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ORIGINAL PAPER

Sewage Sludge Application Enhances the Growth of *Corchorus olitorius* Plants and Provides a Sustainable Practice for Nutrient Recirculation in Agricultural Soils

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Abstract

The current study aimed at evaluating the effects of different sewage sludge (SS) amendment rates as biofertilizers on growth of *Corchorus olitorius* plants and soil properties, with an emphasis on heavy metal (HM) allocation in plant parts and postharvest soil. Then, the soil was supplemented with various SS rates (0, 10, 20, 30, and 40 g kg^{-1}). The effects of these SS amendment rates on different growth parameters of *Corchorus olitorius* and soil properties were investigated. The SS amendment rate of 20 g kg^{-1} triggered the highest growth rates of *Corchorus* plants. Micronutrient HMs, including Co, Cu, Mn, and Ni, increased in the shoots of plants grown in soils amended with 20 g kg^{-1}, but with levels sufficient for normal plant growth and below the phytotoxic limits. The sludge application significantly increased the content of organic matter in postharvest soil from 1.38 to 4.83% at the amendment rate of 20 g kg^{-1}. Furthermore, our data showed that the quantities of the estimated HMs remaining in postharvest soils were below (Cu, Fe, Mn, Mo, Zn, and Pb) or within (Co, Ni, Cd, and Cr) the maximum permissible concentrations in agricultural soils at all of the SS amendment rates. Taken together, our findings suggest that soil application of SS can provide a sustainable safe practice for SS disposal and improve plant growth, while exerting no environmental threats provided there is no accumulation of HMs to toxic levels in shoots of the grown plants or in the amended soils.

Keywords Sewage sludge · Soil amendment · Environmental sustainability · Plant growth · Heavy metals · Nutrient recirculation

1 Introduction

The current increase in industrial and urban development, in addition to the excessive increase in the world population, has led to the production of massive quantities of various solid wastes, including sewage sludge (SS) (Singh et al. 2015). On a global basis, it is estimated that approximately 4 billion tons of solid wastes are generated every year (Vaish et al. 2016; reviewed by Sharma et al. 2017). One of the major ecological concerns worldwide is the safe disposal of these large amounts of SS. There are several common alternatives for SS disposal, including dumping at sea, landfilling, incineration, and soil application (Sanchez Monedero et al. 2004; Gude 2015; Zhang et al. 2017).

In the past, most of the towns and cities on the banks of rivers or shores of seas were allowed to dispose of their sewage in nearby water streams. Recently, several environmental legislations have been passed in many countries to ban the discharge of effluents and sewage into rivers or seas (Singh and Agrawal 2008), which in turn has led to an increasing accumulation of larger amounts of SS than before. On the other hand, the landfill disposal of SS is known to disturb the leachate and increase CO_2 emissions to the air (Barberio et al. 2013). Regarding the incineration technology of SS disposal, it is costly and mainly recommended when the country or region has legislation that

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forbids its soil application, especially in the case of sludge types that have a high content of toxic components (Kacprzak et al. 2017). Accordingly, land application of SS represents one of the most appropriate disposal alternatives. Indeed, the efficient recirculation of plant nutrients is a prerequisite for sustainable agriculture. A successful recirculation strategy of these nutrients could occur through soil amendment as a result of well-managed applications of SS to arable land (Kirchmann et al. 2017).

As SS is rich in organic and inorganic plant nutrients, it can be used for soil fertilization and remediation. It was reported that the yield of crops growing in adequately sludge-amended soils is largely enhanced compared with well-fertilized controls (Singh and Agrawal 2008; Mehrotra et al. 2016; Asgari Lajayer et al. 2019). Furthermore, this application of SS is ecologically sustainable and economically feasible compared with the other disposal strategies (Kacprzak et al. 2017). On the other hand, the disposal of biosolids to agricultural soils has to be carefully investigated to avoid environmental problems such as groundwater contamination or degradation of soil quality (Sharma et al. 2017). SS is a complex mixture of several components, including organic and inorganic matter and microorganisms in dissolved or suspended states (Raheem et al. 2018; Gul et al. 2015). In general, dried SS contains, on average, 50–70% organic matter and 30–50% mineral constituents (including 1–4% inorganic carbon), in addition to high concentrations of macronutrients, including nitrogen (3.4–4.0%) and phosphorus (0.5–2.5%), and considerable amounts of micronutrients (Fytili and Zabaniotou 2008; Samolada and Zabaniotou 2014; Tyagi and Lo 2013). The application of SS in agriculture has become a common practice and is a worldwide accepted source of organic matter and valuable plant nutrients, including micronutrients and macronutrients (Cerqueira et al. 2012; Sharma et al. 2017). Nonetheless, SS also contains various contaminants, such as heavy metals, including chromium (Cr), copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), arsenic (As), mercury (Hg), and selenium (Se), that pose serious threats to plants and food chains if present in high concentrations (Singh and Agrawal 2008; Kasim et al. 2014; Kacprzak et al. 2017; Raheem et al. 2018). In this regard, different countries and environmental organizations have determined maximum safe limits of heavy metal concentrations for use of SS in agriculture (He et al. 2005). Thus, bearing these safe limits of heavy metals in mind, the side effects of SS application could be abolished or minimized.

