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Enhancing Zinc and Copper Stabilization Using Date Palm Waste Compost and the Phytoremediation Potential of *Medicago sativa*

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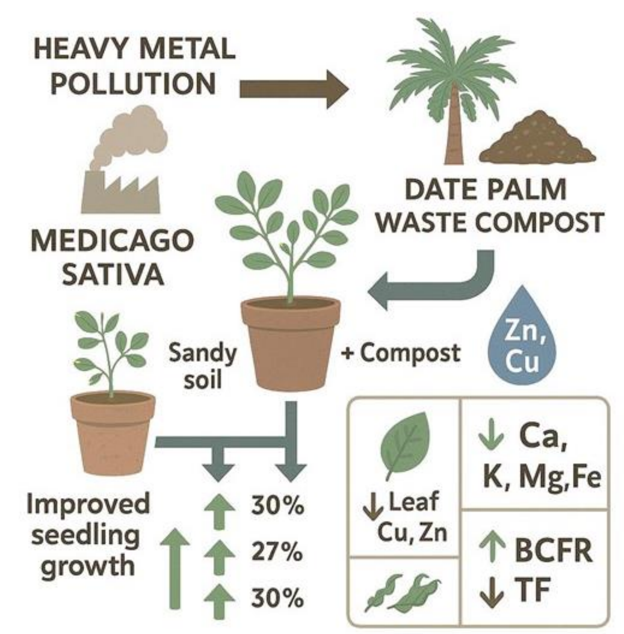
Graphical abstract



Abstract

Heavy metal (HM) pollution poses a serious threat to plant growth and productivity. This study investigated the potential of date palm waste compost to enhance Medicago sativa tolerance to Zn and Cu stress and support phytoremediation. Plants were grown in sandy soil with or without 50% compost and irrigated with nutrient solutions containing high levels of Zn and Cu. Compost amendment significantly improved growth, increasing leaf, stem, and root biomass by 30%, 27%, and 30%, respectively. Despite elevated soil Zn and Cu levels, metal accumulation in leaves decreased, indicating reduced bioavailability. Compost also enhanced nutrient uptake (Ca, K, Mg, Fe) in both leaves and roots. Root bioconcentration factors increased, while shoot translocation factors decreased, suggesting improved phytostabilization. Compost treatments increased microbial colonies, with Zn and Cu addition boosting bacterial counts by 60% and 70%, respectively, though Cu reduced fungal numbers by 70%. Microbial diversity also shifted, notably in bacterial communities, due to the compost's high organic matter and carbon content. PCA analysis revealed distinct mineral distribution patterns across plant tissues under compost treatment. These findings highlight the potential of date palm compost to improve soil quality, support beneficial microbes, and enhance *M. sativa* phytoremediation capacity under heavy metal stress.

Key words: Date palm waste compost, heavy metal stress, *Medicago sativa*, microbial biomass, phytoremediation

1. Introduction

Soil pollution with heavy metal (HM) has become the world's most severe environmental hazard, which induce growth reduction (Kumpiene *et al.* 2008). HM can reach the food chain through contaminated soil, water, and air, and their mobility among environmental compartments is also a great threat to human health (Emami Bistgani *et al.* 2019). The majority of HMs bind to fine-grained particles after entering the riverine environment and deposit in the sediment, causing adverse biological impacts (Bibi *et al.* 2007; Brady *et al.* 2014). The availability of heavy metals (HMs) in soil is influenced by soil pH and the plant's absorption capacity (Shi *et al.* 2016). Copper (Cu) and zinc (Zn) are common soil pollutants that cause growth suppression, leaf chlorosis, and reduced photosynthesis in plants (Hattab *et al.* 2016). To prevent HM accumulation in plants and the food chain, various remediation approaches have been proposed, including physical, chemical, and biological methods (Silva *et al.* 2020). However, inorganic chemical methods may have long-term environmental impacts (Garau *et al.* 2007; Farrell and Jones 2010). In contrast, phytoremediation offers a cost-effective, eco-friendly alternative with multiple benefits, such as plant biomass conversion to bioenergy, biodiversity preservation, and soil stabilization (Silva *et al.* 2020). Plants act as reservoirs for metals, potentially transporting them through the food chain (Hattab *et al.* 2016).

One of the many phytoremediation methods is phytostabilization, which involves using plants that can withstand metals to store the metals in their roots and rhizosphere. *Medicago* is a leguminous genus that contains economically important species. Among 83 species, *M. sativa* is an economically important leguminous plant that can thrive and tolerate a variety of environmental conditions (Raklami *et al.* 2021). Due to its quick growth, metal tolerance, its vigor roots to accumulate and ability to create a symbiosis with rhizobacteria, *M. sativa* has received interest in remediation strategies (Raklami *et al.* 2021).These plants serve as metal excluders, collecting metals mostly in the roots with low accumulation and transfer to the surface (Raklami *et al.* 2021).

Moreover, it has become evident over the past decade that soil and rhizosphere microorganisms can significantly support phytoremediation efforts by enhancing HM bioavailability and facilitating plant uptake (Ahmad *et al.* 2019). Plants naturally interact with microorganisms, which can influence metal mobility, availability, and uptake. Microbial groups like mycorrhizal fungi and nitrogen-fixing bacteria help plants tolerate metal-contaminated soils by producing growth-promoting compounds and improving stress tolerance (Doornbos *et al.* 2012). To support plant growth in such environments, amendments are often used, a strategy known as "assisted phytostabilization. HMs are immobilized in soil through precipitation, sorption, and complexation reactions (Kiikkilä *et al.* 2001). Organic amendments like compost are effective tools to reduce HM bioavailability and improve soil quality (Akram *et al.* 2018) Pulford and Watson 2003). However, excessive use of compost can also lead to HM accumulation in soil and plants, posing risks to human health and the environment (Ghosh *et al.* 2015).

