

INFLUENCES OF SEWAGE SLUDGE-AMENDED SOIL ON HEAVY METAL ACCUMULATION, GROWTH AND YIELD OF ROCKET PLANT (*ERUCA SATIVA*)

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(Received 22nd Nov 2019; accepted 12th Feb 2020)

Abstract. The application of sewage sludge (SS) in agriculture is a wastewater recycling method for soil fertilization. The aim of this study was to assess the influences of SS amendment rates (0, 10, 20, 30, 40 and 50 g kg⁻¹) on the green-salad leafy plant *Eruca sativa*. The SS amendment increased soil salinity and organic matter and decreased soil pH. The heavy metal (HM) contents were below the permissible limits as recommended by the Council of the European Communities. An increase in *E. sativa* morphometric parameters was detected. The leaf, root and total biomasses of *E. sativa* increased at amendment rates 10, 20 and 30 g kg⁻¹. The applications of 40 and 50 g kg⁻¹ negatively affected the biomass. The highest concentration of the measured HMs in the plant was detected at the 50 g kg⁻¹ amendment rate. The bioaccumulation factors of Cd, Co, Cr, Fe, Mn, Ni, Pb and Zn were < 1.0, whereas those of Cu and Mo were > 1. The translocation factors for all HMs were < 1.0 except for Cd and Pb. Even though, SS increased the biomass of *E. sativa*, SS is not recommended as a fertilizer for *E. sativa*, since translocation factors of Cd and Pb were >1.

Keywords: soil pollution, rocket plant, health hazards, environmental risks, metal bioaccumulation

Introduction

Sewage sludge (SS) is a by-product of domestic wastewater treatment processes (Eid and Shaltout, 2016). SS has a variety of elements that are considered nutrients for plants in addition to organic components that increase agricultural soil fertility (Singh and Agrawal, 2008). However, the physical, chemical and microbial properties of SS have to be analyzed before use as agricultural soil fertilizers to avoid any harmful effects on plants, animals and humans (Carbonell et al., 2009). The effect of heavy metals (HMs) on crops differs according to the metal and/or plant (Hara and Sonoda,

1979). Some HMs are required by plants for physiological processes, including Mn, Fe, Cu, Zn, and Mo, while other HMs are phytotoxic, such as Cd and Pb (Raskin et al., 1994).

The concerns in using SS as soil fertilizer relate to the possible transfer and accumulation of significant HMs in the food chain and, subsequently, their effect on human health (Shrivastava and Banerjee, 2004). Several factors influence the toxic HM accumulation in soil and plants, such as the origin and properties of the SS and agricultural soil, amendment level, metal availability, and plant species (Kabata-Pendias, 2011; Dede and Ozdemir, 2016). Even though use of SS in agriculture is an effective and economically beneficial method of disposal, the potential environmental and health issues should be considered.

Rocket (*Eruca sativa* Mill.) is a vegetable leafy crop belonging to the *Brassicaceae* family. Its fresh leaves are used as a green salad food because it is a fast-growing species, and is available nearly all year (Nail et al., 2017). The nutritional and medicinal benefits of *E. sativa* are documented as an anticarcinogenic, antimicrobial and antioxidant agent (Hassan et al., 2017). The biochemical constituents of *E. sativa* and their bio positive effects on health were explained in several studies (Garg and Sharma, 2014; Jilani et al., 2015). In the current study, SS was used as an amendment to agricultural soil for growing *E. sativa*. The objectives were to (1) evaluate the effect of SS on the growth and biomass of *E. sativa* and (2) assess the HM accumulation in *E. sativa* parts and in the agricultural soil.

Materials and Methods

Plant materials, sewage sludge treatments and experimental design

Eruca sativa seeds were obtained from a local market in Abha. The agricultural soil used in the experiment was collected at a depth of 0-20 cm from neighbouring cultivated fields. The SS was obtained from the Abha municipal sewage treatment plant, Aseer region, Saudi Arabia. The agricultural soil and SS were air-dried for 2 weeks and sieved through a sieve with 2-mm pore diameter. The experiment was performed in the greenhouse of the Biology Department, King Khalid University, Abha, Saudi Arabia.

The SS was mixed with agricultural soil at rates of 0 (the control soil), 10, 20, 30, 40 and 50 g kg⁻¹. Each treatment consisted of six replicates of a plastic pot (6-L volume), and each plastic pot was filled with 4 kg of the respective treatment. Twenty *E. sativa* seeds were planted in each pot. The experimental units were arranged in a complete randomized design. The plants were grown for 40 days, starting from the planting day of January 2nd, 2018 and were harvested on February 10th, 2018, in the greenhouse with a natural day/night regime and irrigated as needed. *Figure 1* shows the experiment setup and postharvest analyses.

Plant morphometric parameters and biomass

From the 120 plants in each treatment, 30 plants (5 plants/6 replicates) were used for morphological and biomass measurements. *E. sativa* plants were washed using running water. Shoot height and root length were measured, and the number of leaves were counted. Leaf area was measured using a leaf area metre (Dynamax AM 300, Dynamax Inc, USA). The absolute growth rate (AGR) was calculated according to the formula by Radford (1967) (Eq. 1):

$$\text{AGR (g DM individual}^{-1} \text{ day}^{-1}) = (W_2 - W_1)/(t_2 - t_1) \quad (\text{Eq.1})$$

where W_1 and W_2 are the total biomasses (g DM individual⁻¹) at times (days) t_1 and t_2 , respectively. The value of t_1 is zero and t_2 is 40 which is the growing period of the plants.