Sludge application to the soil improves its physical, chemical, and biological properties (Singh and Agrawal 2008). Approximately 50% of the solid SS is organic matter; it is known to improve the physical properties of soil, such as increasing the bulk density, soil porosity, and water-holding capacity, and it is also known to form stable organic heavy metal complexes and reduce metal availability in contaminated soil (Ramulu 2002; Kumpiene et al. 2008; Kominko et al. 2017). In addition to improving soil physicochemical properties, the addition of sludge to agricultural land has been reported to have a prominent positive effect on plant growth (Singh and Agrawal 2008; Eid et al. 2017a,b; Eid et al. 2018; Eid et al. 2019; Guoqing et al. 2019), thus increasing yield production and the nutritional value of crop plants (Sharma et al. 2017). It was reported that SS amendment improved the growth and yield of various plants. For example, the grain yield of barley increased significantly under repeated SS application (Antolín et al. 2005). Municipal sludge was used as a substrate for the growth of *Ailanthus altissima* plants, and compared with garden soil, the substrate mixed with sludge led to higher nutrient contents, resulting in better growth of *A. altissima* (Liu et al. 2019). Among various solid wastes including municipal waste compost, and poultry, cow, and sheep manures, municipal waste compost gives the highest improvement in tomato growth and yield (Mehdizadeh et al. 2013). In an experiment conducted to study the usefulness of SS amendment for *Helianthus annuus*, it was reported that organic matter, total N, available P, and exchangeable Na, K, and Ca increased in SS-amended soils in comparison with unamended ones, resulting in increased root and shoot length, number of leaves and biomass, in addition to antioxidant activities of sunflower plants (Belhaj et al. 2016). A number of previous studies by our research group indicated that SS application improved soil fertility and crop production of various plant species, including a leafy crop (spinach, Eid et al. 2017a), a fruiting crop (cucumber, Eid et al. 2017b), a legume crop (broad bean, Eid et al. 2018), and a cereal crop (wheat, Eid et al. 2019).

In the current study, we examined the possibility of improving the growth of the commonly consumed leafy vegetable crop *Corchorus olitorius* and amending soil quality through the application of different SS rates (10, 20, 30, and 40 g kg^{-1}) to agricultural soil. The obtained results showed that an amendment rate of 20 g kg^{-1} improved soil quality and enhanced plant growth.

2 Materials and Methods

2.1 Study Materials

The study was conducted in the greenhouse of the Biology Department, King Khalid University, Abha, Saudi Arabia (lat. 18° 15′ 04.65″ N, long. 42° 33′ 31.48″ E). Plastic pots with a volume of 6 L were used for all experiments. The agricultural soil used was collected from adjacent cultivated fields (lat. 18° 14′ 36.37″ N, long. 42° 33′ 58.25″ E). The soil was collected from the surface layer at a depth of approximately 0–20 cm. The SS material applied in the current study was collected from Abha's wastewater treatment plant (lat. 18° 13′ 59.19″ N, long. 42° 31′ 16.35″ E) in Aseer District, Saudi Arabia.

This wastewater treatment plant treats approximately 41,275 m^3 of wastewater per day, producing approximately 90 t day^{-1} of dry SS (personal communication).

2.2 Experimental Procedures

The sewage sludge (SS) was mixed with agricultural soil at rates of 0, 10, 20, 30, 40, and 50 g kg^{-1}. A dose of 50 g kg^{-1} was excluded because the percentage of germination was close to zero. Each plastic pot was filled with 4 kg of the respective soil-sludge treatment. In Saudi Arabia and a large number of other countries around the world, *Corchorus* is a commonly consumed leafy vegetable that is known to be rich in iron and is used for preparing a variety of meals (Ndlovu and Afolayan 2008; Onwordi et al. 2009). In the present study, 20 *Corchorus* seeds (Al-Kamal seeds, Giza, Egypt) were hand sown in each pot, with six biological replicates per treatment. The plants were grown for 57 days (starting from 4 January 2018) in a greenhouse with a natural day/night regime. Throughout the entire growth period, periodic irrigation was applied to maintain a uniform moisture level in each pot.

2.3 Analyses of Physicochemical Properties of Soils and Sewage Sludge

For physicochemical analyses, the soil and SS samples were air-dried for 2 weeks and then ground and sieved through a 2-mm sieve. The dried samples were analyzed for organic matter content using a loss-on-ignition method at 550 °C for 2 h (Wilke 2005). Electrical conductivity (EC) and pH were measured in soil-water extracts at 1:5 (Allen 1989). For heavy metal quantifications, from 0.5 to 1.0 g of each soil, SS or plant samples were digested using a mixed-acid digestion method (HNO_3 and $HClO_4$; 3:1, *v*/*v*) (Eid and Shaltout 2016). A microwave sample preparation system was used for digestion (PerkinElmer Titan MPS, PerkinElmer Inc., USA). Blank samples were included to verify the accuracy and precision of the digestion procedure and subsequent analyses. Various heavy metals, including cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn), cadmium (Cd), chromium (Cr), and lead (Pb), were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Scientific iCAP 7000 Plus Series; Thermo Fisher Scientific, USA) according to procedures outlined by Allen (1989). The detection limits of heavy metals (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. The instrument setting and operational conditions followed the manufacturer's specifications. Standard solutions with known concentrations of different heavy metals were prepared for the standardization of the system.

2.4 Quality Assurance and Quality Control

A certified reference material (SRM 1573a, tomato leaves) was used to verify the accuracy of the heavy metal determinations. This reference material was digested and analyzed using the same methods as those applied to the *Corchorus* plant samples. The digestion and measurement of heavy metals were performed in triplicate. Accuracy was determined by comparing the measured concentration with the certified value and then expressed as a percentage. The recovery rates ranged from 94.8 to 103.7% for SRM 1573a.

2.5 Measurements of Plant Growth Criteria

To measure various growth criteria, plant samples were collected from 57-day-old *Corchorus* plants. The measured growth parameters included shoot height, root length, dry weights of shoots and roots, number of leaves per plant, and leaf area (single sided, using a leaf area meter, Dynamax AM 300, Dynamax Inc., USA). For measuring dry weights, the plants were separated into shoots and roots, oven-dried at 60 °C for 1 week (fully dried), and ground using a metal-free plastic mill. The total biomass refers to the summation of the shoot and root biomasses. The absolute growth rate (AGR) was calculated according to Radford (1967): AGR (g DM $individual^{-1}$ day^{-1}) = $(W_2 - W_1) / (t_2 - t_1)$, where W_1 and W_2 are the total biomasses (g DM/individual) at times (days) t_1 and t_2, respectively.

