ECO-FRIENDLY MITIGATION OF SOIL HEAVY METAL POLLUTION IN SHEIKHUPURA INDUSTRIAL REGION

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ABSTRACT: Developing nations like Pakistan, which is lagging in enforcing pollution regulations and standards, are particularly vulnerable to soil contamination from rising industrial tendencies and waste production, both of which are known to cause cancer. The goal of this study was to investigate the elevated heavy metal concentration in the soil of the Sheikhupura district's industrial zone and to determine efficacy of co-pyrolyzed biochar for the immobilization of detected heavy metals. Additionally, this research also aimed to investigate the effects of biochar on the growth response of spinach in polluted soil. The properties of produced biochar were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) and proximate analysis. Pre analysis of collected soil samples indicated that concentrations of Cu, Cr, Pb, Cd and Zn were found higher than the permissible limits issued by WHO. The results of the pot trail experiment indicated that significant immobilization of all the detected heavy metals was observed in treatment T3 having 4% biochar level and 20% leaf compost. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 58.3%, 56.5%, 60.7%, 58.3% and 58.3%, respectively in SS-1. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 57.7%, 54.2%, 62.6%, 57.7% and 57.2%, respectively in SS-2. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 55.3%, 55.3%, 55.3%, 55.3% and 55.3%, respectively in SS-3. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 63.3%, 57.4%, 68.7%, 63.3% and 66.3%, respectively in SS-4. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 53.9%, 54.4%, 53.9%, 53.9% and 51.2%, respectively in SS-5. Maximum growth attributes of spinach were also observed in treatment T3 in all the soil samples. Therefore, it can be inferred from the conducted research that co-pyrolyzed biochar is an efficient and cost-effective remediation strategy for the immobilization of heavy metals from the polluted soil thereby enhancing the morphological features of different plant species.

Keywords: Soil; Heavy metals; Contamination; Remediation; Biochar.

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INTRODUCTION

The contamination of soil by heavy metals has emerged as a pressing environmental problem in the world and it has caused very serious impacts to the ecosystem and human being. As a basic component of the terrestrial ecosystem, soil has a crucial function in the growth of plants, the cycling of nutrients, and the filtration of water and is a crucial component of agricultural performance and ecological balance (Irfan et al., 2021). But it is the purity of this invaluable resource that is being jeopardized by the infiltration of heavy metal. Of these pollutants, the risks of heavy metals are especially dangerous because of its toxicity and persistence, as well as its prevalence and a high risk to the environment and human population (Priya, 2023).

The level of heavy metal contamination in the soil has become alarming in most parts of the world due to anthropogenic processes like industrial operations, emission of automobiles and improper handling of damaging materials (Peterson et al., 2023). Contamination of heavy metal in South Asian countries such as Pakistan has many sources which are usually interrelated. About 45 per cent of soil heavy metal contamination is due to industrial emissions especially in the manufacture of lead-acid batteries, metal smelting and paint production (Premalatha et al., 2023). Although the phasing of lead gasoline has been abandoned, automobile emissions still lead to approximately 30% of the soil lead, mostly because of the deposition of the past, and also because some regions still use lead fuel (Pourrut et al., 2011). The use of heavy metal-contaminated phosphate fertilizers and sewage sludge is one source of 15% of the metal contamination in soil. The other 10 percent is attributed to poor disposal of electronic wastes and products with high amount of heavy metal (Qin et al., 2022).

The median concentration of various heavy metals in topsoil is around 15 mg/kg in the globe, but in cities such a range is much higher and sometimes more than ten times the median (Shi et al., 2021). In some countries, such as Pakistan, the high levels of the toxic heavy metals are mostly found in the urban and industrial areas, with soil and plants having a maximum contaminant of 300 µg/g and 0.30 µg/g respectively. In more localized studies, still more extreme cases are found; a sample of soil and pasture grass taken close to battery recycling smelters in Hyderabad, Pakistan contained Pb levels as high as 70 and 1310 mg kg^{-1} (Siddika et al., 2022).

Plants are highly affected by exposure to heavy metal contaminants. Heavy metal poisoning in plants that include Pb, Cd, Cr, Cu and Zn poisoning interferes with physiological processes in plants that include photosynthesis, chlorophyll synthesis and absorption of food (Shi et al., 2021). Studies have been done in Pakistan and have revealed that exposure of metals can seriously affect the ability of the staple crop of the region to generate roots and shoots (Qin et al., 2022). Also, the exposure to these heavy metals compromises the capacity of plants to photosynthesize (Pourrut et al., 2011). Doubling and tripling of reactive oxygen species conditions oxidative stress that develops as a result of metal accumulation and further compromises the production and health of plants.

Plant heavy metal poisoning is a complex process that involves many processes. Recent studies also have discovered the molecular mechanisms of stress responses triggered by metals (Gupta et al., 2024). Pb leads to the proliferation of reactive oxygen species (ROS), which damages membrane, and leads to lipid peroxidation. Moreover, lead ions inhibit important enzymes such as ribulose-1, 5 -bisphosphate carboxylase/oxygenase (RuBisCO) which are involved in chlorophyll production and carbon fixation (Feng et al., 2023). The competition between lead and required cations (e.g., Ca^{2+}, Mg^{2+}, Fe^{2+}) causes nutrient imbalances (Sebastian, 2022). These heavy metals may cause genotoxicity at high levels that lead to reduced mitotic activity and chromosomal aberration. It also alters the proportion of the plant hormones and more specifically the levels of auxin and cytokinin which are vital and development and growth (Ettinger et al., 2020).

The impacts of lead contamination have far flung effects on human health, which extend beyond ecological effects (Feng et al., 2023). Lead is a potent neurotoxin, particularly among children, where 52 of the 53 children (98%), with a concentration of lead in blood stream of more than 10 µg /dL with a mean of 21.60 mcg/dl. These concentrations are much above the 5 ug/dL limit at which the U.S. Centers for Disease Control and Prevention (CDC) recommend action, which demonstrates the extent of lead exposure in the urban area. Recent research also concluded that the minor concentration of Pb in blood, which was previously believed to be harmless, also have a connection with neurological disorders, such as an average reduction of 7.4 IQ points per 10 µg/dL of lifetime blood lead content (Ettinger et al., 2020).

Immediate demand is need to be efficient and long-lasting remediation techniques since there is pressing need to control Pb pollution. To illustrate, soil washing is not deemed economically viable to be used on a broader scale due to its potentially high price (between 50 to 200 dollars per cubic meter treated soil) of application (Miceli et al., 2024). Consequently, other remediation methods that are cost-effective and environmentally friendly are increasingly gaining popularity. The application of organic amendments has become the topic of discussion in the last couple of years as a promising alternative to remedial use in heavy metal-contaminated soils (Ettinger et al., 2020). Organic amendments including biochar and compost have been reported to have significant potential on immobilization of heavy metals, increasing soil fertility and growth of plants. A carbon-rich substance formed by the pyrolysis of organic material, biochar, has been suggested to decrease bioavailable lead in soil up to 97% because of its high surface area, which can frequently be greater than 192 m^2/g) and offers many adsorption sites to the lead ions (Chatterjee et al., 2020).

Compost as soil amendment although has several advantages. It has the ability to increase the primal content of soil upto 2% and improve cation exchange capacity due to the improvement of the ability of soils to retain nutrients and immobilize heavy metals (Richardville et al., 2022). Also, the synergistic effect of compost and biochar together has beneficial effects on soil health and lowers the supply of lead. A study conducted on a mixture of biochar and compost enhances the fertility and growth of plants and full yield and nutrient uptake compared to organic fertilizers (Nobile et al., 2022). Another breakthrough work also helped to decode processes behind this synergy (Zhang et al., 2021).

