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EFFECT OF SEWAGE SLUDGE AND BARNYARD MANURE ON GROWTH, NUTRIENT UPTAKE, AND HEAVY METAL ACCUMULATION IN LETTUCE

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Abstract: This study investigated the effects of barnyard manure and different doses of sewage sludge on yield performance, micronutrient uptake, and heavy metal accumulation in lettuce (*Lactuca sativa* L.) under pot conditions. The treatments included: (1) Control, (2) Barnyard manure (BYM, 5 ton ha^{-1}), (3) Sewage sludge at 1.25 ton ha^{-1} (SS1), (4) 2.5 ton ha^{-1} (SS2), (5) 5.0 ton ha^{-1} (SS3), and (6) 7.5 ton ha^{-1} (SS4). According to the Soil Water Protection Regulation, the heavy metal concentrations in the sewage sludge remain below the permissible limit for applying sewage sludge to the soil. Results demonstrated that the highest sludge dose (7.5 ton ha^{-1}) significantly enhanced fresh biomass of both shoots and roots compared to BYM. Moreover, sewage sludge treatments improved the concentrations of Fe, Zn, Cu, and Mn in both roots and leaves. However, they also led to elevated accumulation of Cd, Pb, and Cr in root tissues. The observed increase in plant growth is likely attributed to the high nitrogen content and low C/N ratio of the sewage sludge, which accelerated organic matter mineralization and enhanced nutrient availability through increased microbial activity. Despite the rise in heavy metal uptake, their concentration per unit of biomass was reduced due to the dilution effect. Overall, the application of sewage sludge to soils with low organic matter significantly improved plant development and nutrient acquisition, albeit with a potential risk of heavy metal accumulation.

Keywords: Lettuce *(Lactuca sativa L.),* Biomass production, Micronutrient uptake, Sewage sludge application, Barnyard manure

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1. Introduction

The progressive decline in the organic matter content of agricultural soils has detrimental effects on both the physical and chemical properties of the soil. Organic fertilizers, therefore, play a multifaceted role in mitigating these effects (Canal et al., 2023; Canal et al., 2022a). Due to increasing environmental concerns and rising market prices of chemical fertilizers, there is a growing necessity to reduce the dependence on industrial synthetic fertilizers. Simultaneously, the decreased availability of traditional farmyard manure has elevated the significance of alternative organic fertilization sources. Agriculture, as a system in which nutrients are continually removed through harvest and not naturally replenished at the same rate, inherently requires external nutrient inputs to maintain soil fertility and productivity (Gowing et.al., 2020). Sustainable agricultural practices are increasingly focusing on nutrient recycling and organic amendments to close this input-output gap (Yılmaz and Yılmaz, 2025). Among these alternatives, the agricultural application of sewage sludge has gained attention due to its potential environmental and economic benefits, particularly through the reuse of essential nutrients and organic matter contained in this by-product. However, the use of sewage sludge is not without risk. Its application may lead to the accumulation of hazardous components such as heavy metals, salts, and pathogens in the soil and crops, thereby introducing these contaminants into the food chain (Canal et al., 2022b). Heavy metals are of particular concern, as their concentration in sewage sludge depends heavily on the origin of the waste and the specific treatment processes employed. Furthermore, the environmental impact of heavy metals is influenced by the characteristics of the soil receiving the sludge application. Organic compounds introduced to the soil via sludge have been shown to improve various soil properties, including structure, water retention capacity, cation exchange capacity, and aggregate stability (Singh ve Agrawal, 2008; Van den Berg et. al., 2020). In addition to organic components, digested primary sludge contains approximately 30–60% inorganic mineral fractions that can interact with heavy metals. The phytoavailability of these metals, particularly those adsorbed by inorganic phases, is not expected to increase over time and may actually decrease as surfaces become occluded and less reactive (Metcalf et al., 1991). Numerous studies have examined the agricultural potential and associated risks of sewage sludge. Some studies have reported positive effects on crop growth and yield (Bozkurt et. al., 2020; Ragonezi et al., 2020) and other beneficial effects on the physical, chemical, and/or biological properties of the soil (Dhanker et.al., 2021). Although most of the reported risks are associated with soil and/or plant contamination with heavy metals, most studies have noted that the levels of contamination remain below the threshold values set by the relevant national legislation. The lettuce plant can be grown in all regions of Turkey because its growing period is short. In regions where winters are not harsh, it is grown throughout the year. Lettuce quickly develops in soil rich in organic matter and reaches harvest maturity in a short time (Vural et. al., 2000). This study aims to investigate the effects of sewage sludge derived from wastewater treatment plants on the growth of lettuce (*Lactuca sativa L.).* In addition to assessing shoot and root micronutrient concentrations, the research also examines the accumulation of heavy metals in the roots. Lettuce was chosen as the test plant due to its sensitivity to nitrogen availability and its effectiveness as a bioindicator of heavy metal presence.

