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Research Article

Synergistic Enhancement of Heavy Metal Phytoextraction in Okra (*Abelmoschus esculentus*) Using Biochar and Chelating Agents

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ABSTRACT

Okra (*Abelmoschus esculentus*) is an annual vegetable consumed for its pleasant and spicy flavor. Heavy metal stress adversely affects human health, ecological balance and biodiversity throughout the globe. A pot experiment was conducted using heavy metal-contaminated soils to assess the phytoextraction potential of *A. esculentus*. To create the biochar, we pyrolyzed rice husk, peanut shell, *Asphodelus tenuifolius*, and *Suaeda fruticosa* in a locally fabricated pyrolysis machine at 500–550°C under anaerobic conditions. Our research suggested that the application of biochars and chelating agents significantly enhanced the absorption and transport of heavy metals into plant tissues as compared to untreated plants. Further, all treatments indicated that the root-to-shoot translocation factor was >1, indicating the significant improvement in metal mobilization, plant biomass production, plant height and chlorophyll contents. These findings suggested that the integration of biochar with chelating agents can serve as an effective strategy for the remediation of heavy metal-contaminated soils via phytoextraction using *A. esculentus*. This research will help to develop a sustainable approach for managing agricultural and plant-based waste materials through biochar production.

Keywords: *Asphodelus tenuifolius*, Chelating agents, Citric acid, Okra, Peanut shell biochar, Phytoremediation, Phytoextraction; Rice husk biochar, *Suaeda fruticosa*

INTRODUCTION

Pakistan's agricultural soils have been shown to have excessive amounts of various toxic metals (Pollmann *et al.*, 2019). Due to the presence of several harmful pollutants in soil, soil chemistry keeps on altering and radical changes have been recorded (Haider *et al.*, 2021). Metal toxicity concerns the agricultural environment, because of incurable soil pollution and hinders plant development and metabolism, ultimately affecting crop yield (Bilen *et al.*, 2021; Tepecik and Irget, 2021; Yigider *et al.*, 2021). Contaminants are non-biodegradable and bio-accumulating substances, a major reason for the considerable threat to the climate, even harmful at an exceptionally low rate (Vardhan *et al.*, 2019). Heavy metals pose a serious threat to environmental because of their high toxicity and persistence in ecosystems as these metals are non-biodegradable and can accumulate in living organisms (Ali *et al.*, 2019; Srivastava *et al.*, 2020; Ukhurebor *et al.*, 2021).



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Among the numerous remediation techniques phytoremediation is more considerable because it is more sustainable, economical and environment friendly. So, phytoremediation has emerged as a promising approach for the removal of heavy metals from contaminated soils. Phytoremediation employs certain plant species, that are commonly known as hyperaccumulators. These hyperaccumulators have the tendency to absorb and translocate the heavy metals from soil into their tissue (Awa and Hadibarata, 2020). However, many natural hyperaccumulators have limited practical utility because of their poor biomass production and slow development rates eliminate their metal hyperaccumulation ability. Plentiful research reports have been made to enhance the effectiveness of phytoextraction using natural and artificial chelating agents. Bioavailability of heavy metals in the rhizosphere has been improved by the use of soluble metal chelating complexes that also increase their absorption and movement in plants (DalCorso *et al.*, 2019). Significant research is done on chelating agents like citric acid and ethylenediaminetetraacetic acid (EDTA). Citric acid is naturally biodegradable substitute having quite lower environmental risk, while EDTA being a synthetic chelator poses great binding stability and extensively used in industrial and environmental settings (Kwon *et al.*, 2020). A number of properties of plant species like metals tolerance, their biomass yield, capability of heavy metals accumulation and move into above ground areas play vital role for successful phytoremediation. Furthermore, bioavailable fraction of metals in soil performs a significant role for examining the effectiveness of phytoextraction, which is controlled by the chelating agents and soil chemistry (Shah and Daverey, 2020).

The advancements in the integration of chelating agents with high biomass crop species and soil additives like compost or biochar have improved the ability of metal uptake and support the sustainable remediation techniques. More scientific attention has been drawn towards biochar's significant role in heavy metals removal because it works without having any potential environmental risk. Numerous studies have shown that biochar's made from plant-based residues and agricultural wastes have used for heavy metals adsorption and immobilization from wastewater system (Wang *et al.*, 2021).

Biochar significantly affects the physical, biological and chemical characteristics of soil and its adsorption abilities that consequently change microbial activity, nutrient availability and soil structure (Quin *et al.*, 2014; Van Zwieten *et al.*, 2014; Yildirim *et al.*, 2021; Kul *et al.*, 2022; Mustafa *et al.*, 2026). Biochar is a porous substance rich in carbon, that is made by thermal breakdown of organic materials with little oxygen production (Moradi-Choghamarani *et al.*, 2019). There are mainly three approaches have been used in phytoremediation including usage of hyper accumulator plants to tolerate and accumulate high concentration of heavy metals, application of chelating agents to increase uptake and mobility of metals and the third one is the addition of biochar to increase metal bioavailability and to improve the soil condition (Rostami and Azhdarpoor, 2019). Okra, or *Abelmoschus esculentus* L., is a lucrative crop that is grown primarily in tropical and subtropical areas and belongs to the Malvaceae family (Samiksha *et al.*, 2021). Okra is well-known for its versatility, high yield, and ease of cultivation. It also has hyperaccumulative tendency, which allows it to take up higher levels of some heavy metals from contaminated soils.