Humic substances in compost combine with organic lead molecules, which results in massive lead immobilization but on the other hand, biochar binds inorganic lead ions largely. The mixture promotes the development of favorable soil microorganisms, such as arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria, that boost the nutrient intake by plants and are involved in the process of lead phytostabilization (Wang et al., 2022). The dual amendment is very beneficial in the improvement of porosity and soil aggregation and it reduces the lead mobility by reducing leaching and erosion. The fundamental character of biochar and organic acid generation in the stabilization of compost creates a balanced pH environment to lower the lead solubility (Richardville et al., 2022). These findings provide a great scientific basis of joint application of compost and biochar in the lead remediation strategy.

The main aim of the conducted research was to (i) evaluate heavy metal content in the soil samples of different industrial sector near Sheikhupura district; (ii) to characterize the produced biochar and determine its efficiency in immobilizing heavy metal content from soil samples used in this study; and (iii) to determine the influence of different levels of biochar on growth attributes of spinach under heavy metal contaminated soil.

MATERIALS AND METHODOLOGY

Geology of the Area: The soil of Sheikhupura is a mixture of loamy texture. The Bar, the northwest region of the province, has flat prairie land with relatively flat soil and places called Missies. The Ravi riverine have a light loam soil type in the low lands. Due to high salt concentration, the stiff soil is further classified as Rohi or Kallarathi in Punjab (2016).

Sample collection and preparation: The soil samples were collected from the topsoil layer (0-40 cm) of five different industrial sites using a wooden slab during the month of March. To ensure that no cross-contamination occurred, the slab was sterilized with deionized water after each collection. After that, the samples were moved to the environmental biology lab in the pre-labeled plastic zip lock bags. The soil samples were air-dried for four days at room temperature. After drying, the samples were grounded and sieved through 2mm mesh for ensuring uniform particle size.

Soil digestion for pre-analysis: Soil samples were digested according to the procedure prescribed by Wolf (1982) using di-acid mixture (9:4 ratio) of concentrated nitric acid (HNO_3) and per-chloric acid ($HClO_4$) at 350°C on a hot plate. After the sample was digested, it was cooled at room temperature. Then, 50 mL of distilled water was added and filtered with Whatman No. 42 filter paper. The sample was stored and quantified for lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) using atomic absorption spectrophotometer (AAS) (SP-IAA4530, China).

Biochar production: Rice husk and bagasse were chosen as a feedstock material for biochar production through co-pyrolysis. After three days of sun drying, the feedstocks were crushed and sieved until they reached a particle size of 2 mm. Next, they were blended in a 1:1 ratio and sealed in zip lock bags. A laboratory scale pyrolysis reactor was used for biochar production at 450°C for 1 hour at a rate of 10 °C/min in an inert environment. After production, biochar sample was sieved at 2 mm and sealed in zip-lock bags.

Proximate and spectral analysis of biochar: Biochar yield was calculated by using formula prescribed by Chandra and Bhattacharya (2019). Proximate analysis of biochar was conducted according to the standard procedure (ASTM, 1762). Fourier-transform infrared spectroscopy (FTIR) (Presto-21 Shimadzu IR spectrometer) was conducted to determine the presence of functional groups lying on the surface of biochar sample pH and EC of biochar sample was also determined. According to procedures prescribed by ASTM (1762), proximate analysis of biochar was conducted which include determining moisture content, ash content, volatile matter, and fixed carbon content. Following are the formulas that are used for the determination of yield, moisture content, ash content, volatile matter, and fixed carbon content, respectively;

$$\text{Biochar Yield \%} = \frac{\text{mass of biochar}}{\text{mass of feedstock}} \times 100$$

$$\text{Moisture Content \%} = \frac{\text{weight of biochar} - \text{weight of oven dried biochar}}{\text{weight of biochar}} \times 100$$

$$\text{Volatile Matter \%} = \frac{\text{weight of oven dried biochar} - \text{weight of biochar at 950°C}}{\text{weight of oven dried biochar}} \times 100$$

$$\text{Ash Content \%} = \frac{\text{weight of biochar at 950°C} - \text{weight of biochar at 750°C}}{\text{weight of biochar 950°C}} \times 100$$

Fixed Carbon %= 100−Volatile Matter % + Ash Content %

Determination of physicochemical characteristics

pH and electrical conductivity: The pH and electrical conductivity (EC) of the soil samples as well as biochar was determined by portable digital pH and electrical conductivity meter. 1g of sample was added in 5 ml of distilled water for the preparation of solution, which was shaken in an orbital shaker for 60 minutes and was allowed to cool at room temperature (Feng et al., 2023).

Cation exchange capacity (CEC): CEC of the soil samples and biochar was determined. The samples were grounded and sieved (2 mm) into a fine powder and dissolved in 50 mL of 0. 5M hydrochloric acid (HCl) solution and shaken at 110 revolutions per minute (rpm) for 2 hours at room temperature. The samples were filtered and washed with distilled water until the filtrate had a neutral pH. The filtered samples were then mixed with 50 mL of 0. 5 M barium acetate ($Ba(OAc)_2$) solution and shaken at 110 rpm for 1 hour. Finally, the samples were filtered and washed with 100 mL of deionized water. The solid residue was removed by filtration and the filtrate was titrated with 0. 5N sodium hydroxide (NaOH) solution with phenolphthalein as an indicator until a faint pink color was observed.

Compost production and characterization: Compost was prepared by utilizing Kachnar (*Bauhinia variegata*), Conocarpus (*Conocarpus erectus*), Seesham (*Dalbergia sissoo*), Channa (*Cedrela toona*), Alstonia (*Alstonia scholaris*), and Fex (*Ficus religiosa*) plant species. These components were mixed in a ratio 3:1 of degraded plant species to cow manure (on dry weight basis) after organizing and hewing for 12 weeks (Compo, 2023). Leaf compost was characterized by following standard procedures stated by Kome et al. (2018). Proximate analysis was conducted on leaf compost to analyze moisture content, volatile matter, ash content, and fixed carbon. Fourier transform infrared spectroscopy (FTIR) was conducted for the determination of different functional groups. The physicochemical characteristics of the leaf compost including pH, EC, CEC, total nitrogen (TN), available phosphorus (AP), and available potassium (AK) were evaluated. The TN content of leaf compost was determined following Kjedahl's method, while AP and AK were determined and analyzed by atomic absorption spectrophotometer (AAS) according to the procedure stated by Richards (1954).

Pot Trial: The pot trail experiment was conducted in green house of College of Earth and Environmental Sciences (CEES), University of Punjab. Completely randomized design (CRD) was followed with three replications of each treatment. Round pots having diameter of 17 cm and 22 cm height were filled with 3.5 kg of soil. The soil was layered with leaf compost comprising of 20% of the total soil volume. Seven disinfected seeds of spinach were sown in each pot. In this experiment, there were total fifteen treatments across which three different levels of biochar were applied; 0%, 2%, and 4% w/w. The required amount of biochar was mixed to five different soil samples until it was homogeneously distributed and the mixture was moistened and left for two weeks before planting.

Plant analysis: After 1 month of pot trial experiment, plants were harvested. Different morphological attributes of plants including shoot length and root length, root and shoot biomass were measured. Harvested plants were separated into roots and shoots. Plant samples were washed using tap water and then with deionized water to remove suspended soil particles. Root and shoot length was determined using meter ruler and their fresh weights were determined using a weighing balance. The samples were then oven dried at 70°C for 3-4 days until constant weight was achieved. After oven drying, the samples were then digested using di-acid digestion method and transported to laboratory for the determination of heavy metal content using AAS.

