Heavy metals levels in Spinacia oleracea and Daucus carota harvested from soil treated with different amounts of sewage sludge in Pretoria, South Africa

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Abstract

This study investigated the effects of four different rates of sewage sludge amendment (100, 200, 300, and 400g) on *Spinacia oleracea (Leafy vegetable)* and *Daucus carota (Root vegetable)* on the growth and the accumulation of heavy metals. The yield of *S. oleracea* and *D. carota* was measured in terms of weight while heavy metals levels at harvest were measured using ICP-MS. The growth and yield at harvest in *S.oleracea* were in following order 300g > 400g > 200g > 100g while in *D.carota* the order for the yield was 400g > 200g > 300g > 100g. Elemental concentrations showed that iron (Fe) had the highest average concentration for both plants and ranged between 68.60 ± 2.82mg/kg – 1503.00 ± 82.31mg/kg. Differences obtained as a result of the amendments were significant for heavy metals such as Fe, Pb, Cd from the different plant parts. Concentrations of Pb and Cr recorded in the leaves of *S. oleracea* were above the recommended limit for human consumption with the exception from leaves of *S. Olereacea* harvested from soil treated with 400g of sewage sludge. There was no particular trend of heavy metals accumulation noted in the experiment except for the values obtained for trace elements in the roots of *D. caraota* where, roots parts harvested either from soil treated with 300g or 400g recorded the highest concentrations of these elements. The bioaccumulation factor showed that the elements were bioaccumulated from the soil.

Introduction

Food security is a crucial priority for sustainable global development and it has become a universal concern because of its link with human health [1]. However, the agricultural lands have deteriorated in fertility as a result of excessive cropping, anthropogenic or natural processes [2]. In turn, these practices together with inefficient soil conservation measures, have resulted in the disturbances of the chemical, physical and biological properties of the soil, loss of the organic content of the soil, major changes in the nutrient fluctuations and declining soil fertility in general [3, 4]. In addition, the buildup of escalating quantities of sludge emanating from the treatment processes of wastewater is becoming an emerging environmental problem which can be solved by the use of sewage sludge as a natural fertilizer in agriculture [2].

Sewage sludge and biosolids made from domestic wastewater treatment plants have been exploited over the years to address the recovery and reclamation of lands that are degraded so that energy crops can be produced [5]. The use of sewage sludge for agricultural purposes assists with a sustainable way of preventing the disposal of sewage sludge and organic wastes into landfills [6, 7]. In the United Kingdom, the sewage sludge application to agricultural lands is considered to be the most convenient alternative for the recycling of this crucial organic fertilizer [8]. Sewage sludge has been found to be more effective in promoting plant growth compared to commercial fertilizers [9], because it contains other beneficial micronutrients and organic matter that can improve soil health and promote plant growth. Its consumption for agricultural purposes contributes to a reduction in the quantities of chemical fertilizers used for the plants growth while at the same time it also deals with the challenges of increasing quantities of sewage sludge and high costs associated with chemical fertilizers [2]. In addition to a reduction on the dependency on chemical fertilizers when sewage sludge is used for agricultural purposes, sewage sludge contributes to the sequestration of carbon in soils which is crucial for the alleviation of climate change [7]. The preservation of carbon in soil also assists with the improvement of the soil structure, the ability of the soil to hold water and the biological activity of the soil (Kumar et al., 2020). The sewage sludge contains a mixture of materials that are of agrochemical value such as phosphorus, nitrogen, nitrates, potassium [5, 9] and small quantities of trace elements which include magnesium, calcium and Sulphur [2].

Even though the usage of sewage sludge for agricultural purposes signifies a potential nutrient source for the growth of plants and a substance that can be used for enhancement of the of the soil physical properties, it contains a number of possible toxic heavy metals [5, 10, 11]. These heavy metals and other organic pollutants in sewage sludge are dangerous and limit the land application of sewage sludge because they are taken up by the plants from the soil and eventually end up in the animals and humans through the consumption of plants as a source of food [2, 12]).

Heavy metals are toxic to plants and human beings because they interfere with normal cellular function [13]. They can damage cell membranes, disrupt metabolism, and inhibit the growth and reproduction of plants. For human beings, heavy metals can cause a range of health problems, including neurological damage, cancer, kidney and liver damage, and developmental delays [14]. Some common heavy metals that are particularly harmful include lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) [13]. These heavy metals can enter the body through contaminated water or food, or through inhalation of contaminated air [14]. Long-term exposure to these metals can lead to serious health problems and can even be fatal. Although heavy metals are essential micronutrients and serve as cofactors for many metabolic enzymes, many metabolic enzymes become toxic to the plants and the humans feeding on the plants when their concentrations in soil are above the recommended limits especially for essential heavy metals while the non-essential heavy metals are toxic even at minute concentrations [15–17].

The surging demand for certain vegetables and the diminishing fertility of the soil has driven the usage of organic fertilizers such as sewage sludge to enhance soil integrity, resulting in higher growth rates and yields. However, the application of sewage sludge also elevates heavy metal concentrations in vegetable tissues and on their surfaces [18]. Prolonged exposure to excessive levels of these heavy metals can have a negative impact on human health, as they interfere with various biochemical and biological processes [17, 19]. The ability of plants to bioaccumulate heavy metals in the soil differs and depend on variety of factors. For instance, some leafy vegetables have been reported to bioaccumulate heavy metals in the soil and store it in the leaf [20] while other plants may store it in their roots or fruits [21]. Therefore, this study is conducted to examine the differences in the heavy metals content of two different crop plants *S. oleracea and D. carota* harvested from soil treated with sewage sludge in Pretoria, South Africa.

