PLANT SCIENCE TODAY
Vol 12(sp4): 1-10
https://doi.org/10.14719/pst.8620

eISSN 2348-1900

RESEARCH ARTICLE

Does sewage sludge application increase micronutrients uptake and yield of mung bean (*Vigna radiata* L.) under semi-arid conditions?

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Received: 14 April 2025; Accepted: 18 August 2025; Available online: Version 1.0: 22 October 2025

Cite this article: Rachna, Rohtas K, Ankit, Pawan K, Yogesh, Hanuman SJ. Does sewage sludge application increase micronutrients uptake and yield of mung bean (*Vigna radiata* L.) under semi-arid conditions? Plant Science Today. 2025; 12(sp4): 1-10. https://doi.org/10.14719/pst.8620

Abstract

Sewage sludge, a residual, semi-solid organic matter material obtained from wastewater treatment were beneficially used as soil additives/valuable soil conditioner and as fertilizer being nutrient rich which are naturally occurring and are needed by plants. The sewage sludge application effect on micronutrient uptake and yield of mung bean (*Vigna radiata* L.) in semi-arid conditions of Northwestern India had been studied during *Kharif* 2021-22 with three replications based on completely randomized design. The seven treatments of sewage sludge i.e., 0, 2.5, 5, 7.5, 10, 15 and 20 t ha^{-1} were imposed. Grain yield was increased 44.9 % over control (no sewage sludge) after application of the sewage sludge at 20 t ha^{-1}. The maximum grain and straw yield of mung bean recorded with the application of sewage sludge at 20 t ha^{-1}. The uptake of diethylenetriaminepentaacetic acid (DTPA)-extractable micronutrients (zinc, copper, iron and manganese) increased significantly with the increasing rates of sewage sludge application over control. The highest availability of DTPA-extractable micronutrients in soil and their maximum uptake was found in T_7 where 20 t ha^{-1} sewage sludge applied. The results showed that yield of the mung bean, micronutrients content in soil and their uptake by mung bean increased significantly with the increasing rates of sewage sludge in the semi-arid regions of Northwestern India. The principal component analysis revealed that soil pH, Fe and straw yield were the most influential and reliable factors for evaluating soil quality and mung bean crop performance. Two principal components accounted for 88.87 % of the total variance in original data set.

Keywords: micronutrient uptake; mung bean; semi-arid; sewage sludge; yield

Introduction

Sewage sludge is the mixture of both inorganic and organic components from many sources (industries and domestic sewage), run-off from roads, storm water and other paved area, after physical, chemical and biological treatments. It is rich source of plant nutrient and organic matter (1, 2). The primary nutrients are found in range of sewage sludge varied as total nitrogen from 1.54 % to 1.92 %, phosphorus from 0.61 % to 0.92 % and potassium from 0.35 % to 0.43 % (3). Being the source of plant nutrients, it can be used as manure. Various positive effects have been observed by application of the different rates of the sewage sludge as manure for increasing productivity (4). Applying sewage sludge to soil not only addresses the challenge of waste disposal but also enhances soil health (5) and increasing the productivity of crop (6), as well as promotes a cleaner environment (7). Hence, application of sludge in agriculture has become an attractive disposal action. The use of varying rates of sewage sludge as manure has shown numerous positive effects on boosting productivity (4).

The rise in sewage sludge production is being driven by rapid industrialization and urbanization. Waste management has emerged as a significant environmental concern, with land application of sewage sludge considered the most effective disposal method. This approach enhances soil fertility and physical properties by supplying organic matter and recycling of plant nutrients and boosts crop yields (8). The Central Pollution Control Board (CPCB) has estimated that India generates a total of 62000 million liters of sewage per day however, capacity of the country wastewater treatment plant is only 23277 MLD, or 37 % of the sewage produced. Currently, India generates 42 million tons of solid waste per year, with per capita waste ranging from 200 to 600 kg per[1] (9).

In a predominantly vegetarian country like India, pulses serve as the primary source of dietary protein. The major pulse crops cultivated include pigeon pea, chickpea, black gram, green gram (mung bean), soybean, lentil, etc. Mung bean (*Vigna radiata* L.) is one of the most important and widely cultivated pulse crops globally. It is the cultural base of nourishment as it is low fat (1.3 %) source of protein (24-25 %), carbohydrate (56 %), fiber (4.1 %), calcium (124 mg per 100 g) and phosphorus (326 mg per 100 g) Confederation of Indian Industry (10). The total area, production and productivity of mung bean in India was 3.8 million hectares, 1.6 million tons and 420 kg ha^{-1}, respectively (11). In Haryana mung bean is grown in spring or summer during *Kharif* season. Being leguminous in nature it is grown for food, fodder and as green manuring crop. The use of sewage sludge in agriculture as an organic fertilizer is very beneficial for soil health and a variety of crops.

Many studies have investigated the impact of different nutrient management practices on soil properties and nutrient uptake, there is limited research on how sewage sludge application affects crop micronutrient uptake and yield in semi-arid climatic zones. Considering the above facts, in this study we hypothesized that micronutrients content and uptake in soil and mung bean, respectively, may be changed by application of the sewage sludge. Therefore, investigating the impact of different sewage sludge application rates on mung bean yield and micronutrient uptake would provide valuable insights into how sewage sludge influences micronutrient dynamics. Therefore, objective of the present study was to assess potential of the sewage sludge application to enhance mung bean yield, micronutrient uptake by the mung bean and soils micronutrient content in semi-arid regions of Northwestern India.

Materials and Methods

Experimental site

The experimental was conducted in north-western Haryana, located at a latitude of 29°16′N and a longitude of 75°7′E. A pot experiment was carried out on sandy soil (Typic Ustochrept) in the screen house of the Department of Soil Science at Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana, located in the semi-arid region of Northwestern India, during *Kharif* 2021-22. The study area experiences an average annual rainfall of 443 mm, mostly occurring between July and September during the southwest monsoon. The region has an average annual temperature of 24.8 °C and is located within the Indo-Gangetic Plains (IGPs). The soil used for the experiment was collected from a farmer's field in the village of Balsamand, Hisar. It was air-dried, crushed, passed through a 2 mm sieve and then 4 kg of soil was filled to each pot.

The experimental soil is neutral to slightly alkaline (pH_e = 8.5), non-saline (EC_e = 0.11 dS m^{-1}) and has low organic carbon content (OC = 0.30 %) having 0.01 % calcium carbonate ($CaCO_3$). The concentrations of diethylenetriaminepentaacetic acid (DTPA)-extractable micronutrients in the soil were 1.19 mg kg^{-1} for zinc (Zn), 0.28 mg kg^{-1} for copper (Cu), 3.89 mg kg^{-1} for iron (Fe) and 4.8 mg kg^{-1} for manganese (Mn).

