



Influence of biochar amendment on removal of heavy metal from soils using phytoremediation by *Catharanthus roseus* L. and *Chrysopogon zizanioides* L.

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Abstract

Advances in sustainable toxic heavy metal treatment technologies are crucial to meet our needs for safer land to develop an urban resilient future. The heavy metals bioaccumulate in the food chain due to their persistence in the soil, which poses a serious challenge to its removal and control. Utilisation of hyperaccumulators to reduce the mobility, accumulation and toxic impact of heavy metals is a promising and ecologically safe technique. Amendments such as biochar and chelates have been shown to enhance the phytoremediation efficiency. However, the potential soil improvement is influenced by the properties of the amendment, plant and metal heterogeneities. In this study, an organic sugarcane bagasse biochar amendment for the 60-day pot experiment using *Catharanthus roseus* L. (NT) and *Chrysopogon zizanioides* L. (VT) in a heavy metal-contaminated soil was applied. The influence of biochar on the phytoremediation of lead (Pb), zinc (Zn) and cadmium (Cd) from the soil was explored. The plant survival rate enhanced to 100% with biochar amendment, and the biomass increased from 5.83 to 15 g in Zn-contaminated samples. Nutrients such as potassium concentration are directly correlated to the amendment rates, whereas phosphate decreases beyond the 2% biochar amendment rate in both plants. High heavy metal accumulation capacities with improved growth with biochar indicate the sustainability of the process. The translocation factor (TF) > 1 for Zn in NT represents the phytoextraction efficiencies whereas VT indicates high BCF values in the range of 0.5–3.53 for the amended Zn-contaminated soils. The findings indicate that the amendment rate of 2% improves nutrient cycling, plant biomass and heavy metal removal efficiencies. The insights from this study establish that the synergy between biochar amendment and the selected medicinal plants improved the phytoremediation efficiency.

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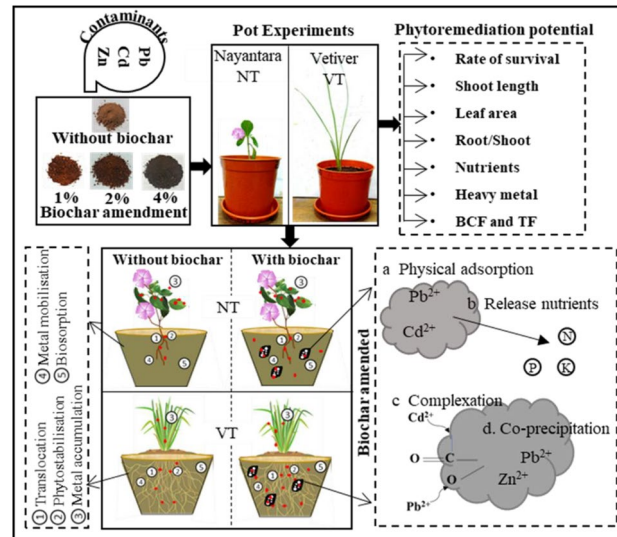
Highlights

- Synergy of sugarcane bagasse biochar with medicinal plants for heavy metal removal is evaluated.
- Pot experiments were conducted using *Catharanthus roseus* L. (NT) and *Chrysopogon zizanioides* L. (VT).
- 2% amendment rate optimised plant growth and heavy metal removal.
- Biochar amendment increased the sustainability of heavy metal phytoremediation.

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Graphical Abstract



Keywords Biochar · Phytoextraction · Heavy metals · Translocation factor · Bioconcentration factor

Introduction

In recent times, there has been an unprecedented surge in the demand for mineral resources, resulting in substantial changes in land utilisation and landscape alteration. The shifts have exacerbated environmental contamination, primarily due to the release of heavy metals into soil and water systems. They pose a serious threat to ecosystems and human health (Narayanan and Ma 2022; Zhang et al. 2023). Within this context, various physicochemical strategies such as vitrification, solidification and biological methods such as bioremediation and phytoextraction have been developed to ameliorate heavy metal contamination, which can be categorized as per their approaches to either mobilise or remove contaminants from the environment (Zhang et al. 2023; Zhong et al. 2024). The physicochemical methods disturb the soil microflora and generate secondary contamination with irreversible changes in the soil. These methods seem futile for low to moderately contaminated soil reclamation. Hence, there is a growing preference for eco-friendly approaches that offer cost-effectiveness and reduced potential for secondary contamination (Ghosh and Maiti 2020; Zhong et al. 2024).

Bioremediation is an attractive, nature-based, low-cost alternative for reducing heavy metal toxicity in soil and water. However, there are chances of toxic element generation with certain microbial interactions (Igiri et al. 2018). Phytoextraction, on the other hand, utilises plants to remove, destroy and stabilise the contaminants, with minimum chances of secondary contamination and sustained

reclamation. The selection of the appropriate plant species is crucial for the phytoextraction process. The hyperaccumulators persist in locations with heavy metal (HM) stress without showing signs of phytotoxicity. The need for HMs by the hyperaccumulators allows them to sequester significant amounts of HM compounds, compared to non-accumulators. To increase contaminant bioavailability, plants secrete phytosiderophores in the rhizosphere, which solubilise and chelate the soil-bound metals. Further, plants resist metal damage either through compartmentalisation, precipitation or chelation (Thomas et al. 2024; Yan et al. 2020). The major limitation of this method is the slow-growing process and adaptability of the plants to poor nutrient conditions (Khan et al. 2020; Rees et al. 2020; Sun et al. 2023). To improve the pace of plant growth and its removal efficiency, various amendments like biochar, compost and other chelating agents such as ethylenediamine tetraacetic acid (EDTA), zeolites, lime, phosphate chemicals, dolomites, oxides of iron and manganese have been suggested (Gul et al. 2020; Kafle et al. 2022; Kumar et al. 2023). Such amendments are capable of improving the metal accumulation in the plant and medium. Biochar is preferred over chemical chelators due to the evidence of secondary contaminants generated from these chemical chelators (Hasan et al. 2019; Ogundiran et al. 2018; Wang et al. 2019).

Biochar is a solid carbon-rich organic material with high porosity, low density and high specific surface area with high metal immobilisation capacity (Otunola et al. 2023; Pandey et al. 2022; Patwa et al. 2023b; Sun et al. 2023). It is derived from the thermochemical decomposition of waste

biomass in a closed container with low to no oxygen content (Patwa et al. 2022b). The biochar has alkaline metal cations, electrons and surface functional groups, and the porous structure of the material provides binding sites for soil organic and inorganic contaminants (Guo et al. 2020; Pandey et al. 2022). It is capable of improving plant growth through reduced nutrient loss and improved soil fertility with the release of essential macro and micronutrients such as potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn) and Zn in addition to improved water absorption capacities (Fellet et al. 2014; Rees et al. 2020; You et al. 2021; Kumar et al. 2023; Zhang et al. 2023). The application of biochar in a metal-contaminated environment modifies microbial activities. However, with plant growth, there are likely changes in the effects of the biochar on heavy metal removal. For the preparation of biochar, locally available lignocellulosic waste biomass is often preferred, as it significantly aids in the reduction of transport-related costs and waste valorisation (Muigai et al. 2021; Patwa et al. 2022a). Sugarcane bagasse is a by-product generated in huge amounts from the sugar and alcohol industries. The global production is estimated to be approximately around 1010MT, with India being the second highest producer (Ajala et al. 2021). Previous studies have indicated the biochar's efficiency in removing

heavy metal contaminants such as lead, cadmium, chromium and other organic contaminants.