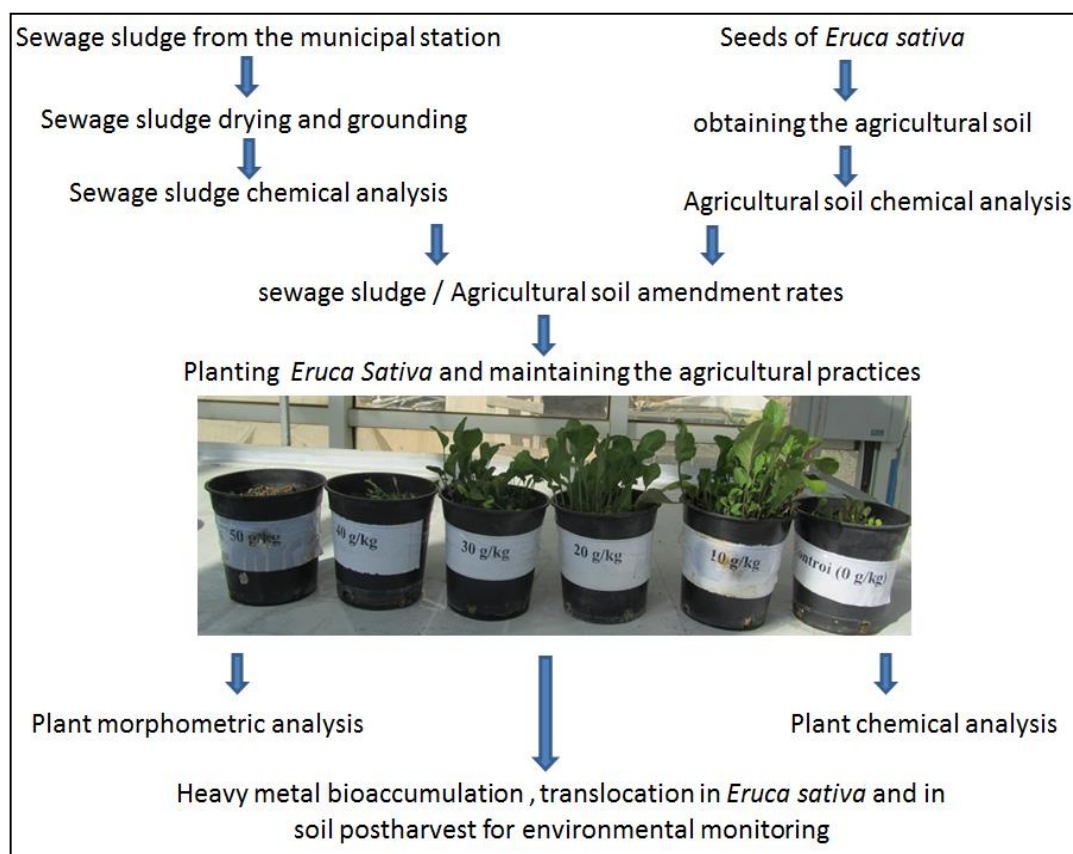


Figure 1. A graphical demonstration of the experiment steps with pots represents the treatments of sewage sludge-soil amendment rates

The plant leaves and roots were oven-dried at 60°C for one week and ground using a plastic mill. The biomass of the leaves and roots were determined. The total biomass refers to the summation of the leaf and root biomass.

Sample analyses

Pre-experiment analyses of the SS and agricultural soil were conducted. At the end of the experiment, the amended soil samples representing all treatments were air dried and sieved through a 2-mm sieve. The pre-used agricultural soil, SS and post-harvest amended soil samples (including the control) were analyzed for organic matter content using a loss-on-ignition method at 550°C for two hours (Wilke, 2005). Soil-water extracts at a ratio of 1:5 were prepared for salinity and pH determination, using conductivity (Myron L Model DA-1, Myron L Company, USA) and pH metres (ICM Model 41150, ICM, USA), respectively. HMs in the pre-experiment agricultural soil, SS, post-harvest amended soil and plant parts samples were detected according to Allen

(1989). HMs were extracted from approximately 0.5 g of each sample using a mixed-acid digestion method (HNO_3 and HClO_4 ; 3:1, v/v). Digestion was performed using a microwave sample preparation system (PerkinElmer Titan MPS, PerkinElmer Inc., USA). Blank samples were used to verify the accuracy. Analytical-grade chemicals were used for sample digestion. Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn were determined using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Scientific iCAP 7000 Plus Series; Thermo Fisher Scientific, USA). The instrument setting and operational conditions followed the manufacturer's specifications. Standard solutions with known concentrations of different HMs were prepared for the standardization of the system.

Estimation of bioaccumulation and translocation factors

Bioaccumulation and translocation factors were calculated as described by Ghosh and Singh (2005). The bioaccumulation factor (BF) was calculated to determine the effectiveness of the plants in accumulating a HM from the soil according the following formula (Eq. 2):

$$BF = \text{conc. of a metal in the roots} / \text{conc. of the same metal in the soil} \quad (\text{Eq.2})$$

The translocation factor (TF) was calculated to determine the ability of the plants to translocate a metal from the roots to the shoot according the following formula (Eq. 3):

$$TF = \text{conc. of a metal in the shoot} / \text{conc. of the same metal in the roots} \quad (\text{Eq.3})$$

Statistical analysis

The data were tested for normality of distribution and homogeneity of variance, and when necessary, the data were log-transformed. The data were assessed using one-way ANOVA. The significant differences between the means of the six amendment rates were identified using the Tukey HSD test at $P < 0.05$. A quadratic regression analysis (Eid et al., 2020) was performed to evaluate the response of the biomass and plant morphometric parameters of *E. sativa* grown in soils amended with different rates of SS. To evaluate the statistical relationships among the content of HM in plant parts and the amendment rate of SS, linear regression procedures were applied according to the following formula (Eq. 4):

$$y = a + bx \quad (\text{Eq.4})$$

where y is the heavy metal concentration (mg kg^{-1}) in *E. sativa* tissue harvested after 40 days, x is the sewage sludge amendment rate (g kg^{-1}) and the regression coefficients used are a and b . Statistical analyses were conducted using Statistica 7.1 (Statsoft, 2007).

