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RESEARCH ARTICLE

Effect of sludge amelioration on yield, accumulation and translocation of heavy metals in soybean grown in acid and alkaline soils

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Abstract

A greenhouse pot experiment was conducted with seven different levels of sludge (0, 5, 10, 20, 40, 80, 160 g kg^{-1}) to assess the potential impact of sludge application on soybean (*Glycine max* (L.) Merr.) productivity, metal accumulation and translocation, and physico-chemical changes in acid and alkaline soils. The outcomes revealed that the application of sludge @ 5.0 to 160 g kg^{-1} resulted in a significant ($p < 0.05$) increase in seed and straw yield in both acid and alkaline soils compared to control. All the assessed heavy metals in soybean were within permissible ranges and did not exceed the phytotoxic limit, except for Fe, Zn, and Cu in the roots from the application of sewage sludge. The values of bioaccumulation factor ($BF_{root/soil}$) and translocation factor i.e., $TF_{straw/root}$ and $TF_{seed/straw}$ were < 1.0 for Ni, Pb and Cr. Overall, for all the sludge application doses the soil pH was observed to increase in the acid soil and decline in alkaline soil when compared to the control. All the investigated heavy metals (Fe, Mn, Zn, Cu, Ni, Cd, Pb, and Cr) in the different plant tissues (root, straw and seed) of soybean were correlated with the soil variables. The study finds that sludge can be a potential organic fertilizer and function as an eco-friendly technique for the recycling of nutrients in the soil while keeping a check on the heavy metals' availability to plants.

Keywords Micronutrients · Pollutant elements · Soybean · Sludge · Translocation factor

Introduction

Sludge is a waste material produced when sewage treatment plants process municipal wastewater (Golui et al. 2016; Choudhary et al. 2022). A considerable increase in the production of sludge has been documented in recent years as a result of rapid population growth, urbanization, and industrialization. The safe management and disposal of sludge are one of the major worldwide challenges that must be addressed immediately (Krahn et al. 2023). According to CPCB (2021), India's total daily sewage produced by metropolitan areas is predicted to reach 72,368 MLD, while the nation's treatment facilities can currently only handle 31,841 MLD, or 43.9%, of that amount. In India, 615 sewage treatment facilities (STPs) are now operating throughout the nation. The total amount of sewage sludge produced daily from all STPs is about 120,000 metric tonnes (DJB 2021). Sludge was mainly rich in organic matter (OM), including nitrogen (N), phosphorus (P), potassium (K) and micronutrients, which are crucial for plant growth and development (Majhi et al. 2022).

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Apart from essential nutrients, a substantial number of pollutants and pathogenic microbes, such as *Escherichia coli* were reported to be present in sludge originating from different sources (Roy et al. 2019; Devi et al. 2021). Pollutant levels in sludge are mainly influenced by the source from where it is received. When compared to sludge generated from industrial effluents, domestic sludge contains less heavy metal. The variance in heavy metal load in sludge is also determined by the nature of industries (Roy et al. 2013). Sludge produced from STPs varies considerably in terms of heavy metal content, *viz*. copper (Cu: 105–1058 mg kg^{-1}), zinc (Zn: 134–2821 mg kg^{-1}), cadmium (Cd: <3.0–154 mg kg^{-1}), nickel (Ni :< 25.0–518 mg kg^{-1}) and lead (Pb: 41.2–413 mg kg^{-1}) (Saha et al. 2017; Choudhary et al. 2022). Apart from the sources, significant differences in heavy metal content in sludge concerning seasons have also been reported (Roy et al. 2013).

The use of sludge in agriculture is a cost-effective technique that provides OM to boost crop productivity, improve soil health, and reduce soil erosion (Verma et al. 2021). Sludge application has been shown to increase yields of a wide range of field crops (for example rice, wheat, maize, sorghum, and barley), as well as vegetables such as broad bean, cucumber, palak, and others (Sharma et al. 2018; Eid et al. 2019; Verma et al. 2021). However, the presence of trace harmful heavy metals and metalloids in sludge limits its usage in agriculture. The usage of sludge in agriculture has recently drawn a lot of attention due to its high toxicity in soil, availability in plants' systems, and persistence in the environment. Concerns about heavy metal toxicity to plant and soil microbial populations as well as food chain contamination arise from the long-term use of sludge in agriculture (Gomes et al. 2019). The food cultivated in the urban and peri-urban areas may surpass statutory and advisory limitations, whether assessed as the heavy metal concentration in produce or stated as anticipated daily intakes, according to a growing body of evidence (Rattan et al. 2005; Meena et al. 2016). Many illnesses and problems in humans and livestock are brought on by excessive metal concentrations or heavy metal toxicity. Severe toxicity of Cd causes heart disease, kidney disease, and bone brittleness, while Cr, Ni, and Pb cause mutagenesis, lung cancer, convulsions, brain damage, and other problems (Golui et al. 2020). Rarely are higher quantities of Cu and Zn harmful to mammals.

Soybean (*Glycine max* (L.) Merr.) is an important leguminous crop grown in India. It is India's third-largest produced oil seed, with a total area of around 12.8 million hectares and production of around 12.9 million tonnes with an average national productivity of 921 kg ha^{-1} (DES 2021). Due to the unpredictable distribution of monsoonal rains, uneven utilization of major and minor nutrients, and low organic carbon state of the soil, soybean production in India is quite low (less than 1 t ha^{-1}). The phytostabilization potential of soybean by remediating heavy metals like Cu, Pb, Ni, Zn, Cd, etc., from contaminated sites, has been documented by numerous researchers (Rai et al. 2019; Liu et al. 2022) apart from higher levels of Ni and Cd also being recorded in the edible portion of soybean (Enengl et al. 2022). Numerous elements were examined in this study, namely Cd, Cr, Fe, Pb, Mn, Ni and Zn. Due to increased industrialization and urbanization, the vast amount of sludge created by several STPs in Delhi and surrounding areas has become a disposal concern. Sludge is currently being used in agriculture by farmers from urban areas as both organic manure and a source of plant nutrients. To the best of our knowledge, no evidence supports the environmental sustainability of soybean under rationalized use of sludge in contrasting soil conditions. So, in the present investigation, the potential impact of sludge application on soybean productivity, accumulation and transfer of metals by soybean plants grown on varying soil pH conditions was evaluated.

Materials and methods

Collection and chemical characteristics of sludge and soils

Sludge sample was collected manually from the Okhla sewage treatment plants (STP), Delhi in June 2018 as per the protocol outlined by Delhi Jal Board (DJB 2021). During sample collection, five representative samples were taken from one heap using an auger and then mixed to have a composite sample with three replicates. The sludge sample was air-dried before being homogenized using a pestle and mortar. Processed sludge was further used for the analysis of chemical parameters as per standard methods given in the "chemical analysis of sludge, soil and plant" section. The sludge was acidic, having high salt content and organic carbon (Table 1). The Council of the European Communities and U.S. Environmental Protection Agency standards for heavy metals content are met in the sludge, which was utilized in this study (US EPA 2018). This can be ascribed to the effective pre-treatment provided by New Delhi's sewage treatment facilities.

In this study two bulk soil samples were collected from different locations, specifically acid soil from the Palampur district in Himachal Pradesh (32°6’1´ N, 76°32´ E) and alkaline soil from the experimental farm of ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28°30´ N, 77°10´ E). The soil samples were taken from the surface layer (0-15 cm) to execute a pot culture study under greenhouse conditions (Fig. S1). Then the collected soils were processed and passed through a 2 mm sieve before being employed in a pot experiment. Initial characteristics

Table 1 Physico-chemical properties of pre-cultivation acid and alkaline soils and sludge used in pot experiment

Parameters	Acid soil	Alkaline soil	Sludge
pH	5.35	7.82	6.47
Electrical conductivity (dS m_{-1})	0.11	0.15	4.46
Mechanical composition			
Clay (%)	48	12	-
Silt (%)	20	14	-
Sand (%)	32	64	-
Texture	Clay loam	Sandy loam	-
Cation exchange capacity [cmol (p^+) kg_{-1}]	29.2	10.2	-
Organic carbon (g kg^{-1})	9.96	4.50	-
Total content			
C (%)	1.20	0.95	25.4
N (%)	0.38	0.26	1.94
P (%)	-	-	1.73
K (%)	-	-	0.30
Fe (%)	3.40	1.31	1.56
Zn (mg kg_{-1})	70.0	48.0	1188
Cu (mg kg_{-1})	27.0	12.6	369
Mn (mg kg_{-1})	644	467	184
Ni (mg kg_{-1})	37.6	21.6	42.0
Cd (mg kg_{-1})	0.49	0.35	23.2
Pb (mg kg_{-1})	23.8	15.3	29.4
Cr (mg kg_{-1})	31.3	21.4	39.0

of soil samples was carried out as per the standard protocol (Table 1).

Greenhouse pot experiment

An experiment was carried out at the Division of SSAC, ICAR-IARI, New Delhi, (28° 38' 24.864" N; 77° 10' 18.12" E) (Fig. 2S), to evaluate the impact of sludge on biomass yield and heavy metal accumulation by soybean (*G. max*) grown on two contrasting soil pH conditions.