Statistical analysis: The obtained data were statistically inspected by one-way analysis of variance (ANOVA) using DSASTAT software, followed by Duncan's multiple range test for finding significance between means of each treatment ($p < 0.05$).

RESULTS

Characteristics of Soil: The soil samples have a clay loamy texture having an alkaline pH ranging from 7.1 to 8.1 and EC ranging from 310 to 770 µS cm^{-1}. The CEC of the soil samples ranged from 6-15 cmol kg^{-1}. The OM content in dry soil samples ranged from 0.5-4%. Tabulated in table 1 are the soil physicochemical attributes. In SS-1, the concentration of Cu, Pb, Cr, Cd and Zn were found to be 0.87 mg kg^{-1}, 0.68 mg kg^{-1}, 0.0062 mg kg^{-1}, 6.58 mg kg^{-1} and 0.90 mg kg^{-1}, respectively. In SS-2, the concentration of Cu, Pb, Cr, Cd and Zn were found to be 0.12 mg kg^{-1}, 0.10 mg kg^{-1}, 0.02 mg kg^{-1}, 0.80 mg kg^{-1} and 0.53 mg kg^{-1}, respectively.

In SS-3, the concentration of Cu, Pb, Cr, Cd and Zn were found to be 0.22 mg kg^{-1}, 0.12 mg kg^{-1}, 0.01 mg kg^{-1}, 0.89 mg kg^{-1} and 0.69 mg kg^{-1}, respectively. In SS-4, the concentration of Cu, Pb, Cr, Cd and Zn were found to be 0.39 mg kg^{-1}, 0.14 mg kg^{-1}, 0.02 mg kg^{-1}, 3.8 mg kg^{-1} and 8.32 mg kg^{-1}, respectively. In SS-5, the concentration of Cu, Pb, Cr, Cd and Zn were found to be 0.35 mg kg^{-1}, 0.17 mg kg^{-1}, 0.02 mg kg^{-1}, 1.8 mg kg^{-1} and 4.2 mg kg^{-1}, respectively as presented in Table 1. Concentrations of Cu, Pb, Cr and Zn in all the soil samples were found above permissible limits issued by WHO (EU _ 2002, WHO/FAO, 2007) (Table 2).

Table 1. Physicochemical attributes of soil samples used in this study.

Attributes	SS-1	SS-2	SS-3	SS-4	SS-5	Unit
Ph	7.2	7.1	8	8.1	7.5	-
EC	430	770	310	350	520	µS cm^{-1}
CEC	8.3	8.7	8.92	9.2	9.5	cmol kg^{-1}
OM	8.4	8.3	8.9	9.2	9.5	%
Copper	0.87	0.12	0.22	0.39	0.35	mg kg^{-1}
Lead	0.68	0.10	0.12	0.14	0.17	mg kg^{-1}
Cadmium	0.0062	0.02	0.01	0.02	0.02	mg kg^{-1}
Zinc	6.58	0.80	0.89	3.8	1.8	mg kg^{-1}
Chromium	0.90	0.53	0.69	8.32	4.2	mg kg^{-1}

Data is given as mean error values. SS1 is soil sample 1 collected from steel industry; SS2 is soil sample 2 collected from paper industry; SS3 is soil sample 3 collected from pharmaceutical industry; SS4 is soil sample 4 collected from leather industry SS1 is soil sample 1 collected from textile industry. Small alphabets indicate mean

Table 2. Maximum permissible limits for heavy metal concentration in soil and plant (EU _ 2002, WHO/FAO, 2007).

Metals	Symbol	Soil (mg kg^{-1})
Cadmium	Cd	0.3
Chromium	Cr	15
Copper	Cu	14
Zinc	Zn	30
Lead	Pb	30

Biochar Characterization: The yield of biochar was found to be 40%. The biochar obtained in the course of pyrolysis was revealed to be alkaline and saline, with a pH of 9, EC of 1335 ± 10 µS cm^{-1} and CEC of 11.73 cmol kg^{-1}. proximate analysis of biochar revealed that it comprised of 6% moisture content, 21% volatile matter, 19% ash content and 60% fixed carbon content. The physicochemical characteristics of biochar are presented in Table 3.

FTIR of Biochar: The FTIR of biochar (Figure 1) produced from co-pyrolysis revealed combination of functional groups and absorption bands indicative of the biochar's composition and structures. The existence of deep and broad bands between 3400 and 3500 cm^{-1} is indicative of O-H stretching vibrations, which are often associated with hydroxyl groups. Aliphatic and aromatic compounds' stretching of the C-H bond occurs in the gravitational field regions spanning 2900-3000 cm^{-1}. A number of peaks have been identified: 1600–1700 cm^{-1} Chemical bond stretching vibrations of carboxyl and ketone groups absorb these bands. Aromatic systems include frequencies between 1500 and 1600 cm^{-1}, which are linked to C=C stretching. C-O asymmetric stretching from phenolic and ether functional groups corresponds to many peaks at about 1200-1400 cm^{-1}. The space below 1000 cm^{-1} may be attributed to various functional groups' C-H bending and C-O stretching modes.

Table 3. Physiochemical characteristics of biochar and leaf compost.

Parameters	Biochar	Leaf Compost	Units
pH	9.4±0.7	6.5±0.3	-
EC	1335±10	1755±10	µS cm^{-1}
Moisture content	6±1.88	25.32±0.93	%
Volatile matter	21±2.03	26.06±0.56	%
Ash content	19±2.51	18.94±0.48	%
Fixed carbon	60±2.09	55±0.74	%
CEC	11.73±1.79	28±3.89	cmol kg^{-1}

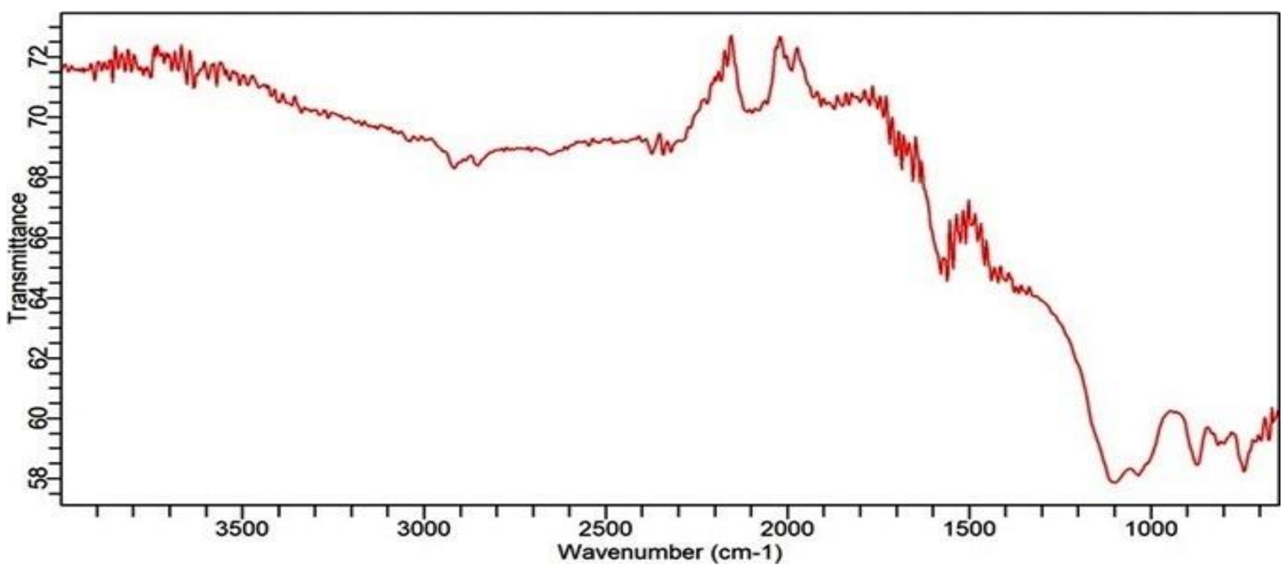

Figure 1. FTIR of Biochar

Physicochemical characteristics of leaf compost: The pH and EC of leaf compost were found to be 6.5±0.3 was 1755±10 µS cm^{-1}. The results of the compost's proximate analysis are shown in table 3. The compost's moisture level was determined to be 25%, while the volatile matter and ash contents were 26%, 18%, and 55%, respectively.

