



Amendment of biochar and thiourea-modified biochar to mitigate chromium bioavailability and toxicity by modulating oxidative stress system in *Vigna radiata* in chromium-contaminated agriculture soil

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Abstract

Chromium (Cr) contamination in agricultural soil poses a significant threat to crop productivity and human health. However, strategies to reduce Cr bioavailability and transfer within the soil–plant system remain limited. The sole application of biochar or Thiourea has been reported to mitigate Cr-induced toxicity in plants. However, the combined application of biochar and thiourea for alleviating Cr toxicity in plants and inhibiting Cr mobility in soil has not yet been reported. Therefore, this study examines the impact of biochar and thiourea biochar amendments on soil fertility and Cr stress tolerance in *Vigna radiata*. Biochar, derived from corn husk, was characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray Spectroscopy (EDS). Physicochemical properties, including pH (9.62 ± 0.109), salinity (0.91 ± 0.005 ppt), and EC (0.859 ± 0.021 mS/cm), were higher in thiourea biochar. FTIR revealed new peaks at 617.9 cm^{-1} and 1110.5 cm^{-1} in thiourea biochar, indicating successful thiourea modification. XRD and SEM analyses confirmed structural changes and increased porosity in thiourea biochar. In this study, a pot experiment was conducted in a completely randomized design with three replications (control, 25 mg kg⁻¹ of Cr contamination soil, 25 mg kg⁻¹ of Cr soil with 5% of biochar, and 25 mg kg⁻¹ of Cr-contaminated soil with 5% of thiourea biochar) to study the mitigating effect of thiourea biochar on *V. radiata* growth and stabilization of Cr bioavailability. A randomized pot experiment showed that Cr (25 mg/kg) treatment alone reduced seed germination and plant growth, while thiourea biochar significantly enhanced seed germination (86.0%), shoot length (8.7 ± 0.173 cm), root length (4.01 ± 0.031 cm), and biomass (0.314 ± 0.002 g and 0.386 ± 0.001 g). Cr accumulation in *V. radiata* roots and shoots decreased significantly with thiourea biochar, and Cr translocation from root to shoot was reduced by 35.5%. Photosynthetic pigment content decreased by 20.0% under Cr stress and increased by 123.8% with thiourea biochar addition. Antioxidative enzyme activities catalase (CAT), peroxidase (POX), and superoxide (SOD) dismutase decreased under Cr stress but were significantly enhanced with thiourea biochar. Thus, thiourea biochar amendment in Cr-contaminated soil is an efficient strategy to promote plant growth, reduce Cr bioavailability, and thereby protect the ecosystem.

Keywords Antioxidative enzymes · Chromium stress · Detoxification mechanism · Soil fertility · Thiourea · *Vigna radiata* growth

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Introduction

The contamination of hazardous heavy metals (HHMs) in agricultural land is a foremost global concern. Flaming of industrial runoff, mining activities, domestic waste, and more uses of agrochemicals are the main sources for HHMs accumulation in soil (Saleem et al. 2024). Among the HHMs, Cr is considered a highly toxic metal in the environment because of its properties: persistence, non-biodegradability, nonessentiality, carcinogenicity, loss of soil fertility, high mobility, and easy bioaccumulation in the food chain (Gholami et al. 2020). The leftover accumulation of Cr in agricultural soil poses severe problems in crop plants, such as inhibition of nutrient uptake, photosynthesis, growth, and decrease of agricultural crop yield (Ali et al. 2023). Furthermore, Cr accumulation in crop plants enriches the formation of reactive oxygen species (ROS), such as hydroxyl radical ($\text{OH}^\bullet$), superoxide radical ($\text{O}_2^\bullet$), and hydrogen peroxide (H_2O_2) (Rajendran et al. 2019a). Nevertheless, plants have efficient antioxidant defence systems, including SOD, CAT, ascorbate peroxidase (APX), and POX for scavenging ROS under Cr-induced stress (Haider et al. 2022; Rajendran et al. 2019b). On the other hand, HHMs accumulated in plants are the primary sources of human exposure to the food chain (Haider et al. 2022) and cause several health risks (Bandara et al. 2017; Manikandan et al. 2016). Therefore, there is an urgent need to develop a technique to remedy the Cr contamination and stabilization of Cr bioavailability in the rhizosphere to limit its uptake by crop plants (Wang et al. 2019). The stabilization of HHMs in farmland is vital for ensuring food chain safety.

Recently, various remediation techniques, including electrochemical (Figuerola et al. 2016), soil flushing (Reddy et al. 2011), and chemical immobilization (Tajudin et al. 2016), have been formulated to limit the bioavailability of HHMs in farmlands. However, these techniques are unsuitable for agricultural land, creating secondary pollution and expensive techniques (Cang et al. 2012). Among these techniques, the in situ stabilization technique with biowaste-modified non-hazard materials has great potential to limit the HHMs bioavailability and sequential accumulation in crop plants (Saleem et al. 2024). In recent times, organic biochar or biochar-modified amendments have been a good choice for stabilizing Cr in agricultural soil (Cui et al. 2016). Biochar is an organic fine porous material produced by pyrolysis under oxygen-free conditions (Beesley et al. 2014) and improves the soil fertility, water-holding capacity, and microbial population, limits the loss of nutrients, and inhibits the bioavailability of HHMs in soil (Ahmad et al. 2020; Baigorri et al. 2020). Moreover, biochar has different minerals such as oxides,

phosphates (Lu et al. 2012) and various functional groups (carboxylic, hydroxyl, chromone, and ketone) (Kamran et al. 2019) which bind with HHMs and precipitate in soil (Choppala et al. 2016). For example, adding hardwood biochar can inhibit the Cr, Cu, Pb, and Mn mobility and bioavailability in contaminated soil and uptake by *Oryza sativa* (Khan et al. 2020). Similarly, biochar supplementation can enhance the maize plant growth, biomass, and photosynthetic pigments and reduce the Cr accumulation in plant tissues by limiting the bioavailability of Cr in soil (Bashir et al. 2021). On the other hand, compared to biochar alone, the application of inorganic-modified biochar amendments may be a better choice for reducing Cr bioaccumulation in plants and improving soil fertility (Cui et al. 2016). Therefore, some recent research reported that the inorganic modified biochar supplementation effectively improves HHMs mobility and bioavailability in soil (Guo et al. 2018). For instance, the thiourea-modified biochar effectively immobilized the Cd, Cu, Cr, Pb, Ni, and Zn in Gadap town and Malir cantonment contaminated soil. It enhanced the mustard plant growth and biomass (Saleem et al. 2024). Furthermore, biochar with thiourea addition has improved maize growth in Cd-polluted soil by decreasing Cd in shoots and roots (Haider et al. 2022). Thiourea is a growth-promoting hormone that is majorly involved in plant biochemical changes and enhances metabolism (Haider et al. 2022; Perveen et al. 2015). Meanwhile, the effects of biochar and thiourea alone or integrated biochar with thiourea have been reported to reduce the HHMs bioavailability and increase plant growth. However, the addition of biochar or thiourea alone has alleviated the toxic heavy metals bioavailability and increased the plant growth and productivity. However, no study report is available on thiourea biochar application on Cr mobility and bioavailability in agricultural soil and plant growth and physiological and biochemical changes. Therefore, the present study aimed to investigate the effect of biochar alone and thiourea biochar supplementation on mitigating the Cr mobility, bioavailability, and uptake in *Vigna radiata* by evaluating the seed germination, plant growth, biomass, and antioxidative enzyme activities in *V. radiata* grown in Cr-contaminated soil.