Treatments and experimental design

The experiment was conducted with seven graded doses of sludge @ 0, 5, 10, 20, 40, 80, and 160 g kg^{-1} applied to two soils (acid and alkaline). The experiment was done using a factorial completely randomized design (f-CRD). In the experiment, each of the 14 treatment combinations (2 soils × 7 sludge treatments) was replicated three times, resulting in a total of 42 experimental units. In the pot experiment, plastic pots with a 5 kg capacity were employed. Each pot was filled with 4 kg of soil from the respective acid or alkaline soils that were processed and passed through a 2 mm sieve as described earlier. After filling the pots with soil, each pot was irrigated with distilled water to ensure that the soil was moist. The pots were then kept at field capacity moisture, which is the maximum amount of water that the soil can hold, and allowed to equilibrate for 7-10 days after the sludge application.

Equilibration is the process by which the soil reaches a steady-state condition following any external disturbance. This waiting period made it possible for the soil to stabilize and for any potential effects of the sludge application to be fully integrated into the soil system before the soybean plants were sown. During July, soybean (Pusa Soybean 9712) seeds (10 per pot) were sown in the top 2-3 cm of soil in each pot and watered initially. Ten seeds were sown in each pot to ensure adequate germination, and the soil was further watered to provide moisture for the seeds. After 10 days of germination, thinning was carried out to maintain a uniform plant population of 5 plants per pot. The soybean plants were then allowed to grow until the middle of November. The soybean crop was harvested 116 days after sowing (DAS). At this point, the biomass yield and heavy metal accumulation in the harvested plants were measured. The choice of harvest time was likely based on, firstly, the developmental stage of the soybean plant and secondly, duration of the growth period needed to achieve maximum biomass yield and heavy metal accumulation. The timing of the harvest is an important experimental consideration because it can dictate the quality and quantity of the data obtained.

After harvesting, the fresh weight of the soybean plant samples was taken to determine the initial biomass yield. The plants were then cleaned using a series of washing steps. First, they were washed with tap water to remove any visible dirt or debris. Then, they were washed with a diluted hydrochloric acid solution to remove any residual soil particles and to help remove any heavy metal contaminants that may have accumulated on the plants' surface. Finally, they were submerged in distilled water and allowed to dry. The roots of the plants were also cleaned thoroughly by washing them several times under running tap water to remove soil particles from the surface of the roots. They were then immersed in a dilute hydrochloric acid solution and washed again with distilled water. After cleaning, the stem, leaves, shoots, and seeds of the soybean plants were separated and dried in a hot air oven at 65°C until a constant weight was reached. This allowed us to accurately determine the biomass yield of the plants. Once the plants were completely dried, they were ground into a fine powder using a mortar and pestle, and the resulting plant material was used for further chemical analysis. The drying and grinding of plant samples is a common practice in plant analysis, as it enables accurate measurement of the plant's nutrient and heavy metal content.

Chemical analysis of sludge, soil and plant

For pH determination in sludge samples, a 1:5 sludge-to-water suspension was used and pH was measured according to the method described by Allen (1989). For soil samples, a 1:2 soil-to-water suspension was used and pH was measured according to the method described by Jackson (1973). The electrical conductivity (EC) of sludge and soils was estimated in the supernatant liquid of the same extracts at 25°C using a conductivity meter, following the method described by Richards (Richard 1954). Total organic carbon and nitrogen (N) in sludge were determined using the dry combustion method with the help of a CHNS analyzer, as described by Nelson and Sommers (1982). Dichromate-oxidizable organic carbon (OC) in soil was determined using the Walkley and Black method (Walkley and Black 1934). The heavy metal content in sludge and plant samples was analyzed by digesting the samples with nitric acid and hydrogen peroxide (HNO_3:H_2O_2:: 9:2) and then analyzed with inductively coupled plasma mass spectrometry (ICP-MS) using a PerkinElmer NexION 300, according to the method described by Miller (2019). Aqua regia (HNO_3: HCl:: 1:3) extractable Fe, Cu, Mn, Zn, Cd, Cr, Ni, and Pb in soil were also analyzed by AAS after digesting the samples with the same acid mixture, as described by Jackson (1973). Total P and K in sludge were determined by digesting the samples with nitric acid (HNO_3) and perchloric acid ($HClO_4$) at a ratio of 9:4, according to Jackson (1973). Standard reference material (tomato leaves, National Institute of Standard and Technology, USA, SRM 1573a) served to validate the digestion and was analyzed by ICP-MS in triplicate. On average, the recovery percentage was recorded as 91.1 ± 0.49% for Zn, 92.4 ± 4.02% for Cu, 97.5 ± 0.97% for Ni and 92.3 ± 10.1% for Cd.

Calculation of bioaccumulation and translocation factor

The bioaccumulation factor (BF) is computed as the ratio of the concentration of the heavy metal in the roots of plants to the concentration of the same heavy metal in the soils. The translocation factor (TF) is calculated as the ratio of the concentration of the heavy metal in the plant tissues (shoot or seed) to the concentration of the same heavy metal in the roots. The factor values indicate the ability of the plant to take up and transport heavy metals from soil to different plant parts. More specifically, bioaccumulation and translocation factors were calculated (Kabata-Pendias and Mukherjee 2007) as follows:

$$BF_{root/soil} = \frac{Croot}{Csoil}$$

$$TF_{straw/root} = \frac{Cstraw}{Croot}$$

$$TF_{grain/straw} = \frac{Cgrain}{Cstraw}$$

Where, $BF_{root/soil}$, $TF_{straw/root}$, $TF_{grain/straw}$ are the bioaccumulation and translocation factors for soil to root, root to straw and straw to grain, respectively. Meanwhile C_{soil}, C_{root}, C_{straw}, and C_{grain} are the total metal concentrations (mg kg^{-1}) in the soil, root, straw and grain, respectively.

Data analysis

The data were first checked for homogeneity of variance and normality of distribution. If necessary, the data were log-transformed to meet these assumptions. Then, a one-way analysis of variance (ANOVA) was undertaken to evaluate the effect of sludge on various parameters, including biomass yield, heavy metal content in plant tissues, bioaccumulation, translocation factor, and soil chemical properties. The results of the analysis were presented as the average value with standard deviation. To identify significant differences between treatment means, the Duncan multiple range test (DMRT) was used at a significance level of $p < 0.05$. Pearson's correlation coefficient was used to evaluate the correlation between soil parameters, such as pH, organic carbon, heavy metals, and plant tissue metal content. This was done to determine the effect of these soil parameters on heavy metal accumulation in plant tissue. All statistical analyses were performed using IBM SPSS Statistics 20.

Results and discussion

Yield of soybean

The seed yield of soybean significantly increased by 12.8 to 137% and 26.8 to 156% in acid and alkaline soil, respectively, compared to control due to the application of graded doses of sludge @ 5 to 160 g kg^{-1} (Fig. 1). Application of sludge @ 160 g kg^{-1} enhanced straw yield of soybean to the tune of 67.7 and 56% in acid and alkaline soils, respectively, compared to the control (Table 1 in Suplimenatry Information). The mean seed and straw yield of soybean was higher in acid soil in comparison to the alkaline soil, which may be due to the inherent higher fertility level of acid soil owing to the relatively high easily oxidizable organic carbon. Soil organic carbon (SOC) plays a crucial role in maintaining soil health and promoting plant growth by improving soil structure, water retention, nutrient cycling, and biological activity (Pawar et al. 2017). SOC provides a source of energy and nutrients to soil microorganisms, which in turn

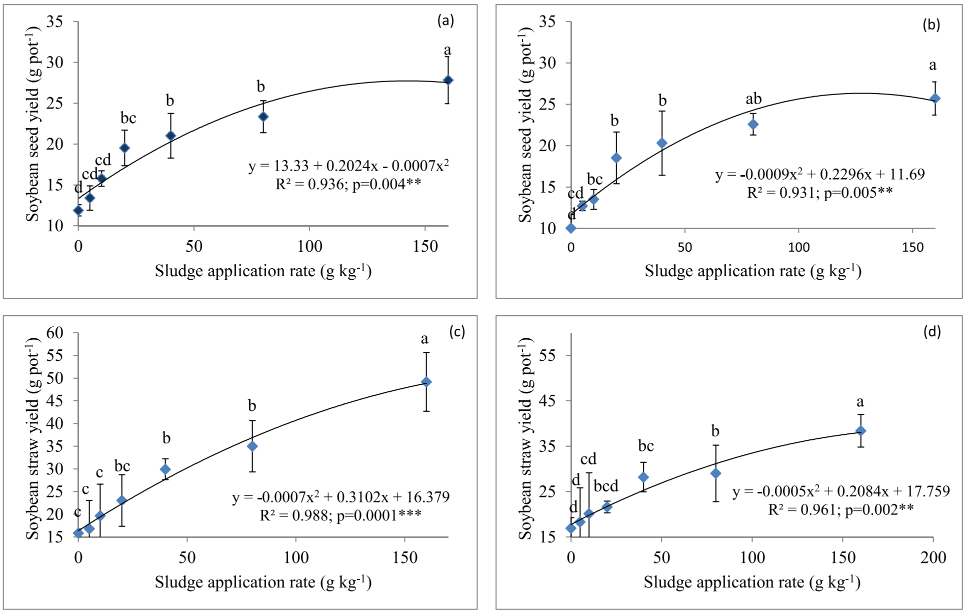

Fig. 1 Effects of sludge application on the seed yield of soybean grown on (a) acid soil and (b) alkaline soil, and straw yield of soybean grown on (c) acid soil, (d) alkaline soil (mean ± standard deviation, n = 21). The p values represent one-way ANOVA, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

help to break down organic matter and release nutrients that can be taken up by plants (Dwivedi and Datta 2012). The addition of sludge could improve overall nutrient-supplying capacity and soil physical health, thus enhancing crop yield. The application of sludge has been reported to substantially increase the production of *Brassica napus* compared to the control (Zaier et al. 2010). The application of sludge (i.e. 5 to 160 g kg^{-1}), particularly in acid soil, increased the soil pH (from 5.31 to 6.13) significantly leading to a favorable scenario for plant growth. The literature is replete with studies reporting the increase in biomass yield of a wide range of crops (Eid et al. 2017; Saha et al. 2017; Eid et al. 2019; Roy et al. 2019) including wheat (Latare et al. 2014) and tomato (Dhir 2016).