Sewage sludge

Sewage sludge (SS) was brought from the sewage treatment plant of CCS HAU, Hisar. Before analysis, the sewage sludge was air dried, crushed and pass through 2 mm sieve. The pH of sewage sludge used in the pot-experiment was 7.7; electrical conductivity 3.0 dS m^{-1} and organic carbon 2.7 %. The N, P and K content in sewage sludge was found to be 1.24, 0.30 and 0.45 %, respectively and DTPA-extractable content of Zn, Cu, Fe and Mn was found to be 6.95, 5.31, 12.05 and 13.60 mg kg^{-1}, respectively.

Experimental details

In total twenty-one pots were filled soil, each pot having 4 kg of soil. The seven treatments consisting of T_1: 0 (without sewage sludge), T_2: 2.5 ton SS ha^{-1} which was equal to 4.4 g pot^{-1}, T_3: 5.0 ton SS ha^{-1} which was equal to 8.9 g pot^{-1}, T_4: 7.5 ton SSha^{-1} which was equal to 13.3 g pot^{-1}, T_5: 10 ton SSha^{-1} which was equal to 17.8 g pot^{-1}, T_6: 15 ton SSha^{-1} which was equivalent to 26.7 g pot^{-1} and T_7: 20 ton SSha^{-1} which was equivalent to 35.6 g pot^{-1} (Table 1). The soil and sewage sludge were mixed well. These pots were replicated thrice. The experiment was designed using a Completely Randomized Design (CRD).

In the first week of July, ten mung bean seeds (MH 421) were sown in each pot. Prior to sowing, a basal dose of N + P + K (urea: 28.5 g and KH_2PO_4: 64 g) was applied as a 50 ppm liquid solution to each pot. The mung bean crop was allowed to grow until maturity and was harvested on 5th October 2021. The grain yield from each pot was recorded (grams per pot). The straw yield was calculated by subtracting the grain yield from biological yield of each pot and recorded in grams per pot. After harvesting, plant and soil samples were collected from each pot. The plant samples were washed with a 0.2 % detergent solution, followed by 0.1 N hydrochloric acid (HCl) and double-distilled water (DDW), then dried in a hot air oven at 60 ± 2 °C for 48 hr. The soil samples were air-dried, crushed, passed through a 2 mm sieve and stored in polythene bags.

Chemical analysis

The soil $pH_{1:2}$ was measured using a glass electrode through the potentiometric method as described by Jackson (12). After the suspension settled, supernatant analyzed for the electrical conductivity ($EC_{1:2}$) by using conductometric method (12).

Soil organic carbon (SOC) content was determined using wet digestion method (13), which involved oxidizing the soil samples with 1N potassium dichromate ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4). The excess $K_2Cr_2O_7$ was titrated with a 0.5N standard ferrous ammonium sulphate solution using diphenylamine as an indicator and sodium fluoride as a catalyst to measure the oxidized organic carbon.

The dry plant samples and sewage sludge were grinded finely and then digested in a di-acid mixture ($HClO_4$:HNO_3 in a 4:1 v/v ratio). Soil and sewage sludge were extracted using DTPA (14) and the concentrations of Zn, Cu, Fe and Mn were determined using an atomic absorption spectrophotometer (UNICAM-969). The DTPA-extractable micronutrients (Zn, Cu, Fe and Mn) in the digests were measured using atomic absorption spectrophotometry.

Table 1. The treatment details of sewage sludge

Treatment	Symbol	Sewage sludge	
		Rate of application (g pot^{-1})	Equivalent ton ha^{-1}
T_1	Control	0	0
T_2	$SS_{2.5}$*	4.4	2.5
T_3	SS_5	8.9	5.0
T_4	$SS_{7.5}$	13.3	7.5
T_5	SS_{10}	17.8	10
T_6	SS_{15}	26.7	15
T_7	SS_{20}	35.6	20

*SS: sewage sludge.

Statistical analysis

The statistical analysis of the data for comparing mean and correlation among different soil parameters were performed using the SPSS version 20 software (SPSS Inc., Chicago, IL) (15). The significance of differences between treatments was determined using Duncan's multiple range test (DMRT) at a 95 % confidence interval. The principal component analysis (PCA) was conducted to assess variance explained by principal components (PCs) using the Origin (Pro) Version 2024 (16). The most significant two principal components, which accounted for the largest portion of the variance, were displayed visually on a two-dimensional plot.

Results and Discussion

Yield parameters

The number of branches $plant^{-1}$, pods $plant^{-1}$, plant height and pod length increased with higher rates of sewage sludge application (Fig. 1). All treatments showed significant increment in these parameters as compared to the control. The highest number of branches $plant^{-1}$ (10.00) was observed in treatment T_7 (SS_{20}), while the lowest (4.33) was recorded in the control (T_1) where no sewage sludge was applied. The treatment T_7 was statistically like T_6 (SS_{15}). Similarly, the maximum number of pods $plant^{-1}$ (22.66) was recorded in T_7 (SS_{20}) and the minimum (7.63) was recorded in the control. Treatment T_4 ($SS_{7.5}$) was statistically similar to T_5 (SS_{10}) and T_6 (SS_{15}). The significant increases in branches, pods, plant height and pod length can be attributed to the higher sludge application rates, which enhanced organic matter content and improved soil physical properties such as aggregate stability, reduced bulk density, increased water-holding capacity and greater nutrient availability (17). Similar findings have been reported in the previous studies (18, 19). As organic matter of sewage sludge breaks down, it steadily releases nutrients, ensuring a consistent availability of essential elements for plants during the growing season. Nitrogen, especially, supports vegetative development by stimulating cell division and elongation, which contributes to increased plant height and improved branching (20).

The plant height and pod length varied from 41.00 to 60.00 cm and 5.00 to 11.07 cm, respectively. The height of plant and pod length recorded maximum (60.00 and 11.07 cm, respectively) in treatment T_7 (SS_{20}) and minimum (41.00 and 5.00 cm, respectively) in treatment T_1 (control). This may be due to the nutrient from sewage sludge were more readily available and better utilized and balanced nutrient availability aids in pod formation and filling, leading to longer pods and a higher number of pods per plant. Organic amendments such as sewage sludge further enhance this process by stimulating metabolic functions and enzyme activities associated with pod development (21). The pod length of treatment T_6 (SS_{15}) was at par with T_5 (SS_{10}). The number of branch $plant^{-1}$, pod $plant^{-1}$, pod length and plant height in treatment T_7were found 2.31, 2.97, 2.21 and 1.46 times higher as compared to control, respectively. This improvement could be attributed to the enhanced rate of nutrient release in the soil, facilitated by the decomposition of sludge (22, 23).