Results and discussion

The application of SS as an amendment to agricultural soils has been reported to play a role in soil fertilization (Jitariu et al., 2011; Eid et al., 2019). Analyses of SS and soil showed that SS had high organic matter content and acidity and was more saline compared

to the cultivated soil. The analysis of the ten HMs' content in the SS showed the following order: Fe > Pb > Zn > Mn > Cr > Cu > Ni > Co > Cd > Mo. However, the order of these metals in the agricultural soil was as follows: Fe > Mn > Zn > Cr > Co > Ni > Cu > Cd > Pb > Mo (Table 1). The properties of soils amended with sludge depend on the initial constituents of both the soil and SS (Baghina et al., 2014). The HM contents in SS were under the allowed limits for soil application of SS recommended by the Council of the European Communities (1986). The HM contents in the SS were low because of the effective pre-treatment and monitoring of wastewater treatment processes in Saudi Arabia.

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

Properties	Sewage sludge	Cultivated fields soil
Salinity (mS cm ⁻¹)	1.39 $\pm$ 0.10	0.07 $\pm$ 0.00
pH	6.98 $\pm$ 0.02	8.68 $\pm$ 0.02
Organic matter (%)	65.0 $\pm$ 0.9	0.9 $\pm$ 0.2
Cd (mg kg ⁻¹)	1.17 $\pm$ 0.08	2.91 $\pm$ 0.05
Co (mg kg ⁻¹)	25.86 $\pm$ 1.31	35.49 $\pm$ 1.13
Cr (mg kg ⁻¹)	168.09 $\pm$ 4.45	44.11 $\pm$ 0.35
Cu (mg kg ⁻¹)	162.56 $\pm$ 2.32	15.01 $\pm$ 0.57
Fe (mg kg ⁻¹)	24410.0 $\pm$ 450.0	17120.0 $\pm$ 160.0
Mn (mg kg ⁻¹)	560.70 $\pm$ 9.81	340.43 $\pm$ 7.04
Mo (mg kg ⁻¹)	0.91 $\pm$ 0.04	0.40 $\pm$ 0.02
Ni (mg kg ⁻¹)	138.73 $\pm$ 3.71	23.20 $\pm$ 0.65
Pb (mg kg ⁻¹)	671.11 $\pm$ 6.22	1.51 $\pm$ 0.59
Zn (mg kg ⁻¹)	667.62 $\pm$ 13.44	70.59 $\pm$ 1.07

The chemical properties of the soil were monitored post-harvest at all the SS amendment rates (Table 2). Organic matter and salinity significantly increased, however, salinity gradually increased; the significant increasing was detected only at a SS amendment rate 50 g kg⁻¹. The soil pH significantly decreased at all SS application rates, reaching 7.33 at the highest amendment rate. These results agree with those of our previous study on the leafy plant spinach (Eid et al., 2017) and other studies (Indoria et al., 2013; Kumar et al., 2016). The decreasing soil pH post-addition of SS could be a result of organic acid production resulting from SS decomposition and/or nitrification and mineralization of sulphur-rich compounds (Singh and Agrawal, 2008). All HMs content a significant gradually increased in the soil at all SS amendment rates except Mo (Table 2). A significant increase in Cd, Co, Cu and Zn concentrations was detected at an amendment rate of 30 g kg⁻¹. However, Pb and Ni significantly increased at amendment rates of 40 and 50 g kg⁻¹, respectively. Although, the chemical composition of the applied SS and soil were analysed (Table 1) before planting *E. sativa*, the concentrations of HM in the soil 40 days after growing *E. sativa* were different according to the SS amendment rate.

The plant *E. sativa* was selected because it is grown in most cases on small farms around and within urban areas and is directly consumed as a fresh green plant. Effects of SS amendments on the morphological parameters of *E. sativa* are shown in Figure 2. A significant gradual increase in shoot length, leaf number, whole plant leaf area, single leaf area and AGR was observed in the SS application until an amendment rate of 30 g kg⁻¹,

with the highest growth improvement at 20 g kg⁻¹, followed by a decreasing trend at a SS application rate of 40 and 50 g kg⁻¹. However, the root length decreased at all SS amendment rates compared to that of the control plants. A similar trend was observed in terms of the *E. sativa* biomass, and a significant increase in leaf, root and total biomass was detected at amendment rates of 10, 20 and 30 g kg⁻¹. The *E. sativa* leaf/root ratio significantly increased at SS amendment rates of 10 and 20 g kg⁻¹. The application rates of 40 and 50 g kg⁻¹ caused significant negative effects on the biomass measurements of *E. sativa* compared to those of the control (Figure 3). These findings agree with those of Indoria et al. (2013), who reported enhancement of *E. sativa* growth parameters when grown in sludge-amended soil, as well as our previous studies on the effect of SS applications on spinach growth (Eid et al., 2017). Plant growth improvement was a result of increased organic matter, macro- and micro-nutrients contents and availability, soil porosity and bulk density (Antolín et al., 2005). The reduction of the growth at high amendments rates may refer the high HM concentrations in soil which inhibit absorption of beneficial macronutrients such as K and Ca (Burzynski, 1987).