Metal content in soybean

The amounts of metal in the root, straw and seed of soybean are presented in Table 2. In general, all heavy metals increased significantly in different parts of soybean. Graded sludge application does exhibit a noticeable increase in heavy metal content. However the change is not significant except for higher dose, i.e. Zn content of straw in acid and alkaline soils, the Pb content of straw in acid soil, and the Cr content of root in acid soils. The largest concentration of all determined heavy metals in soybean was recorded when sludge @ 160 g kg^{-1} was applied, except Fe (straw) and Ni (root) in acid soil. The concentration of Fe exceeded the phytotoxic limit (500 mg kg^{-1}) in roots at a sludge dose of 40 (522 ± 60.8 mg kg^{-1}), 80 (656 ± 108 mg kg^{-1}), and 160 g kg^{-1} (698 ± 145 mg kg^{-1}) in acid soil, while in the alkaline soil, Fe content exceeded the critical limit in root (580 ± 103 mg kg^{-1}) at the highest dose of sludge application. In Zn, the concentrations in soybean were noticeably higher than the phytotoxic limit (100 mg kg^{-1}) in roots at a sludge dose of 80 (182 ± 39.3 mg kg^{-1}), and 160 g kg^{-1} (288 ± 46.9 mg kg^{-1}) in acid soil, whereas in alkaline soil such higher Zn concentration was observed only at the highest sludge dose.

Similarly, the Cu content in root was also seemingly higher and exceeded the critical limit (30 mg kg^{-1}) at sludge dose of 80 (33.3 ± 3.91 mg kg^{-1}), and 160 g kg^{-1} (43.9 ± 2.13 mg kg^{-1}) in acid soil, and at 160 g kg^{-1} in alkalines soil. In general, acid soil had a higher mean heavy metal content in soybean tissues than in alkaline soil. However, all measured heavy metal concentrations (except for Fe, Zn and Cu in the roots and Fe content in the straw of acid soils) were within the usual range and did not attain maximum phytotoxic levels. All the analyzed heavy metal concentrations were highest in the root, followed by straw and seed. Heavy metal accumulation increased significantly in different parts of soybean as sludge application rates increased. It was quite natural because of the appreciable amount of heavy metals present in the sludge.

The most important factor governing heavy metal availability to plants is pH (Bose and Bhattacharyya 2008). In the case of acid soil, the addition of sludge (@ 0-160 g kg^{-1}) increased the pH (5.13 to 6.13) (on an average of one unit) of the soil (Table 4). However, in the case of alkaline soil, by and large, the increase in heavy metal concentration with the rising sludge application rates was higher than acid soil. This could be attributed to the reduction and increase in pH in sludge-amended alkaline and acid soils, respectively. On one hand, heavy metals were added to the soil through sludge in acid soil, while on the other hand, the solubility of heavy metals is curtailed due to the increase in pH of acid soil as a result of sludge addition. Therefore, two opposing effects

Table 2 Effects of sludge application on heavy metal concentrations (mg/ kg) in seed, straw and roots of soybean plants that were grown on acid and alkaline soils

Metal	Soils	Tissue	Sludge application rate (g kg^{-1})							*F*-value	Safe limit[#] (mg kg^{-1})	Phytotoxic range[†] (mg kg^{-1})
			0	5	10	20	40	80	160			
Fe	Acid	Seed	43.2 ± 5.07^{c}	47.4 ± 6.22^{bc}	52.1 ± 2.32^{bc}	59.1 ± 1.90^{bc}	82.7 ± 13.7^{b}	145 ± 42.2^{a}	156 ± 22.1^{a}	18.7***	500	100-500
		Straw	129 ± 5.61^{d}	143 ± 20.1^{d}	260 ± 17.5^{c}	395 ± 43.8^{b}	463 ± 55.5^{ab}	505 ± 73.3^{a}	501 ± 64.1^{a}	36.9***		
		Root	285 ± 56.8^{d}	297 ± 26.5^{d}	335 ± 78.4^{d}	437 ± 56.9^{cd}	522 ± 60.8^{bc}	656 ± 108^{ab}	698 ± 145^{a}	12.2***		
	Alkaline	Seed	28.2 ± 1.91^{c}	41.3 ± 3.29^{c}	43.6 ± 2.71^{c}	45.4 ± 2.78c	69.1 ± 23.0^{bc}	89.6 ± 51.9^{ab}	118 ± 20.3^{a}	5.82**		
		Straw	96.6 ± 22.4^{d}	138 ± 40.8^{d}	253 ± 38.5^{c}	261 ± 37.9c	293 ± 48.0^{bc}	332 ± 16.0^{b}	414 ± 24.1^{a}	30.4***		
		Root	231 ± 28.8^{b}	235 ± 7.90^{b}	279 ± 16.3^{b}	297 ± 49.4b	302 ± 51.4^{b}	337 ± 71.1^{b}	580 ± 103^{a}	13.6***		
Mn	Acid	Seed	21.8 ± 3.98^{d}	22.7 ± 1.27^{d}	29.6 ± 1.18^{cd}	30.1 ± 4.48^{cd}	36.6 ± 2.18^{bc}	40.7 ± 2.43^{b}	54.9 ± 9.95^{a}	18.8***	20	20-300
		Straw	23.3 ± 4.58^{d}	28.0 ± 2.00^{cd}	33.2 ± 2.04^{bc}	37.5 ± 5.81^{b}	37.2 ± 7.55^{b}	51.8 ± 1.57^{a}	55.7 ± 6.51^{a}	17.8***		
		Root	39.3 ± 5.33^{b}	39.4 ± 11.5b	43.6 ± 6.79^{b}	54.2 ± 7.14^{b}	57.9 ± 8.91^{b}	84.6 ± 30.3^{a}	98.8 ± 12.7^{a}	8.08***		
	Alkaline	Seed	12.6 ± 1.44^{d}	25.6 ± 5.23c	28.6 ± 3.14^{c}	28.1 ± 6.61^{c}	33.3 ± 2.89^{abc}	36.0 ± 5.32^{ab}	39.9 ± 0.85^{a}	13.5***		
		Straw	10.7 ± 2.56^{d}	12.0 ± 0.92d	18.1 ± 0.85^{c}	21.6 ± 2.12^{b}	23.2 ± 0.76^{b}	23.8 ± 1.37^{b}	28.0 ± 3.61^{a}	30.4***		
		Root	23.2 ± 5.12^{d}	37.6 ± 7.27c	42.7 ± 3.20^{cc}	39.7 ± 11.9^{c}	53.2 ± 3.36^{ab}	56.9 ± 5.49^{a}	59.7 ± 5.10^{a}	11.6***		
Zn	Acid	Seed	25.7 ± 1.53^{d}	26.8 ± 1.92^{d}	30.9 ± 3.83^{d}	38.8 ± 4.48^{c}	43.2 ± 6.24^{bc}	49.0 ± 3.00^{b}	66.3 ± 7.09^{a}	31.4***	50	27-150
		Straw	54.6 ± 15.6^{c}	50.9 ± 10.1^{bc}	57.6 ± 8.13^{abc}	59.0 ± 9.42^{abc}	61.7 ± 5.47^{abc}	68.4 ± 2.19^{ab}	74.0 ± 4.32^{a}	2.39^{NS}		
		Root	102 ± 9.85^{c}	112 ± 3.21c	125 ± 1.60^{c}	132 ± 4.10^{c}	146 ± 18.3^{bc}	182 ± 39.3^{b}	288 ± 46.9^{a}	20.4***		
	Alkaline	Seed	21.6 ± 2.38^{d}	22.8 ± 2.43^{cd}	24.2 ± 1.93^{cd}	29.1 ± 4.62^{c}	30.2 ± 2.03^{c}	38.9 ± 3.56^{b}	46.0 ± 7.54^{a}	15.5***		
		Straw	41.5 ± 10.3^{b}	48.7 ± 11.5^{ab}	49.1 ± 7.22^{ab}	49.9 ± 8.75^{ab}	61.7 ± 8.79^{a}	62.5 ± 3.97^{a}	65.3 ± 14.2^{a}	2.56^{NS}		
		Root	72.8 ± 14.2^{d}	94.1 ± 8.98^{cd}	82.4 ± 12.2^{cd}	98.7 ± 8.72^{bc}	102 ± 6.57^{bc}	119 ± 8.11^{b}	194 ± 20.8^{a}	32.3***		
Cu	Acid	Seed	12.9 ± 0.74^{b}	13.0 ± 1.70^{b}	14.3 ± 1.21^{b}	14.4 ± 0.32^{b}	14.9 ± 0.86^{b}	19.6 ± 1.78^{a}	19.9 ± 3.21^{a}	9.89***	30	25-30
		Straw	12.4 ± 0.80^{d}	13.1 ± 1.32^{cd}	13.7 ± 0.12^{bcd}	15.0 ± 0.90^{bcd}	15.4 ± 2.46^{bc}	16.6 ± 0.51^{b}	21.5 ± 2.80^{a}	11.4***		
		Root	17.2 ± 1.89^{e}	17.6 ± 1.30e	21.7 ± 2.08^{d}	24.0 ± 1.00^{cd}	26.6 ± 1.70^{c}	33.3 ± 3.91^{b}	43.9 ± 2.13^{a}	57.2***		
	Alkaline	Seed	9.97 ± 2.44^{c}	12.9 ± 1.95b	15.2 ± 1.11^{ab}	13.8 ± 0.76^{ab}	16.2 ± 0.87^{a}	15.3 ± 0.95^{ab}	16.7 ± 2.15^{a}	6.35**		
		Straw	10.8 ± 2.51^{b}	12.1 ± 0.70b	12.7 ± 1.99^{b}	12.8 ± 0.67^{b}	13.5 ± 0.83^{b}	14.1 ± 2.86^{b}	19.1 ± 3.23^{a}	4.80*		
		Root	11.0 ± 1.05^{d}	13.4 ± 0.52d	14.3 ± 1.21^{d}	14.6 ± 1.13^{d}	19.9 ± 3.11^{c}	25.1 ± 3.41^{b}	30.3 ± 4.18^{a}	24.3***		
Ni	Acid	Seed	0.25 ± 0.01^{d}	0.27 ± 0.05^{d}	0.32 ± 0.03^{d}	0.36 ± 0.03^{c}	0.45 ± 0.05^{c}	0.76 ± 0.06^{b}	1.07 ± 0.16^{a}	57.6***	1.5	10-50
		Straw	1.54 ± 0.14^{e}	1.60 ± 0.32^{e}	1.80 ± 0.17^{de}	2.23 ± 0.20^{cd}	2.37 ± 0.40^{c}	3.21 ± 0.39^{b}	4.36 ± 0.36^{a}	34.4***		
		Root	2.64 ± 0.44^{b}	2.69 ± 0.63b	3.74 ± 1.15^{b}	4.01 ± 1.39^{ab}	5.53 ± 2.52^{ab}	7.58 ± 2.69^{a}	7.57 ± 3.08^{a}	3.48*		
	Alkaline	Seed	0.15 ± 0.04^{e}	0.18 ± 0.03de	0.29 ± 0.05^{cde}	0.35 ± 0.01^{cd}	0.48 ± 0.05^{bc}	0.63 ± 0.13^{b}	0.92 ± 0.24^{a}	19.2***		
		Straw	1.06 ± 0.21^{e}	1.42 ± 0.20de	1.64 ± 0.42^{d}	2.12 ± 0.19^{c}	2.17 ± 0.11^{c}	2.66 ± 0.25^{b}	3.26 ± 0.39^{a}	22.9***		
		Root	1.71 ± 0.17^{d}	2.23 ± 0.19cd	2.58 ± 0.24^{bc}	2.61 ± 0.39^{bc}	3.02 ± 0.12^{abc}	3.35 ± 0.53^{ab}	3.76 ± 0.90^{a}	7.21***		

