



Article

Planned Application of Sewage Sludge Recirculates Nutrients to Agricultural Soil and Improves Growth of Okra (*Abelmoschus esculentus* (L.) Moench) Plants

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Abstract: The aim of this study was to investigate the feasibility of using sewage sludge (SS) biosolids as a low-cost soil fertilizer to improve soil characteristics and crop yields. Okra (*Abelmoschus esculentus* (L.) Moench) plants were grown in soil supplemented with different concentrations of SS (0, 10, 20, 30, 40, and 50 g/kg). The results showed that SS soil application led to improved soil quality with a 93% increase of organic matter (at SS dose of 10 g/kg), decreased pH (a reduction from 8.38 to 7.34), and enhanced macro- and micro- nutrient contents. The levels of all the investigated heavy metals (HMs; Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the postharvest SS-amended soil were within the prescribed safe limits. The application of SS to soil considerably enhanced the growth parameters of okra plants. Total biomass increased 13-fold and absolute growth rate increased 10-fold compared to plants grown in nonamended (control) soils. Among the applied SS doses, the 10 g/kg SS dose led to the highest values of the measured growth parameters, compared to those of plants grown in control soils. The induced growth at 10 g/kg SS was accompanied by a substantial increase in metal content in roots, stems, leaves, and fruits; however, all levels remained within safe limits. Consequently, the data presented in this study suggest that SS could be used as a sustainable organic fertilizer, also serving as an ecofriendly method of SS recycling.

Keywords: agriculture; bio-fertilizers; soil amendment; okra plant; heavy metals

1. Introduction

Among the numerous types of solid waste, sewage sludge (SS) is an important type of organic waste. Due to population expansion, dwindling natural resources, and the current energy crisis, the relevance of establishing a sustainable strategy for eco-friendly solid waste management cannot be overstated. The inappropriate disposal of SS and other biowastes poses a major threat to environmental quality, resulting in issues such as groundwater pollution and soil deterioration [1].

The use of SS as a soil fertilizer is gaining popularity. SS can be utilized to improve the physical and chemical properties of soil, such as pH, fertility, porosity, bulk density,

and water retention capacity [2,3]. In addition, SS, as an organic amendment, can have a significant impact on the floral and faunal populations of the soil [4]. Before incorporating these wastes into the soil as fertilizers, the total concentration of heavy metals (HMs) should be considered [5,6]. The presence of HMs in SS and soils treated with it can be determined by a variety of parameters [3]. As a negative side effect of SS application to agricultural soils, the buildup occurs of several hazardous HMs from both urban and industrial sources, such as Cu, Zn, Ni, Cd, Cr, Pb, and Hg, leading to subsequent absorption by plants [7]. Furthermore, SS may be a source for various pathogenic strains of microorganisms [8]. Accordingly, the use of SS in agriculture raises concerns regarding soil and water contamination, and possible hazards to living creatures [9]; hence, there must be a balance between improved soil fertility and potential risks from SS treatment.

The application of SS has various positive effects on soil quality and crop yield. It increases the quantity of organic matter (OM) and enhances the availability of micronutrients. Plants have specialized and efficient ways to acquire important plant micronutrients from soil, even when they are present in minute quantities [10]. Plants can solubilize and absorb micronutrients at extremely low concentrations in the soil, even from precipitates that are almost insoluble, owing to chelating agents, pH shifts, and other redox processes that help plant roots [11]. Plants have evolved unique systems to absorb, translocate, and store toxic HMs, which mimic the chemical characteristics of plants that are specific to vital nutrients [11]. All these transport systems are involved in the uptake of a variety of mineral ions from the soil for plant development.

The effect of SS application on the growth performance and yield of various plants has been investigated in many studies [12–14]. The use of SS clearly improves the production of rice, wheat, maize, barley, lentil, cucumber, spinach, and sunflower [12–14]. Micronutrient absorption in plants increases as SS application doses increase, showing that SS enhances micronutrient availability in the soil [15].

Okra (*Abelmoschus esculentus* (L.) Moench) is an annual vegetable crop of the *Malvaceae* family. Globally, this vegetable is mostly cultivated in tropical and warm, temperate regions. The edible component of this vegetable is the fibrous, mucilaginous fruit with white seeds. The okra fruit is rich in energy, minerals, fiber, vitamins, and carbohydrates [16]. The current study was designed to assess the possible positive and negative effects of SS application on soil quality and growth of *A. esculentus*. The results of this study showed that the application of SS to soil led to improved soil quality with higher and enhanced the growth and yield parameters of okra plants. The data recommend the application of SS as an environmentally friendly strategy to dispose of SS while also improving soil fertility and enhancing plant growth.

2. Materials and Methods

2.1. Plant Materials, Sewage Sludge Treatments, and Experimental Design

The cultivated field soil used in the experiment (coarse sandy loam; Typic Torriorthents: [6]) was obtained at a depth of 0–20 cm from freshly reclaimed, adjacent lands (latitude: 18.2434, longitude: 42.5661). Dewatered, activated SS was obtained from the Abha City Municipal Wastewater Treatment Plant (latitude: 18.2331, longitude: 42.5212), which serves around 760,000 inhabitants and treats an estimated 41,275 m³ of wastewater each day, with daily dumping of approximately 90 ton/day of SS following aerobic tertiary treatment [6]. The cultivated field soil and SS samples were air-dried for two weeks before being crushed and sieved through a 2 mm sieve. The experiment was conducted at the Biology Department greenhouse at King Khalid University. The SS was mixed evenly with the cultivated field soil at rates of 0 (control), 10, 20, 30, 40, and 50 g/kg, based on a preliminary experiment. These were comparable to 0, 30, 60, 90, 120, and 150 t/ha, respectively. Each treatment consisted of six 6 L plastic pots containing 4 kg of the chosen treatment, as well as ten *A. esculentus* seeds (Clemson Spineless, West Hills Seeds, Sutter, CA, USA). A completely randomized design was used to arrange the experimental units. The plants were cultivated in the greenhouse for 62 days (beginning on 10 February 2018) under a natural day-night

cycle and watered with tap water as required to maintain a water content of 40–50% in each pot. Weeding was performed manually as required. After 15 days, the plants were carefully trimmed to one plant per pot.

