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

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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 its 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, compost and chelates have shown to enhance the phytoremediation efficiency. The potential soil improvement is influenced by the properties of the amendment, plant and metal heterogeneities. In this study, an organic biochar amendment for the 60-day pot experiment using *C. roseus, Chrysopogon zizanioides* in a heavy metal contaminated soil was applied. The influence of biochar prepared from sugarcane bagasse biochar on phytoremediation of Pb, Zn and Cd from the soil was explored. Biochar amendment rate of 2% had significant effect on the improvement of the morphological characteristics of the plants in comparison to the control. The soil potassium concentrations increased with amendment rates, while the ammonia and phosphorous reduced beyond an optimum biochar content. Heavy metal concentrations in the soil were consistently lower in the amended soil for both plants. The translocation factor (TF) and bioconcentration factor (BCF) exhibited the phytoextraction and phytostabilisation potential of the plants. However, it remains specific to each contaminant, plant and amendment rate. The insights from this study establishes that the synergy between biochar amendment and the selected medicinal plants improved the phytoremediation efficiency.

Highlights

- The biochar assisted phytoremediation for the decontamination of soil is studied
- Biochar amendment rate impacts phytoremediation efficacy differently across plants
- 2% amendment rate is optimum for phytoextraction, phytostabilisation of heavy metal
- Synergistic interaction can be used for sustainable reclamation of contaminated soil

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. Such persistent pollutants pose serious risks to ecosystems and human health (Narayanan et al., 2022; Zhang et al., 2023). Within this context, various physicochemical strategies such as vitrification, solidification and biological methods such as bioremediation and phytoremediation 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, 2021; 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 elements generation with certain microbial interactions (Igiri et al., 2018). Phytoremediation, 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 phytoremediation 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 (Yan et al., 2020; Thomas et al., 2024). 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 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 the chemical chelators due to the evidences of secondary contaminants generated from these chemical chelators (Ogundiran et al., 2018; Hasan et al., 2019; 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 (Pandey et al., 2022; Otunola et al., 2023; Patwa et al., 2023; 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., 2022). The biochar has alkaline metal cations, electrons, surface functional groups and 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 water absorption capacities (Fellet et al., 2014; Rees et al., 2020; Zhang et al., 2023). The pyrolysed waste improves soil fertility with the release of essential macro and micronutrients such as K, Ca, Mg, Zn and Mn (You et al., 2021; Kumar 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 the 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., 2022). 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 a HM contaminated soil. From the 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., 2023). 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* (Pandey et al., 2020; Dorafshan et al., 2023; Soumya and Kiranmayi, 2023). Literature indicates the high HM removal efficiencies of the *C roseus and Chrysopogon zizanioides* plants (Subhashini and Swamy, 2017; Nugroho et al., 2021; Soumya and Kiranmayi, 2023). 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 conjunction with sugarcane bagasse biochar, to address the sustainable remediation of Pb, Cd and Zn contamination in soil.

Table 1
Literature review on phytoremediation of heavy metal contaminated soil

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

The novelty of this research lies in the recognition of the phytoremediation potential of the medicinal *C roseus (locally known as* Nayantara: NT*), 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 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. (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.

Materials and Methodology

2.1.1 Soil and plants

The locally accessible residual soil from the Assam state of North eastern part of India was used in this study. The soil samples were oven dried and the impurities like dead leaf, organic matter and stones were physically removed. The soil was sieved through 4.75mm sieve for homogenization. Table 2 presents the soil index properties determined as per the ASTM procedures (ASTM D854, 2014; ASTM D422-63, 2007; ASTM D4318, 2010; ASTM D698, 2012). The background values of the HMs (Pb, Zn, Cd) in the soil were negligible in comparison to the artificially spiked concentrations.

Table 2
Basic physico-chemical 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 D698-12, 2012
Maximum dry density (MDD)	1.579g/cc	
Optimum moisture content (OMC)	22.7%	
Specific gravity	2.71 ± 0.03	ASTM D854, 2010
pH	6.68	ASTM D4972, 2018
Organic matter	5.45%	ASTM D2974, 1987
Consistency limits		
Liquid limit	54.42 ± 1.58	ASTM C128-15, 2015
Plastic limit	35.25 ± 1.18	
Shrinkage limit	30.12 ± 1.08	
USCS classification	MH	ASTM D2487-11

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 (Dorafsan et al., 2023). The NT on the other hand, indicates enhanced alkaloids, phenols and flavonoids accumulation under HM stress, which increases the medicinal value (Soumya and Kiranmayi, 2022; Qurban et al., 2023). The ornamental plant is suited to different climates, soil, and enhances the aesthetic value of the surroundings. Such drought resistance along with high metal tolerance are essential characteristics for efficient metal uptake and overall phytoremediation success.

2.1.2 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 the seven days, they were cut to 30mm size for uniform and complete pyrolysis of the biomass. Thereafter, it was oven dried at 60°C for 72hours 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. (2022). Table 3 provides the properties of the sugarcane bagasse biochar used in this study. The high pH value of the biochar could be attributed to the base cation concentrations and high EC was due to the presence of higher soluble salts. Figure 1 represents the honeycomb structure of the sugarcane bagasse biochar as observed in the SEM micrograph images. The presence of lignin, cellulose and hemicellulose biopolymer generates the particular structure of the intra-pores (Patwa et al., 2022). 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., 2020).

Table 3
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[a]	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^2g^{-1})	21.787
Average pore diameter (nm)	8.423
Total pore volume (cm^3g^{-1})	0.045

2.2 Experimental design

To analyse the effects of HM contamination, the soil was artificially spiked with individual 500mg/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 in accordance with the recommended optimum range by Gao et al. (2021) for plant growth rate. Table 4 lists the different treatments for the pot experiments. 1.7kg of the prepared soil samples were collected in plastic pots of dimensions 15×15×14cm and the biochar amendment rate was measured as a percentage of dry soil weight. All the pots were paced with trays, so as 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 two 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 pots were placed randomly in a shed of translucent fiber sheets for protection against rainfall, wind and for uniform ambient conditions. Table 5 shows the average pH, EC, ORP at the initial stage for both plants under different treatments. The pH of the control samples is lower in comparison to the biochar amended samples due to the higher alkalinity in biochar. The variations were low compared to the control samples due to the low amendment rates. The EC variations with biochar was due to the higher surficial charged particles. The oxidation reduction potential shows a reduction with 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.

Table 4
Different treatments of heavy metal contaminated and amended soil samples

Treatments	HM	Biochar amendment rate (%)	NT	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

Table 5
Properties of soil at the time of transplantation

Contaminant	Soil type	pH	EC (mS)	ORP (mV)
Pb	CK	4.72	67.14	533
	SB-1	4.44	57.97	480
	SB-2	4.37	55.34	487
	SB-4	4.98	83.54	494
Cd	CK	4.13	122.3	538
	SB-1	4.56	134.4	536
	SB-2	4.73	96.29	535
	SB-4	4.55	112.2	457
Zn	CK	4.09	125.1	532
	SB-1	4.86	177	491
	SB-2	4.56	178.5	446
	SB-4	4.63	235.4	453

2.3 Analytical testing

The plant growth parameters were recorded at regular intervals during the experiment period. Rate of survival indicates the number of plants surviving in the contaminated environment till the end of the experiment with respect to 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 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 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 hours at 80$^{\circ}C$. The oven dried biomass is separated and weighed as the root and shoot biomass to calculate the root to shoot ratio (R/S). The root-to-shoot ratio (RSR) is a stable indicator of the above and below-ground 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 hours at 60$^{\circ}C$ for further analysis. The nutrients in the soil was 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 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., 2021), but did not have any significant effect on the NH_4^+ and PO_4^{3-} content. In general, the phosphorous sorption is affected by the acidic pH and higher metal oxide content (Wu et al., 2022).