Table 2 (continued)

Metal	Soils	Tissue	Sludge application rate (g kg^{-1})							*F*-value	Safe limit[#] (mg kg^{-1})	Phytotoxic range[†] (mg kg^{-1})
			0	5	10	20	40	80	160			
Cd	Acid	Seed	0.04 ± 0.01^{d}	0.05 ± 0.01^{d}	0.05 ± 0.01^{d}	0.07 ± 0.01^{cd}	0.08 ± 0.01^{c}	0.12 ± 0.02^{b}	0.16 ± 0.03^{a}	30.9^{***}	1.5	1.5-30
		Straw	0.31 ± 0.04^{c}	0.35 ± 0.02^{c}	0.40 ± 0.05^{c}	0.45 ± 0.02^{bc}	0.60 ± 0.06^{b}	0.80 ± 0.04^{a}	0.89 ± 0.23^{a}	17.8^{***}		
		Root	0.26 ± 0.03^{e}	0.31 ± 0.04^{de}	0.45 ± 0.04^{d}	0.70 ± 0.09^{c}	0.82 ± 0.17^{c}	1.17 ± 0.05^{b}	1.39 ± 0.11^{a}	68.0^{***}		
	Alkaline	Seed	0.02 ± 0.00^{d}	0.03 ± 0.01^{d}	0.03 ± 0.01^{d}	0.04 ± 0.01^{cd}	0.05 ± 0.01^{bc}	0.07 ± 0.02^{b}	0.10 ± 0.02^{a}	17.9^{***}		
		Straw	0.17 ± 0.04^{d}	0.21 ± 0.05^{d}	0.27 ± 0.03^{d}	0.33 ± 0.05^{cd}	0.46 ± 0.10^{bc}	0.59 ± 0.12^{b}	0.86 ± 0.14^{a}	23.6^{***}		
		Root	0.23 ± 0.13^{c}	0.29 ± 0.06^{c}	0.34 ± 0.08^{bc}	0.35 ± 0.01^{bc}	0.37 ± 0.04^{bc}	0.47 ± 0.04^{b}	0.84 ± 0.09^{a}	22.0^{***}		
Pb	Acid	Seed	0.05 ± 0.01^{d}	0.06 ± 0.01^{d}	0.09 ± 0.02^{c}	0.09 ± 0.02^{c}	0.13 ± 0.02^{b}	0.16 ± 0.01^{b}	0.21 ± 0.02^{a}	40.1^{***}	2.5	30-300
		Straw	0.49 ± 0.02^{b}	0.57 ± 0.03^{b}	0.57 ± 0.04^{b}	0.58 ± 0.01^{b}	0.56 ± 0.02^{b}	0.58 ± 0.03^{b}	0.73 ± 0.19^{a}	2.83^{NS}		
		Root	0.72 ± 0.09^{f}	0.81 ± 0.16^{ef}	0.91 ± 0.02^{de}	1.02 ± 0.03^{cd}	1.11 ± 0.06^{c}	1.62 ± 0.13^{b}	2.35 ± 0.12^{a}	100^{***}		
	Alkaline	Seed	0.02 ± 0.01^{e}	0.02 ± 0.01^{e}	$0.05 \pm .01^{d}$	0.06 ± 0.00^{cd}	0.08 ± 0.01^{c}	0.11 ± 0.01^{b}	0.13 ± 0.01^{a}	96.9^{***}		
		Straw	0.42 ± 0.06^{d}	0.47 ± 0.07^{cd}	0.49 ± 0.06^{bcd}	0.51 ± 0.07^{bcd}	0.57 ± 0.04^{abc}	0.60 ± 0.05^{ab}	0.63 ± 0.03^{a}	5.18^{*}		
		Root	0.52 ± 0.09^{d}	0.63 ± 0.04^{cd}	0.67 ± 0.09^{cd}	0.78 ± 0.10^{c}	0.82 ± 0.08^{c}	1.07 ± 0.19^{b}	1.91 ± 0.11^{a}	56.1^{***}		
Cr	Acid	Seed	0.06 ± 0.01^{e}	0.08 ± 0.02^{de}	0.10 ± 0.01^{de}	0.11 ± 0.02^{d}	0.16 ± 0.02^{c}	0.22 ± 0.04^{b}	0.26 ± 0.01^{a}	45.1^{***}	20	10-100
		Straw	0.82 ± 0.09^{d}	0.77 ± 0.10^{d}	1.12 ± 0.20^{cd}	1.42 ± 0.15^{c}	1.32 ± 0.10^{c}	2.05 ± 0.37^{b}	2.74 ± 0.27^{a}	35.7^{***}		
		Root	2.51 ± 0.67^{b}	3.13 ± 0.78^{b}	3.77 ± 1.32^{b}	5.18 ± 1.80^{ab}	6.48 ± 4.45^{ab}	8.41 ± 5.81^{ab}	10.8 ± 5.37^{a}	2.17^{NS}		
	Alkaline	Seed	0.04 ± 0.01^{d}	0.04 ± 0.01^{d}	0.05 ± 0.01^{cd}	0.08 ± 0.01^{bc}	0.11 ± 0.02^{b}	0.15 ± 0.01^{a}	0.17 ± 0.04^{a}	22.5^{***}		
		Straw	0.54 ± 0.04^{d}	0.67 ± 0.05^{cd}	$0.93 \pm 0.18b^{cd}$	1.05 ± 0.30^{bcd}	1.13 ± 0.43^{bc}	1.47 ± 0.38^{ab}	1.69 ± 0.43^{a}	5.49^{*}		
		Root	1.61 ± 0.21^{d}	1.97 ± 0.09^{d}	2.15 ± 0.27^{cd}	2.78 ± 0.61^{bc}	3.35 ± 0.32^{b}	3.42 ± 0.55^{b}	4.93 ± 0.56^{a}	22.3^{***}		

[*] *F*-values represent a one-way ANOVA, [†]Means (means ± standard deviation) in the same row followed by different superscript letters are significantly different at $p < 0.05$ according to Duncan Multiple Range Test (DMRT). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: not significant (*i.e.,* $p > 0.05$). [†]Rattan and Goswami, (2009); [#] Indian standard (Awashthi 2000)

of sludge addition on the availability of heavy metals in acid soils are evident at the same time. Conversely, it is quite interesting to note that both the magnitude and solubility of heavy metals in alkaline soil increased due to a subsequent decrease in soil pH through the addition of sludge. It is well known that the solubility of divalent heavy metal cations (like Cd^{2+}, Pb^{2+} and Zn^{2+}) decreases 100 times with a one-unit increase in soil pH (Lindsay 1979).