A few research reports have been done to investigate the effect of chelating agent and biochar together to affect okra under heavy metal stress, regardless of earlier reports focusing on their independent effects on metal uptake in plants. *Therefore, the present study was designed to explore the integrated impact of chelating agents (EDTA and citric acid) and different biochars (derived from rice husk, peanut shell, *Asphodelus tenuifolius*, and *Suaeda fruticosa*) on the physicochemical properties of soil and the growth performance of *A. esculentus* L. when exposed to synthetic heavy metal contamination from lead, copper, and chromium.

MATERIALS AND METHODS

Collection of Biomass and Biochar Production

For biochar synthesis, four types of biomass were manually collected from various regions of Punjab, Pakistan: *Asphodelus tenuifolius* and *Suaeda fruticosa* from Kasur, peanut shells from Chakwal, and rice husk from Lahore. The collected biomass was air-dried and stored in clean polyethylene bags until further use. Each biomass type was separately subjected to pyrolysis in a locally designed, cost-effective pyrolyzing unit. The process was conducted under limited oxygen conditions at a temperature range of 500–550°C for four hours, using a slow pyrolysis method in a charcoal kiln. During pyrolysis, the feedstock was intermittently stirred using an external rotary rod to ensure even heat distribution. Once the unit cooled (typically by the following day), the resulting biochar was collected and stored in airtight zip-lock bags for subsequent analyses.

Characterization of Biomass and Biochar

Physicochemical properties of biomass and resulting biochar, including pH, electrical conductivity (ECe), total dissolved solids (TDS), and NaCl content, were assessed using the protocols described by Enders and Lehmann

(2012) and Rajkovich *et al.* (2012). Moisture content was measured following ASTM Method D-1762-84 (2007), and volatile matter was determined using Zimmerman's (2010) protocol. Total Organic Carbon (TOC) and Total Organic Matter (TOM) were derived from volatile content calculations using the following formulas:

Biochar yield (%) = (Weight of produced biochar / Weight of dried feedstock) × 100

Bulk density (g/cm³) = Mass of dry sample (g) / Volume of container (cm³)

Moisture content (%) = $((W_1 - W_2) / W_1) \times 100$

Ash content (%) = $(W_1 / W_2) \times 100$

Volatile content (%) = 100 – % Ash

TOC (%) = % Volatile content / 1.8

TOM (%) = 1.703 + (0.502 × TOC %)

Where W_1 = initial weight and W_2 = final weight after drying or ashing.

Preparation and Application of Chelating Agents

Two chelating agents—ethylenediaminetetraacetic acid (EDTA) and citric acid (CA)—were prepared in double-distilled water to avoid salt precipitation. These were applied at two concentrations: 1 mM and 5 mM. Chelating solutions were applied separately to respective pots, while control plants received no treatment.

Heavy Metal Solution Preparation and Application

Stock solutions (1000 ppm) of cadmium chloride, chromium chloride, and lead nitrate were prepared using analytical-grade salts dissolved in distilled water. These stocks were then diluted to a working concentration of 50 ppm for soil application.

Soil Sampling and Physicochemical Analysis

Soil was collected from the Botanical Garden of Punjab University, Lahore. A saturated soil paste was prepared by mixing 500 g of air-dried soil with deionized water until saturation. The paste was equilibrated overnight. The extract was obtained using a vacuum suction pump. Parameters such as pH, EC, TDS, moisture content, bulk density, ash content, volatile matter, TOC, and TOM were measured from triplicate samples and averaged.

Pot Experiment Setup

A 65-day pot experiment was conducted in the Environmental Biotechnology Research Laboratory, Institute of Botany, University of the Punjab, Lahore, to assess the effects of chelators (EDTA, citric acid) and biochars (from rice husk, peanut shell, *A. tenuifolius*, and *S. fruticosa*) on the growth and heavy metal uptake of *Abelmoschus esculentus* L. under induced heavy metal stress (Pb, Cd, Cr). Medium-sized pots were filled with 2 kg of air-dried, sieved soil. A completely randomized design (CRD) with three replications per treatment was followed. Seeds of *A. esculentus* were procured from Pride Seeds, Gulberg, Lahore. After pre-soaking, three seeds were sown per pot. At 12 days post-germination, the healthiest seedling in each pot was retained.

Assessment of Plant Growth Parameters

Upon harvest, morphological and physiological parameters were recorded. Fresh weights of roots, shoots, and leaves were measured separately, followed by drying in an oven at 60°C for 24 hours to determine dry weight. Plant height, shoot length, root length, and total plant length were recorded in centimeters. The number of leaves was manually counted. Chlorophyll content was assessed using a SPAD chlorophyll meter (SPAD-502, Japan).

Heavy Metal Analysis and Translocation Factor

Plant tissues were analyzed for heavy metal content (Cd, Pb, Cr) using an atomic absorption spectrometer (GBC SAVAANT AA, Australia). Calibration and measurements were performed in accordance with the manufacturer's operational manual. The translocation factor (TF), representing the efficiency of metal movement from root to shoot, was calculated as: $TF = \text{Metal concentration in shoot} / \text{Metal concentration in root}$.