The date palm (*Phoenix dactylifera* L.) as an perennial fruit tree and alfalfa (*M. sativa* L.) as a perennial feed crop dominate the agriculture system in south Tunisia oases. Date palm wastes have a high potential for use as plant growth culture material in hydroponic systems. Compost has also been used for soil HMs immobilizing by moving speciation from highly bioavailable forms (i.e. free metals) to fractions associated with carbonates, metal oxides or organic matter (OM) (Mohammadi 2011; Amirjani 2011), which are substantially less bioavailable (Walker and Bernal 2008). These amendments are known to immobilize the HMs due to the humic acids in them, which can interact with a wide range of metal(loid)s (Madejón *et al.* 2018). Compost has long been used as an OA to immobilize HMs during soil remediation. While previous studies have demonstrated the potential of various composts such as those derived from leaves, straw, olive mill waste or municipal organic matter to enhance phytoremediation, these typically involve different organic amendments and soil conditions. In contrast, our study uniquely explores how date palm compost influences both metal immobilization and rhizosphere microbial dynamics, particularly under Zn and Cu contamination. For example, green waste compost application in lettuce reduced shoot Cu and Zn uptake by 22–59% and 16–25%, respectively (Ullah *et al.* 2025). Similarly, compost from rice straw improved soil pH, organic matter, and potassium availability while lowering HM bioavailability (Tang *et al.* 2020) and Olive-mill waste compost also enhanced shoot growth and decreased Cu, Zn, and Mn concentrations in both shoots and soil (Walker *et al.* 2004). The novelty of our study lies in using *date palm waste compost*, abundant in arid regions like Tunisia, to improve phytoremediation outcomes. Unlike previous studies, we focus on how compost-mediated shifts in microbial biomass and diversity contribute to HM immobilization and plant stabilization. Specifically, we assess its ability to enhance Zn and Cu exclusion capacity of *M. sativa* and promote stable vegetation on contaminated soils. Our objectives were: (i) to evaluate *M. sativa* phytoremediation performance using date palm compost, and (ii) to study how Zn and Cu additions affect rhizosphere microbial communities in compost-amended soil.

2. Materials and methods

2.1. Plant material and growth conditions

M. sativa seeds were obtained from the Institute of Arid Regions in Gabes. Following collection, the seeds underwent a 30-minute stirring process in a 4% $NaClO$ solution, followed by three washes with deionized water. Subsequently, the sterilized seeds were placed in petri dishes and subjected to dark conditions at a temperature of 25°C for germination. After a week, the emerging Medicago saplings were transplanted into distinct pots. These pots were filled with a sandy soil derived from desert dune-sand to provide an environment conducive to further development. This soil was supplemented with certified high-quality compost obtained from date palm waste in one portion (CMP, 50% of the pot volume) (**Table 1**). This proportion was chosen based on its ability to enhance soil fertility, support microbial activity, and aid in the stabilization of heavy metals, particularly for phytoremediation. The 50% compost level balances enriching the soil with organic matter without negatively affecting plant growth or nutrient uptake. Additionally, this ratio has been shown to improve soil properties such as moisture retention, porosity, and microbial diversity, which are critical for *M. sativa* growth in metal-contaminated soils. The date palm leaves were first gathered from the Cheneni and Chenchou Oasis (Gabes, south region of Tunisia). It was physically crushed to a particle size of 5-10 cm before being soaked in tap water for 5–7 d in a 4 m x 4 m basin with a depth of 2 m. The swelling of date palm waste improved their biodegradation during the composting process. The physicochemical properties of date palm waste compost are presented in **Table 1.** The moisture content was measured by drying samples at 95 °C for 24 h. Organic matter content in soil was calculated by multiplying Corg by 1.72, whereas OM in organic amendments was determined by ashing at 430 °C. Soil pH was measured in saturated paste extracts, while pH of organic amendments was determined in 1:10 (w/v) amendment-to-water extracts. Electrical conductivity (EC) was measured in 1:5 (w/v) aqueous extracts. Minerals were determined using atomic absorption spectrophotometer assay (Method 2.3). Total nitrogen was analyzed following the methods described by Walker and Bernal (2004). Greenhouse conditions: temperature 25 ± 2 °C and light intensity (PAR): 500–700 µmol m^{-2} s^{-1}. Plants were irrigated every 4 d with a complete nutrient solution. The metallic stress was imposed after a month of acclimatization by adding the doses of Zn and Cu in the irrigation solution to 6 and 4 mM, respectively. To avoid osmotic shock, the Zn or Cu dose was gradually increased by 2 mM each day until the appropriate concentrations were achieved. he stability and uptake of Zn and Cu in the soil–plant system was confirmed by analyzing metal concentrations in soil and plant tissues using atomic absorption spectrophotometer (Method 2.3). The experimental design was conducted with 8 seedlings in five pots for each treatment. Four weeks after beginning the treatments, these plants were harvested. The leaves were weighed and then separated into two groups. The initial group was preserved at 80°C for subsequent biochemical analyses. Meanwhile, the second group underwent a drying process at 80°C for 48 hours, after which it was finely powdered to facilitate ion analyses.

Table 1. Main Physical and Chemical Properties Measured in Date Palm Waste Compost and Soil: pH, EC, Moisture, Organic Matter, and Nutrient Content

Parameters	Date palm waste	Soil
pH	7.7±0.3	6.8 ±0.04
EC (mS/cm)	5.9±0.04	0.92± 0.03
Moisture (%)	9.9±0.52	2.12±0.1
Dry matter (%)	63±0.50	18.7±4.0
OM(%)	25.7±0.2	4.4±0.2
OC(%)	52.6±0.3	10±0.23
N (%)	0.6±0.01	0.1±0.02
K (mg/g dw) DM)	18.2±0.2	15.6± 0.2
Ca (mg/g dw)	20.9± 1.1	8.6± 0.03
Mg (mg/g dw)	3.0± 0.3	3.4± 0.07
Na (mg/g dw)	10.9±1.0	3.5 ±0.1
Fe (mg/g dw)	1.75± 0.1	2.0±0.00

pH, EC (Electrical Conductivity), Moisture (%) (Moisture Content), Dry Matter (%) (Dry Matter Content), OM (%) (Organic Matter Content), OC (%) (Organic Carbon Content), N (%) (Nitrogen Content), K (mg/g dw) (Potassium Content), Ca (mg/g dw) (Calcium Content), Mg (mg/g dw) (Magnesium Content), Na (mg/g dw) (Sodium Content), and Fe (mg/g dw) (Iron Content).

2.2. Soil and compost processing, and characterization

The pH of the soil suspension was read with the aid of a pre calibrated pH meter (*ISO 10390*). 20 g compost was stirred in distilled water to measure the EC by a conductivity meter (*ISO 11265*). Dry matter content was measured through the gravimetric method, drying the sample at 105 ± 2°C in hot air oven until fulfilling constant mass. Moisture content was determined as the weight difference between fresh and dry matter. After calcining a dried sample at 550°C for 4 hours in a muffle furnace, the organic OM was measured. The amount of OC was calculated according to numerous researchers, OC % = OM %/1.8 (Kolka *et al.* 2008). The total nitrogen (N) was determined using the Afnor standard Kjeldahl method (Sáez-Plaza *et al.* 2013).