2. Materials and Methods

2.1. Material

In the pot expreriment, experiment soil, sewage sludge, and barn yard manure carecteristics are given in Table 1. This soil is characterized by medium calcareous, alkaline pH, low organic matter, and low nitrogen (N). The DTPA-Cd, Cr, Pb levels in the soil are low (Alpaslan et al. 1998).

Table1. Properties experiment soil, sewage sludge and barn yard manure

Carecteristics	Soil	Sewage sludge	Barnyard manure
pH (1:2.5 water)	8.56	6.11	
Organic matter, %	1.55	47.3	55.0
C/N	21.92	9.73	21.3
Total N, %	0.041	2.82	1.50
available P,%	0.034	0.76	0.34
K, mg kg^{-1}	245	288	552
Ca, mg kg^{-1}	2980	695	2152
Mg,, mg kg^{-1}	160	222	194
Na, mg kg^{-1}	982	1217	1454
DTPA Extractable cations (mg kg^{-1})			
Fe	4.0	10.5	4.0
Mn	13.3	19.8	17.5
Zn	0.69	18.0	1.33
Cu	0.97	2.60	0.97
Cd	0,005	0,35	0.009
Pb	0.36	10.7	0.30
Cr	0.004	0.67	-

2.2. Pot Experiment

In this study, a pot experiment was conducted under greenhouse conditions. Lettuce *(Lactuca sativa* L.*)* seedlings initially grown in seedling trays (violas) were transplanted into 1 kg capacity pots filled with field soil. Barnyard manure and sewage sludge were applied to the soil surface at a depth of 0–20 cm according to the designated treatment doses. The experiment was arranged in a randomized plot design with four replications. Each replication consisted of three pots, resulting in 12 pots per treatment and a total of 72 pots across six treatments. In the control treatment, nitrogen was applied at a rate of 300 mg N kg^{-1} as ammonium sulfate, phosphorus at 80 mg P kg^{-1} as triple superphosphate, and potassium at 80 mg K kg^{-1} as potassium sulfate.

The six treatments were as follows:

1. Control
2. Barnyard manure (BYM, 5 ton da^{-1})
3. Sewage sludge 1 (SS1, 1.25 ton da^{-1})
4. Sewage sludge 2 (SS2, 2.5 ton da^{-1})
5. Sewage sludge 3 (SS3, 5.0 ton da^{-1})
6. Sewage sludge 4 (SS4, 7.5 ton da^{-1})

2.3. Analysis of the Experiment Soil, Sewage Sludge, And Barn Yard Manure

In the soil of the experiment area, texture was analyzed using the hydrometer method (Bouyoucos, 1965). Soil pH was determined through potentiometric analysis of a 1:2.5 soil-to-water suspension (Jackson, 1958). Organic matter content was quantified colorimetrically using the modified Walkley-Black method (Walkley, 1947). Lime content was calculated using Scheiblercalcimeter (Hızalan and Ünal, 1966). A sodium bicarbonate extract and the molybdenum blue technique were used to measure the amount of available phosphorus (P) (Olsen et al. 1954). Total N was analyzed by the Kjeldahl method (Kacar,1994). Using a solution (pH 7.3) containing 0.005 M diethylenetriamine pentaacetic acid (DTPA), Fe, Mn, Zn, and Cu were extracted (Lindasy and Norvell, 1978). The concentrations of these elements in the extracts were determined by atomic absorption spectrophotometer. Through digestion process, the overall amounts of heavy metals in the soil, sludge, and manure were determined. (Khan and Frankland, 1983).

2.4. Analysis of Heavy Metals, Macro, And Micronutrients In Plant Samples

Harvested plant samples were dried in an oven at 70 °C until reaching a constant weight and then ground using a laboratory mill. Nitrogen content was determined using the Kjeldahl method. Phosphorus was analyzed according to the yellow colorimetric method (Kacar, 1994), following wet digestion of the plant material. Concentrations of Cd, Pb, Cr, Fe, Zn, Cu, and Mn were measured using an atomic absorption spectrophotometer after the air-dried samples were digested with a mixture of HNO_3 and $HClO_4$ acids (İbrikçi et al., 1994).