Grain yield

The mung bean grain yield (Fig. 2) increased significantly with application of various rates of sewage sludge to the soil, showing an upward trend with each incremental sludge application. Grain yield ranged from 4.43 to 6.42 g pot^{-1}, with the highest yield recorded in treatment T_7 (SS_{20}), representing a 44.9 % increase over the control (T_1). This may be due to sewage sludge provides substantial quantities of vital nutrients such as particularly nitrogen, phosphorus and potassium that directly promote plant growth and grain development. These nutrients contribute to vigorous vegetative growth, increasing biomass or straw yield, while also enhancing reproductive processes, thereby improving overall grain yield (24). But lowest grain yield (4.43 g pot^{-1}) was observed in the control treatment without sludge application. However, higher rates of sewage sludge (T_7: SS_{20}) did not show a significant advantage over treatments T_5 (SS_{10}) and T_6 (SS_{15}). The grain yield in T_6 (SS_{15}) was statistically similar to T_4, T_5 and T_7. The increase in grain yield may be attributed to an enhanced rate of the nutrient release from higher sludge doses, which likely improved the soil's oxidation status. A similar trend was observed, with rice yield increasing by 60, 111, 125, 134 and 137 % at sewage sludge

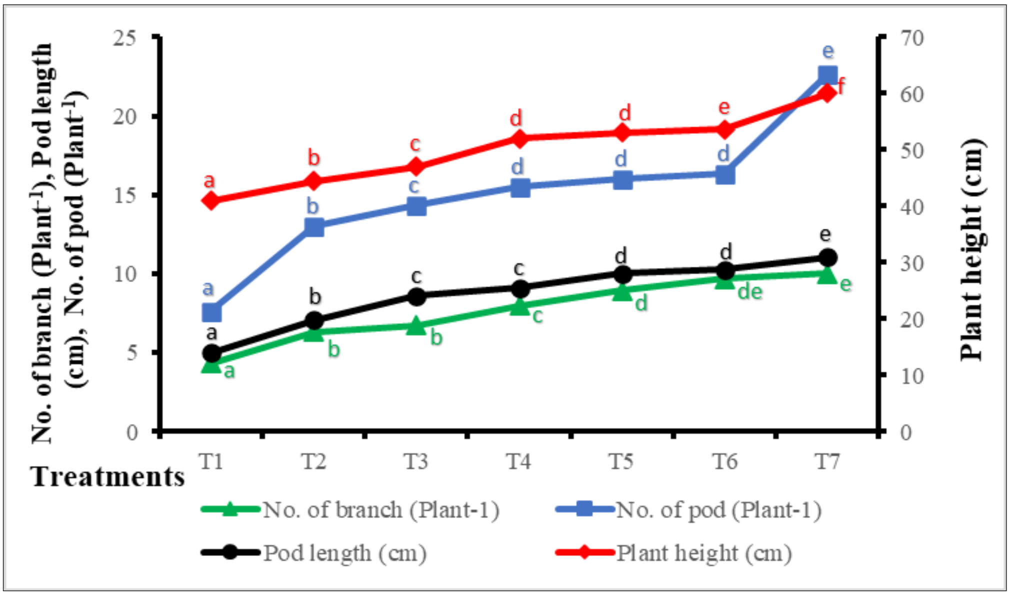

Fig. 1. Impact of different sewage sludge application rates on the yield characteristics of mung bean in semi-arid regions of Northwestern India. Mean value with same letters were non-significant ($p < 0.05$). The values with different letter were significantly different at $p < 0.05$. The treatments: T_1 - control (no sewage sludge); T_2 - 2.5 t ha^{-1} sewage sludge (SS); T_3 - 5.0 t ha^{-1} SS; T_4 - 7.5 t ha^{-1} SS; T_5 - 10 t ha^{-1} SS; T_6 - 15 t ha^{-1} SS; T_7 - 20 t ha^{-1} SS.

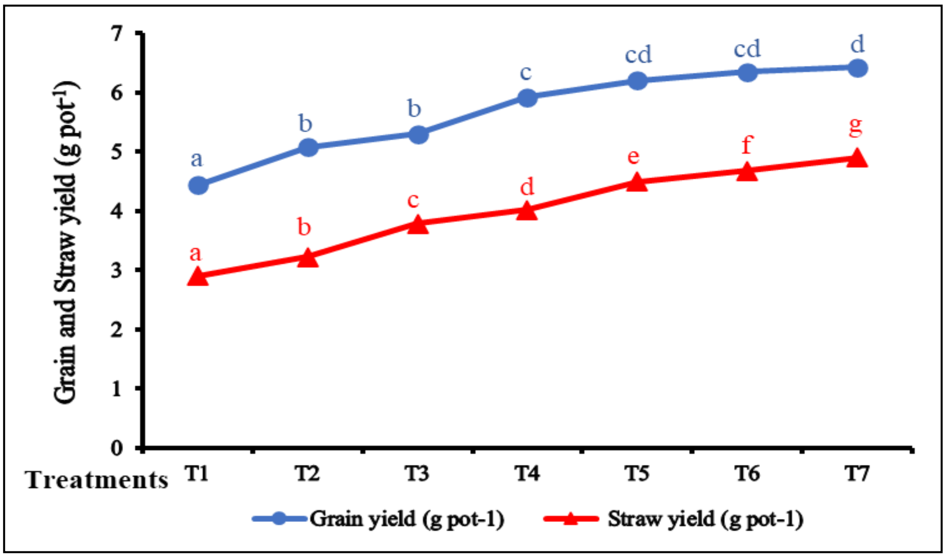

Fig. 2. Impact of different sewage sludge application rates on grain and straw yields of mung bean in the semi-arid regions of Northwestern India. Mean value with same letters were non-significant ($p < 0.05$). The values with different letter were significantly different at $p < 0.05$. The treatments: T_1 - control (no sewage sludge); T_2 - 2.5 t ha^{-1} sewage sludge (SS); T_3 - 5.0 t ha^{-1} SS; T_4 - 7.5 t ha^{-1} SS; T_5 - 10 t ha^{-1} SS; T_6 - 15 t ha^{-1} SS; T_7 - 20 t ha^{-1} SS.

amendment rates of 3, 4.5, 6, 9 and 12 kg m^{-2}, respectively, compared to the control (17). Comparable increases in grain yield have also been reported with sewage sludge applications (25, 26).