Materials and methods

Soil sample collection and physicochemical analysis

The soil sample was collected from agricultural land near Periyar University, Salem, Tamil Nadu, India. The collected soil sample was air dried and sieved by 20-mesh for the pot experiment, and physicochemical properties such as pH, electrical conductivity (EC), salinity, available phosphorus,

and nitrogen contents were determined (Reeuwijk et al. 1995) and APHA et al. (1999) adapted methods. In addition, Cr and Pb concentrations were determined by an atomic absorption spectrophotometer (AAS), and the results are presented in Table 1.

Biochar and thiourea-modified biochar preparation and characterization

The corn husk was collected from the local market in Salem, Tamil Nadu, India, and dried at 70 °C for 48 h. The dried corn husk sample was cut into small pieces and pyrolyzed at 500 °C for 3 h under an oxygen-free environment using a muffle furnace. The thiourea-modified biochar was prepared using the following method (Gholami et al. 2020). Briefly, 2 g of corn husk biochar was added into 50 ml of 1 N thiourea solution and mixed by magnetic stirring for 12 h at 40 °C. After that, the mixture was filtered and oven-dried at 45 °C to obtain thiourea biochar. The prepared biochar and thiourea biochar surface functional group was determined by FTIR, crystal nature was measured by XRD, and morphology and elements were observed using SEM with EDX. Additionally, developed biochar and thiourea biochar physicochemical properties were analyzed.

Experiment design

The pot experiment was studied in the greenhouse at Periyar University. The soil used in this study was collected from agricultural land near Periyar University, Salem, Tamil Nadu, India. The collected soil sample physicochemical properties were analyzed and presented in Table.

1. The soil sample was air-dried and sieved through 5-mm mesh, and Cr-contaminated soil was prepared by adding $K_2Cr_2O_7$ (25 mg kg⁻¹) and then placed in a greenhouse for 20 days. After 25 days of incubation, the Cr-contaminated soil was used for *Vigna radiata* seed germination and growth. This experiment included six treatment groups with

three replicates for each treatment. The treatment groups include (i) control (without the addition of Cr, Biochar and thiourea biochar, only agricultural soil), (ii) 25 mg kg⁻¹ of Cr contamination agricultural soil, (iii) 5% of biochar with agricultural soil, (iv) 25 mg kg⁻¹ of Cr contamination agricultural soil with 5% of biochar, (v) 5% of thiourea biochar with agricultural soil, and (vi) 25 mg kg⁻¹ of Cr contamination agricultural soil with 5% of thiourea biochar. Each treatment contains 1 kg of agricultural soil, and proper biochar and thiourea biochar were supplemented manually according to the respective treatments. Prior to sowing, a basal dose of NPK (20:20:20) fertilizer was uniformly applied at a rate of 50 mg/kg soil to all treatments to ensure standard nutrient availability across all pots. Based on this preliminary study, 5% biochar and 5% thiourea biochar showed the best results on the fixation of Cr-contaminated agricultural soil. *Vigna radiata* seeds were washed in distilled water, sterilized with 3% sodium hypochlorite, and washed with sterile distilled water. Then, seven seeds were sown to germinate in each treatment. During this experiment, the soil was irrigated with deionized water to maintain its moisture content. After five days of treatment, seed germination was calculated as follows (Scott et al. 1984):

$$\text{Seed germination \%} = \frac{\text{Total Number of seeds germinated}}{\text{Total seed sown in soil}} \times 100 \quad (1)$$

Determination of plant length, biomass, and Cr accumulation

After 30 days of treatment, *V. radiata* plants were harvested and washed with distilled water. The shoot and root were then separated for further analysis. After harvesting, the shoot and root lengths were measured immediately. Shoots and roots fresh weight was measured and dried at 65 °C for 48 h in a hot air oven for dry weight measurement and biomass production (Manikandan et al. 2016). Moreover, 0.5

Table 1 Physicochemical properties of soil, biochar, and thiourea biochar used in the present study

Physicochemical parameters	Soil samples	Before treatment		After treatment	
		Biochar	Thiourea biochar	Biochar	Thiourea biochar
pH	7.4 ± 0.151 ^c	8.83 ± 0.033 ^b	9.62 ± 0.109 ^a	8.00 ± 0.033 ^a	8.60 ± 0.057 ^a
EC (mS/cm)	2.32 ± 0.037 ^a	0.677 ± 0.028 ^b	0.859 ± 0.021	2.183 ± 0.028 ^{ab}	2.636 ± 0.038 ^a
Salinity (ppt)	0.208 ± 0.006 ^b	0.787 ± 0.029 ^a	0.91 ± 0.005 ^c	0.987 ± 0.029 ^a	0.261 ± 0.001 ^b
Nitrogen (kg/hectare)	1.22 ± 0.323 ^c	1.96 ± 0.000 ^b	3.74 ± 0.671 ^a	0.85 ± 0.008 ^b	1.38 ± 0.003 ^c
Phosphorus (kg/hectare)	0.75 ± 0.000 ^c	5.20 ± 0.849 ^a	4.71 ± 0.593 ^b	10.33 ± 1.002 ^a	8.94 ± 0.906 ^b
Cr (mg/kg)	0.054 ± 0.003 ^a	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Pb (mg/kg)	1.2 ± 0.036 ^a	0.03 ± 0.000 ^b	0.01 ± 0.000 ^c	0.014 ± 0.001 ^b	0.005 ± 0.000 ^c

Mean ± SE (*n* = 3). The different letters after the value within a row represent significant differences at *P* < 0.05

EC electrical conductivity, Cr chromium, Pb lead

g of dried shoot and root tissues were ground and digested in 10 ml of HCl and HNO₃ (7:3 v/v) at 70 to 90 °C until a clear solution was obtained. After digestion, the suspensions were filtered with Whatman No. 1 filter paper and a 20 ml solution with sterile distilled water to determine Cr accumulation in shoot and root tissues by atomic absorption spectrometry (Shimadzu, AA-7000F). On the other hand, the internal transfer of Cr in *V. radiata* plant tissues was determined by translocation factor (TF), which is an index of the Cr transfer ratio in root-to-shoot tissues of *V. radiata* (Das and Maiti 2007).

$$\text{TF (\%)} = \frac{\text{Cr accumulation in shoot tissue}}{\text{Cr accumulation in root tissue}} \times 100 \quad (2)$$