Materials and Methods

Study area and sampling

The soil samples used for the study were collected at 0 to 10cm depth using a spade from an area that has not been used previously for agricultural purposes at Sefako Makgatho Health Sciences University situated in Ga-Rankuwa in the north of Pretoria in Gauteng, South Africa, with the coordinates (S 25° 36' 53" & E 28° 1' 25"). The soil was categorized as sandy clay loam. The sewage sludge was first air dried and applied only once before cultivation. The collected soil samples were placed and allowed to fill terrocota pot plants (22cm diameter and 28cm height) and treated with different quantities (100g, 200g, 300g and 400g) of sewage sludge collected from a treatment plant at the university. The sewage sludge was thoroughly mixed with the soil sample in the plots and allowed to mineralize after a period of about 8 weeks before planting of the plants. During the 9th week after the introduction of the plants to the pots, the growth in terms of the leaf length was measured for *S. oleracea*. At about 80 and 90 days respectively for *S. oleracea* and *D. carota* samples were harvested, thoroughly washed with tap water to remove the soil particles in preparation for heavy metals analysis. The yield for both *Spinacia olearacea* and *D. carota* was measured by weighing the harvested vegetables with a weighing scale. The harvested vegetables were divided into leaves, stalks and roots for *S. oleracea* while in *D. carota* they were divided into leaves and roots before they were dried up in an oven at 70°C until a constant weight was determined [22]. The experiment was carried out twice for the purpose of repeatability of the results obtained in this study. The dried samples were then crushed using mortar and pestle, and stored until being digested using the wet digestion method and analysed for heavy metals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Blanks for the plant samples were prepared for the quality assurance. Certified reference materials for plant was used for the purpose of result reliability and assurance.

Wet digestion

One gram (1 g) of the dried residue from each sample of *Spinacia oleracea* and *Daucus carota* samples (leaves, roots and stalks) was weighed into a porcelain crucible and put in a muffle furnace, where the temperature was gradually raised over the course of two hours to between 500 and 550°C and then maintained for six hours. After being digested with concentrated HNO_3, the ashed samples were examined for targeted heavy metals using ICP-MS.

Statistical Analysis

The data was statistically analyzed using SPSS 28.0 for windows. Analysis of Variance (ANOVA) was performed to determine if there were significant differences between the levels of the heavy metals recorded for the different quantities of the sewage sludge and the control for *S. oleracea* and *D. carota* harvested.

Results and Discussion

The results of the *D. carota* yield at harvest, as depicted in Fig. 1, indicated that the highest yield (451.3g) was produced when *D. carota* was cultivated in soil amended with 400g sewage sludge. This was followed by yields of 451.97g, 451.13g, 337.97g and 70.94g for soil amended with 200g, 300g, 100g and control, respectively. As seen in Fig. 2, the highest yield of *S. oleracea* (555.72g) was observed in the soil amended with 300g sewage sludge, with yields of 534.00g, 369.27g, 209.14g and 55.74g for 400g, 200g, 100g and control, respectively. Figure 3 further portrays the growth performance of *S. oleracea*, with the greatest leaf length (30.23 ± 2.483 cm) observed in *S oleracea* cultivated in soil amended with 300g sewage sludge from week 1 and the order for growth remained the same throughout the experiment. The growth of *S. oleracea* from soil amended with 200g sewage sludge showed increase in growth rate until harvest in week 9. The growth and yield of *S.oleracea* and *D.carota* harvested from the soil amended with sewage sludge was significantly better than the control plants. This could be attributed to the elevated levels of essential plant nutrients, including nitrogen (N), phosphorus (P) and potassium (K) found in the sewage sludge, as indicated in Tables 1 and 2. These findings agree with those reported by Aina et al. [23].

Variations in the properties of sludge-amended soils are contingent on the properties of the sludge and soil [24]. Applying excessive amounts of sewage sludge can cause plant toxicity due to the buildup of heavy metals in soils [25] as well as the rising salt content [26]. In the present study, Tables 1–5 below shows the levels of heavy metals in the leaves, roots and stalks of *S. oleracea* and *D. carota* harvested from soil treated with different quantities of sewage sludge. Of all the heavy metals measured, iron (Fe) showed the highest mean concentration of heavy metals in both harvested *S. oleracea* and *D. carota*. The highest mean concentration (555.30 ± 45.30 mg/kg) of Fe in *D. carota* was recorded in the leaves harvested from soil treated with 300g of sewage sludge and the lowest concentration (305.67 ± 64.82 mg/kg) was recorded in the roots of *D. Carota* harvested from soil treated with 200g of sewage sludge (Tables 1 and 3). For *S. oleracea*, the highest concentration (1503. 00 ± 82.31 mg/kg) of Fe was recorded in the leaves harvested from soil treated with 200g of sewage sludge whereas the lowest concentration (68.67 ± 6.97 mg/kg) was recorded in the roots of *S. oleracea* harvested from soil treated with 300g sewage sludge (Tables 2 and 4). The plant harvested from the amended soil showed better growth and yield compared with the plant harvested from untreated soil. The differences obtained in the concentrations of Fe as a result of the treatment in the leaves and roots of all the plants was significant ($P < 0.05$). In plants, Fe plays an important role of chlorophyll synthesis and contributes to the green colour in plants [27]. According to Miano,[28] and Olowoyo et al. [29], *S. oleracea* leaves are a rich source of Fe that protects from osteoporosis while the deficiency of Fe results in anaemia in human beings. Fe is also crucial for the functioning of the female and male reproductive systems [30]. The deficiency of Fe in plants results in chlorosis of immature leaves which can also be transferred to the older leaves as the severity of the shortage of Fe increases [30]. Even though Fe is present in trace quantities, it is required by the body and is essential for biological systems and also for sustaining aerobic life [31]. Fe consumption in excess has been associated with accidental death in young children aged under six years [31]. In the current study, the levels of Fe in both *S. oleracea* and *D. carota* were above WHO permissible limit (450 mg/kg) [32]. The results of the current study are in agreement with the results in Swain et al. [7] and Kumar et al. [33] where the leaves of *S. oleracea* harvested from soil treated with sewage sludge showed the highest uptake of heavy metals like Fe and other micronutrients as a result of the increase in the application quantities of sewage sludge.