2.3. Analysis of mineral contents

Two hundred mg of each *M. sativa* sapling (leaves, stem and roots) was digested in solution containing HNO_3/H_2O for evaluation of macro and micro-elements. The concentrations of minerals elements (Ca, Na, K, Mg, Zn and Cu) were determined with a standard of 1% nitric acid using an atomic absorption spectrophotometer (Abdelgawad *et al.* 2015; Alotaibi *et al.* 2021; Saleh *et al.* 2020). The mineral composition of soil and soil mixed with compost was measured using atomic absorption spectrophotometer.

2.4. Heavy Metal Translocation Index

2.4.1. Translocation factor (TF)

This factor indicates the transfer of Zn or Cu from the soil to the root, and from the root to the shoot (Phusantisampan *et al.* 2016):

$$TF = C_{shoot}/C_{root}$$

where C_{root} and C_{shoot} is the Zn or Cu concentration in soil, shoot or root.

2.4.2. Bioconcentration coefficient (BCFR)

It implies the accumulation of heavy metal by roots (Phusantisampan *et al.* 2016):

$$BCFR = C_{roots}/C_{soil}$$

where $C_{\_soil}$ and $C_{\_root}$ indicate is the concentration of Zn or Cu in soil or root.

2.5. Bacteria and fungi count

Microbial counts of bacteria and fungi were conducted from 1 g of soil or compost sample suspended in 9 mL of sterile 0.9% NaCl solution. A tenfold serial dilution series (ranging from 10^{-1} to 10^{-6}) was prepared to reduce microbial concentrations to countable levels. For bacterial enumeration, 100 µL aliquots from the 10^{-4} dilution were plated onto Plate Count Agar (PCA). The plates were incubated at 28°C for 48 hours, after which colony-forming units (CFUs) were counted. For fungal enumeration, a preliminary test was performed to determine the optimal dilution range. Based on these trials, the 10^{-2} dilution was selected. From this dilution, 100 µL aliquots were plated onto Potato Dextrose Agar (PDA), supplemented with chloramphenicol to suppress bacterial growth. Plates were incubated at 25°C for 75 hours. These dilution factors (10^{-4} for bacteria and 10^{-2} for fungi) was selected based on preliminary trials to ensure the optimal countable range of colonies. Incubation times and temperatures were chosen to maximize the growth of both bacterial and fungal communities, as recommended by previous studies (Lionel Ranjard , Sylvie Nazaret , Francois Gourbiere *et al.* 2000). The soil microbial count represents the number of colony forming units (CFUs) per gram of dry soil (Lionel Ranjard , Sylvie Nazaret , Francois Gourbiere *et al.* 2000).

2.6. Statistical analyses

The statistical analyses were carried out using the R statistics software (ggplot2, agricolae_1.3-5). One-way analysis of variance (ANOVA) was performed, using Tukey's Test (P 0.05) as a post-hoc test for mean separations. Prior to performing the one-way analysis of variance (ANOVA), data normalization was applied to ensure comparability across treatments and eliminate any potential scale-related biases. The number of replicates per treatment was consistently maintained at five (n = 5) for all experiments, ensuring the reliability of the data. Each experiment was repeated at least three times to ensure robust and reproducible results. All data were measured in appropriate units, which are explicitly stated in the results section. Pearson correlation analysis was also performed on all experimental data using the corrplot_0.92 package in R, and hierarchical clustering was constructed to group similar data points. Principal component analysis (PCA) was also carried specifically to reduce the dimensionality of the data, allowing for the visualization of underlying trends and interactions. By using R (ggplot2, agricolae_1.3-5), we were able to visualize how different treatments related to each other and to other experimental factors, helping to highlight the key influences on the system. Additionally, correlation analysis was used to quantify the strength and direction of relationships between key variables.

3. Results

3.1. Zn and Cu effect on the study soils

In comparison to the sandy soil, the pH of the soil in composted pots was dramatically increased (around neutral, pH 7.4, **Table 2**). These increases can be explained by increased Zn and Cu doses that raised the pH of the soil from 6.8 to 7.8 and 8, respectively. In pots treated with 50 % of date palm waste compost, increased Zn lowered the soil pH, while higher Cu increased pH of former soil. The highest value of EC (1.74 dS/m) was recorded for soil mixed by compost and Cu. The highest moisture content (23%) was recorded for soil combined with compost. The high doses of Zn and Cu did affect soil moisture. When compost was applied instead of just soil, organic matter increased to 46%. In all types of soils, Zn and Cu reduced organic matter, but it still higher in soil mixed with compost (29% and 32%). Total N increased by 35% in soil amendment with palm waste compost. Compared to the single effect of Zn or Cu, their interaction with compost boosted total nitrogen by 50% and 60%, respectively. Date palm waste compost increased the OC carbon to 25%. But the addition of Zn and Cu decreased the OC in target soils.

Table 2. Physico-chemical properties of sandy soil and compost-amended soil treated with zinc (Zn) and copper (Cu).

	SS	SC	SS+Zn	SS +Cu	SC+ Zn	SC +Cu
pH	6,85±0.1c	7,43±0.00b	7,91±0.04a	8,01±0.05a	7,79±0.00b	7,59±0.01b
EC (dS/m)	0,92±0.01b	1,61±0.02a	0,98±0.01b	1,05±0.00b	1,68±0.00a	1,74±0.00a
Moisture (%)	2.14±0.00d	23,06±0.2a	2,26±0.00d	3,00±0.01c	14,68±0.2b	18,12±0.2b
OM (%)	3.12±0.01d	46,5±0.3a	4,04±0.02c	3,11±0.01d	29,41±0.5b	32,16±0.4a
Total azote (%)	0.18±0.00c	0,35±0.00b	0,2±0.00c	0,22±0.00b	0,91±0.00a	0,87±0.00a
OC(%)	1.73±0.01d	25,83±0.2a	2,24±0.00c	1,72±0.00d	17,16±0.01b	17,87±0.3b

OC: Organic Carbon, TN: Total Nitrogen, OM: Organic Matter, SS: Sandy Soil, SC: Sandy Soil + Compost, SS+Zn: Sandy Soil treated with Zn, SS+Cu: Sandy Soil treated with Copper (Cu), SC+Zn: Compost-amended Soil treated with Zinc, SC+Cu: Compost-amended Soil treated with Cu.The superscript lowercase letters (a- d) indicate statistically significant differences between the samples. The statistical significance of the relative abundances was determined by Tukey's post hoc test. with $p < 0.05$ (n=5)

3.2. The impact of compost, Zn and Cu on soil mineral compositio006E

The compost application to the soil enriched it with high concentrations of Ca, K, Mg, Fe, and Zn. (**Figure 1**). The application of compost resulted in a significant increase in the levels of Ca, K, Na, and Mg by 20%, 205%, 50%, and 15% respectively. However, the Cu content appeared to remain unchanged with the use of compost. As a result, the introduction of a high Zn concentration led to an increase in the Zn content in sandy soil, whereas in compost, the Zn level was lower. Notably, the presence of high Zn did not lead to a reduction in the levels of K and Fe in compost compared to sandy soil. Following a similar trend, the inclusion of Cu results in a greater increase in the content of Ca, K, Mg, and Fe in sandy soil compared to compost.