Straw yield

The mung bean straw yield (Fig. 2) increased significantly with sewage sludge application, ranging from 2.90 to 4.90 g pot^{-1}. The highest straw yield (4.90 g pot^{-1}) was observed in T_7 (SS_{20}), which was higher than T_6 (SS_{15}) by 4.7 %, T_5 (SS_{10}) by 9.1 %, T_4 ($SS_{7.5}$) by 21.9 %, T_3 (SS_5) by 29.6 %, T_2 ($SS_{2.5}$) by 52.2 % and T_1 (control) by 68.9 %. A significant increase in straw yield was observed across treatments, from T_1 (control) to T_7 (SS_{20}). This improvement in straw yield with increasing sewage sludge application rates may be attributed to higher organic carbon content and enhanced cation exchange capacity (CEC) of the soil. And, the slow breakdown of organic matter in sewage sludge ensures a steady release of nutrients throughout the mung bean growing season, supporting uniform and vigorous plant growth (27). These factors likely created a more favourable environment for root growth, improved nutrient availability (both macro and micro) and promoted overall plant growth. Similar positive effects of sewage sludge on straw yield have also been reported (17, 23, 28).

Properties of post-harvest soil

Soil pH

The soil pH ranged between 8.02 and 8.47 (Table 2). While in control (T_1) pot, soil pH decreased to 8.47 (in control) from its initial value (8.50) and all treatments with sewage sludge application exhibited further reductions in pH as the application rate increased. The lowest pH (8.02) was recorded in the treatment T_7, which received the highest sludge application rate of 20 t ha^{-1}. This decrease in soil pH is likely due to the high organic matter content in sewage sludge, which, upon decomposition, releases organic acids such as humic acid, thereby lowering the pH. Sewage sludge typically contains considerable levels of ammonium (NH_4^+), which is converted by soil microbes into nitrate (NO_3-) through nitrification. This process releases hydrogen ions (H^+), leading to soil acidification and a decrease in pH, particularly when sludge is applied in large quantities (29). Similar findings have also been reported (30-32).

Electrical conductivity

The EC of the soil increased slightly in all treatments from its initial value of 0.11 dS m^{-1}, except in the control (T_1) and T_2, which had EC values of 0.09 and 0.10 dS m^{-1}, respectively (Table 2). The

Table 2. Effect of the different rates of sewage sludge application on properties of post-harvest soil and DTPA extractable micronutrient (Zn, Cu Fe and Mn) content of soil (mean of three replicates) in semi-arid regions of Northwestern India

Treatment	pH	EC (dS m^{-1})	OC (%)	Available micronutrients in post-harvest soil (mg kg^{-1})			
				Zn	Cu	Fe	Mn
T_1	8.47 ± 0.13^{a}	0.09 ± 0.01^{a}	0.42 ± 0.01^{a}	1.06 ± 0.02^{a}	0.27 ± 0.01^{a}	3.06 ± 0.21^{a}	3.80 ± 0.26^{a}
T_2	8.43 ± 0.22^{a}	0.10 ± 0.01^{a}	0.51 ± 0.01^{b}	1.46 ± 0.09^{b}	0.35 ± 0.01^{ab}	4.00 ± 0.10^{b}	4.96 ± 0.07^{b}
T_3	8.37 ± 0.09^{a}	0.12 ± 0.00^{ab}	0.56 ± 0.03^{c}	1.76 ± 0.09^{c}	0.43 ± 0.01^{ab}	4.36 ± 0.06^{b}	5.30 ± 0.12^{bc}
T_4	8.30 ± 0.06^{a}	0.13 ± 0.01^{bc}	0.58 ± 0.02^{c}	1.93 ± 0.09^{cd}	0.57 ± 0.02^{ab}	5.16 ± 0.18^{c}	5.63 ± 0.09^{c}
T_5	8.20 ± 0.17^{a}	0.14 ± 0.01^{cd}	0.64 ± 0.03^{d}	2.16 ± 0.09^{de}	0.73 ± 0.05^{b}	5.93 ± 0.09^{d}	6.06 ± 0.09^{d}
T_6	8.17 ± 0.20^{a}	0.14 ± 0.01^{cd}	0.74 ± 0.01^{e}	2.36 ± 0.08^{ef}	1.41 ± 0.13^{c}	6.33 ± 0.09^{de}	6.50 ± 0.06^{e}
T_7	8.02 ± 0.03^{a}	0.16 ± 0.01^{d}	0.84 ± 0.01^{f}	2.56 ± 0.08^{f}	2.63 ± 0.32^{d}	6.66 ± 0.12^{e}	6.93 ± 0.09^{f}

Mean ± standard error. Mean value with same letters were non-significant ($p < 0.05$). The values with different letter were significantly different at $p < 0.05$. The treatments: T_1 - control (no sewage sludge); T_2 - 2.5 t ha^{-1} sewage sludge (SS); T_3 - 5.0 t ha^{-1} SS; T_4 - 7.5 t ha^{-1} SS; T_5 - 10 t ha^{-1} SS; T_6 - 15 t ha^{-1} SS; T_7 - 20 t ha^{-1} SS.

soil EC increased from 0.09 dS m^{-1} (control) to 0.16 dS m^{-1} (T_7: SS_{20}) with increasing rates of the sewage sludge application from 0 to 20.0 t ha^{-1}. The minimum soil EC (0.09 dS m^{-1}) was recorded in the control (T_1), while the maximum EC was recorded in treatment T_7 with the highest sludge application rate of 20.0 t ha^{-1}. The soil EC increased significantly in all treatments compared to the control, except in T_2. The increase in soil EC may be attributed to the accumulation of soluble salts and presence of alkaline substances in sewage sludge (33). Similar increases in soil EC with sewage sludge application have been reported in the earlier studies (34). With the higher application rates, greater amounts of salts were added to the soil, leading to a proportional and occasionally exponential increase in EC of soil (35).

Organic carbon

The application of varying rates of sewage sludge significantly increased the OC content in the soil from 0.42 % to 0.84 % (Table 2). All treatments showed a significant increase in OC compared to the control, while treatments T_3 (SS_5) and T_4 ($SS_{7.5}$) being statistically similar. The highest OC content (0.84 %) was observed in treatment T_7, which received the maximum sludge application rate of 20.0 t ha^{-1}. The increase in soil OC content is likely due to the organic matter present in the sewage sludge and a considerable portion of the organic carbon in sewage sludge resists rapid mineralization and instead becomes part of stable soil organic matter (humus), gradually contributing to long-term increases in soil organic carbon (36). Similar enhancements in soil organic carbon with sewage sludge application have been reported in the earlier studies (37-39).