FTIR of leaf compost: The FTIR of leaf compost showed a combination of absorption bands indicative of the compost's organic composition. Since water, hydroxyl groups, and carboxylic acids all have O-H stretching vibrations, there is a wide absorption band between 3400 and 3500 cm^{-1}. aliphatic and aromatic C-H stretching vibrations occur between 2900 and 3000 cm^{-1}. Carboxyl, ketone, and ester groups' C=O stretching vibrations cause the absorption band to range from 1600 to 1700 cm^{-1}. Aromatic ring C=C stretching vibrations, which occur between 1500 and 1600 cm^{-1} phenolic, ether, and ester groups all contribute to 1200-1400 cm^{-1} by C-O stretching vibrations. aliphatic and aromatic groups' C-H bending vibrations, 1000-1200 cm^{-1}.

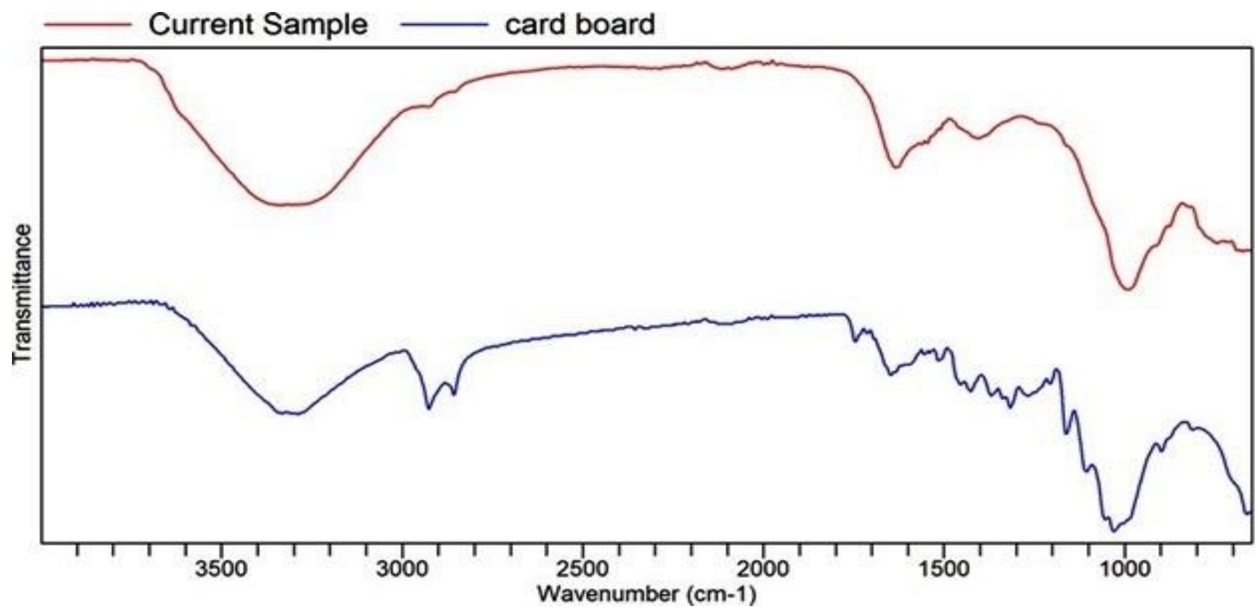

Figure 2. FTIR of leaf compost

Effect of biochar and leaf compost on immobilization of heavy metals

Copper (Cu): Influence of different levels of biochar (0%, 2% and 4%) and leaf compost (20%) on the remediation of heavy metals was analyzed. The efficiency of biochar and leaf compost in the reduction of Cu metal in soil samples including SS-1, SS-2, SS-3, SS-4 and SS-5 under treatment T2 was found to be 44.7%, 49.3%, 45.2%, 54.1% and 47.8%, respectively. Similarly, in treatment T3, the reduction of 58.3%, 57.7%, 55.3 %, 63.3% and 53.9% was recorded in SS-1, SS-2, SS-3, SS-4 and SS-5, respectively. The results indicated that the maximum removal of Cu was shown by treatment T3 in all the soil samples in comparison to treatment T1 followed by treatment T2.

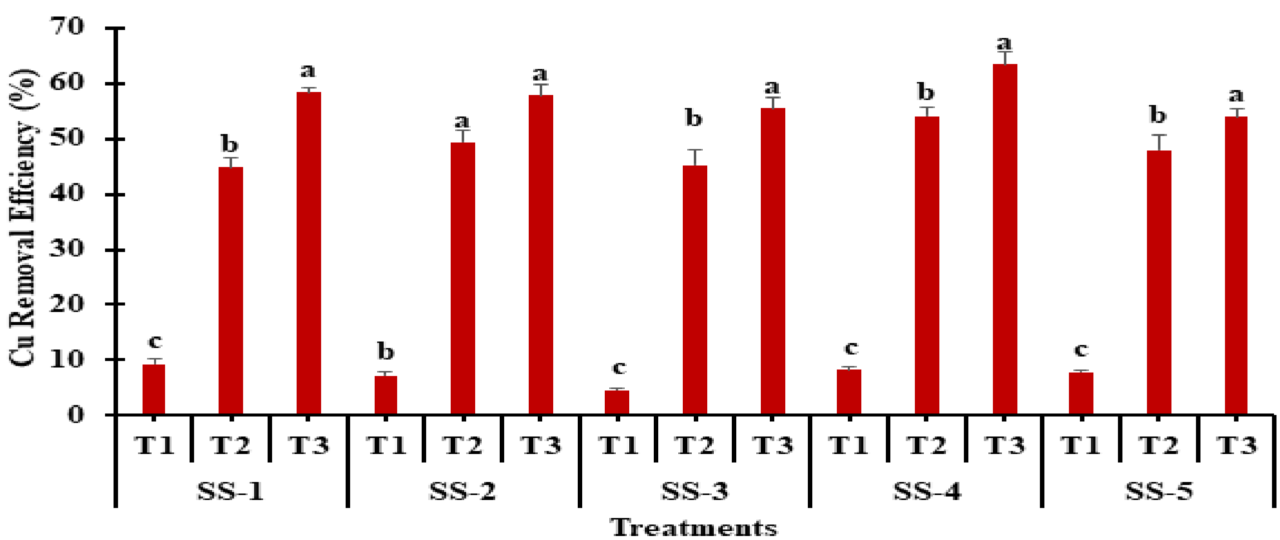

Figure 3. Influence of different levels of biochar on Cu immobilization from different soil samples. SS-1 represents soil sample collected from steel industry; SS-2 represents soil sample collected from paper industry; SS-3 represents soil sample collected from pharmaceutical industry; SS-4 represents soil sample collected from leather industry; SS-5 represents soil sample collected from textile industry. Small alphabets on each bar indicates significant differences among each treatment.