The concentration of manganese (Mn) in the leaves and roots of harvested *D. carota* ranged from 30.00 ± 1.95 mg/kg to169.80 ± 0.50 mg/kg (Tables 1 and 3). The leaves of *D.carota* harvested from the amended soil with 300g showed the highest concentration (226.10 ± 2.17 mg/kg) (Table 1) while the least value of Mn was recorded in the roots harvested from the soil amended with 200g (30.00 ± 1.95 mg/kg) (Table 3). With *S.oleracea*, the leaves harvested from 100g treated soil recorded the highest concentration of 90.57 ± 3.36 (Table 2) while the lowest concentration of Mn for *S. oleracea* (21.70 ± 0.56 mg/kg) was recorded in the roots of 300g amended soil. The differences obtained in the concentrations of Mn in the leaves and roots of the plants were all significant ($p < 0.05$). All the plants of *D. carota* and *S. oleracea* showed lower levels of Mn than the permissible limit for Mn stipulated by WHO (500 mg/kg)[34]. Mn is a crucial trace element for plant growth and is a component of photosynthesis (Alejandro et al., 2020) However, an excess of Mn in plants can interfere with their ability to absorb and use other minerals, affect their energy metabolism, lower their photosynthetic rates, and cause oxidative stress (Fernando & Lynch, 2015; Tavanti et al., 2020).

The concentration of zinc (Zn) in the roots and leaves of *S. oleracea* ranged from 62.23 ± 1.60 mg/kg – 120.60 ± 0.69 mg/kg. Generally, with Zn, in *S. oleracea*, the concentrations obtained for the leaves were higher than those recorded in the roots. Zn concentration harvested from plants treated with 400g of amended sewage sludge had the highest concentrations for Zn. The differences obtained in the concentrations of Zn as a result of the amendment used were all significant ($p < 0.05$). In the present study, all the samples recorded higher Zn concentrations compared to the concentration of 0.02 mg/kg as recommended by WHO [34]. Zn is the transition metal that is present in the greatest abundance in living things [35], because of its structural, catalytic, and activating properties. Zn is essential for plant development, reproduction, and signaling [36]. Zn is a necessary element for plants, but too much of it can seriously harm them [37]. Zn is one of the main pollutants that are discharged into the environment as a result of industrial activity, mining, smelting, and sewage sludge, as well as the ongoing use of Zn fertilizers [38].

Copper (Cu) concentration in the leaves were generally higher than the concentrations obtained for the roots with the exception of roots harvested from soil treated with 400g of the sewage sludge. The concentrations of Cu in *S. oleracea* varied between 5.68 ± 0.42 mg/kg – 27.57 ± 3.64 mg/kg. The highest value for Cu from *S. oleracea* was recorded from the roots of the soil amended with 400 g of the sewage sludge. The differences obtained in the values recorded for Cu from the different treatments were not significant either in the leaves or the roots (Tables 2 and 4). For *D. carota*, the range of the Cu concentration was from 5.76 ± 0.13 mg/kg to 11.80 ± 0.46 mg/kg. Unlike the *S. oleracea*, the concentrations obtained in the roots were more than those recorded for the leaves and the differences obtained in the concentrations of Cu as a result of the treatment either from the values recorded in the roots or leaves were also significant ($p < 0.05$). An increase in the concentration of sewage sludge increases the concentration of Cu both for the leaves and roots of *D. carota* (Tables 1 and 3). Cu is essential for the proper growth and development of plants, as it is involved in numerous morphological, physiological, and biochemical roles [39]. However, an excessive amount of Cu in the soil can have a detrimental effect on the production and growth of plants [40]. To counter this, plants are able to tolerate Cu toxicity by immersing the excess amounts into parts which can be harvested, such as leaves, cell walls, and the vacuolar membrane of the root cortex (Zandi et al., 2020).

The concentrations of Cr ranged from 0.60 ± 0.04 mg/kg – 3.80 ± 0.06 mg/kg in *D.carota* (Tables 1 and 3) while it ranged from 1.37 ± 0.08 mg/kg – 4.57 ± 0.14 mg/kg in *S. oleracea.* Values obtained in the leaves of both plants were more than values obtained in the roots of both plants respectively (Tables 1–4). With *S. oleracea* the differences obtained for Cr was only significant in plants harvested from soil treated with 100g of the sewage sludge when compared with other treatments ($p < 0.05$). The WHO recommended limit for Cr was set to be 1.30 mg/kg. In the present study, the highest concentration of Cr (4.57 ± 0.14 mg/kg) was recorded in the roots of *S.oleracea* harvested from the soils treated with 200g of sewage sludge whereas the least concentration (0.60 ± 0.04 mg/kg) was found in the roots of carrot harvested from the soils treated with 100g of sewage sludge. It has been shown that there is no known biological function of Cr in plant physiology [41]. However, it is generally accepted that excessive levels of Cr in plant tissues can cause a range of morphological, physiological and biochemical changes in plants [42, 43]. This is because heavy metal toxicity is usually the result of complicated interactions between the heavy metals, genetic processes, signal transduction pathways, and cellular macromolecules [44].

