + 0.064 \times Fe_{soil} - 3187.528 \times pH + 628.992 \times OM$	0.61	0.48	0.20	0.05	0.68	0.51
$Mn_{root} = 4344.638 + 0.337 \times Mn_{soil} - 513.371 \times pH - 1.470 \times OM$	0.84	0.84	0.13	0.00	0.04	0.97
$Mo_{root} = 16.434 + 1.769 \times Mo_{soil} - 1.980 \times pH + 0.725 \times OM$	0.65	0.70	0.26	0.06	0.98	0.34
$Ni_{root} = 32.315 + 0.250 \times Ni_{soil} - 3.556 \times pH + 0.822 \times OM$	0.82	0.84	0.09	0.02	0.88	0.39
$Pb_{root} = 33.307 - 0.452 \times Pb_{soil} - 3.559 \times pH - 0.082 \times OM$	0.36	0.26	0.48	0.07	0.63	0.54
$Zn_{root} = 48.039 + 1.463 \times Zn_{soil} - 14.568 \times pH + 9.521 \times OM$	0.83	0.83	0.19	0.00	0.05	0.96

R^2 : coefficient of determination, ME: model efficiency, MNAE: mean normalized average error, MNB: mean normalized bias

The build-up of HMs in plants is a complex process that depends on various soil properties, SS composition and application rate, species phenology and physiology, rhizosphere biochemistry, climatic factors, chelating effects and chemical speciation of

HMs (Dolgen et al., 2007). The root system is considered the main accumulator of HMs and filter for HM uptake; it slowly accumulates HMs (particularly if present in high concentrations), thus preventing or diminishing their transport to the above-ground biomass (Eid and Shaltout, 2016; Sawidis et al., 2011). The findings of the present study confirm this observation, as almost all the estimated HMs were higher in the roots of *P. vulgaris* than in its stems, leaves and pods.

Singh et al. (2004) reported that the hyper-accumulation of HMs in a root system may be ascribed to complexation of HMs with sulfhydryl groups, resulting in less HM translocation to the shoot system. Furthermore, higher accumulation of an HM in the root of a certain plant than in its above-ground tissues may occur because the root is the first tissue to come into contact with HMs; therefore, greater accumulation occurs there (Eid and Shaltout, 2014). In a plant such as *P. vulgaris*, this is a preferential behaviour that prevents toxic levels of HMs from occurring in the edible shoot parts, particularly the pods, which are cooked in immature and dried stages (Huxley, 1999; Tindall, 1978).

The three HMs with the highest concentrations in the four tissues of *P. vulgaris* and the supporting soil-sludge mixture were $\text{Fe} > \text{Mn} > \text{Zn}$, suggesting their easy uptake as a result of their higher levels in the soils and because they are essential for the growth of the studied plant (Dolgen et al., 2007; Lopes et al., 2012). In contrast, Pb and Cd tend to accumulate poorly in plant tissues and, if their uptake occurs, they accumulate in the root system (Latare et al., 2014).

The addition of SS to cultivated soil often leads to soil pollution by HMs and HM accumulation and phytotoxicity in the food supply (Yilmaz and Temizgül, 2012). The HM contents in the soil mixed with SS in the current work, except the Cd and Fe contents, were in the normal range reported by Allen (1989) and Kabata-Pendias (2011). In addition, most of the HM concentrations in *P. vulgaris* tissues were not in the critical ranges proposed by Kabata-Pendias (2011); however, some of them were within the normal range reported by Allen (1989) (e.g., concentrations of Cu, Fe, Mn, Pb and Zn). Thus, although the mixing of cultivated soil with SS may be practical for some crops, it might not be practical for others, such as *P. vulgaris*. Nevertheless, regular checking of HM levels in crop products that are cultivated in soil mixed with SS is recommended to avoid toxic effects of HMs in the food chain.

The BCF (root:soil ratio) is a rough estimate of HM uptake but reflects fewer details about site conditions (Zeng et al., 2011). In the current work, soil pH had a negative effect on BCF values, while soil OM had a positive effect. Significant negative r coefficients between soil pH and BCF of HMs in the root system of *P. vulgaris* could be interpreted in the view that alkalinity decreases the availability of HMs in soil solutions while acidity increases their availability (Du Laing et al., 2008; Novotná et al., 2015; Zeng et al., 2011). In addition, the presence of significant positive r coefficients between soil OM and BCF of HMs in the root system of the studied plant may be interpreted in the view that OM increases HM mobility in soil and HM uptake by plants (Singh and Agrawal, 2010). This finding is comparable to that of some previous related studies (Boshoff et al., 2014; Chaudri et al., 2007; Zeng et al., 2011). The BCF was < 1 for some metals (e.g., Fe, Cr, Ni, Cd, Co and Cu), which means that either the accumulation capacity of the root system was low for these HMs or they were transferred in low concentrations (see Novotná et al., 2015). In contrast, the BCF was > 1 for Mn, Mo, Pb and Zn, which means that *P. vulgaris* is a hyper-accumulator and may be used as a phytoremediator for these metals. Regarding the TF (aerial

tissue:root ratio), all the values were < 1 , except that for Mo, which means that the root is an effective bio-filter for these elements, while it is less effective for Mo.