Lead (Pb): Influence of different levels of biochar (0%, 2% and 4%) and leaf compost (20%) on the remediation of heavy metals was analyzed. The efficiency of biochar and leaf compost in the reduction of Cu metal in soil samples including SS-1, SS-2, SS-3, SS-4 and SS-5 under treatment T2 was found to be 46.4%, 51.3%, 50.3%, 56.1% and 50.6%, respectively. Similarly, in treatment T3, the reduction of 60.7%, 62.6%, 55.3 %,68.7 % and 53.9% was recorded in SS-1, SS-2, SS-3, SS-4 and SS-5, respectively The results indicated that the maximum immobilization of Pb was shown by treatment T3 in all the soil samples in comparison to treatment T1 followed by treatment T2.

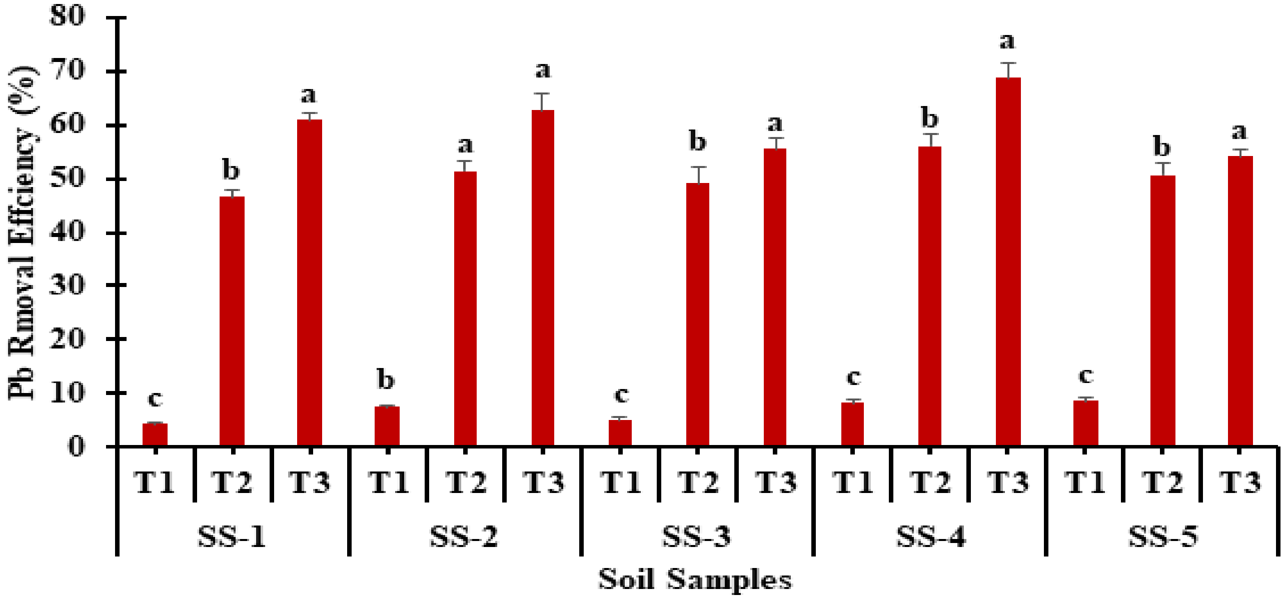

Figure 4. Influence of different levels of biochar on Pb immobilization from different soil samples. SS-1 represents soil sample collected from steel industry; SS-2 represents soil sample collected from paper industry; SS-3 represents soil sample collected from pharmaceutical industry; SS-4 represents soil sample collected from leather industry; SS-5 represents soil sample collected from textile industry. Small alphabets on each bar indicates significant differences among each treatment.

Cadmium (Cd): Influence of different levels of biochar (0%, 2% and 4%) and leaf compost (20%) on the remediation of heavy metals was analyzed. The efficiency of biochar and leaf compost in the reduction of Cu metal in soil samples including SS-1, SS-2, SS-3, SS-4 and SS-5 under treatment T2 was found to be 44.7%, 49.3%, 45.2%, 54.1% and 47.8%, respectively. Similarly, in treatment T3, the reduction of 58.3%, 57.7%, 55.3%, 63.3% and 53.9% was recorded in SS-1, SS-2, SS-3, SS-4 and SS-5, respectively. The results indicated that the maximum removal of Cd was shown by treatment T3 in all the soil samples in comparison to treatment T1 followed by treatment T2.

Chromium (Cr): Influence of different levels of biochar (0%, 2% and 4%) and leaf compost (20%) on the remediation of heavy metals was analyzed. The efficiency of biochar and leaf compost in the reduction of Cu metal in soil samples including SS-1, SS-2, SS-3, SS-4 and SS-5 under treatment T2 was found to be 44.7%, 47.7%, 45.2%, 53.6% and 47.8%, respectively. Similarly, in treatment T3, the reduction of 56.5%, 54.2%, 55.3%, 57.4% and 54.4 % was recorded in SS-1, SS-2, SS-3, SS-4 and SS-5, respectively. The results indicated that the maximum removal of Cr was shown by treatment T3 in all the soil samples.

Zinc (Zn): Influence of different levels of biochar (0%, 2% and 4%) and leaf compost (20%) on the remediation of heavy metals from five different soil samples was analyzed. The efficiency attained by treatment T2 in the reduction of Zn metal from SS-1 was found to be 44.7%, 50.3% by SS-2, 45.2% by SS-3, 56.2% by SS-4 and 48.8% by SS-5. Similarly, in treatment T3, the reduction of 58.3%, 57.2%, 55.3%, 66.3% and 51.2% was recorded in SS-1, SS-2, SS-3, SS-4 and SS-5, respectively. The results indicated that the maximum removal of Zn was shown by treatment T3 in all the soil samples.

Post treatment values of heavy metals in soil: The table 4 provided shows the concentrations of various heavy metals (Cu, Pb, Cd, Zn, and Cr) in soil samples collected from different types of industries. These concentrations are measured in milligrams per kilogram (mg kg^{-1}) and provide an insight into the level of contamination associated with each industry.