Table 1 mentions some of the studies conducted to evaluate the role of biochar-amended phytoremediation (BAP) efficiency in HM-contaminated soil. From Table 1, it is evident that the specific plant species and biochar type play a pivotal role in determining the efficiency of phytoremediation. Although various edible plant species are popular hyperaccumulators, recent studies suggest an increasing demand for the identification of aromatic and medicinal plants globally (Pandey et al. 2016; Pirzadah et al. 2019; Qurban et al. 2021; Deka et al. 2024). They are increasingly promoted due to their potential application in soil health recovery. Some of the previously investigated plants include *Carantheus Roseus*, *Vetiveria zizanioides*, *Ocimum basilicum*, *Cymbopogon flexuosus* (Dorafshan et al. 2023; Pandey and Singh 2020; Soumya and Kiranmayi 2023). Literature indicates the high HM removal efficiencies of the *C. roseus* and *Chrysopogon zizanioides* plants (Nugroho et al. 2021; Soumya and Kiranmayi 2023; Subhashini and Swamy 2013). However, very few studies have compared the behaviour of diverse species in the presence of different HM contaminants in soil. Therefore, this study focuses on the deliberate selection of specific medicinal/aromatic plant species, in

Table 1 Literature review on phytoremediation of heavy metal contaminated soil

HM	Biochar	Plant	Conclusions/metal removal	Reference
Cd, Pb, Zn	<i>Miscanthus giganteus</i> straw	<i>Agrostis capillaris</i> L., <i>Lupinus albus</i> L	Immobilisation of heavy metals using biochar is dependent on the rhizosphere pH	Houben and Sonnet (2015)
Cd	<i>Eucalyptus</i> , poultry litter	<i>Amaranthus tricolor</i> L	BAP improved soil biochemical properties	Lu et al. (2015)
Pb	Rice husk, ground shell	<i>M. oleifera</i>	Rice husk improved shoot growth and ground shell improved Pb removal	Ogundiran et al. (2018)
Cd	Wheat straw and sugarcane bagasse	<i>Spinacia oleracea</i>	Wheat straw is more effective in Cd stabilization at 2% application rate	Bashir et al. (2019)
Cd	Tea waste	<i>Boehmeria nivea</i> (L.)	Biochar changed Cd speciation and subcellular distribution. Reduction in oxidative stress	Gong et al. (2019)
Cr, Pb	Poplar wood (PWB), sugarcane bagasse (SBBC)	<i>Lactuca sativa</i>	Optimum content of SBBC was more effective in increasing biomass, reducing intake, accumulation and health risk in contrast to PWB	Khan et al. (2020)
Cd	Pine needle	<i>Bidens pilosa</i> L	Accumulation enhanced with biochar. Plant stress indicates increased proline concentration and reduced chlorophyll	Manori et al. (2021)
Pb, Cd	Sugarcane bagasse	<i>Zea mays</i>	SBBC improves heavy metal stabilisation	Rassaei (2023)
Cd, Zn	Rice straw	<i>Quercus</i> spp.	Increased growth biomass in <i>Q. fabri</i> and improved bioconcentration, biomass in <i>Q. taxena</i> with amendment	Li et al. (2023)
Cd, Pb, Zn	Corn straw	<i>Lolium perenne</i> L	Change in bioavailability of heavy metals increased Cd and Zn but decreased Pb accumulation	Zhang et al. (2023)

conjunction with sugarcane bagasse biochar, to address the sustainable remediation of Pb, Cd and Zn contamination in soil. The detailed list of the abbreviations used in this study is mentioned in Table 2.

The novelty of this research lies in the recognition of the phytoremediation potential of the medicinal *C. roseus* (locally known as Nayantara: NT) and *Chrysopogon zizanioides* (locally known as Vetiver Grass: VT) plants, which exhibit remarkable characteristics to identify as exceptionally well-suited for our study area. These plants and sugarcane bagasse biomass are found worldwide with wide prospects in phytoremediation. To date, there are limited studies assessing the synergy between sugarcane bagasse biochar (SB) and the two medicinal plants for HM removal. Hence, the present study explores the influence of sugarcane bagasse biochar on the VT and NT plants in severely HM-contaminated soil. The objectives of the work are threefold: (1) to evaluate the effect of biochar on the growth, survival and HM accumulation in the plant; (2) to assess the soil HM concentrations and its physicochemical properties; and (3) to investigate the relationship between the amendment rates and the performance of NT and VT in the remediation efficiency of HM-contaminated soil. The findings of this study will provide deeper insights and broader scopes in developing the phytoremediation potential of medicinal plants and the role of biochar in enhancing HM removal mechanisms and plant performances.

Table 2 List of abbreviations in the study

Abbreviations	Definition
BCF	Bioconcentration factor
Cd	Cadmium
CK	Control contaminated unamended soil
EC	Electrical conductivity
HM	Heavy metal
K	Potassium
LA	Leaf area
Mn	Manganese
NT	<i>Catharanthus roseus</i> L. (Nayantara)
ORP	Oxidation–reduction potential
Pb	Lead
RSR	Root-to-shoot ratio
SB	Sugarcane bagasse biochar
SB-1	1% sugarcane bagasse biochar amended soil
SB-2	2% sugarcane bagasse biochar amended soil
SB-4	4% sugarcane bagasse biochar amended soil
SEM	Scanning electron microscope
TF	Translocation factor
VT	<i>Chrysopogon zizanioides</i> L. (Vetiver grass)
Zn	Zinc

Materials and methodology

Soil and plants

The locally accessible residual soil from the Assam state of the North-eastern part of India was used in this study. The soil samples were oven dried as air drying enhances the organic matter mineralisation, and the impurities like dead leaf, organic matter and stones were physically removed. The soil was sieved through a 4.75-mm sieve for homogenisation. Table 3 presents the soil index properties determined as per the ASTM procedures (ASTM D.854 2014; ASTM D422-63 2007; ASTM D4318 2010; ASTM D698 2012). The detailed procedure for soil characterization is mentioned in Patwa et al. (2023a). The background values of the HMs (Pb, Zn, Cd) in the soil were negligible in comparison to the artificially spiked concentrations.

The saplings of the two selected medicinal crops, NT and VT, were collected from the IIT Guwahati campus (26.1847°N and 91.6672°E). The VT grass characteristics include high metal tolerance with minimum growth and development effects, reduction of soil erosion and evapotranspiration (Otunola et al. 2023). The fast-growing and essential oil-producing grass requires low maintenance and provides additional soil reinforcement (Dorafshan et al. 2023). The NT, on the other hand, indicates enhanced alkaloid, phenol and flavonoid accumulation under HM stress,

Table 3 Basic physicochemical properties of soil

Property	Value	Code
Grain size distribution (mm)		ASTM D422-63, 2007
Coarse Sand (4.75–2)	0.8%	
Medium Sand (2–0.425)	12.1%	
Fine Sand (0.425–0.075)	9.1%	
Silt (0.075–0.002 mm)	39.74%	
Clay (<0.002 mm)	38.26%	
Compaction characteristics		ASTM D698, 2012
Maximum dry density (MDD)	1.579 g/cc	
Optimum moisture content (OMC)	22.7%	
Specific gravity	2.71 ± 0.03	ASTM D.854, 2014
pH	4.68	ASTM D4972-19, 2019
Organic matter	5.45%	ASTM D2974-14, 2014
Consistency limits		
Liquid limit	54.42 ± 1.58	ASTM D4318, 2010
Plastic limit	35.25 ± 1.18	
Shrinkage limit	30.12 ± 1.08	
USCS classification	MH	ASTM D2487-11, 2011

which increases the medicinal value (Qurban et al. 2021; Soumya and Kiranmayi 2023). The ornamental plant is suited to different climates and soil along with enhancing the aesthetic value of the surroundings. Such drought resistance and high metal tolerance are essential characteristics for efficient metal uptake and overall phytoremediation success.

Biochar

The biochar used in this study was prepared from the biomass of sugarcane bagasse. The feedstock was collected from the local markets of Amingaon, Assam, India (26.1847°N, 91.6672°E). To remove dirt and contaminants, the feedstock was rinsed thoroughly in running water. After sun-drying at room temperature for 7 days, they were cut to 30-mm size for uniform and complete pyrolysis of the biomass. Thereafter, it was oven-dried at 60 °C for 72 h and stored in an airtight box. The process for the generation of biochar from the pyrolysis of the oven-dried biomass is mentioned in Patwa et al. (2022b). Initially, thermogravimetric analysis (TGA) was performed on both raw biomasses to determine the optimal pyrolysis temperature. The biomass was then pyrolyzed at 500 °C, with a heating rate of 10 °C per minute and a holding time of 45 min in a fixed batch reactor under an argon atmosphere. The resulting biochar from the pyrolysis process was pulverized, sieved through a 2-mm sieve and stored in airtight containers for further analysis. Table 4 provides the properties of the sugarcane bagasse biochar (SB) used in this study. The high pH value of SB could be attributed to the base cation concentrations, and the high electrical conductivity (EC) was due to the presence of higher soluble salts. Figure 1 represents the honeycomb structure of the SB as observed in the scanning electron microscope (SEM) micrograph images. The presence of lignin, cellulose and hemicellulose biopolymer generates the particular structure of the intra-pores (Patwa et al. 2022b). The biochar exhibits significant water-holding capacity, which can be due to the higher surface area, intra-pores and various functional groups (Khan et al. 2020; Muigai et al. 2021).