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	CK	1.77	1.30	0.07	0.07	2.05	1.67
	SB-1	1.12	1.08	0.04	0.04	4.1	1.37
	SB-2	1.41	1.31	0.06	0.14	7.84	8.08
	SB-4	1.45	1.35	0.06	0.04	10.56	13.6
Cd	CK	1.09	1.01	0.04	0.04	1.73	1.8
	SB-1	0.93	1.16	0.07	0.07	3.56	4.84
	SB-2	0.96	1.25	0.07	0.07	13.28	7.53
	SB-4	1.17	1.24	0.07	0.07	13.78	11.56
Pb	CK	0.40	0.86	0.19	0.06	4.49	1.65
	SB-1	0.98	0.93	0.05	0.07	3.37	4.53
	SB-2	0.70	0.62	0.11	0.04	7.91	8.48
	SB-4	0.90	0.80	0.04	0.08	11.26	19.42

2.4 Heavy metal analysis

For the HM analysis, the soil and plant samples were oven dried at 60°C for 48 hours and ground to fine powder. Approximately, 1g soil samples were 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 42mm filter paper and diluted to 100ml using deionised water. The diluted extracts were analysed for concentration of HM content using atomic absorption spectrometer (AAS). The translocation from the shoots to roots was evaluated using the translocation factor (TF) given by:

$$\text{TF}=\frac{Heavymetalconcentrationinshoots(mg/kg)}{Heavymetalconcentrationinroots(mg/kg)}$$

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

$$\text{BCF}=\frac{Heavymetalconcentrationinplant(mg/kg)}{Heavymetalconcentrationinsoil(mg/kg)}$$

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.

Results and discussions

3.1 Effects of biochar amendment on the plant

3.1.1 Germination and sapling survival rate

Figure 4 presents the variation of plant survival rate and the germination rate with respect to different treatments mentioned in Table 4. 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%, 80% and 100% with increase in biochar amendment rates to 1%, 2% and 4% respectively. Similar patterns were observed for the NT plants in the Cd contaminated soil where the survival increased from 10% without amendment to 80% at 4% biochar amendment. The percentage of germination in Pb contaminated NT indicates an increase from 30% in control samples (CK) to 90% with 4% biochar amendment, while survival was observed to be 100% at amendment rates of 2% and 4%. The germination of VT in Zn contaminated soil indicated a steady increase with biochar amendment from 60–100%. 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 (Subhasini 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 75% 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 positive effects of biochar on plant survival can be attributed to the presence of basic cations and nutrients on its surface. They are capable of occupying the exchangeable sites of the contaminated soil by replacing harmful cations from the soil. Furthermore, they are bioavailable and beneficial for promoting optimal plant growth (Deal et al., 2012; Ogundiran et al., 2018).

3.1.2 Responses of the plant morphological characteristics

a. Leaf Area (LA)

Figure 5 presents the variation of LA with 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 of time. 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 the 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 with respect to the canopy area. However, it improves the lateral growth of the leaf, resulting in canopy expansion (Qian et al., 2019; Guo et al., 2023).

b. Shoot length

Figure 6 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 the plants after 40 days of the experiment. The biochar-amended samples indicated consistently high values in both the plants (Fig. 6c). 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 high average plant height, with the maximum of 80cm in T4 treatment. For the Cd contaminated VT plants, the maximum plant height increased by 18.75% in comparison to control with 2% biochar amendment. In both the plants, 2% amendment rate indicated 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 belowground 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. The biochar addition reduces the soil bulk density and increases porosity with enhanced root penetration and extension (Zhang et al., 2012). The modified root morphology with increased nutrient pools may have improved shoot growth and yield in comparison to unamended control samples (Ali et al., 2021).

c. 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 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 (0.62g) 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. 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 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). The total biomass enhancement with biochar can be attributed to the significant abridging of metal stress on the plant along with improved chlorophyll content (Rathika et al., 2021).

3.2 Effect of biochar amended phytoremediation on contaminated soil

a. Nutrient concentrations

Figure 8 shows the nutrient concentrations in the soil at the end of the experiment period. In general, the bioavailability 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 19.5mg/l in Zn, 2.77 to 13.6mg/l in Cd and 2.33 to 18.18mg/l in Pb contaminated NT soil samples. Additionally, for VT plants, Zn shows an increase from 1.58 to 16.04mg/l, whereas Cd and Pb indicate an increase from 1.5 to 12.48mg/l and 2.29 to 11.02mg/l respectively. The elevated 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 C/N ratio with biochar amendment, leading to biological fixation of the nutrients (Lehman et al., 2003; Li et al., 2021).

In both plants, the nutrients generally indicate minimum bioavailability at a 2% biochar amendment rate, which can be an indication of higher adsorption capacities. However, the possible reason for the increased bioavailability at higher biochar additions could be that the enhanced negative charges in the soil may limit the availability of sorption sites for PO_4^{3-} (Wu et al., 2022). Moreover, other molecular organic acids released by biochar might compete for the adsorption sites. 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.

b. 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, 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 the values in the concentration range of 181 to 463mg/kg. The 2% amended treatments experienced low Zn concentrations in the narrow range of 106–123mg/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 23.06% and 12.85% lower metal concentrations than the control. For the Cd contaminated soil, the treatment with NT decreased metal concentration by 83.5% with 2% amendment. Hence, the biochar amendment rate of 2% was found to have comparatively low HM concentrations in all treatments, for both NT and VT species.

The precipitation of Pb, Zn and Cd due to the adsorption on the porous biochar surface may reduce the bioavailability of the metals (Namgay et al., 2010; Ahmad et al., 2012). The sugarcane bagasse biochar 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 (-COOH) on the biochar and positively charged metal ions could lead to chelation or coordinate adsorption (Ghosh and Maiti, 2020). These electrostatic 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 enhanced inner sphere and surface metal complexation. Hence, the addition of biochar 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).

3.3 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 plant after the 60-day experiment. Results shown in Fig. 10b, c, indicated that a wide range of TF values were evident in NT (0.12 to 2.86) and VT (0.09 to 1.49) plants. The values lesser than 1.0 indicate the restriction in the translocation from roots to aboveground biomass. 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 reduced translocation can be attributed to lowered bioavailability of the metals with biochar, or the accumulation of the metals in the vacuoles of the roots and cell membranes (Peng and Gong, 2014). The 1% biochar amendment increased the Zn translocation by 49.68% in VT, while decreased from 2.18 (control) to 0.30 in NT plants.