DTPA-extractable micronutrients in post-harvest soil

The content of DTPA-extractable micronutrients (Zn, Cu, Fe and Mn) in soil significantly increased across all treatments compared to the control with higher rates of sewage sludge application (Table 2). In the control, the content of these micronutrients declined compared to their initial concentration, whereas all the other treatments showed an increment in soil. Specifically, in the control, Zn decreased from 1.19 to 1.06 mg kg^{-1}, Cu from 0.28 to 0.27 mg kg^{-1}, Fe from 3.89 to 3.06 mg kg^{-1} and Mn from 4.80 to 3.08 mg kg^{-1} (Table 2). The DTPA-extractable Zn content ranged from 1.06 to 2.56 mg kg^{-1}, with the highest level observed in treatment T_7 (SS_{20}). Treatment T_6 showed similar results to T_5 and T_7. For DTPA-extractable Cu, the content ranged from 0.28 to 2.63 mg kg^{-1}, with the maximum observed in T_7 (2.63 mg kg^{-1}), where the highest sewage sludge rate (20.0 t ha^{-1}) was applied. The DTPA-extractable Cu concentration increased significantly across all treatments compared to the control, except for T_2, T_3 and T_4. The DTPA-extractable Fe content in post-harvest soil varied from 3.06 to 6.66 mg kg^{-1}, with the highest recorded in T_7 (6.66 mg kg^{-1}) and the lowest in T_1 (3.06 mg kg^{-1}). The soil under treatment T_6 showed similar Fe content to T_7. In soil content of DTPA-extractable Mn, ranged was 3.80 to 6.93 mg kg^{-1}, with the highest concentration in T_7 (6.93 mg kg^{-1}) and the lowest in T_1 (3.80 mg kg^{-1}), where no sewage sludge was applied. The increased concentration of DTPA-extractable Zn, Cu, Fe and Mn in the soil with higher rates of sewage sludge application can be attributed to the significant quantities of these micronutrients in the sludge, which led to their accumulation in the soil. This pattern is consistent with previous findings (25, 40, 41). The breakdown of sewage sludge can lead to a reduction in soil pH, which in turn increases the solubility and availability of DTPA-extractable micronutrients (Zn, Cu, Fe and Mn) by releasing metals bound to organic matter. This process enhances plant uptake and increases the overall micronutrient content in the soil (42). Additionally, a polynomial relationship of SOC was observed with DTPA-extractable Zn (R^2 = 0.926), Mn (R^2 = 0.962), Fe (R^2 = 0.943) and Cu (R^2 = 0.900) in soils after mung bean harvest (Fig. 3).

Micronutrient uptake in grain and straw

Zn uptake in grain and straw

The Zn uptake in grain ranged from 97.11 to 195.05 µg pot^{-1} (Fig. 4A), with the highest value (195.05 µg pot^{-1}) recorded in treatment T_7 (SS_{20}) and the lowest (97.11 µg pot^{-1}) in T_1 (control, no sewage sludge). The T_7 treatment (SS_{20}) increased Zn uptake by 2.01 times compared to T_1. The Zn uptake in T_7 was significantly greater than in T_1 (control), T_2 ($SS_{2.5}$), T_3 (SS_5), T_4 ($SS_{7.5}$) and T_5 (SS_{10}), while at par with T_6 (SS_{15}). In mung bean straw (Fig. 4B), Zn uptake ranged from 48.91 to 122.41 µg pot^{-1}, with the highest (122.41 µg pot^{-1}) observed in T_7 (SS_{20}) and the lowest in T_1 (control, no sewage sludge applied). The uptake of Zn was significantly higher in treatments T_3 (SS_5), T_4

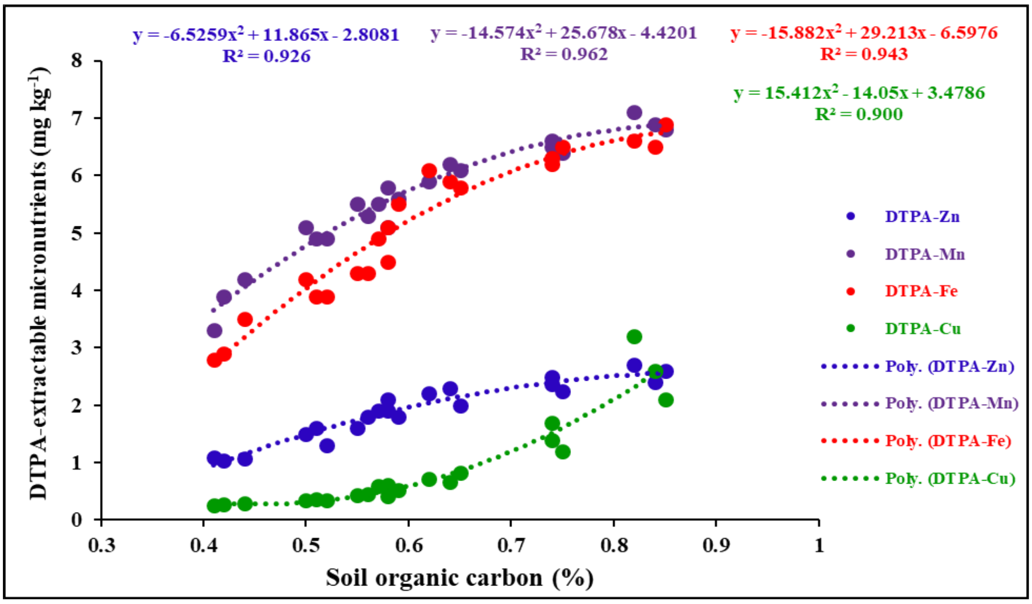

Fig. 3. Relationship between soil organic carbon (SOC) and DTPA-extractable micronutrients (Zn, Mn, Fe and Cu) in soils after harvesting of mung bean in semi-arid regions of Northwestern India.

($SS_{7.5}$), T_5 (SS_{10}), T_6 (SS_{15}) and T_7 (SS_{20}) compared to T_1, while T_2 ($SS_{2.5}$) showed similar results to T_1. The T_7 (SS_{20}) treatment showed 150.3 % higher Zn uptake as compared to T_1. In the treatment T_7, Zn uptake was significantly higher than in T_1, T_2, T_3, T_4 and T_5, but at par with T_6. The enhanced Zn uptake in grain and straw can be attributed to the high concentration of micronutrients in sewage sludge, which likely forms chelated complexes, releasing nutrients in the soil to meet plant requirements as needed (2). This may be because compounds present in sewage sludge enhance zinc bioavailability by inhibiting its precipitation and adsorption onto soil minerals. Dissolved organic fractions, including organic acids, humic acids and fulvic acids, increasing Zn mobility in the soil and promoting its uptake by plant roots (43).