Determination of photosynthetic pigments content

The photosynthetic pigments, namely, chlorophyll a, b, total chlorophyll, and carotenoid contents, were determined by the method (Arnon 1949). After treatment, fresh leaves were collected from each treatment and homogenized in 2 ml of 80% (v/v) ice-cold acetone. The supernatant was transferred into a sterile microcentrifuge tube (2 ml) and spun at 6000 rpm for 5 min. After the spun supernatant was collected and determined, the chlorophyll a and b, total chlorophyll, and carotenoid contents were measured at different wavelengths: 663, 645, and 470 nm. The determined photosynthetic pigment content was calculated by following the formula (Venkatachalam et al. 2017) and expressed as mg g⁻¹ FW.

$$\text{Chl a} = 12.25 \times A_{663} - 2.79 \times A_{645} \quad (3)$$

$$\text{Chl b} = 21.50 \times A_{645} - 5.10 \times A_{663} \quad (4)$$

$$\text{Chl a} + \text{b} = 7.15 \times A_{663} - 18.71 \times A_{645} \quad (5)$$

$$\text{Car} = (1000 \times A_{470} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}) / 198 \quad (6)$$

Assay of antioxidative enzyme activities

After the treatment, fresh leaves were collected and homogenized with 2 ml of 50 mM phosphate buffer in a chilled mortar and pestle and spun at 8000 rpm for 10 min. After centrifugation, supernatants were collected and used as enzyme extract for antioxidative enzyme activity. CAT activity was determined by the (Aebi 1984) modified method. The 3 ml reaction mixture contained phosphate buffer (50 mM, pH-7.8), H₂O₂ (75 mM), distilled water, and enzyme extract. The CAT activity was started by adding H₂O₂ (75 mM), and absorbance was noted at 240 nm

using a UV–visible spectrophotometer. POX activity was assayed according to the method of (Castillo et al. 1984). The reaction mixture of the POX assay consisted of phosphate buffer (50, pH-6.1), 16 mM of guaiacol, 2 mM of H₂O₂, enzyme extract, and distilled water. The guaiacol oxidation was determined at 470 nm using a UV–visible spectrophotometer. Furthermore, SOD activity was measured by (Chauhan et al. 2022) proposed method. According to this method, the reaction mixture contained phosphate buffer (50 mM, pH 7.8), methionine (13.33 mM), nitroblue tetrazolium (2.25 mM), EDTA (0.1 mM), Na₂CO₃ (50 mM), riboflavin (60 mM), distilled water, and enzyme extract. The reduction of nitroblue tetrazolium was absorbed at 560 nm by a UV–visible spectrophotometer. Each enzyme activity was expressed as U mg⁻¹ FW.

Determination of lipid peroxidation, H₂O₂, and total soluble protein content

The lipid peroxidation level was expressed as malondialdehyde (MDA) content according to (Frankic and Hershner 2003) described method. The MDA concentration was calculated by (Michael and Krishnaswamy 2011) proposed formula.

$$\text{MDA } (\mu\text{mol/g}) = (6.4 \times (A_{532} - A_{600}) - (0.56 \times A_{450})) \times Vt/W \quad (7)$$

where $Vt = 0.002$ L and $W = 0.2$ g.

In addition, the H₂O₂ level was measured by (Sergiev et al. 1997) modified method, and the content was expressed as nmol g⁻¹ FW. Furthermore, the total soluble protein concentration was determined by (Bradford 1976) modified method and content was expressed in mg kg⁻¹ FW.

Statistical analysis

A one-way ANOVA was performed using GraphPad Prism 5 software to determine the statistical significance among treatments. The Student–Newman–Keuls (SNK) test was used as a post hoc comparison at a 5% significance level ($P < 0.05$). Prior to analysis, normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was verified using Levene's test via Past statistical software. In addition, Pearson's correlation and principal component analysis were performed using past statistical software. Pearson's correlation analysis was used to study the direct and indirect relationship between the effects of biochar and thiourea biochar on plant growth, biomass, Cr accumulation, photosynthetic pigment content, antioxidative enzymes activity, MDA, HO, and total soluble protein content in *Vigna radiata* under the Cr treatment.

Results and discussion

To enhance clarity and facilitate comparison, treatment effects in the following sections are presented both as absolute values (mean $\pm$ SD) and as percentage changes relative to the Cr-only treatment.

Characterization of biochar and thiourea biochar

The basic physicochemical properties of biochar and thiourea biochar are shown in Table 1. The pH value of thiourea biochar (9.62 ± 0.109) was found to be higher than biochar (8.83 ± 0.033). This is due to the presence of some amine functional groups in thiourea, which increased the alkalinity on the thiourea-modified biochar surface (Tang et al. 2018). Similarly, the salinity (0.91 ± 0.005 ppt) and EC (0.859 ± 0.021 mS/cm) values of thiourea biochar were found to be higher than those of biochar (0.787 ± 0.029 ppt and 0.677 ± 0.028 mS/cm). These results indicated the presence of hydroxyl, carboxyl, and thiol active functional groups on the thiourea biochar surface, which may increase the EC and salinity values in thiourea biochar (Ding et al. 2016). The available nitrogen contents are also higher in thiourea biochar compared to biochar (Table 1). This may be attributed to thiourea's ability to produce nitrogen and sulfur elements (Xu et al. 2021). Meanwhile, the phosphorus value in biochar was found to be higher than that of thiourea biochar. On the other hand, Cr and Pb concentrations were not detected in biochar and thiourea biochar. Similarly, the higher levels of pH, EC, and other physiochemical properties of thiourea-modified biochar compared to carrot pulp biochar (Gholami et al. 2020). The present study performed FTIR analysis to identify the functional groups in biochar and thiourea biochar (Fig. 1). The peaks 3425.0 cm^{-1} ,

2924.8 cm^{-1} , and 1640.6 cm^{-1} are represented the stretching vibrations of hydroxyl (-OH) (Goswami et al. 2016), methylene (C-H) (Liu et al. 2018), and C=C groups (Xu et al. 2021) shown in biochar. However, new peaks 617.9 cm^{-1} and 1110.5 cm^{-1} corresponded to the stretching vibrations of -C-S and -C=S (thiocarbonyl) groups (Goswami et al. 2016; Wu et al. 2019) observed in thiourea biochar. Nevertheless, these new peaks were not found in biochar. In this study, thiourea biochar has nitrogen, oxygen, and sulfur-containing functional groups compared to biochar, which indicates that thiourea can increase the active functional groups on the surface of biochar to stabilize HHMs in soil. The specific surface area (SSA) of the biochars was analyzed using BET analysis. Thiourea biochar exhibited a surface area of $35.6\text{ m}^2/\text{g}$, compared to $21.8\text{ m}^2/\text{g}$ for unmodified biochar. Chromium adsorption capacity was measured via batch equilibrium studies, revealing a higher maximum adsorption capacity (q_{max}) of 31.2 mg/g for thiourea biochar versus 18.4 mg/g for biochar (Du et al. 2023).