Experimental design

To analyse the effects of HM contamination, the soil was artificially spiked with individual 500 mg/kg concentrations of Pb, Zn and Cd. The stock solutions of lead nitrate, cadmium nitrate and zinc nitrate were added to the oven-dried soil while maintaining the optimum moisture content. The soil and soil biochar–contaminated composites were stored for 7 days to develop proper soil and metal reaction in a closed container. The biochar amendment rates of 1%, 2% and 4% were selected following the recommended optimum range by Gao et al. (2021) for plant growth rate. Table 5 lists the different treatments for the pot experiments. The terms CK,

Table 4 Properties of biochar

Properties	Value
Feedstock	Sugarcane bagasse
Pyrolysis process	Slow pyrolysis
Pyrolysis temperature (°C)	500
Proximate analysis (%)	
Moisture content	6.09
Volatile matter	15.42
Ash content	3.82
Fixed carbon	74.67
Ultimate analysis (%)	
Carbon (C)	78.93
Hydrogen (H)	3.57
Nitrogen (N)	0.74
Oxygen (O)	12.94
Atomic ratios	
H/C	0.54
O/C	0.12
C/N	124.44
Specific gravity	0.605
pH	9.16
EC (mS cm ⁻¹)	1.52
Zeta potential (mV)	−64
Water holding capacity (%)	874.08
Specific surface area (m ² g ⁻¹)	21.787
Average pore diameter (nm)	8.423
Total pore volume (cm ³ g ⁻¹)	0.045

SB-1, SB-2 and SB-4 denote the different rates of biochar amendment of 0%, 1%, 2% and 4% respectively under different contaminant and plant treatments. For uniformity and clear representation of the amended and contaminated samples, they are denoted by treatments of T1–T12 (Table 5).

Of the prepared soil samples, 1.7 kg was collected in plastic pots of dimensions 15 × 15 × 14 cm, and the biochar amendment rate was measured as a percentage of dry soil weight and the compaction characteristics of the composites are mentioned in Table S3. All the pots were paced with trays, to avoid the mixing of the leachates. A photograph of the test set-up is presented in Fig. 2. The plant saplings were grown for 20 days in clean soil prior to transplanting into the contaminated samples. The saplings were planted in the experimental soils after the completion of the equilibration period. Plants were regularly irrigated with distilled water, and the excess water was poured back from the tray. The planted pots were allowed to develop the contaminated soil and plant equilibrium for 2 weeks, and thereafter, the experimental analysis of the percentage germination, survival, shoot length and leaf area was initiated. All of the experimental analyses were conducted in triplicates, and the average values with statistical deviations were plotted. The

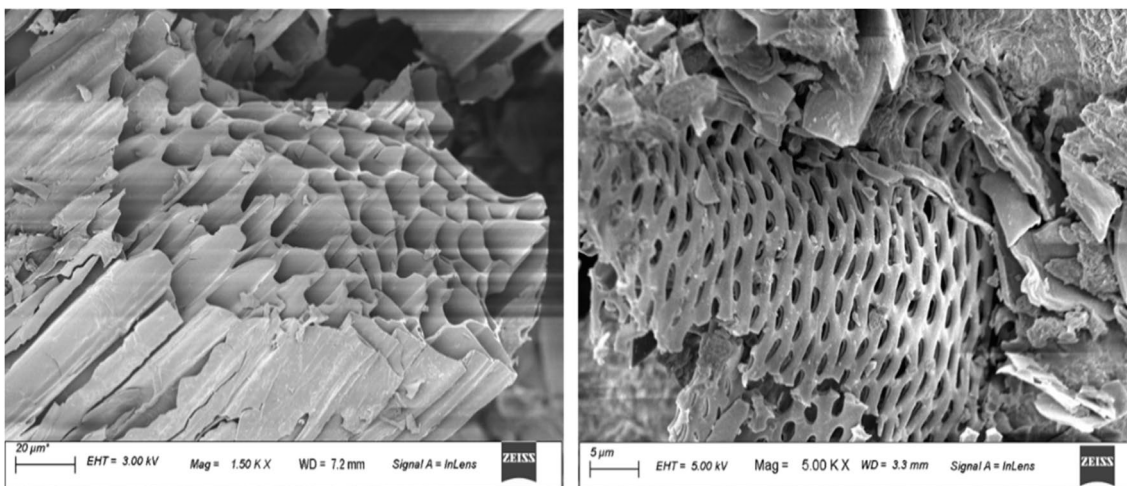


Fig. 1 SEM micrograph images of sugarcane bagasse biochar

Table 5 Different treatments of heavy metal contaminated and amended soil samples

Treatments	Heavy metal (HM)	Biochar amendment rate (%)	Nayantara (NT)	Vetiver (VT)
T1	Zinc	0	Zn-CK-NT	Zn-CK-VT
T2	Zinc	1	Zn-BC1-NT	Zn-BC1-VT
T3	Zinc	2	Zn-BC2-NT	Zn-BC2-VT
T4	Zinc	4	Zn-BC4-NT	Zn-BC4-VT
T5	Cadmium	0	Cd-CK-NT	Cd-CK-VT
T6	Cadmium	1	Cd-BC1-NT	Cd-BC1-VT
T7	Cadmium	2	Cd-BC2-NT	Cd-BC2-VT
T8	Cadmium	4	Cd-BC4-NT	Cd-BC4-VT
T9	Lead	0	Pb-CK-NT	Pb-CK-VT
T10	Lead	1	Pb-BC1-NT	Pb-BC1-VT
T11	Lead	2	Pb-BC2-NT	Pb-BC2-VT
T12	Lead	4	Pb-BC4-NT	Pb-BC4-VT

pots were placed randomly in a shed of translucent fibre sheets for protection against rainfall, wind and for uniform ambient conditions. Table S1 shows the average pH, EC and oxidation reduction potential (ORP) at the initial stage for both plants under different treatments. The pH for all the CK soils under different contaminants indicates unamended soil samples. There was no significant variation in the metal-contaminated and clean soil samples. The possible reason may be that the heavy metals Pb, Cd and Zn considered in this study do not undergo extensive hydrolysis such as aluminium (Al), iron (Fe) and Mn in acidic soils (Shetty et al. 2021; Ngoune Tandzi et al. 2018). So, they do not have a direct impact on the pH, but their toxicity affects the microbial activities, which may indirectly impact the soil pH. However, in this study, the weight of amended biochar is significantly low to increase the pH values. The soil pH over the period of the experiment slightly changes but remains in

the acidic range at the end of the experiment as shown in the supplementary table S2, indicating the chances of possible high mobility of the metal cations. The slight increase from the initial pH values can be due to the pH neutralisation with biochar amendment (Narayanan and Ma 2022). The EC variations with biochar were due to the higher surficial charged particles. The ORP shows a reduction with an increase in the amendment rate, as biochar acts as a reductant and lowers the redox potential (Joseph et al. 2021). The acidic pH and reducing condition favour metal solubilisation in the soil.

Analytical testing

The plant growth parameters were recorded at regular intervals during the experiment period. The rate of survival indicates the number of plants surviving in the contaminated environment till the end of the experiment with respect to

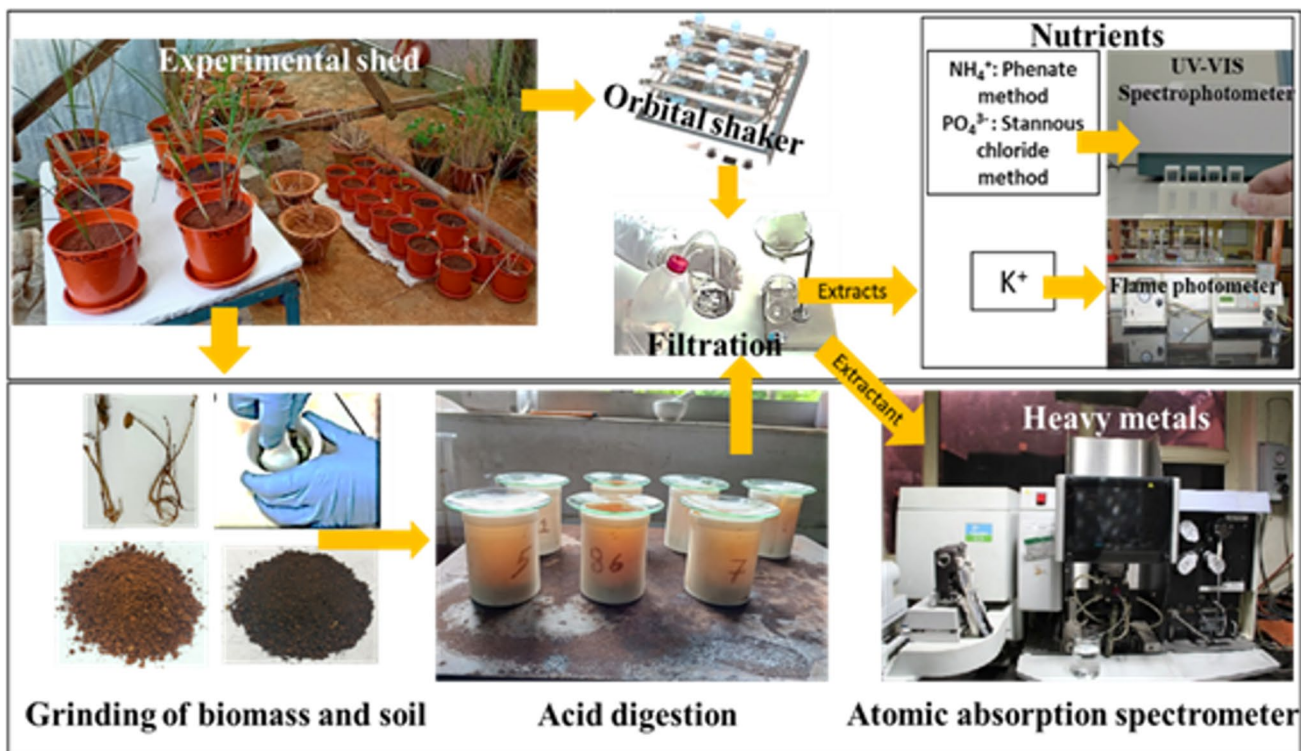


Fig. 2 Overall methodology for HM and nutrient analysis in plants and soil

the transplanted saplings. On the other hand, the rate of germination refers to the saplings that showed the growth of green leaves after transfer to the contaminated soil in the first week. The shoot length was considered from the collar of