Statistical Analysis

Data were subjected to statistical analysis using SPSS (Version 23). Treatment means were compared using the Least Significant Difference (LSD) test at a 5% significance level. Duncan's Multiple Range Test (DMRT) was employed to assess significant differences among treatment groups. All experiments were conducted in triplicate to ensure data reliability.

RESULTS

Proximate and Physicochemical Analysis of Feedstock

In the present study we used *A. tenuifolius*, *S. fruticosa*, Rice husk and Peanut shell to compare the physicochemical and proximate analysis of feedstock. Results of proximate analysis of feedstock indicated that peanut shell contained highest moisture contents (18.48±0.32) whereas lowest MC (15.05±0.64) was reported in *A. tenuifolius*.

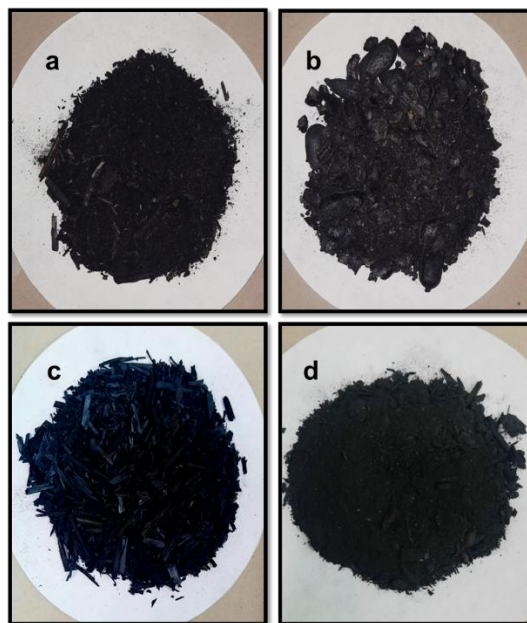


Figure 1. Biochar prepared at 500-550°C (a) Peanut shell (b) *A. tenuifolius*(c) *S. fruticosa* (d) Rice husk.

Table 1. Proximate and physiochemical analysis of feedstock.

Parameters	MC (%)	AC (%)	VC (%)	TOC (%)	TOM (%)	pH	EC (µS/cm)	NaCl (%)	TDS (ppm)	BD (g/cm ³)
<i>A. tenuifolius</i>	15.05 ±0.64	84.95 ±2.76	15.05 ±0.33	8.36 ±0.98	5.90 ±0.44	6.81 ±0.24	1344.24 ±0.02	2.0 ±0.14	475 ±2.18	0.04 ±0.07
Peanut shell	18.48 ±0.32	81.52 ±1.39	18.48 ±0.45	10.27 ±0.67	6.86 ±0.22	6.76 ±0.06	1395.03 ±0.02	0.72 ±0.35	324.52 ±0.16	0.18 ±0.26
Rice husk	17.58 ±0.87	79.42 ±2.33	20.58 ±1.28	11.43 ±0.32	7.44 ±1.02	6.99 ±0.14	1537.60 ±0.14	1.23 ±0.19	471.61 ±1.23	0.15 ±0.004
<i>S. fruticosa</i>	18.29 ±0.93	81.71 ±3.65	18.29 ±0.72	10.16 ±0.45	6.80 ±0.33	5.656 ±0.19	1664.34 ±0.27	3.42 ±0.36	715.6 ±3.42	0.06 ±0.31
CV (%)	3.36	2.05	0.48	0.79	0.77	0.73	1.42	0.84	0.29	0.45
LSD _(0.05)	0.0180	0.0711	0.0352	0.0411	0.0201	0.5432	6.2727	0.3333	2.3790	0.2215

MC: moisture content, AC: ash content, VC: volatile content, TOC: total organic carbon, TOM: total organic matter, G. mean: grand mean, ±: standard deviation, CV: coefficient of variance, LSD: least significance deviation.

However, *A. tenuifolius* showed highest AC (84.95±2.76) percentage which was lowest (79.42±2.33) in rice husk. It was also noted that there was a slight difference in AC% among all the variables. Percentage of volatile contents was higher (20.58±1.28) in rice husk and least (15.05±0.33) in *A. tenuifolius*. Total organic carbon (TOC) and total organic matter (TOM) were noted highest in rice husk and minimum in *A. tenuifolius* as shown in Table.1. Physiochemical properties such as pH, electric conductivity (EC), NaCl contents, total dissolved solids (TDS), and bulk density (BD) of feedstock (Figure 1). From the results it was observed that rice husk showed the highest (6.99±0.14) pH value whereas minimum pH was expressed by *S. fruticosa* (5.656±0.19) as shown in Table. 1. Highest EC (1664.34±0.27), NaCl (3.42±0.36) and TDS value (715.6±3.42) was observed by *S. fruticosa*. Peanut shell showed maximum BD (0.18±0.26) as compared to other variables followed by rice husk (0.15±0.004).