BCF of any plant in a contaminated soil is recognised as a significant measure of human health risk (Zhang et al., 2023; Bashir et al., 2020). The metal accumulation and bioremediation can be estimated using the BCF in the HM polluted soil. The BCF values as shown in Fig. 10d, e indicated that the biochar amendment improved the translocation from soil to plants, which is in accordance with our previous results. For Zn and Cd, the BCF values indicated a significant increase with biochar, while the Pb-treated NT plants demonstrated a noticeable reduction with the amendment. However, the reduction of BCF with amendment in Pb indicates NT plants as potential phytostabilisers of the metal. The VT plants showed low Cd accumulation capacity (BCF: 0.28–1.209), while the highest accumulation was observed for Zn (BCF:0.38–3.496) across all the treatments. When the VT plants were exposed to 2% biochar amendment, the BCF for Pb enhanced by 16.23% and by 3.36 times for Cd and by 9.2 times for Zn as compared to the control. The increase in BCFs with biochar indicates decrease in the plant defense response in supressing the HM translocation. From our findings, it is evident that NT can act as 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 possibilities for metal mobilisation and their reduced bioavailability due to metal complex formation. The improved survival and lower contaminant in the amended soil are signs of enhanced phytoremediation mechanisms.

A comparison between the NT and VT plants in unamended and amended pots shows that the VT grass exhibited better growth characteristics. The total HM and nutrient concentrations in VT were consistent with NT, but the high biomass production in VT emphasises its role as a hyperaccumulator. 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 above ground biomass, but increased the contaminant removal from the soil. The biochar application beyond the appropriate content, 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 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 amount 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.

Conclusions

The current study elucidates the efficiency of phytoremediation using medicinal crops with different biochar amendment rates of 1%, 2% and 4% in a HM contaminated soil. For developing the synergy of phytoremediation with biochar, the plant germination, survival, shoot length and LA were evaluated. Also, the findings from the nutrient and heavy metal concentrations in the soil and translocation in the plants elaborate the mechanisms involved in the biochar amended phytoremediation. The major findings of the study include:

- Biochar amendment rates of 2% and 4% enhance NT plant survival to 100% in Pb polluted soil, while Cd contamination exhibits the maximum survival of 75% and germination of 80% at 4% biochar amendment rate. For the Zn contaminated samples, the germination in NT shows a steady increase from 45–75% and VT indicates an increment from 50–100% with the as the amendment rate rose from 0–4%. The improved plant survival and germination in biochar-amended soil can be attributed to the reduced physiological and metabolic effects from HM stress.
- The maximum shoot length in NT is shown by 2% biochar amended Cd contaminated samples, whereas VT contaminated with Zn at 4% amendment indicated the highest shoot length of 80cm. The plants without biochar amendment indicated lower shoot lengths than the amended samples. The LA results showed a similar trend, with values gradually declining after 40–50 days in unamended samples. Biochar amendment rate of 2% indicated an increase in the total biomass of VT in Zn from 5.83 to 15.12g and the Pb samples demonstrated 73.52% increase in comparison to control. The R/S variations indicate an amelioration effect of biochar on the HM contamination.
- The ammonia and phosphate nutrient concentrations indicate no significant differences with biochar amendment rates. However, the K values are directly related to the amendment rates, with Zn contaminated soil showing 19.5 mg/l in 4% amendment and 1.6 mg/l without biochar amendment in NT. Similarly, Cd indicated an increase from 2.77 to 13.6 mg/l and Pb contaminated samples indicated an increase from 2.33 to 18.18 mg/l. However, on an average, biochar amendment rate of 2% indicates an enhanced nutrient cycling with biomass increase, which is an indication of improved plant growth.

- The TF values in Cd and Zn indicate a reduction from 2.86 to 0.08 and 2.18 to 0.056 respectively for NT plants. TF values lesser than 1 indicates the restriction of HM from roots to shoots as observed for the VT plants. On the other hand, BCF values indicated an enhanced translocation with biochar amendment, except for the Pb contaminated NT plants. VT plants in Cd contamination indicate an increase of 52.63% with 2% amended biochar amended soil in comparison to the control, whereas the Zn contaminated soil showed an increase from 0.287 to 1.209. The TF and BCF values represent the phytostabilisation Pb and Cd by VT and phytoextraction potential of NT. The results from this study indicate the need for an optimum biochar application of 2% to improve the phytoremediation efficiency of the plants in a 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. It is to be mentioned that the performance of the proposed plants and optimum biochar content is dependent on the particular soil and environmental conditions. Moreover, the efficiency of the amended soils is critical to be assessed for the sustained reclamation of the heavy metal contaminated sites. The potential implementation of proposed application should be investigated via pilot scale experiments for field applications.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Author contributions according to CRediT

Dhritilekha Deka: Conceptualization, Investigation, Formal analysis; Methodology; Writing-original draft, Writing-review & editing. **Deepak Patwa**: Investigation, Formal analysis, Writing – original draft. **Archana M Nair**: Resources; Supervision. **K Ravi:** Resources; Supervision, Writing-review & editing.