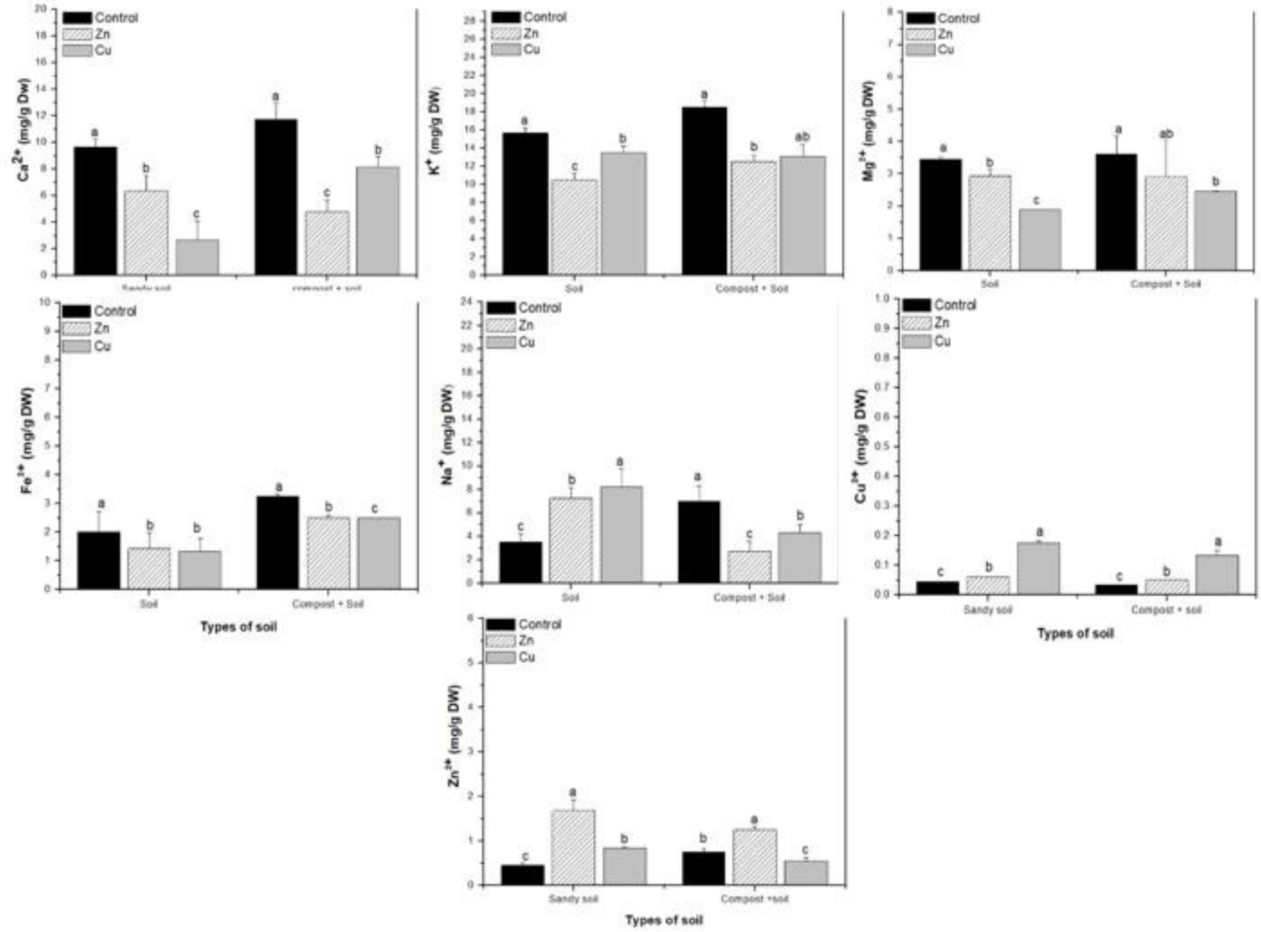


Figure 1. The effect of Zn and Cu on mineral composition sandy soil and soil mixed with compost. SS: Sandy Soil, SC: Sandy Soil + Compost, SS+Zn: Sandy Soil treated with Zn, SS+Cu: Sandy Soil treated with Copper (Cu), SC+Zn: Compost-amended Soil treated with Zinc, SC+Cu: Compost-amended Soil treated with Cu .Values are represented by mean ± standard deviation of at least three independent replicates. Within the same organs, different letters on the bars indicate significant differences at *$p < 0.05$*

3.3. The effect of compost, Zn and Cu on biomass accumulation of M. Sativa

The application of compost sharply increased biomass of leaves, stem and roots of plants by 30%, 27% et 30%, respectively (**Figure 2**). But Zn and Cu addition decreased the biomass of all *Medicago* organs in both types of soil. In fact, the highest concentration of Zn caused a significant decrease in leaves biomass by 50% as compared to control. The addition of compost mitigates the decrease in leaf biomass. Cu decreased the leaves biomass by 70% in sandy soil, but compost decreased it by 50% as compared to control. The fresh weight of stems reduced by 60% in response to high Zn and Cu concentrations, whereas the reduction did not reach 40% in plants grown in soil amended with compost. In addition, roots biomass showed the greatest decline in response to increasing dosages of Zn and Cu.

3.4. The influence of compost, Zn and Cu on M. sativa mineral nutrition

Fe, Mg and K concentrations in leaves of plants cultivated with compost increased by 47% 17%, and 25%, respectively (**Figure 3**). The absorption of mineral elements was altered by the uptake of Zn and Cu concentrations. For instance, Ca, K, Mg, Fe and Na content reduced in all *Medicago* organs in response to high doses of Cu and Zn. Compost reduced mineral level, while the Zn and Cu accumulation was increased. The findings demonstrated that roots significantly accumulated more Cu and Zn than roots of plants cultivated in soil.