the root to the tip of the plant (Fig. 3a, b). The leaf area (LA) is the leaf pixel area determined with the colour threshold technique from the histogram of the leaves and their reflection using the ImageJ software (Fig. 3c). The cropped image

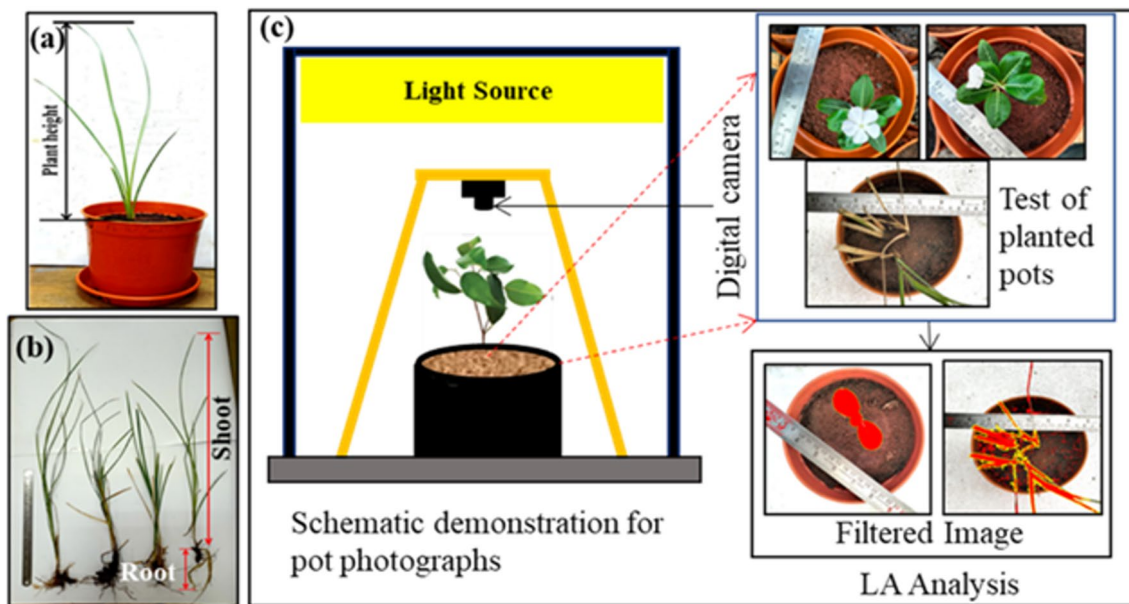


Fig. 3 Plant morphological parameters: a shoot length, b shoot and root biomass, c leaf area

was adjusted for hue, saturation and brightness to select the pixels that fall under the leaf colour. This parameter plays a critical role in plant respiration, photosynthesis, precipitation and interception. It is the major site for volatilisation and excretion, which is an essential detoxification mechanism for contaminants (Liu et al. 2018). The fundamental component of global vegetation, LA was analysed from the initial to the final stage of the experiment. At the end of the experiment, the plants were harvested and separated into leaves, stems and roots. The plant parts were rinsed thoroughly using deionised water to remove soil and dried for 72 h at 80 °C. The oven-dried biomass is separated and weighed as the root and shoot biomass to calculate the root-to-shoot ratio (RSR). The RSR is a stable indicator of the above- and belowground plant biomass produced in harsh environments (Xia 2004). It is a standard parameter used in the phyto-recurrent studies for the selection of different plant species (Rogers et al. 2019). Soil samples were collected from the rhizosphere region and oven dried for 72 h at 60 °C for further analysis. The nutrients in the soil were analysed to check if the non-availability of nutrients affected the growth characteristics.

The concentrations of the soil nutrients: ammonium, potassium and phosphate were examined at the beginning and end of the experiment. The soil solutions were prepared using an orbital shaker, and the filtered extractants were subsequently analysed. The exchangeable ammonium and phosphate concentration were determined using the phenate method and stannous chloride method respectively (APHA et al. 2012) using a UV/VIS spectrophotometer. The stannous chloride and ammonium molybdate were used as the colouring reagents. The potassium concentration in the extractant was analysed using a Flame photometer (APHA et al. 2012). Table 6 shows the average initial soil nutrient concentrations under different treatments. The

application of biochar increased the potassium concentration (Farrar et al. 2022), but did not have any significant effect on the ammonia (NH₄⁺) and phosphate (PO₄³⁻) content. In general, phosphorous sorption is affected by the acidic pH and higher metal oxide content (Wu et al. 2022).

Heavy metal analysis

For the HM analysis, the soil and plant samples were oven-dried at 60 °C for 48 h and ground to fine powder. Approximately, 1 g of soil samples was weighed in a Teflon beaker and digested using the modified aqua regia method (nitric acid, hydrogen peroxide, hydrochloric acid) (USEPA 3051: 1997) by using a hot plate (Fig. 2). The digested samples were filtered using Whatman 42-mm filter paper and diluted to 100 ml using deionised water. The diluted extracts were analysed for concentration of HM content using an atomic absorption spectrometer (AAS). The translocation from the shoots to roots was evaluated using the translocation factor (TF) given by

$$TF = \frac{\text{Heavy metal concentration in shoots (mg/kg)}}{\text{Heavy metal concentration in roots (mg/kg)}}$$

The HM translocated from soil to the plants was evaluated in terms of bioconcentration factor (BCF), which is given by

$$BCF = \frac{\text{Heavy metal concentration in plant(mg/kg)}}{\text{Heavy metal concentration in soil (mg/kg)}}$$

The concentration ratios were evaluated in the treated and control samples in triplicates. The mean and standard deviations of the experimental samples were used to report the findings.

Table 6 Nutrient concentrations in the soil at the time of transplantation

HM	Treatment	NH ₄ ⁺		PO ₄ ³⁻		K ⁺	
		NT	VT	NT	VT	NT	VT
Zn	T1	1.77	1.30	0.07	0.07	2.05	1.67
	T2	1.12	1.08	0.04	0.04	4.1	1.37
	T3	1.41	1.31	0.06	0.14	7.84	8.08
	T4	1.45	1.35	0.06	0.04	10.56	13.6
Cd	T5	1.09	1.01	0.04	0.04	1.73	1.8
	T6	0.93	1.16	0.07	0.07	3.56	4.84
	T7	0.96	1.25	0.07	0.07	13.28	7.53
	T8	1.17	1.24	0.07	0.07	13.78	11.56
Pb	T9	0.40	0.86	0.19	0.06	4.49	1.65
	T10	0.98	0.93	0.05	0.07	3.37	4.53
	T11	0.70	0.62	0.11	0.04	7.91	8.48
	T12	0.90	0.80	0.04	0.08	11.26	19.42

Results and discussions

Effects of biochar amendment on the plant

Germination and sapling survival rate

Figure 4 presents the variation of plant survival and the germination rate with respect to different treatments mentioned in Table 5. The red markers indicate the percentage germination, and the black columns indicate the percentage of plants surviving till the end of the experiment (Fig. 4). The survival rates of VT plants in Zn contaminated soils increased from 50% at control to 75% and 100% with an increase in the biochar amendment rates to 1 to 2%. Similar patterns were observed for the NT plants in the Cd-contaminated soil where the survival increased from 17% without amendment to 75% at 4% biochar amendment. The percentage of germination in Pb contaminated NT indicates an increase from 30% in control samples (T1) to 90% with 4% biochar amendment, while survival increased to 100%. The germination of VT in Zn contaminated soil indicated a steady increase with biochar amendment from 60 to 100%. Plants in all the treatments did not significantly show signs of HM stress upon biochar amendment.