Table 2. Selected chemical properties (means $\pm$ standard error, $n = 6$) of soil at different sewage sludge amendment rates after harvesting *Eruca sativa* that had been grown for 40 days

Properties	Sewage sludge amendment rate (g kg ⁻¹)						F-value
	0	10	20	30	40	50	
Salinity (mS cm ⁻¹)	0.29 $\pm$ 0.01a	0.31 $\pm$ 0.02a	0.37 $\pm$ 0.04ab	0.38 $\pm$ 0.03ab	0.41 $\pm$ 0.03ab	0.49 $\pm$ 0.03b	5.5**
pH	8.49 $\pm$ 0.01f	8.15 $\pm$ 0.05e	7.95 $\pm$ 0.03d	7.73 $\pm$ 0.03c	7.57 $\pm$ 0.06b	7.33 $\pm$ 0.05a	106.6***
Organic matter (%)	0.98 $\pm$ 0.14a	2.52 $\pm$ 0.05b	3.57 $\pm$ 0.15c	4.20 $\pm$ 0.16d	5.19 $\pm$ 0.09e	6.56 $\pm$ 0.06f	286.3***
Cd (mg kg ⁻¹)	2.02 $\pm$ 0.03a	2.07 $\pm$ 0.05a	2.18 $\pm$ 0.01ab	2.33 $\pm$ 0.01bc	2.48 $\pm$ 0.03cd	2.49 $\pm$ 0.08d	23.4***
Co (mg kg ⁻¹)	24.37 $\pm$ 0.08a	25.65 $\pm$ 0.29ab	25.87 $\pm$ 0.86ab	26.60 $\pm$ 0.09b	26.76 $\pm$ 0.17b	26.99 $\pm$ 0.64b	4.4**
Cr (mg kg ⁻¹)	56.79 $\pm$ 0.32a	63.54 $\pm$ 0.89b	65.04 $\pm$ 3.77bc	70.73 $\pm$ 0.16c	88.49 $\pm$ 1.31d	104.32 $\pm$ 1.31e	104.7***
Cu (mg kg ⁻¹)	3.48 $\pm$ 0.08a	3.57 $\pm$ 0.15a	4.45 $\pm$ 0.15a	6.49 $\pm$ 0.48b	7.64 $\pm$ 0.45bc	9.17 $\pm$ 0.88c	26.2***
Fe (mg kg ⁻¹)	17303.1 $\pm$ 170.0a	19739.4 $\pm$ 1001.0b	21360.7 $\pm$ 120.1b	21558.4 $\pm$ 396.1b	26821.0 $\pm$ 493.4c	32585.9 $\pm$ 755.3d	91.7***
Mn (mg kg ⁻¹)	244.29 $\pm$ 5.87a	269.07 $\pm$ 12.06ab	289.88 $\pm$ 0.42b	299.52 $\pm$ 4.81b	365.16 $\pm$ 9.94c	457.60 $\pm$ 12.04d	82.2***
Mo (mg kg ⁻¹)	0.55 $\pm$ 0.08a	0.63 $\pm$ 0.04a	0.67 $\pm$ 0.06a	0.74 $\pm$ 0.06a	0.78 $\pm$ 0.19a	0.89 $\pm$ 0.08a	1.5 ^{ns}
Ni (mg kg ⁻¹)	30.63 $\pm$ 0.08a	31.43 $\pm$ 0.22ab	31.99 $\pm$ 1.39ab	32.24 $\pm$ 0.20ab	32.29 $\pm$ 0.03ab	33.84 $\pm$ 0.37b	3.2*
Pb (mg kg ⁻¹)	5.85 $\pm$ 0.23a	5.94 $\pm$ 0.09a	6.28 $\pm$ 0.09a	6.71 $\pm$ 0.18a	8.55 $\pm$ 0.67b	8.68 $\pm$ 0.29b	15.7***
Zn (mg kg ⁻¹)	67.44 $\pm$ 0.54a	70.34 $\pm$ 1.22a	80.21 $\pm$ 1.96ab	93.84 $\pm$ 5.67b	120.45 $\pm$ 0.71c	130.07 $\pm$ 7.98c	40.6***

F-values represent one-way ANOVA; degrees of freedom (df) = 5. Means in the same row followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD test. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant (i.e., $P > 0.05$)