Nickel (Ni) concentration in the leaves and roots of *D.carota* varied between 1.27 ± 0.31 mg/kg – 4.61 ± 0.48 mg/kg. The roots of *D.carota* harvested from 300g sewage sludge amended soil showed the highest concentration (4.61 ± 0.48 mg/kg) while the least concentration (1.27 ± 0.31 mg/kg) was found in the roots of the control soil. For *S.oleracea*, the highest concentration (19.17 ± 0.37mg/kg) of Ni was found in the roots of plants harvested from the 400g sewage sludge amended soils and the least concentration (2.34 ± 0.20 mg/kg) was found in the leaves of the soils treated with 200g of sewage sludge. Comparing this study to the WHO accepted limit of (10.00 mg/kg), the study showed that the plants were within the acceptable limit. Nickel (Ni) is an essential trace element for plant growth, without which the plant cannot complete its life cycle [45]. However, excessive amounts of this heavy metal can have a devastating effect on the plant's physiology, disturbing processes such as photosynthesis, root growth, enzyme activity, and mineral nutrition [46]. The differences obtained for the values of Ni from the leaves of *S.oleracea* was significant with plants harvested from 400g of soil ($p < 0.05$). However, with carrot, the differences obtained as a result of the treatment were not significant ($p > 0.05$).

The concentration of Cd in the leaves and roots of *D.carota* ranged from 0.23 ± 0.002 mg/kg– 0.68 ± 0.03 mg/kg (Table 1). The highest concentration (0.68 ± 003 mg/kg) of Cd was recorded from the leaves harvested from the soils amended with 300g of sewage sludge while the least concentration (0.23 ± 0.00 mg/kg) was recorded from the roots and leaves harvested from soils treated with of 200g and 300g of sewage sludge amended soils respectively. The concentration of Cd in *S.oleracea* harvested from soil amended with 100g of sewage sludge showed the highest concentration of 0.36 ± 0.01 mg/kg (Table 2) whereas the lowest concentration (0.13 ± 0.01 mg/kg) was recorded in the roots of *S. oleracea* harvested from the 100g soils (Table 4). Differences obtained in the concentrations of Cd in both plants either in the roots or leaves were not significant ($p > 0.05$). The combustion of zinc mines, non-ferrous smelting, phosphate fertilizers, fossil fuels and sewage sludge application are the main sources that are linked to the accumulation of Cd in soil and plants [47]. Exposure to high levels of Cd can cause a decrease in growth rate, weak mineralization of bones, hypertension, anemia, and damage to the renal tubules [47], while chronic exposure to Cd at lower levels can lead to problems in the skeletal system, kidneys, and respiratory system [44]. The values recorded for both *S. oleracea* and *D. carota* in the current study in some of the plant parts were slightly above the [32] permissible limit 0.3 mg/kg.

The concentration of Pb in the leaves and roots of *D.carota* ranged from 0.25 ± 0.00 mg/kg– 1.05 ± 0.03 mg/kg. The highest concentration of Pb with a value of 1.05 ± 0.03 mg/kg was found in the leaves of soil amended with 300g of sewage sludge and the lowest concentration value of 0.025 ± 0.001 mg/kg in the roots of the plant harvested from soils treated with of 200g of sewage sludge (Tables 1 and 3). For *S.oleracea* the highest concentration of 1.47 ± 0.00 mg/kg was recorded in the roots of soils treated 200g sewage sludge compared to the lowest value of 0.31 ± 0.00 mg/kg in the leaves of the vegetable harvested from the soils treated with 300g of sewage sludge. All the values obtained for Pb in the roots of *S. oleracea* were above the recommended limit by WHO. Excess Pb may cause acute and chronic poisoning. It also has an effect on the functioning of the kidney and liver [48].

Table 1

Heavy metals (mg/kg) in the leaves of *Daucus carota* harvested from soil treated with different amounts of sewage sludge.

Plant	Sewage sludge (g)	Heavy Metals (mg/kg)								
		Fe*	Mn*	Zn*	Cu**	Cr *	Ni**	Sr	Cd**	Pb**
Daucus carota	Control	306.00 ± 11.14	77.97 ± 2.97	29.23 ± 1.95	7.14 ± 0.24	1.28 ± 0.67	1.27 ± 0.31	31.84 ± 0.28	0.23 ± 0.02	0.51 ± 0.03
	100	535.70 ± 36.25	137.50 ± 6.50	53.70 ± 3.97	7.51 ± 0.51	3.59 ± 0.14	2.14 ± 0.74	46.53 ± 0.71	0.25 ± 0.01	0.51 ± 0.01
	200	364.30 ± 14.01	68.07 ± 5.25	62.33 ± 1.68	8.43 ± 0.55	2.73 ± 0.04	1.79 ± 0.36	44.95 ± .0.48	0.27 ± 0.02	0.55 ± 0.02
	300	555.30 ± 45.36	226.10 ± 2.17	106.50 ± 2.74	10.53 ± 0.50	3.53 ± 0.10	3.76 ± 0.36	55.47 ± 0.46	0.68 ± 0.03	1.05 ± 0.03
	400	434.00 ± 7.94	169.80 ± 0.50	103.90 ± 2.16	9.43 ± 0.48	3.80 ± 0.06	3.49 ± 0.15	47.34 ± 0.27	0.43 ± 0.02	0.36 ± 0.00

*: Significant differences in the values obtained for this element at different rates of sewage sludge application ($P < 0.05$).