2.2. Plant Morphology and Biomass

A. esculentus individuals were collected after 62 days. Shoot height (measured from the clipped base of the shoot to the tip of the highest leaf), root and fruit lengths, and the number of leaves and fruits per individual were measured using a measuring tape. A leaf area meter was used to measure the single-sided leaf area (Dynamax AM 300, Dynamax Inc., Houston, TX, USA). At each SS amendment rate, the total leaf area (cm²) per plant was determined by multiplying the number of leaves per shoot by the mean leaf area (cm²/leaf). All plant components were cleaned under running water and then in deionized water before being separated into roots, fruits, stems, and leaves. The partitioned plant components were dried for a week at 60 °C. Their masses were recorded before they were crushed in a plastic mill (Philips HR2221/01, Philips, Shanghai, China) and stored until later use. The aerial portions of the plant (sum of the fruit, stem, and leaf biomass) were included in the aboveground biomass, and the total biomass (sum of the aboveground biomass and root biomass) was computed. The absolute growth rate (AGR; g DM/ind./day) was calculated as follows:

$$\text{AGR} = (W_2 - W_1) / (t_2 - t_1) \quad (1)$$

where W_1 and W_2 are the total biomass (g DM/ind.) at times t_1 and t_2 , respectively.

2.3. Sample Analysis

At the completion of the experiment, soil samples from each replicate were collected, air-dried for two weeks, crushed, and sieved through a 2 mm sieve. In addition to the SS and cultivated field soil, soil samples from all treatments were analyzed for OM and N content using a CHN Elemental Analyzer (Yanako CHN Corder MT-5, Kyoto, Japan and Auto Sampler MTA-3, Kyoto, Japan) [17]. Electrical conductivity (EC) and pH were measured in 1:5 soil-water extracts [17] using a portable calibrated salinity multiparameter instrument (Hanna HI 9811-5, Hanna Instruments Italia Srl, Italy). A sample of 0.5–1.0 g each of soil, SS, cultivated field soil, and plant sample (just HMs) were digested using a tri-acid mix digestion technique (HNO₃:H₂SO₄:HClO₄; 5:1:1, v/v/v; Sigma-Aldrich, Merck SA, Darmstadt, Germany) to estimate the P, K, and HM concentrations [17]. For digestion, a microwave device was employed (PerkinElmer Titan MPS, PerkinElmer Inc., Waltham, MA, USA) [17], till a transparent color appeared, then soil, SS, cultivated field soil, and plant digests were filtered and diluted to 25 mL with double deionized water. Blank samples were used to ensure that the digestion technique and subsequent tests were accurate and precise. Inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 7000 Plus Series; Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the concentrations of nine HMs (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) [16]. The detection limits of HMs (in g/L) were 6.0, 2.0, 2.0, 2.0, 1.0, 1.0, 1.0, 0.3, and 0.1 for Ni, Co, Cr, Cu, Fe, Pb, Zn, Mn, and Cd, respectively. K was measured using an atomic absorption spectrophotometer (Shimadzu AA-6300, Shimadzu Co., Ltd., Kyoto, Japan) [17], and P was determined using the ammonium molybdate technique [17] with a spectrophotometer (CECIL CE 1021, Cecil Instruments Limited, Milton, Cambridge, UK). The manufacturer's instructions were followed for the instrument settings and operational conditions. Standard solutions with known HM levels were prepared to calibrate the apparatus.

2.4. Quality Assurance and Quality Control

To ensure the accuracy of the HM determinations, a certified reference sample (SRM 1573a, tomato leaves; NIST, Gaithersburg, MD, USA) was used. The same techniques used to digest and analyze the *A. esculentus* samples were used for the reference material. Triplicate HM digestion and measurements were performed. By comparing the measured

content to the certified value, the accuracy was determined, and the result was expressed as a percentage. The recovery rates ranged from 93.8% to 106.8%.

3. Data Analyses

The bioaccumulation factor (BF) is used to assess the capacity of *A. esculentus* to acquire HMs in its roots, while the translocation factor (TF) is used to assess the ability of *A. esculentus* to transfer HMs from roots to aerial shoot tissues (stems, leaves, and fruits). BF and TF were calculated as follows [18]:

$$BF = \frac{\text{Heavy metal content in the root (mg/kg)}}{\text{Heavy metal content in the soil (mg/kg)}} \quad (2)$$

while

$$TF_{stem} = \frac{\text{Heavy metal content in the stem (mg/kg)}}{\text{Heavy metal content in the root (mg/kg)}} \quad (3)$$

and

$$TF_{leaf} = \frac{\text{Heavy metal content in the leaf (mg/kg)}}{\text{Heavy metal content in the root (mg/kg)}} \quad (4)$$

and

$$TF_{fruit} = \frac{\text{Heavy metal content in the fruit (mg/kg)}}{\text{Heavy metal content in the root (mg/kg)}} \quad (5)$$

Before performing the analysis of variance (ANOVA), the data were tested for their normality of distribution using Shapiro-Wilk's W test and homogeneity of variance using Levene's test. When necessary, data were log-transformed. Significant differences between the measured parameters (soil properties, biomass, plant morphometric parameters, BF, TF and HMs data for *A. esculentus* tissues) at different SS amendment rates (0, 10, 20, 30, 40, and 50 g/kg) were determined using a one-way ANOVA. Moreover, the disparities between the nine different HMs for each SS amendment rate were identified by applying an one-way ANOVA to the BFs and TFs data. Significant differences between treatment means were identified using Tukey's Honest Significant Difference (HSD) test at $p < 0.05$. SPSS 15 (Armonk, NY, USA) was used for all statistical analyses [19].

4. Results

4.1. Chemical Properties of the Sewage Sludge and Precultivation Soil Used in the Pot Experiment

The concentrations of OM, pH, EC, and HM were assessed in the precultivation soil and SS. Table 1 reveals that the agricultural soil used had less than 1% OM, with a basic pH of 8.7 and a low EC of 0.1 mS/cm. The soil also contained low levels of several micronutrients. In contrast, the results in Table 1 indicate favorable agronomic properties of SS, such as high OM content (65%) and EC (1.4 mS/cm). The SS had high amounts of macronutrients, such as N and P, with values of 56.4 and 16.2 (g/kg), respectively, as well as certain critical micronutrients such as Cu, Fe, Mn, Ni, and Zn, in comparison with the target soil. The results also indicated that SS contained relatively high levels of toxic HMs, such as Pb, but these were within the permissible limits for SS (Table 1).

4.2. Selected Chemical Properties of Postharvest Soils at Different Doses of Sewage Sludge Amendment

One of the major concerns about utilizing SS as a soil amendment is the buildup of HM to lethal levels in agricultural soils. The data presented in Table 2 depict the impact of a gradual increase in SS doses on soil OM, pH levels, macro- and micro-nutrients, and HMs. The results showed that increasing the SS rate resulted in a significant increase in OM in the SS-amended soil, recording 2.7% at the recommended SS amendment rate (10 g/kg), compared to 1.74% in the control soil (Table 2). The SS application decreased soil pH from 8.38 to 7.34 at the recommended SS amendment rate (Table 2). Furthermore, increasing the SS amendment doses increased the concentrations of macronutrients (N, P, and K) and various micronutrient minerals (Co, Cr, Cu, Fe, Mn, Ni, and Zn) in postharvest soils.