Data Availability Statement

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

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Figures

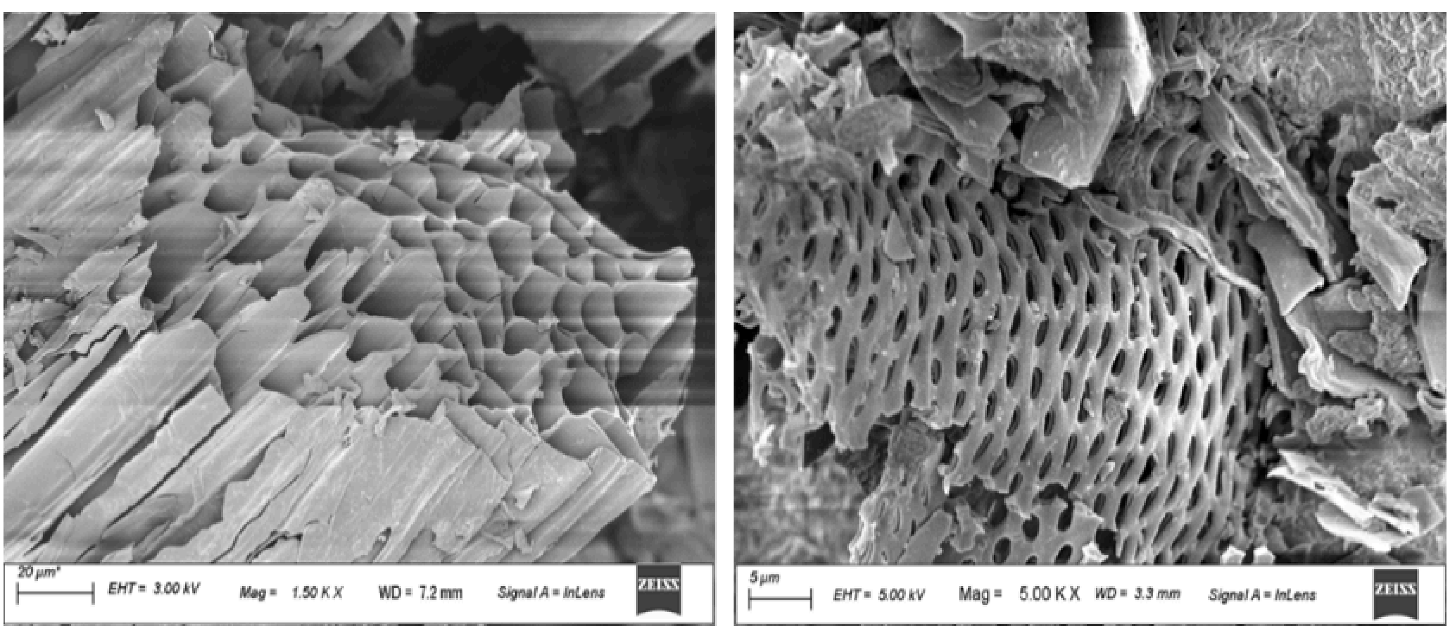

Figure 1

SEM micrograph images of sugarcane bagasse biochar

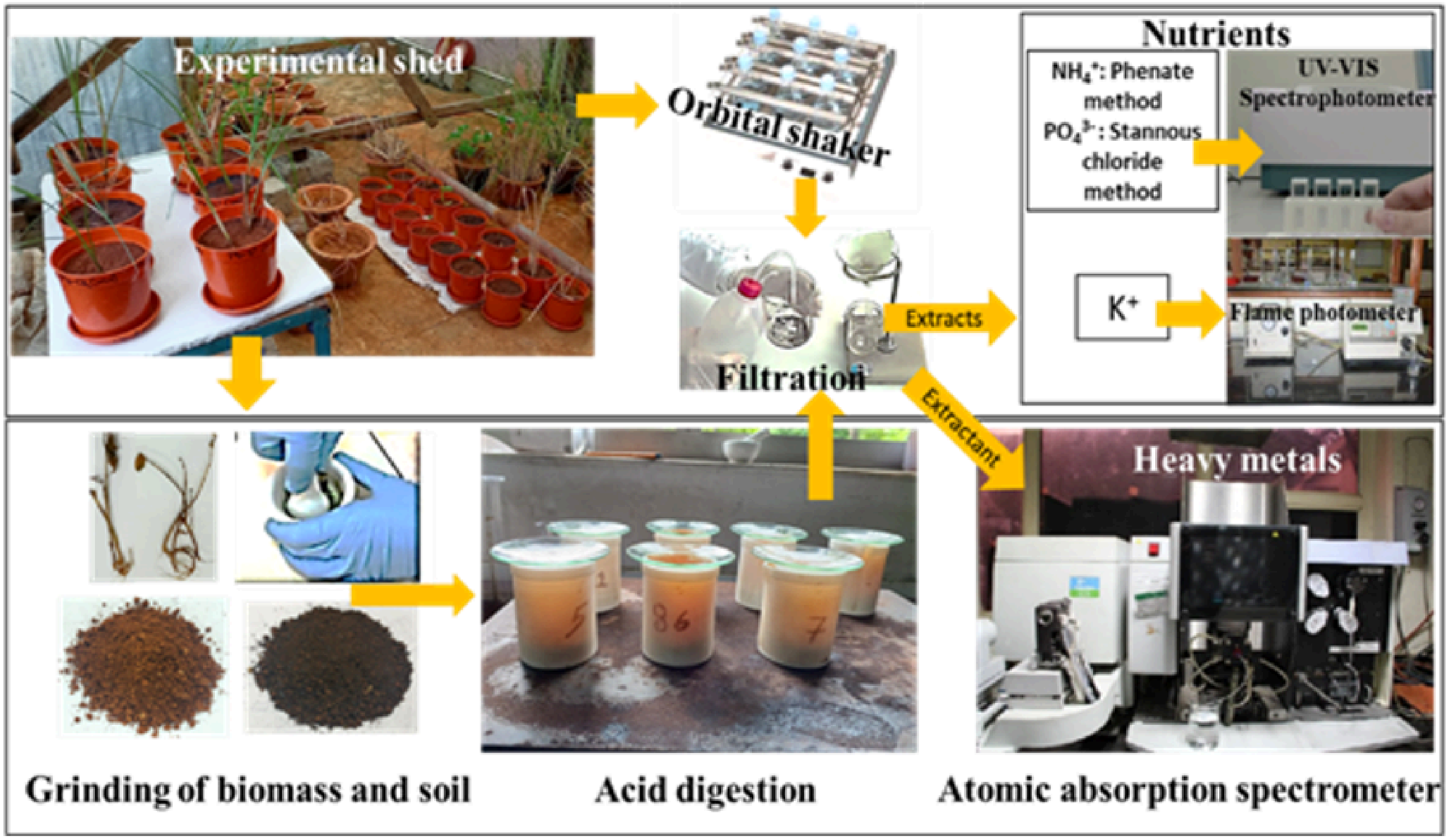

Figure 2

Overall methodology for HM and nutrient analysis in plants and soil

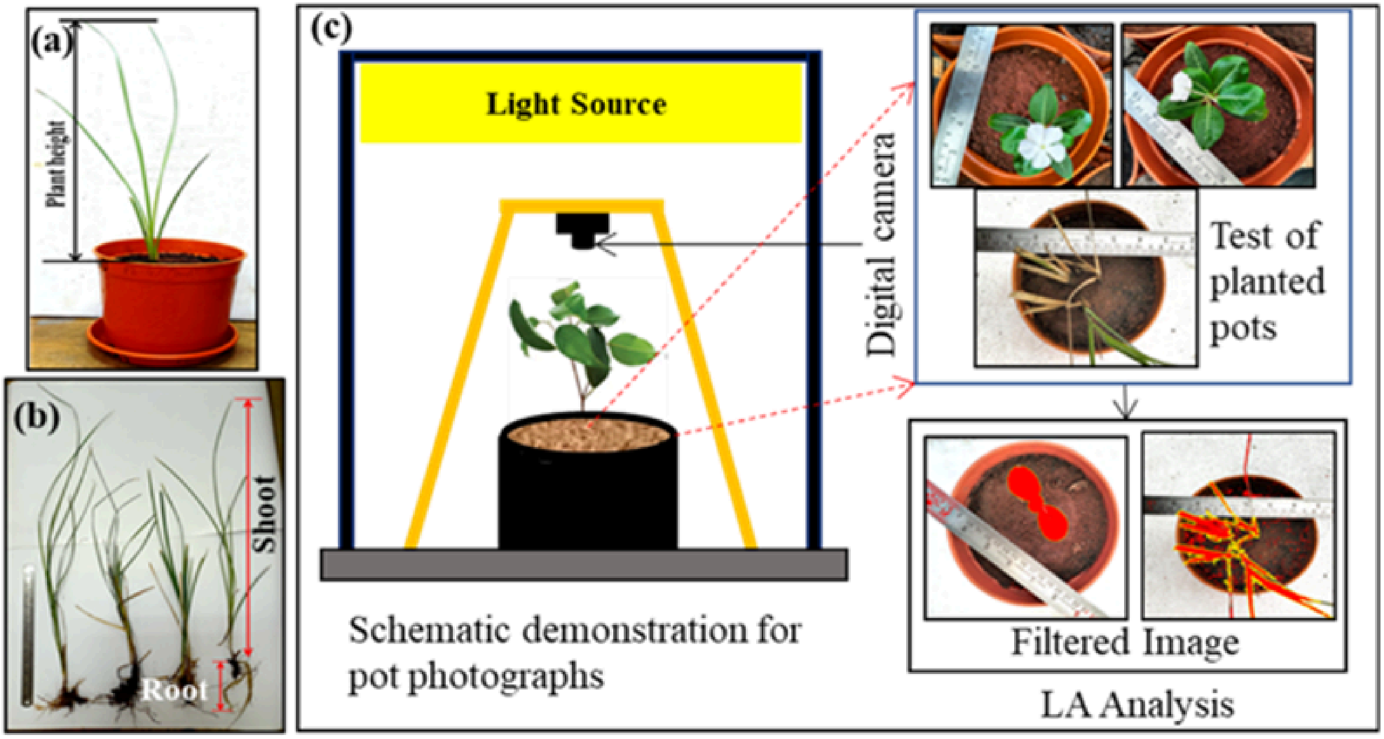

Figure 3

Plant morphological parameters: a) Shoot length (cm) b) Shoot and root biomass, (c) Leaf area