Proximate And Physiochemical Analysis of Biochar

It was noted that rice husk biomass possessed maximum moisture content i.e., followed by *S. fruticosa*, *A. tenuifolius* and peanut shell. Maximum (93.88±0.22) ash content was observed in peanut shell followed by *A. tenuifolius* (90.60±0.27), *S. fruticosa* (89.89±0.65), and rice husk (88.07±0.45). Percentage of VC, TOC and TOM was observed higher in rice husk as compared to other variables as shown in Table.2. Physiochemical analysis of biochar of carried out using *A. tenuifolius* Peanut shell, rice husk and *S. fruticosa*.

Results of Table.2 indicated that rice husk biochar possessed highest (10.13 ± 0.38) pH value followed by peanut shell. Maximum (1382.66 ± 0.34) EC was exhibited by *S. fruticose* and lowest (1166 ± 0.03) by *A. tenuifolius*. Further it was observed that NaCl, TDS and BD were also maximum in *S. fruticose* biochar indicating that *S. fruticose* biochar is of high value regarding these contents.

The physicochemical characterization of soil used in this study indicated the following values: pH of 6.53, electrical conductivity (EC) of $418.6 \mu\text{S}/\text{cm}$, total dissolved solids (TDS) of 0.83 ppm, and NaCl concentration of 212%. Other soil parameters included a moisture content of 0.32%, bulk density of $0.36 \text{ g}/\text{cm}^3$, ash content of 91.88%, volatile content of 8.13%, total organic matter (TOM) of 9.94%, and total organic carbon (TOC) of 4.52%, as presented in Table. 2.

Table 2. Proximate and physicochemical analysis of Biochar

Parameters	MC (%)	AC (%)	VC (%)	TOC (%)	TOM (%)	pH	EC ($\mu\text{S}/\text{cm}$)	NaCl (%)	TDS (ppm)	BD (g/cm^3)
<i>A.s tenuifolius</i>	9.40 ± 0.45	90.60 ± 0.27	9.40 ± 0.36	5.22 ± 0.12	4.33 ± 0.48	8.93 ± 0.02	1166 ± 0.03	4.16 ± 0.25	546 ± 4.18	0.23 ± 0.05
Peanut shell	6.12 ± 0.36	93.88 ± 0.22	6.12 ± 0.42	3.40 ± 0.35	3.41 ± 0.24	9.71 ± 0.32	1186.43 ± 0.61	3.39 ± 0.11	522.82 ± 2.16	0.31 ± 0.14
Rice husk	11.93 ± 0.65	88.07 ± 0.45	11.93 ± 0.22	6.63 ± 0.84	5.03 ± 0.36	10.13 ± 0.38	1234.12 ± 0.23	1.98 ± 0.12	582.66 ± 19.14	0.27 ± 0.17
<i>S. fruticosa</i>	10.11 ± 0.48	89.89 ± 0.65	10.11 ± 0.06	5.61 ± 0.37	4.52 ± 0.57	8.16 ± 0.02	1382.66 ± 0.34	6.1 ± 0.1	892.66 ± 5.57	0.29 ± 0.02
CV (%)	0.83	0.95	0.65	0.99	0.22	0.17	2.54	0.04	2.37	0.16
LSD _(0.05)	0.0215	0.1395	0.2406	0.0463	0.0251	0.6423	5.3589	0.4247	4.8333	0.2423

MC: moisture content, AC: ash content, VC: volatile content, TOM: total organic matter, TOC: total organic carbon. G. mean: grand mean, \pm : standard deviation, CV: coefficient of variance, LSD: least significance deviation.

Table 3. Comparison of means for shoot and root length in *A. esculentus* grown under different stress conditions.