2.6 Statistical Analysis

All statistical analyses in the current study were carried out using Statistica 7.1 (Statsoft 2007). The data were tested for normality of distribution and homogeneity of variance to determine whether they required log transformation before performing one-way analysis of variance (ANOVA). Significant differences in various measured parameters under different SS amendment rates were assessed using one-way ANOVA. Significant differences between the means of the five amendment rates were identified using the Tukey HSD test at $P < 0.05$.

3 Results

3.1 Physicochemical Properties of Agricultural Soil and Sewage Sludge

For prior testing of the quality of the used agricultural soil and sewage sludge (SS), the organic matter, pH value, electrical conductivity (EC), and contents of 10 common heavy metals (HMs) were analyzed. The data presented in Table 1 show that the agricultural soil from the study region is poor in fertility because it contains less than 1% organic matter. The pH of this soil is 8.68 (basic), which reduces the availability of most nutrients (Sharma et al. 2017), with EC equal to 0.07, indicating poor contents of mineral ions. Additionally, the contents of various micronutrient elements, including Cu, Fe, Mn, Mo, Ni, and Zn, in the investigated agriculture soil (Table 1) are lower than the adequate limits for normal plant growth (Kabata-Pendias 2011).

To improve the fertility of this poor soil, SS from a nearby wastewater treatment plant was collected for application to soil. Before application to soil, the physicochemical properties of this SS were analyzed (Table 1). The data in Table 1 show that the investigated SS is very rich in organic matter (65%) and has a pH value of 6.98, with EC equal to 1.39. The HM contents in the SS are within the acceptable limits for biosolids to be applied to agricultural soils (Table 1). Indeed, the analyzed SS is rich in micronutrients that are low in the investigated agricultural soil, including Cu, Mn, Ni, and Zn. Regarding toxic heavy metals, including Cd, Cr, and Pb, their contents in the investigated SS are even lower than the permissible limits in biosolids that are intended to be applied to agricultural soils (Table 1) (Kabata-Pendias 2011).

3.2 Effects of Sludge Application on the Growth of *Corchorus* Plants

To determine an appropriate amendment rate of SS that can improve soil fertility and *Corchorus* plant growth, and at the same time does not cause toxicity to soil or to the crop plant as a human food source, we applied different rates, i.e., 0, 10, 20, 30, and 40 g SS per kg soil. The results presented in Fig. 1 show that the amendment rates of 10 and 20 g kg^{-1} increased the plant height and root length, whereas the higher doses (30 and 40 g kg^{-1}) did not cause any significant increases compared with control soils. The other measured growth criteria, including number of leaves per plant, leaf area, shoot and root dry weights, and total biomass, all showed enhanced growth of *Corchorus* plants at all amendment rates (Fig. 1). In addition, our results show that the absolute growth rate increased at all SS amendment rates (Fig. 1). Importantly, our data show that the SS amendment rate of 20 g kg^{-1} achieved the highest value for all of the measured growth parameters, and this amendment rate is recommended for field application as a fertilizer for *Corchorus* plants.

3.3 HM Contents in Shoots and Roots of *Corchorus* Plants Grown in SS-Amended Soils

The results presented above suggest that SS soil amendment enhances the growth of *Corchorus* plants, especially at the amendment rate of 20 g kg^{-1}. However, it is crucial to determine how many HMs accumulate in plant parts and to decide whether they are within the sufficient levels for plant metabolism and do not exceed the safe limits for human nutrition. Indeed, the most limiting factor related to the toxic effect of HMs on plant metabolism is the accumulation of HMs to toxic levels in plant leaves or shoots. Here, we analyzed the quantities of 10 HMs in the shoots and roots of *Corchorus* plants at the different applied SS amendment rates. The data presented in Table 2 show that at the amendment rate of 20 g kg^{-1}, the

Table 1 Physicochemical properties of agricultural soil and sewage sludge used in the pot experiment (means ± standard error, $n = 3$)

Properties	Agricultural soil		Sewage sludge	
	Measured values	Average normal limits[a]	Measured values	Permissible limits[b]
Organic matter (%)	0.9 ± 0.2	NA	65.0 ± 0.9	NA
pH	8.68 ± 0.02	NA	6.98 ± 0.02	NA
EC	0.07 ± 0.00	NA	1.39 ± 0.10	NA
Co (mg kg^{-1})	35.49 ± 1.13	35	25.86 ± 1.31	–
Cu (mg kg^{-1})	15.01 ± 0.57	105	162.56 ± 2.32	1000–1750
Fe (mg g^{-1})	17.12 ± 0.16	39.2	24.41 ± 0.45	–
Mn (mg kg^{-1})	340.43 ± 7.04	775	560.70 ± 9.81	–
Mo (mg kg^{-1})	0.40 ± 0.02	7	0.91 ± 0.04	–
Ni (mg kg^{-1})	23.20 ± 0.65	40	138.73 ± 3.71	300–400
Zn (mg kg^{-1})	70.59 ± 1.07	200	667.62 ± 13.44	2500–4000
Cd (mg kg^{-1})	2.91 ± 0.05	3	1.17 ± 0.08	20–40
Cr (mg kg^{-1})	44.11 ± 0.35	125	168.09 ± 4.45	900
Pb (mg kg^{-1})	1.51 ± 0.59	160	671.11 ± 6.22	750–1200

NA not applicable

[a] Kabata-Pendias (2011)

[b] He et al. (2005)

levels of all of the investigated micronutrients in shoots significantly increased compared with those grown in control soil, except for Mo and Zn, for which there were no significant changes. In addition, the accumulated levels of these elements in the shoots of *Corchorus* plants grown in soil amended with SS up to 20 g kg^{-1} were within the sufficient levels for plant metabolism and far below the phytotoxic limits, except for Fe, which reached a level higher than the sufficient limit at this SS amendment rate (Kabata-Pendias 2011) (Table 2). Regarding the toxic HMs, the data presented in Table 2 show that the levels of Cd and Pb in shoots were below the toxic limits at all of the SS amendment rates. However, Cr was around the non-toxic limit only at the amendment rate of 10 g kg^{-1}. The roots of plants grown in the SS-amended soils accumulated large amounts of HMs, especially at high SS amendment rates (30 and 40 g kg^{-1}), compared with the roots of plants grown in unamended soil.