Increasing the SS doses resulted in an increase in non-nutritive HMs such as Cd and Pb in the agricultural soil, but their levels did not exceed the permitted limits (Table 2).

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

Properties	Precultivation Soil		Sewage Sludge	
	Measured Values	Average Normal Limits *	Measured Values	Permissible Limits
Electrical conductivity (mS/cm)	0.1 ± 0.0	NA	1.4 ± 0.1	NA
pH	8.7 ± 0.1	NA	7.0 ± 0.1	NA
Organic matter (%)	0.9 ± 0.2	NA	65.0 ± 0.9	NA
N (g/kg)	0.3 ± 0.0	NA	56.4 ± 3.9	NA
P (g/kg)	2.7 ± 0.1	NA	16.2 ± 0.1	NA
K (g/kg)	14.8 ± 0.6	NA	6.1 ± 0.2	NA
Cd (mg/kg)	2.9 ± 0.1	3.0	1.2 ± 0.1	20.0–40.0 **
Co (mg/kg)	35.5 ± 1.1	35.0	25.9 ± 1.3	-
Cr (mg/kg)	134.3 ± 0.7	125.0	176.2 ± 1.9	900.0 ***
Cu (mg/kg)	15.0 ± 0.6	105.0	162.6 ± 2.3	1000.0–1750.0 **
Fe (mg/g)	42.4 ± 0.5	39.2	24.1 ± 0.5	-
Mn (mg/kg)	677.3 ± 3.2	1500.0–3000.0	560.7 ± 9.8	-
Ni (mg/kg)	68.1 ± 3.7	40.0	138.7 ± 3.7	300.0–400.0 **
Pb (mg/kg)	3.5 ± 0.4	160.0	671.1 ± 6.2	750.0–1200.0 **
Zn (mg/kg)	77.2 ± 1.9	200.0	667.6 ± 13.4	2500.0–4000.0 **

*: Kabata-Pendias [20], **: Council of the European Communities [21], ***: He et al. [22], NA: not available.

4.3. Morphological Characters and Biomass Components of Okra Plants at Different Doses of Sewage Sludge Amendment

The data presented in Figure 1 show that all the applied SS doses resulted in improved morphological characteristics of the okra plants, including root length, shoot height, leaf area, fruit length, and number of leaves and fruits, as well as improved biomass components including root, stem, and leaf biomasses, absolute growth rate, and total biomass, compared to the control values. The SS amendment rate of 10 g/kg demonstrated the best performance for all the assessed growth parameters (Figure 1). Specifically, the number of fruits and their biomass were highest at the 10 g/kg SS amendment rate, whereas higher rates had negative effects on the fruit parameters. As a result, a 10 g/kg amendment rate is recommended for use as a fertilizer for okra plants in the field.

4.4. Effects of Different Rates of Sewage Sludge on HM Levels in Fruits, Leaves, Stems, and Roots of Okra Plants

The results shown in Table 3 indicate that at a 10 g/kg amendment rate, the levels of all the investigated micronutrients in the roots, stems, leaves, and fruits increased significantly when compared to those of plants produced in the control soil. Excluding the Fe level in the roots, the accumulated levels of these elements in the roots, stems, leaves, and fruits of okra plants were sufficient for plant metabolism and far below the phytotoxic limits at the SS rate of 10 g/kg. In terms of hazardous HMs, the levels of Cd and Pb in the shoots were below the toxic limits for all SS amendment doses (Table 3). At a 10 g/kg amendment rate, Cr, on the other hand, was near the nontoxic limit. In comparison to the roots of plants grown in control soil, the roots of plants grown in SS-amended soils received significant amounts of HMs, particularly at the highest SS amendment doses (40 and 50 g/kg).

Table 2. Selected chemical properties of soil at different doses of sewage sludge amendment after harvesting *Abelmoschus esculentus* grown for 62 days (means \pm standard error, $n = 6$).

Properties	Sewage Sludge Amendment Rate (g/kg)						F-Value	Maximum Permissible Limits in Agricultural Soil	
	0	10	20	30	40	50		Kabata-Pendias [20]	Council of the European Communities [21]
Electrical conductivity (mS/cm)	0.27 \pm 0.02 a	0.36 \pm 0.02 b	0.39 \pm 0.02 bc	0.40 \pm 0.00 bc	0.43 \pm 0.00 c	0.60 \pm 0.01 d	61.6 ***	NA	NA
pH	8.38 \pm 0.03 c	7.34 \pm 0.01 b	7.31 \pm 0.02 b	7.30 \pm 0.03 b	6.83 \pm 0.02 a	6.76 \pm 0.02 a	608.1 ***	NA	NA
Organic matter (%)	1.74 \pm 0.01 a	1.74 \pm 0.09 a	3.82 \pm 0.23 b	5.14 \pm 0.04 c	5.85 \pm 0.27 d	7.27 \pm 0.08 e	209.6 ***	NA	NA
N (g/kg)	0.38 \pm 0.02 a	0.59 \pm 0.06 a	1.93 \pm 0.14 b	2.78 \pm 0.03 c	3.32 \pm 0.17 d	4.35 \pm 0.05 e	252.1 ***	NA	NA
P (g/kg)	2.41 \pm 0.01 a	2.66 \pm 0.03 b	2.73 \pm 0.01 bc	2.77 \pm 0.03 cd	2.83 \pm 0.01 d	2.92 \pm 0.01 e	71.9 ***	NA	NA
K (g/kg)	8.91 \pm 0.17 a	9.09 \pm 0.22 a	11.14 \pm 0.16 b	12.40 \pm 0.03 c	12.99 \pm 0.23 c	15.75 \pm 0.12 d	238.6 ***	NA	NA
Cd (mg/kg)	2.2 \pm 0.1 a	2.7 \pm 0.1 b	2.9 \pm 0.1 bc	3.0 \pm 0.1 bc	3.1 \pm 0.1 c	3.2 \pm 0.1 c	12.9 ***	1–5	1–3
Co (mg/kg)	27.5 \pm 0.9 a	29.3 \pm 1.3 ab	31.0 \pm 1.6 ab	31.8 \pm 1.7 ab	33.0 \pm 1.7 ab	35.4 \pm 2.3 b	2.8 *	20–50	-
Cr (mg/kg)	83.5 \pm 7.4 a	87.7 \pm 7.2 a	91.7 \pm 7.5 a	95.9 \pm 7.6 a	103.4 \pm 6.9 a	110.2 \pm 6.5 a	1.8 ns	50–200	-
Cu (mg/kg)	8.7 \pm 1.2 a	10.3 \pm 1.5 ab	15.2 \pm 2.2 abc	17.4 \pm 2.3 bcd	19.2 \pm 2.1 cd	24.9 \pm 3.2 d	7.5 ***	60–150	50–140
Fe (mg/g)	26.6 \pm 1.9 a	29.2 \pm 1.9 ab	30.2 \pm 1.9 ab	31.4 \pm 2.0 ab	32.8 \pm 2.4 ab	36.5 \pm 2.1 b	2.5 *	20–40 †	-
Mn (mg/kg)	428.1 \pm 31.2 a	447.3 \pm 33.3 a	467.7 \pm 31.9 a	479.5 \pm 33.3 a	504.4 \pm 31.9 a	554.4 \pm 34.7 a	1.7 ns	>3000	-
Ni (mg/kg)	28.9 \pm 0.6 a	30.9 \pm 0.9 ab	31.3 \pm 0.4 ab	33.9 \pm 0.6 bc	34.5 \pm 0.8 c	38.0 \pm 1.5 d	14.2 ***	20–60	30–75
Pb (mg/kg)	3.9 \pm 0.2 a	4.2 \pm 0.2 a	4.5 \pm 0.2 a	4.8 \pm 0.2 ab	5.6 \pm 0.3 bc	6.1 \pm 0.3 c	11.0 ***	20–300	50–300
Zn (mg/kg)	68.5 \pm 1.2 a	72.8 \pm 2.2 a	90.6 \pm 3.3 b	97.7 \pm 1.7 b	110.7 \pm 1.9 c	123.2 \pm 4.4 d	66.4 ***	100–300	150–300