Biochars	Treatments	Mean \pm SE					
		Shoot length (cm)			Root length (cm)		
		Cd	Cr	Pb	Cd	Cr	Pb
Control	-	33.31 \pm 1.22a	33.31 \pm 0.75a	33.31 \pm 0.81a	3.67 \pm 0.15abc	3.67 \pm 0.14abc	3.59 \pm 0.12a
<i>A. tenuifolius</i>	EDTA 1mM	32.62 \pm 1.0ab	34.32 \pm 0.99a	33.21 \pm 0.92a	3.87 \pm 0.06abc	3.87 \pm 0.11ab	3.49 \pm 0.02a-d
	EDTA 5mM	33.11 \pm 1.35a	34.11 \pm 0.46a	34.54 \pm 1.02a	3.92 \pm 0.08abc	4.12 \pm 0.12a	3.63 \pm 0.03a
	CA 1mM	35.17 \pm 1.15a	33.23 \pm 1.00a	34.31 \pm 1.48a	4.16 \pm 0.18ab	3.66 \pm 0.15abc	3.52 \pm 0.02a-d
	CA 5mM	35.38 \pm 0.73a	32.57 \pm 1.3ab	35.15 \pm 0.95a	3.99 \pm 0.08abc	3.99 \pm 0.16a	3.61 \pm 0.01a
Peanut shell	EDTA 1mM	32.8 \pm 1.06ab	31.2 \pm 1.13abc	33.75 \pm 0.46a	3.55 \pm 0.07abc	3.61 \pm 0.18abc	3.63 \pm 0.02a
	EDTA 5mM	34.72 \pm 0.52a	32.44 \pm 0.7ab	33.34 \pm 0.92a	3.99 \pm 0.07abc	3.88 \pm 0.08ab	3.49 \pm 0.01a-d
	CA 1mM	31.2 \pm 1.35ab	31.72 \pm 0.8ab	33.58 \pm 0.95a	3.54 \pm 0.19bc	3.18 \pm 0.10bcd	3.51 \pm 0.04a-d
	CA 5mM	32.5 \pm 1.35ab	33.54 \pm 1.14a	35.52 \pm 1.41a	4.22 \pm 0.16a	3.78 \pm 0.05abc	3.58 \pm 0.02ab
Rice husk	EDTA 1mM	33.21 \pm 1.71a	34.21 \pm 1.38a	34.43 \pm 1.16a	4.12 \pm 0.13ab	3.62 \pm 0.22abc	3.51 \pm 0.08a-d
	EDTA 5mM	33.15 \pm 2.07a	34.15 \pm 0.47a	35.31 \pm 0.70a	3.94 \pm 0.16abc	3.14 \pm 0.07cd	3.32 \pm 0.03bcd
	CA 1mM	34.58 \pm 1.28a	33.42 \pm 0.90a	34.15 \pm 0.84a	3.89 \pm 0.19abc	3.89 \pm 0.13ab	3.38 \pm 0.02a-d
	CA 5mM	34.43 \pm 1.75a	35.43 \pm 1.16a	35.55 \pm 1.07a	3.49 \pm 0.06bc	3.68 \pm 0.20abc	3.57 \pm 0.03abc
<i>Seuda fruticosa</i>	EDTA 1mM	23.62 \pm 0.44c	24.82 \pm 0.29d	26.22 \pm 0.71b	3.43 \pm 0.10c	3.43 \pm 0.18a-d	3.29 \pm 0.09d
	EDTA 5mM	24.41 \pm 0.88c	26.61 \pm 1.40cd	25.61 \pm 0.88b	3.35 \pm 0.14c	3.55 \pm 0.16abc	3.46 \pm 0.06a-d
	CA 1mM	24.74 \pm 0.88c	25.44 \pm 0.53d	25.41 \pm 0.62b	3.95 \pm 0.11abc	3.25 \pm 0.11bcd	3.30 \pm 0.02cd
	CA 5mM	26.63 \pm 0.70bc	28.1 \pm 0.65bcd	25.74 \pm 0.54b	3.39 \pm 0.09c	2.79 \pm 0.05d	3.53 \pm 0.07a-d
Critical value for comparison		6.438	4.982	4.995	0.6727	0.7260	0.2722

Means sharing similar letters in a column are statistically non-significant (PP>0.05).

Table 4. Comparison of means for plant height and No. of leaves in *A. esculentus* grown under different stress conditions.

Biochars	Treatments	Mean±SE					
		Plant Height			No. of leaves		
		Cd	Cr	Pb	Cd	Cr	Pb
Control	-	37.0±0.68ab	36.98±0.61a	36.98±1.33a	6.67±0.3a-d	6.67±0.3abc	6.67±0.33ab
A. <i>tenuifolius</i>	EDTA 1mM	36.49±2.0ab	38.19±0.79a	36.73±1.32a	6.33±0.3bcd	7.00±0.0abc	6.67±0.33ab
	EDTA 5mM	37.03±0.5ab	38.23±1.09a	38.22±1.00a	7.33±0.3abc	7.33±0.33ab	7.33±0.33a
	CA 1mM	39.33±0.52a	36.89±0.22a	37.83±0.66a	7.33±0.3abc	6.67±0.3abc	6.67±0.33ab
	CA 5mM	39.37±1.47a	36.56±0.6ab	38.79±0.74a	8.00±0.00ab	6.67±0.3abc	7.33±0.33a
Peanut shell	EDTA 1mM	36.31±0.6ab	34.87±2abc	37.37±0.87a	8.33±0.33a	7.33±0.33ab	6.67±0.33ab
	EDTA 5mM	38.71±1.95a	36.32±1.4ab	36.81±2.02a	7.33±0.3abc	7.33±0.33ab	7.67±0.33a
	CA 1mM	34.7±1.8abc	34.90±1abc	37.02±0.59a	7.33±0.3abc	6.33±0.3abc	6.67±0.33ab
	CA 5mM	36.8±1.25ab	37.32±1.50a	39.11±1.25a	8.00±0.58ab	6.67±0.3abc	7.33±0.33a
Rice husk	EDTA 1mM	37.33±1.6ab	37.83±1.56a	37.81±0.40a	6.67±0.3a-d	6.67±0.3abc	7.00±0.00a
	EDTA 5mM	37.1±1.07ab	37.29±0.94a	38.58±1.04a	6.67±0.3a-d	7.33±0.3ab	7.33±0.33a
	CA 1mM	38.47±0.69a	37.31±0.94a	37.49±0.10a	5.67±0.33cd	7.33±0.33ab	6.67±0.33ab
	CA 5mM	37.92±3.01a	39.11±1.48a	39.14±0.70a	6.67±0.3a-d	8.00±0.58a	7.67±0.33a
<i>Seuda fruticosa</i>	EDTA 1mM	27.05±1.44d	28.25±0.81d	29.35±0.42b	5.33±0.33d	5.67±0.33bc	5.33±0.33b
	EDTA 5mM	27.76±0.2cd	30.1±0.77cd	28.95±0.85b	6.33±0.3bcd	5.33±0.33c	6.67±0.33ab
	CA 1mM	28.7±1.55cd	28.69±0.54d	28.68±1.50b	5.67±0.33cd	6.33±0.3abc	6.33±0.33ab
	CA 5mM	30.0±1.1bcd	30.9±0.4bcd	29.41±0.33b	7.00±0.0a-d	6.67±0.3abc	7.00±0.00a
Critical comparison	value for	7.355	5.757	5.311	1.758	1.808	1.651

Means sharing similar letters in a column are statistically non-significant (PP>0.05).