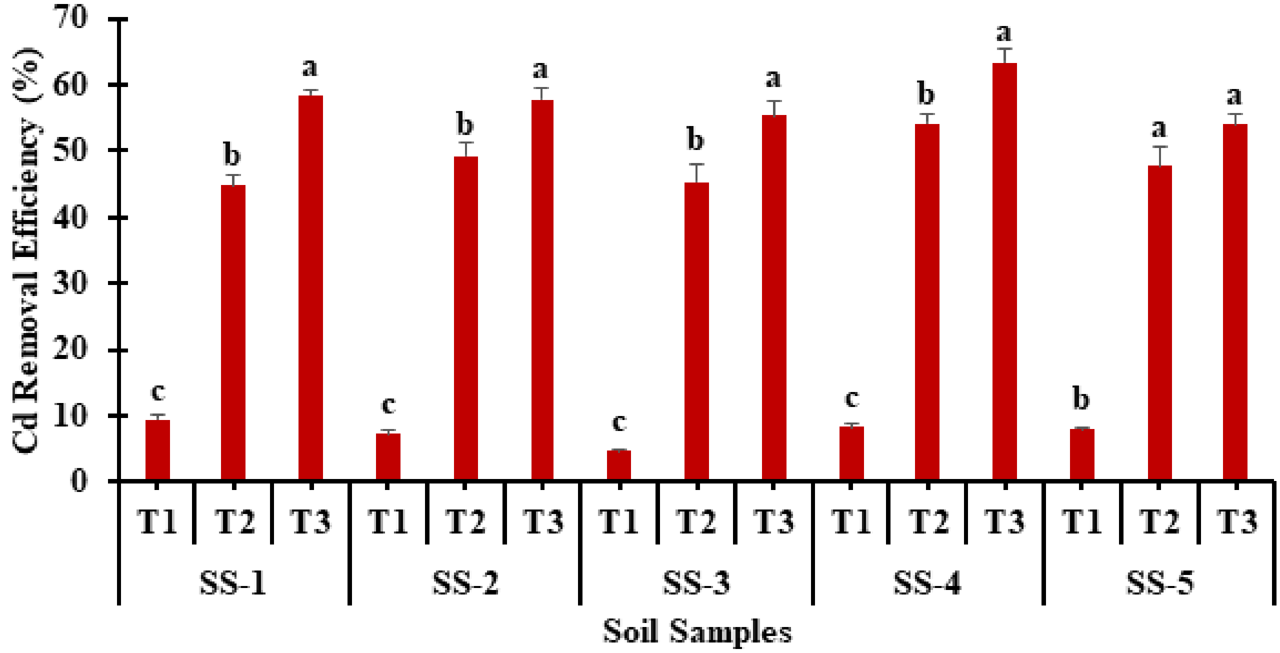

Figure 5. Influence of different levels of biochar on Cd immobilization from different soil samples. SS-1 represents soil sample collected from steel industry; SS-2 represents soil sample collected from paper industry; SS-3 represents soil sample collected from pharmaceutical industry; SS-4 represents soil sample collected from leather industry; SS-5 represents soil sample collected from textile industry. Small alphabets on each bar indicates significant differences among each treatment.

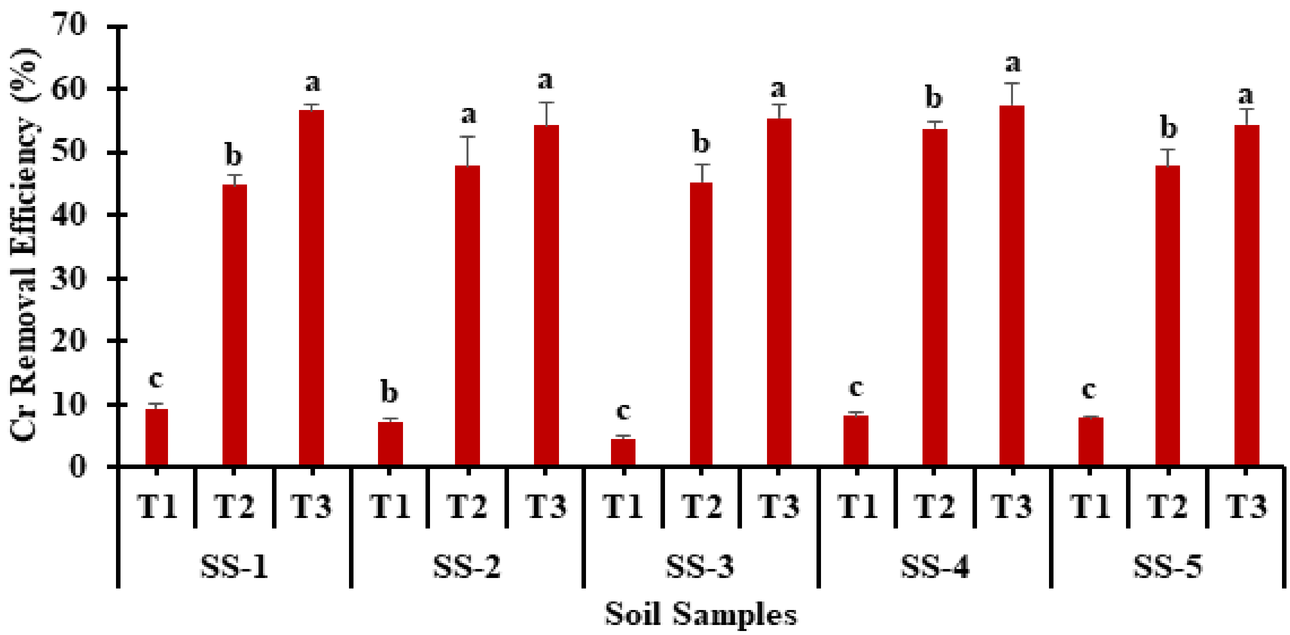

Figure 6. Influence of different levels of biochar on Cr immobilization in different soil samples. SS-1 represents soil sample collected from steel industry; SS-2 represents soil sample collected from paper industry; SS-3 represents soil sample collected from pharmaceutical industry; SS-4 represents soil sample collected from leather industry; SS-5 represents soil sample collected from textile industry. Small alphabets on each bar indicates significant differences among each treatment.

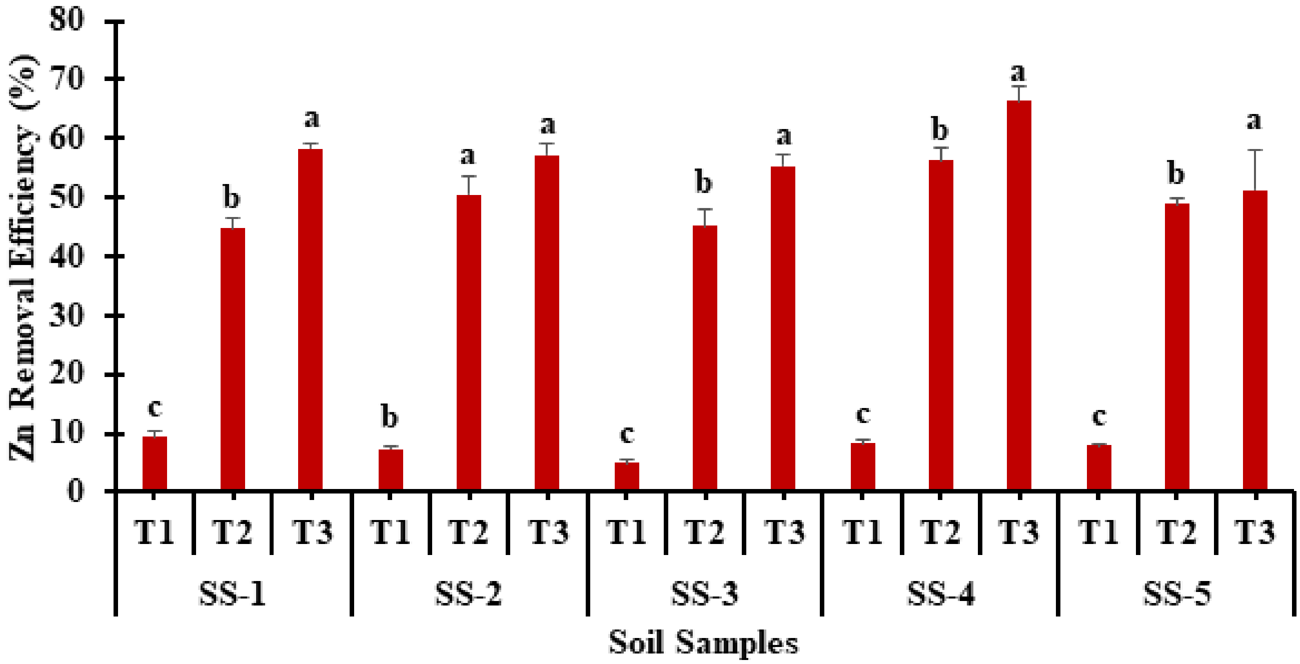

Figure 7. Influence of different levels of biochar on Zn immobilization in different soil samples. SS-1 represents soil sample collected from steel industry; SS-2 represents soil sample collected from paper industry; SS-3 represents soil sample collected from pharmaceutical industry; SS-4 represents soil sample collected from leather industry; SS-5 represents soil sample collected from textile industry. Small alphabets on each bar indicates significant differences among each treatment.