F-values represent one-way ANOVA, degrees of freedom = 5. Means in the same row followed by different letters are significantly different at $p < 0.05$, according to Tukey's Honest Significant Difference (HSD) test. *: $p < 0.05$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$), †: Cornell and Schwertmann [23], NA: not available.

Table 3. Effects of different doses of sewage sludge amendment on heavy metal contents (mg/kg) in the fruits, leaves, stems, and roots of *Abelmoschus esculentus* harvested after 62 days (means \pm standard error, $n = 6$).

Metal	Tissue	Sewage Sludge Amendment Rate (g/kg)						F-Value	Safe Limits †	Phytotoxic Range †
		0	10	20	30	40	50			
Cd	Fruit	0.3 \pm 0.0 a	0.3 \pm 0.0 a	0.4 \pm 0.1 a	0.8 \pm 0.0 b	1.9 \pm 0.1 c	2.1 \pm 0.0 c	529.7 ***	up to 0.5	5.0–30.0
	Leaf	0.3 \pm 0.0 a	0.5 \pm 0.0 b	0.7 \pm 0.0 c	0.7 \pm 0.0 c	1.1 \pm 0.0 d	1.1 \pm 0.0 d	257.3 ***		
	Stem	0.1 \pm 0.0 a	0.2 \pm 0.0 b	0.2 \pm 0.0 b	0.3 \pm 0.0 c	0.4 \pm 0.0 d	0.6 \pm 0.0 e	124.3 ***		
	Root	0.1 \pm 0.0 a	0.3 \pm 0.0 b	0.4 \pm 0.0 c	0.4 \pm 0.0 c	0.5 \pm 0.0 d	0.6 \pm 0.0 e	51.8 ***		
Co	Fruit	0.8 \pm 0.0 a	0.9 \pm 0.0 a	0.9 \pm 0.1 a	1.0 \pm 0.0 a	1.6 \pm 0.1 b	1.7 \pm 0.0 b	46.8 ***	0.2–1.0	15.0–50.0
	Leaf	0.8 \pm 0.0 a	1.0 \pm 0.0 b	1.5 \pm 0.0 c	1.5 \pm 0.1 c	2.1 \pm 0.0 d	2.2 \pm 0.0 e	310.8 ***		
	Stem	0.3 \pm 0.0 a	0.8 \pm 0.0 ab	1.4 \pm 0.1 bc	1.5 \pm 0.2 bc	1.8 \pm 0.0 c	2.9 \pm 0.4 d	18.5 ***		
	Root	3.8 \pm 0.6 a	6.7 \pm 1.0 b	10.8 \pm 0.9 c	12.8 \pm 0.2 c	16.1 \pm 0.8 d	22.1 \pm 1.0 e	69.5 ***		
Cr	Fruit	0.1 \pm 0.0 a	0.1 \pm 0.0 a	0.1 \pm 0.0 a	0.4 \pm 0.1 b	0.8 \pm 0.1 c	0.9 \pm 0.0 c	55.3 ***	5.0	10.0–100.0
	Leaf	1.8 \pm 0.1 a	2.2 \pm 0.1 a	4.0 \pm 0.1 b	5.7 \pm 0.0 c	8.2 \pm 0.0 d	12.3 \pm 0.2 e	1410.8 ***		
	Stem	0.8 \pm 0.0 a	0.8 \pm 0.1 a	1.4 \pm 0.1 b	1.5 \pm 0.1 b	2.2 \pm 0.1 c	3.3 \pm 0.2 d	57.2 ***		
	Root	14.4 \pm 2.5 a	36.0 \pm 5.5 a	103.4 \pm 6.0 b	153.2 \pm 3.0 c	166.3 \pm 14.4 c	168.5 \pm 16.2 c	50.2 ***		
Cu	Fruit	5.6 \pm 0.5 a	7.3 \pm 0.2 b	9.0 \pm 0.2 c	12.5 \pm 0.1 d	13.6 \pm 0.6 d	16.0 \pm 0.6 e	93.6 ***	40.0	20.0–100.0
	Leaf	2.7 \pm 0.0 a	5.7 \pm 0.1 b	6.3 \pm 0.2 c	8.5 \pm 0.0 d	8.6 \pm 0.2 d	10.8 \pm 0.1 e	530.2 ***		
	Stem	0.9 \pm 0.0 a	2.7 \pm 0.1 a	7.0 \pm 0.1 b	7.6 \pm 1.1 b	11.0 \pm 0.2 c	11.5 \pm 0.5 c	72.9 ***		
	Root	9.2 \pm 1.1 a	15.7 \pm 3.1 b	19.4 \pm 0.9 b	25.4 \pm 0.9 c	27.1 \pm 0.6 c	33.8 \pm 1.2 d	32.2 ***		

Table 3. Cont.