3.4 HM Levels in Postharvest SS-Amended Soil

We showed that SS enhances the growth of *Corchorus* plants and that HM levels in the plant shoots were within physiological limits for the plants. However, one of the main concerns of using SS for soil amendment is the expected accumulation of HM in agricultural soils to toxic levels. In the current study, in addition to the organic matter and pH levels, we investigated how many HMs were leftover in the postharvest soils. The data presented in Table 3 show that the organic matter in the SS-amended soil significantly increased at all amendment rates. At the SS amendment rate of 20 g kg^{-1}, the organic matter in postharvest soil was 4.83% compared with 1.38% in unamended soil (Table 3). The SS application decreased the soil pH from 8.33 to 7.61 at the amendment rate of 20 g kg^{-1} (Table 3). Regarding HMs, the data presented in Table 3 show that the quantities of HMs remaining in postharvest soils were

Fig. 1 Effects of SS amendment rates on various growth criteria and the absolute growth rate of *Corchorus* plants. *F* values represent one-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$. Means with different letters are significantly different at $P < 0.05$ according to Tukey's HSD test

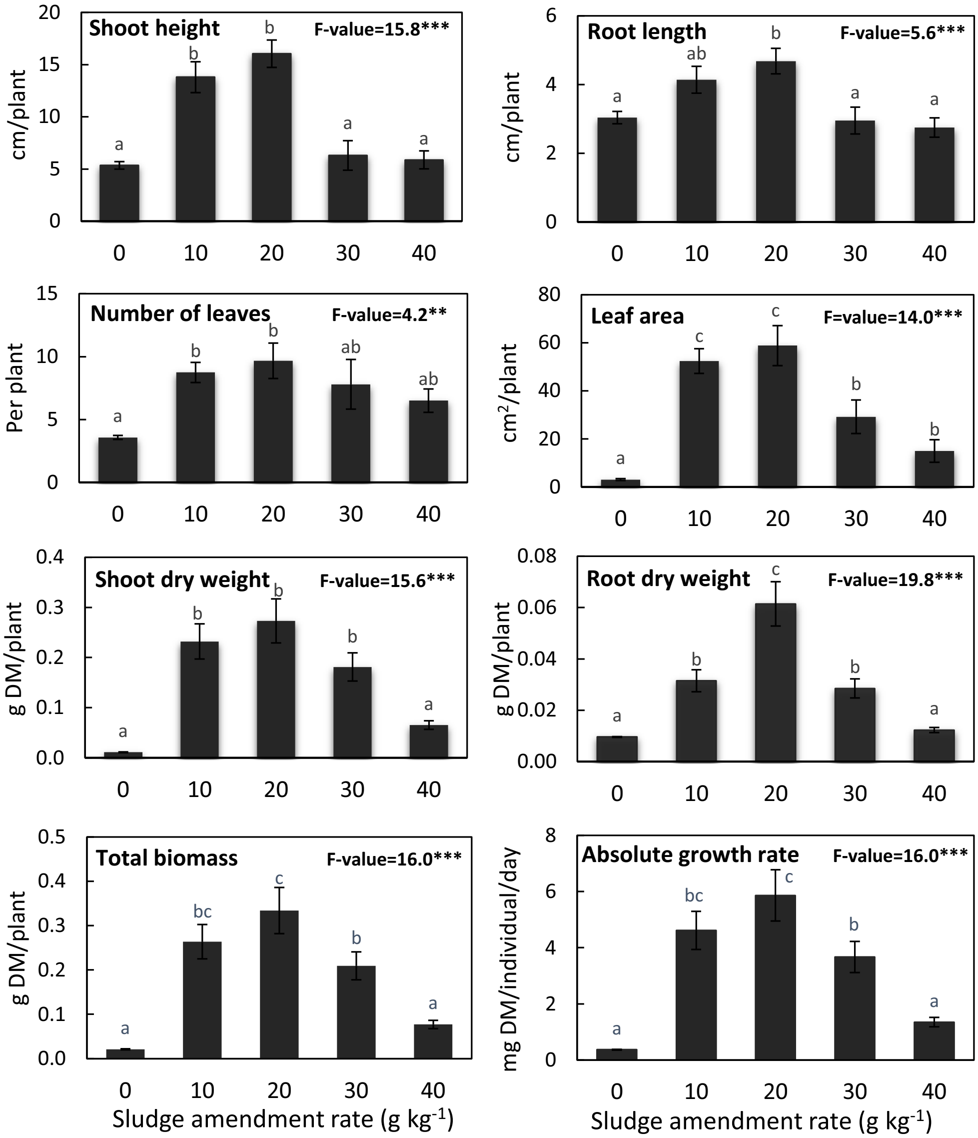

Table 2 Heavy metal concentrations (mg kg^{-1}) in shoots and roots of 57-day-old *Corchorus* plants grown in SS-amended soils (means ± standard error, $n = 6$)