The high survival rates in NT plants exposed to Pb contaminated samples may be due to the high metal tolerance of the plant (Subhashini and Swamy 2013). Also, Cd- and Zn-contaminated VT and NT samples indicated an increase in survival rates of biochar-amended samples in comparison to the control. Survival rates lower than 50% in the Cd and Zn samples can be due to the alteration of physiological, biochemical and metabolic activities of the plants owing to the absorption and translocation of beyond-limit essential and

non-essential metal concentrations. The presence of basic cations and nutrients on the biochar surface is capable of occupying the exchangeable sites of the contaminated soil by replacing harmful cations from the soil, thereby increasing the plant survival rate. Furthermore, they are bioavailable and beneficial for promoting optimal plant growth (Deal et al. 2012; Ogundiran et al. 2018).

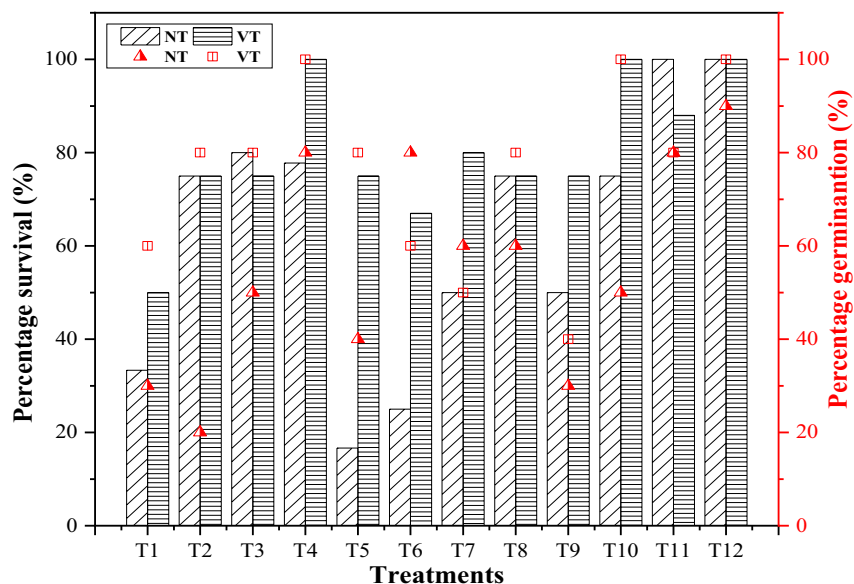
Responses of the plant morphological characteristics

1 Leaf area (LA)

Figure 5 presents the variation of LA with the number of days for both the plant species during the experiment period. The trends from the LA analysis under different treatments indicate a gradual initial increase followed by a decline or stagnant growth over the period. The maximum LA values were achieved between 40 and 50 days. Following the maximum value, the control samples indicated a significant decline of 34.65%, 41.57% and 52.69% in the Pb, Zn and Cd samples of NT respectively. LA values reached a maximum value of 10.28 in the T2 treatment for NT species. Similarly, the VT samples showed a significant increase with the amendments, with the highest LA of 66.37 in the T6 treatment.

Hence, it can be observed that biochar modulates leaf development, as evident from the increase in LA with amendment. Similar observations were corroborated in the study by Wan et al. (2023). Biochar is capable of mitigating the effects of contaminants, increasing water retention and improving nutrient cycling efficiency (Ilyas et al. 2021; Li et al. 2021). The higher biochar content may not enhance the leaf area concerning the canopy area. However, it improves the lateral growth of

Fig. 4 Rate of survival and germination of NT and VT in different soil treatments



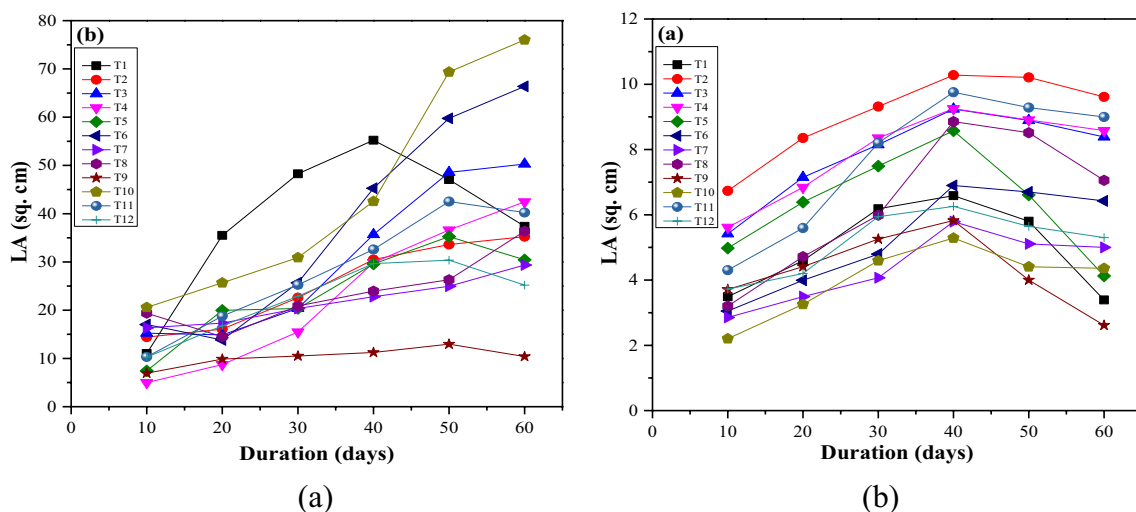


Fig. 5 Variation in LA analysis for different treatments during the experiment period (a) NT, (b) VT

the leaf, resulting in canopy expansion (Guo et al. 2023; Qian et al. 2019).

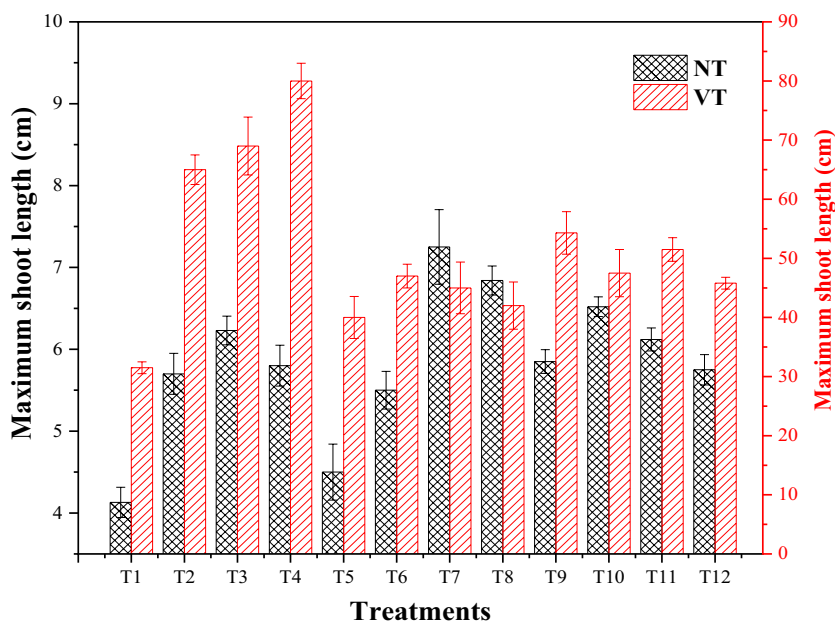
2 Shoot length

Fig. S1 demonstrates the variation in the plant heights of NT and VT during the experiment period. The performance of the plant species in the control and amended contaminated samples is indicated through the plant biomass and shoot length. The shoot length variations stabilise for both plants after 40 days of the experiment. The biochar-amended samples indicated consistently high values in both the plants (Fig. 6). For the NT samples, 2% biochar amendment showed a significant increase of 33.7% in Zn-contaminated soil and 37.93% for the Cd-

contaminated soil in comparison to the control. On the other hand, the VT shows a high average plant height, with a maximum of 80 cm in T4 treatment. For the Cd-contaminated VT plants, the maximum plant height increased by 18.75% in comparison to the control with 2% biochar amendment. In both plants, a 2% amendment rate indicated a considerably high maximum shoot length.

From the observations, it was evident that biochar enhances shoot elongation. Low shoot lengths in the control soil indicate signs of plant metabolism inhibition due to HM stress. It has been suggested that the allometric relationships between aboveground and below-

Fig. 6 Maximum shoot length for NT and VT at the end of the 60-day experiment



ground traits respond to the growth conditions of the plant (Wan et al. 2023). The root morphology is critical in the plant-soil system and can directly influence shoot development. Biochar addition reduces soil bulk density and increases porosity, enhancing root penetration and extension (Zhang et al. 2012). This modified root morphology, with increased nutrient pools, may improve shoot growth and yield compared to unamended control samples (Ali et al. 2021).