2.5. Statistical Analysis

The experiment was designed as randomized plots with 4 replications per treatment. The variations among the treatments were analyzed using a one-way analysis of variance (ANOVA), with post-hoc comparisons conducted via Duncan's multiple range test to identify significant differences between the treatment groups. To ensure robust and reliable results, statistical analysis was conducted using SPSS software version 13.0. (Hilbe 2005). Correlation analysis was performed by eliminating endogenous correlations among the studied traits, ensuring that correlations were calculated exclusively between characteristics. Pearson's coefficient was employed for the correlation analyses, and data visualization was carried out using the 'corrplot' package (Wei et al., 2017).

3. Results and Discussion

3.1. Plant Growth of Lettuce

Significant increases in the fresh weight of lettuce were observed in the SS2, SS3, and SS4 treatments compared to the control (Figure 1). Specifically, fresh weight increased by 7.6 %, 8.1 %, and 43.4 % with SS2, SS3, and SS4 applications, respectively. In contrast, barnyard manure application led to a 27.3 % decrease in fresh weight compared to the control. The highest increase was recorded with the SS4 treatment. In terms of root fresh weight, SS2 and SS3 treatments showed similar results to the control, whereas SS4 led to a 92.7% increase in root dry weight (Figure 2). On the other hand, barnyard manure caused a 48.7% reduction in root fresh weight compared to the control. As shown in Figure 3, the N, P, and K content in the SS4 treatment was comparable to the control. Among the tested sewage sludge doses, the application of 7.5 ton da^{-1} significantly improved nitrogen, phosphorus, and potassium accumulation as well as root dry matter yield. Conversely, barnyard manure appeared insufficient to support optimal plant growth at the given dose. The carbon-to-nitrogen (C/N) ratio of organic amendments plays a critical role in mineralization (Dikici et al., 2017; Myrold and Bottomley, 2008). Sewage sludge used in this study had a relatively low C/N ratio (9.73) and a high total nitrogen content (2.83%), both of which favor mineralization and nutrient availability (Weil et al., 2017). Enhanced mineralization not only supports nitrogen uptake but also stimulates microbial activity, thereby improving soil fertility and plant development (Bhatia and Sindhu, 2024). In this context, the low C/N ratio and high nitrogen content of the sewage sludge contributed positively to the nitrogen requirements of lettuce, resulting in higher fresh and root biomass compared to barnyard manure. These findings are consistent with those reported by Arrobas et al. (2024), who found that sewage sludge with a low C/N ratio enhanced N and P uptake and improved dry matter yield in lettuce.

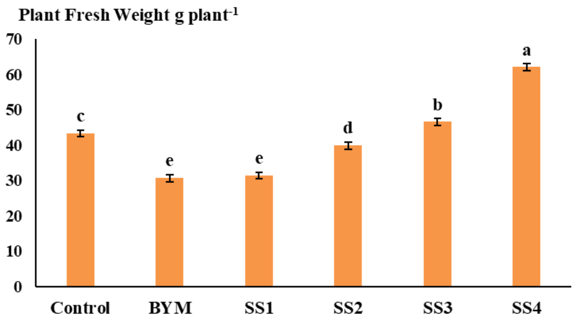

Figure1. Effect of different levels of applied sewage sludge on lettuce fresh weight. Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 5 ton/da, SS1: 1.25 ton da-1,SS2: 2.5 ton da-1, SS3: 5.0 ton da-1,SS4: 7.5 ton da-1.

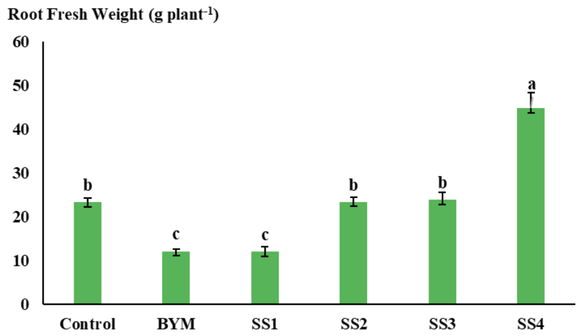

Figure2 Effect of different levels of applied sewage sludge on lettuce root fresh weight. Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 0.5 ton/da,SS_1: 1.25 ton da^{-1},SS_2: 2.5 ton da^{-1}, SS_3: 5.0 ton da^{-1},SS_4: 7.5 ton da^{-1}.