Table 4. Post treatment values of heavy metals in soil

Industry	Cu (mg l^{-1})	Pb (mg l^{-1})	Cd (mg kg^{-1})	Zn (mg kg^{-1})	Cr (mg kg^{-1})
Steel Industry	0.5450	0.3407	0.0025	5.1391	0.3608
Paper Industry	0.0753	0.0548	0.0000	0.6291	0.2157
Pharmaceutical Industry	0.1396	0.0630	0.0000	0.6948	0.2788
Leather Industry	0.2465	0.0748	0.0000	2.9688	3.3293
Textile Industry	0.2213	0.0856	0.0000	1.4089	1.7200

Influence of biochar and leaf compost on growth of Spinach: Influence of three different levels of biochar and leaf compost on the growth attributes of spinach was analyzed**.** The results have been demonstrated below.

Root length: Results indicated that shoot length of 2.1 cm, 2.62 cm and 4.2 cm was shown by treatment T1, T2 and T3 under SS-1, root length of 2.3 cm, 2.53 cm and 4.7 cm was shown by treatment T1, T2 and T3, respectively, under SS-2, root length of 2.2 cm, 2.37 cm and 4.57 cm was shown by treatment T1, T2 and T3, respectively, under SS-3, root length of 2.4 cm, 2.42 cm and 4.32cm was shown by treatment T1, T2 and T3, respectively, under SS-4, while in SS-5, the root length of 1.98 cm, 2.61 cm and 4.62 cm was recorded in treatment T1, T2 and T3, respectively. Results indicated that the maximum root length of spinach plant was observed in treatment T3 in all the soil samples.

Shoot length: Results indicated that shoot length of 2.6 cm, 2.9 cm and 3.87 cm was shown by treatment T1, T2 and T3 under SS-1, root length of 2.7 cm, 2.72 cm and 3.73 cm was shown by treatment T1, T2 and T3, respectively, under SS-2, root length of 2.31 cm, 2.43 cm and 3.32 cm was shown by treatment T1, T2 and T3, respectively, under SS-3, root length of 2.53 cm, 2.52 cm and 3.91 cm was shown by treatment T1, T2 and T3, respectively, under SS-4, while in SS-5, the root length of 2.1 cm, 2.37 cm and 3.41 cm was recorded in treatment T1, T2 and T3, respectively. It was observed that maximum shoot length was shown by spinach plant grown in treatment T3 in all the soil samples.

Root biomass: Under SS-1, root biomass of 1.5 g, 1.43 g, and 3 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-2, root biomass of 1.34 g, 1.42g, and 3.3 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-3, root biomass of 1.12 g, 1.56 g, and 3.47 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-4, root biomass of 1.37 g, 1.7 g, and 3.63 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-5, root biomass of 1.43 g, 1.82 g, and 3.17 g was shown by treatment T1, treatment T2 and treatment T3, respectively. It was observed that in all the soil samples maximum increment in root biomass was shown by treatment T3.

Shoot biomass: Under SS-1, shoot biomass of 1.35 g, 1.51 g, and 2.57 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-2, shoot biomass of 1.45 g, 1.54 g, and 2.9 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-3, shoot biomass of 1.33 g, 1.61 g, and 2.83 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-4, shoot biomass of 1.42 g, 1.76 g, and 2.79 g was shown by treatment T1, treatment T2 and treatment T3, respectively. Under SS-5, shoot biomass of 1.57 g, 1.83 g, and 2.64 g was shown by treatment T1, treatment T2 and treatment T3, respectively. It was observed that in all the soil samples maximum increment in shoot biomass was shown by treatment T3.

Table 5. Morphological attributes of spinach plant

Parameters	SS-1	SS-2	SS-3	SS-4	SS-5
			Treatment T1		
Root length (cm)	2.1±0.13	2.3±0.16	2.2±0.09	2.4±0.14	1.98±0.12
Shoot length (cm)	2.6±0.3	2.7±0.17	2.31±0.08	2.53±0.13	2.1±0.22
Root biomass (g)	1.5±0.23	1.34±0.43	1.12±0.13	1.37±0.23	1.43±0.07
Shoot biomass (g)	1.35±0.32	1.45±0.22	1.33±0.18	1.42±0.05	1.57±0.37
			Treatment T2		
Root length (cm)	2.62±0.28	2.53±0.31	2.37±0.21	2.42±0.13	2.61±0.03
Shoot length (cm)	2.9±0.09	2.72±0.07	2.43±0.23	2.52±0.11	2.37±0.23
Root biomass (g)	1.43±0.13	1.42±0.65	1.56±0.39	1.7±0.18	1.82±0.08
Shoot biomass (g)	1.51±0.86	1.54±0.73	1.61±0.13	1.76±0.29	1.83±0.28
			Treatment T3		
Root length (cm)	4.2±0.7	4.7±0.173	4.57±0.23	4.32±0.13	4.62±0.14
Shoot length (cm)	3.87±0.31	3.73±0.38	3.32±0.36	3.91±0.33	3.41±0.23
Root biomass (g)	3±0.13	3.3±0.17	3.47±0.16	3.63±0.18	3.17±0.33
Shoot biomass (g)	2.57±0.47	2.9±0.43	2.83±0.07	2.79±0.09	2.64±0.03

DISCUSSION

Heavy metal pollution is regarded as one of the major environmental issues that poses severe threats to the development, production, and well-being of plants in all levels of food chain (Chang et al., 2023). The issue of heavy metals in the polluted soils is becoming vulnerable due to its alarming concentration in the polluted soils. Even though some mitigation methods can be applied, a combination of compost and biochar can be a possible solution to the immobilization of heavy metals in soils. The impact of both of these amendments on plant growth attributes was also studied using a comprehensive approach**.**

The production process of biochar greatly determines the nature and effectiveness of biochar. Biochar in this research was produced by co-pyrolyzing both the feedstock materials at 450°C, which is considered slow pyrolysis (300-700°C), according to Wijitkosum (2023). The pyrolysis temperature influences biochar yield and carbon content whereby high temperature minimizes the yield but maximizes the carbon content (Khater et al., 2024a). The balance between yield and carbon stability was observed at 450°C. Similar findings were reported by Khater et al. (2024a) and Wijitkosum (2023), who reported maximum yield of different kinds of biochar at the temperature ranging from 300-700°C**.**

pH and EC of biochar were also analyzed in order to determine its role in immobilization. It was observed that pH of biochar also rises with pyrolysis temperature resulting in alkaline properties (Chatterjee et al., 2020). Biochar that was made at the temperature of 450°C had an alkaline pH of 9.4, which are considered beneficial in neutralizing acidic soils (Roshan et al., 2023). Pyrolysis temperature is also associated with EC of biochar. The EC values range of 505.6 to 1414.4 mg/L at 400°C according to Khater et al. (2024b), whereas the biochar used in the research had an EC of 1335 mg L^{-1}. The moisture content of biochar was found to be 6%, indicating that it has low water holding capacity, which is favorable in terms of structural stability in the soil.

The content of volatile matter was found to be 21% indicating a stable structure of carbon leading to long-term storage of carbon in the soil. The ash content of biochar was 19%, which is an indicator of the nutrient availability while fixed carbon content was 60%, indicating that the biochar can be used in carbon sequestration and stability in the soil. Our findings coincide with the findings of Roshan et al. (2023) and Chatterjee et al. (2020), who conducted proximate analysis of biochar having higher fixed carbon content indicating biochar's structural stability.