**: No significant difference in the values obtained for this element at different rates of sewage sludge application ($P > 0.05$).

Table 2
Heavy metals (mg/kg) in the leaves of *Spinacia oleracea* harvested from soil treated with different amounts of sewage sludge.

Plant	Sewage sludge (g)	Heavy Metals (mg/kg)								
		Fe*	Mn**	Zn*	Cu*	Cr**	Ni **	Sr**	Cd**	Pb**
Spinacia oleracea	Control	1294.00 ± 72.51	86.87 ± 5.19	118.60 ± 4.84	10.90 ± 0.98	2.23 ± 0.07	3.67 ± 0.56	39.58 ± 0.61	0.17 ± 0.01	0.55 ± 0.01
	100	1431.00 ± 89.06	90.57 ± 3.36	78.47 ± 0.85	8.97 ± 0.47	1.64 ± 0.03	2.97 ± 0.53	33.46 ± 0.42	0.36 ± 0.01	0.37 ± 0.02
	200	1503.00 ± 82.31	82.70 ± 2.25	109.10 ± 3.82	14.90 ± 0.36	1.37 ± 0.08	2.34 ± 0.20	35.48 ± 0.35	0.19 ± 0.02	0.35 ± 0.00
	300	1252.00 ± 56.16	79.70 ± 1.73	111.3 ± 1.81	16.07 ± 0.38	1.86 ± 0.18	4.94 ± 0.05	23.15 ± 0.32	0.17 ± 0.01	0.31 ± 0.00
	400	616.00 ± 20.00	89.03 ± 0.64	120.60 ± 0.69	14.03 ± 0.65	1.58 ± 0.07	6.71 ± 0.33	25.05 ± 0.19	0.30 ± 0.02	0.39 ± 0.02

*: Significant differences in the values obtained for this element at different rates of sewage sludge application ($P < 0.05$).

**: No significant difference in the values obtained for this element at different rates of sewage sludge application ($P > 0.05$).

Table 3
Heavy metals (mg/kg) in the roots of *Daucus carota* harvested from soil treated with different amounts of sewage sludge.

Plant	Sewage sludge (g)	Heavy Metals (mg/kg)								
		Fe*	Mn*	Zn*	Cu*	Cr*	Ni*	Sr**	Cd**	Pb**
Daucus carota	Control	505.00 ± 24.98	50.20 ± 2.62	31.53 ± 2.63	5.76 ± 0.13	1.39 ± 0.13	2.32 ± 0.42	11.83 ± 0.11	0.28 ± 0.01	0.51 ± 0.01
	100	368.67 ± 36.74	55.57 ± 3.76	42.40 ± 2.63	8.08 ± 1.06	0.60 ± 0.04	1.38 ± 0.18	14.09 ± 0.13	0.33 ± 0.02	0.29 ± 0.01
	200	305.67 ± 64.82	30.00 ± 1.95	51.00 ± 3.54	8.96 ± 0.12	1.08 ± 0.08	3.19 ± 0.20	13.16 ± 0.05	0.23 ± 0.02	0.25 ± 0.01
	300	417.00 ± 7.81	48.23 ± 3.10	57.10 ± 5.33	10.37 ± 0.40	2.25 ± 0.05	4.61 ± 0.48	14.67 ± 0.05	0.29 ± 0.00	0.59 ± 0.02
	400	513.00 ± 22.72	57.30 ± 3.83	67.67 ± 1.27	11.80 ± 0.46	1.67 ± 0.04	4.13 ± 0.11	13.88 ± 0.16	0.30 ± 0.01	0.25 ± 0.01

*: Significant differences in the values obtained for this element at different rates of sewage sludge application ($P < 0.05$).

**: No significant difference in the values obtained for this element at different rates of sewage sludge application ($P > 0.05$).

Table 4
Heavy metals (mg/kg) in the roots of *Spinacia oleracea* harvested from soil treated with different amounts of sewage sludge.

Plant	Sewage sludge (g)	Heavy Metals (mg/kg)								
		Fe*	Mn*	Zn*	Cu*	Cr**	Ni*	Sr**	Cd**	Pb*
Spinacia oleracea	Control	518.00 ± 40.85	31.20 ± 0.36	63.33 ± 0.68	6.04 ± 0.3	3.01 ± 0.10	3.14 ± 0.20	27.37 ± 0.30	0.29 ± 0.03	1.10 ± 0.02
	100	276.00 ± 9.85	27.40 ± 1.95	72.1 ± 4.23	6.11 ± 0.33	2.56 ± 0.07	6.74 ± 0.26	29.35 ± 0.32	0.13 ± 0.01	1.29 ± 0.24
	200	106.70 ± 2.08	21.90 ± 0.26	62.23 ± 1.6	5.68 ± 0.42	4.57 ± 0.14	6.42 ± 0.08	34.08 ± 0.35	0.18 ± 0.01	1.47 ± 0.00
	300	68.60 ± 2.82	21.70 ± 0.56	74.47 ± 2.61	6.45 ± 0.36	4.56 ± 0.01	5.85 ± 0.27	29.99 ± 0.27	0.18 ± 0.02	1.35 ± 0.01
	400	96.67 ± 6.97	35.50 ± 0.36	90.47 ± 1.12	27.57 ± 3.64	3.25 ± 0.13	19.17 ± 0.37	27.73 ± 0.08	0.24 ± 0.01	0.78 ± 0.00

*: Significant differences in the values obtained for this element at different rates of sewage sludge application ($P < 0.05$).