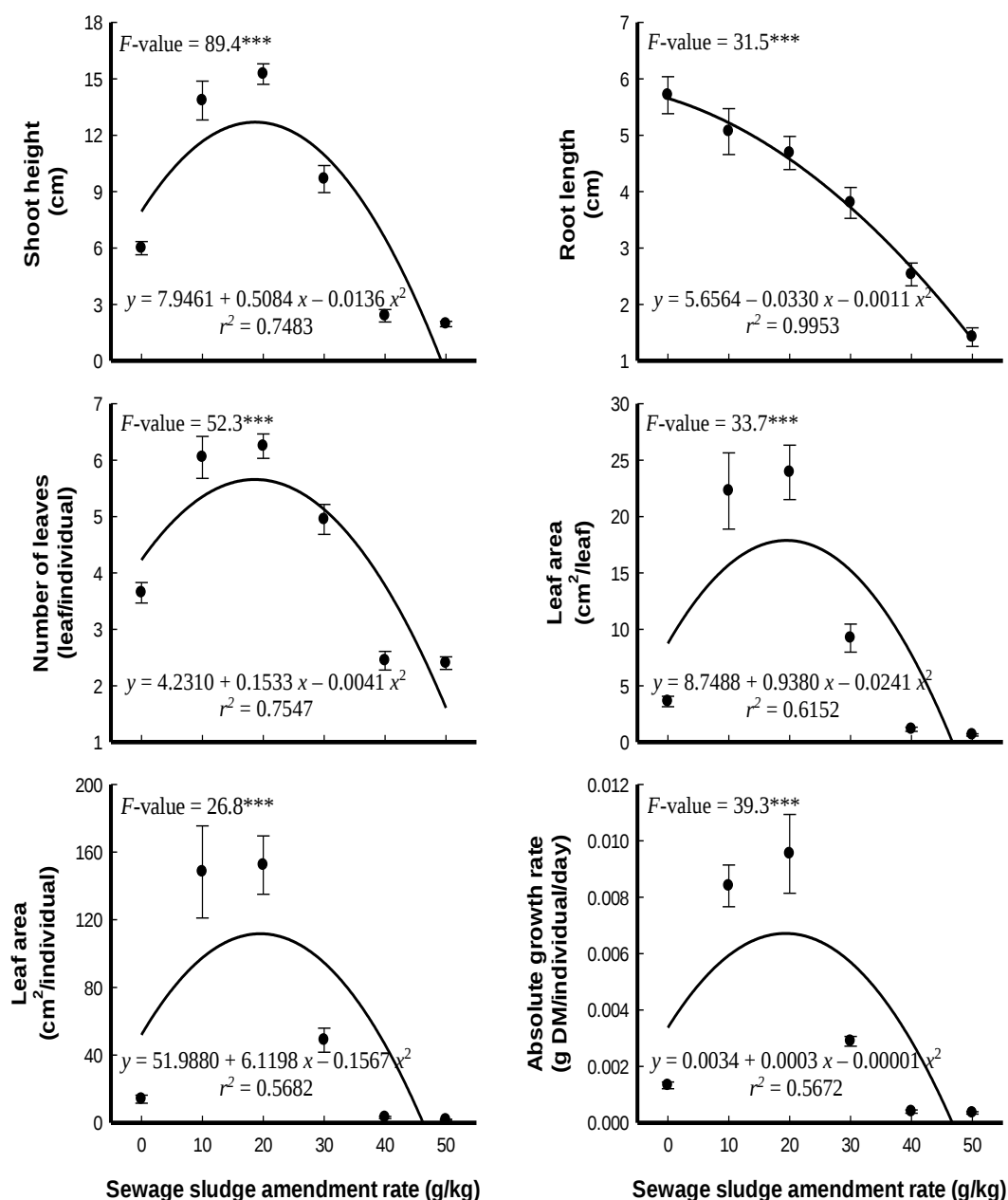


Figure 2. Effects of different sewage sludge amendment rates on the morphometric parameters of *Eruca sativa* harvested after 40 days (means $\pm$ standard error, $n = 30$). The F-values represent one-way ANOVA and a degree of freedom (df) = 5. *** = $P < 0.001$

The HMs uptake is a complicated method that is affected by numerous factors, comprising soil characteristics and type, environmental conditions, plant species, physiology and phenology, rhizosphere biochemistry and chelating effects of other HMs (Basta et al., 2005). In the current study, SS resulted in increasing shoot and root mass of the measured HMs at all the amendments rates (Table 3). The highest concentration of HMs was detected at a 50 g kg^{-1} as the shoot contents of Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn increased to 304, 207, 210, 491, 380, 156, 246, 239, 363 and 323%, respectively, compared to the control plants. The high concentration of HMs in plants

negatively affects several physiological processes (Kabata-Pendias, 2011). Substitution of Mg in chlorophyll by HMs results in the plants being incapable of photosynthesis (Kupper et al., 1996). HM toxicity in plants reduces the transpiration and photochemical light reactions (Singh and Agrawal, 2010).

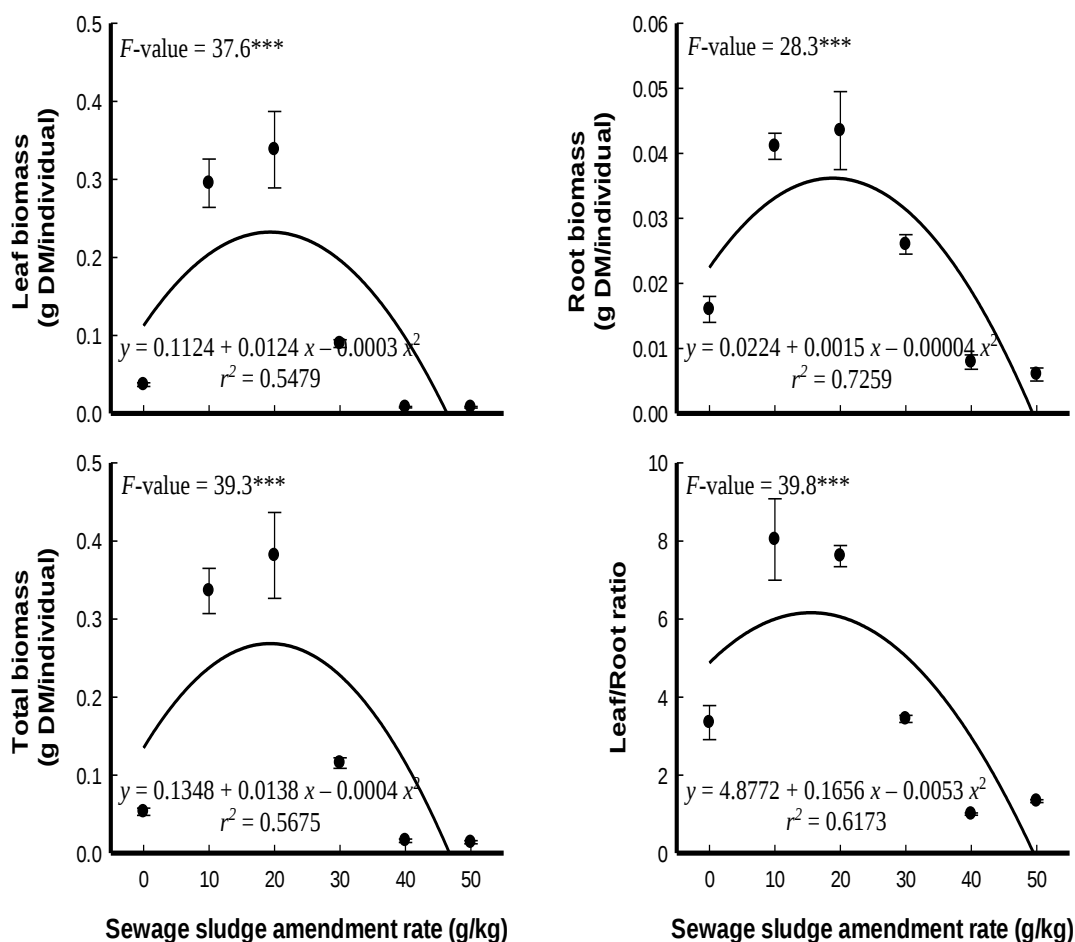


Figure 3. Effects of different sewage sludge amendment rates on the biomass of *Eruca sativa* harvested after 40 days (means $\pm$ standard error, $n = 30$). F -values represent a one-way ANOVA and degrees of freedom (df) = 5. $^{***} = P < 0.001$