Figure 2 revealed the crystal structure of biochar and thiourea biochar, which were determined by XRD analysis. The peaks 23.4° and 42.8° correspond to (002) and (100) planes of amorphous carbon and carbons (Ding et al. 2020) shown in biochar (Fig. 2B). On the other hand, the thiourea-modified biochar (thiourea biochar) showed some peaks located at 25.3° , 44.2° , 59.0° , and 76.1° that could correspond to (002), (100), (200) and (203) planes of graphite structure (Fig. 2A), which indicates that thiourea was successfully loaded onto biochar (McMurdie et al. 1986; Yu et al. 2020).

The biochar and thiourea biochar morphological characterization was examined by using SEM, and element content was determined by element mapping with EDS. Figure 3 shows the SEM images and EDS element mapping of biochar and thiourea biochar. The biochar surface morphology was shown to be smooth, but thiourea biochar morphology

Fig. 1 FTIR spectra of corn husk biochar and thiourea biochar

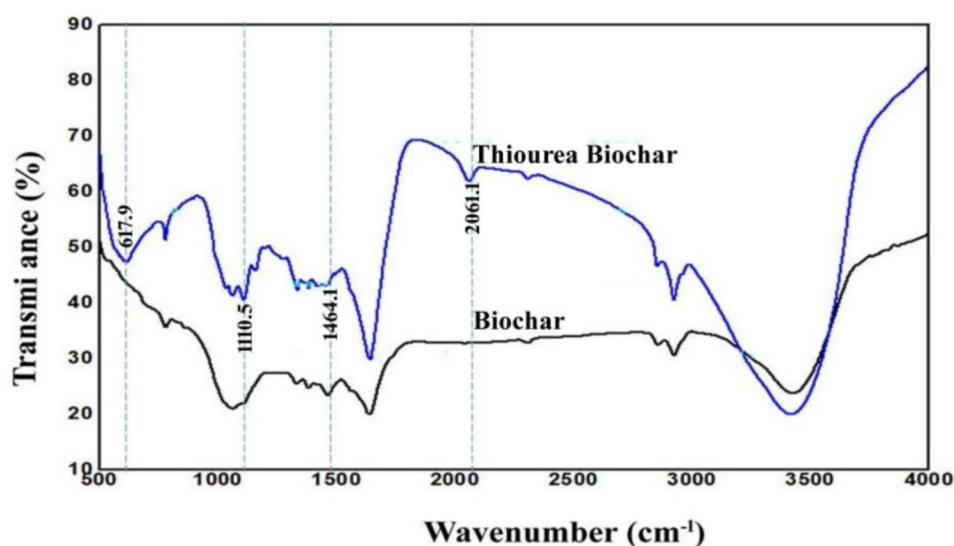
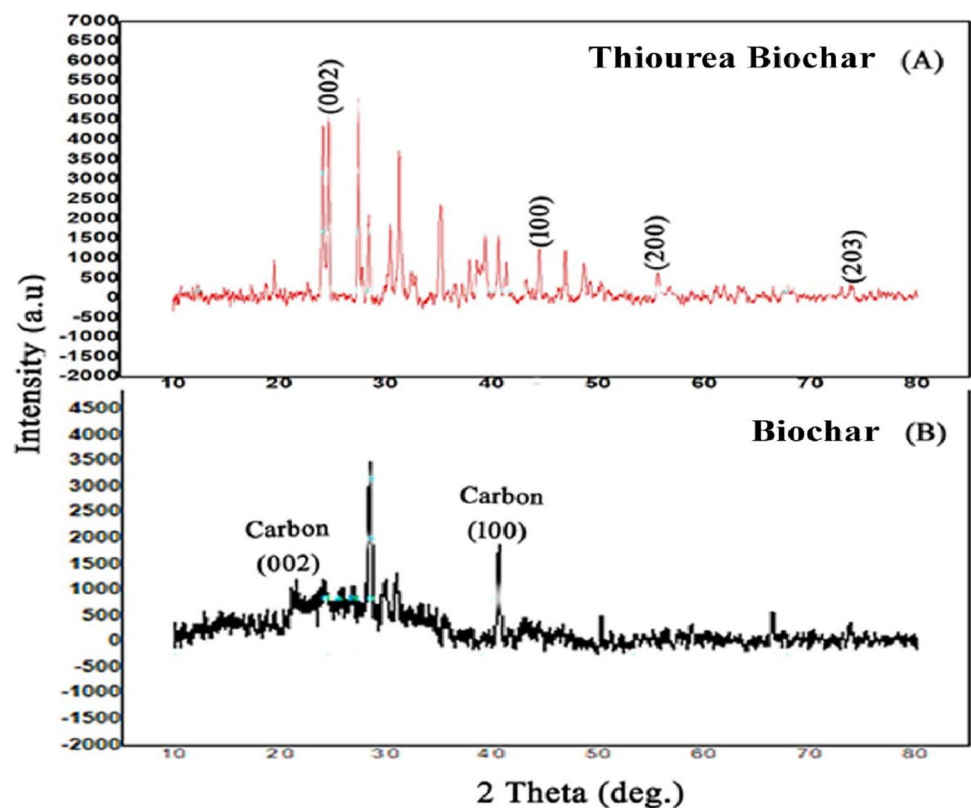


Fig. 2 XRD spectra of thiourea biochar (A) and biochar (B)



was seen as a rugged surface with rich porous structures. This result suggested that the presence of porous structures on carbon particles of thiourea biochar enhanced more binding sites for heavy metals (Liu et al. 2018). Moreover, by EDS spectrum analysis, it was seen that biochar and thiourea biochar contain carbon, oxygen, potassium, iron, and chloride elements (Fig. 3C, D). However, it was shown that the sulfur element was successfully doped in thiourea biochar (Fig. 3D). Similar results were reported by Xu et al. (2021), where the S and N elements were successfully doped in S-BC.

Effects of biochar and thiourea biochar on soil physicochemical properties

Variations in the physicochemical properties of soil after incubation with biochar and thiourea biochar are presented in Table 1. During the treatment period, soil physicochemical properties slightly changed after the addition of biochar and thiourea biochar. The amendment of thiourea biochar in soil significantly increased the pH, EC, and N values (Table 1) when compared to the control and biochar-added soil. These results indicate the increased soil pH levels could enhance the competition for heavy metals and nutrients in the rhizosphere of plants for immobilization of heavy metals in soil (Chen et al. 2024b). Similarly, available N content was also found to be higher in thiourea biochar amendment

soil than in control and biochar-added soil (Table 1). Xu et al. (2021) reported that thiourea can provide S and N elements in soil, which may increase soil N content (Chen et al. 2024a). Post-treatment Cr concentrations in soil were assessed. The Cr-only treatment maintained a concentration of 25 mg/kg, while biochar and thiourea biochar reduced these levels to 13.4 mg/kg and 9.6 mg/kg, respectively, demonstrating their remediation potential (Saleem et al. 2024).