**: No significant difference in the values obtained for this element at different rates of sewage sludge application ($P > 0.05$).

Table 5
Transfer factors in the leaves of *S. oleracea* and *D. carota* harvested from soil treated with different quantities of sewage sludge.

Plant		**Fe**	**Mn**	**Zn**	**Cu**	**Cr**	**Ni**	**Sr**	**Cd**	**Pb**
S. oleracea	Control	0.61	1.55	0.93	1.24	0.92	0.55	2.69	0.82	1.00
	100	1.45	2.47	1.27	0.93	5.98	1.55	3.30	0.76	1.76
	200	1.19	2.27	1.22	0.94	2.53	0.56	3.42	1.17	2.20
	300	1.33	4.69	1.87	1.02	1.57	0.82	3.78	2.34	1.78
	400	0.85	2.96	1.54	0.80	2.28	0.85	3.41	1.43	1.44
D. carota	Control	2.50	2.78	1.87	1.80	0.74	1.17	1.45	0.59	0.50
	100	5.18	3.31	1.09	1.47	0.64	0.44	1.14	2.77	0.29
	200	14.09	3.78	1.75	2.62	0.30	0.36	1.04	1.06	0.24
	300	18.25	3.67	1.49	2.49	0.41	0.84	0.77	0.94	0.23
	400	6.37	2.51	1.33	0.51	0.49	0.35	0.90	1.25	0.50

Table 5 showed the transfer factor which is an indication of bioaccumulation potentials of the plants. From Table 6, with the exception of some values reported for Cr, most of the heavy metals determined in the study were bioaccumulated from the soil ((TF > 1). In the present study, from Table 1, 2 and 3, an increase in the concentrations of the sewage sludge especially when increased between 300g and 400g might have resulted in an increase in the concentrations of heavy metals in the plant tissues. This is similar to the findings of Koutroubas et al. [49] where an increase in soil amendments led to an increase in the concentrations of the heavy metals in the plant tissue. Application of sewage sludge on agricultural land can boost crop output by enhancing soil fertility and physical characteristics [49]. Many factors have been attributed to the cause of uptake of heavy metals by the planted plants such as soil organic matter, and the soil pH [29]. The pH less than 6 has been reported to enhance leaching of heavy metals, making them available for plants uptake [50].

Liu et al. (2013) and Bi et al. (2018) reported high metal uptake in vegetables because of higher levels in the background soil as a result of industrial activities. Other studies have reported similar findings of higher heavy metal uptake because of higher levels in sewage sludge [50–52], which are in accordance with the present result. Contrary to these findings, Ok et al. [53] recorded lower metal uptake as a result of higher organic matter content and soil conductivity which immobilized heavy metals. Demirezen and Ahmet,[54] and Sharma et al. [55] have attributed the uptake of heavy metals to the wastewater used for irrigation. One of the main ways that heavy metals from sewage sludge-amended soils enter the food chain is through plant uptake. The plants in the food chain might take in enough heavy metals to endanger consumers' health. The increase in yield brought about by the application of sewage sludge in the current study is consistent with previous studies showing that sewage sludge has the potential to increase the crop yield [56, 57]. Sewage sludge's high nutrient content, which favorably affects soil structure, improves soil aeration, and fosters the activities of living organisms within the soil, is responsible for the increase in yield as a result of its use[58] .

Transportation of heavy metals in soils are facilitated by different factors including the concentration of heavy metals in the soil, the nature of organic matter and the acidic nature of the soil used for planting. In this stud, the soil pH were slightly acidic which might have favored the transportation of the heavy metals in the soil. The characterization and profiling of the soil also showed that the soil was sandy clay loam soil which could have also facilitated the movement of the heavy metals in the soil. In addition, from the current study, looking at the control sites, the concentrations of heavy metals in the harvested plants differed from those that were harvested either from pots added with 300g or 40g of sewage sludge used in the study. This might have been the impact of raw sewage sludge used in the study. The report of Buta et al. [59] showed that raw sewage sludge has the capacity of introducing heavy metals in the soil owing to their sources. Generally, from the current study, the two crop plants have a great bioaccumulation potential hence the current levels of heavy metals in their tissues.

Conclusion

This study revealed that *D. carota* and *S. oleracea* have the potentials to bioaccumulate heavy metals from soil as noted with the transfer factor. The use of sewage sludge in this study might have increased the plant productivity but with an increase in heavy metals bioaccumulation. Sewage sludge used as an amendment to the soil may be a good option for *S. oleracea* and *D. carota* due to the sewage sludge's high soil fertilizer value and the plant's favorable response in terms of growth and yield. However, lower sewage sludge amendment rates might be more beneficial to preserve soil health, maximize plant yields, prevent food chain contamination, and reduce human health risks. It is recommended that farmers and the general public should both be actively educated about the different kinds of organic materials such as sewage sludge that can be used in agriculture as well as the best times, places, and methods for doing so. It is necessary to improve and refocus on the removal of persistent organic pollutants such as heavy metals from the soil, water, and wastewater that are used for agricultural purposes.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

L.L. Mugivhisa: Visualization, Writing original draft, Methodology, Editing, Supervision; R. Thabo: Data collection, Editing; O.M. Oladeji: Writing, Review& Editing; J.O. Olowoyo: Conceptualization, Methodology, Editing and Supervision