The regression analysis showed that the increase in the HM contents was correlated with an increase in the SS amendment rates (Table 4). The highest R^2 values in the *E. sativa* roots and shoots were 0.942 and 0.936 for the HMs Cr and Zn, respectively. However, the lowest R^2 in the roots and shoots were 0.400 and 0.481 for Zn and Co, respectively (Table 4). This variation in R^2 could be related to the physiological role of each element in the plants. Decreasing soil pH increases HM bioavailability (Eid and Shaltout, 2016) as observed in the current study; thus, a follow-up study of the HM concentrations in edible parts of plants grown in sludge-amended soil is a necessity to avoid HM risks. Prediction models of HM concentrations in *Eruca sativa* grown in sewage sludge-amended soil have been developed by our research group and published recently (Eid et al., 2020).

Table 3. Effects of different sewage sludge amendment rates on heavy metal concentrations (mg kg^{-1}) in the shoots and roots of *Eruca sativa* harvested after 40 days (means $\pm$ standard error, $n = 6$)

Metal	Tissue	Sewage sludge amendment rate (g kg^{-1})						F-value	Safe limit ⁺	Phytotoxic range [‡]
		0	10	20	30	40	50			
Cd	Shoot	0.43 $\pm$ 0.03a	0.47 $\pm$ 0.05ab	0.54 $\pm$ 0.00b	0.67 $\pm$ 0.03c	1.14 $\pm$ 0.02d	1.31 $\pm$ 0.01e	184.3***	0.3	5-30
	Root	0.23 $\pm$ 0.00a	0.35 $\pm$ 0.05ab	0.40 $\pm$ 0.03ab	0.43 $\pm$ 0.09ab	0.48 $\pm$ 0.06b	0.56 $\pm$ 0.00b	5.0**		
Co	Shoot	1.26 $\pm$ 0.35a	1.28 $\pm$ 0.02a	1.36 $\pm$ 0.33ab	1.57 $\pm$ 0.04ab	2.12 $\pm$ 0.02bc	2.61 $\pm$ 0.01c	7.9***	-	30-40
	Root	3.03 $\pm$ 0.01a	6.06 $\pm$ 0.11b	6.38 $\pm$ 0.30b	7.72 $\pm$ 0.55c	10.20 $\pm$ 0.10d	11.27 $\pm$ 0.06e	130.3***		
Cr	Shoot	7.65 $\pm$ 0.41a	8.15 $\pm$ 0.03a	9.02 $\pm$ 0.51ab	9.81 $\pm$ 0.03b	14.86 $\pm$ 0.56c	16.10 $\pm$ 0.14c	103.4***	5	10-100
	Root	17.99 $\pm$ 0.28a	31.71 $\pm$ 3.38b	46.03 $\pm$ 3.74c	58.62 $\pm$ 3.78d	73.09 $\pm$ 0.05e	86.49 $\pm$ 0.50f	98.3***		
Cu	Shoot	5.45 $\pm$ 0.07a	5.47 $\pm$ 0.77a	6.34 $\pm$ 0.07a	6.85 $\pm$ 1.12a	10.41 $\pm$ 0.19b	26.76 $\pm$ 0.10c	219.8***	40	20-100
	Root	7.66 $\pm$ 0.02a	9.93 $\pm$ 1.50ab	11.56 $\pm$ 2.34ab	13.83 $\pm$ 2.09bc	18.34 $\pm$ 0.04c	19.26 $\pm$ 0.09c	10.6***		
Fe	Shoot	869.9 $\pm$ 81.7a	1215.5 $\pm$ 108.3b	1353.6 $\pm$ 35.7b	1854.1 $\pm$ 29.0c	2383.1 $\pm$ 74.9d	3312.2 $\pm$ 13.7e	191.1***	450	> 1000
	Root	3760.8 $\pm$ 278.2a	7141.0 $\pm$ 468.9b	9666.9 $\pm$ 446.8c	11403.1 $\pm$ 1065.9c	15364.9 $\pm$ 19.9d	17477.9 $\pm$ 98.2e	94.9***		
Mn	Shoot	78.08 $\pm$ 2.71a	79.38 $\pm$ 0.45a	98.31 $\pm$ 0.67b	102.74 $\pm$ 0.44b	102.80 $\pm$ 3.85b	122.06 $\pm$ 0.20c	71.4***	-	> 400
	Root	99.91 $\pm$ 2.55a	216.75 $\pm$ 9.71b	219.18 $\pm$ 18.73b	228.37 $\pm$ 8.67b	388.26 $\pm$ 1.98c	395.58 $\pm$ 0.29c	146.9***		
Mo	Shoot	1.16 $\pm$ 0.02a	1.82 $\pm$ 0.01b	1.98 $\pm$ 0.16b	1.98 $\pm$ 0.12b	2.61 $\pm$ 0.10c	2.86 $\pm$ 0.03c	53.4***	10	135
	Root	0.71 $\pm$ 0.08a	0.94 $\pm$ 0.04b	1.03 $\pm$ 0.01b	1.13 $\pm$ 0.07bc	1.26 $\pm$ 0.07cd	1.41 $\pm$ 0.01d	20.5***		
Ni	Shoot	3.00 $\pm$ 0.62a	3.43 $\pm$ 0.03a	3.82 $\pm$ 0.04a	3.98 $\pm$ 0.91a	6.36 $\pm$ 0.26b	7.18 $\pm$ 0.10b	13.7***	20	40-246
	Root	7.27 $\pm$ 0.30a	13.11 $\pm$ 1.28b	15.12 $\pm$ 1.76b	18.99 $\pm$ 0.76c	22.89 $\pm$ 0.05d	26.60 $\pm$ 0.14e	53.9***		
Pb	Shoot	0.86 $\pm$ 0.06a	0.87 $\pm$ 0.09a	1.11 $\pm$ 0.05a	1.31 $\pm$ 0.01ab	1.93 $\pm$ 0.21b	3.13 $\pm$ 0.38c	22.1***	5	30-300
	Root	0.23 $\pm$ 0.05a	0.38 $\pm$ 0.04a	0.42 $\pm$ 0.02a	0.57 $\pm$ 0.03ab	0.99 $\pm$ 0.28b	1.03 $\pm$ 0.01b	7.7***		
Zn	Shoot	25.02 $\pm$ 1.86a	40.42 $\pm$ 1.60b	47.05 $\pm$ 0.10c	49.40 $\pm$ 1.37c	70.98 $\pm$ 0.11d	80.94 $\pm$ 0.10e	315.9***	60	100-500
	Root	54.19 $\pm$ 6.09a	56.82 $\pm$ 7.63a	65.41 $\pm$ 9.79ab	70.92 $\pm$ 0.67ab	77.60 $\pm$ 0.04ab	83.02 $\pm$ 0.22b	4.1**		