An increase in the solubility of heavy metals with decreasing soil pH due to sludge application was reported by many researchers (Golui et al. 2014; Eid and Shaltout 2016; Jalali et al. 2023). Application of sludge enhances heavy metal content in seed/grain, straw and root of various crops (Bose and Bhattacharyya 2008; Carbonell et al. 2011; Roy et al. 2013). In our study the heavy metal content in the plant parts was in the following order: Fe>Zn>Mn>Cu>Cr>Ni>Pb>Cd in the root, Fe>Zn>Mn>Cu>Ni>Pb>Cr>Cd in straw and Fe>Zn>Mn>Cu>Ni>Cr>Pb>Cd in seed (Table 2). Higher root heavy metal content could be due to the low mobility of heavy metals from root to shoot (Kashem and Singh 2001). Plant compounds such as phytokeratin made a complex with heavy metals in roots or leaves, preventing their movement and transmission to seeds (Marschner 1995). Metal compartmentalization and translocation in the vascular system cause variations in heavy metal content in different parts of soybean (Bose and Bhattacharyya 2008).

Heavy metals including Cd, Ni, and Pb can accumulate in root cells due to some compounds like phytochelatins, phytosiderophores, siderophores, organic acids, and amino acids. These act as metal chelators, leading to higher sequestration in the roots of soybean (Eid and Shaltout 2016; Eid et al. 2019). Interestingly, plants have developed defence mechanisms to restrict the movement of heavy metals to their edible shoots, thereby reducing the risk of toxic heavy metal accumulation in the edible parts of the plant. The root barrier is one such defence mechanism that prevents the translocation of heavy metals from the roots to the above-ground plant parts. In addition to the root barrier, plants use metal chelation in the cytoplasm or storage in vacuoles to detoxify heavy metals. These defence mechanisms have been observed in sunflower plant grown in sludge-amended soils, as reported by Belhaj et al. (2016). Furthermore, heavy metal uptake and distribution in root, stem, shoot and seed or grains can vary depending on a variety of factors like water content, soil parameters, chemical characteristics of applied materials, plant types, climate, etc. (Page and Feller 2015).

Bioaccumulation and translocation of metals in soybean

The bioaccumulation factor (BF) and translocation factor (TF) were assessed to study the pattern of heavy metal translocation from soil to roots and subsequent plant tissues including the edible portion of crops. In Table 3, the values of BFs and TFs of numerous heavy metals computed are presented. Results revealed that the ratio of BFs for measured metals was <1 (except Zn). Mean value of $TF_{straw/root}$ for all the heavy metals was <1.0 (except Fe and Cd) which followed the order Cd > Fe > Cu > Ni > Pb > Mn > Zn > Cr. However, $TF_{seed/straw}$ was >1 for Mn and Cu (Table 3). Higher BF and TF associated with Zn, Fe, Cd, Cu and Mn, is an indication of higher heavy metal mobilization and phytoextraction potential of plants for these heavy metals from soil to root, straw, and seeds. In the meantime, a BF value of <1 may be attributed to the tolerance mechanism used by plants, resulting in phytostabilization of these metals in roots and restricted translocation to above-ground plant parts (Eid et al. 2017). Similar results were also reported earlier, where the transfer factor was more than one in the case of Zn, Cu and Cd (Eid et al. 2017, 2019; Sharma et al. 2018). Soybean crop being a hyper-accumulator plant sequesters a large amount of heavy metals in plant tissues over cereals (rice and wheat) (Delil et al. 2020). The higher translocation efficiency of Cd and Zn was also documented by Eid and Shaltout (2016) in some native plant species grown at polluted sites. The translocation of heavy metals from soil to root is an intricate process that is influenced by several factors like soil type, pH, organic carbon, moisture content, soil texture, $CaCO_3$ content, etc. (Samal et al. 2023).

Current investigation showed that the content of Fe in the soil was very high causing significant Fe uptake by roots. However, the TF was still below one, confirming the low translocation capacity of Fe from root to straw to grain. Low translocation capacity of Fe, Cr, and Pb was reported in spinach, fababean, cucumber, and wheat under sludge treatments (Kashem and Singh 2001; Shahbazi et al. 2017; Eid et al. 2019). According to the findings, Cd has the highest potential for entry into the food chain, followed by Zn, Mn, and Cu in both acid and alkaline soils. The results documented by Rattan et al. (2005) suggested that soils receiving sewage irrigation have a consistent pattern of heavy metal transfer from the soil to the plants. This information is important because it can help in assessing the risk of growing food crops on soils with elevated levels of heavy metals.

Soil properties after harvest of soybean

A significant increase in pH from 5.31 to 6.13 was recorded in acid soil, whereas the opposite trend was observed in the case of alkaline soil with a substantial decline in pH value from 7.76 to 6.90 due to the application of sludge from 0 to 160 g kg^{-1} (Table 4). The increase in pH of acid soil may be ascribed to the buffering capacity of organic matter (sludge) and the presence of a huge amount of bicarbonates (HCO_3^-) ions in sludge (Whalen et al. 2000). Dominantly, organic acids present in sludge are instrumental in affecting the

Table 3 Effects of sludge application on bioaccumulation factors (BFs), from soil to roots, and translocation factors (TFs), from roots to straw and seed of heavy metals in soybean plants grown on acid and alkaline soils