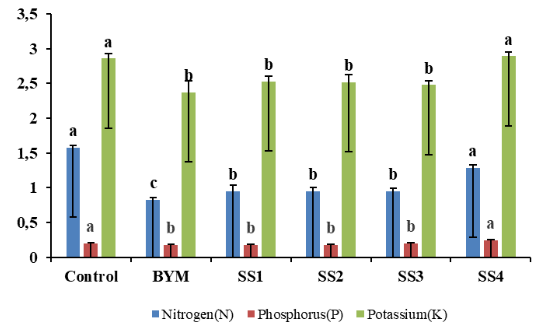

Figure 3. Effect of different levels of applied sewage sludge on lettuce macro nutrients (N, P, K). Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 0.5 ton/da,SS1: 1.25 ton da-1, SS2: 2.5 ton da-1, SS3: 5.0 ton da-1,SS4: 7.5 ton da-1.

3.2. Micro Nutrients of Lettuce (*Lactuca Sativa* L.)

In the pot experiment, it was observed that the leaf and root Fe content in the SS1, SS2, and SS3 treatments did not differ significantly from the control. However, in the SS4 treatment, leaf Fe content increased from 136 mg kg^{-1} (control) to 160 mg kg^{-1}, while root Fe content increased from 3353 mg kg^{-1} to 4853 mg kg^{-1}. The concentrations of Zn and Cu in both leaves and roots were elevated in all sludge treatments (SS1, SS2, SS3 and SS4) compared to the control. Specifically, leaf Zn content increased from 8.37 mg kg^{-1} (control) to 24.8 mg kg^{-1} (SS1), 25.9 mg kg^{-1} (SS2), 24.1 mg kg^{-1} (SS3), and peaked at 40.9 mg kg^{-1} (SS4). Similarly, root Zn content rose from 36 mg kg^{-1} (control) to 141 mg kg^{-1} (SS1), 139 mg kg^{-1} (SS2), 156 mg kg^{-1} (SS3), and 183 mg kg^{-1} (SS4) (Table 2). The availability of micronutrients in the soil solution depends largely on environmental conditions such as soil pH and aeration (Sparrow & Uren, 2014). Application of sewage sludge influences the mineralization process, which in turn alters soil pH, significantly affecting micronutrient uptake. Furthermore, sludge application increases soil organic matter content (Zhang et al., 2018; da Silva et al., 2021), enhancing the cation exchange capacity and chelation potential. These changes improve the solubility and availability of essential micronutrients like Fe, Zn, Cu, and Mn (Eid et al., 2017). In this study, the highest accumulation of Fe, Zn, and Cu in both roots and leaves was observed at the SS4 (7.5 ton da^{-1}) treatment level. These findings are in agreement with Rodrigues et al. (2024), who reported that sewage sludge application enhanced Fe and Mn concentrations in lettuce plants. They also noted that while aeration had limited influence, changes in soil pH played a significant role in the bioavailability of metallic micronutrients (Table 2). Impact of different levels of applied sewage sludge and single dose BYM on lettuce micronutrient (Fe, Zn, Cu, Mn) content (mg kg-1).

Table 2. Micro nutrients of Lettuce

	Applications	Fe	Zn	Cu	Mn
Leaf micro nutrients	Control	136±7.77b	8.37±0.97c	2.47±0.13c	33±5.51
	BYM	112±6.06c	10.2±0.53c	2.89±0.13b	28±1.00
	SS_1	136±3.61b	24.8±0.49b	2.94±0.03b	23±0.88
	SS_2	130±8.66bc	25.9±0.17b	2.69±0.07bc	20±4.26
	SS_3	125±4.91bc	24.1±0.52b	2.67±0.08bc	20±4.41
	SS_4	160±0.33a	40.9±3.76a	3.39±0.04a	28±5.67
F_{value}		7.444** P≤0.01	53.50** P≤0.01	12.57** P≤0.01	NS
	Applications	Fe	Zn	Cu	Mn
Root micro nutrients	Control	3353±236b	36±2.00d	13.37±0.27d	131±4.91ab
	BYM	1937±239c	53±1.20d	11.53±0.38d	110±7.22b
	SS_1	2267±118c	141±3.46c	17.17±0.90c	109±6.66b
	SS_2	2650±333bc	139±2.91c	16.0±0.36c	110±5.90b
	SS_3	3190±225b	156±6.66b	21.03±1.25b	118±4.70b
	SS_4	4853±176a	183±5.70a	44.47±0.47a	141±4.04a
F_{value}		20.198*** P<0.001	206*** P<0.001	301** P≤0.01	5.441** P≤0.01

Averaged values within a column, followed by different lowercase letters (a, b, c, d, e), indicate significantdifferences among treatments at BYM:Barn yard manure, SS Sewage sludge, NS: non significant, *P<0.05, **P<0.01,***P<0.001 significance level.