F-values represent one-way ANOVA, degrees of freedom (df) = 5. Means in the same row followed by different letters are significantly different at $P < 0.05$, according to Tukey's HSD test. **: $P < 0.01$, ***: $P < 0.001$, +: FAO/WHO standard (Codex Alimentarius Commission, 2011), ‡: Kabata-Pendias (2011)

Table 4. Linear regression equations of the form $y = a + bx$, where y is the heavy metal concentration (mg kg^{-1}) in *Eruca sativa* tissue harvested after 40 days and x is the sewage sludge amendment rate (g kg^{-1})

y	a	SE	b	SE	R^2	P
Cd	Shoot	0.296	0.041	0.019	0.847	0.000
	Root	0.258	0.035	0.006	0.436	0.000
Co	Shoot	1.023	0.146	0.027	0.481	0.000
	Root	3.515	0.241	0.157	0.920	0.000
Cr	Shoot	6.419	0.436	0.180	0.822	0.000
	Root	18.081	1.760	1.370	0.942	0.000
Cu	Shoot	1.504	1.482	0.348	0.598	0.000
	Root	7.324	0.989	0.244	0.622	0.000
Fe	Shoot	673.197	78.526	46.329	0.904	0.000
	Root	4017.229	379.026	271.409	0.933	0.000
Mn	Shoot	76.180	1.912	0.843	0.840	0.000
	Root	115.001	12.638	5.720	0.847	0.000
Mo	Shoot	1.291	0.072	0.031	0.834	0.000
	Root	0.756	0.038	0.013	0.760	0.000
Ni	Shoot	2.496	0.359	0.085	0.603	0.000
	Root	8.054	0.676	0.371	0.890	0.000
Pb	Shoot	0.482	0.165	0.042	0.637	0.000
	Root	0.177	0.085	0.017	0.519	0.000
Zn	Shoot	25.618	1.447	1.067	0.936	0.000
	Root	52.854	3.855	0.606	0.400	0.000

SE: standard error, $n = 36$

HM accumulation in plants depends on the soil properties, SS origin and composition, amendment rate, plant species, plant physiology, climatic factors and metal chemical form (Mahdy et al., 2007). The bioaccumulation factors (BFs) and translocation factors (TFs) of all ten detected HMs in *E. sativa* were calculated (Table 5). The BFs of Cd, Co, Cr, Fe, Mn, Ni, Pb and Zn were < 1.0 , whereas the BFs of Cu and Mo were > 1 . The highest BF was detected for Cu at a SS amendment rate of 10 g kg^{-1} ; however, there were no significant differences in the BFs of Cu and Mo between the control and all the SS amendment rates. The lowest BFs were found in the cases of Cd and Pb in roots of *E. sativa* grown in the control soil. The translocation factor (TF) varied among the HMs and soil treatments (Table 5). The TFs for all HMs were < 1.0 except for the HMs Cd and Pb. The accumulation of the majority of HMs was detected in roots rather than in the shoots (Tables 3 and 5). HMs, and particularly Cd, are inducible factors of phytochelatin which are low-molecular-weight proteins that form various complexes with Cd and as a result, prevent it from circulating as free Cd^{+2} inside the cytosol, subsequently aiding in the complexation and HM accumulation in roots (Clemens, 2006).

Table 5. Bioaccumulation factors (BFs), from soil to roots, and translocation factors (TFs), from roots to shoots, of heavy metals in *Eruca sativa* grown in soil at different sewage sludge amendment rates (means $\pm$ standard error, $n = 6$)