Table 5. Comparison of means for shoot and root length in *A. esculentus* grown under different stress conditions

Biochars	Treatments	Mean±SE					
		Fresh shoot + leaves weight (mg)			Fresh root weight (mg)		
		Cd	Cr	Pb	Cd	Cr	Pb
Control	-	145±65.9ab	1450±38.5abc	1450±65a-e	126.7±5b-g	126.7±6.3abc	126.7±3.05a
A. <i>tenuifolius</i>	EDTA 1mM	1420±53.2ab	1494±33.02ab	1445±78a-e	133.6±5.0a-f	133.6±7.99ab	121.5±2.9abc
	EDTA 5mM	1441±72.5ab	1484±68.39ab	1503±96.2ab	135.3±2a-e	142.2±4.89a	125.4±5.15ab
	CA 1mM	1531±13.68a	1446±29.9abc	1493±73abc	143.6±6.9ab	126.3±2.0abc	123.2±4.4abc
	CA 5mM	1506±92.76a	1417±104abc	1530±65.63a	137.7±5abc	137.7±5.22ab	125.7±3.69ab
Peanut shell	EDTA 1mM	1459±39.1ab	1360±17.77a-e	1469±62a-e	123±6.7c-g	124.6±4.7abc	125.0±3.15ab
	EDTA 5mM	1511±50.21a	1412±78.82a-d	1451±82a-e	137.7±6abc	133.9±6.21ab	119.8±0.9abc
	CA 1mM	1359±53abc	1380±37.65a-e	1461±63a-e	122.2±8c-g	109.8±8.10bc	118.8±1.6abc
	CA 5mM	1417±52.5ab	1460±44.8abc	1546±89.19a	145.7±6.24a	130.5±6.82ab	123.9±3.89ab
Rice husk	EDTA 1mM	1445±39ab	1489±62.32ab	1498±65.2ab	142.2±7.ab	125.0±8abc	116.7±2.2a-d
	EDTA 5mM	1443±79.6ab	1486±55.18ab	1537±53.83a	136.0±5a-d	108.4±5.18bc	112.9±3.56cd
	CA 1mM	1505±50.24a	1454±96.3abc	1486±80a-d	134.3±8a-e	134.3±7.95ab	115.3±5.1bcd
	CA 5mM	1498±51.32a	1542±55.85a	1547±23.52a	120.5±5d-g	127.0±5.4abc	123.9±5.50ab
S. <i>fruticosa</i>	EDTA 1mM	1028±25.40d	1080±52.94e	1141±94b-e	118.4±7efg	118.4±3.9abc	108.1±4.19d
	EDTA 5mM	1062±70.2cd	1158±76.4cde	1115±85.2de	115.6±5.21g	122.5±6.6abc	115.3±3.2bcd
	CA 1mM	1077±51.4cd	1107±36.88de	1106±50.94e	136.4±5a-d	112.2±6.2abc	112.9±2.08cd
	CA 5mM	1159±63bcd	1224±20.22b-e	1120±21cde	117.0±4.2fg	96.3±4.24c	126.7±4.25a
Critical comparison	value for	303.15	309.87	373.37	31.51	32.32	19.40

Means sharing similar letters in a column are statistically non-significant (PP>0.05).

Table 6. Comparison of means for shoot and root length in *A. esculentus* grown under different stress conditions.