Metal	Soils	Tissue	Sludge application rate (g kg^{-1})							F-value
			0	5	10	20	40	80	160	
Fe	Acid	$BF_{root/soil}$	0.03 ± 0.01^{ab}	0.02 ± 0.00^{a}	0.02 ± 0.01^{a}	0.03 ± 0.00^{ab}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{b}	0.03 ± 0.01^{ab}	1.87^{NS}
		$TF_{straw/root}$	0.46 ± 0.09^{a}	0.49 ± 0.10^{a}	0.81 ± 0.24^{b}	0.91 ± 0.09^{b}	0.89 ± 0.08^{b}	0.78 ± 0.12^{b}	0.73 ± 0.13^{b}	5.74^{**}
		$TF_{seed/straw}$	0.34 ± 0.02^{b}	0.34 ± 0.09^{b}	0.20 ± 0.01^{a}	0.15 ± 0.02^{a}	0.18 ± 0.03^{a}	0.28 ± 0.06^{b}	0.31 ± 0.01^{b}	9.11^{***}
	Alkaline	$BF_{root/soil}$	$0.03 \pm .01^{a}$	0.03 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{b}	1.82^{NS}
		$TF_{straw/root}$	0.42 ± 0.08^{a}	0.59 ± 0.17^{ab}	0.91 ± 0.13^{c}	0.89 ± 0.18^{c}	0.97 ± 0.07^{c}	1.01 ± 0.19^{c}	0.73 ± 0.16^{bc}	6.79^{**}
		$TF_{seed/straw}$	0.30 ± 0.06^{a}	0.31 ± 0.08^{a}	0.17 ± 0.02^{a}	0.18 ± 0.03^{a}	0.25 ± 0.12^{a}	0.27 ± 0.14^{a}	0.29 ± 0.06^{a}	1.35^{NS}
Mn	Acid	$BF_{root/soil}$	0.07 ± 0.01^{a}	0.07 ± 0.02^{a}	0.07 ± 0.02^{a}	0.09 ± 0.02^{ab}	0.09 ± 0.01^{ab}	0.11 ± 0.04^{b}	0.09 ± 0.01^{ab}	1.77^{NS}
		$TF_{straw/root}$	0.59 ± 0.06^{a}	0.76 ± 0.24^{a}	0.78 ± 0.17^{a}	0.70 ± 0.10^{a}	0.65 ± 0.16^{a}	0.68 ± 0.28^{a}	0.56 ± 0.01^{a}	0.66^{NS}
		$TF_{seed/straw}$	0.98 ± 0.33^{a}	0.81 ± 0.03^{a}	0.90 ± 0.09^{a}	0.81 ± 0.16^{a}	1.01 ± 0.19^{a}	0.79 ± 0.07^{a}	1.01 ± 0.30^{a}	0.72^{NS}
	Alkaline	$BF_{root/soil}$	0.05 ± 0.00^{a}	0.08 ± 0.02^{b}	0.09 ± 0.01^{b}	0.08 ± 0.03^{b}	0.11 ± 0.01^{b}	0.10 ± 0.02^{b}	0.09 ± 0.02^{b}	4.05^{*}
		$TF_{straw/root}$	0.46 ± 0.05^{ab}	0.32 ± 0.04^{a}	0.43 ± 0.04^{ab}	0.60 ± 0.28^{b}	0.44 ± 0.04^{ab}	0.42 ± 0.03^{ab}	0.47 ± 0.06^{ab}	1.59^{NS}
		$TF_{seed/straw}$	1.21 ± 0.19^{a}	2.14 ± 0.44^{b}	1.59 ± 0.25^{a}	1.31 ± 0.37^{a}	1.44 ± 0.17^{a}	1.53 ± 0.32^{a}	1.44 ± 0.20^{a}	3.20^{*}
Zn	Acid	$BF_{root/soil}$	1.85 ± 0.34^{b}	1.84 ± 0.16^{b}	1.74 ± 0.15^{ab}	1.63 ± 0.36^{ab}	1.68 ± 0.36^{ab}	1.26 ± 0.32^{a}	1.40 ± 0.29^{ab}	1.73^{NS}
		$TF_{straw/root}$	0.54 ± 0.13^{b}	0.46 ± 0.10^{b}	0.46 ± 0.07^{b}	0.44 ± 0.06^{b}	0.43 ± 0.06^{b}	0.39 ± 0.07^{ab}	0.26 ± 0.04^{a}	3.21^{*}
		$TF_{seed/straw}$	0.50 ± 0.15^{a}	0.55 ± 0.17^{a}	0.55 ± 0.14^{a}	0.68 ± 0.18^{ab}	0.71 ± 0.17^{ab}	0.72 ± 0.03^{ab}	0.89 ± 0.05^{b}	2.94^{*}
	Alkaline	$BF_{root/soil}$	1.75 ± 0.31^{c}	1.85 ± 0.06^{c}	1.34 ± 0.24^{b}	1.26 ± 0.05^{ab}	1.00 ± 0.24^{ab}	0.92 ± 0.06^{a}	1.15 ± 0.18^{ab}	10.5^{***}
		$TF_{straw/root}$	0.57 ± 0.04^{b}	0.51 ± 0.10^{ab}	0.61 ± 0.13^{b}	0.51 ± 0.08^{ab}	0.61 ± 0.12^{b}	0.53 ± 0.05^{b}	0.34 ± 0.10^{a}	2.75^{NS}
		$TF_{seed/straw}$	0.54 ± 0.13^{a}	0.49 ± 0.16^{a}	0.50 ± 0.10^{a}	0.61 ± 0.20^{a}	0.50 ± 0.10^{a}	0.62 ± 0.09^{a}	0.71 ± 0.09^{a}	1.22^{NS}
Cu	Acid	$BF_{root/soil}$	0.62 ± 0.08^{ab}	0.57 ± 0.01^{a}	0.72 ± 0.08^{bc}	0.77 ± 0.09^{c}	0.80 ± 0.08^{c}	0.62 ± 0.09^{ab}	0.58 ± 0.03^{a}	5.16^{**}
		$TF_{straw/root}$	0.73 ± 0.13^{b}	0.75 ± 0.13^{b}	0.64 ± 0.06^{ab}	0.63 ± 0.06^{ab}	0.58 ± 0.08^{ab}	0.50 ± 0.06^{a}	0.49 ± 0.07^{a}	3.84^{*}
		$TF_{seed/straw}$	1.05 ± 0.12^{a}	1.00 ± 0.22^{a}	1.04 ± 0.08^{a}	0.96 ± 0.04^{a}	0.99 ± 0.19^{a}	1.18 ± 0.12^{a}	0.95 ± 0.26^{a}	0.71^{NS}
	Alkaline	$BF_{root/soil}$	0.71 ± 0.08^{bc}	0.84 ± 0.06^{c}	0.63 ± 0.01^{ab}	0.57 ± 0.04^{ab}	0.57 ± 0.13^{ab}	0.58 ± 0.11^{ab}	0.53 ± 0.05^{a}	5.43^{**}
		$TF_{straw/root}$	0.97 ± 0.18^{c}	0.91 ± 0.09^{bc}	0.90 ± 0.20^{bc}	0.88 ± 0.11^{bc}	0.68 ± 0.06^{ab}	0.57 ± 0.16^{ab}	0.64 ± 0.16^{a}	3.59^{*}
		$TF_{seed/straw}$	0.95 ± 0.25^{a}	1.06 ± 0.12^{a}	1.20 ± 0.11^{a}	1.09 ± 0.09^{a}	1.21 ± 0.12^{a}	1.13 ± 0.31^{a}	0.90 ± 0.22^{a}	1.17^{NS}
Ni	Acid	$BF_{root/soil}$	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}	0.11 ± 0.04^{ab}	0.11 ± 0.04^{ab}	0.14 ± 0.06^{ab}	0.18 ± 0.06^{b}	0.17 ± 0.06^{ab}	2.20^{NS}
		$TF_{straw/root}$	0.60 ± 0.16^{a}	0.63 ± 0.24^{a}	0.52 ± 0.21^{a}	0.60 ± 0.18^{a}	0.51 ± 0.28^{a}	0.49 ± 0.28^{a}	0.65 ± 0.29^{a}	0.22^{NS}
		$TF_{seed/straw}$	0.16 ± 0.02^{a}	0.18 ± 0.05^{a}	0.18 ± 0.01^{a}	0.16 ± 0.02^{a}	0.19 ± 0.02^{ab}	0.24 ± 0.04^{b}	0.24 ± 0.02^{b}	4.67^{**}
	Alkaline	$BF_{root/soil}$	0.12 ± 0.01^{a}	0.14 ± 0.02^{ab}	0.15 ± 0.02^{ab}	0.14 ± 0.03^{ab}	0.16 ± 0.02^{ab}	0.15 ± 0.04^{ab}	0.17 ± 0.05^{b}	1.18^{NS}
		$TF_{straw/root}$	0.62 ± 0.08^{a}	0.64 ± 0.06^{a}	0.64 ± 0.20^{a}	0.83 ± 0.17^{ab}	0.72 ± 0.04^{ab}	0.81 ± 0.13^{ab}	0.89 ± 0.13^{b}	2.22^{NS}
		$TF_{seed/straw}$	0.14 ± 0.05^{a}	0.13 ± 0.04^{a}	0.19 ± 0.09^{ab}	0.17 ± 0.02^{a}	0.22 ± 0.04^{ab}	0.24 ± 0.06^{ab}	0.29 ± 0.10^{b}	2.42^{NS}

Table 3 (continued)