Metal	Factor	Sewage sludge amendment rate (g kg ⁻¹)						F-value
		0	10	20	30	40	50	
Cd	BF	0.114 $\pm$ 0.002a	0.172 $\pm$ 0.026ab	0.181 $\pm$ 0.015ab	0.183 $\pm$ 0.041ab	0.196 $\pm$ 0.025ab	0.231 $\pm$ 0.005b	3.4*
	TF	1.887 $\pm$ 0.116ab	1.382 $\pm$ 0.060a	1.414 $\pm$ 0.106ab	2.196 $\pm$ 0.559ab	2.548 $\pm$ 0.314b	2.331 $\pm$ 0.009ab	3.2*
Co	BF	0.124 $\pm$ 0.001a	0.237 $\pm$ 0.007b	0.250 $\pm$ 0.020bc	0.290 $\pm$ 0.022c	0.381 $\pm$ 0.002d	0.426 $\pm$ 0.008e	83.8***
	TF	0.418 $\pm$ 0.116b	0.212 $\pm$ 0.001a	0.203 $\pm$ 0.042a	0.209 $\pm$ 0.018a	0.208 $\pm$ 0.002a	0.232 $\pm$ 0.001a	2.8*
Cr	BF	0.317 $\pm$ 0.007a	0.503 $\pm$ 0.060b	0.737 $\pm$ 0.100c	0.829 $\pm$ 0.053c	0.827 $\pm$ 0.012c	0.838 $\pm$ 0.009c	19.3***
	TF	0.424 $\pm$ 0.016c	0.272 $\pm$ 0.028b	0.207 $\pm$ 0.028ab	0.171 $\pm$ 0.011a	0.203 $\pm$ 0.008ab	0.186 $\pm$ 0.003a	26.3***
Cu	BF	2.206 $\pm$ 0.041a	2.714 $\pm$ 0.306a	2.708 $\pm$ 0.619a	2.310 $\pm$ 0.492a	2.443 $\pm$ 0.143a	2.359 $\pm$ 0.158a	0.4 ^{ns}
	TF	0.711 $\pm$ 0.011a	0.556 $\pm$ 0.008a	0.681 $\pm$ 0.132a	0.491 $\pm$ 0.007a	0.568 $\pm$ 0.010a	1.389 $\pm$ 0.006b	37.3***
Fe	BF	0.218 $\pm$ 0.018a	0.373 $\pm$ 0.043b	0.453 $\pm$ 0.023bc	0.527 $\pm$ 0.043cd	0.574 $\pm$ 0.011d	0.547 $\pm$ 0.009cd	25.6***
	TF	0.246 $\pm$ 0.039b	0.169 $\pm$ 0.004a	0.141 $\pm$ 0.003a	0.170 $\pm$ 0.015a	0.155 $\pm$ 0.005a	0.189 $\pm$ 0.001ab	4.4**
Mn	BF	0.411 $\pm$ 0.020a	0.822 $\pm$ 0.073b	0.756 $\pm$ 0.065b	0.761 $\pm$ 0.017b	1.067 $\pm$ 0.029c	0.885 $\pm$ 0.017b	26.3***
	TF	0.788 $\pm$ 0.047c	0.370 $\pm$ 0.019ab	0.464 $\pm$ 0.037b	0.454 $\pm$ 0.019b	0.265 $\pm$ 0.010a	0.309 $\pm$ 0.001a	47.7***
Mo	BF	1.601 $\pm$ 0.397a	1.528 $\pm$ 0.147a	1.628 $\pm$ 0.162a	1.610 $\pm$ 0.211a	2.116 $\pm$ 0.423a	1.544 $\pm$ 0.108a	0.7 ^{ns}
	TF	1.735 $\pm$ 0.181a	1.948 $\pm$ 0.078a	1.917 $\pm$ 0.156a	1.819 $\pm$ 0.206a	2.100 $\pm$ 0.118a	2.033 $\pm$ 0.028a	0.9 ^{ns}
Ni	BF	0.237 $\pm$ 0.009a	0.419 $\pm$ 0.044b	0.489 $\pm$ 0.076bc	0.589 $\pm$ 0.027cd	0.709 $\pm$ 0.001de	0.793 $\pm$ 0.007e	35.6***
	TF	0.399 $\pm$ 0.069b	0.275 $\pm$ 0.029ab	0.272 $\pm$ 0.033ab	0.202 $\pm$ 0.040a	0.278 $\pm$ 0.011ab	0.269 $\pm$ 0.001ab	2.9*
Pb	BF	0.037 $\pm$ 0.008a	0.064 $\pm$ 0.007ab	0.067 $\pm$ 0.004ab	0.086 $\pm$ 0.005bc	0.106 $\pm$ 0.025bc	0.122 $\pm$ 0.003c	9.2***
	TF	4.744 $\pm$ 0.835a	2.347 $\pm$ 0.304a	2.712 $\pm$ 0.250a	2.302 $\pm$ 0.112a	4.078 $\pm$ 1.551a	3.045 $\pm$ 0.365a	1.7 ^{ns}
Zn	BF	0.800 $\pm$ 0.084a	0.818 $\pm$ 0.123a	0.833 $\pm$ 0.142a	0.772 $\pm$ 0.054a	0.644 $\pm$ 0.004a	0.680 $\pm$ 0.029a	0.9 ^{ns}
	TF	0.513 $\pm$ 0.092a	0.761 $\pm$ 0.074ab	0.809 $\pm$ 0.119b	0.698 $\pm$ 0.026ab	0.915 $\pm$ 0.002b	0.975 $\pm$ 0.003b	5.6**
<i>F-value</i> _{BF}		31.9***	45.2***	14.2***	15.7***	30.2***	60.5***	
<i>F-value</i> _{TF}		24.3***	56.6***	55.7***	20.7***	7.1***	82.5***	

F-values represent one-way ANOVA, degrees of freedom (*df*) = 5. Means in the same row followed by different letters are significantly different at $P < 0.05$, according to Tukey's HSD test. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant (i.e., $P > 0.05$)

Conclusions

The results of the current investigation indicate that a SS amendment to agricultural soils up to rate of 30 g kg⁻¹ could be beneficial as a fertilizer for *E. sativa*. SS amendment rates of 40 and 50 g kg⁻¹ caused negative effects on the growth, biomasses of *E. sativa*. The translocation factors for all the detected HMs were < 1.0 except for Cd and Pb. However, SS sludge used in the current study increased the biomass productivity of *E. sativa*, it is not recommended to be used as biofertilizer as the *E. sativa* is a green-salad freshly consumed plant. Also, the repeated application of SS at the same site may pose more environmental and health risks of consuming *E. sativa* plants. Thus, regular assessment and monitoring of HMs in agricultural products originating from plants grown in sludge-amended soils is recommended to avoid HM accumulation in the food chain. The future research should consider studying alteration of the crop rotation in cultivated areas that apply SS to introduce crops with low accumulation ability of HMs. 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. 1/73/40.

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