Metal	Tissue	Sewage Sludge Amendment Rate (g/kg)						F-Value	Safe Limits ‡	Phytotoxic Range †
		0	10	20	30	40	50			
Fe	Fruit	54.1 ± 3.8 a	70.5 ± 0.2 a	102.2 ± 7.9 a	211.3 ± 21.6 b	452.6 ± 18.7 c	459.5 ± 16.8 c	178.7 ***	450.0	>1000.0
	Leaf	362.2 ± 12.1 a	495.4 ± 31.4 b	577.4 ± 14.6 c	806.8 ± 5.2 d	1145.5 ± 7.4 e	1474.1 ± 9.4 f	716.9 ***		
	Stem	153.2 ± 21.1 a	155.2 ± 14.8 a	242.3 ± 25.2 b	292.0 ± 16.7 b	393.6 ± 38.5 c	653.7 ± 5.8 d	69.1 ***		
	Root	6715.3 ± 1417.0 a	7147.7 ± 1215.4 a	12,743.4 ± 1184.5 ab	14,434.9 ± 404.3 b	18,484.3 ± 1843.9 b	19,136.7 ± 3173.3 b	9.3 ***		
Mn	Fruit	37.6 ± 1.7 a	70.5 ± 2.9 b	92.7 ± 0.1 b	174.4 ± 3.5 c	374.9 ± 3.8 d	383.5 ± 14.0 d	609.0 ***	-	400.0–1000.0
	Leaf	137.3 ± 3.2 a	186.8 ± 2.8 b	355.3 ± 11.8 c	376.6 ± 4.1 c	553.6 ± 12.9 d	664.4 ± 10.0 e	565.5 ***		
	Stem	10.9 ± 0.5 a	28.9 ± 2.2 ab	61.6 ± 0.9 b	141.4 ± 23.5 c	163.7 ± 2.7 c	178.9 ± 8.2 c	50.1 ***		
	Root	108.4 ± 20.4 a	170.9 ± 28.3 a	368.7 ± 41.4 b	396.0 ± 8.1 b	417.4 ± 13.9 b	861.2 ± 27.1 c	107.0 ***		
Ni	Fruit	0.1 ± 0.0 a	0.5 ± 0.0 ab	0.8 ± 0.0 b	1.7 ± 0.1 c	3.3 ± 0.3 d	3.5 ± 0.1 d	96.4 ***	20.0	40.0–246.0
	Leaf	0.0 ± 0.0 a	0.0 ± 0.0 a	0.9 ± 0.0 b	1.7 ± 0.1 c	3.1 ± 0.0 d	4.5 ± 0.2 e	316.9 ***		
	Stem	0.2 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.1 ab	0.6 ± 0.0 ab	0.7 ± 0.2 b	4.1 **		
	Root	8.2 ± 1.5 a	14.8 ± 0.8 a	56.1 ± 3.3 b	78.7 ± 1.5 c	80.5 ± 7.4 c	85.7 ± 7.6 c	55.5 ***		
Pb	Fruit	0.2 ± 0.1 a	0.2 ± 0.1 a	0.2 ± 0.0 a	0.3 ± 0.1 ab	0.4 ± 0.1 b	0.6 ± 0.0 c	14.0 ***	up to 10.0	30.0–300.0
	Leaf	0.0 ± 0.0 a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.1 ± 0.0 b	0.2 ± 0.0 c	0.3 ± 0.0 d	33.2 ***		
	Stem	0.1 ± 0.0 a	0.1 ± 0.0 a	0.3 ± 0.0 ab	0.5 ± 0.1 ab	1.0 ± 0.0 b	2.7 ± 0.4 c	32.9 ***		
	Root	1.1 ± 0.3 a	1.6 ± 0.4 ab	2.2 ± 0.2 bc	2.8 ± 0.0 c	3.0 ± 0.2 c	3.2 ± 0.2 c	11.2 ***		
Zn	Fruit	23.3 ± 0.6 a	31.2 ± 2.3 b	61.8 ± 1.4 c	73.6 ± 0.4 d	74.5 ± 1.0 d	96.3 ± 3.5 e	219.6 ***	60.0	100.0–500.0
	Leaf	21.7 ± 0.4 a	25.6 ± 0.7 b	31.0 ± 0.1 c	52.4 ± 1.1 d	74.6 ± 1.9 e	75.0 ± 1.2 e	493.3 ***		
	Stem	12.2 ± 0.8 a	18.0 ± 1.6 a	33.7 ± 0.9 b	56.6 ± 8.5 c	65.8 ± 2.3 c	87.2 ± 3.3 d	55.7 ***		
	Root	39.5 ± 4.8 a	67.6 ± 16.2 a	103.4 ± 3.1 b	116.3 ± 10.2 b	118.7 ± 2.7 b	157.2 ± 6.2 c	23.2 ***		

F-values represent one-way ANOVA, degrees of freedom = 5. Means in the same row followed by different letters are significantly different at $p < 0.05$, according to Tukey's Honest Significant Difference (HSD) test. **: $p < 0.01$, ***: $p < 0.001$, †: FAO/WHO standard (Codex Alimentarius Commission [24]), ‡: Kabata-Pendias [20].

4.5. Bioaccumulation Factors (BFs) and Translocation Factors (TFs) of Heavy Metals

The results shown in Table 4 demonstrate that increasing the SS amendment doses in the soil considerably increased the BF of HMs from the soil to the roots of the okra plants, except Cu, for which the increase in BF was nonsignificant. BF was generally larger for Ni, with a mean value of 1.788, followed by Cu, Zn, Cr, Mn, Pb, Fe, Co, and Cd.

Table 4. Bioaccumulation factors (BFs) of heavy metals from the soil to roots in *Abelmoschus esculentus* grown for 62 days in soil with different doses of sewage sludge amendment (means ± standard error, $n = 6$).