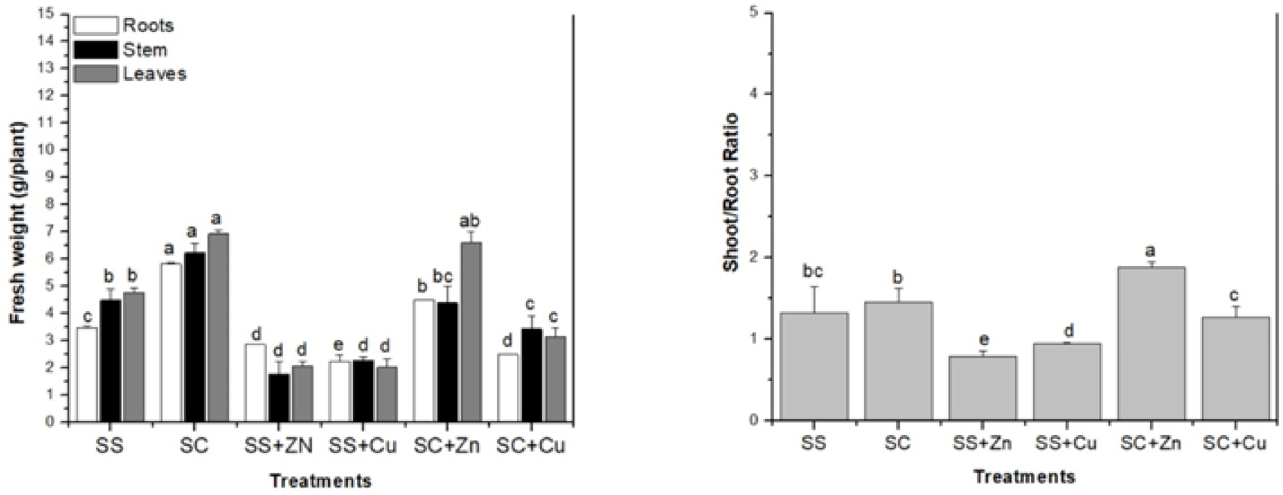


Figure 2. The effect of compost, Zn, and Cu on the growth parameters (roots (g/plant), stems (g/plant), leaves (g/plant), and shoot/root ratio) of *Medicago sativa*. Treatments include SS: Sandy Soil, SC: Sandy Soil + Compost, SS+Zn: Sandy Soil treated with Zn, SS+Cu: Sandy Soil treated with Copper (Cu), SC+Zn: Compost-amended Soil treated with Zinc, and SC+Cu: Compost-amended Soil treated with Cu. Values are represented as the mean ± standard deviation of at least three independent replicates. Within the same species, different letters on the bars indicate significant differences at $p < 0.05$.

3.5. Translocation Inde0078

TF and coefficients represent *Medicago*'s ability to take up and accumulate Zn and Cu into shoots and roots. TF values of *Medicago* saplings in sandy soil treated with Zn were less than 1, and declined but remained higher level in soil mixed with compost (0.78, **Table 3**). However, Cu reduced TF values for roots, except for Cu treatment. TF values below 1 indicate that Zn or Cu is retained in the roots, preventing translocation to the shoots, which is beneficial for phytoremediation. In Medicago saplings grown in sandy soil with Zn, TF values were below 1, and further reduced in compost-amended soil (0.78, **Table 3**), suggesting that compost helps limit metal mobility within the plant. Compost treatment decreased BCFR to 0.39. Compared to all types of Zn treated soil (BCFR values >1), a high dose of Cu decreased BCFR to 0.39 in soil mixed with date palm waste compost. Compost treatment decreased BCFR to 0.39, indicating reduced metal transfer from roots to shoots. In contrast, all Zn-treated soils had BCFR values greater than 1, while a high dose of Cu reduced BCFR to 0.39 in compost-amended soil, suggesting that compost limits metal translocation in the plant.

Table 3. Translocation factor (TF) and bioconcentration coefficient for roots (BCFR) in the *Medicago sapling*.

		TF	BCFR
	Metal		
Sandy Soil	Zn	1.39±0.10a	1.00±0.17a
	Cu	0.31±0.07c	0.42±0.02b
Soil + Compost	Zn	0.70±60.07b	1.27±0.13a
	Cu	0.15±0.01d	0.39±0.04b

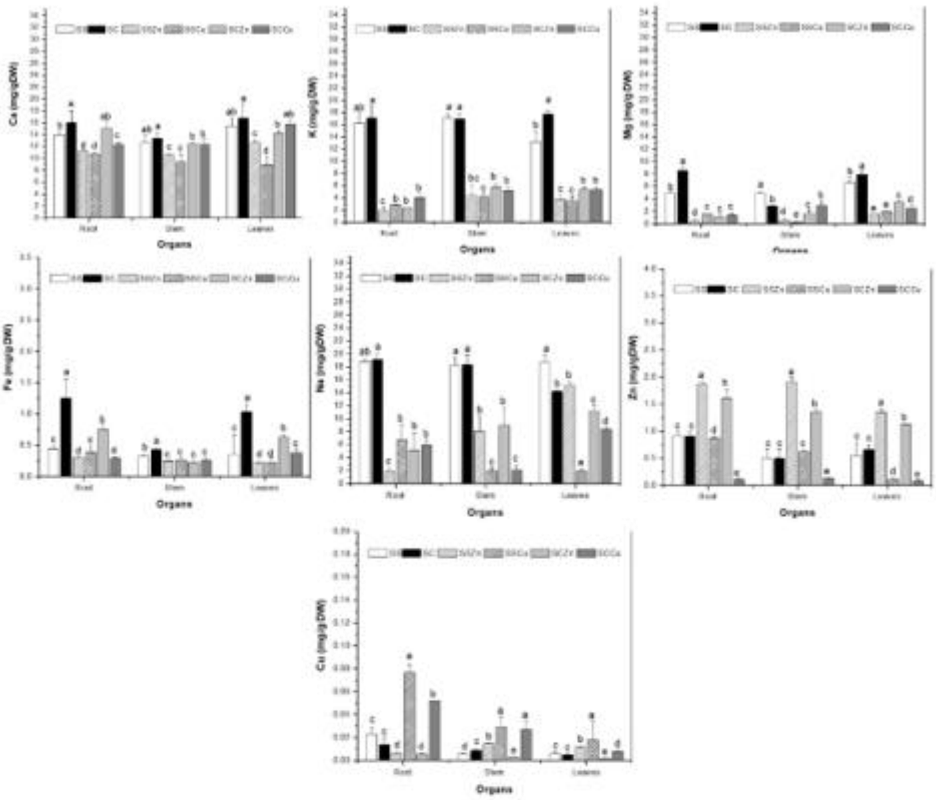


Figure 3. The effect of compost, Zn, and Cu on the mineral composition (mg/g DW) of the roots, stems, and leaves of *Medicago sativa*. Treatments are the same as described in Figure 2. Values are represented as the mean ± standard deviation of at least three independent replicates. Within the same organs, different letters on the bars indicate significant differences at $p < 0.05$.