Metal	Tissue	Sewage sludge amendment rate (g kg^{-1})					*F* value	In mature leaf tissues	
		0	10	20	30	40		Sufficient or normal[a]	Excessive or toxic[a]
Co	Shoot	1.47 ± 0.17a	1.90 ± 0.04ab	2.27 ± 0.04bc	2.37 ± 0.14bc	2.51 ± 0.19c	9.6***	0.2–1	15–50
	Root	4.99 ± 0.09a	6.44 ± 0.07a	7.19 ± 1.14a	9.85 ± 0.69b	12.50 ± 0.80c	25.1***		
Cu	Shoot	7.98 ± 0.97a	10.08 ± 0.04b	10.18 ± 0.27b	10.51 ± 0.12bc	12.00 ± 0.09c	9.9***	40	20–100
	Root	16.16 ± 0.26a	16.35 ± 0.44a	20.48 ± 1.31a	29.05 ± 4.93b	52.78 ± 0.22c	44.7***		
Fe	Shoot	228.2 ± 10.4a	265.7 ± 12.2a	1292 ± 69.6b	2117 ± 68.9c	2880 ± 252.3d	91.3***	450	> 1000
	Root	4089 ± 9.5a	4546 ± 122.4a	5003 ± 344.4a	8934 ± 15.9b	14,931 ± 211.8c	17.2***		
Mn	Shoot	141.0 ± 11.3a	148.2 ± 8.9ab	149.3 ± 4.6ab	171.8 ± 4.1b	173.1 ± 2.8b	4.3**	30–300	400–1000
	Root	118.6 ± 2.2a	151.7 ± 1.2a	253.5 ± 52.9b	311.4 ± 21.4b	438.2 ± 0.6c	25.3***		
Mo	Shoot	0.61 ± 0.15a	0.75 ± 0.03a	0.77 ± 0.03a	0.79 ± 0.04a	1.61 ± 0.18b	14.6***	0.2–5	10–50
	Root	0.46 ± 0.10a	1.20 ± 0.02b	1.34 ± 0.14b	1.57 ± 0.17b	3.13 ± 0.10c	81.6***		
Ni	Shoot	1.67 ± 0.26a	2.18 ± 0.03a	5.81 ± 0.06b	7.54 ± 0.09c	9.77 ± 0.22d	465.8***	0.1–5	10–100
	Root	8.65 ± 0.01a	9.39 ± 1.86a	9.60 ± 0.11a	18.75 ± 0.20b	25.20 ± 0.92c	62.8***		
Zn	Shoot	27.49 ± 0.08a	28.31 ± 0.48a	29.31 ± 0.97a	29.69 ± 0.67a	39.85 ± 0.72b	59.9***	27–150	100–400
	Root	46.05 ± 0.44a	58.20 ± 0.21a	63.75 ± 5.03a	106.4 ± 19.54b	177.1 ± 10.20c	35.2***		
Cd	Shoot	0.37 ± 0.05a	0.51 ± 0.02b	0.59 ± 0.00b	0.60 ± 0.00b	0.60 ± 0.01b	18.0***	Up to 0.5	5–30
	Root	0.47 ± 0.10a	0.75 ± 0.02b	0.77 ± 0.03b	0.86 ± 0.01b	2.08 ± 0.06c	165.9***		
Cr	Shoot	2.03 ± 0.07a	2.18 ± 0.05a	9.08 ± 0.36b	13.89 ± 1.24c	20.74 ± 0.42d	171.9***	Up to 2	5–30
	Root	18.51 ± 0.20a	19.73 ± 4.84a	20.76 ± 1.11a	36.81 ± 0.22a	63.37 ± 9.33b	16.4***		
Pb	Shoot	0.24 ± 0.04a	0.65 ± 0.04ab	0.87 ± 0.12b	1.13 ± 0.11bc	1.62 ± 0.28c	12.4***	Up to 10	30–300
	Root	1.20 ± 0.15a	1.45 ± 0.05a	2.09 ± 0.06b	2.53 ± 0.25b	3.82 ± 0.22c	38.4***		

F values represent one-way ANOVA, degrees of freedom (*df*) = 4. Means in the same row followed by different letters are significantly different at $P < 0.05$, according to Tukey's HSD test

[a] Kabata-Pendias (2011)

$^{**}P < 0.01$; $^{***}P < 0.001$

below (Cu, Fe, Mn, Mo, Zn, and Pb) or within (Co, Ni, Cd, and Cr) the maximum permissible concentrations at any of the SS amendment rates (Kabata-Pendias 2011).

4 Discussion

Among the several disposal strategies, the application of SS to agricultural soils has a myriad of benefits, including improvement of soil physicochemical properties, increase of organic matter, and, most importantly, recycling of valuable nutrients for plants and various food chains (Martinez et al. 2002; Sharma et al. 2017; Kirchmann et al. 2017). In the current study, we investigated the value of the soil application of SS in improving the growth of *Corchorus* plants and in amending soil fertility without threats to the environment. The prior analysis of the used agricultural soil showed that it contains very low contents of organic matter and micronutrient metals (Table 1) and can thus receive SS application. Furthermore, our analyses indicated that the proposed SS is rich in organic matter (65%) and contains relatively high amounts of HMs that do not exceed critical concentrations for agricultural soil (He et al. 2005; Kabata-Pendias 2011).