Cu uptake in grain and straw

The application of sewage sludge had a significant impact on Cu uptake in mung bean grain (Fig. 4A). The highest Cu uptake by grain (32.33 µg pot^{-1}) was observed in treatment T_7 (SS_{20}), while the lowest uptake (17.52 µg pot^{-1}) was recorded in T_1, where no sewage sludge was applied. Treatment T_7 was statistically similar to T_5 and T_6 but showed significantly higher Cu uptake as compared to T_1, T_2, T_3 and T_4. Similarly, T_5 was at par with T_4, T_6 and T_7 but treatment T_4 significantly differed from T_1, T_2, T_3, T_6 and T_7. In mung bean straw, Cu uptake ranged from 10.15 to 23.34 µg pot^{-1} (Fig. 4B). The highest Cu uptake (23.34 µg pot^{-1}) in straw was also observed in T_7 (SS_{20}), while the lowest was in T_1 (no sewage sludge). Treatment T_7 exhibited significantly higher Cu uptake than all other treatments and an increasing trend in Cu uptake was observed as the sewage sludge dose increased from 0 to 20 t ha^{-1}. This might be due to organic constituents in sewage sludge which promote the formation of soluble and exchangeable Cu fractions. As the organic matter breaks down, Cu that was initially bound to organic compounds is gradually released into more plant-available forms, enhancing its uptake by mung bean root (44). This increase in Cu uptake in both grain and straw can be attributed to enhanced micronutrient availability due to sludge application. Similar findings have also been reported (45), showing increased Cu uptake in wheat grain and straw with sewage sludge application.

Fe uptake in grain and straw

The uptake of Fe by both grain and straw increased significantly across all treatments with the application of varying rates of sewage sludge (Fig. 4). The highest Fe uptake in grain (455.17 µg pot^{-1}) was recorded in treatment T_7 (SS_{20}), while the lowest was recorded in T_1 (control). The treatment T_7 exhibited significantly

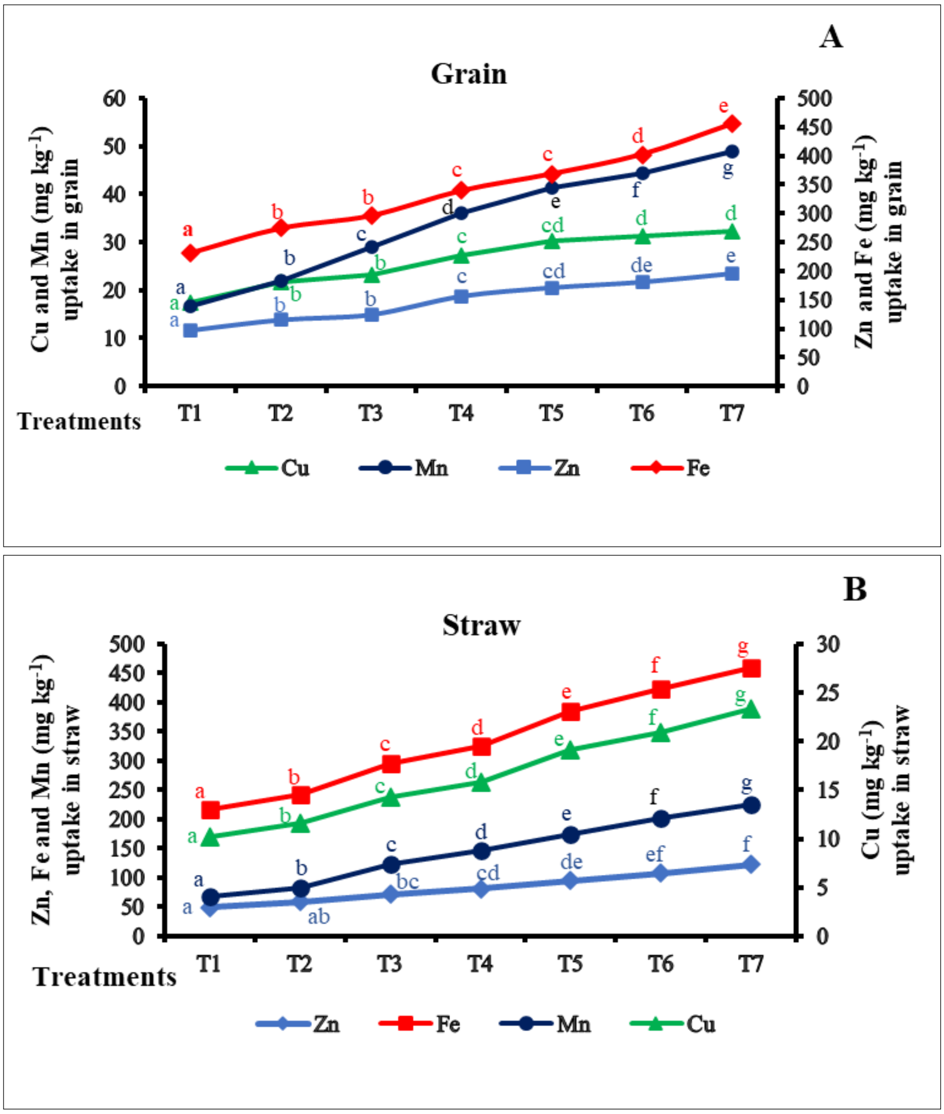

Fig. 4. Impact of different sewage sludge application rates on micronutrients uptake (mg kg^{-1}) in mung bean grain (A) and straw (B) in semi-arid regions of Northwestern India. Mean value with same letters were non-significant ($p < 0.05$). The values with different letter were significantly different at $p < 0.05$. The treatments: T_1 - control (no sewage sludge); T_2 - 2.5 t ha^{-1} sewage sludge (SS); T_3 - 5.0 t ha^{-1} SS; T_4 - 7.5 t ha^{-1} SS; T_5 - 10 t ha^{-1} SS; T_6 - 15 t ha^{-1} SS; T_7 - 20 t ha^{-1} SS.

greater Fe uptake as compared to all other treatments, showing that Fe uptake in grain increased with increasing sewage sludge application rates (Fig. 4A). In mung bean straw (Fig. 4B), Fe uptake ranged from 216.53 to 459.15 µg pot^{-1}, with the highest concentration (459.15 µg pot^{-1}) in treatment T_7 (SS_{20}) and the lowest (216.53 µg pot^{-1}) recorded in treatment T_1. Increasing the sewage sludge application from 0 to 20 t ha^{-1}, T_7 resulted in the highest Fe uptake among all other treatments. Applying sewage sludge often results in a decrease in soil pH. Since Fe becomes more soluble and bioavailable under acidic conditions, this pH reduction enhances the availability of Fe in forms that are more readily absorbed by mung bean roots (46). The higher sewage sludge doses may enhance the availability of micronutrients by facilitating their gradual adsorption and release for plant uptake. Similar findings have been reported (45), indicating increased Fe uptake with sewage sludge application.