Metal	Soils	Tissue	Sludge application rate (g kg^{-1})							F-value
			0	5	10	20	40	80	160	
Cd	Acid	$BF_{root/soil}$	0.50 ± 0.05^{a}	0.46 ± 0.07^{a}	0.57 ± 0.12^{a}	0.56 ± 0.05^{a}	0.50 ± 0.15^{a}	0.62 ± 0.09^{a}	0.59 ± 0.11^{a}	0.97^{NS}
		$TF_{straw/root}$	1.19 ± 0.15^{b}	1.13 ± 0.12^{b}	0.88 ± 0.09^{a}	0.65 ± 0.06^{a}	0.76 ± 0.25^{a}	0.69 ± 0.06^{a}	0.63 ± 0.12^{a}	8.68^{***}
		$TF_{seed/straw}$	0.14 ± 0.03^{a}	0.15 ± 0.01^{a}	0.13 ± 0.03^{a}	0.15 ± 0.01^{a}	0.13 ± 0.01^{a}	0.15 ± 0.01^{a}	0.19 ± 0.07^{a}	1.15^{NS}
	Alkaline	$BF_{root/soil}$	0.69 ± 0.41^{c}	0.67 ± 0.11^{bc}	0.66 ± 0.17^{bc}	0.34 ± 0.05^{ab}	0.27 ± 0.02^{a}	0.31 ± 0.02^{a}	0.45 ± 0.07^{abc}	3.23^{*}
		$TF_{straw/root}$	0.82 ± 0.25^{ab}	0.75 ± 0.30^{a}	0.82 ± 0.21^{ab}	0.95 ± 0.16^{ab}	1.26 ± 0.37^{b}	1.27 ± 0.32^{b}	1.02 ± 0.05^{ab}	2.00^{NS}
		$TF_{seed/straw}$	0.13 ± 0.03^{a}	0.14 ± 0.04^{a}	0.12 ± 0.03^{a}	0.13 ± 0.04^{a}	0.12 ± 0.05^{a}	0.12 ± 0.04^{a}	0.12 ± 0.04^{a}	0.13^{NS}
Pb	Acid	$BF_{root/soil}$	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	3.62^{*}
		$TF_{straw/root}$	0.69 ± 0.08^{c}	0.71 ± 0.10^{c}	0.62 ± 0.06^{bc}	0.57 ± 0.01^{b}	0.51 ± 0.01^{b}	0.36 ± 0.03^{a}	0.31 ± 0.08^{a}	17.8^{***}
		$TF_{seed/straw}$	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	0.15 ± 0.04^{a}	0.16 ± 0.03^{ab}	0.23 ± 0.03^{bc}	0.27 ± 0.02^{c}	0.30 ± 0.08^{c}	11.9^{***}
	Alkaline	$BF_{root/soil}$	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.00^{a}	1.09^{NS}
		$TF_{straw/root}$	0.82 ± 0.25^{c}	0.75 ± 0.30^{bc}	0.82 ± 0.21^{bc}	0.95 ± 0.16^{bc}	1.26 ± 0.37^{bc}	1.27 ± 0.32^{b}	1.02 ± 0.05^{a}	7.73^{***}
		$TF_{seed/straw}$	0.04 ± 0.01^{a}	0.05 ± 0.02^{a}	0.11 ± 0.01^{b}	0.13 ± 0.02^{b}	0.13 ± 0.00^{b}	0.18 ± 0.02^{c}	0.21 ± 0.02^{d}	45.9^{***}
Cr	Acid	$BF_{root/soil}$	0.11 ± 0.03^{a}	0.11 ± 0.04^{a}	0.09 ± 0.03^{a}	0.11 ± 0.04^{a}	0.12 ± 0.08^{a}	0.15 ± 0.11^{a}	0.16 ± 0.09^{a}	0.42^{NS}
		$TF_{straw/root}$	0.34 ± 0.06^{a}	0.26 ± 0.06^{a}	0.34 ± 0.19^{a}	0.29 ± 0.08^{a}	0.27 ± 0.14^{a}	0.31 ± 0.15^{a}	0.32 ± 0.22^{a}	0.16^{NS}
		$TF_{seed/straw}$	0.08 ± 0.01^{a}	0.10 ± 0.02^{ab}	0.09 ± 0.02^{ab}	0.08 ± 0.02^{a}	0.12 ± 0.00^{b}	0.11 ± 0.04^{ab}	0.10 ± 0.01^{ab}	1.87^{NS}
	Alkaline	$BF_{root/soil}$	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.02^{a}	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.57^{NS}
		$TF_{straw/root}$	0.34 ± 0.05^{a}	0.34 ± 0.02^{a}	0.43 ± 0.03^{a}	0.41 ± 0.20^{a}	0.34 ± 0.15^{a}	0.45 ± 0.18^{a}	0.35 ± 0.13^{a}	0.39^{NS}
		$TF_{seed/straw}$	0.07 ± 0.03^{ab}	0.06 ± 0.01^{ab}	0.06 ± 0.01^{a}	0.08 ± 0.01^{ab}	0.10 ± 0.03^{ab}	0.11 ± 0.04^{c}	0.10 ± 0.01^{bc}	3.10^{*}

$^{*}F$-values represent a one-way ANOVA, †Means (means ± standard deviation) in the same row followed by different superscript letters are significantly different at $p < 0.05$ according to Duncan Multiple Range Test (DMRT). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, NS: not significant (*i.e.*, $p > 0.05$).

Table 4 Effects of sludge application on chemical properties of soil after harvesting soybean plants that were grown on acid and alkaline soils

Soils properties	Soil	Sludge application rate (g kg^{-1})							*F*-value	Maximum permissible limits in agricultural soil†
		0	5	10	20	40	80	160		
pH	Acid	5.31 ± 0.06^{g}	5.47 ± 0.06^{f}	5.60 ± 0.04^{e}	5.68 ± 0.04^{d}	5.82 ± 0.04^{c}	5.93 ± 0.03^{b}	6.13 ± 0.05^{a}	121^{***}	NA
	Alkaline	7.76 ± 0.03^{g}	7.69 ± 0.02^{f}	7.57 ± 0.03^{e}	7.51 ± 0.03^{d}	7.45 ± 0.01^{c}	7.24 ± 0.06^{b}	6.90 ± 0.03^{a}	260^{***}	
EC (dS m^{-1})	Acid	0.12 ± 0.02^{a}	0.18 ± 0.01^{ab}	0.21 ± 0.03^{b}	0.38 ± 0.05^{c}	0.41 ± 0.04^{c}	0.55 ± 0.06^{d}	0.72 ± 0.05^{e}	91.2^{***}	NA
	Alkaline	0.16 ± 0.03^{a}	0.20 ± 0.04^{ab}	0.24 ± 0.04^{bc}	0.27 ± 0.02^{cd}	0.30 ± 0.01^{d}	0.47 ± 0.03^{e}	0.56 ± 0.05^{f}	60.8^{***}	
OC (g kg^{-1})	Acid	8.15 ± 0.78^{d}	8.60 ± 0.79^{cd}	9.43 ± 0.62^{cd}	9.84 ± 0.95^{bc}	11.3 ± 1.15^{b}	12.9 ± 0.91^{a}	14.2 ± 0.76^{a}	20.4^{***}	NA
	Alkaline	5.26 ± 0.48^{c}	5.53 ± 0.75^{c}	6.18 ± 0.81^{c}	6.57 ± 0.80^{c}	8.01 ± 1.07^{b}	8.78 ± 0.53^{ab}	9.77 ± 1.07^{a}	13.3^{***}	
Fe (g g^{-1})	Acid	11.2 ± 0.66^{d}	12.9 ± 0.62^{d}	15.4 ± 1.28^{c}	16.5 ± 1.75^{bc}	17.0 ± 2.08^{bc}	18.4 ± 1.37^{b}	22.6 ± 1.37^{a}	21.3^{***}	20-40
	Alkaline	7.41 ± 0.52^{e}	7.91 ± 0.72^{de}	8.41 ± 1.70^{de}	9.08 ± 1.66^{cde}	10.0 ± 2.50^{bc}	11.1 ± 1.28^{b}	13.7 ± 1.50^{a}	9.76^{***}	
Mn (mg kg^{-1})	Acid	556 ± 94.1^{c}	606 ± 84.7^{bc}	612 ± 88.8^{bc}	625 ± 84.7^{bc}	668 ± 57.1^{bc}	750 ± 37.5^{b}	1069 ± 141^{a}	11.4^{***}	< 450
	Alkaline	431 ± 86.0^{b}	450 ± 56.0^{b}	494 ± 96.0^{b}	494 ± 39.0^{b}	500 ± 39.0^{b}	550 ± 28.5^{ab}	648 ± 90.9^{a}	3.42^{*}	
Zn (mg kg^{-1})	Acid	55.8 ± 6.29^{e}	60.8 ± 3.82^{de}	72.7 ± 6.43^{cde}	83.7 ± 15.0^{cd}	89.7 ± 17.6^{c}	146 ± 5.29^{b}	209 ± 20.6^{a}	59.6^{***}	100–300
	Alkaline	41.7 ± 3.82^{e}	50.8 ± 3.82^{e}	61.7 ± 5.20^{de}	78.3 ± 8.78^{d}	106 ± 28.4^{c}	130 ± 9.01^{b}	170 ± 11.5^{a}	39.5^{***}	
Cu (mg kg^{-1})	Acid	28.0 ± 1.75^{c}	31.0 ± 3.00^{c}	30.4 ± 2.90^{c}	31.3 ± 2.16^{c}	33.5 ± 2.36^{c}	54.4 ± 4.65^{b}	76.1 ± 3.33^{a}	107^{***}	60–150
	Alkaline	15.7 ± 2.15^{e}	16.1 ± 1.01^{e}	22.6 ± 1.35^{d}	25.6 ± 2.82^{d}	35.9 ± 5.66^{c}	43.5 ± 3.77^{b}	56.9 ± 2.72^{a}	71.3^{***}	
Ni (mg kg^{-1})	Acid	32.4 ± 1.19^{e}	33.6 ± 0.99^{e}	35.6 ± 1.70^{de}	37.4 ± 2.19^{cd}	40.2 ± 1.75^{bc}	41.5 ± 1.79^{b}	45.4 ± 3.33^{a}	16.2^{***}	20–60
	Alkaline	14.8 ± 0.60^{d}	16.4 ± 0.65^{cd}	17.3 ± 1.28^{c}	18.3 ± 0.76^{bc}	19.5 ± 1.29^{b}	22.4 ± 1.93^{a}	22.1 ± 0.81^{a}	18.9^{***}	
Cd (mg kg^{-1})	Acid	0.54 ± 0.04^{d}	0.68 ± 0.05^{d}	0.82 ± 0.12^{d}	1.26 ± 0.12^{c}	1.71 ± 0.20^{b}	1.92 ± 0.22^{b}	2.40 ± 0.30^{a}	50.1^{***}	1.0–5.0
	Alkaline	0.35 ± 0.03^{f}	0.44 ± 0.02^{ef}	0.53 ± 0.08^{e}	1.04 ± 0.13^{d}	1.36 ± 0.06^{c}	1.51 ± 0.05^{b}	1.89 ± 0.08^{a}	203^{***}	
Pb (mg kg^{-1})	Acid	22.8 ± 0.42^{f}	24.8 ± 0.82^{f}	28.4 ± 1.18^{e}	32.8 ± 1.66^{d}	36.7 ± 1.32^{c}	45.7 ± 1.69^{b}	56.2 ± 3.10^{a}	158^{***}	20–30
	Alkaline	10.1 ± 1.99^{e}	14.6 ± 0.91^{d}	16.2 ± 1.25^{d}	16.8 ± 1.56^{cd}	20.4 ± 3.61^{c}	27.7 ± 1.93^{b}	41.9 ± 2.41^{a}	75.9^{***}	
Cr (mg kg^{-1})	Acid	23.6 ± 2.21^{f}	29.0 ± 3.76^{e}	39.6 ± 3.87^{d}	48.8 ± 2.25^{c}	55.6 ± 2.05^{b}	56.7 ± 1.81^{b}	68.1 ± 3.15^{a}	95.0^{***}	50–200
	Alkaline	15.6 ± 1.08^{f}	17.6 ± 1.00^{f}	21.3 ± 1.57^{e}	25.5 ± 1.75^{d}	31.6 ± 2.26^{c}	36.6 ± 0.89^{b}	47.5 ± 1.46^{a}	174^{***}	