4.1 Soil Application of SS Enhances the Growth of *Corchorus* Plants

Our results showed that the application of a series of SS amendment rates, i.e., 10, 20, 30, and 40 g kg^{-1}, to agricultural soil improved the growth of *Corchorus* compared with plants grown in control soil, with an amendment rate of 20 g kg^{-1} providing the highest enhanced growth. The increased growth parameters included shoot and root lengths, leaf area, dry weights, and total biomass (Fig. 1). Similarly, it was found that SS application at the rates of 80, 160, and 320 t per hectare increased the dry weight of sunflower plants (*Helianthus annuus*) (Morera et al. 2002). A significant increase in the grain yield of barley was reported with repeated application of biosolids (Antolín et al. 2005). Biosolid application increased the biomass of two native grasses (*Zoysia japonica* and *Poa annua*) at amendment rates of 15 and 60 t per hectare compared with plants grown in unamended soils (Wang et al. 2008). In comparison with plants grown in unamended soils, lady's finger (*Abelmoschus esculentus*) yield increased significantly by 75% and 135% at 20 and 40% SS application, respectively (Singh and Agrawal 2009). The SS application improved the crop production of spinach plants (Eid et al.

2017a). These reports and results presented here (Table 1) propose that enhancements of plant growth due to soil application of biosolids are mainly due to their high contents of organic matter and micronutrients that serve as good sources of nutrients for plant growth (Logan and Harrison 1995; Singh and Agrawal 2009; Eid et al. 2019).

4.2 The HM Contents in Plant Shoots Are Below the Phytotoxic Limits at the SS Amendment Rate of 20 mg kg^{-1}

One of the most significant concerns surrounding SS application to agricultural soils is the possible accumulation of HMs in plant shoots to toxic levels that in turn negatively affect plant metabolism and food chains. In the current study, we analyzed the quantities of 10 common HMs in the shoots and roots of *Corchorus* plants grown in SS-amended soil (Table 2). The investigated HMs may be divided into micronutrients that are essential to plant growth when available at sufficient concentrations (including Co, Cu, Fe, Mn, Mo, Ni, and Zn) and toxic metals (including Cd, Cr, and Pb) that negatively affect living organisms (He et al. 2005). Our data showed that, at the SS amendment rate of 20 g kg^{-1}, which provided the highest growth of *Corchorus* plants, the accumulated levels of the studied micronutrients in shoots significantly increased compared with the control, except for Mo and Zn, which did not change significantly. However, the shoot contents of both elements were already within the sufficient limits for normal plant metabolism (Table 2). Several studies reported increased concentrations of micronutrient HMs due to soil application of SS. For example, the concentrations of Fe and Ni were higher in flax plants grown in sludge-amended soil compared with control (10:1) (Tsakou et al. 2002). A single application of 112, 225, and 450 t per hectare of biosolids linearly increased Cu and Zn concentrations in snap beans (*Phaseolus vulgaris*) with increasing amendment doses (Dowdy et al. 1978; Singh and Agrawal 2008).

Importantly, our results indicated that the contents of the micronutrients that showed significantly increased levels in the shoots of plants grown in SS-amended soils were within sufficient levels for plant metabolism and far below the phytotoxic limits, except for Fe, which reached a level higher than the general sufficient limit for plants (Kabata-Pendias 2011) (Table 2). However, the recorded high growth of *Corchorus* plants obtained at the 20 g kg^{-1} amendment rate (Fig. 1) suggests that the estimated high level of Fe had no noticeable toxic effects on *Corchorus* growth. Indeed, *Corchorus* plants are commonly consumed leafy vegetables that are well known to be rich in iron (Ndlovu and Afolayan 2008; Onwordi et al. 2009) and thus have mechanisms that may have developed in their ancestors, enabling them to accommodate high levels of Fe in their shoots.

Table 3 Physicochemical properties of postharvest soils at different sewage sludge amendment rates and heavy metal contents measured in mg kg^{-1} soil (means ± standard error, $n = 6$)

Properties	Sewage sludge amendment rate (g kg^{-1})					*F* value	Maximum permissible limits in agricultural soil (mg kg^{-1})[a]
	0	10	20	30	40		
Organic matter (%)	1.38 ± 0.12a	2.74 ± 0.17b	4.83 ± 0.09c	5.88 ± 0.12d	6.97 ± 0.19e	252.7***	NA
pH	8.33 ± 0.02e	7.90 ± 0.01d	7.61 ± 0.02c	7.48 ± 0.04b	7.24 ± 0.03a	289.8***	NA
EC (mS cm^{-1})	0.43 ± 0.02a	0.62 ± 0.04b	0.63 ± 0.04b	0.66 ± 0.01b	0.76 ± 0.04b	13.7***	NA
Co	23.83 ± 0.26a	23.95 ± 0.27a	24.76 ± 0.50a	25.08 ± 0.25ab	26.45 ± 0.71b	5.8**	20–50
Cu	2.45 ± 0.15a	2.97 ± 0.07b	3.23 ± 0.12b	5.44 ± 0.04c	5.99 ± 0.03d	292.6***	60–150
Fe	14,518 ± 77a	15,104 ± 86b	15,927 ± 147c	16,761 ± 262d	16,906 ± 124d	45.0***	20,000–40,000[b]
Mn	207.3 ± 0.5a	207.9 ± 1.9a	228.2 ± 4.3b	229.0 ± 6.1b	236.2 ± 1.9b	13.9***	< 450[c]
Mo	0.31 ± 0.03a	0.55 ± 0.03ab	0.64 ± 0.12b	0.78 ± 0.04b	1.21 ± 0.10c	20.3***	4–10
Ni	28.51 ± 0.30a	28.54 ± 0.05a	28.68 ± 0.27a	30.39 ± 1.36ab	30.91 ± 0.25b	3.2*	20–60
Zn	57.94 ± 0.83a	63.56 ± 1.12ab	70.84 ± 1.21b	89.62 ± 4.29c	103.90 ± 0.52d	82.9***	100–300
Cd	2.46 ± 0.05a	2.51 ± 0.01ab	2.54 ± 0.05ab	2.56 ± 0.07ab	2.70 ± 0.06b	3.0*	1–5
Cr	48.39 ± 0.61a	50.73 ± 0.07b	51.27 ± 0.08b	54.22 ± 0.26c	55.31 ± 0.81c	35.4***	50–200
Pb	3.91 ± 0.08a	4.31 ± 0.34a	4.39 ± 0.09a	4.40 ± 0.04a	4.49 ± 0.17a	1.6^{ns}	20–300