Metal	Sewage Sludge Amendment Rate (g/kg)						F-Value
	0	10	20	30	40	50	
Cd	0.044 ± 0.009 a	0.094 ± 0.005 b	0.131 ± 0.015 cd	0.125 ± 0.007 bc	0.163 ± 0.009 d	0.201 ± 0.008 e	34.4 ***
Co	0.139 ± 0.021 a	0.230 ± 0.034 b	0.346 ± 0.028 c	0.404 ± 0.007 cd	0.487 ± 0.023 d	0.625 ± 0.028 e	48.5 ***
Cr	0.172 ± 0.029 a	0.410 ± 0.063 a	1.128 ± 0.065 b	1.596 ± 0.031 c	1.608 ± 0.139 c	1.529 ± 0.147 c	47.5 ***
Cu	1.054 ± 0.123 a	1.524 ± 0.303 a	1.274 ± 0.058 a	1.459 ± 0.049 a	1.407 ± 0.033 a	1.361 ± 0.049 a	1.4 ns
Fe	0.252 ± 0.053 a	0.245 ± 0.042 a	0.422 ± 0.039 ab	0.460 ± 0.013 ab	0.563 ± 0.056 b	0.524 ± 0.087 b	6.5 ***
Mn	0.253 ± 0.048 a	0.382 ± 0.063 a	0.788 ± 0.089 b	0.826 ± 0.017 b	0.827 ± 0.028 b	1.553 ± 0.049 c	70.9 ***
Ni	0.285 ± 0.051 a	0.478 ± 0.024 a	1.788 ± 0.105 b	2.323 ± 0.046 c	2.331 ± 0.213 c	2.253 ± 0.201 bc	52.9 ***
Pb	0.288 ± 0.069 a	0.369 ± 0.101 ab	0.489 ± 0.046 ab	0.604 ± 0.007 b	0.542 ± 0.028 b	0.522 ± 0.035 b	4.4 **
Zn	0.577 ± 0.069 a	0.929 ± 0.222 ab	1.141 ± 0.035 b	1.190 ± 0.105 b	1.073 ± 0.024 b	1.277 ± 0.050 b	5.4 **
F-value	24.7 ***	10.8 ***	80.1 ***	262.2 ***	57.9 ***	50.2 ***	

F-values represent one-way ANOVA. Means in the same row followed by different letters are significantly different at $p < 0.05$, according to Tukey's Honest Significant Difference (HSD) test. **: $p < 0.01$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$).

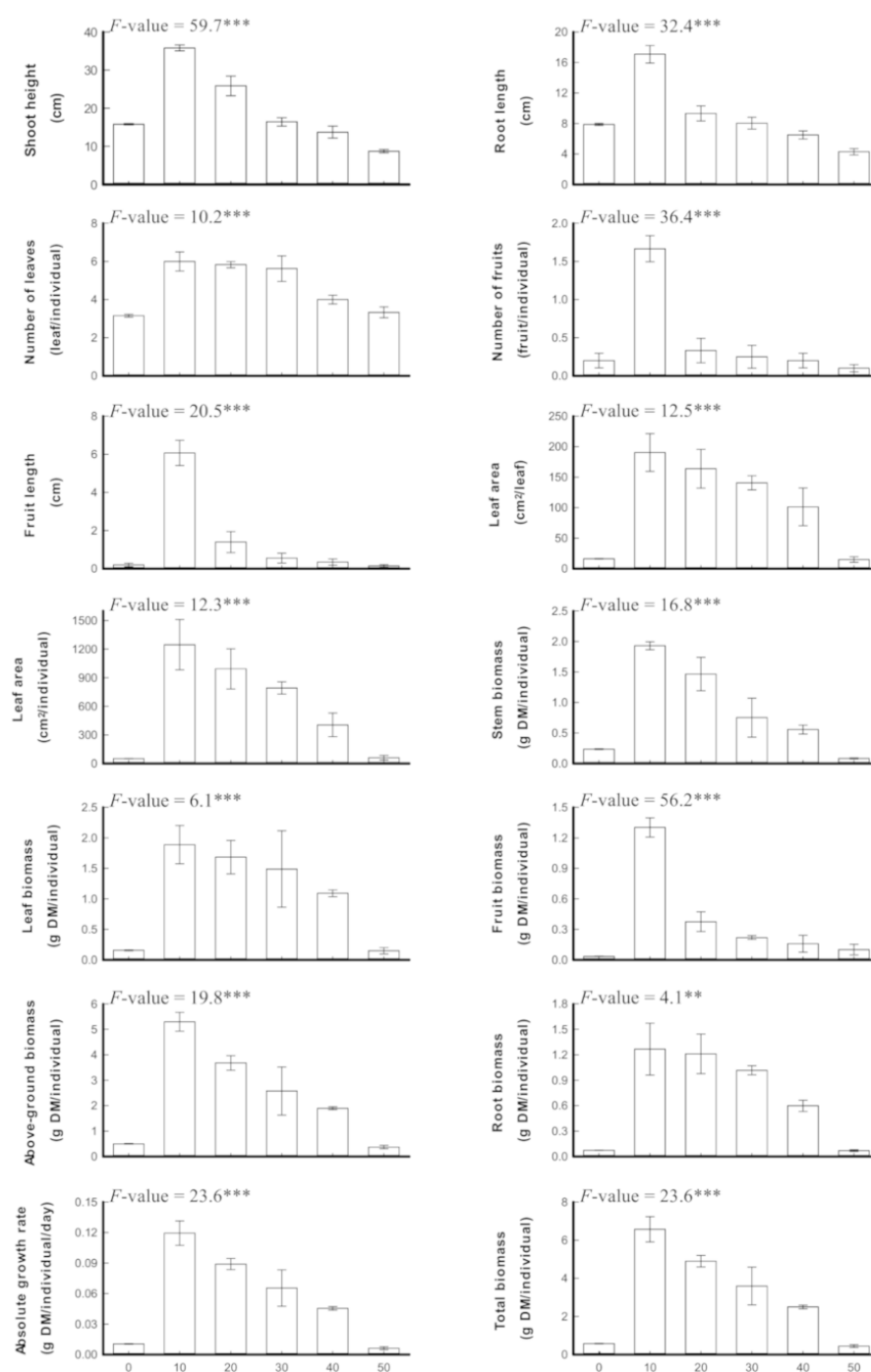


Figure 1. Effect of different doses of sewage sludge amendment on the morphometric parameters and biomass components of okra (*Abelmoschus esculentus*) plants grown for 62 days (mean \pm standard error, $n = 6$). F-values represent one-way ANOVA, degrees of freedom = 5. **: $p < 0.01$, ***: $p < 0.001$.

Table 5 displays the TF which was used to assess the plant capacity to translocate HMs from roots to aerial shoot tissues (stems, leaves, and fruits). The calculated TFs for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in *A. esculentus* revealed that the TF varied between plant tissues, and that the TF values for nonessential metals such as Cd and Pb were equivalent to those for essential elements. For Cd, root-leaf translocation was high at low SS amendment doses (10 and 20 g/kg) but low at high doses (30, 40, and 20 g/kg). The translocation of Pb was greatest in the root-fruit at the lowest SS amendment rate (10 g/kg) and highest in the

root-stem at higher SS amendment doses (20, 30, 40, and 50 g/kg). The micronutrients Cr, Cu, Co, Fe, Mn, Zn, and Ni, were mostly translocated in the root-leaf and root-stem systems.

Table 5. Translocation factors (TFs) of heavy metals from the roots to stems, leaves, and fruits in *Abelmoschus esculentus* grown for 62 days in soil with different doses of sewage sludge amendment (mean \pm standard error, $n = 6$).