Mn uptake by grain and straw

The uptake of Mn by mung bean grain (Fig. 4A) ranged from 16.58 to 48.81 µg pot^{-1}. The highest uptake of Mn (48.81 µg pot^{-1}) was observed with treatment T_7 (SS_{20}), which showed a 1.94 times higher uptake of Mn as compared to T_1 (no sewage sludge). Treatment T_7 significantly enhanced Mn uptake in grain compared to all other treatments and all treatments showed higher Mn uptake than the control. With increasing sludge application, the total input of Mn to the soil increases accordingly. This expands the nutrient reservoir available for plant uptake, leading to greater accumulation of Mn in both the grain and straw (47). This may be due to that increased decomposition of sewage sludge at higher doses likely improved Mn availability in soil. The uptake of Mn in mung bean straw (Fig. 4B), ranged from 68.63 to 225.24 µg pot^{-1}. The lowest uptake (68.63 µg pot^{-1}) was recorded in the control (T_1), while the highest (225.24 µg pot^{-1}) was observed in T_7 (SS_{20}), representing a 2.28 times higher Mn uptake than T_1. The treatment T_6 (SS_{15}) and T_5 (SS_{10}) also showed 1.94 and 1.55 times, respectively, higher Mn uptake in straw as compared to T_1. These findings resulted that higher sewage sludge application gradually enhanced the micronutrient availability for plant uptake. Studies have reported that sewage sludge application improves Mn uptake (45) and enhanced the nutrient release pattern in the soil. This may be explained the increased micronutrient uptake in both grain and straw of mung bean when sewage sludge was applied. Additionally, the soil micronutrients content enhanced by the application of sewage sludge (48).

Correlation between soil pH, EC, SOC, DTPA-extractable micronutrients and yield of mung bean crop after harvesting

The correlation matrix was developed to analyze the relationships between soil pH, EC, SOC, DTPA-extractable micronutrients (Zn, Mn, Fe and Cu) and the yield of mung bean crop after harvest (Table 3). The results revealed that soil pH had a significant negative correlation with soil EC (r = -0.455*; $p < 0.05$), SOC (r = -0.479*; $p < 0.05$), DTPA-extractable Zn (r = -0.534*; $p < 0.05$), Mn (r = -0.486*; $p < 0.05$), Fe (r = -0.589**; $p < 0.01$), Cu (r = -0.472*; $p < 0.05$), grain yield (r = -0.533*; $p < 0.05$) and straw yield (r = -0.553**; $p < 0.01$). Conversely, soil EC was positively and significantly correlated with SOC (r = 0.836**; $p < 0.01$), DTPA-extractable Zn (r = 0.852**; $p < 0.01$), Mn (r = 0.868**; $p < 0.01$), Fe (r = 0.866**; $p < 0.01$), Cu (r = 0.707**; $p < 0.01$), grain yield (r = 0.893**; $p < 0.01$) and straw yield (r = 0.886**; $p < 0.01$). Similarly, SOC also exhibited a strong positive correlation with DTPA-extractable Zn (r = 0.894**; $p < 0.01$), Mn (r = 0.938**; $p < 0.01$), Fe (r = 0.922**; $p < 0.01$), Cu (r = 0.888**; $p < 0.01$), grain yield (r = 0.856**; $p < 0.01$) and straw yield (r = 0.938**; $p < 0.01$). All DTPA-extractable micronutrients (Zn, Mn, Fe and Cu) were significantly positively correlated ($p < 0.01$) with each other and also with grain and straw yields of the mung bean crop. Furthermore, the grain yield showed a significant positive correlation with straw yield (r = 0.938**; $p < 0.01$) of the mung bean crop.

Principal component analysis (PCA)

Principal component analysis (PCA) plots display the positions of different treatments (Fig. 5A) and various soil properties (Fig. 5B) in orthogonal space. The PCA of different soil properties-such as pH, EC, SOC, grain and straw yield, DTPA-extractable micronutrients (Zn, Mn, Fe and Cu) and their uptake in mung bean grain and straw revealed that the first two principal components (PCs) had eigenvalues greater than 1.0 and accounted for 88.87 % of the total variance in the dataset (Table 4). PC1 had three variables with high weightings: Fe, straw yield and SOC with loading values of -0.260, -0.260 and -0.259, respectively. PC1 explained 84.13 % of the total variance in the data, with an eigenvalue of 14.30. PC2 explained 4.74 % of the variance, with an eigenvalue of 1.16. The highest loading value for PC2 was 0.788 for soil pH, followed by -0.333 for SOC and 0.331 for copper uptake in grain. The variance explained by each PC varied between 0.05 % and 0.95 %.

Table 3. Correlation matrix (Pearson's correlation cofficients) depicting realtionship between soil variables of post-harvest soil, DTPA-extractable miceonutrients in soil and mung bean yield in semi-arid regions of Northwestern India

Variables	pH	EC	SOC	DTPA-Zn	DTPA-Mn	DTPA-Fe	DTPA-Cu	Grain yield	Straw yield
pH	1								
EC	-0.455*	1							
SOC	-0.479*	0.836**	1						
DTPA-Zn	-0.534*	0.852**	0.894**	1					
DTPA-Mn	-0.486*	0.868**	0.938**	0.952**	1				
DTPA-Fe	-0.589**	0.866**	0.922**	0.954**	0.963**	1			
DTPA-Cu	-0.472*	0.707**	0.888**	0.781**	0.789**	0.760**	1		
Grain yield	-0.533*	0.893**	0.856**	0.929**	0.948**	0.956**	0.672**	1	
Straw yield	-0.553**	0.886**	0.938**	0.966**	0.959**	0.979**	0.776**	0.938**	1

EC: electrical conductivity; SOC: soil organic carbon; DTPA: diethylenetriamine pentaacetate; Zn: zinc; Mn: manganese; Fe: iron; Cu: copper.