Regression models are complex approaches with which we can predict the HM contents in plant tissues using some soil variables, e.g., HMs, pH and OM (Chaudri et al., 2007; Waegeneers et al., 2011). The results of the studied species indicated that the constructed models performed well for most of the estimated HM concentrations in the tissues of *P. vulgaris*, taking into account the parameters that indicate the high performance of the models (R^2 , ME , $MNAE$ and p -value). All the measured soil factors (HM, pH and OM) consistently contributed to HM concentrations in the plant tissues. Similar findings were reported by other investigators (e.g., Bešter et al., 2013; Boshoff et al., 2014; dos Santos-Araujo et al., 2017; Gan et al., 2017).

To the best of the researchers knowledge, to this day, no study has been performed where regression models have been developed to forecast of HMs uptake by *P. vulgaris* from the soil amended with SS. The mathematical models for the uptake of HMs by the roots, stems, leaves and pods of *P. vulgaris* constructed here are comparable to those produced for some other crops cultivated in soil amended with SS. For example, the variability (R^2) in Cd in the tissues of cucumber was 49-76% (Eid et al., 2018a), in garden pea was 25-67% (Eid et al., 2020c), in *Eruca sativa* was 49-81% (Eid et al., 2020b), in spinach was 83-88% (Eid et al., 2018b), compared with 44-61% for the *P. vulgaris* tissues. In their study on the Cd models of some vegetables, Bešter et al. (2013) reported R^2 values of 41% for tomato and 90% for endive. Regarding the same element, Boshoff et al. (2014) reported an R^2 range of 10-47% for *Urtica dioica* and 31-38% for *Agrostis* and *Poa* species. In Brazil, dos Santos-Araujo et al. (2017) recorded an R^2 of 45% for lettuce and 47% for carrot. A possible reason for the lower R^2 values of some HM models in previous studies (Table 6) may be the large number of replicates used in model construction which results in a large amount of noise in the datasets that may influence the predictive ability of the models (Römkens et al., 2004). The pollution levels, physico-chemical characteristics of the soil at the sampling sites, soil texture, soil microbial activity, soil types with different origins and mineralogy, distinct land management practices and analytical methods used in digestion of sample materials (Du Laing et al., 2003, 2009; Kabata-Pendias, 2011) could be other reasons for the different R^2 values between HM models.

Table 6. Regression models for predicting the concentration of Cd in plants based on the concentration of Cd in soil and soil properties

Plant	n	Model	R^2	Reference
Kidney bean	18	$Cd_{pod} = -3.576 - 0.054 \times Cd_{soil} + 0.460 \times pH + 0.099 \times OM$ (%)	54%	Present study
	18	$Cd_{leaf} = 0.930 + 0.004 \times Cd_{soil} - 0.093 \times pH - 0.002 \times OM$ (%)	44%	
	18	$Cd_{stem} = 1.770 - 0.037 \times Cd_{soil} - 0.174 \times pH + 0.019 \times OM$ (%)	51%	
	18	$Cd_{root} = 11.361 - 0.138 \times Cd_{soil} - 1.263 \times pH - 0.073 \times OM$ (%)	61%	
Agrostis and Poa species	37	$\log Cd_{plant} = -0.56 + (0.58 \times \log Cd_{soil})$	38%	Boshoff et al. (2014)
	37	$\log Cd_{plant} = 0.08 + (0.27 \times \log CaCl_2 [Cd]_{soil})$	31%	
Barley	90	$\log Cd_{grain} = 0.04 + 0.21 \times \log Cd_{soil} - 0.23 \times pH$	22%	Adams et al. (2004)
Cabbage	16	$Cd_{plant} = 0.007 + 0.002 \times Cd_{soil}$	44%	Bešter et al. (2013)
Carrot	54	$Cd_{plant} = 0.107 + 0.017 \times Cd_{soil} - 0.00007 \times Mn_{soil}$	47%	Bešter et al. (2013)
Carrot	238	$Cd_{plant} = -0.19 + 0.46 \times Cd_{soil}$	33%	dos Santos-Araujo et al. (2017)
	238	$Cd_{plant} = 0.89 + 0.42 \times Cd_{soil} - 0.17 \times pH$	45%	
	238	$Cd_{plant} = 0.90 + 0.42 \times Cd_{soil} - 0.17 \times pH - 0.01 \times OM$ (%)	45%	
	238	$Cd_{plant} = 0.92 + 0.43 \times Cd_{soil} - 0.18 \times pH - 0.01 \times OM$ (%) $- 0.04 \times Clay$ (%)	45%	