3.6. Bacteria and fungi count

Compost treatments had more microbial colony forming than the control (**Figure 4**), which was sandy soil (the data was not presented because no microbial colony forming was detected). Furthermore, adding Zn and Cu to soil mixed with compost significantly enhanced microbial colony forming by 60% and 70%, respectively. Furthermore, Zn had no effect on the colony forming of the fungi. As a result of the Cu application, the total fungus count decreased by 70%.

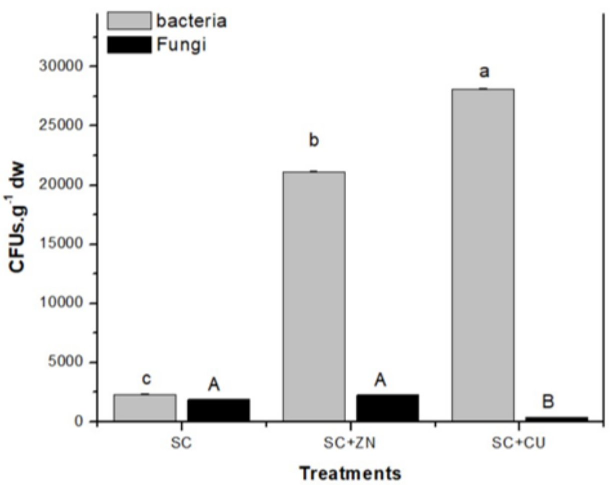


Figure 4. The effect of compost, Zn, and Cu application on bacterial and fungal biomass (CFU/gDW). The treatments are as follows: SS: Sandy Soil, SC: Sandy Soil + Compost, SS+Zn: Sandy Soil treated with Zn, SS+Cu: Sandy Soil treated with Copper (Cu), SC+Zn: Compost-amended Soil treated with Zinc, SC+Cu: Compost-amended Soil treated with Cu. Values are represented as the mean ± standard deviation of at least three independent replicates. Within the same treatment, different letters on the bars indicate significant differences at $p < 0.05$.

3.7. Principal Component Analysis (PCA) indicated specific organ and treatment responses

PCA was employed to investigate the variation in mineral composition and microbial community structure among different plant organs and treatment conditions. PCAs of samples taken from plants cultivated in two types of soil contaminated with Zn and Cu revealed a very comparable distribution of the seven elements in the roots and stem of the *Medicago* seedling cultivated under the different four treatments. The mineral composition of *Medicago* seedling leaves, on the other hand, moved from other organs in plants grown in soil mixed with compost (**Figure 5**). In addition to mineral composition, PCA was also used to explore the relationships between environmental variables, bacterial communities, and fungal populations (**Figure 6**). A clear separation of bacterial and fungal diversity was observed between soil treatments with compost and its interactions with Zn and Cu, with the heavy metals being separated along Principal Component 1 (PC1). This suggests that compost application significantly influenced microbial community composition in the soil, particularly in the presence of heavy metals. Notably, bacterial diversity showed a distinct separation based on OM and OC content, likely due to the high OM and OC levels in the compost. In the organic and control treatments, bacterial and fungal populations were highly comparable. This indicates that compost not only serves as a source of nutrients but also plays a crucial role in shaping the soil microbiome, particularly the bacterial communities. Bacterial communities showed greater variability in compost-treated soils compared to fungal populations, indicating a stronger response to the compost and heavy metals. In contrast, microbial populations in the organic and control treatments were similar, highlighting the impact of compost and heavy metals on microbial diversity. These PCA results underscore the complex interactions between soil amendments, metal contamination, and microbial communities, emphasizing the need to consider both mineral composition and microbial diversity in evaluating soil amendments' effectiveness.