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Figures

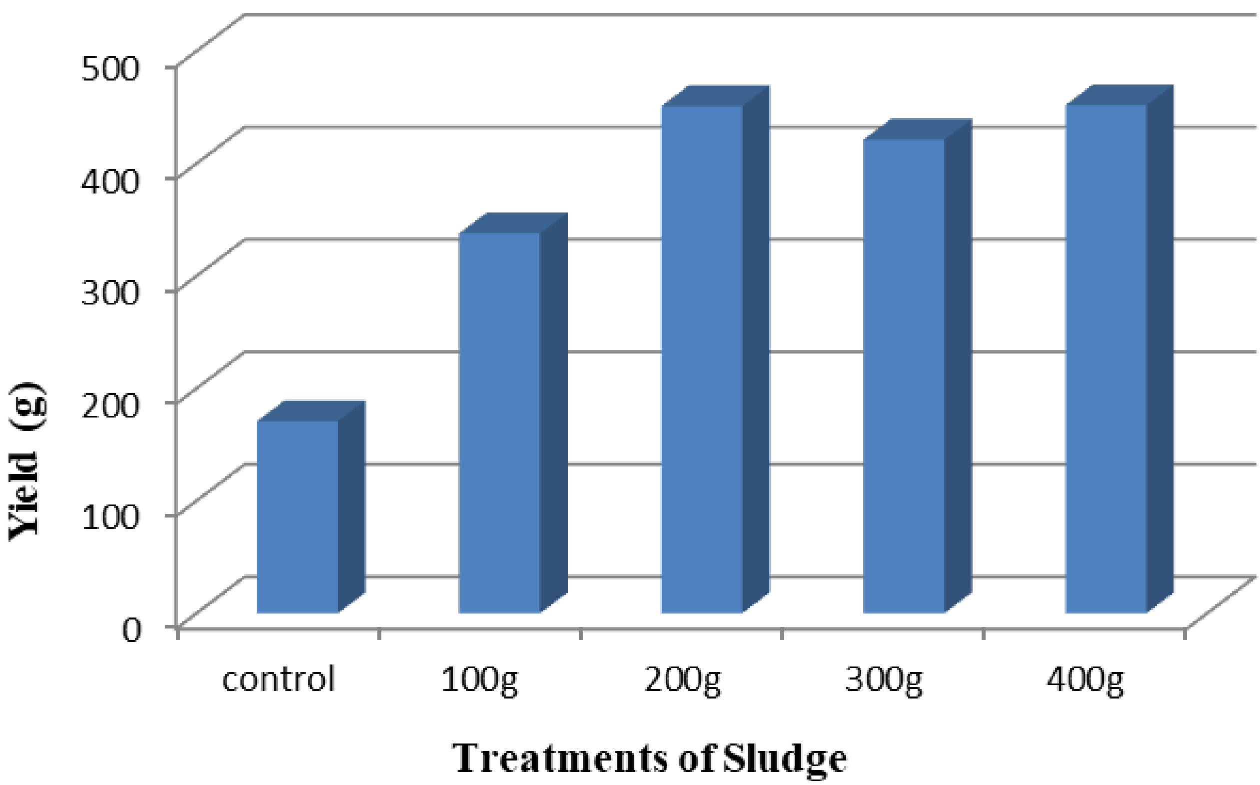

Figure 1

Yield of *D.carota*harvested from soil treated with different amounts of sludge.