Metal	Factor	Sewage Sludge Amendment Rate (g/kg)						F-Value
		0	10	20	30	40	50	
Cd	TF _{fruit}	2.938 \pm 0.313 bc	1.309 \pm 0.076 a	1.112 \pm 0.259 a	2.276 \pm 0.117 b	4.016 \pm 0.277 d	3.287 \pm 0.128 cd	28.0 ***
	TF _{leaf}	2.426 \pm 0.239 a	2.026 \pm 0.110 a	1.894 \pm 0.242 a	1.803 \pm 0.133 a	2.132 \pm 0.064 a	1.736 \pm 0.069 a	2.5 ns
	TF _{stem}	1.485 \pm 0.304 b	0.816 \pm 0.047 a	0.689 \pm 0.073 a	0.751 \pm 0.021 a	0.731 \pm 0.063 a	0.947 \pm 0.045 a	5.0 **
Co	TF _{fruit}	0.235 \pm 0.032 b	0.140 \pm 0.015 a	0.084 \pm 0.006 a	0.079 \pm 0.002 a	0.106 \pm 0.006 a	0.080 \pm 0.005 a	16.0 ***
	TF _{leaf}	0.231 \pm 0.037 b	0.177 \pm 0.031 ab	0.147 \pm 0.013 a	0.120 \pm 0.005 a	0.128 \pm 0.004 a	0.103 \pm 0.004 a	5.2 **
	TF _{stem}	0.079 \pm 0.009 a	0.134 \pm 0.016 a	0.131 \pm 0.007 a	0.114 \pm 0.017 a	0.109 \pm 0.005 a	0.139 \pm 0.027 a	2.1 ns
Cr	TF _{fruit}	0.005 \pm 0.001 b	0.004 \pm 0.001 ab	0.001 \pm 0.000 a	0.003 \pm 0.001 ab	0.005 \pm 0.001 b	0.005 \pm 0.001 b	4.9 **
	TF _{leaf}	0.147 \pm 0.022 b	0.066 \pm 0.009 a	0.039 \pm 0.002 a	0.037 \pm 0.001 a	0.051 \pm 0.004 a	0.077 \pm 0.009 a	15.0 ***
	TF _{stem}	0.067 \pm 0.014 b	0.022 \pm 0.000 a	0.014 \pm 0.002 a	0.009 \pm 0.001 a	0.014 \pm 0.001 a	0.021 \pm 0.003 a	12.5 ***
Cu	TF _{fruit}	0.621 \pm 0.023 a	0.564 \pm 0.099 a	0.473 \pm 0.029 a	0.494 \pm 0.014 a	0.506 \pm 0.029 a	0.475 \pm 0.021 a	1.6 ns
	TF _{leaf}	0.319 \pm 0.039 a	0.439 \pm 0.081 a	0.324 \pm 0.007 a	0.339 \pm 0.009 a	0.319 \pm 0.011 a	0.320 \pm 0.010 a	1.6 ns
	TF _{stem}	0.100 \pm 0.009 a	0.203 \pm 0.034 ab	0.366 \pm 0.014 c	0.306 \pm 0.053 bc	0.408 \pm 0.007 c	0.343 \pm 0.027 c	15.9 ***
Fe	TF _{fruit}	0.011 \pm 0.003 a	0.012 \pm 0.002 a	0.009 \pm 0.001 a	0.015 \pm 0.001 a	0.025 \pm 0.001 b	0.028 \pm 0.005 b	9.6 ***
	TF _{leaf}	0.067 \pm 0.013 a	0.085 \pm 0.019 a	0.048 \pm 0.006 a	0.056 \pm 0.002 a	0.065 \pm 0.006 a	0.089 \pm 0.015 a	1.9 ns
	TF _{stem}	0.025 \pm 0.002 a	0.023 \pm 0.002 a	0.021 \pm 0.004 a	0.020 \pm 0.001 a	0.023 \pm 0.004 a	0.039 \pm 0.006 b	3.7 *
Mn	TF _{fruit}	0.403 \pm 0.059 ab	0.461 \pm 0.059 b	0.268 \pm 0.030 a	0.441 \pm 0.008 b	0.905 \pm 0.038 c	0.447 \pm 0.017 b	28.4 ***
	TF _{leaf}	1.569 \pm 0.323 b	1.252 \pm 0.192 ab	1.009 \pm 0.081 ab	0.954 \pm 0.027 ab	1.339 \pm 0.074 ab	0.774 \pm 0.021 a	3.3 *
	TF _{stem}	0.118 \pm 0.018 a	0.184 \pm 0.018 a	0.179 \pm 0.021 a	0.354 \pm 0.055 b	0.395 \pm 0.016 b	0.210 \pm 0.017 a	15.2 ***
Ni	TF _{fruit}	0.019 \pm 0.003 ab	0.033 \pm 0.002 bc	0.014 \pm 0.001 a	0.021 \pm 0.001 ab	0.045 \pm 0.008 c	0.043 \pm 0.004 c	10.1 ***
	TF _{leaf}	0.001 \pm 0.000 a	0.001 \pm 0.000 a	0.016 \pm 0.001 b	0.021 \pm 0.002 b	0.040 \pm 0.004 c	0.056 \pm 0.007 d	39.1 ***
	TF _{stem}	0.023 \pm 0.004 b	0.012 \pm 0.001 a	0.003 \pm 0.001 a	0.004 \pm 0.001 a	0.007 \pm 0.000 a	0.007 \pm 0.003 a	12.3 ***
Pb	TF _{fruit}	0.304 \pm 0.122 a	0.258 \pm 0.099 a	0.116 \pm 0.013 a	0.118 \pm 0.019 a	0.151 \pm 0.026 a	0.198 \pm 0.014 a	1.4 ns
	TF _{leaf}	0.027 \pm 0.011 a	0.100 \pm 0.036 b	0.057 \pm 0.015 ab	0.049 \pm 0.003 ab	0.049 \pm 0.003 ab	0.098 \pm 0.004 b	3.1 *
	TF _{stem}	0.092 \pm 0.029 a	0.142 \pm 0.040 a	0.159 \pm 0.010 a	0.154 \pm 0.049 a	0.325 \pm 0.018 b	0.853 \pm 0.077 c	44.2 ***
Zn	TF _{fruit}	0.645 \pm 0.094 a	0.701 \pm 0.201 a	0.602 \pm 0.031 a	0.659 \pm 0.061 a	0.629 \pm 0.016 a	0.617 \pm 0.029 a	0.1 ns
	TF _{leaf}	0.587 \pm 0.063 b	0.513 \pm 0.113 ab	0.301 \pm 0.009 a	0.465 \pm 0.031 ab	0.629 \pm 0.021 b	0.479 \pm 0.015 ab	4.3 **
	TF _{stem}	0.319 \pm 0.018 a	0.409 \pm 0.121 a	0.328 \pm 0.017 a	0.538 \pm 0.119 a	0.554 \pm 0.016 a	0.563 \pm 0.044 a	2.5 ns
F-value _{fruit}		59.9 ***	23.7 ***	17.8 ***	261.7 ***	182.9 ***	539.7 ***	
F-value _{leaf}		37.6 ***	59.7 ***	54.9 ***	164.6 ***	474.6 ***	459.1 ***	
F-value _{stem}		21.0 ***	29.5 ***	69.4 ***	26.6 ***	125.4 ***	99.7 ***	