3 Plant biomass

Figure 7 represents the variations in plant biomass in terms of root-to-shoot ratio to represent the response to variations in biochar amendments. In general, previous studies have indicated an increase in RSR with high contaminant concentrations. Except for the Pb-contaminated VT and Zn-contaminated NT plants, the RSR values were higher than the control. The RSR in the T9 samples of VT was 2.78, whereas the T11 indicated a value of 2.49. Significant variations have been observed in the 2% biochar-amended samples for both plant species. The total biomass content for VT in T3 treated samples was 29% higher than the control, whereas, for Cd, it was 64.05% higher than the control. The NT species indicated higher biomass at 4% amendment rates (0.62 g) for Zn-contaminated samples, whereas there was no significant increase in the biomass from the Pb and Cd samples.

Biochar amendment increases the overall yield and shoot biomass which is reflected in the lower RSR. This can be attributed to the significant abridging of metal stress on the plant along with improved chlorophyll content (Rathika et al. 2021). The reduced RSR in the amended samples is an indication of amelioration of the contaminant effect in the

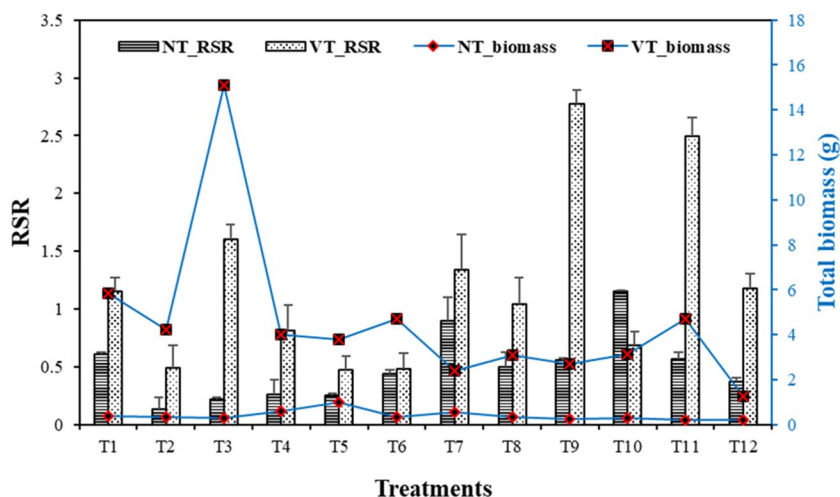
shoots (Xia 2004). On the contrary, the increased values represent the promotion of the growth rate of the roots, which are the primary accumulation sites and can be an indication of improved contaminant removal potential. However, RSR cannot be objectively used to demonstrate the amendment effects (Wan et al. 2023).

Effect of biochar amended phytoremediation on contaminated soil

1 Nutrient concentrations

Figure 8 shows the nutrient concentrations in the soil at the end of the experiment period. In general, the bio-availability of ammonium and phosphate is lower in biochar-amended samples for both plant species. Biochar provides adsorption sites for the nutrients, reducing their leaching and ensuring better plant growth characteristics. In the case of phosphate, the VT grass showed a similar trend in Pb- and Cd-contaminated samples, whereas Zn-treated soil showed an increment in nutrient availability with biochar amendment. As in a study by Li et al. (2021), the nutrients showed lower concentrations than the control but increased with the higher amendment rates. For the NT species, phosphate concentrations showed similar trends in Zn and Pb, whereas ammonia variations in the Zn- and Cd-treated samples were fairly alike (Fig. 8). The most significant direct relationship was observed between the biochar doses and the K concentrations. The increase in biochar amendment rate from control (CK) to 4% indicated an increase in K values from 1.6 to 19.5 mg/l in Zn, 2.77 to 13.6 mg/l in Cd and 2.33 to 18.18 mg/l in Pb-contaminated NT soil samples. Additionally, for VT plants, Zn shows an increase from 1.58 to 16.04 mg/l, whereas Cd and Pb indicate an increase from 1.5 to 12.48 mg/l and from 2.29 to 11.02 mg/l respectively. The elevated

Fig. 7 Plant biomass (root and shoot) for NT and VT under different treatments



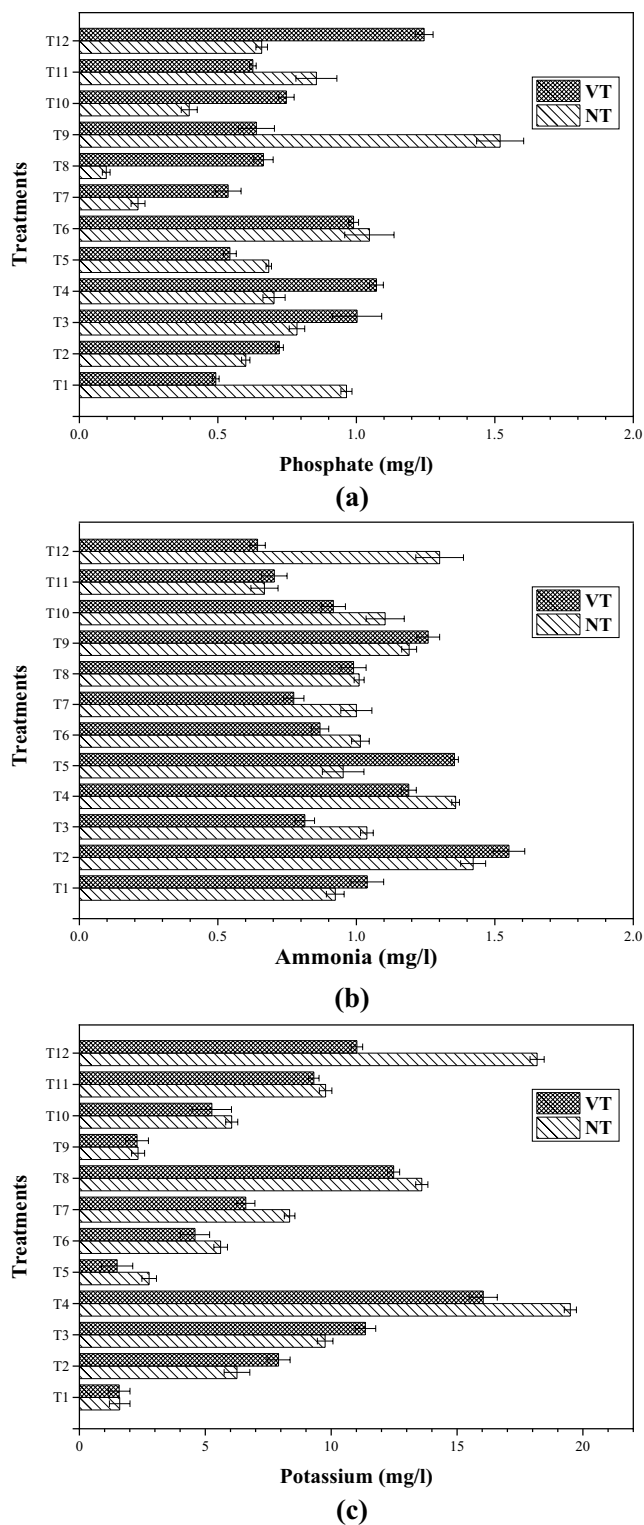


Fig. 8 Concentrations of (a) phosphate, (b) ammonia, and (c) potassium for NT and VT in the soil after the 60-day experiment period

K concentration is due to the higher retention and continuous cycling of the nutrient with the porous and high surficial structure of biochar (Farrar et al. 2022; Li et al.

2021). The lower available PO_4^{3-} and NH_4^+ values can be due to the enhanced carbon/nitrogen (C/N) ratio with biochar amendment, leading to biological fixation of the nutrients (Lehmann et al. 2003; Li et al. 2021).