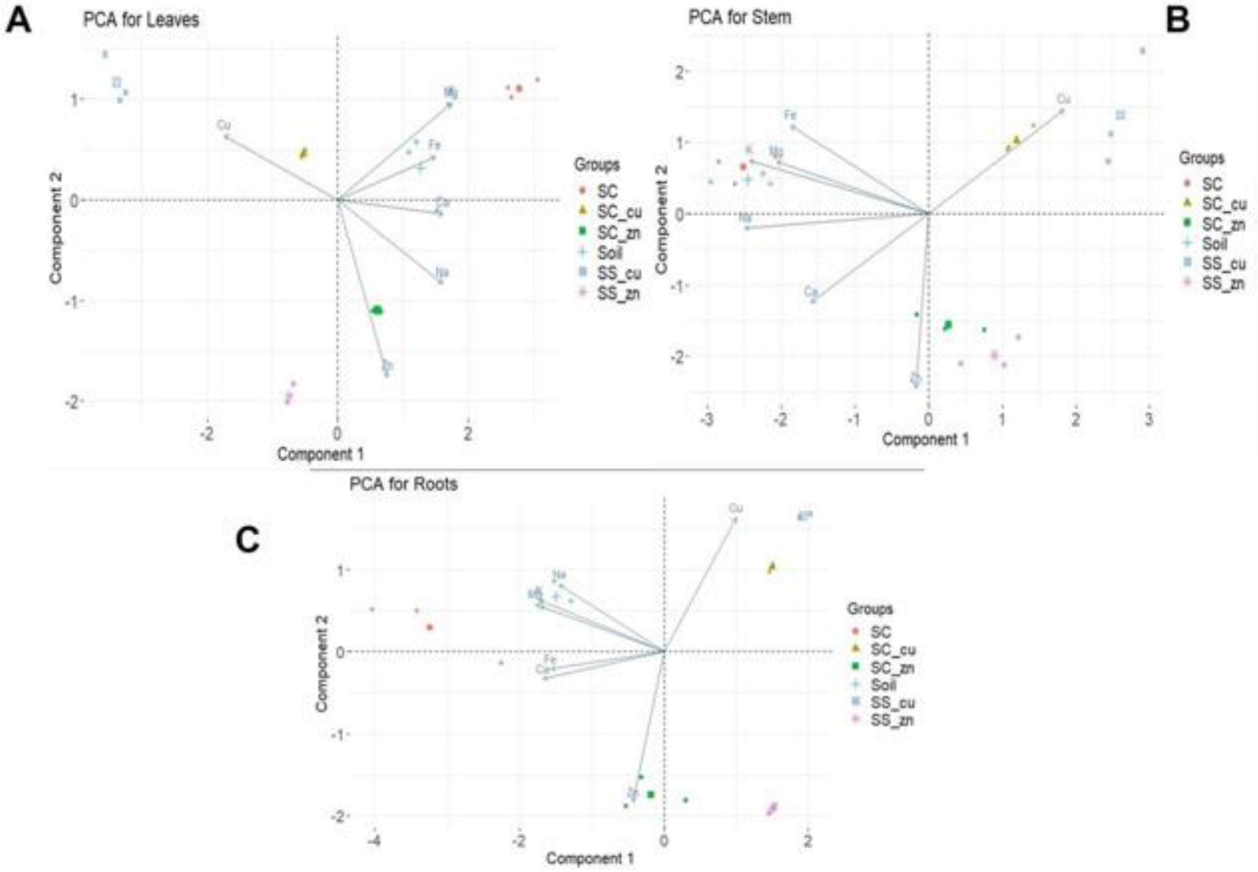


Figure 5. *Principal Component Analysis (PCA) Biplot depicting the relationship of various parameters from the leaves (A), stems (B), and roots (C) of* Medicago sativa.

3.8. Chemical characteristics and biological activity correlations

According to Pearson's correlation coefficients between biological and chemical factors (**Figure 7**), pH was the strongest predictor, with substantial correlations between all variables. MO and OC were negatively connected ($P < 0.05$), while Cu and Zn were positively correlated ($P < 0.01$). MO, OC, Ca and K are all favorably associated with total fresh weight. HMs concentrations were highly found inversely associated to both bacterial and fungal biomass. In compost treated soil, however, both bacterial and fungal biomass were adversely linked with MO, OC and N ($P < 0.05$).

4. Discussion

Date palm waste compost has attracted considerable research interest due to its beneficial effects on soil structure, functioning, and its role as an effective fertilizer (Ali 2008). In this study, the use of date palm waste compost significantly improved the growth of *M. sativa*. A previous study found that *M. sativa* exhibited a higher survival rate when planted in compost-amended soil compared to control soil (Marchand *et al.* 2017). Green compost individually or in combination with arbuscular mycorrhizal fungi as an effective strategy to improve alfalfa productivity under stress conditions (Ben-Laouane *et al.* 2020). In agreement with our results, soil amendments have been shown to enhance plant growth and yields in various species, such as barley (Agegnehu *et al.* 2016) and poplar trees (Guarino *et al.* 2020). Furthermore, increased application rates of date palm waste compost positively influenced the growth characteristics of *M. sativa*, likely due to improved nutrient mineralization and organic matter decomposition (Rady *et al.* 2016). The increase in *Medicago* yield appeared to be linked to mineral nutrient absorption. According to our findings, applied compost can operate as a nutrient reservoir, allowing nutrients to gradually release to roots of plant or into the soil (Yahaghi *et al.* 2019). Compost, rich in nutrients, humus, and microorganisms, positively affects soil biota metabolism, nutrient uptake, soil properties, and enzymatic activity, which enhances plant growth and productivity (Farrell and Jones 2010). In our study, compost, either alone or combined with Zn or Cu, significantly improved Medicago growth, suggesting it can mitigate Zn and Cu uptake and toxicity. The increased organic matter OM, nutrient concentrations, and OC in date palm compost were linked to improved *M. sativa* growth. Our study uniquely explores how date palm compost influences both metal immobilization and rhizosphere microbial dynamics. Similarly, rice straw compost improved soil pH, organic matter, and potassium availability while reducing heavy metal bioavailability. Olive-mill waste compost also enhanced shoot growth and decreased Cu, Zn, and Mn concentrations in both shoots and soil (Tang *et al.* 2020; Walker *et al.* 2004).

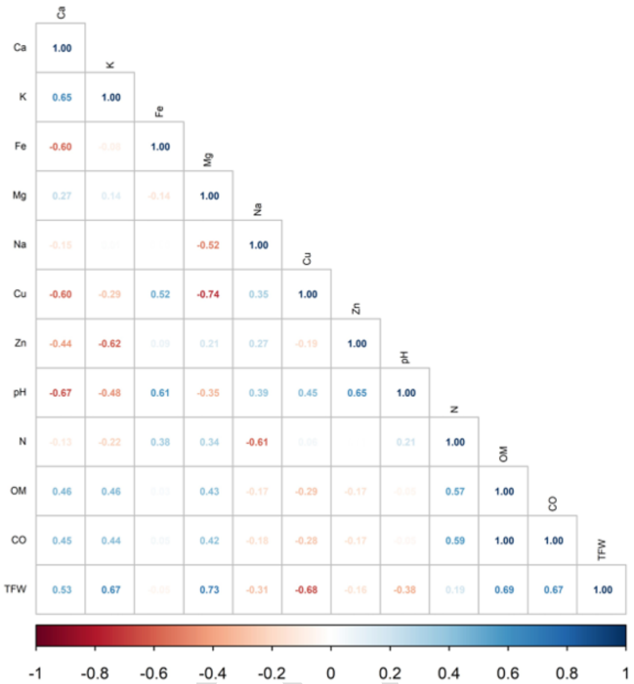


Figure 6. Correlation analysis of variance between Organic Matter (OM), Organic Carbon (OC), Nitrogen (N), pH, heavy metals, essential elements, and total fresh weight of *Medicago sativa* saplings. The blue and red colors represent positive (0<r<1) and negative (1<r<0) correlations, respectively.

Compost also indirectly boosts plant growth by influencing soil microbial activity (Rady *et al.* 2016). *M. sativa* ability to phytostabilize and phytoextract Cu and Zn has been well-documented (Baldantoni *et al.* 2014). TF and BCFR are critical indicators of metal uptake, with TF > 1 and BCFR > 1 signaling efficient metal translocation and accumulation. According to Meeinkuirt *et al.* (2012), TF values of 1 and BCFR > 1 indicate good metal tolerance. Our results showed BCFR values above 1, indicating effective metal uptake, while TF values for Zn in sandy soil were below 1, suggesting limited translocation. The addition of compost further reduced TF, implying better Zn retention in roots. This is consistent with the role of compost in enhancing metal immobilization. While *M. sativa* did not meet the hyperaccumulator threshold (TF > 1, BCFR > 10), its ability to concentrate metals in roots and improve soil retention through compost suggests strong potential for phytostabilization, similar to findings in other studies (e.g., Baldantoni *et al.* 2014). This suggests the potential for phytostabilization, where compost helps stabilize Zn in roots and limits its transport to the stem and leaves (Taeprayoon *et al.* 2022; García Martín *et al.* 2020) Concerning the phytomanagement of Cu, it's likely that *M. sativa* has a higher ability to tolerate and accumulate Cu than other Cu-bioaccumulators like *Brassica juncea* (Qadir *et al.* 2004) and could be used for phytoremediation (Rosca *et al.* 2021). Moreover, Brassica species are also effective for phytoattenuation, particularly for reducing the bioavailability of HMs in contaminated soils through phytoextraction (Meers *et al.* 2010).