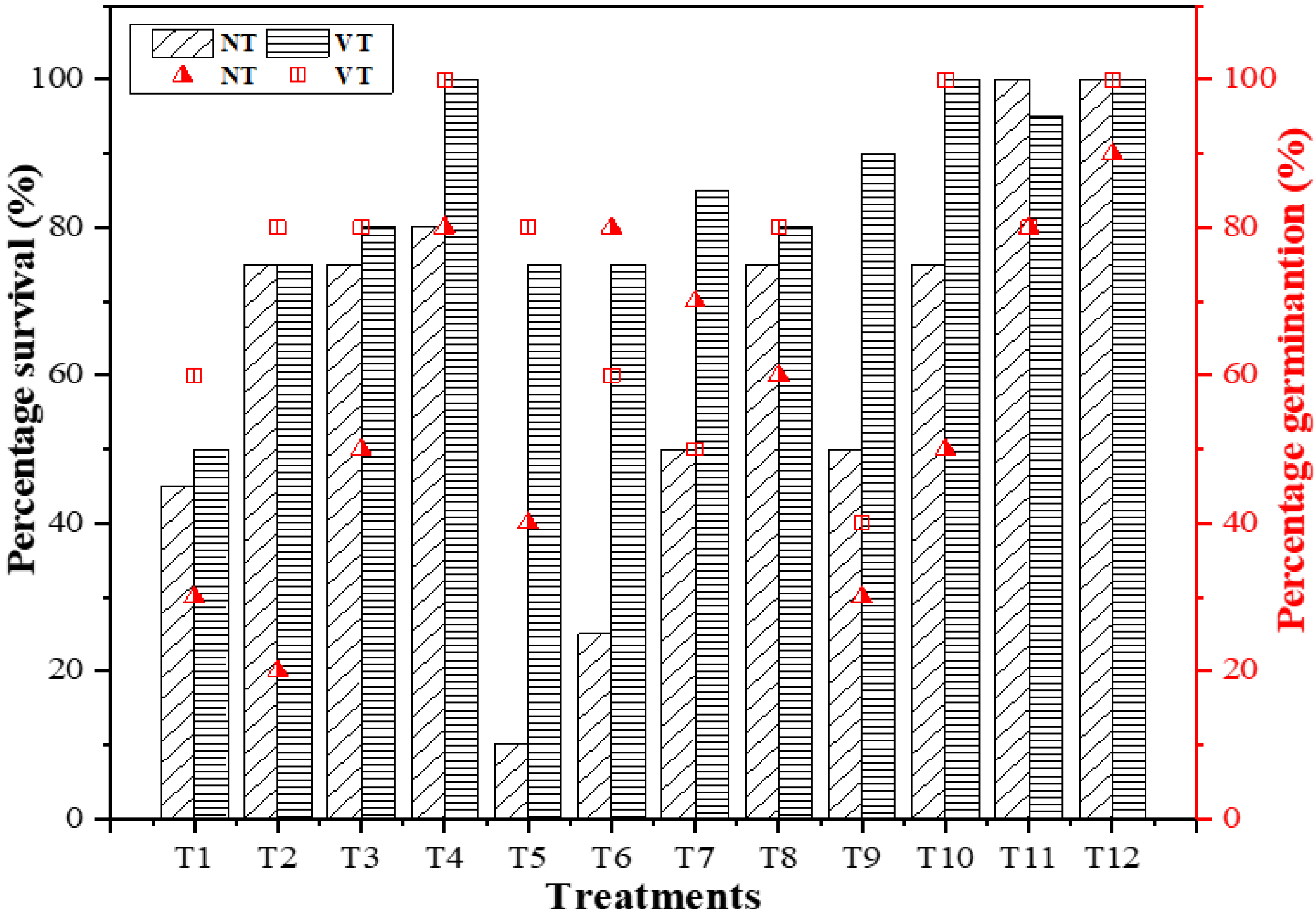

Figure 4

Rate of survival and germination of NT and VT in different soil treatments

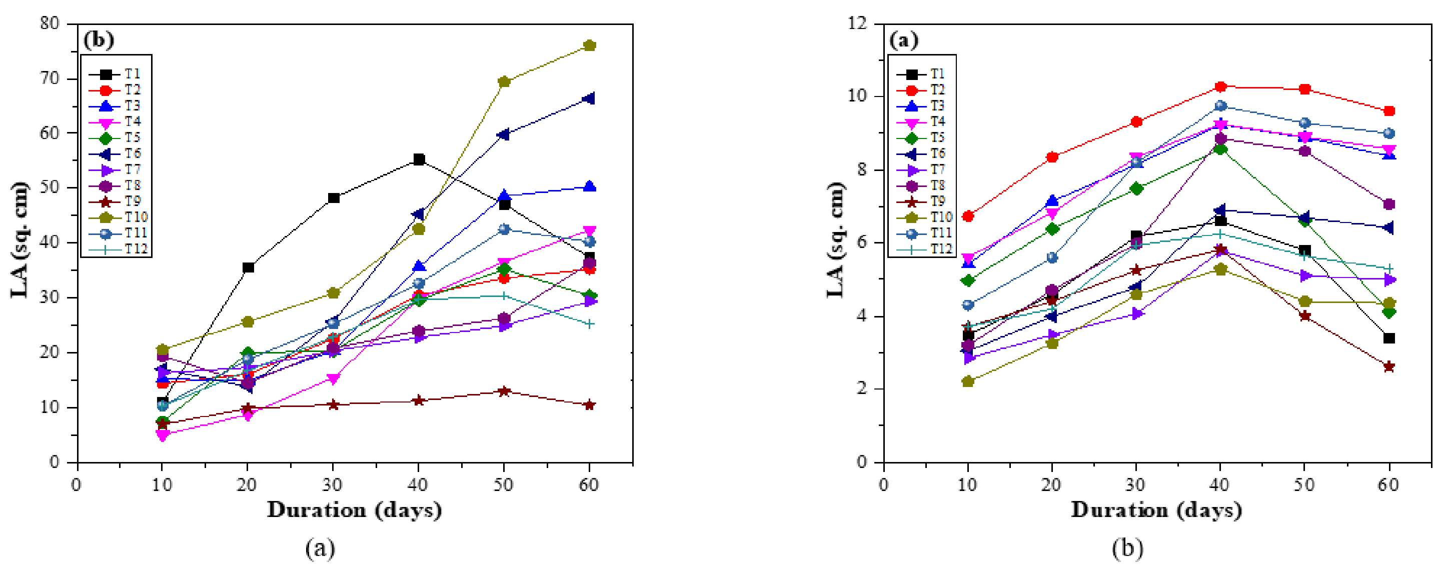

Figure 5

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

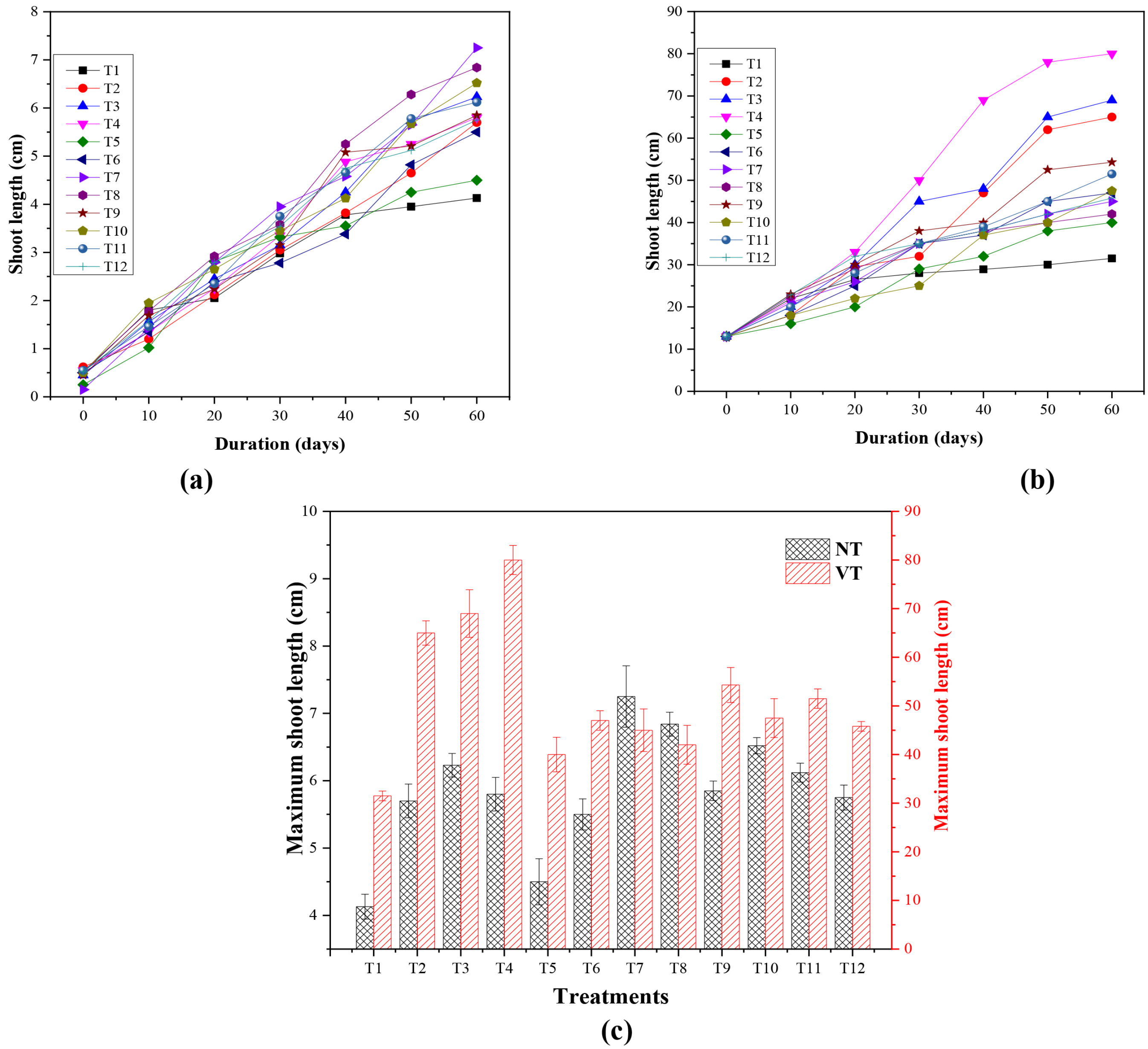

Figure 6

Shoot lengths for (a) NT and (b) VT, (c) maximum shoot length for the 60-day experiment