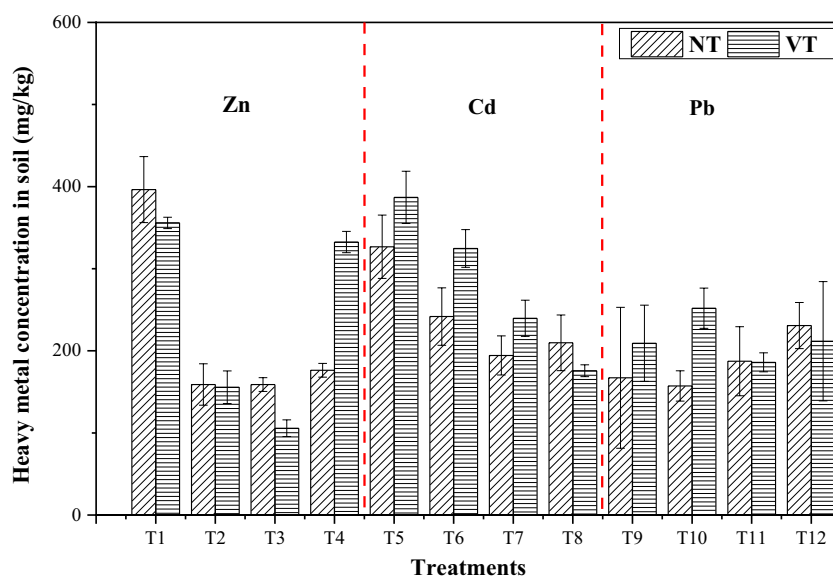
In both plants, the minimum bioavailability of nutrients at a 2% biochar amendment rate can be an indication of higher adsorption capacities. At higher biochar additions, the increased negative charges in the soil may limit the availability of sorption sites for PO_4^{3-} , and other molecular organic acids released by biochar might compete for these adsorption sites (Wu et al. 2022). Further, the dissolved black carbon released with biochar amendment increases the total organic carbon, which competes with the phosphate ions for sorption sites (Wu et al. 2022). In all cases, the nutrient concentrations have increased from the initial stage, which can be attributed to plant growth and biomass production.

2 Heavy metal concentrations

Figure 9 shows the range of Pb, Cd and Zn levels in the amended and unamended soil for the two plant species. The difference in the residual metal content in the pots could be due to variations in plant uptake (Fig. S2), mobilisation, leaching potential, biochar interactions and type of plant (Guo et al. 2019; Pandey et al. 2022). Following the harvest, the VT plants showed minimum concentrations of Pb and Zn in the T3 and T11 treatments. The Cd concentrations in the soil for both species can be ranked as $T5 > T6 > T7 > T8$, with values in the concentration range of 175 to 386 mg/kg. The 2% amended treatments experienced low Zn concentrations in the narrow range of 106–159 mg/kg, due to its role as an essential metal for plant growth. Compared to the soil without biochar treatment, there was no significant influence on the Pb concentration in the amended soil. The total Zn concentration with 1% biochar amendment in NT and VT plants showed 59.8% and 56.25% lower metal concentrations than the control. For the Cd-contaminated soil, the treatment with NT decreased metal concentration by 40.51% with the 2% amendment. Hence, the biochar amendment rate of 2% was found to have comparatively low soil HM concentrations in all treatments, for both the plant species.

The precipitation of Pb, Zn and Cd due to the adsorption on the porous biochar surface may reduce the bioavailability of the metals (Ahmad et al. 2012; Namgay et al. 2010). The SB structure is capable of removing HMs through physical and chemical processes along with electrostatic interactions. The pore volume and surface area of the biochar adsorbent determine the potential covalent bond formations for the removal of HMs. Further, electrostatic interaction between the hydroxymethyl ($-\text{COOH}$) on the biochar and positively charged metal ions could lead to chelation

Fig. 9 Soil heavy metal concentrations in amended and treated soil after 60-day experiment period



or coordinate adsorption (Ghosh and Maiti 2020). These interactions enhance the ion exchanges between the metal cations and the proton (H⁺)-emitted carboxyl (–COOH) or hydroxyl (–OH) groups. The phenolic (lignin), hemicellulose and cellulose of the biochar bind with HMs, leading to the enhanced inner sphere and surface metal complexation. Hence, biochar amendment enhances the electronegative charges, which improves the adsorption ability, reduces the phytotoxicity of the metals and releases essential nutrients in the soil (Bashir et al. 2019).

Translocation and bioconcentration of heavy metals in biochar-amended phytoremediation

Figure 10 represents the translocation and bioconcentration of heavy metals within the plant and from the soil to the plant after the 60-day experiment. Results shown in Fig. 10a, b indicated that a wide range of TF values was evident in NT (0.12 to 2.44) and VT (0.09 to 1.13) plants. Since the shoot biomass is predominant in the NT plants, the TF corresponds to higher values in comparison to VT. For Pb, the biochar amendment of 1% increased the TF, while for Cd, the TF decreased with increasing rates of biochar amendment. The translocation factor (TF) values less than 1.0 indicate restricted movement of metals from roots to aboveground biomass. This restriction can be attributed to the lowered bioavailability of metals due to biochar amendment or their accumulation in the vacuoles of roots and cell membranes (Peng and Gong 2014). The 1% biochar amendment increased the Zn translocation by 52.01% in VT, while it decreased from 2.44 (control) to 1.18 in NT plants.

BCF of any plant in contaminated soil is recognised as a significant measure of human health risk (Bashir et al. 2019; Zhang et al. 2023). It calculates the extent of HM

accumulation by the plant biomass from a HM-polluted soil. The BCF values as shown in Fig. 10c, d indicated that the biochar amendment improved the translocation from soil to plants, which is in accordance with our previous results. For all the HMs, the BCF values indicated a significant increase with biochar. The VT plants showed low Cd accumulation capacity (BCF: 0.31–1.53), while the highest accumulation was observed for Zn of 3.53 across all the treatments. When the VT plants were exposed to 2% biochar amendment, the BCF for Pb enhanced by 10% and by 3.05 times for Cd and by 9.31 times for Zn as compared to the control. The increase in BCFs with biochar indicates a decrease in the plant defence response in suppressing the HM translocation. From our findings, it is evident that NT can act as an effective phytoextractor of Cd, Zn and Pb (TF > 1 and BCF > 1), while VT can be a potential candidate for the phytostabilisation (Banerjee et al. 2016; Nugroho et al. 2021) of Pb and Cd and extraction of Zn.

Figure 11 provides a conceptual diagram to illustrate the role of biochar in enhancing plant growth and heavy metal removal. It can be considered that amendment had a more prominent effect on the metal translocation from soil to plants, than from roots to shoots. Furthermore, varying extraction capacities for different HMs by specific plant species speculate selective extraction of HMs for different treatments and conditions. It has been stated that the HMs are predominantly taken by the root tips through the root apex mainly at the meristematic elongation zone, then transported to the plant aboveground tissues via xylem (Yan et al. 2020). The decreased metal toxicity and improved soil characteristics with biochar amendment enhanced the uptake and accumulation of metal by the plant roots. The increased porosity with biochar in the soil composite allows the plant roots to grow, allowing

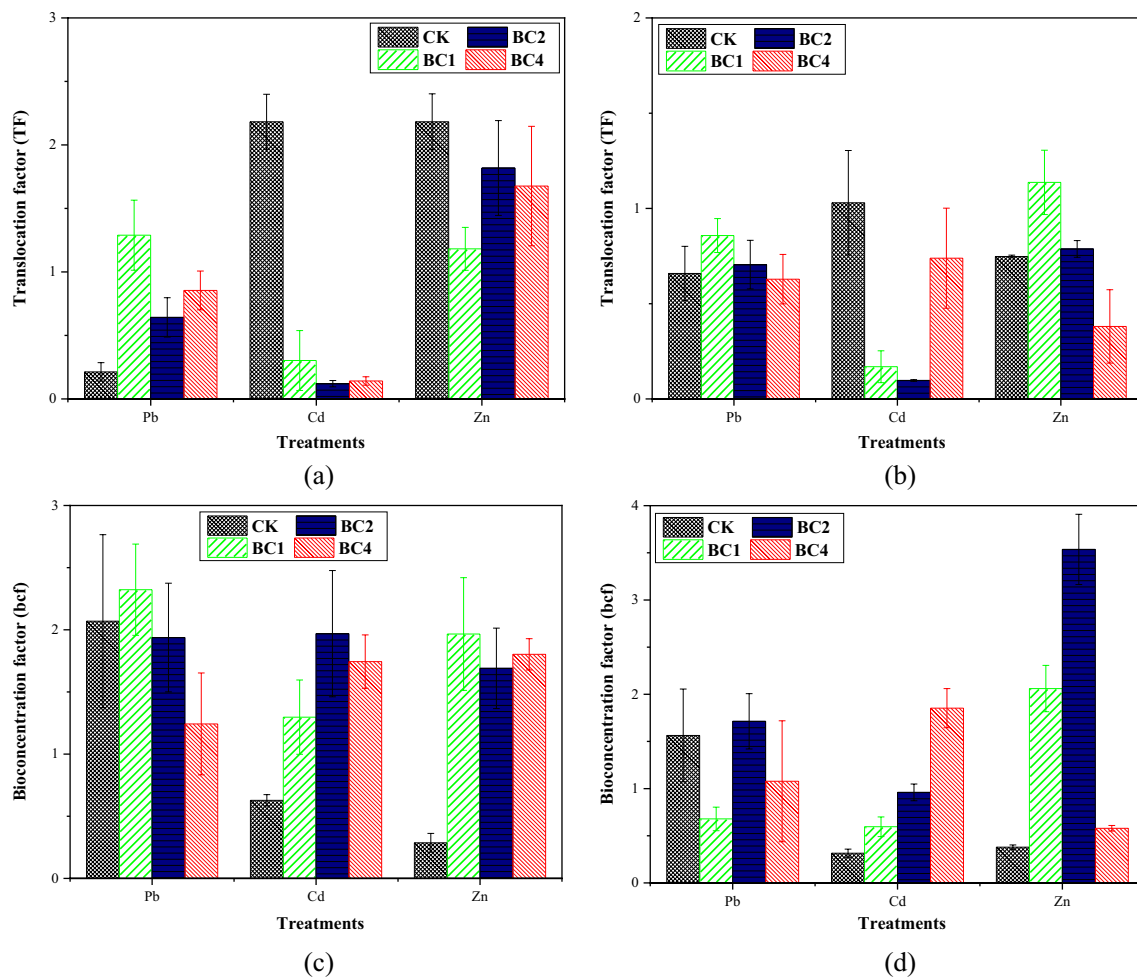


Fig. 10 Heavy metal translocation by plants (a) TF in NT, (b) TF in VT, (c) BCF in NT, (d) BCF in VT at the end of the experiment period

possibilities for metal mobilisation and reduced bioavailability due to metal complex formation.