3.3. Correlation Analysis Between Micro Nutrients And Yield Criteria

This study presents a comprehensive analysis of the relationships between various physiological and biochemical parameters in plants. Correlation analysis reveals several important and significant interactions. In particular, there is a positive and statistically significant correlation. A significant positive correlation was found between the fresh weight and root fresh weight of the lettuce plant (r=0.95, P<0.01). Furthermore, a significant (r= 0.93 P<0.01) positive correlation was found between root dry weight (RFW) and root iron (R-Fe) content. Moreover, a significant positive correlation was found between root fresh weight (RFW) and root manganese (R-Mn) content (r=0.73, P<0.01), as well as between root copper (R-Cu) content (r=0.88, P<0.01). This means that the increase in root iron, copper, and manganese content has led to a significant increase in root wet weight (Figure 4).

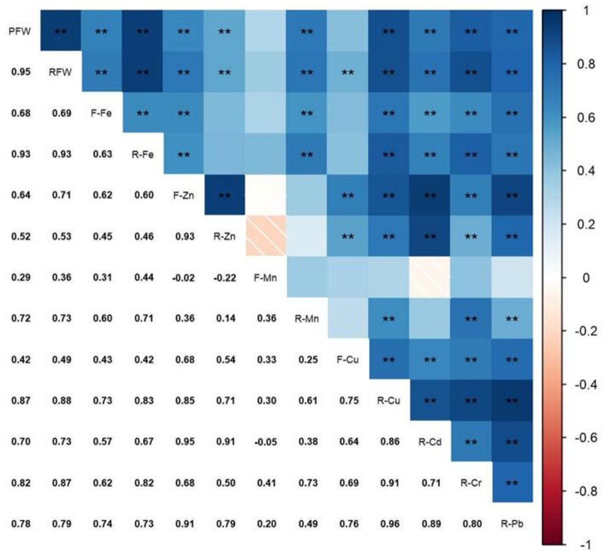

Figure 4. Correlation relationship between growth parameters, micro nutrients and heavy metal of plant materials obtained from sewage sludge applications. Correlations between the studied characteristics in rapeseed. *, **, and *** indicates significance at P≤0.05, P≤0.01, and P≤0.001, respectively. PFW:plant fresh weight, RFW:root fresh weight, R-Fe:Root iron, R-Zn:Root zinc, F-Fe:Foliar(leaf) iron, F-Zn: Foliar(leaf) zinc R-Cu:Root copper R-Cd: Root cadmium. R-Pb: Root lead.

3.4. Heavy Metals in Lettuce Root

The concentrations of cadmium (Cd) in the roots of lettuce plants treated with control and barnyard manure (BYM) were statistically grouped together, with values of 0.06 and 0.08 mg kg^{-1}, respectively. However, a progressive increase in root Cd concentration was observed with increasing doses of sewage sludge. Specifically, the Cd contents were 0.16, 0.17, 0.18, and 0.28 mg kg^{-1} for SS1, SS2, SS3, and SS4 treatments, respectively. Among all treatments, the SS4 dose (7.5 ton da^{-1}) led to the highest root Cd accumulation (Figure 5). Similarly, root lead (Pb) concentrations in the control and BYM groups did not differ significantly, whereas increasing doses of sewage sludge resulted in marked Pb accumulation in lettuce roots. The Pb concentrations for SS1, SS2, SS3, and SS4 were 2.37, 2.40, 2.07, and 6.51 mg kg^{-1}, respectively, with the SS4 treatment again showing the highest accumulation (Figure 6). For chromium (Cr), the control, BYM, SS1, SS2, and SS3 treatments formed a statistically similar group. However, a significant increase in root Cr concentration was observed under the SS4 treatment (Figure 7), indicating a dose-dependent effect of sewage sludge on heavy metal accumulation in plant tissues. The application of sewage sludge to agricultural soils has been reported to increase both biomass production and heavy metal accumulation in various crops (Tandi et al., 2004; Jat Baloch et al., 2023). These effects largely depend on the application rate and frequency. In this study, the SS4 treatment significantly elevated Cd, Pb, and Cr levels in the root zone of lettuce, while BYM remained comparable to the control. Despite the increase in heavy metal accumulation under high-dose sewage sludge application, improved plant growth was observed. This may be attributed to the concurrent rise in essential macro- (N, P, K) and micronutrients (Fe, Zn, Cu, Mn) in the growth medium. Chu et al., (2017) Who reported that the optimum application rate of sewage sludge compost (SSC), i.e., 30% SSC with 70% soil, significantly increased plant height, soil diameter and plant fresh weight of plant (M. persiciforma) and effectively reduced the concentrations of heavy metals (Cu, Zn, Pb and Cd). Notably, the accumulation of heavy metals did not increase proportionally with biomass production. This inverse relationship is explained by the "dilution effect," wherein enhanced plant growth leads to a reduced concentration of heavy metals per unit of dry weight (Bourioug et al., 2015). Hence, although SS4 elevated heavy metal uptake in absolute terms, their concentration in plant tissue was mitigated by vigorous vegetative development.