Effects of biochar and thiourea biochar on seed germination and plant growth

Generally, plant growth and metabolism were affected when grown in Cr-contaminated agricultural soil. To reduce the Cr-induced toxicity in plants, there is an urgent need to control the Cr uptake and accumulation (Ullah et al. 2023). In the present study, *V. radiata* seed germination, growth, and biomass were impaired under Cr treatment (Fig. 4). Thiourea biochar significantly enhanced seed germination by 86.0%, representing a 61.1% increase over Cr treatment alone. Shoot length improved by 72.5%, root length by 84.3%, and biomass by 76.8% in thiourea biochar + Cr treatment compared to Cr-only. The present study results indicated that the amendment of biochar and thiourea biochar, along with Cr contamination, showed positive effects (Ghandali et al. 2024) on seed germination, growth, and biomass of *V. radiata* plants by mitigating the Cr mobility

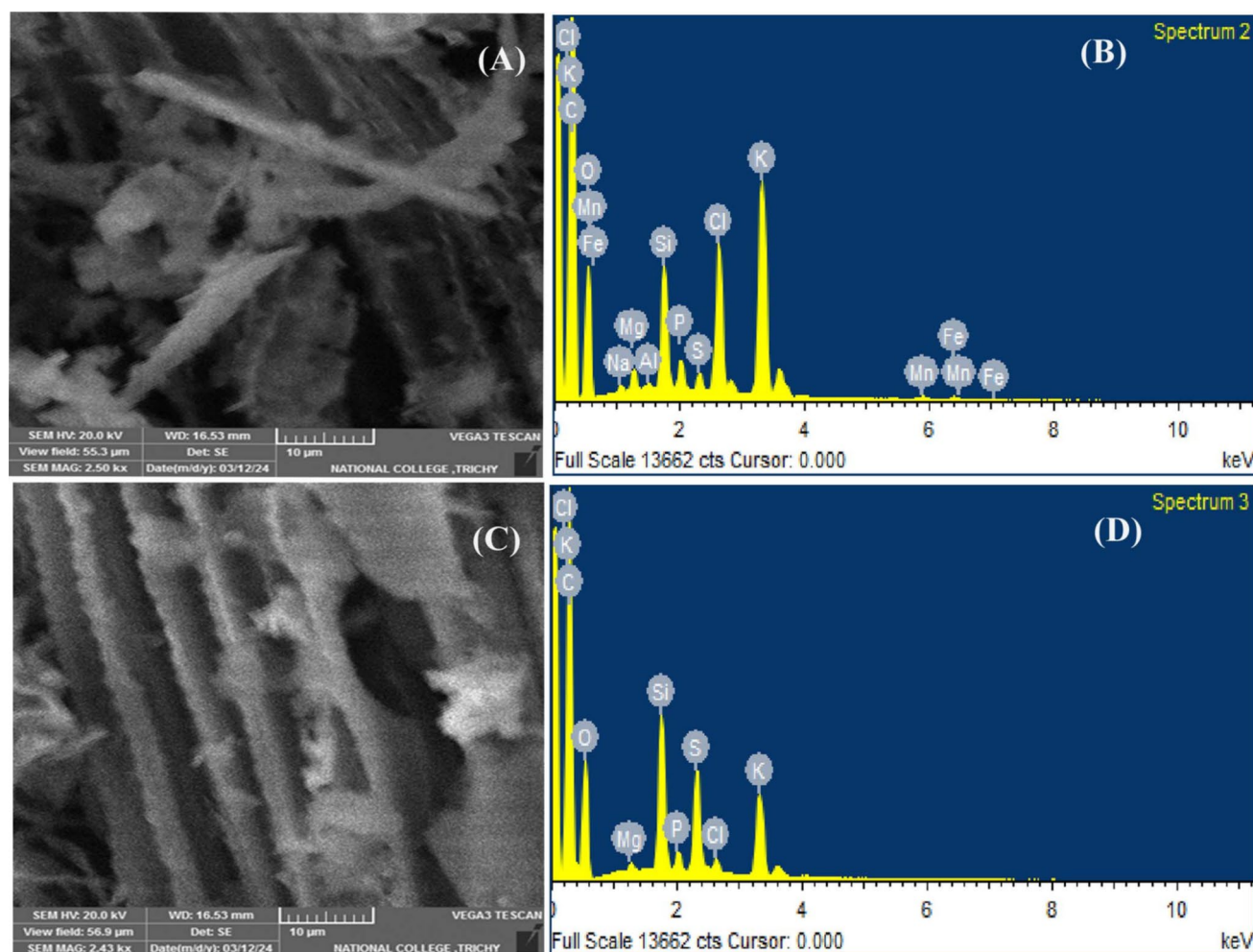


Fig. 3 SEM images and EDX maps of biochar (A, B) and thiourea biochar (C, D)

and bioavailability in soil. Moreover, thiourea and biochar addition could increase the growth and biomass of maize plants in Cd-contaminated soils.

Effects of biochar and thiourea biochar on Cr accumulation

The maximum concentration of Cr accumulation notices was 203.1 ± 1.086 and 97.42 ± 0.952 $\mu\text{g/g}$ DW for roots and shoot tissues, respectively, at Cr alone treatment (Table 2). On the other hand, Cr accumulation in root and shoot was decreased by 124.0 ± 0.055 and 50.13 ± 0.00 $\mu\text{g g}^{-1}$ DW with biochar amendments and by 94.8 ± 0.031 and 33.71 ± 0.053 $\mu\text{g g}^{-1}$ DW with thiourea biochar addition, respectively, in Cr-contaminated soil (Table 2). Concomitantly, the thiourea biochar amendment significantly reduced the Cr accumulation in *V. radiata* plants in Cr-contaminated soil. Moreover, the translocation of the Cr concentration root to shoot increased by

48.0% at the Cr alone treatment. However, it was maximum decreased (Mng'ong'o et al. 2023) by 35.5% after supplementation of thiourea biochar in Cr-contaminated soil (Table 2). The lower accumulation of Cr in shoot and root tissues of *V. radiata* plant under the amendment of thiourea biochar can be attributed to the limit of the Cr mobility, bioavailability, and uptake in Cr-contaminated soil (Ceballos et al. 2023). The superior performance of thiourea biochar in reducing chromium bioavailability is primarily due to its enhanced surface chemistry and structural properties. FTIR spectral analysis revealed distinct peaks at 617.9 cm^{-1} and 1110.5 cm^{-1} , corresponding to thiol (-SH) and thiocarbonyl (-C=S) functional groups introduced by thiourea modification. These sulfur- and nitrogen-containing groups, along with amine (-NH₂) moieties, exhibit strong affinity for heavy metal ions such as Cr through complexation, chelation, and electrostatic interactions. These functional groups provide multiple