*Correlation is significant at $p < 0.05$ level (2 tailed).

**Correlation is significant at $p < 0.01$ level (2 tailed).

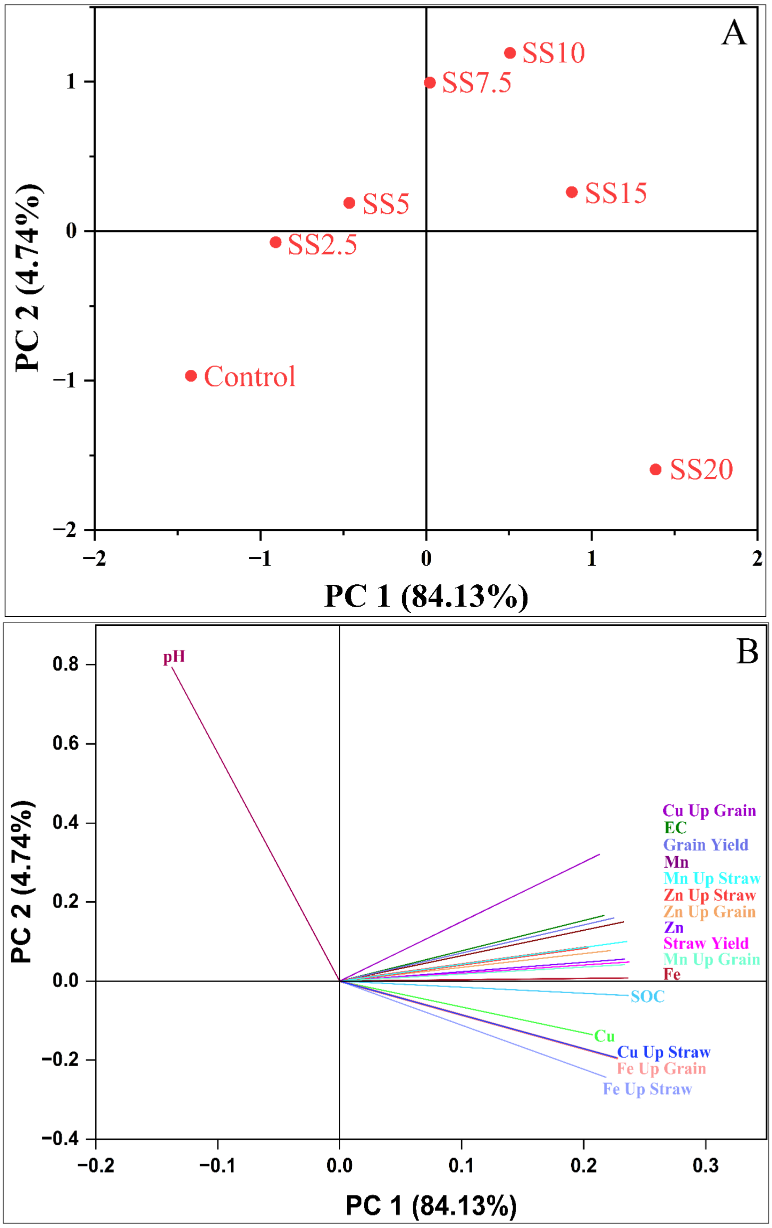

Fig. 5. The plots of principal component analysis (PCA) on the treatments (A) and soil properties (B) including soil pH, electrical conductivity (EC), soil organic carbon (SOC), grain and straw yield, DTPA-extractable micronutrients (Zn: zinc; Mn: manganese; Fe: iron; Cu: copper) and their uptake (Up) in grain and straw of mung bean in semi-arid regions of Northwestern India. PC: principal component; SS: sewage sludge.

Table 4. Loading values of soil variables and percentage contribution of principal components on the axis determined by principal component analysis (PCA)

Soil variables	PC1		PC2	
	Loading values	Contribution of variables (%)	Loading values	Contribution of variables (%)
pH	0.153	2.334	0.788	62.143
EC	-0.237	5.621	0.172	2.957
SOC	-0.259	6.686	-0.333	0.110
Zn	-0.256	6.561	0.060	0.362
Mn	-0.255	6.521	0.156	2.441
Fe	-0.260	6.740	0.016	0.025
Cu	-0.225	5.049	-0.138	1.902
Grain yield	-0.248	6.129	0.169	2.865
Straw yield	-0.260	6.772	0.055	0.299
Zn uptake grain	-0.243	5.895	0.080	0.646
Zn uptake straw	-0.222	4.923	0.087	0.765
Mn uptake grain	-0.257	6.597	0.049	0.241
Mn uptake straw	-0.258	6.646	0.107	1.142
Fe uptake grain	-0.249	6.188	-0.192	3.680
Fe uptake straw	-0.239	5.719	-0.242	5.854
Cu uptake grain	-0.233	5.452	0.331	10.964
Cu uptake straw	-0.248	6.167	-0.190	3.605
Eigen value		14.30		1.16
Variability (%)		84.13		4.74
Cumulative variability (%)		84.13		88.87

PC: principal component; EC: electrical conductivity; SOC: soil organic carbon; Zn: zinc; Mn: manganese; Fe: iron; Cu: copper.

Conclusion

The results indicated that the use of sewage sludge boosted the content and uptake of micronutrients in both soil and mung bean plants, respectively. Moreover, the application of sewage sludge significantly improved both the grain and straw yields of mung bean. Our results also revealed that increasing the amount of sewage sludge applied enhances the soil's organic carbon content, which seems to have a complementary role in managing it in agriculture. The multivariate analysis (PCA) effectively identified soil variables that significantly responded to the increased application of sewage sludge. The effective rate of sewage sludge application can improve mung bean yield, as well as micronutrient uptake and soil content. This research advocates for the use of sewage sludge to improve soil micronutrients content and their uptake and providing valuable insights for policymakers. This study also encourages farmers, particularly in semi-arid regions, to adopt sewage sludge application with 20 t ha^{-1} being the most effective rate for increasing micronutrient availability, yield and micronutrients uptake in mung bean crops.

Acknowledgements

Thanks are due to the Head, Department of Soil Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, for providing necessary laboratory and pot facilities. The guidance and financial assistance for this research from authorities of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India is thankfully acknowledged.

Authors' contributions

R conceptualized the study and, together with RK and A, developed the methodology. R and RK carried out the validation, while R, A and PK performed the formal analysis. Data curation was managed by R, RK and Y. R, A and Y wrote the original draft of the paper and PK, Y and HSJ handled the review and editing. Supervision was provided by R, RK, PK and HSJ. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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