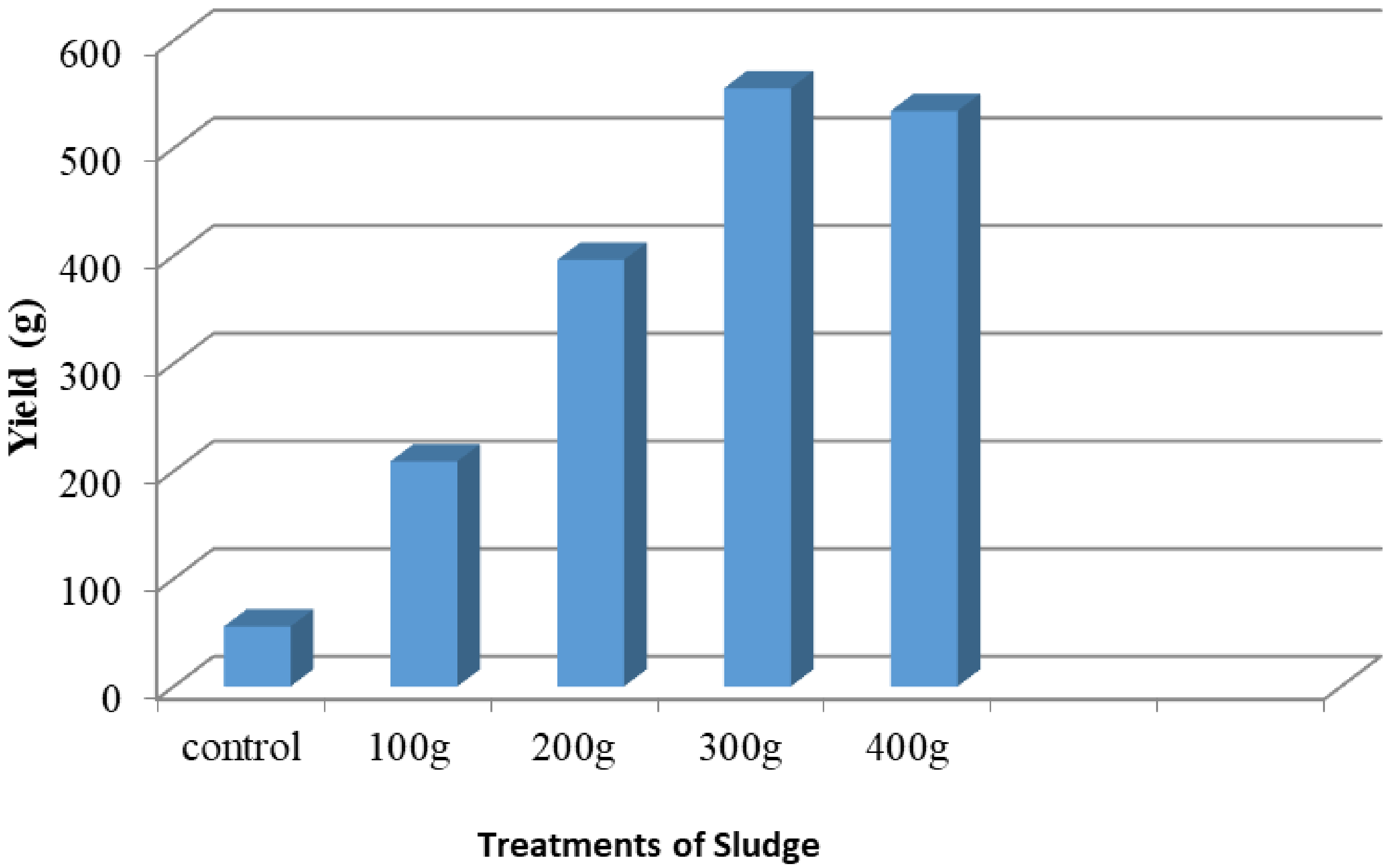

Figure 2

Yield of *S.oleracea*harvested from soil treated with different amounts of sludge.

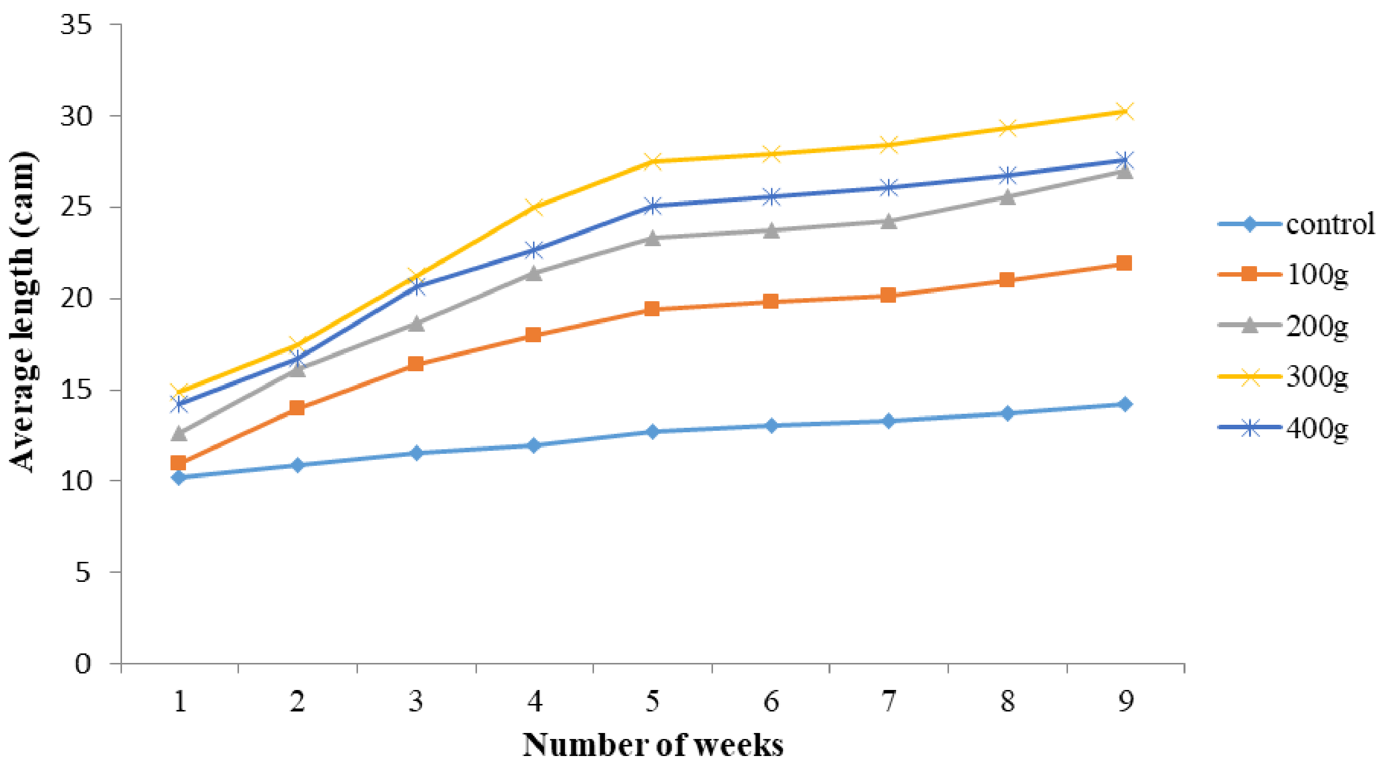

Figure 3

Average weight of *S. oleracea* plants fertilized with different amounts of sewage sludge.