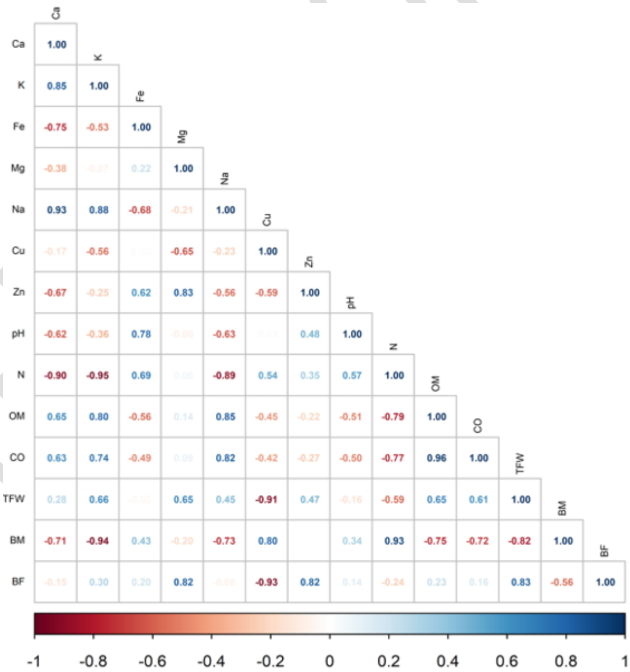


Figure 7. Correlation analysis of variance between Organic Matter (OM), Organic Carbon (OC), Nitrogen (N), pH, heavy metals, essential elements, and microbial and fungal biomass. The blue and red colors represent positive (0<r<1) and negative (1<r<0) correlations, respectively.

M. sativa responded differently to Cu and Zn in compost-amended soils. BCFR values were lower in these soils, and TF approached 1, indicating limited metal translocation. The roots accumulated 10 times more Cu than *M. indica*, confirming ability of *M. sativa* to extract Cu (Bélanger 1992). This is likely due to metal binding in root cell walls and storage in vacuoles, mechanisms used to avoid metal toxicity (Krzesłowska 2011). Although *M. sativa* transferred Cu to aerial parts, the low leaf Cu content suggests it acts as a Cu excluder. The addition of date palm waste compost enhanced this exclusion. Soil pH, OM content, and nutrient availability influence Cu and Zn phytoavailability (Tariq Rafiq *et al.* 2014). The compost increased pH (7.5–7.7), reducing metal bioavailability by enhancing metal sorption (Sukreeyapongse *et al.* 2002; Kazlauskaitė-Jadzevičėa *et al.* 2013). The high OM content in the compost further decreased metal availability, promoting phytostabilization (Cornu *et al.* 2016; Stewart *et al.* 2008). These findings indicate that *M. sativa*, in compost-amended soils, enhances phytostabilization, especially for Cu, by improving soil quality and reducing metal toxicity.

Compost significantly enhanced soil microbial diversity, particularly among bacteria and fungi, crucial for nutrient cycling and plant growth. These microbial shifts are key indicators of soil quality and fertility changes, often occurring faster than organic matter content. Compost, rich in beneficial microorganisms, boosts nutrient uptake, soil structure, and enzymatic activities, promoting plant productivity (Farrell *et al.* 2010; Rady *et al.* 2016). The increased microbial counts result from the carbon substrate and microorganisms in compost, with date palm waste providing nutrients that support beneficial microbes (Ren *et al.* 2018). Despite the presence of Zn and Cu, microbial biomass remained stable, suggesting compost improved microbial resistance to heavy metal stress (Azarbad *et al.* 2013). In compost-enriched soils, Zn and Cu had a minimal impact on microbial community structure. The higher microbial counts in contaminated soils likely stem from compost's high nitrogen and carbon content. However, the microbial balance shifted: bacterial resistance improved, while fungal communities became more sensitive. These shifts have ecological implications, affecting nutrient competition and soil health (Fones and Preston 2013; Hudek *et al.* 2013). Thus, compost not only supports plant growth under contamination but also reshapes microbial consortia, enhancing phytoremediation by improving plant–microbe interactions (Farrell *et al.* 2010; L. Wood *et al.* 2016; Kamran *et al.* 2017). This study shows that applying date palm waste compost to contaminated soils improves soil health, promotes microbial diversity, and helps immobilize metals, reducing their bioavailability. Compost-assisted phytoremediation is a promising strategy for restoring contaminated soils while maintaining ecological stability.

Overall, the present study highlights the viability of integrating locally available organic waste, such as date palm compost, into phytomanagement strategies for metal-contaminated soils. This approach not only enhances plant growth and soil microbial activity but also contributes to metal stabilization, offering a low-cost and environmentally friendly alternative to conventional remediation methods. The use of agricultural waste within a circular bioeconomy framework aligns with sustainable development goals and has strong potential in arid and semi-arid regions, where resource recovery and soil rehabilitation are critical. On the other hand, this study was conducted under controlled conditions and for a limited duration, which may constrain the applicability of results to long-term, field-scale scenarios. While changes in microbial abundance were observed, deeper functional insights (*e.g.,* metagenomics) are needed. Nonetheless, our findings support the combined use of organic compost and phytoremediation as a promising strategy for soil restoration. Future research should focus on field validation, economic feasibility, and life-cycle analysis to advance this sustainable approach.

5. Conclusion

The use of date palm waste compost amendment to *Medicago sativa* saplings reduced Zn translocation and increased its retention by roots. The compost enhanced Cu phytoextraction by roots and its immobilization, likely due to its high organic matter content, organic carbon, and lower pH. The microbial consortia in the compost, particularly fungi, also contributed to improved phytoremediation outcomes. This compost-based strategy, which benefits from enhancing soil structure and microbial diversity while stabilizing metals, presents a promising and cost-effective approach for soil remediation. Its applicability extends beyond *M. sativa*, offering a sustainable solution for contaminated soils, especially in arid and semi-arid regions with abundant date palm waste.

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