EC: electrical conductivity, OC: organic carbon, [*]*F*-values represent a one-way ANOVA, [†]Means (means ± standard deviation) in the same row followed by different superscript letters are significantly different at $p < 0.05$ according to Duncan Multiple Range Test (DMRT). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: not significant (*i.e.,* $p > 0.05$), [†]Kabata-Pendias (2011).

chemical reaction occurring in the soil (Brown et al. 2008). The addition of organic manure to acid soils can induce higher soil pH values due to H^+ ion adsorption. Further, the application of pig slurry induced the accumulation of Ca^{2+} and Mg^{2+} in the surface soil layers, increasing base saturation and decreasing Al^{3+} saturation (Lourenzi et al. 2011). Much published literature has reported an increase in soil pH due to sludge addition (Kumar and Chopra 2014; Paradelo and Barral 2017).

On the contrary, a decrease in the pH of alkaline soil due to sludge application in our study (Table 4) may be attributed to the acidic pH of sludge and the production of organic acids during the decomposition of sludge under aerobic conditions. It may also be attributed to the mineralization of the organic N and S added by sludge, which produces H^+ ions leading to the decrease in soil pH (Eid et al. 2019). It can be concluded that high nutrient content was associated with a neutral pH range because the pH of acid soil increased and the pH of alkaline soil decreased due to sludge application. Overall, the effects of sludge application on soil pH can have important ramifications for agriculture and environmental management. Understanding the factors that affect soil pH and the mechanisms by which sludge can influence soil pH can help to maximize the use of this valuable resource and minimize its potentially serious impacts on soil and environmental health. Electrical conductivity (EC) of post-harvest soil of soybean increased significantly with increasing rates of sludge application (Table 4). The EC ranged from 0.12 to 0.72 and 0.16 to 0.56 dS m^{-1} in acid and alkaline soil, respectively. The increased EC in both soils as a result of the addition of increasing rates of sludge may be due to the high amount of soluble and disassociated ions released from sludge. A higher value of EC was recorded in alkaline soil compared to acid soil (Table 4), which can be attributed to the inherent characteristics of these two contrasting soils. In this study, even the application of sludge @ 160 g kg^{-1} could not induce the soil salinity conditions. Usually, the critical value of EC more than 4.0 dS m^{-1} has been considered as one of the criteria for judging saline soil (Choudhary and Kharche 2018; Othman et al. 2023). However, in the present study the maximum value of EC has been recorded as 1.84 dS m^{-1}, which is far less than the above-mentioned critical value.

In addition to pH, organic carbon has been identified as an important indicator of soil quality (Table 4) because it serves as a storehouse of essential nutrients for plants and microorganisms and plays an important role in nutrient dynamics (Rattan et al. 2005). However, the addition of sludge may be more effective in increasing organic carbon in acid soil than in alkaline soil. It can be attributed to the higher clay content in acid soil (48%) as compared to that in alkaline soil (12%), which confers higher adsorption capacity. This has proved to be a conducive condition for the formation of clay organic complexes (Hamdi et al. 2019). In this study, sludge contains around 25.4% organic carbon, and when sludge is added to soil, the organic carbon content in the soil increases. This increase in soil organic carbon content is believed to have a positive impact, especially under tropical conditions where organic carbon continuously diminishes due to a higher decomposition rate. The organic carbon contained in sludge can be a valuable source of nutrients and energy for soil microorganisms, and its addition to soil can lead to improvements in soil structure, water-holding capacity, and nutrient availability. As a result, sludge application can enhance soil fertility, leading to increased crop yields and improved plant growth (Golui et al. 2014).

Sludge contains a considerably large amount of heavy metal content. Our results show that the total metal content in both soils significantly increased due to sludge application (Table 4). Application of sludge @ 5.0–160 g kg^{-1} could enhance Fe, Mn, Zn, Cu, Ni, Cd, Pb, and Cr content by 15.2–102, 8.93–92.2, 8.96–275, 10.7–172, 3.70–40.1, 25.9–344, 8.77–146 and 22.9–189%, respectively, in acid soil, and 6.75–84.9, 4.41–50.3, 21.8–308, 2.55–262, 10.8–49.3, 25.7–440, 8.77–315 and 22.9–204%, respectively, in alkaline soil compared to the control. The mean value of heavy metal content was higher in the acid soil as compared to alkaline soil. However, the increase in extractable heavy metal content in alkaline soil was higher compared to acid soil. Higher heavy metal content in alkaline soil due to sludge application may be explained by the decreased solubility of metals at higher pH (Lindsay 1979; Saraswat et al. 2023). All treatments consisting of graded doses of sludge application showed heavy metal content below the permissible levels, except Pb which exceeded the permissible levels, particularly at the higher rate of sludge application. Such similar results concerning the increase in extractable heavy metal content with sludge application have also been reported by Roy et al. (2013) and Golui et al. (2014).

Relationship between plant metal content and soil properties

Pearson's correlation analysis evaluates the relationship between heavy metal content in soybean plant tissues and soil chemical properties (Table 2 in Supplementary Information). The pH of acid soil had significant ($p < 0.01$) positive correlations ($r = 0.58–0.95$) with all heavy metals in the soybean plant, while the pH of alkaline soil showed a negative ($r = -0.64$ to -0.96 at $p < 0.01$) correlation. A strong negative correlation observed in alkaline soil strongly suggests the increased heavy metal availability to plants with a decrease in pH due to sludge application. To prevent the build-up of heavy metals in soil and their transfer within plants, it is important to carefully manage the use of sludge as a soil amendment. The analyzed heavy metals in roots, straw and seed of soybean had significant positive correlations with soil heavy metals, with r values varying between 0.46 to 0.97. Galal et al. (2017) heavy metal concentrations in plants can increase with increasing heavy metal levels in the soil, the specific relationship between soil heavy metal levels and plant uptake can be complex and influenced by many factors.

Proper management of heavy metal contamination in soil is important to minimize any potential negative outcomes for human and environmental health. The use of soybean as a test plant could be considered a bio-indicator for heavy metals in sludge-amended soils. This is supported by the significantly positive correlations between the majority of heavy metals in the soil and soybean tissues (Galal and Shehata 2015). However, it must be taken into consideration that bioavailability, mobility, and uptake of heavy metals by plants is a complex process that is influenced by several different factors. Included here are the type and form of heavy metal present in the soil, the pH of the soil, the presence of other chemical compounds in the soil, and specific plant species. Some plants may be more effective at taking up heavy metals than others, and some heavy metals may be more readily taken up by plants than others. The uptake of heavy metals by plants can have serious consequences for human health and the environment, particularly if the plants are consumed by humans or animals. Therefore, it is important to carefully monitor and manage soils' heavy metal levels so that the safe levels for human and environmental health are not exceeded. Organic carbon had a significant positive correlation with all heavy metals analyzed. The outcome of our study was consistent with earlier reports by Golui et al. (2014) and Eid et al. (2017, 2019).

Conclusions

This study discovered that the use of sludge as an alternative source of organic fertilizer can increase soybean yield and subsequent heavy metal accumulation in soybean tissue. The levels of all heavy metals measured in the edible plant parts were within the safe limits according to Indian standards, except for Fe, Zn, and Cu in the roots and Fe content in the straw of acid soils. The heavy metal content in soybean tissues was strongly correlated with soil pH, soil organic carbon, and soil extractable metals. The study also revealed that the use of sludge in alkaline soil would be safer than in acid soil, as there was a negative correlation between the pH of alkaline soil and the heavy metal content in soybean. Based on the results, the safe dose of sludge for soybean was determined to be 40 and 80 g kg^{-1} for acid and alkaline soil, respectively. However, it is important to note that these limits are indicative only and need to be further evaluated under field conditions. The uptake of heavy metals by plants from sludge-amended soil should also be monitored, preferably when conducting a long-term field study. To prevent the inflow of potentially toxic elements through sludge into the soil-plant-human system, appropriate regulations should be established and enforced to check the sources and seasons of heavy metal content in sludge.

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Data availability All data underlying the results are generated and analyzed by the authors and are available as part of the article. No additional source data are included in the manuscript. Statement regarding materials availability is not applicable.

Declarations

Ethical Approval Not applicable

Consent to Participate Not applicable

Consent to publish Not applicable

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