F values represent one-way ANOVA, degrees of freedom (*df*) = 4. Means in the same row followed by different letters are significantly different at $P < 0.05$ according to Tukey's HSD test. *Corchorus* plants were harvested after 57 days

ns not significant, *NA* not applicable

[a] Reviewed by Kabata-Pendias (2011)

[b] Cornell and Schwertmann (2003)

[c] Adriano (2001)

The reported increase of several micronutrients in the shoots of plants grown in SS-amended soils compared with the unamended control (Table 2) is suggested to contribute to the enhanced growth of *Corchorus* plants shown here (Fig. 1). Indeed, sufficient levels of various micronutrients in plant leaf tissues are crucial for many physiological functions and plant metabolism. For example, Co is involved in N_2 fixation and stimulation of chlorophyll and protein synthesis (Palit et al. 1994; Kabata-Pendias 2011). Cu is essential for a number of key physiological functions, including photosynthesis, protein and carbohydrate metabolism, oxidation, valence changes, and cell wall metabolism (Kabata-Pendias 2011). Fe is very important for photosynthesis, N_2 fixation, and valence modifications (Kabata-Pendias 2011; Rout and Sahoo 2015).

Regarding the investigated toxic HMs, our study showed that the levels of Cd and Pb in shoots were below the toxic limits at all of the SS amendment rates. Plant roots, in general, do not absorb large amounts of Pb from soils, and there is a strong belief that it is the least available metal to plants (Kabata-Pendias 2011). Additionally, Cd is highly adsorbed to organic matter in soil and is less available to plants (Arenas-Lago et al. 2013). The Cr level in shoots was relatively high at the amendment rate of 20 g kg^{-1}. However, its measured content (9.08 mg kg^{-1}) was already below the average excessive limits for this element (~ 17 mg kg^{-1}) (Table 2). Concerning the levels of HMs accumulated in roots of plants grown in SS-amended soils, our results showed that *Corchorus* plants efficiently accumulated significant amounts of HMs in their roots compared with the amounts transported to shoots (Table 2). The accumulation of large quantities of HMs in roots, which are nonedible parts of *Corchorus* plants, represents a phytoremediation practice that cleans the soil after successive SS application cycles (Sewelam et al. 2014).

4.3 The SS Soil Application Improved the Physicochemical Properties of Postharvest SS-Amended Soil with HM Contents Within the Permissible Limits

The applications of SS to agricultural soils are expected to change the physicochemical properties of the postharvest SS-amended soils, including organic matter, pH, and HM contents. The data presented here showed a significant increase in organic matter from 1.38% in unamended soil to 4.83% in amended soil at the SS rate of 20 g kg^{-1} (Table 3), suggesting the use of SS at this amendment rate as a replacement for chemical fertilizers. In addition to its direct contribution to the enhancement of plants grown in SS-amended soils (Fig. 1), SS organic matter also has beneficial effects on many soil physical properties, including soil porosity and soil aggregation, and the increasing retention and movement of water (Kominko et al. 2017). Furthermore, the increase of organic matter in SS-amended soils can reduce the bioavailability of HMs to plants due to the ability of metals to form stable complexes with organic matter (Park et al. 2011; Wasilkowski et al. 2017).

In addition to the properties of both soil and SS and the applied amendment rates, the pH value of the sludge-soil mixtures represents a limiting factor, especially for the availability of metals in sludge-amended soils (Parkpain et al. 1998; Denny 2002; Kabata-Pendias 2011). The results presented here showed that the increase in the SS amendment rate linearly decreased the soil pH, with a change from 8.33 to 7.61 at the amendment rate of 20 g kg^{-1} (Table 3). This change of pH towards acidity may be due to the increase of the humic acid content, which may occur due to biodegradation of the increased organic matter in the SS-amended soil (Singh et al. 2011). Another important environmental concern about the application of SS to agricultural soils is the expected accumulation of HMs to toxic levels in the postharvest SS-amended soils. In this regard, the data presented in Table 3 show that the quantities of HMs remaining in postharvest soils were below (Cu, Fe, Mn, Mo, Zn, and Pb) or within (Co, Ni, Cd, and Cr) the maximum permissible concentrations in agricultural soils (Kabata-Pendias 2011) at any of the SS amendment rates.

4.4 The Application of SS to Agricultural Soil Is Environmentally Safe and Sustainable

To conclusively show the extent to which the application of SS to agricultural soil in the current study is environmentally safe and sustainable, we used the data presented in Tables 1 and 3 to compare the organic matter and HM concentrations in agricultural soils before and after cultivation, without and with SS application, against permissible limits of HMs in agricultural soils. The comparisons presented in Fig. 2 indicate that the organic matter content increased from less than 1% before cultivation to 1.38% and 4.83% in the postharvest soils

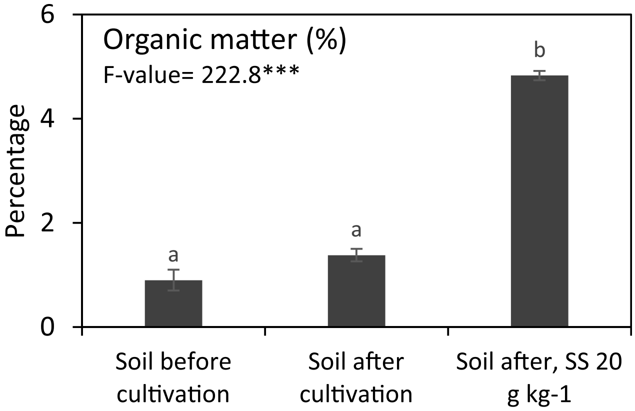

Fig. 2 A comparison between the organic matter contents in the soil before and after cultivation, with or without SS amendment at a rate of 20 g kg^{-1}. *F* values represent one-way ANOVA. ***$P < 0.001$. Means with different letters are significantly different at $P < 0.05$ according to Tukey's HSD test

without or with application of an SS amendment rate of 20 g kg^{-1}, respectively. This finding proposes that scheduled cycles of SS applications could improve the fertility of agricultural soil.