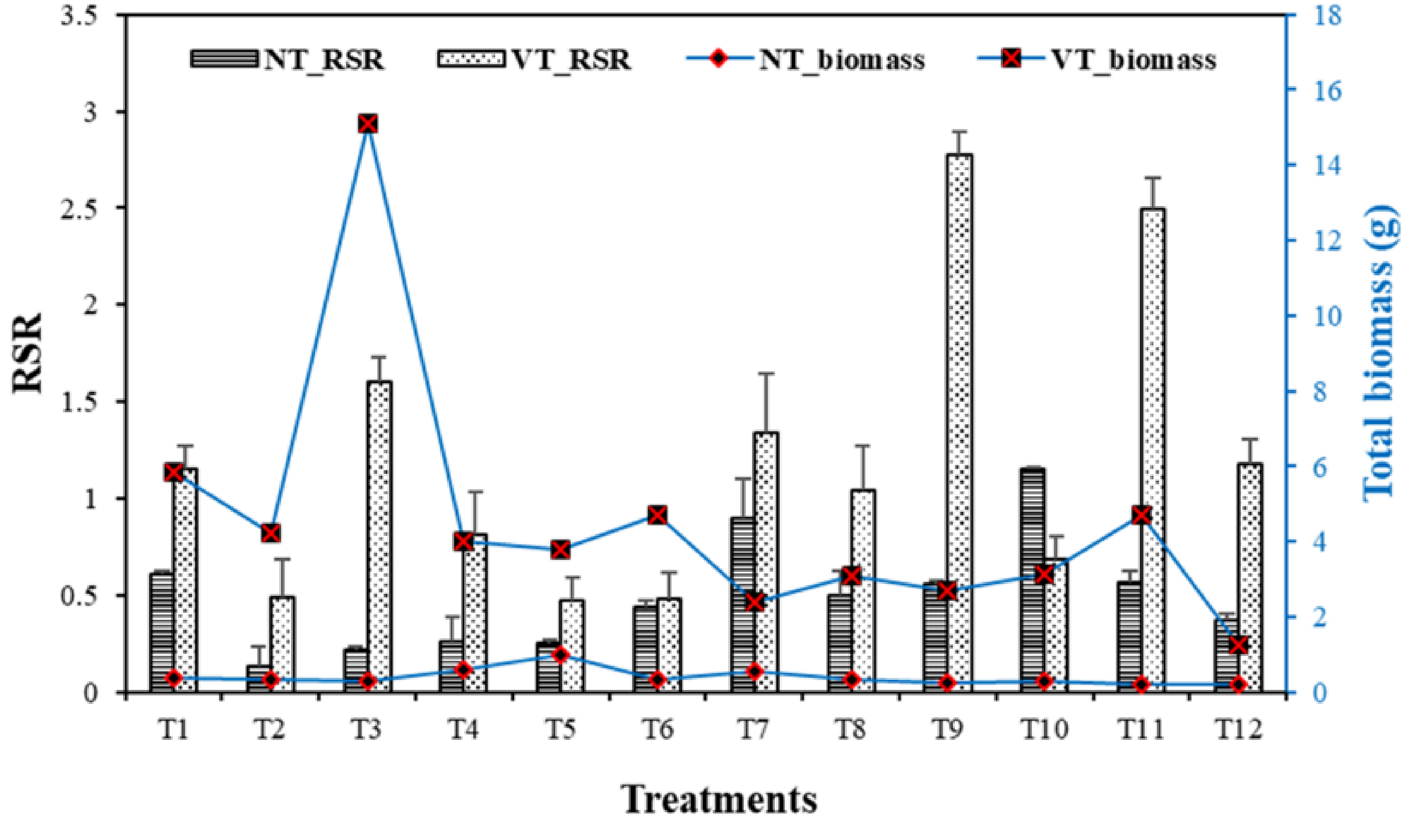

Figure 7

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

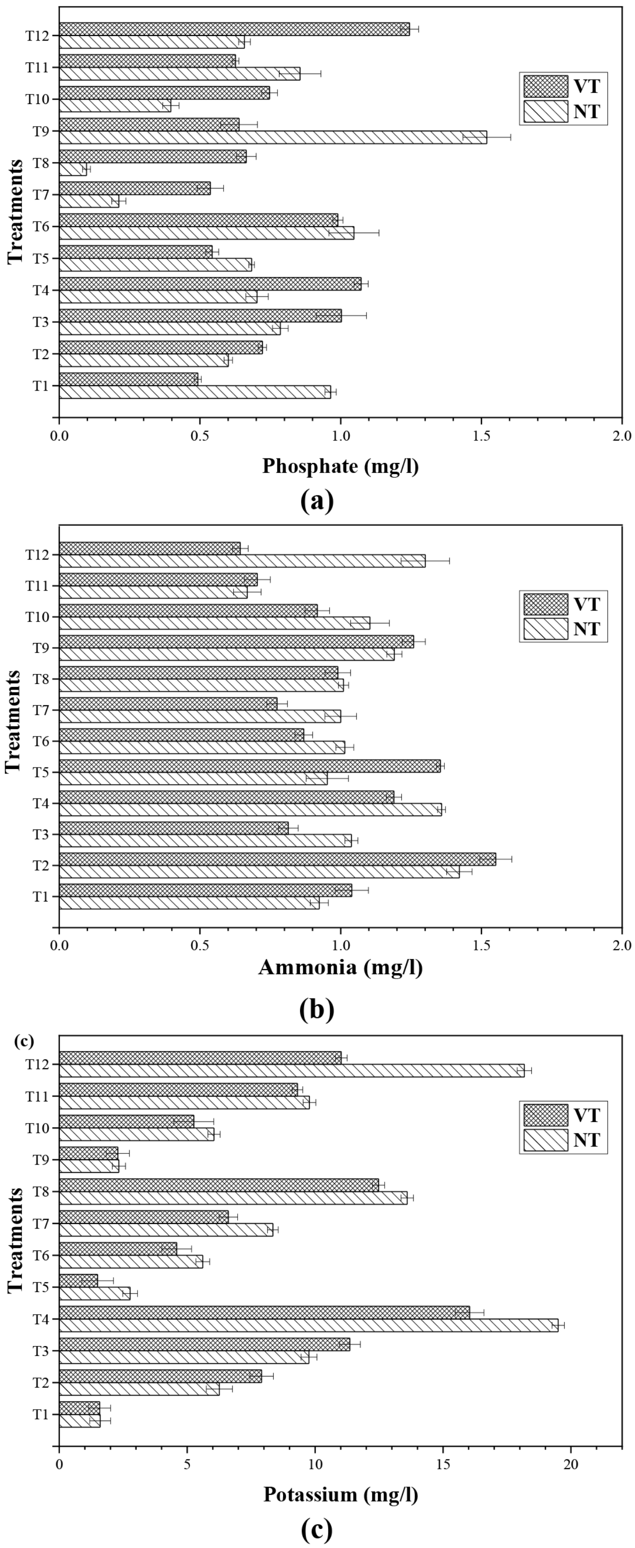

Figure 8

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

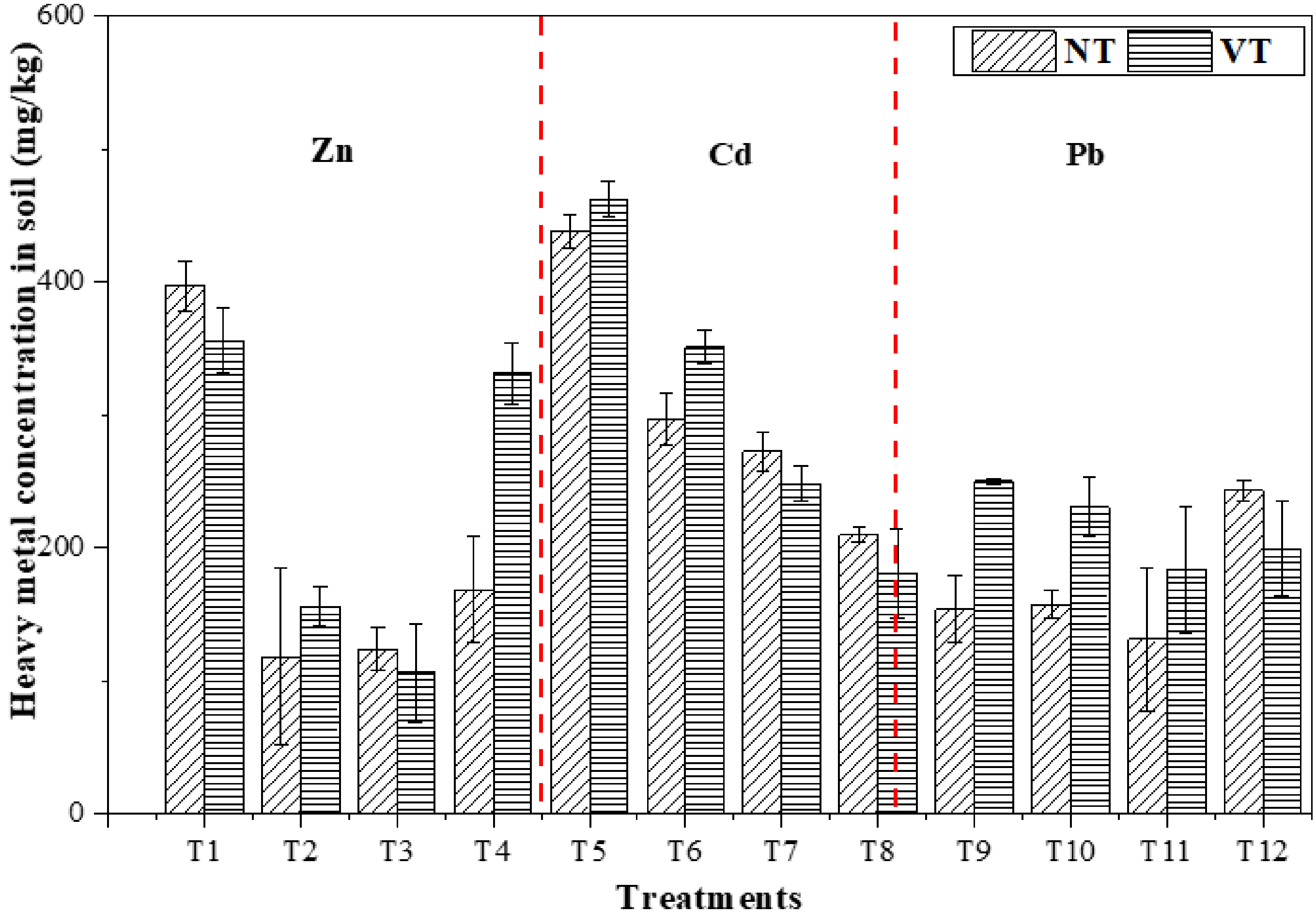