Physical characteristics of compost were also determined. It was observed that compost addition enhances soil aggregation, which has a positive effect on aeration and water retention. According to Sullivan et al. (2018), the pH of compost was between 6.0 and 7.5 that is comparable to the observed pH of compost used in this study having value of 6.6. This pH is considered suitable for soil application in terms of nutrient availability and microbial activity. The moisture content of compost was found to be 25.3%, 26% volatile matter, 18.9% ash and 55% fixed carbon. Similar findings were reported by Sullivan et al. (2018) who conducted proximate analysis of compost.

The soil analysis displayed some of the important properties that are pertinent to its remediation and plant growth. All the samples of the soil were alkaline in nature which affects the availability of nutrition and the performance of the growth of plants. The soil samples used in this research had an EC of 310-770 µS cm^{-1} which showed the salinity level of the soil. CEC of the soil was found to be 16.67cmol kg^{-1} with is considered moderate. The analysis of soil is in accordance to the study conducted by Smith and Doran (2013) who reported the similar findings regarding the soil contaminated with heavy metal content.

When higher levels of biochar (4% in treatment T3) were used, growth parameters of spinach plant were drastically improved. This enhanced efficacy of biochar on heavy metal toxicity has been in line with the recent discoveries (Yu et al., 2019), who indicated comparable development reactions in vegetables cultivated in heavy metal-heavy soils with added biochar. The dose-dependent effect in the research indicates that the biochar efficacy in reduction of heavy metal stress is concentration-dependent, which (Ali and Nas, 2018b) also observe in their article on biochar use in polluted agricultural soils.

Heavy-metal contamination of agricultural soils commonly reduces plant growth by disrupting root development, decreasing shoot elongation, and lowering biomass accumulation through oxidative stress, nutrient imbalances and root membrane damage. In the present study different growth attributes of spinach (*Spinacia oleracea*) were analyzed that were grown in contaminated soil. The results showed differential phytotoxic effects on growth attributes of spinach including root length, shoot length, and fresh biomass reflecting both the metal load and metals' speciation. As expected, the spinach grown in untreated contaminated soils i.e., treatment T1 showed minimum root lengths, reduced shoot elongation and the lowest root and shoot fresh biomass relative to the plants that were grown in treatments T2 and T3. This reduction likely results from metal-induced root apical damage and inhibited cell elongation, which together limit water and nutrient uptake and consequently above-ground growth. The improved toxicity of biochar on the toxicity of heavy metals is in line with the reports in recent studies (Palansooriya et al., 2022) had had found the same level of growth improvement in vegetables that were cultivated in heavy metal-contaminated soils that were added with biochar. The dose response effect of biochar of the study indicates that the effect of biochar in alleviating heavy metal stress is dependent on the concentration which also appears to be the situation with (Ali and Nas, 2018) in their study of the biochar applications on the polluted agricultural soils.

Addition of co-pyrolyzed (rice-husk and sugarcane-bagasse) biochar at two different levels i.e., 2% and 4% (w/w) significantly mitigated the negative effects of detected heavy metals, with 4% biochar level generally executed better growth performance than 2%. Plants grown in biochar-amended pots showed better root and shoot length and higher fresh biomass in both root and shoot tissues leading to significant growth performance in comparison to the unamended pots. This enhancement might be due to biochar characteristics that reduces metal's phyto-availability through sorption to surface functional groups, complexation with oxygenated moieties, promotion of metal precipitation and by increasing soil pH and cation exchange capacity. These characteristics might be responsible for the reduction of metal sorption at the root surface. These mechanisms are consistent with meta-analyses and reviews (Ejaz et al., 2023; Palansooriya et al., 2022) showing that biochar additions commonly lower the bioavailable fractions of Pb, Cd, Zn, Cr and Cu and improve plant growth in contaminated soils.

Although 4% biochar level was more prone to increasing root and shoot length and biomass more, some soils had a diminishing effect i.e. the increase in biomass between 2% and 4% was less than the increase between 0% and 2%. This trend probably represents two complementary processes, including greater degrees of sorption sites and pH buffering with amendments rate and possible nutrient dilution or modification to the soil physical structure at higher amendment rates that may restrict additional improvements. Similar findings were reported in the majority of studies, where an optimal range of biochar rates was found to be 1-5%. According to Huang et al. (2018), the use of silicon in Cd and Zn contaminated soil led to an enormous growth of the shoot and root mass of rice plant.

The root length also grew significantly by the addition of amendment and these presented evidence of reduced root apical toxicity and regained soil exploration ability. The longer roots of the pots treated with biochar would most probably be explained by reduced rhizosphere metal activity, as well as by the fact that biochar-treated soils have better soil structure (more porosity, more aeration), and this allows the roots to grow longer, not stay short. Better root systems followed and enhanced the growth of shoot and the accumulation of fresh biomass. According to Mohamed et al. (2015), application of bamboo derived biochar at the rate of 1.5% increased the growth of cabbage and maize in Cd contaminated soil.

Impact of biochar on immobilization of Pb, Cd, Cr, Cu and Zn heavy metals in soil samples was determined. The results indicated that maximum immobilization of all the detected heavy metals was observed in treatment T3. This immobilization might have occurred due to surface functionality of biochar having porous, carbon-based material of greater cation-exchange capacity and rich in oxygen-containing functional groups. The properties make metal ions adsorbable and stabilize it by surface complexation, precipitation and electrostatic attraction, which decrease their mobility and bioavailability. The differences in metal-binding efficacy among the soils across the various industries imply that pH, organic matter, and original levels of metals are likely to influence immobilization efficacy, however, the general findings suggest that co-pyrolyzed bagasse-husk biochar is an efficient, inexpensive amendment to alleviate heavy-metal pollution and enhance the long-term soil health. Antonangelo et al. (2023) reported immobilization of heavy metals from rye grass utilizing biochar amendment. Du et al. (2023) also reported Cd immobilization in tobacco seedlings utilizing biochar produced through novel approach.

Conclusion: Soil pollution is increasing tremendously due to rapid industrialization and urbanization. There is a need to develop sustainable and economically viable methods that can reduce soil pollution. The co-pyrolyzed biochar used in this study showed the moisture content of 6%, volatile content of 21%, ash content of 19% and fixed carbon content of 60%. The FTIR of biochar and leaf compost were also conducted. The results indicated that in all the soil samples the maximum immobilization of detected heavy metals i.e., Cu, Cr, Pb, Cd and Zn was recorded in treatment T3 comprising of 4% biochar with 20% leaf compost. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 58.3%, 56.5%, 60.7%, 58.3% and 58.3%, respectively in SS-1. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 57.7%, 54.2%, 62.6%, 57.7% and 57.2%, respectively in SS-2. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 55.3%, 55.3%, 55.3%, 55.3% and 55.3%, respectively in SS-3. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 63.3%, 57.4%, 68.7%, 63.3% and 66.3%, respectively in SS-4. The removal efficiency of Cu, Cr, Pb, Cd and Zn was found to be 53.9%, 54.4%, 53.9%, 53.9% and 51.2%, respectively in SS-5. Maximum growth attributes of spinach were also recorded in treatment T3 in all the soil samples. Thus, it can be inferred that addition of co-pyrolyzed sugarcane bagasse and rice husk biochar to heavy metal contaminated soils can be applied safely as a sustainable and eco-friendly approach for the immobilization of heavy metals in polluted soils and for the enhancement of growth attributes of plant species being grown in polluted soils.

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