Fig. 4 Effects of Biochar and Thiourea Biochar on seed germination (A), shoot length (B), root length (C), and biomass (D) in *V. radiata* L. under the Cr treatment. Mean $\pm$ SD ($n=3$). The different letters above the bars are representing significant difference at $P<0.05$. Cr (chromium). BC, biochar; TU, thiourea

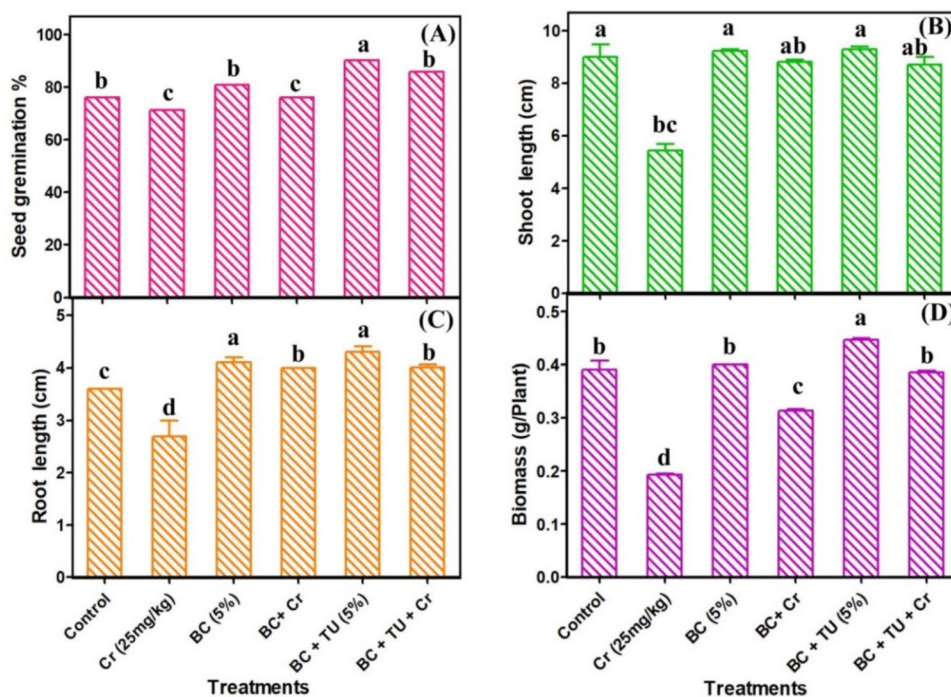


Table 2 Effects of biochar and thiourea biochar on Cr accumulation in shoot and root and translocation factor in *Vigna radiata* L. under the Cr treatment

Treatments	Shoot Cr (µg/g DW)	Root Cr (µg/g DW)	Translocation factor (%)
Control	BDL	BDL	0
Cr (25 mg/kg)	97.42	203.1	48
Biochar (5%)	BDL	BDL	0
Biochar+Cr	50.13	124	40.4
Thiourea biochar (5%)	BDL	BDL	0
Thiourea biochar+Cr	33.71	94.8	35.5

*BDL below detectable limit

active sites for binding Cr, thereby immobilizing it in the soil matrix. Additionally, SEM imaging confirmed a more porous and irregular surface morphology in the thiourea biochar, which increases the number of physical adsorption sites. BET analysis further supported this by showing a significantly higher specific surface area (35.6 m²/g) compared to unmodified biochar (21.8 m²/g). Together, these chemical and physical enhancements allow thiourea biochar to more effectively capture and retain Cr ions, minimizing their uptake by plants and reducing their mobility in the soil (Yang et al. 2025; Zhu et al. 2020). Likewise, it was similarly reported that Cr content in Mustard plant parts was reduced by 84.49 with the addition

of thiourea-modified biochar in toxic metal-contaminated soils (Saleem et al. 2024).

Effects of biochar and thiourea biochar on photosynthetic pigments

Heavy metal-contaminated soil led to significant photosynthetic pigment levels by chlorosis and inhibition of Hill reaction in plants (Mukherjee et al. 2023). For example, in this study, Chl-content decreased by 20.0% at Cr treatment in the *V. radiata* plant, compared to the control. In comparison to the Cr-only treatment, chlorophyll-a increased by 116.6% and 123.8% with biochar+Cr and thiourea biochar+Cr treatments, respectively. Similarly, chlorophyll-b content rose by 106.4% and 113.0%, and total chlorophyll by 118.3% and 124.5%. Carotenoids improved by 122.1% and 136.0%, respectively (Fig. 5D). This result indicated that the Cr stress was more damaging to *V. radiata* photosynthetic pigments, while biochar and thiourea biochar supplementation (Saud et al. 2022) effectively counteracted the Cr-induced stress in the *V. radiata* plant. In line with present research, additionally photosynthetic pigments were enhanced in *Zea mays* by thiourea supplementation under Cd contamination (Razzaq et al. 2024).

Effects of biochar and thiourea biochar on antioxidative enzyme activity

Heavy metal-induced stress inhibits the plant's growth and development by enhancing ROS at the cellular level (Altaf

Fig. 5 Effects of biochar and thiourea biochar on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) in *V. radiata* L under the Cr treatment. Mean $\pm$ SD ($n=3$). The different letters above the bars are representing significant difference at $P<0.05$. Cr (chromium). BC, biochar; TU, thiourea

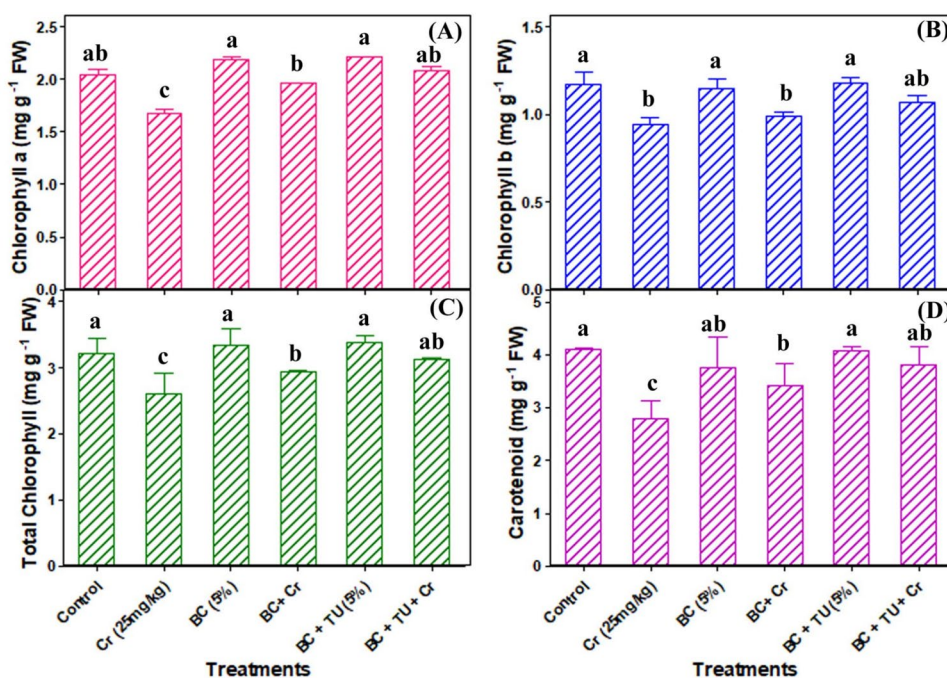


Fig. 6 Effects of Biochar and Thiourea Biochar on anti-oxidative enzyme activities (SOD (A), CAT(B), POX(C), non-anti-oxidative enzyme activities (MDA (D), H₂O₂ (E)) and total soluble protein content (F) in *V. radiata* L under Cr stress