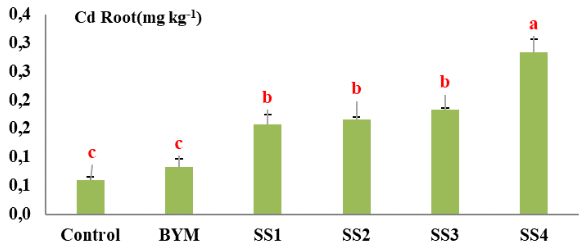

Figure 5. Impact of different levels of applied sewage sludge and single dose BYM on Cd content of lettuce root. Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 5 ton/da,SS1: 1.25 ton da-1,SS2: 2.5 ton da-1, SS3: 5.0 ton da-1,SS4: 7.5 ton da-1.

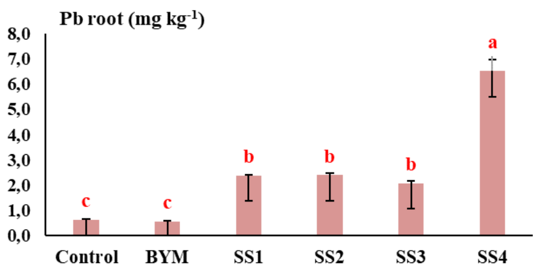

Figure 6 Impact of different levels of applied sewage sludge and single dose BYM on Pb content of lettuce root. Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 5 ton/da,SS1: 1.25 ton da-1,SS2: 2.5 ton da-1, SS3: 5.0 ton da-1,SS4: 7.5 ton da-1.

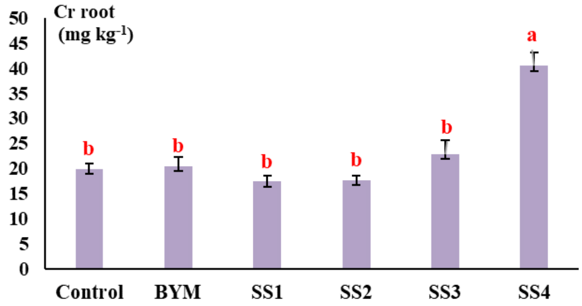

Figure 7. Impact of different levels of applied sewage sludge and single dose BYM on Cr content of lettuce root. Different letters top of the bars indicate significant differences according to Duncan test, **(P≤0.01). BYM: 5 ton/da, SS1: 1.25 ton da-1,SS2: 2.5 ton da-1, SS3: 5.0 ton da-1,SS4: 7.5 ton da-1.

4. Conclusion

The high nitrogen (N) content and low C/N ratio of the sewage sludge likely promoted rapid substrate mineralization, enhancing microbial activity and nutrient cycling in the soil. This process substantially contributed to increased fresh biomass production in lettuce. In contrast, the barnyard manure (BYM) released nitrogen more slowly, resulting in limited effects during the short growth cycle of lettuce and performing similarly to the control. Application of sewage sludge at a dose of 7.5 tons per hectare significantly enhanced both shoot and root fresh weight, as well as the accumulation of micronutrients in root and leaf tissues. However, this highest dose also led to a notable increase in root concentrations of Cd, Pb, and Cr. Despite this, the concentration of heavy metals per unit of plant biomass decreased due to the dilution effect, as nutrient uptake outpaced heavy metal accumulation. Overall, the 7.5 tons da^{-1} sewage sludge application positively influenced lettuce growth and micronutrient uptake, but its use should be carefully managed due to the associated rise in heavy metal content in root tissues.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	S.B.C.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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