F-values represent one-way ANOVA. Means in the same row followed by different letters are significantly different at $p < 0.05$, according to Tukey's Honest Significant Difference (HSD) test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant (i.e., $p > 0.05$).

5. Discussion

As OM and mineral nutrients are constantly cycling through all living creatures, SS as a soil fertilizing treatment for boosting crop growth and performance is becoming increasingly important. Indeed, a comprehensive risk assessment including chemical and ecological analyses is a prerequisite for a realistic evaluation of the application of biosolids to agricultural soils [25]. In the current study, precultivation soil and SS compositions were investigated. The pretreatment soil showed poor fertility with low OM, macro- and micro-

nutrients, and EC, with a basic pH (8.7), compared to typical values. The applied SS was rich in OM with a high EC and a relatively low pH value (Table 1). The HM concentrations in the SS used in the current work were compared to the allowed HM values for SS used in agriculture. As per the Statutory Instruments No. 267/2001 ([21]: Co: 10–100 mg/kg; Cr: 50–1750 mg/kg; Cu: 1000–1750 mg/kg; Ni: 300–400 mg/kg; Pb: 750–1200 mg/kg; and Zn: 2500–4000 mg/kg), the levels of all HMs were below the allowable limits (Table 1). The application of SS greatly improved the properties of the postharvest soil, with higher OM content and EC levels, and lower pH values (Table 2).

The amount of OM in the soil influences the N cycle as well as the long-term sustainability of soil fertility. As a result, soil OM content might be an excellent indicator of its fertility [13,26]. Soil pH affects nutrient recycling, mobility, bioavailability, and translocation in plants, as well as the distribution and elimination of hazardous chemicals in the environment [27]. In agricultural soils, optimal nutrient phytoavailability is typically attained in a pH range of 6.0 to 7.0. Alkaline soils are known to restrict crop yield due to low phytoavailability of micronutrients such as Fe, Zn, Mn, and Cu, and thus, are regarded as less fertile soils [6]. The mineralization of SS-supplied organic nitrogen creates protons through nitrification and sulfur-rich compound mineralization, leading to a decrease in soil pH [13]. This might also be ascribed to the organic acid generation as a result of enhanced SS break-down under aerobic soil conditions [28]. The highly acidic soil conditions make the situation even worse and may cause higher mobility of metals from SS [25,29].

The data presented in the current study reveal that increasing the SS doses significantly increases the EC of the soil (Table 2). A soil with a high EC increases the potential forces that retain water in the soil, making it more difficult for plant roots to absorb water [29,30]. The use of SS in agriculture may result in HM accumulation [31]. In the current investigation, the use of SS significantly increased Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn concentrations. Because of the low pH of the SS used, the addition of SS lowered the pH of the amended soil and increased the availability of HMs. In this regard, it has been reported that in a slightly alkaline soil, the highest SS amendment rate results in significant increases in the soil availability of HMs [32].

The results from the current study showed that an amendment rate of 10 g SS per kg soil considerably enhanced most of the biomass and growth parameters of okra plants, when compared to the control. Similarly, SS has been shown to improve the growth and yield of various crop plants, including rice, wheat, maize, barley, lentil, cucumber, spinach, and sunflower [12–14]. The ability of SS to improve soil nutritional quality, aeration, and soil water-holding capacity may be responsible for the increase in okra biomass and morphometric characteristics [33]. The improved plant growth with SS application is further aided by increased OM, macro- and micro-nutrient content and availability, soil porosity, and bulk density [34]. The enhanced plant growth at 10 g/kg SS dosage can be ascribed to the rich micronutrient content of SS, which contains Cu, Fe, Mn, Ni, and Zn [6]. Furthermore, greater SS treatment doses resulted in a limited growth rate and biomass when compared to the significant improvements at the SS rate of 10 g/kg. In this regard, a comparable growth retardation, as evidenced by reductions in the length and dry mass of barley plants, was observed as a dose-dependent response, particularly at higher SS dosages [35,36]. The decrease in growth doses at high HM levels may be attributable, at least in part, to the oxidative stress caused by their interference with normal plant metabolic processes [37]. Furthermore, the slowed development caused by high levels of deposited HMs might be attributed to either an obstruction in cell division and elongation, or a reduction in photosynthetic performance and antioxidant enzyme activity [38]. Thus, SS amendment as a soil fertilizer should be evaluated in light of many factors such as SS quality, dose, field soil characteristics, and crop responses [6].

Soil physiochemical characteristics, SS origin and composition, amendment rate, plant species, plant physiology, climatic factors, and metal chemical form and concentration all have an impact on HM accumulation in plants [39,40]. It has been suggested that increased HM contents in SS-amended soils (Table 2) increase HM supply to plant roots and improve

the ability of HMs to accumulate (Table 3). Similarly, the translocation of HMs to aerial components (stems, leaves, and fruits) was clearly enhanced for nearly all nutritive metals (Cu, Fe, Mn, Ni, and Zn) and substantially higher compared to the control [41]. The lowest BF for Cd and Pb, in the roots of okra plants, was observed at the highest SS amendment rate (50 g/kg). BF was typically greater for Ni with a mean value of 1.788 at an amendment rate of 10 g/kg, followed by Cu, Zn, Cr, Mn, Pb, Fe, Co, and Cd. The TF varied between the HM and soil treatments (Table 5).

6. Conclusions

The current study indicated that soil amendment with SS is an efficient approach for improving soil quality as well as the growth and yield of okra (*Abelmoschus esculentus* (L.) Moench). An SS dosage of 10 g/kg is recommended to safely increase the growth, nutritive value, and yield of okra plants. Future studies may be required to further investigate the long-term effects of SS on soil quality. Such studies may attempt to determine appropriate cultivation rotation cycles for the application of SS, as well as for the cultivated crop species. Furthermore, in future work, the present study may be extended to explore the effects of SS application on soil microflora.

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