Chicory	29	$Cd_{plant} = 0.016 + 0.017 \times Cd_{soil}$	60%	Bešter et al. (2013)
Cucumber	18	$Cd_{root} = 0.11 + 0.23 \times Cd_{soil} - 0.04 \times pH + 0.07 \times OM$ (%)	49%	Eid et al. (2018a)
	18	$Cd_{stem} = -0.56 + 0.02 \times Cd_{soil} + 0.07 \times pH + 0.03 \times OM$ (%)	55%	
	18	$Cd_{leaf} = -0.14 - 0.002 \times Cd_{soil} + 0.02 \times pH + 0.03 \times OM$ (%)	56%	
	18	$Cd_{fruit} = 0.74 + 0.50 \times Cd_{soil} - 0.15 \times pH + 0.17 \times OM$ (%)	76%	
Endive	26	$Cd_{plant} = 0.089 + 0.032 \times Cd_{soil} - 0.014 \times OM$ (%)	90%	Bešter et al. (2013)
<i>Eruca sativa</i>	18	$Cd_{leaf} = 0.326 + 0.204 \times Cd_{soil} - 0.070 \times pH + 0.136 \times OM$ (%)	81%	Eid et al. (2020b)
	18	$Cd_{root} = 1.518 - 0.298 \times Cd_{soil} - 0.088 \times pH + 0.067 \times OM$ (%)	49%	
Garden pea	15	$Cd_{pod} = 4.373 - 0.052 \times Cd_{soil} - 0.480 \times pH - 0.051 \times OM$ (%)	60%	Eid et al. (2020c)
	15	$Cd_{shoot} = 1.366 + 0.003 \times Cd_{soil} - 0.133 \times pH - 0.020 \times OM$ (%)	25%	
	15	$Cd_{root} = -1.455 + 0.249 \times Cd_{soil} + 0.144 \times pH + 0.042 \times OM$ (%)	67%	
Hop	13	$Cd_{plant} = 0.061 \times Cd_{soil} - 0.28 \times OM$ (%)	51%	Novotná et al. (2015)
Lettuce	293	$Cd_{plant} = -0.06 + 0.39 \times Cd_{soil}$	35%	dos Santos-Araujo et al. (2017)
	293	$Cd_{plant} = 1.10 + 0.44 \times Cd_{soil} - 0.18 \times pH$	42%	
	293	$Cd_{plant} = 1.35 + 0.48 \times Cd_{soil} - 0.18 \times pH - 0.28 \times OM$ (%)	44%	
	293	$Cd_{plant} = 1.11 + 0.39 \times Cd_{soil} - 0.14 \times pH - 0.22 \times OM$ (%) $- 0.14 \times Clay$ (%)	47%	
Maize	79	$Cd_{shoot} = 90.1 + 0.24 \times Cd_{soil} - 12.9 \times pH$	17%	Tudoreanu and Phillips (2004)
Onion	35	$Cd_{plant} = 0.208 + 0.005 \times Cd_{soil} - 0.002 \times OM$ (%) $- 0.027 \times pH$	85%	Bešter et al. (2013)
Potato	29	$Cd_{plant} = 0.042 + 0.007 \times Cd_{soil}$	76%	Bešter et al. (2013)
Potato	17	$Cd_{plant} = -0.018 + 2.46 \times Cd_{soil} - 0.0041 \times Clay$ (%) $+ 0.036 \times Zn_{soil} + 0.021 \times pH:OM$ (%) $- 0.0056 \times Zn_{soil}:pH - 0.37 \times Cd_{soil}:pH$	60%	Novotná et al. (2015)
Red beet	20	$Cd_{plant} = 0.017 + 0.026 \times Cd_{soil}$	67%	Bešter et al. (2013)
Rice	33	$\log Cd_{grain} = 0.473 \times \log Cd_{soil} - 0.157 \times pH + 0.445 \times \log OM$ (g/kg) $- 0.984$	66%	Mu et al. (2020)
Rye grass	156	$Cd_{shoot} = 35.3 + 0.37 \times Cd_{soil} - 4.9 \times pH$	13%	Tudoreanu and Phillips (2004)
Spinach	12	$Cd_{leaf} = 0.402 + 0.014 \times Cd_{soil} - 0.047 \times pH + 0.043 \times OM$ (%)	88%	Eid et al. (2018b)
	12	$Cd_{root} = 2.144 + 0.060 \times Cd_{soil} - 0.294 \times pH + 0.130 \times OM$ (%)	83%	
Tomato	51	$Cd_{plant} = 0.020 + 0.002 \times Cd_{soil} - 0.000008 \times Mn_{soil}$	41%	Bešter et al. (2013)
<i>Urtica dioica</i>	66	$\log Cd_{plant} = 0.26 + (0.24 \times \log CaCl_2 [Cd]_{soil})$	10%	Boshoff et al. (2014)
	68	$\log Cd_{plant} = -0.13 + (0.69 \times \log Cd_{soil}) - (0.87 \times \log clay$ %)	47%	
Wheat	162	$\log Cd_{grain} = 0.28 + 0.44 \times \log Cd_{soil} - 0.18 \times pH$	49%	Adams et al. (2004)
Wheat	100	$\log Cd_{plant} = -1.75 + 0.59 \times Cd_{soil} - 0.23 \times OM$ (%)	64%	Novotná et al. (2015)
Wheat	14	$\log Cd_{grain} = 1.386 + \log Cd_{soil} - 0.279 \times pH$	85%	Liu et al. (2015)