Figure 9

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

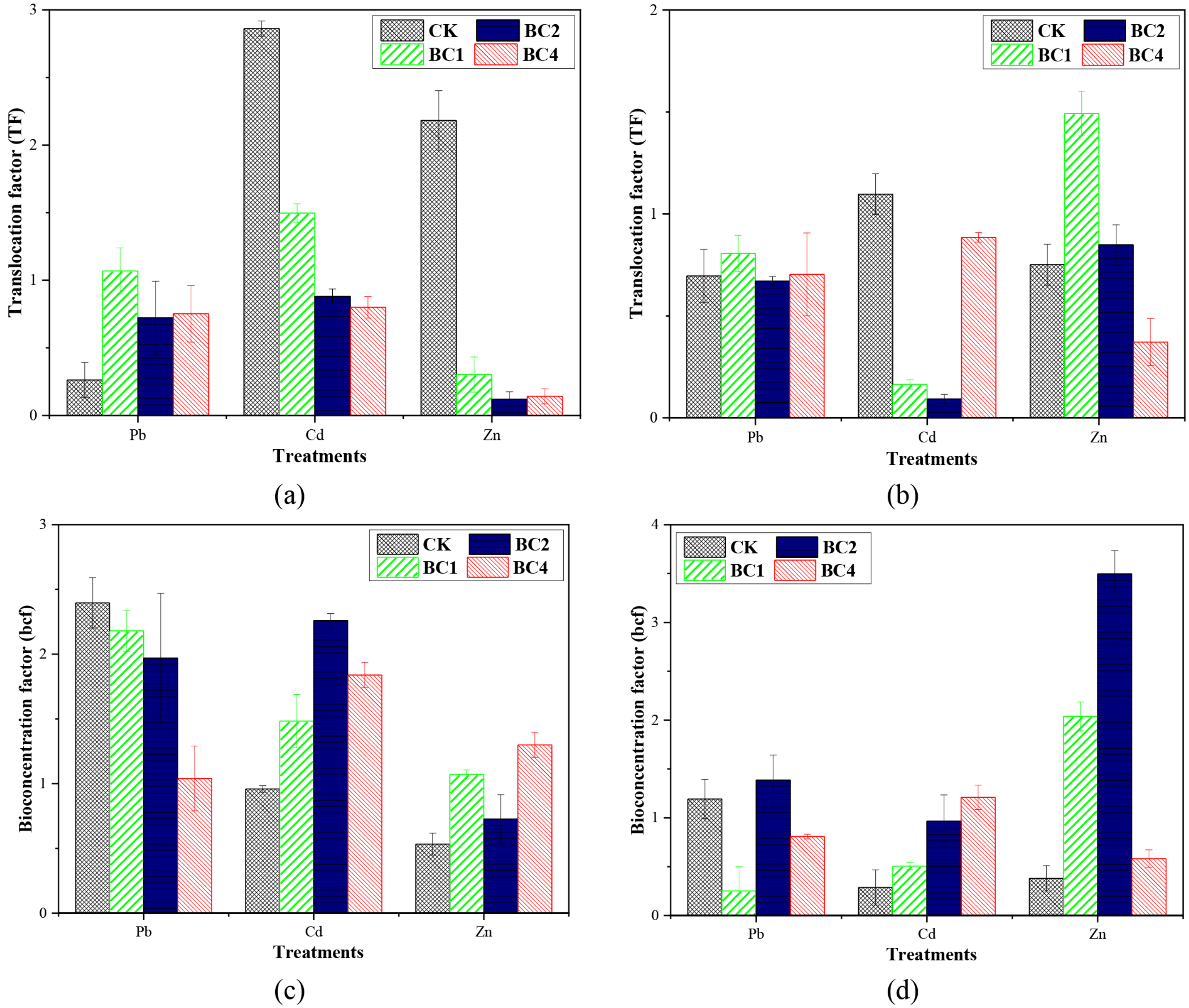

Figure 10

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

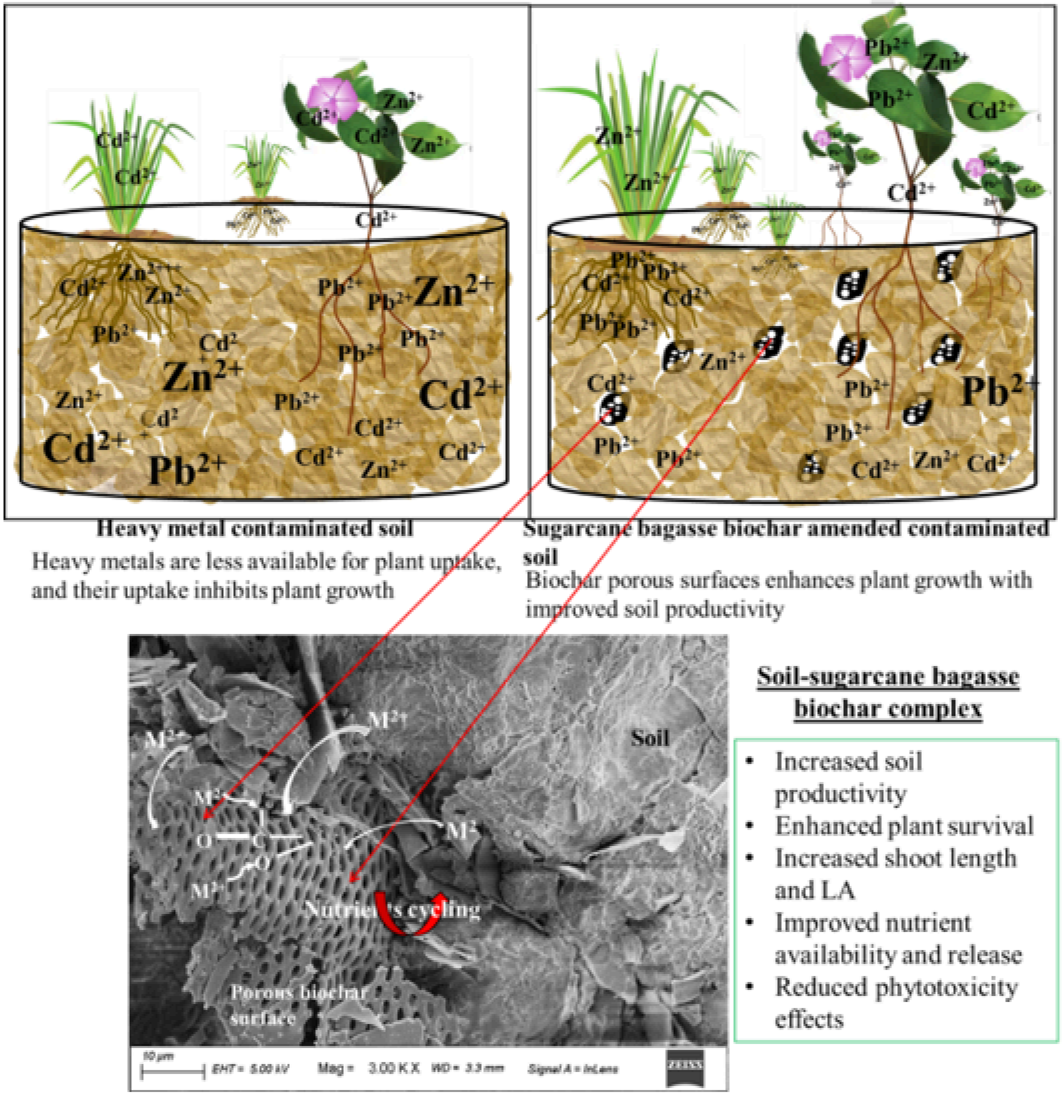

Figure 11

Conceptual diagram representing the effect of biochar amendment on plant responses to heavy metal contamination with and without biochar

Supplementary Files

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- Graphicalabstract.png