Notably, the data presented in Fig. 3 show that the levels of almost all of the micronutrients in postharvest soils without SS amendment were lower than those of the precultivation soils. This finding suggests that after several cultivation cycles without SS amendment or chemical fertilizers, the micronutrient elements would be depleted from the soil. Furthermore, the levels of all of the investigated HMs in postharvest soils amended with an SS dose of 20 g kg^{-1} remained far below the average allowable concentration (AAC) in agricultural soils (Fig. 3). Taken together, these findings show that land application of SS to agricultural soil can provide a sustainable way to securely remove SS, recirculate nutrients in agricultural soils, improve plant growth by replacing chemical fertilizers, and at the same time cause no environmental threats

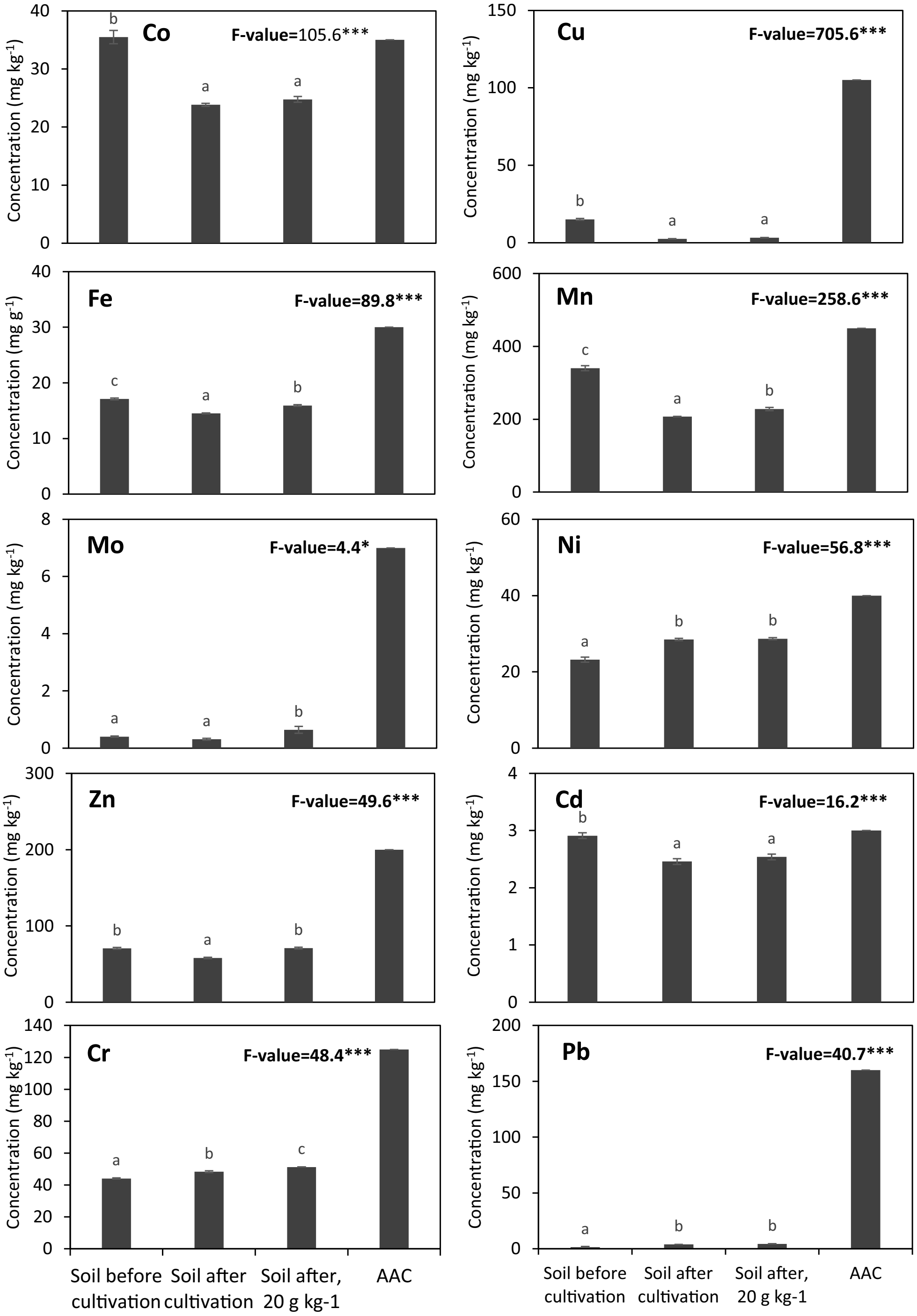

Fig. 3 A comparison between the HM contents in soil before and after cultivation, with or without SS amendment at a rate of 20 g kg^{-1}, and the average allowable concentration (AAC) in agricultural soils (Kabata-Pendias 2011). *F* values represent one-way ANOVA. $^{*}P < 0.05$, $^{***}P < 0.001$. Means with different letters are significantly different at $P < 0.05$ according to Tukey's HSD test

provided there is no accumulation of toxic levels of HMs in shoots of the grown plants or the amended soils. 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. This future study may suggest suitable agricultural rotation cycles for the application of SS, as well as for the cultivated crop species. Additionally, in future work, this type of study may be extended to investigate the effects of SS application on soil microorganisms.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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