n: number of samples, *R*²: coefficient of determination

Conclusion

The HM concentrations in the different tissues of *P. vulgaris* showed that most of the investigated HMs were accumulated in the plant roots rather than in the other tissues. The SS is a rich source of OM, can increase solubility of soil HMs, and therefore the use of SS as amendment must be applied carefully to avoid the accumulation of these HMs in the soil and their transfer to plants. All the TFs values were less than unity, except that for Mo, which means that the root is an effective bio-filter for these HMs, while it is less effective for Mo. Prediction models for HMs concentrations proved that pH, OM and HMs concentrations of the soil were good predictors for the uptake of HMs by *P. vulgaris*. These models functioned well and demonstrated great levels of efficacy and low rates of error, and can be employed in examining *P. vulgaris* plants grown in soil amended with SS. Applying the *t* test, there are no significant differences between the actual and predicted values of the ten HMs in *P. vulgaris* tissues, which reflect the best-fitting of these equations for predicting the uptake of these HMs. An

extended field study for several growth seasons using the same SS may be required to confirm whether the repeated application of SS causes environmental problems in the future. Additionally, in future work, this study could be extended to investigate the microbial effects of SS application on soil quality and plant growth. Also, investigations of suitable methods for SS treatments are needed before the application in the agricultural system to avoid the health and environmental hazards.

Acknowledgements. This work was supported by the Deanship of Scientific Research at King Khalid University under Grant number R.G.P. 1/109/40.

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APPENDIX

Appendix 1. Selected chemical properties of sewage sludge and agricultural soil used in the pot experiment (means $\pm$ standard error, $n = 3$)

Properties	Agricultural soil	Sewage sludge
pH	8.7 $\pm$ 0.02	7.0 $\pm$ 0.02
Organic matter (%)	0.9 $\pm$ 0.2	65.0 $\pm$ 0.9
Cd (mg kg ⁻¹)	2.9 $\pm$ 0.1	1.2 $\pm$ 0.1
Co (mg kg ⁻¹)	35.5 $\pm$ 1.1	25.9 $\pm$ 1.3
Cr (mg kg ⁻¹)	134.3 $\pm$ 0.7	176.2 $\pm$ 1.9
Cu (mg kg ⁻¹)	15.0 $\pm$ 0.6	162.6 $\pm$ 2.3
Fe (mg g ⁻¹)	42.4 $\pm$ 0.5	24.1 $\pm$ 0.5
Mn (mg kg ⁻¹)	677.3 $\pm$ 3.2	560.7 $\pm$ 9.8
Mo (mg kg ⁻¹)	1.1 $\pm$ 0.0	0.9 $\pm$ 0.0
Ni (mg kg ⁻¹)	68.1 $\pm$ 3.7	138.7 $\pm$ 3.7
Pb (mg kg ⁻¹)	3.5 $\pm$ 0.4	671.1 $\pm$ 6.2
Zn (mg kg ⁻¹)	77.2 $\pm$ 1.9	667.6 $\pm$ 13.4