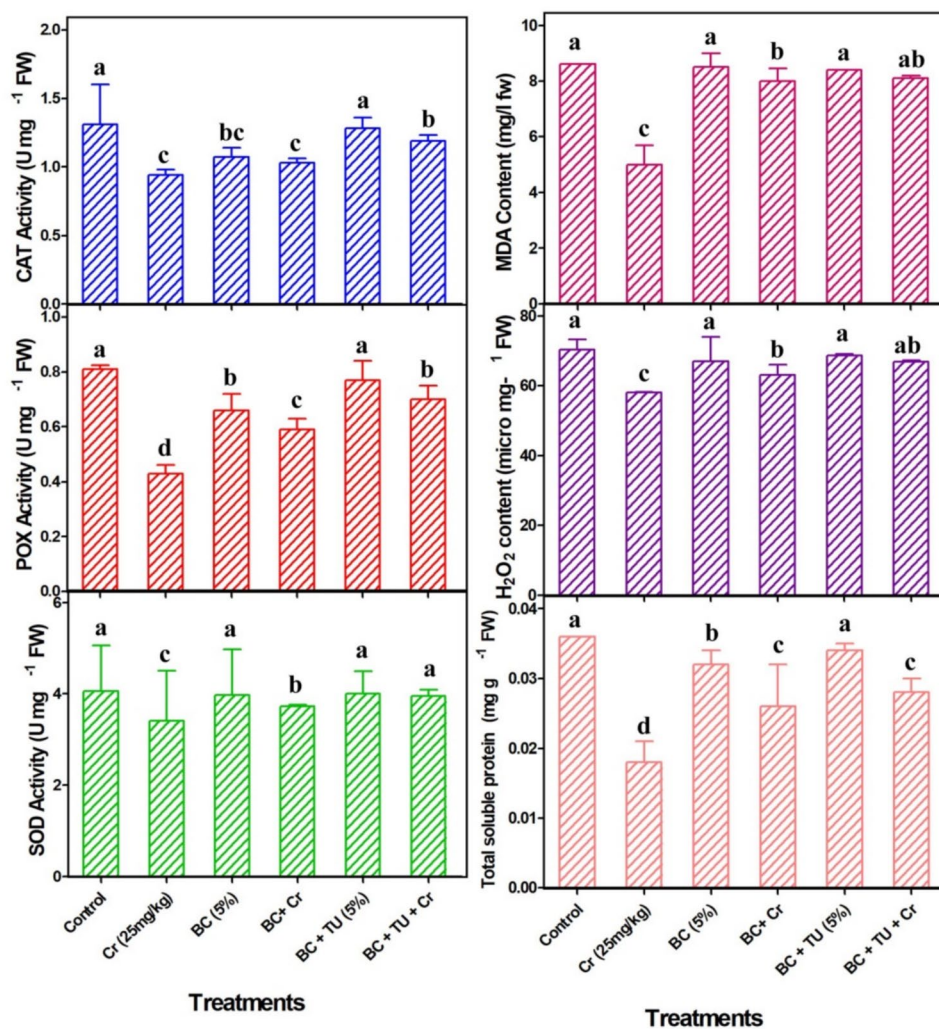


Table 3 Correlation analysis between shoot length (SL), root length (RL), biomass, Cr accumulation in root (Root Cr), Cr accumulation in shoot (Shoot Cr), chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll (Total Chl.), carotenoids (Car.), catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), malondialdehyde (MDA), hydrogen peroxide (H₂O₂), and protein content in *Vigna radiata* L. under Cr stress

	SL	RL	Biomass	Shoot Cr	Root Cr	Chl a	Chl b	Total Chl	Car	CAT	SOD	POX	MDA	H ₂ O ₂	Protein
SL		0.93316	0.92361	− 0.90187	− 0.84475	0.92965	0.77726	0.90451	0.89433	0.67649	0.9124	0.84444	0.9925	0.8654	0.87589
RL			0.8773	− 0.77136	− 0.70296	0.91829	0.6426	0.84643	0.76336	0.52529	0.7782	0.68695	0.8877	0.7021	0.7024
Biomass				− 0.95284	− 0.91846	0.97822	0.91684	0.98208	0.96241	0.81976	0.9618	0.92009	0.9196	0.9384	0.9223
Shoot Cr					0.99121	− 0.93764	− 0.96821	− 0.97816	− 0.95292	− 0.78955	− 0.9634	− 0.91529	− 0.9161	− 0.956	− 0.9805
Root Cr						− 0.90341	− 0.9823	− 0.96183	− 0.92343	− 0.77244	− 0.9277	− 0.8877	− 0.8600	− 0.934	− 0.9738
Chl a							0.87822	0.98427	0.89585	0.68441	0.9181	0.82835	0.9119	0.870	0.8711
Chl b								0.94796	0.93021	0.83605	0.9227	0.90649	0.7980	0.942	0.9590
Total Chl									0.93222	0.75364	0.9432	0.87792	0.8992	0.919	0.9310
Car										0.92138	0.9848	0.99087	0.9175	0.992	0.9716
CAT											0.8605	0.95904	0.7160	0.915	0.8655
SOD												0.96403	0.9402	0.987	0.9602
POX													0.8783	0.987	0.9587
MDA														0.900	0.9026
H ₂ O ₂															0.9782
Protein															

et al. 2023). The present study results have revealed that Cr-induced stress decreased the CAT (28.2%), POX (47.0%), and SOD (16.0%) activities in *V. radiata* when compared to control (Fig. 6A–C). However, biochar and thiourea biochar supplementation played a significant role in decreasing Cr stress. Moreover, CAT, POX, and SOD activities increased by 109.5%, 137.2%, and 109.3% under biochar + Cr treatment and by 126.5%, 162.7%, and 116.0% with thiourea biochar + Cr, respectively, compared to Cr-only (Fig. 6A–C). The increased SOD activity indicated that converting $O_2^{\bullet-}$ into H_2O_2 and O_2 is the first line of the plant defence mechanism against heavy metal-induced oxidative stress (Basit et al. 2023). Further, CAT plays a significant role in converting H_2O_2 into H_2O and improving plant metabolism (Li et al. 2023).