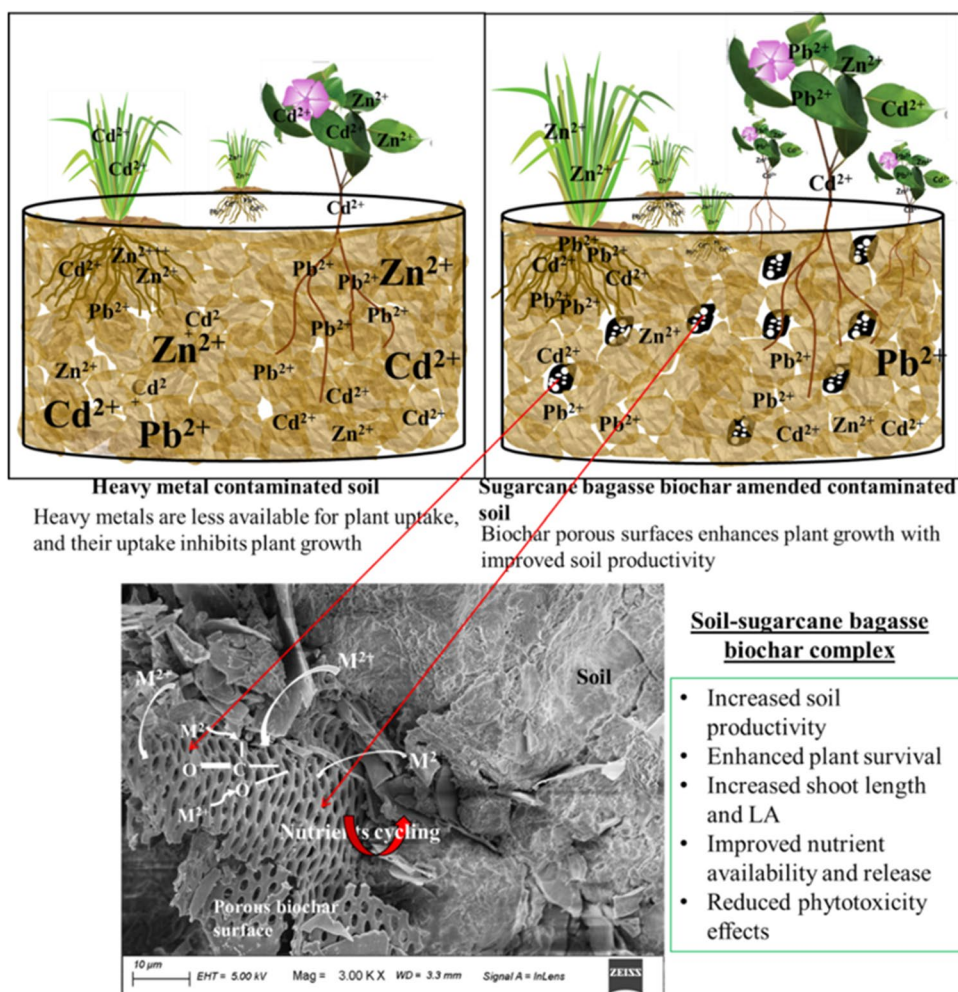
A comparison between the NT and VT plants in unamended and amended pots shows that the VT grass exhibited enhanced growth and survival characteristics with higher HM and biomass production in the amended soil. The membrane transporter proteins can transport HMs due to the chemical similarity of the ions. This similarity can lead to competition thereby affecting the uptake and transport of nutrients in contaminated soil. These proteins are species-dependent and might be the reason behind the high TF values in NT plants. Moreover, the lower TF values in VT can help restrict the exposure to the food chain, and the high BCF can reduce the HM leaching towards the underground water sources.

Our findings revealed that the effects of biochar on the soil and plants were closely related to the amendment rates. An optimum amount can improve the soil structure, promote soil aggregation and stability (Li et al. 2021). As per our findings, the 2% amendment rate limited

the translocation towards the aboveground biomass but increased the contaminant removal from the soil. The improved survival and lower contaminant levels in the amended soil are signs of enhanced phytoremediation mechanisms. However, excessive biochar application can inhibit plant growth. This can be attributed to the high water retention and less oxygen in the pore space leading to an anaerobic state of the plant roots. Also, the high adsorption capacity at the early stages reduces the release of nutrients thereby leading to a lack of fertilisers in the later stages (Shi et al. 2022).

It is remarkable to note that the biochar improvement of HM accumulation and translocation can establish it as an effective and nature-based alternative to enhance phytoremediation. Adding biochar at an optimum rate can generate additional benefits in comparison to the expensive and risk-prone chemicals and complexing agent applications. The biochar-augmented soil functions include increased storage of organic carbon, water retention and lower risk of nutrient leaching.

Fig. 11 Conceptual diagram representing the effect of biochar amendment on plant responses to heavy metal contamination



Conclusions

The current study elucidates the efficiency of phytoremediation using medicinal plants amended with 1%, 2% and 4% sugarcane bagasse biochar in HM-contaminated soil. The major findings of the study include:

- Biochar amendment rates of 2–4% improve the survival rate to 100% in Pb NT samples. The improved plant survival and germination in biochar-amended soil can be attributed to the reduced physiological and metabolic effects of HM stress.
- VT contaminated with Zn at 4% amendment indicated the highest shoot length of 80 cm. The LA results showed a similar trend, with values gradually declining after 40–50 days in unamended samples. The amendment rate of 2% indicated an increase in the total biomass of VT in Zn from 5.83 to 15.12 g, and the Pb samples demonstrated a 73.52% increase in comparison to CK.
- Potassium values are directly related to the amendment rates, with Zn NT soil showing 19.5 mg/l in 4% amend-

ment but 1.6 mg/l in CK. Similarly, Cd indicated an increase from 2.77 to 13.6 mg/l, and Pb samples indicated an increase from 2.33 to 18.18 mg/l.

- The TF values in Cd and Zn indicate a reduction from 2.18 to 0.012 and from 2.44 to 1.18 respectively for NT plants with biochar amendment. TF values lesser than 1 indicate the restriction of HM from roots to shoots as observed for the VT plants. On the other hand, BCF values indicated an enhanced translocation from soil to plants with amendment, with the highest increase in the Zn-contaminated samples. VT plants in Pb samples indicate an increase of 9.5% with 2% amended soil in comparison to the CK, whereas the Cd-contaminated soil showed an increase from 0.31 to 0.96.

The results from this study indicate that the biochar amendment rate of 2% represents an enhanced nutrient cycling with biomass increase, denoting improved plant growth in an HM-contaminated environment. Although the findings provide insights into the development of biochar-amended phytoremediation for the removal of heavy metals, these are limited

to laboratory conditions. The potential implementation of the proposed application should be investigated via pilot-scale experiments for field applications. Moreover, the present study only accounts for the quality of soil under heavy metal (HM) contamination. The effect of HM on the water leached from the pots, necessary for the partitioned assessment has not been considered in this study. Bioconcentration factor (BCF) is only indicative of the phytoremediation of the medicinal plants existing in an HM-contaminated biochar-amended soil. The aspects related to precipitation, adsorption affecting HM mobility will require a more controlled experimental leaching setup and can be incorporated into future studies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-024-34734-4>.

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Author contribution Dhritilekha Deka: conceptualization, investigation, formal analysis; methodology; writing (original draft), writing (review and editing). Deepak Patwa: investigation, formal analysis, writing (original draft). Archana M Nair: resources, supervision. K Ravi: resources; supervision, writing (review and editing).

Data availability Data generated or analysed during this study are provided in full within the published article.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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