Biochars	Treatments	Mean±SE					
		Fresh plant weight (mg)			Dry plant weight (mg)		
		Cd	Cr	Pb	Cd	Cr	Pb
Control	-	1,576±34.0ab	1,576±109abc	1,576±61.34a	114.84±6.17a	114.84±4.5ab	114.84±2.24a
<i>A. tenuifolius</i>	EDTA 1mM	1,553±63.6ab	1,627±53.6ab	1,567±52.23a	113.16±3.7ab	118.55±3.78a	114.15±5.39a
	EDTA 5mM	1,576±99.4ab	1,627±47.1ab	1,630±89.41a	114.84±2.43a	118.51±2.28a	118.77±4.27a
	CA 1mM	1,674±37.81a	1,573±77abc	1,615±54.13a	121.97±4.14a	114.56±6.2ab	117.64±2.17a
	CA 5mM	1,677±64.58a	1,555±62abc	1,655±60.50a	122.21±6.56a	113.30±7abc	120.60±4.95a
Peanut shell	EDTA 1mM	1,548±50.7ab	1,485±47a-d	1,594±54.66a	112.80±5.5ab	108.19±3a-d	116.11±6.02a
	EDTA 5mM	1,649±34.73a	1,546±65abc	1,571±71.81a	120.12±2.77a	112.61±3abc	114.44±2.50a
	CA 1mM	1,481±55abc	1,490±50a-d	1,580±35.11a	107.89±5abc	108.57±5a-d	115.12±3.38a
	CA 5mM	1,563±37.4ab	1,590±54abc	1,670±58.80a	113.85±3.6ab	115.85±3.7ab	121.65±7.02a
Rice husk	EDTA 1mM	1,588±81.6ab	1,614±59.6ab	1,615±51.83a	115.66±3.31a	117.57±4.24a	117.67±3.05a
	EDTA 5mM	1,579±63.9ab	1,595±60abc	1,650±67.19a	115.02±2.54a	116.17±1.8ab	120.18±3.52a
	CA 1mM	1,639±87.96a	1,589±72abc	1,602±48.09a	119.42±5.51a	115.75±2.9ab	116.68±4.40a
	CA 5mM	1,619±63.41a	1,669±54.91a	1,671±50.48a	117.94±3.90a	121.59±4.71a	121.74±3.99a
<i>S. fruticosa</i>	EDTA 1mM	1,146±23.94d	1,199±50.11d	1,249±61.17b	83.52±2.96d	87.32±1.53e	91.01±2.48b
	EDTA 5mM	1,178±57.8cd	1,281±52.5cd	1,230±57.44b	85.82±2.69d	93.30±2.9cde	89.60±2.75b
	CA 1mM	1,213±53.7cd	1,219±54.70d	1,219±63.97b	88.38±2.34cd	88.83±2.40de	88.79±4.04b
	CA 5mM	1,276±54bcd	1,320±42bcd	1,247±40.17b	92.96±3bcd	96.17±2.5b-e	90.84±3.43b
Critical value for comparison		316.46	324.03	309.88	21.76	20.82	5.775

Means sharing similar letters in a column are statistically non-significant (PP>0.05).

Table 7. Comparison of means for in Chlorophyll content in *A. esculentus* grown under different stress conditions.

Biochars	Treatments	Mean ± SE		
		Chlorophyll content		
		Cd	Cr	Pb
Control	-	33.20±0.60abc	33.20±1.11bcd	33.20±1.05bc
<i>A. tenuifolius</i>	EDTA 1mM	28.56±1.74cd	34.74±0.67bc	30.89±0.89b-e
	EDTA 5mM	33.33±0.50abc	36.29±1.22abc	34.16±1.76ab
	CA 1mM	34.23±1.18abc	31.75±0.42cde	35.39±1.31ab
	CA 5mM	37.64±1.95a	26.82±0.56e-h	33.87±1.09abc
Peanut shell	EDTA 1mM	38.14±1.31a	36.29±1.37abc	34.91±1.00ab
	EDTA 5mM	33.50±0.88abc	25.32±1.31gh	28.18±1.02c-f
	CA 1mM	33.25±1.46abc	32.68±0.87bcd	30.55±0.79b-f
	CA 5mM	36.94±1.58ab	28.92±0.60d-g	32.39±1.12bcd
Rice husk	EDTA 1mM	32.19±1.49abc	35.39±0.95bc	30.39±0.82b-f
	EDTA 5mM	29.46±0.59cd	31.44±0.33c-f	33.90±0.43abc
	CA 1mM	28.76±0.97cd	37.13±1.52ab	30.55±0.48b-f
	CA 5mM	34.99±2.15abc	40.83±0.80a	39.27±2.11a
<i>S. fruticosa</i>	EDTA 1mM	23.71±0.51d	28.11±0.85d-g	24.82±1.18f
	EDTA 5mM	30.46±1.76bcd	22.68±0.97h	26.18±0.54ef
	CA 1mM	28.62±1.25cd	26.43±1.31fgh	26.76±1.24def
	CA 5mM	32.99±1.15abc	27.22±0.57e-h	32.15±0.55bcd
Critical value for comparison		7.040	5.127	5.273

Means sharing similar letters in a column are statistically non-significant (PP>0.05)

Plant Growth Parameters

To evaluate the impact of heavy metal stress on plant development, *A. tenuifolius* was grown under cadmium (Cd), chromium (Cr), and lead (Pb) exposure. Key growth parameters including root length, shoot length, total plant height, fresh and dry biomass of shoots and whole plants were recorded. Chelating agents citric acid (CA) and ethylenediaminetetraacetic acid (EDTA) were applied at concentrations of 1 mM and 5 mM to soils amended with various biochars. The data in tables 3-7 showing the means of various plant traits under diverse stress conditions. indicated that the highest shoot length under Cd, Cr and Pb were observed (35.38 ± 0.73) in peanut shell 5mM treatment, (35.43 ± 1.16) in rice husk 5mM treatment and (35.55 ± 1.07) rice husk 5mM treatment respectively. Root length also peaked under the same EDTA 5 mM treatment in *A. tenuifolius*. Highest root length (4.22 ± 0.16) was observed in Cd stress while treated with peanut shell CA5mM treatment. Highest No of leaves (8.33 ± 0.33) were recorded under Cd stress while using the peanut shell as biochar at EDTA 1mM. Total plant height, fresh shoot and plant weight, and dry plant weight were recorded under chromium stress when EDTA 5 mM was applied in combination with rice husk biochar. Similarly, in lead-stressed conditions, peanut shell biochar in combination with EDTA 5 mM resulted in the best overall growth parameters, except for root length, which again was maximized under EDTA 5 mM with *A. tenuifolius*. Maximum fresh plant weight ($1,677 \pm 64.58$) was noted under Cd stress in *A. tenuifolius* during level CA 5mM. Further it was observed that dry plant weight (122.21 ± 6.56) was also maximum under same stress and similar treatment conditions. Highest chlorophyll contents were recorded (40.83 ± 0.80) in rice husk CA 5mM treatment during the Cr stress as shown in Table. 7.