These results showed that the biochar and thiourea biochar supplementation had a positive role under Cr toxicity conditions. Similar results were reported in wheat (*Triticum aestivum*) under cobalt stress with the addition of sulfur-rich thiourea (Ahmad et al. 2024) and in maize plants under moisture stress conditions with thiourea (Zahid et al. 2024).

Effects of biochar and thiourea biochar on MDA, H_2O_2 , and total soluble protein contents

Another important factor in ameliorating Cr toxicity in *V. radiata* is the capacity of biochar and thiourea biochar, in addition to decreasing oxidative stress by improving anti-oxidative activity in plants. Heavy metal toxicity in plants

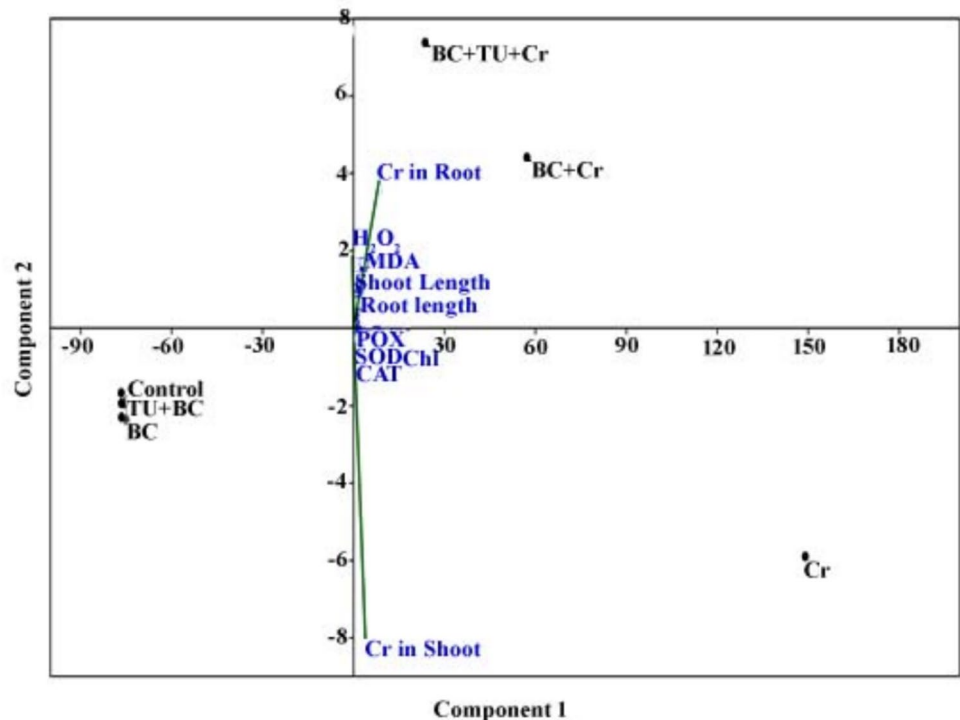
stimulates ROS, MDA, and H_2O_2 , finally leading to oxidative stress. ROS formation, in turn, initiates the antioxidant systems like APX, CAT, SOD, and POX to alleviate H_2O_2 and lipid peroxidation accumulation (Singh et al. 2023).

Compared to Cr-only treatment, the supplementation of biochar increased MDA by 160.0%, H_2O_2 by 108.4%, and protein content by 144.4%. Thiourea biochar further enhanced MDA by 162.0%, H_2O_2 by 115.0%, and protein by 155.5% (Fig. 6D–F). A previous study also reported that sulfur-thiourea application was able to reduce heavy metal toxicity and increase MDA and H_2O_2 levels in *Triticum aestivum* (Zahid et al. 2024). Also, the addition of biochar and thiourea decreases Cd stress and improves MDA and H_2O_2 content in the maize plant (Yasin et al. 2024).

Correlation and principal component analysis of studied parameters

Pearson's correlation analysis showed a positive correlation between plant growth (root and shoot length), photosynthetic pigments (Chl. a, b, total chlorophyll and carotenoids), anti-oxidative enzymes (CAT, SOD, and POX), and non-antioxidative enzymatic activities (Zhang et al. 2021) such as MDA and H_2O_2 (Table 3). This positive correlation indicated the alleviation of Cr-induced toxicity in *V. radiata*. In contrast, Cr accumulation in *V. radiata* was negatively correlated with plant growth, photosynthetic pigments, antioxidative enzyme activities, MDA, and H_2O_2 (Table 3). Moreover, there is a high correlation between maize plant growth and

Fig. 7 Principal component analysis showed parameters shoot length (SL), root length (RL), biomass, Cr accumulation in root (Root Cr), Cr accumulation in shoot (Shoot Cr), chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll (Total Chl.), carotenoids (Car.), catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and protein content in *V. radiata* L under Cr stress. Cr, chromium; BC, biochar; TU, thiourea



antioxidative enzyme activities under Cr stress (Akhtar et al. 2023). On the other hand, the principal component analysis showed that both biochar and thiourea biochar supplementation decreased Cr stress in *V. radiata* (Fig. 7). This result is by who studied that thiourea-modified biochar significantly reduced (Kapoor and Zdarta 2024) the Cd and Pb toxicity in Chinese cabbage and improved the soil microorganism population.

Conclusion

The present study concluded that thiourea biochar amendment can increase the *V. radiata* growth under Cr stress and reduce Cr bioavailability. The Cr toxicity affected the seed germination, plant growth, biomass, and photosynthetic pigments and increases the antioxidative enzyme activities and Cr accumulation. The alleviation role of biochar and thiourea biochar supplementation in Cr-stressed *V. radiata* could enhance the seed germination, growth, biomass, and photosynthetic pigments and decrease the antioxidative enzyme activities. These results confirm the ROS level reduction in thiourea biochar combination treatment; in addition, thiourea biochar combination amendment inhibited the lipid peroxidation generation by controlling oxidative stress induced by Cr. Moreover, biochar and thiourea biochar additions limit the Cr bioavailability in the rhizosphere, accumulation, and translocation in *V. radiata* tissues and promote soil fertility. These results confirmed that the ROS generation is being mitigated by thiourea biochar amendment in plants under Cr-contaminated soil. These results together suggested that amendments of biochar and thiourea biochar could enhance the soil fertility and tolerance of *V. radiata* to Cr stress by activating the defence system. To the best of our knowledge, this is the first study that comprehensively describes the biochar alone and thiourea biochar combined effects on Cr stress alleviation mechanisms and accumulation in *V. radiata* and stabilization of Cr in agricultural soil.

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Author contribution Lavanya Muthusamy: writing—original draft—and data collection. Manikandan Rajendran: methodology and validation. Vivekanandan Krishnaswamy Ezhilan: conceptualization, review, and editing. Sabariswaran Kandasamy: methodology, supervision, review, and editing. Gayathri Kaliyannan: data analysis. Rathinam Raja: review and editing. Kavitha Ramamoorthy: writing—review and editing, investigation, and visualization.

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Data availability All data is available upon request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publication Not applicable.

Competing interests The authors declare no competing interests.

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