DISCUSSION

Phytoextraction is an emerging approach where plants are utilized to remove heavy metals or pollutants from soil or water through root observation and translocate them in their shoots or leaves. Cost-effective, environment friendly, and visually attractive characteristics are making the phytoextraction technique more popular (Awa and Hadibarata, 2020). However, typically, hyperaccumulations are not very effective due to ineffective absorption of heavy metals from the soil and comparatively low biomass production. The success of phytoextraction is often limited by the bioavailability of metals in the soil. To overcome these issues, chelating-mediated phytoextraction has become a promising alternative.

This method is used to increase the mobility and solubility of heavy metals in soil using natural or synthetic chelating agents (DalCorso *et al.*, 2019). The usage of organic soil additives like biochar has vital role in soil fertility, enhancing plant development and enabling the uptake or immobilization of heavy metals (Wang *et al.*, 2021). Both chelating agents and biochar have significant role in enhancing plant performance and removal of heavy metals from contaminated soils (Shah and Devere, 2020).

Recently research reports have been designed to investigate the mechanism of elimination of three heavy metals, such as Cd, Cr, and Pb, and improve the growth of *A. esculentus*. It was observed that the pyrolysis of biomass managed a significant decrease in mass due to the loss of volatile components and moisture (Gai *et al.*, 2004). In comparison to their original feedstock, biochar possesses lower moisture content, higher bulk density, and ash content. Our research also demonstrated that ash content varied significantly based on the type of biomass and pyrolysis temperature. Our results were aligned with the findings of Muigai *et al.* (2021) and Sun *et al.* (2017). Additionally, depending on the kind of feedstock, the biochars showed a range of pH values, primarily trending toward alkalinity after pyrolysis. This pattern is consistent with earlier studies by Spokas *et al.* (2012) and Pokharel *et al.* (2020). The results of Lin *et al.* (2021) were supported by the biochars' generally lower electrical conductivity (EC) values when compared to their raw feedstocks.

When compared to untreated control, plant growth performance was considerably better in soil modified with chelating agents and charcoal. All altered treatments showed an increase in chlorophyll content, which suggests that the plants are healthier, more robust, and produce more biomass. Similar results by Agegnechu *et al.* (2016) support this. Nevertheless, certain indications of metal toxicity persisted in spite of the generally better growth, most likely as a result of excessive nutrient or metal buildup that exceeded safe thresholds (Vida *et al.*, 2020).

Biochar showed a significant ability to absorb heavy metals, with the type, concentration, and rate of application all affecting the degree of removal. These results are consistent with those of Komkiene and Baltreinaite (2016), who found that biochars made from pine and birch effectively reduced heavy metals. The current work also demonstrated that using chelating agents and biochar together greatly enhanced metal uptake in a synergistic way, confirming previous findings by Munir *et al.* (2020).

A. esculentus can effectively transfer absorbed metals from roots to shoots, as evidenced by the fact that the translocation factor (TF), which is the ratio of metal concentration in shoot tissues to that in roots, was greater than one for all tested metals (Rezapour *et al.*, 2019; Yap *et al.*, 2022). This lends credence to the plant's potential as a phytoextraction candidate. These results are consistent with those of Ng *et al.* (2016), Wajid *et al.* (2020), and Ismail *et al.* (2019).

However, because marijuana experiments may understate the effectiveness of phytoextraction in the actual world, the paper admits that field-based assessments could offer more thorough insights. The phytoremediation process is further complicated by the fact that several biotic and abiotic factors, such as microbial activity, soil composition, climate, and plant genotype, affect the dynamics of metal uptake and accumulation in plants.

CONCLUSION

It is widely acknowledged that heavy metals are dangerous environmental contaminants that endanger human health and ecological integrity. In comparison to untreated controls, the current work shows that the application of biochar and chelating agents together greatly increases the absorption and translocation of heavy metals in *A. esculentus* L. The potential of this strategy for successful phytoextraction is highlighted by the synergistic interaction that enhances metal absorption from contaminated soils and promotes their transport throughout plant tissues. Future studies should look into evaluating the antioxidant capabilities of a wider variety of biochar types and hyperaccumulator species. However, it is acknowledged that results from controlled pot experiments may not fully represent field conditions. Real-world applications would require field trials, as the dynamics of metal uptake are influenced by numerous biotic and abiotic factors, making phytoremediation a complex yet promising solution for soil decontamination.

AUTHOR CONTRIBUTIONS

GEZ and FEB: Writing – original draft, Writing – review & editing, Formal analysis. MFKP, SZ, and SO: Validation, Resources, Supervision. FB and HMA: Writing – review & editing. MFKP, SZ, and SO: Writing – review & data analysis. FB and HMA: Validation, Visualization. GEZ and FEB: Conceptualization, Methodology, Supervision, Resources, Validation.

CONFLICT OF INTEREST

Conflicts of Interest: Author declares no conflict of interest.

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